

# **Dietary Biopolymers: Fermentation Potentials of a Primitive Gut Ecosystem**

## **Dissertation**

To obtain the Academic Degree

Doctor rerum naturalium

(Dr. rer. nat.)

Submitted to the Faculty of Biology, Chemistry, and Geosciences  
of the University of Bayreuth

by

Lydia Zeibich

Place of Birth: Hagenow

Bayreuth, 2019



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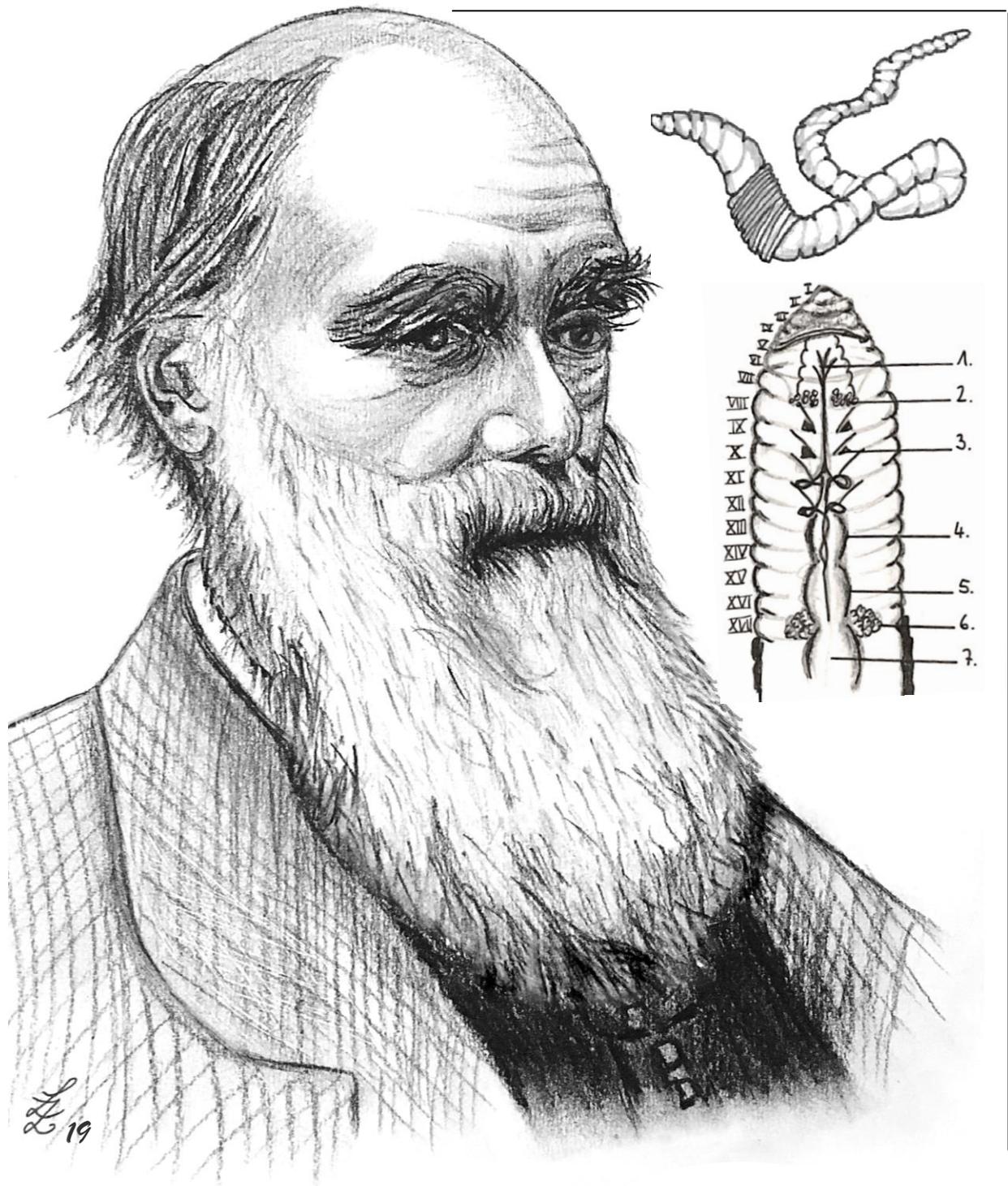
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**"It may be doubted if there are any other animals which have played such an important part in the history of the world as these lowly organized creatures."**

Charles R. Darwin



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## PUBLICATIONS

This dissertation is based in part on data and textual information in the following published peer reviewed papers and manuscripts submitted or in preparation:

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## ABBREVIATIONS

Ar	Argon
ATP	Adenosine triphosphate
B.C.	Before Christ
CBM	Cellulose binding module
cDNA	Complementary DNA
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
CoA	Coenzyme A
ddH <sub>2</sub> O	Deionized double distilled water
DW	Dry weight
e.g.	For example
EDP	Entner–Doudoroff pathway
EMP	Embden-Meyerhof-Parnas pathway
et al.	And others
Fd	Oxidized ferredoxin
Fd <sup>2-</sup>	Reduced ferredoxin
Fe <sup>3+</sup>	Ferric iron
FW	Fresh weight
ΔG	Gibbs free energy
GC	Gas chromatography
GPT	Group phylotype
H <sup>+</sup>	Proton
H <sub>2</sub>	Hydrogen
HPLC	High performance liquid chromatography
i.e.	That is
LCFA	Long chain fatty acid
LDA	Linear discriminant analysis
LefSe	Linear discriminant analysis effect size
N <sub>2</sub>	Dinitrogen
N <sub>2</sub> O	Nitrous oxide
Na <sup>+</sup>	Sodium ion
NAD <sup>+</sup>	Oxidized Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NMDS	Non-metric multidimensional scaling
NO <sub>3</sub> <sup>-</sup>	Nitrate
O <sub>2</sub>	Oxygen
P	Phosphate (as used in certain figures)
THF	Tetrahydrofolate

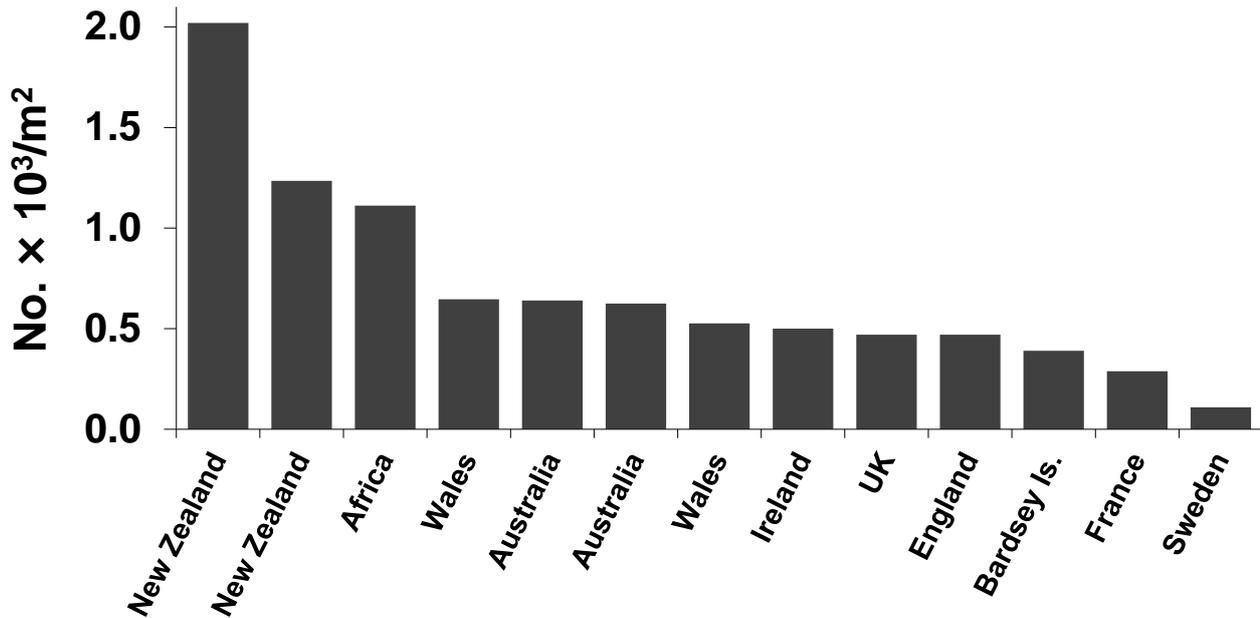
# 1. INTRODUCTION

## 1.1. Importance of earthworms

### 1.1.1. History of earthworms

Intestinal microbes are important to the performance and health of their animal hosts (Shreiner *et al.*, 2015; Blake and Suchodolski, 2016; Fohse *et al.*, 2016; Liang *et al.*, 2018). Based on fossil records, worm-like triploblastic metazoans and annelids existed 0.5 to 1.1 billion years ago (Seilacher, 1998; Morris and Peel, 2008). Aristotle (384 to 322 B.C.) was one of the first historically famous persons understanding the importance of earthworms in soil formation and maintenance of soil structure and fertility. He suitably called them “The Intestine of the Earth” (Yadav, 2017). Approximately three hundred years later Cleopatra VII (69 to 30 B.C.), one of the most famous female rulers in history, was fascinated by these inconspicuous soil creatures and declared them to be sacred after she recognized the strong contribution of earthworms to the Egyptian agriculture (Abul-Soud *et al.*, 2009; Yadav, 2017). At this time, the removal of earthworms from Egypt carried the death penalty (Abul-Soud *et al.*, 2009). However, until the late 1800s, when Charles Darwin published 1881 his book “The Formation of Vegetable Mould through the Action of Worms” (Darwin, 1881), earthworms were commonly underappreciated and considered as garden pest (Brown *et al.*, 2004). Darwin and his work brought finally widespread public attention to the central importance of earthworms in the maintenance of soil structure, aeration, drainage and fertility, including the decomposition of dead plant material and animal matter (Darwin, 1881; Brown *et al.*, 2004).

Soil fertility is defined as the capacity of soil to supply essential nutrients to crops and is strongly associated with the productivity of soils (Stockdale *et al.*, 2002), which is one of the most important aspects regarding the nutrition of 7.7 billion people on the planet, a number which increases year to year (<https://www.worldometers.info>). More than 98% of the world nutrition originates from terrestrial ecosystems (Schinner and Sonnleitner, 1996), demonstrating the importance of these ecosystems and the need for understanding the factors that influence their functions. An ecosystem can be defined as “a unit of interaction among organisms and between organisms and their physical environments, including all living things within a defined area” (Lewis, 1992). In this regard, the earthworm is one such factor that influence the functions of the terrestrial ecosystem. With up to 2,000 individuals per square meter, earthworms represent the most dominant macrofauna in many soils (Figure 1; Edwards and Bohlen, 1996), and their feeding habits result in substantial physical, chemical, and biological alterations of the terrestrial biosphere, including the turnover of elements and diverse effects on plant growth (Tomati *et al.*, 1988; Lavelle *et al.*, 1998; Brown *et al.*, 2000; Bastardie *et al.*, 2003). Since it is known that earthworms lead to alterations in physical structure, nutrient fluxes, and energetic status, earthworms are aptly called soil ecosystem engineers (Jones *et al.*, 1994; Lavelle *et al.*, 1998).



**Figure 1.** Abundance of earthworms in different pastures. A country listed twice represents two different samplings in that country. Figure based on numbers obtained from Edwards and Bohlen, 1996.

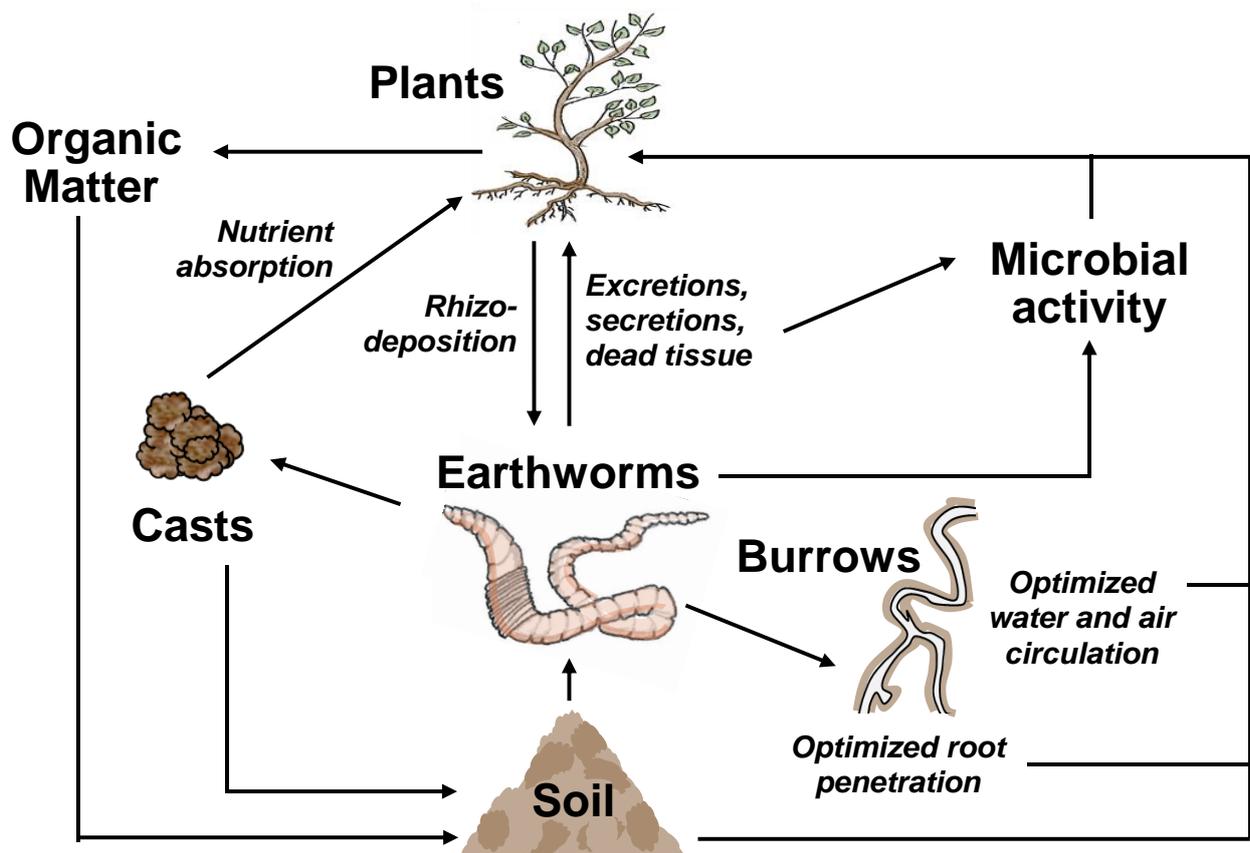
### 1.1.2. Earthworms and the turnover of elements

The important role of earthworms in the breakdown of complex organic matter, for example dead plant biomass and animal material, is attributable to their high abundance in many soils and their propensity to consume high amounts of their habitat (Edwards and Bohlen, 1996). Therefore, earthworms influence organic matter and nutrient cycles on four different levels: (a) during the gut passage, (b) in fresh earthworm cast, (c) in aging cast, and (d) during the long-term genesis of the soil profile (Lavelle and Martin, 1992). In this regard, ingested organic matter that passes through the earthworm gut is broken down into much smaller particles, resulting in a greater surface area of organic matter exposed to further microbial decomposition (Martin, 1991). Previous experiments demonstrated that a 90% decreased earthworm population results in a 43%, 30% and 32% increase of fine, coarse, and total particulate organic matter, respectively (Parmelee *et al.*, 1990). These findings indicate the positive correlation between the annelid biomass and the amount of decomposed organic matter, and furthermore illustrates the high importance of earthworms in the fragmentation and breakdown of complex organic material incorporated in the terrestrial biosphere. The effectivity of organic matter fragmentation and incorporation into soil is dependent on the different feeding habits of earthworms (Section 1.1.4). Anecic earthworms (e.g., *Lumbricus terrestris*) incorporate large amounts of organic matter into soil and are able to ingest large litter fragments by pickling off smaller pieces (Edwards and Bohlen, 1996). In contrast, epigeic and endogeic earthworms either do not incorporate organic matter into soil or feed only on already fragmented material (Ferrière, 1980; Judas, 1992). However, the concomitant occurrence of anecic and endogeic earthworms in many soils, suggesting a synergistic effect on the reallocation of organic matter in the soil profile (Shaw and Pawluk, 1986a, 1986b). Especially in the renewal of forests ecosystems, the mixing and

fragmentation of the litterfall by the activity of earthworms turned out as fundamentally important (Bernier and Ponge, 1994). Beyond that, by the repeated ingestion and turnover of soil and organic matter, earthworms (a) facilitate the rate of mineralization (a process defined as the conversion of organic forms from organic material to plant utilizable inorganic forms) (Edwards and Bohlen, 1996) and (b) enhance nitrogenous gas emission of soil and the nitrogen uptake by plants (Karsten and Drake, 1997; Matthies *et al.*, 1999; Borken *et al.*, 2000; Bertora *et al.*, 2007; Rizhiya *et al.*, 2007; Lubbers *et al.*, 2011).

### 1.1.3. Earthworms and the effect on plant growth

Earthworms share the soil environment with roots and the impact on plant growth and productivity is therefore unavoidable (Figure 2). These impacts on plant growth including root development and productivity can occur on three levels: physically, biologically, and chemically (Figure 2; Edwards, 2004). While the physical and chemical impact on plants is mostly indirect, the biological effect can be either direct or indirect.



**Figure 2.** Simplified model connecting the physical, chemical, and biological effects of earthworms on plant growth and nutrition. Figure modified from Edwards, 2004.

In more detail, earthworms have an indirect biological effect on plants when they (a) disperse or change the populations and activity of plant-beneficial microbes (e.g., plant promoting rhizobacteria or nitrogen fixing root symbionts), plant pests, parasites and pathogens (Dash *et al.*, 1980; Brown, 1995; Nakamura *et al.*, 1995; Brown, 1995; Anderson and Bohlen, 1998; Lavelle *et*

*al.*, 1998; Maraun *et al.*, 1999; Brown *et al.*, 2000), or (b) produce plant promoting or regulating substances (e.g., hormones and vitamins) (Gavrilov, 1963; Nielson, 1965; Harti *et al.*, 2001b, 2001a). In contrast, root abrasion, ingestion of living plant material or seeds, and burial of seeds by earthworms are examples of direct biological effects (Chen and Lui, 1963; Hameed and Bouché, 1993; Barrion and Litsinger, 1997; Brown, 1999). Furthermore, earthworm casts lead to aggregation and crust formation, whereas macropores (larger than 30  $\mu\text{m}$ ) caused by earthworm burrows, (a) enhance the aeration and erosion of soil, (b) facilitate the root infiltration and elongation, and (c) optimize the water retention (Figure 2; Blanchart *et al.*, 1997; Hirth *et al.*, 1997; Kretzschmar, 1998; Jiménez, 1999; Decaëns and Rossi, 2001). These are physical changes in soil structure that influence indirectly the plant growth, root development and productivity. The release or immobilization of plant nutrients, denitrification, and mineralization (processes that influence nutrient availability) can be enhanced by earthworm activities, and result in indirect chemical effects on plants (Barois *et al.*, 1999; Brussaard, 1999; Rangel *et al.*, 1999; Cortez and Hameed, 2001). Although earthworms have diverse positive effects on plant growth, and are of value for vermicomposting (Suthar and Singh, 2008; Domínguez *et al.*, 2010), the invasiveness of this invertebrate may have negative environmental consequences (Migge-Kleian *et al.*, 2006; Addison, 2009).

#### **1.1.4. Morphological features and feeding habits of earthworms**

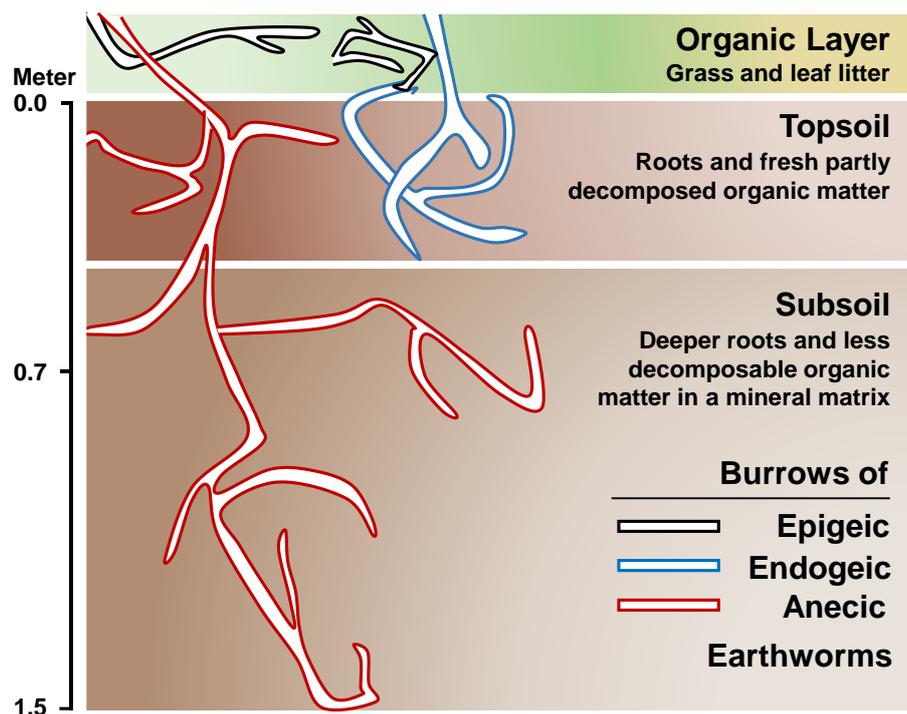
Earthworms (a) belong to the class *Oligochaeta*, consisting of approximately 800 genera and 8000 species, and (b) constitute up to 90% of invertebrate biomass in soil (Edwards, 2004). Dependent on the morphological features, habitats and feeding skills the burrows of earthworm can vary in volume, orientation, tortuosity, stability, and connectivity (Capowiez *et al.*, 2003; Bastardie *et al.*, 2005). Considering the different earthworm lifestyles, earthworms can be divided into three ecotypes, termed as epigeic, endogeic or anecic earthworms (Bouché, 1977).

The epigeic earthworms decomposing litter on the soil surface, whereby only small amounts of soil or no soil is ingested (Palm *et al.*, 2013). Epigeic earthworms are characteristic for their relative small size and heavy ventrally and dorsally pigmentation. Because these worms (a) feed mainly on fresh or partially decomposed litter in the upper organic layer (Figure 3) and (b) form only some horizontally burrow in the upper few centimeters of the top soil (Palm *et al.*, 2013), epigeic earthworms also called litter-dwellers and humus formers (Bouché, 1977; Perel, 1977). Furthermore, they are short lived, grow rapidly and exhibit relatively high reproduction rates (Edwards and Bohlen, 1996).

In contrast, anecic earthworms form humus while feeding on litter and soil (Perel, 1977). They are characteristic for pulling organic plant material into their large permanent and semi-permanent vertical burrow system. In this regard, the anecic earthworm *L. terrestris* is well known for removing significant quantities of litter from forest floors (Curry and Schmidt, 2007). Deduced from the fact that anecic earthworm burrows can extend several meters into the mineral subsoil

(Figure 3), they are called as deep-burrowers. Furthermore, they are relative large, and medium to heavy dorsally pigmented (Perel, 1977).

Endogeic earthworms consume, in contrast to anecic and epigeic earthworms, large amounts of mineral soil with preference for material rich in organic matter (e.g., dead roots) (Curry and Schmidt, 2007). Their activity leads to extensive sub-horizontal highly branched and less stable burrows in the upper 10 to 15 cm of top soil (Figure 3, Palm *et al.*, 2013). Endogeic earthworms are unpigmented or lightly pigmented, exhibit a medium size, and termed soil-dwellers or humus feeders (Perel, 1977; Edwards and Bohlen, 1996).



**Figure 3.** Burrow profile of the different earthworm ecotypes demonstrated at a cross section of soil. Figure based on information obtained from Fraser and Boag, 1998; Schelfhout *et al.*, 2017; Channarayappa and Biradar, 2019.

## 1.2. Alimentary canal of earthworms

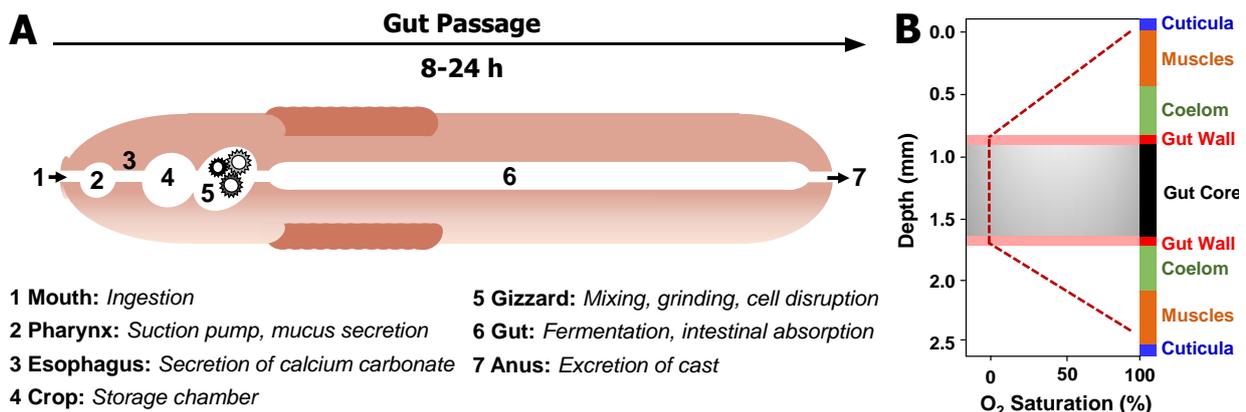
The structure of the alimentary canal of earthworms can be considered to be relatively simple. Nonetheless, these primitive invertebrates (Seilacher, 1998) have survived several extinction events (Barnosky *et al.*, 2011), illustrating in part the durable functionality of their gut ecosystem.

### 1.2.1. Sections of the alimentary canal, related functions, and conditions

The alimentary canal of *L. terrestris* is one of the best described earthworm alimentary canals (Laverack, 1963; Edwards and Fletcher, 1988; Edwards and Bohlen, 1996; Breidenbach, 2002; Doube and Brown, 2004; Storch *et al.*, 2009). Although the gut ecosystem of earthworms

can be considered primitive and less compartmented it can be divided into mouth, cavity, pharynx, esophagus, crop, gizzard, intestine and anus, whereby the simple intestine represent the largest proportion of the alimentary canal (Figure 4 A).

The alimentary passage begins with the ingestion of dietary material that is usually a mixture of plant material, microbes and soil, and ends with the excretion of casts (Edwards and Bohlen, 1996). The time for this passage varies from 8 to 24 h depending on the species of the earthworm and its feeding behavior (Parle, 1963a; Satchell, 1967; Wüst *et al.*, 2011). In more detail, the food enters the alimentary canal via the mouth and is transferred to the buccal cavity and pharynx, both located directly behind the mouth (Edwards and Bohlen, 1996). The pharynx operates as suction pump and facilitate the ingestion of food, whereas pharyngeal glands excrete protease-, glycoprotein-, amylase-, glycoside- and amino acids-containing mucus with several functions (Laverack, 1963; Martin *et al.*, 1987; Trigo *et al.*, 1999). Thus, the mucus (a) facilitates the transport of the relative dry ingested material through the gut system, (b) initiates the hydrolysis of several biopolymers (Urbášek and Pilž, 1991) and (c) activates ingested soil fermenters (Section 1.2.2; Brown *et al.*, 2000; Edwards, 2004; Huang and Xia, 2018).



**Figure 4.** Sections of the earthworm alimentary canal and their functions (A), and *in vivo* microsensor-derived O<sub>2</sub> profile of the midgut of *Lumbricus rubellus* (B). Panel A: Figure based on information obtained from Edwards and Bohlen, 1996. Panel B: The right axis identifies the anatomical regions of a cross section of the earthworm. The absence of detectable O<sub>2</sub> in the gut core of the alimentary canal (crop/gizzard, foregut, midgut, and hindgut) was confirmed with *Apporectoedeia caliginosa* and *L. terrestris* (Horn *et al.*, 2003; Wüst *et al.*, 2009b). Figure modified from Horn *et al.*, 2003.

Behind the pharynx is the esophagus with calciferous glands which produce calcium carbonate that is presumed to regulate (a) the pH and carbon dioxide (CO<sub>2</sub>) concentrations, and (b) potentially toxic cations (Dotterweich and Franke, 1936; Robertson, 1936; Crang *et al.*, 1968; Pearce, 1972; Bal, 1977). The crop, situated behind the esophagus and in front of the gizzard, is a thin-walled storage chamber transferring the material successively into the gizzard (Edwards and Bohlen, 1996). Before the ingested material enters the intestine it passes the gizzard, a hard muscular organ that abrasively mixes, grinds, and disrupts ingested material including plant material and large microbial cells (e.g., fungal hyphae [Kristúfek *et al.*, 1994; Schönholzer *et al.*, 1999]). Most of the digestion in the alimentary canal of earthworms occur in the oxygen (O<sub>2</sub>)-free intestine (Figure 4 B), an organ described as mutualistic system in which additional exoenzymes

are produced by ingested intact bacteria (Urbášek and Pilž, 1991; Drake and Horn, 2007). It is assumed, that the activity of the anaerobic gut microbiota (a) strongly enhance the degradation of ingested complex organic material during the gut passage, and (b) increase the capacity of the worm to absorb nutrients (Sampedro *et al.*, 2006; Drake and Horn, 2007). The gut passage ends with the re-absorption of the mucus and associated water, a process followed by the defecation of casts by the anus (Edwards and Bohlen, 1996).

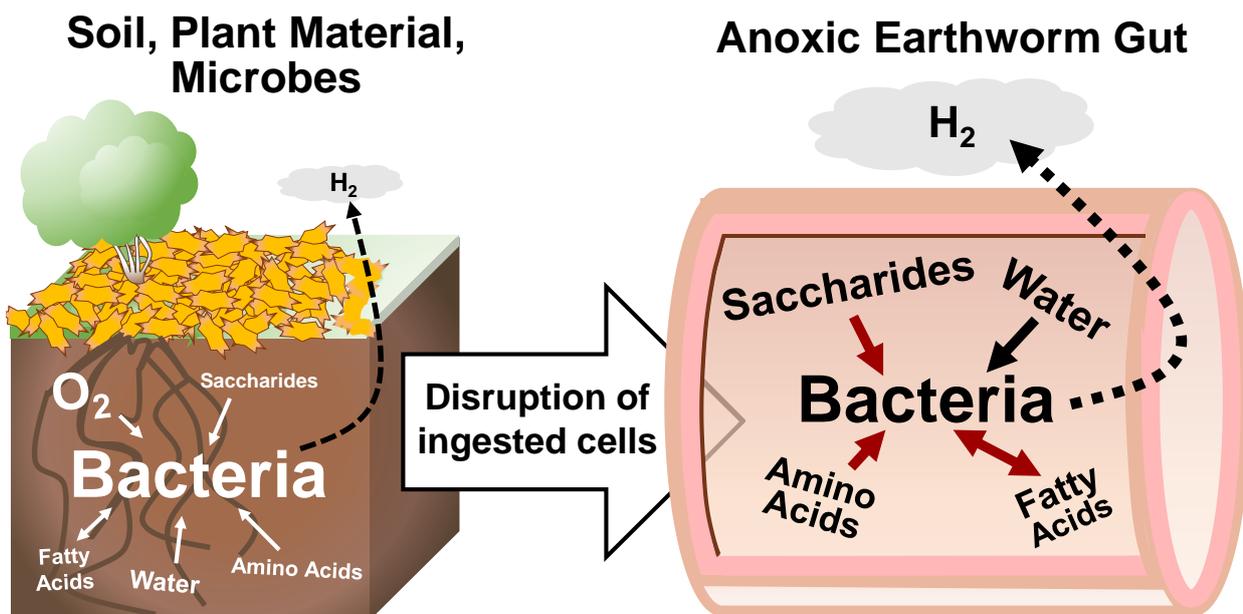
### 1.2.2. The earthworm gut microbiota

Soil contain one of the largest known microbial diversities, with a gram dry weight of soil containing approximately  $10^{10}$  microbial cells (Torsvik *et al.*, 1990; Whitman *et al.*, 1998), a number that illustrates a tremendous phylogenic and physiologic diversity. Furthermore, the cultivable number of soil-related facultative aerobes and anaerobes range from  $10^7$  to  $10^9$  per gram dry weight soil (Karsten and Drake, 1997; Küsel *et al.*, 1999), illustrating the large potential of earthworm-ingested microorganisms to facilitate anaerobic processes in the anoxic alimentary canal of the earthworm. In this regard, several molecular methods revealed similar bacteria in soil, the earthworm gut, and earthworm casts (Bassalik, 1913; Brown, 1995; Furlong *et al.*, 2002; Egert *et al.*, 2004). Although these findings about the nature of the gut microbiota suggest that most microbes in the earthworm are likely ingested and transient, the non-responsiveness of soil microbes to a specific high value gut nutrient and anoxia has made it difficult to demonstrate that responsive gut fermenters are derived from soil. However, other studies demonstrated that earthworms can also harbor potential bacterial symbionts that are strongly associated to this invertebrate and not detected in the earthworm-surrounding material (Pinel *et al.*, 2008; Nechitaylo *et al.*, 2010). Until today, only three such symbionts are recorded, including the *Mycoplasmataceae*-affiliated uncultured *Candidatus* Lumbricincola (Nechitaylo *et al.*, 2009).

Ingested aerated soil is relatively dry, nutrient-poor and exhibit high fluctuations in pH (e.g., pH 4.6 to 7.1; Drake and Horn, 2007). These conditions result in a low activity or a state of dormancy of prokaryotic cells (e.g., as cysts, starving cells or endospores) (Drake and Horn, 2007). In marked contrast, the gut content of earthworms, an anoxic microzone in soils, can reach a water content up to 80%, and is rich on diverse nutrients (Horn *et al.*, 2003; Drake and Horn, 2007). For example, total amino acids can be 170-fold greater in the gut than in soil, and the aqueous phase of the gut contains millimolar concentrations of diverse saccharides, whereas saccharide levels in soil are negligible (Figure 5; Horn *et al.*, 2003; Wüst *et al.*, 2009b). The detectable various water-soluble organic matter can be derived from (a) the breakdown of plant and microbial cells, or (b) the earthworm-produced mucus (Section 1.2.1).

Inactive facultative aerobes and anaerobes (e.g., bacilli and clostridia) are common in nutrient-poor soil (Slepecky and Leadbetter, 1984; Ovreås and Torsvik, 1998; da Silva *et al.*, 2003; Garbeva *et al.*, 2003) and their activation is induced by their ingestion and exposure to the nutrient richness in the anoxic earthworm gut ecosystem (Edwards and Bohlen, 1996; Brown *et*

*al.*, 2000; Drake and Horn, 2007). The maximum recorded densities of earthworms in soil theoretically yield up to 500 ml gut content per square meter of soil (Edwards and Bohlen, 1996; Schulz *et al.*, 2015), indicating the enormous capacity of this anoxic microzone to potentially stimulate high numbers of these soil microbes. In this regard, several anaerobic activities in the gut are related to the emission of nitrous oxide ( $\text{N}_2\text{O}$ ), dinitrogen ( $\text{N}_2$ ), and hydrogen ( $\text{H}_2$ ) by earthworms (Horn *et al.*, 2006a; Wüst *et al.*, 2009a; Depkat-Jakob *et al.*, 2012; Schulz *et al.*, 2015). However, fermentation is presumed to be the dominant anaerobic process in the gut, with the *in situ* amount of reducing equivalents (i.e., electrons) in fermentation-derived fatty acids being over one thousand-fold greater than the *in situ* amount of reducing equivalents in the denitrification-produced gases  $\text{N}_2\text{O}$  and  $\text{N}_2$  (Horn *et al.*, 2006b; Wüst *et al.*, 2009b). Especially the fermentative families *Aeromonadaceae*, *Enterobacteriaceae*, *Bacillaceae*, *Clostridiaceae*, *Lachnospiraceae* and *Peptostreptococcaceae* (a) play a central role in earthworm gut fermentation, and (b) produce a complex fermentation profile, including  $\text{CO}_2$ ,  $\text{H}_2$ , acetate, lactate, butyrate, formate, succinate, propionate, and ethanol (Wüst *et al.*, 2011; Meier *et al.*, 2018). Fermentation-derived fatty acids in the aqueous phase of the gut can exceed 30 mM (Wüst *et al.*, 2009b) and are, like in other animals, absorbed and utilized by the earthworm (Bergman, 1990; Drake and Horn, 2007; Wüst *et al.*, 2009b; Sampedro *et al.*, 2006), illustrating the trophic link between microbial gut fermentation and the earthworm. In this regard, the flow of electrons towards fermentation is essential for these invertebrates since microbial respiration would lead to the fully oxidation of the available organic carbon to  $\text{CO}_2$  and thus be disadvantageous for earthworm nutrition.



**Figure 5.** Hypothetical model illustrating the ingestion and activation of soil fermenters in the anoxic gut of earthworms. The relative concentration of compounds is indicated by the font sizes, and the relative effect of each compound on the production of  $\text{H}_2$  in the gut and its subsequent emission (Wüst *et al.*, 2009b) is indicated by the thickness of the arrow. Figure modified from Horn *et al.*, 2003.

### 1.3. Dietary biopolymers and their hydrolysis

Gut mucus is produced by the earthworm to aid passage of ingested material and can drive fermentation in the alimentary canal (Section 1.2.1) that is linked to the fermentative production of fatty acids that can be absorbed by the earthworm (Drake and Horn, 2007; Wüst *et al.*, 2009b; Sampedro *et al.*, 2006). Although reuse of mucus-derived organic carbon by the earthworm is advantageous, earthworms cannot self-perpetuate by this process. Thus, the sustenance and growth of earthworms is ultimately dependent on the ability of the animal to obtain nutrients from the environment. In this regard, the survival of the earthworm is linked to its consumption of diverse biomass, a feeding activity that affects plant growth and the turnover of organic matter in soil habitats (Section 1.1.2 and Section 1.1.3). These considerations are reinforced by *L. terrestris* that (a) ingests plant-derived biomass (e.g., roots, shoots, and litter) and soil that contains high amounts of microbial cells and (b) has the capacity to consume nearly the entire yearly litter fall; approximately 80 mg of leaves per gram fresh body weight can be incorporated on a daily basis (Needham, 1957; Raw, 1962; Satchell, 1967; Knollenberg *et al.*, 1985; Baylis *et al.*, 1986; Gunn and Cherrett, 1993, 1993). Thus, ingested biomass is subject to disruption during the passage through the crop/gizzard at the anterior portion of the alimentary canal (Section 1.2.1) (Kristůfek *et al.*, 1994; Schönholzer *et al.*, 1999). Furthermore, the potential occurrence of proteases, chitinases, cellulases and many other glycosidic enzymes in the gut (Tracey, 1951; Laverack, 1963; Mishra and Dash, 1980; Loquet and Vincelas, 1987; Edwards and Fletcher, 1988; Urbášek and Pilž, 1991; Lattaud *et al.*, 1997, 1998, 1999; Nozaki *et al.*, 2009) suggests a hydrolysis and utilization of ingested and ruptured plant- and microbial-derived biopolymers. However, little is known about the capacity of fermentative microbes in the earthworm gut to hydrolyze and utilize ingested biopolymers.

#### 1.3.1. Polysaccharides

Many polysaccharides ingested by the earthworm, like cellulose, pectin, and xylan, are produced as structural components of plant cell walls (Table 1). Cellulose and xylan constitute the hemicellulose which is embedded in amorphous pectin polymers and stabilized by structural proteins and phenolic compounds (Figure 7; Ochoa-Villarreal *et al.*, 2012). The main functions of the plant cell wall include (a) the conferment of stabilization, resistance, rigidity and protection of the cell, but also (b) the mediation of nutrients, gases and various intercellular signals to reach the plasma membrane (Ochoa-Villarreal *et al.*, 2012).

In addition, earthworms prefer to feed on microbe-rich material (Cooke and Luxton, 1980; Bonkowski *et al.*, 2000; Jayasinghe and Parkinson, 2009), and fungal hyphae as well as larger bacterial cells are subject to digestion in the earthworm gut (Kristůfek *et al.*, 1994; Schönholzer *et al.*, 1999), indicating that chitin and peptidoglycan are other potential dietary structural polysaccharides. Plant biomass and microbial cells can also contain non-structural energy storage polysaccharides (e.g., starch and glycogen) that could constitute an additional source of

fermentable carbohydrates in the gut after disruption. Furthermore, all microbial and plant cells are surrounded by a phospholipid-bilayer membrane that introduces lipids to the alimentary canal.

**Table 1.** Potentially ingested polysaccharides and the most abundant backbone subunits (Figure 6) from which they are composed.

Polysaccharide	Dry Weight (%)	Subunit	Bond <sup>d</sup>	Reference <sup>e</sup>
<b>Structural<sup>a</sup></b>				
Cellulose	50	Glucose	beta-1,4-glycosidic	1
Peptidoglycan	70	NAG <sup>c</sup> , NAM <sup>c</sup>	beta-1,4-glycosidic	2
Chitin	20	NAG <sup>c</sup>	beta-1,4-glycosidic	3
Pectin	35	Galacturonic Acid	alpha-1,4-glycosidic	1
Xylan	30	Xylose	beta-1,4-glycosidic	1
<b>Non-structural<sup>b</sup></b>				
Starch	30	Glucose	alpha-1,4-glycosidic	4
Dextran	-	Glucose	alpha-1,6-glycosidic	5
Glycogen	50	Glucose	alpha-1,4-glycosidic	6

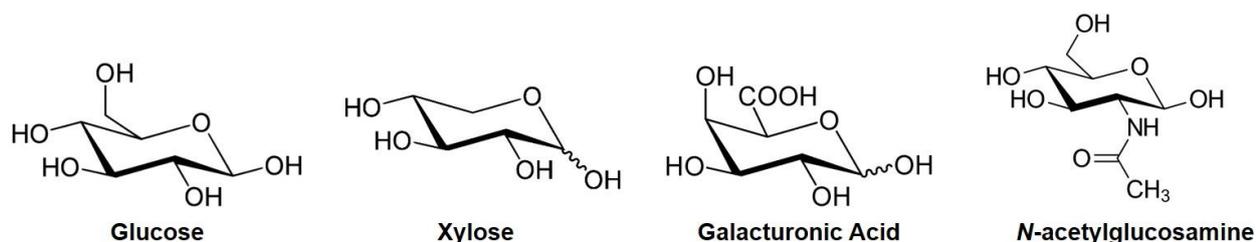
<sup>a</sup>Values of dry weight reflect the maximum amount of the respective structural polysaccharide that was detected in plant or microbial cell walls.

<sup>b</sup>Values of dry weight reflect the maximum amount of the respective non-structural polysaccharide that was detected in plant or microbial cell biomass. Dextran is an extracellular polysaccharide and therefore not quantified.

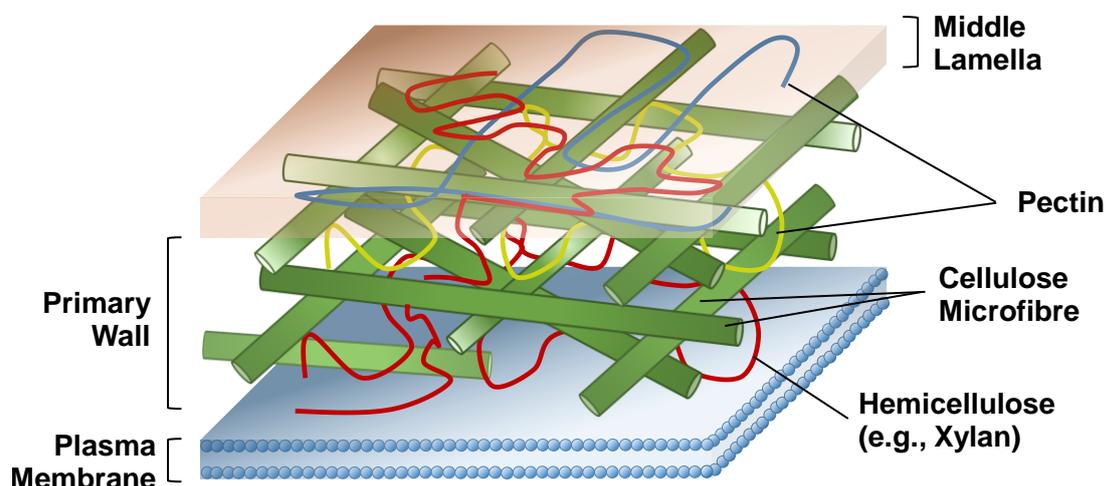
<sup>c</sup>NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid.

<sup>d</sup>Only the most abundant and characteristically bonds were prioritized.

<sup>e</sup>Table based on information obtained from: 1, Fry, 1988; 2, Schleifer and Kandler, 1972; 3, Bowman and Free, 2006; 4, Gravatt and Kirby, 1998; 5, Khalikova *et al.*, 2005; 6, Iglesias and Preiss, 1992.



**Figure 6.** Chair conformations of the dominant backbone-forming subunits in polysaccharides (Table 1). Modified from Dewick, 2006; Langan *et al.*, 2014; Yuzwa and Vocadlo, 2014; Rautiainen *et al.*, 2015.



**Figure 7.** Simplified model of the primary plant cell wall. Figure modified from Xing *et al.*, 2018.

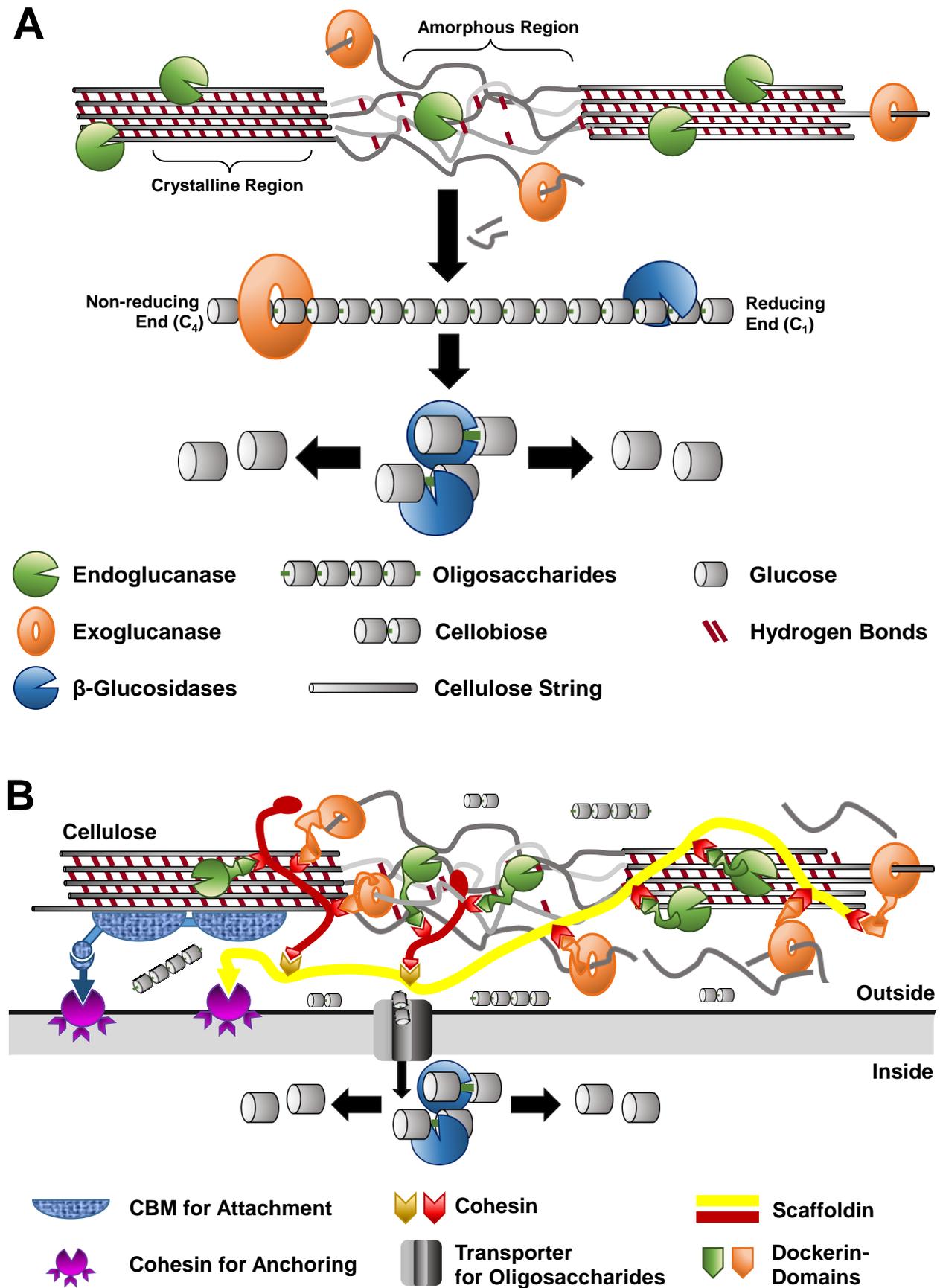
### 1.3.1.1. Structural polysaccharides

#### Cellulose

Cellulose, the most abundant organic biopolymer on earth, is the primary cell wall polymer of plants (Klemm *et al.*, 2005). Chemically, cellulose is a linear beta-1,4-glycosidic linked D-glucan and insoluble (Moon *et al.*, 2011). Whereas glucose is known as the chemical repeating unit, the disaccharide cellobiose is determined as the structural repeating unit of cellulose. The remarkably stability of cellulose attest to the high tendency to form intra- and intermolecular hydrogen bonds between the glucose subunits (Pinkert *et al.*, 2009). These chemical interactions between the individual glucose subunits result in chain formation and aggregation into microfibrils that contain crystalline and amorphous regions (Figure 8; Flint *et al.*, 2008). Dependent on the kind of plant (e.g., hardwood or softwood) and the part of the plant (e.g., leaf, root or stem), the cellulose content can be highly variable (Sjörström, 1993; Smole *et al.*, 2005). For example, the leaves of the model plant *Arabidopsis thaliana* contain a cellulose content of 15%, whereas stem walls of the same plant species contain twice as much cellulose (Smole *et al.*, 2005).

The microbial degradation of cellulose requires the production of different hydrolytic cellulases which belong to the broad superfamily of glycosidases. Cellulases, divided into endo- and exo-glucanases, are specialized to hydrolyze the beta-1,4-glycosidic bonds in cellulose (Bayer *et al.*, 2013). Especially amorphous regions and defects in the crystalline structure were preferred to initiate the process of hydrolysis, reflecting the dependence on accessibility. Based on the structure of endo- and exo-glucanases, endo-acting cellulases are able to produce a new end in the internal proportion of the cellulose chain (Figure 8 A). These ends are than accessible for exo-acting cellulases which cleaving activity leads to the release of the disaccharide cellobiose. The beta-1,4-glycosidic linkage of cellobiose molecules is hydrolyzed by beta-glucosidases, a process resulting in two single glucose molecules (Figure 8 A). Cellulases are typical for cellulose depolymerizing aerobic fungi that are environmentally important to the recycling of plant biomass (Green III and Highley, 1997). For example, the brown-rot fungi (lignin left behind) *Fomitopsis palustris*, *Laetiporus sulphureus*, and *Wolfiporia cocos* are even able to degrade the difficult to access crystalline regions of cellulose (Machuca and Ferraz, 2001; Yoon and Kim, 2005).

In anaerobic microorganisms, the necessary enzymes for cellulose degradation can be cohered and anchored to the microbial membrane as cellulosome, a multienzyme complex first described for *Clostridium thermocellum* (Bayer *et al.*, 1983). This arrangement may have evolved to ensure a more efficient and economic degradation of insoluble polymers and to decrease the competition with other microorganisms for the soluble products of hydrolysis. Cellulosomes can be diverse, but generally consist of a polymer attachment domain, several scaffoldins, and a cohesion-dockerin system that includes the enzymatic active biopolymer hydrolyzing enzymes (Figure 8 B; Flint *et al.*, 2008; Bayer *et al.*, 2013).



**Figure 8.** Simplified model of cellulose-degrading enzymes with different activities (A) and exemplary arrangement of these enzymes in cellulosomes of anaerobic bacteria (B). CBM, cellulose-binding module. Figure based on information obtained from Flint *et al.*, 2008; Bayer *et al.*, 2013.

The interactions of these different compounds guarantee the attachment and anchoring of the hydrolyzing enzymes to the microbial cell wall that prevents the loss of these enzymes. Cellulosomes are widespread within the group of anaerobic microorganism and can additionally contain other glycoside hydrolases than cellulases (e.g., xylanases and mannanases) that optimizes the degradation of other plant-derived polymers (e.g., hemicellulose and pectin) (Flint *et al.*, 2008; Bayer *et al.*, 2013).

## Pectin

Pectin can consist of 17 different monosaccharides linked with more than 20 different bonds and is therefore likely the most complex macromolecule in nature (Voragen *et al.*, 2009). It belongs to the most abundant plant polysaccharides and is localized in the middle lamella which is situated between the primary and secondary plant cell wall (Figure 7; Xing *et al.*, 2018). Like other plant polysaccharides, the pectin content show high variations between different plant species and parts of the plant. Thus, grasses and wood tissues exhibit approximately 2 to 10% of pectin, whereas in dicotyledonous, the pectin content can be up to 35% (Fry, 1988). Pectin is insoluble and consists of approximately 70% of galacturonic acid molecules (Figure 6), that are connected via alpha-1,4-glycosidic bonds and form the pectin backbone (Sundar Raj *et al.*, 2012). In recent years, several repeating structural elements of pectin have been characterized. Although these structural elements can vary slightly, it is assumed that all pectins are composed of these elements (Voragen *et al.*, 2009). For example, homogalacturonan can constitute approximately 60% of pectin and is therefore the most dominant structural element (Mohnen, 2008). Based on the order of frequency (highest to lowest), Xylogalacturonan, Rhamnogalacturonan I, Rhamnogalacturonan II, Arabinan, Arabinogalactan I, and Arabinogalactan II are additional structural elements (Mohnen, 2008). The alpha-1,4-galacturonic acid backbone of homogalacturonan can be methyl esterified or acetylated (Gee *et al.*, 1959; Mort *et al.*, 1993). Non-esterified galacturonic residues are sensitive to calcium ion cross linkages (Garnier *et al.*, 1994) that are, among other linkages, responsible for the stability of pectin. Arabinose, rhamnose, and xylose are examples of other structural repeating elements that can exhibit a large number of different site groups (e.g., methanol, acetyl or ferulic acid), bonds, and cross linkages (Voragen *et al.*, 2009).

The complex structure of pectin affects the number of different enzymes that are necessary for an efficient hydrolysis. The cleavage of the alpha-1,4-linked galacturonic acids backbone requires endo- and exo-polygalacturonase. The activity of these enzymes is influenced by the diverse aforementioned site groups. For example, (a) increasing amounts of methyl-esterifications can lead to a concomitant decrease of endo-polygalacturonase activity (Pařenicová *et al.*, 2000), and (b) rhamnogalacturonan hydrolase that cleaves alpha-1,4-galacturonic acid / alpha-1,2-rhamnose linkages exhibits a intolerance for acetyl-esterifications (Kauppinen *et al.*, 1995).

## Xylan

Xylan is a primary component in the hemicellulose of plant cell walls. The basic structure of xylan is a backbone of beta-1,4-linked xylose subunits (Figure 6; Timell, 1967; Saha, 2003; Smith *et al.*, 2017). This structural polysaccharide is insoluble and can be, like pectin, highly acetylated or extended by diverse glycosidic bonds with several polymeric side chains of arabinose, mannose, galactose, or ferulic acid (Timell, 1967; Saha, 2003; Smith *et al.*, 2017). The efficient degradation requires a complex subset of different enzymes with contrasting activities. Whereas endo-xylanases hydrolyze the beta-1,4 bonds inside the xylose backbone, exo-xylanases cleave glycosidic bonds at the end of the chain and ensure the availability of the disaccharide xylobiose, that can be converted to single xylose molecules via the beta-xylosidase (Saha, 2003). Furthermore, enzymes like alpha-arabinofuranosidase, alpha-glucuronidase, ferulic acid esterase or acetylxylan esterase are necessary to cleave the variable glycosidic bonds between the saccharides of the diverse side chains (Saha, 2003).

## Chitin

Chitin, a insoluble polymer of *N*-acetylglucosamine chains with beta-1,4-glycosidic bonds and inter-chain hydrogen bondings, is the second most dominant polymerized carbon in nature and chemical similar to cellulose (Einbu, 2007; Zargar *et al.*, 2015). This structural polysaccharide is (a) produced by molluscs, crustaceans, insects, algae, and fungi, and (b) comply the same functions that are known for cellulose in plants (Neville and Luke, 1969; Peters, 1972; Childress and Nygaard, 1974; Kapaun and Reisser, 1995; Fesel and Zuccaro, 2016). The cell walls of filamentous fungi, in which chitin is situated directly on the cell membrane, can contain 10 to 20% of chitin (Bartnicki-Garcia, 1968; de Nobel *et al.*, 2000).

Several studies demonstrated that earthworms, including *L. terrestris*, exhibit a feeding preference for certain filamentous fungi (Cooke and Luxton, 1980; Bonkowski *et al.*, 2000; Jayasinghe and Parkinson, 2009). The detection of disrupted soil fungi in cast and gut contents (Domsch and Banse, 1972; Dash *et al.*, 1986; Tiwari *et al.*, 1990; Kristůfek *et al.*, 1994; Schönholzer *et al.*, 1999; Wolter and Scheu, 1999) is consistent with this assumption and suggest the occurrence of fungi-derived chitin in the earthworm gut.

Chitinases are produced by a wide range of organisms (e.g, bacteria, fungi, insects, higher plants, animals), but also in biological agents like viruses (Aam *et al.*, 2010; Hartl *et al.*, 2012). The degradation of chitin is strongly related to that of cellulose (Yan and Fong, 2015). Thus, whereas endo-chitinase cleave the glycosidic bonds within an *N*-acetylglucosamine chain, the exo-chitinases (e.g., *N*-acetyl-beta-glucosaminidase and chitobiosidase) cleave the chitin molecule at a terminal position (Yan and Fong, 2015). The released *N*-acetylglucosamine molecules (Figure 6) are then deacetylated and phosphorylated to glucosamine-6-phosphate that is deaminated and converted to fructose-6-phosphate, a metabolic intermediate of the glycolysis (Yan and Fong, 2015; Section 1.4.1). The occurrence of anaerobic microbial degradation of chitin in soil slurry (Wieczorek *et al.*, 2014), the detection of chitinases in the earthworm gut (Tracey,

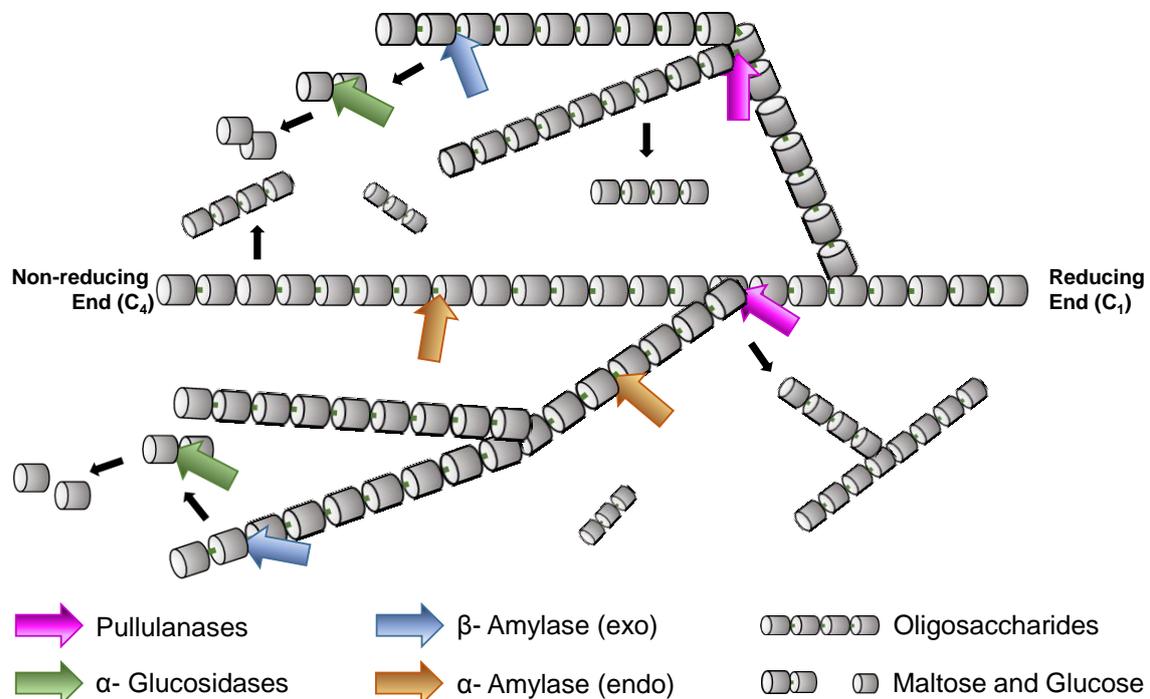
1951; Laverack, 1963; Edwards and Fletcher, 1988; Tiwari *et al.*, 1990), and the expression of an chitinase encoding gene in the gut tissue of earthworms (Kim *et al.*, 2016), suggest a potential chitin hydrolysis in the alimentary canal of earthworms.

### 1.3.1.2. Non-structural polysaccharides

#### Starch

Starch, a non-structural polysaccharide, serves in plants as energy storage polymer and is produced in the leaves, seeds, fruits, stems, and roots (Fraser-Reid *et al.*, 2008; Bertoft, 2017). It consist of glucose subunits that are connected via alpha-1,4-glycosidic bonds, forming approximately 25 % linear amylose and approximately 75% alpha-1,6-branched amylopectin (Fraser-Reid *et al.*, 2008; Bertoft, 2017).

Although starch is a non-structural polysaccharide, the degradation is similar to that of cellulose and chitin, and starch-hydrolyzing amylases can be classified in endo-enzymes, exo-enzymes, and dimer cleaving glucosidases (Figure 9; Fraser-Reid *et al.*, 2008; Horstmann *et al.*, 2017). However, in contrast to the linear chains of structural polysaccharides cellulose and chitin, the efficient hydrolysis of the branched amylopectin requires pullulanases that cleave the alpha-1,6-branches (Fraser-Reid *et al.*, 2008; Horstmann *et al.*, 2017). The hydrolysis of starch leads to a mix of oligomers, also known as maltodextrin (Wang and Wang, 2000).



**Figure 9.** Model of starch degrading enzymes and their activities. Figure based on information obtained from Horstmann *et al.*, 2017.

In nature, amylose and amylopectin form intermolecular and intramolecular hydrogen bonds as well as hydrophobic bonds that hold the molecules together, a process resulting in water-insoluble granules (Fraser-Reid *et al.*, 2008; Horstmann *et al.*, 2017). The amount of starch can

be highly variable between different plants and their parts. For example, starch can constitute approximately 30% of the dry weight of roots obtained from seedling of hardwood trees (Gravatt and Kirby, 1998), whereas the leaves of clover exhibit only a maximum starch content of approximately 5% (Cave *et al.*, 1981). However, especially the occurrence of fine roots in the digestion tract of earthworms (Baylis *et al.*, 1986; Gunn and Cherrett, 1993) reinforce the likelihood that starch might occur in the gut and is therefore subject to hydrolysis and fermentation during the gut passage. Detected amylases in the gut of earthworms (Laverack, 1963; Edwards and Fletcher, 1988; Tiwari *et al.*, 1990), corroborate the assumption that starch is a potential substrate for gut-associated fermenters.

## Glycogen

Several bacteria and fungi can produce substantial amounts of intracellular glycogen, a polymer of glucose subunits with alpha-1,4-glycosidic bonds (Table 1; Holme *et al.*, 1956; Iglesias and Preiss, 1992). Although glycogen is similar to amylopectin it exhibit twice as much alpha-1,6-branches and no intermolecular bonds (Fraser-Reid *et al.*, 2008). The lack of the intermolecular bond results in the absence of crystallinity, that enable the high water solubility of glycogen (Fraser-Reid *et al.*, 2008). That the degradation of glycogen requires the same enzymes as in the starch hydrolysis reflects the structural similarity of these both non-structural polysaccharides. In this regard, pullulanases cleave the alpha-1,6-branches, whereas amylases degrade the chain of alpha-1,4-linked glucose molecules (Djekrif *et al.*, 2016). The resulting disaccharide maltose is hydrolyzed to two glucose molecules by alpha-glycosidases (Djekrif *et al.*, 2016). Glycogen can constitute approximately 20% of the dry weight of microbial cells (Table 1; Roach *et al.*, 2012), and is therefore a potential earthworm-ingested substrate that is released by the activity of the gizzard (Section 1.1.2), and might be therefore subsequently hydrolyzed and fermented by the gut microbiota.

## Dextran

Earthworm-ingested polysaccharides could also include microbial dextran, a polymer of glucose subunits mainly linked by alpha-1,6-glycosidic bonds (Naessens *et al.*, 2005) that has multiple functions including adhesion, protection, and extracellular energy storage (Khalikova *et al.*, 2005). Several strains of *Leuconostoc mesenteroides* (Jeanes *et al.*, 1954), *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus salivarius* use dextransucrases to synthesize dextrans from sucrose (Robyt, 1995). These non-structural polysaccharides are mainly branched by alpha-1,3-glycosidic linkages. In certain *L. mesenteroides* strains, these branches are formed by alpha-1,2- and alpha-1,4-glycosidic linkages (Fraser-Reid *et al.*, 2008).

The efficient degradation of dextran requires diverse dextransases that can be divided, like the other polysaccharide degrading enzymes, into endo- and exo-enzymes. These dextransases causing the release of different hydrolysis products, like glucose, isomaltose, or isomaltotriose (Khalikova *et al.*, 2005). The isomaltose-forming exo-dextranase cleaves alpha-1,6- linkages, but

also alpha-1,2- and alpha1,3- and alpha-1.4-linkages. However, dextranase activities can be also specific, for example, the alpha-1,2-glucosidase found in *Flavobacterium* sp. is strictly specific for alpha-1,2- glycosidic branches (Khalikova *et al.*, 2005).

### 1.3.2. Proteins

The ingestion of plant and microbial cells is linked to the abrasive action of the gizzard that ensures the disruption of larger cells (Section 1.2.1) and thus the release of diverse biopolymers into the earthworm gut. Protein is the primary component of microbial cells and can constitute up to 50% of microbial biomass on a dry weight basis (Table 2; Babel and Müller, 1985; Lange and Heijnen, 2001; Delgado *et al.*, 2013).

**Table 2.** Potentially ingested plant- and microbial-derived biopolymers and the most abundant subunits from which they are composed. Table excludes polysaccharides (see Table 1).

Biopolymer	Dry Weight <sup>a</sup> (%)	Subunit	Bond <sup>b</sup>	Refer- ence <sup>d</sup>
<b><i>Plant-derived</i></b>				
Proteins	25	Amino Acids	peptide	1
Lipids <sup>c</sup>	5	Glycerol, LCFA	ester	2
Nucleic Acids	2	Ribose/Deoxyribose, Nucleobases	phosphodiester- and glycosidic	2
Lignin	36	Phenylpropane	beta-O,4-ether	3, 4
<b><i>Microbial-derived</i></b>				
Proteins	50	Amino Acids	peptide	5, 6, 7
RNA	20	Ribose, Nucleobases	phosphodiester- and glycosidic	6, 7
Lipids <sup>c</sup>	10	Glycerol, LCFA	ester and phosphodiester	6, 7
DNA	3	Deoxyribose, Nucleobases	phosphodiester and glycosidic	6, 7

<sup>a</sup>Values of dry weight reflect the maximum amount of the respective biopolymer that was detected in cell biomass. The maximum amount of lignin is based on cell wall analysis.

<sup>b</sup>Only the most abundant and characteristically bonds were prioritized.

<sup>c</sup>Subunits and bonds concerning glycerophospholipids. LCFA, long chain fatty acids.

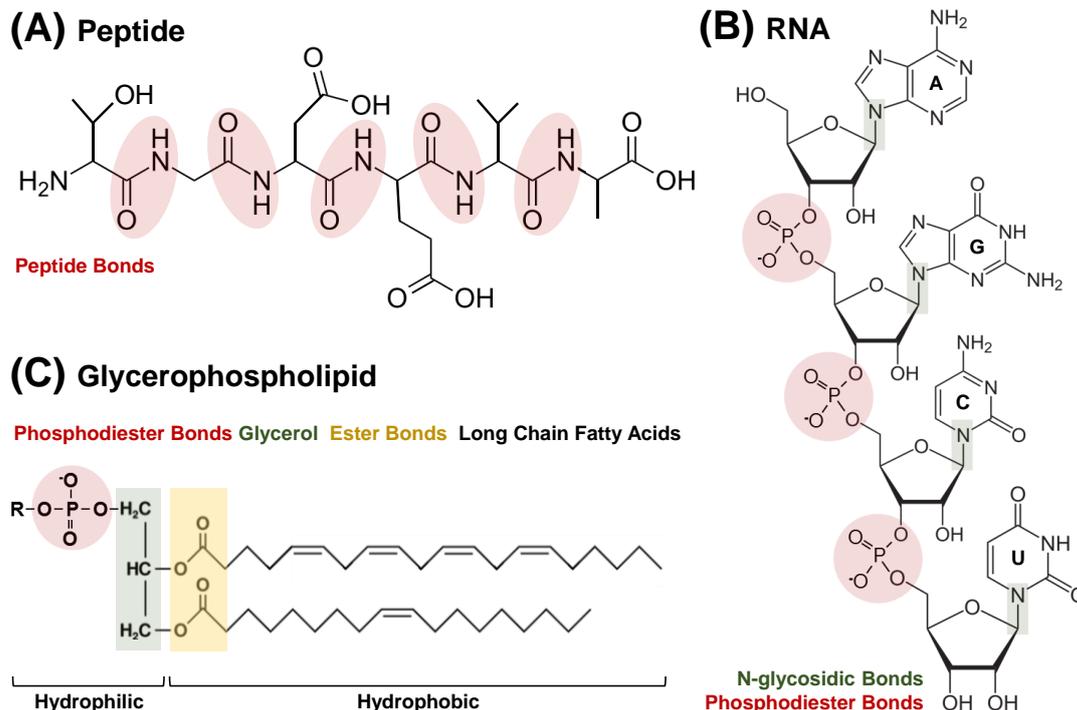
<sup>d</sup>Table based on information obtained from: 1, Andrews *et al.*, 2006; 2, Schink, 1999; 3, Campbell and Sederoff, 1996; 4, Dolgonosov and Gubernatorova, 2010; 5, Lange and Heijnen, 2001; 6, Delgado *et al.*, 2013; 7, Neidhardt *et al.*, 1996.

Indeed, nearly 2 mM amino acids may occur in the aqueous phase of the earthworm gut (Horn *et al.*, 2003) reinforcing the likelihood that protein hydrolysis in the gut yields amino acids that are subject to consumption during gut passage. In this regard and on the assumption that (a) the cytoplasm of a microbial cell is 80% water and on a dry weight basis contains 50% protein,

and (b) the average molecular weight of a representative amino acid in protein is 100, this amount of protein would theoretically yield 1 M polymeric amino acids in the cytoplasm of a gizzard-disrupted cell. As such, a microbial cell in this location of the alimentary canal (i.e., in the immediate vicinity of a ruptured cell) could experience a short-lived ‘tidal wave’ of peptides (Figure 10 A).

The detection of protein and proteases in the anterior part of the alimentary canal and the decrease of protein during the gut passage reinforce the likelihood of protein breakdown to peptides and single amino acids in the alimentary canal of earthworms (Laverack, 1963; Mishra and Dash, 1980; Edwards and Fletcher, 1988; Tillinghast *et al.*, 2001). The earthworm-produced gut mucus also contains proteins that additionally enhances the availability of these components.

Although proteases exhibit a huge diversity in action and structure, they can be classified, like other biopolymer degrading enzymes, into exo- and endo-acting, two subdivisions depending on the cleaving position which appears either at the terminal end (exo-) or within (endo-) the amino acid chain (Rao *et al.*, 1998). Furthermore, proteases can be categorized by their functional group at the active site. For example, serine proteases harbor a serine group in their active site, whereas metalloproteases are dependent on a metal ion for their activity.



**Figure 10.** Exemplary structure and bonds of peptides (A), RNA (B), and glycerophospholipids (C). Panel A: Amino acid sequence from left to right: Thr-Gly-Asp-Glu-Val-Ala. Based on the amino acid conformations in Dewick, 2006. Panel B: A, adenine; G, guanine; C, cytosine; U, uracil. Modified from Gonzalez-Ruiz *et al.*, 2011. Panel C: Arachidonic acid and oleic acid as attached long chain fatty acids. Modified from Timberlake, 2003.

### 1.3.3. Nucleic Acids

Nucleic acids occur in all living cells and are necessary for the long-term storage of genetic information, coding, decoding, regulation and expression of genes (Madigan *et al.*, 2015). In

consideration of the abundance of DNA and RNA in plant and microbial cells, RNA proves as the more dominant component, constituting up to 20% of microbial biomass on a dry weight basis (Babel and Müller, 1985; Delgado *et al.*, 2013), whereas DNA constitute less than 3% (Table 2; (Neidhardt *et al.*, 1996; Delgado *et al.*, 2013; Schink, 1999). The relative high abundance of RNA in microbial cells, the disruption of larger microbial cells by the gizzard (Section 1.2.1; Kristůfek *et al.*, 1994; Schönholzer *et al.*, 1999), and the production of extracellular RNases by soil microbes (Greaves and Wilson, 1970; Mishra *et al.*, 2017) reinforce the likelihood that RNA is subject to hydrolysis and fermentation during gut passage.

RNA, a biopolymer of an ribose-phosphate backbone and attached purines and pyrimidines (Figure 10 B), differ from double-stranded DNA in the three following points: (a) RNA is single stranded, (b) the ribose of the backbone contains a hydroxyl group that is attached to the second carbon atom, and (c) thymine is replaced by uracil (Lehninger *et al.*, 2008; Madigan *et al.*, 2015). The fermentation of RNA is dependent on its initial degradation by hydrolytic or phosphorolytic RNases that yield monophosphorylated or diphosphorylated nucleotides, respectively, which can be further metabolized and yield ribose (in either a phosphorylated or non-phosphorylated form), purines, and pyrimidines (Deutscher, 2006).

### 1.3.4. Additional

#### 1.3.4.1. Lignin

Lignin is an aromatic polymer, located in the primary cell wall and constituting up to 36% of hardwood (Table 2; Campbell and Sederoff, 1996). It is highly branched and consist of aromatic phenylpropane subunits that are randomly linked via carbon-carbon and ether bonds (Dolgonosov and Gubernatorova, 2010). The bond energy of an ether bond is 360 kJ/mol and the microbial cleavage is therefore a difficult barrier for biodegradation that effects the biological mineralization (White *et al.*, 1996; Blanksby and Ellison, 2003). In this regard, the microbial degradation of lignin and aromatic compounds was thought, for a long time, to be strictly aerobic (Evans, 1963; Ornston and Stanier, 1964), whereby O<sub>2</sub> is required as terminal electron acceptor and for hydroxylation and ring fission (Sugumaran and Vaidyanathan, 1978; Colberg, 1988). In this regard, aerobic basidiomycetes (fungi in the division *Basidiomycota*) are efficient degraders of lignin and environmentally very important to the overall turnover of plant biomass (Eggert *et al.*, 1997). For example, the white-rot fungi (cellulose left behind) *Dichomitus squalens*, *Phanerochaete chrysosporium*, *Pycnoporus cinnabarinus*, and *Sporotrichum pulverulentum* are able to degrade lignin and its aromatic constituents by producing laccases, phenol oxidases, and peroxidases (Ander and Eriksson, 1976; Gold *et al.*, 1989; Périé and Gold, 1991; Eggert *et al.*, 1997; Arora and Gill, 2000).

Nonetheless, the anaerobic degradation of lignin-derived aromatic compounds was first described in 1934, when lignin was converted after 600 days to CO<sub>2</sub> and methane (CH<sub>4</sub>) (Boruff and Buswell, 1934). Subsequent studies confirmed that the anaerobic decomposition of

monoaromatic lignin can yield CO<sub>2</sub> and CH<sub>4</sub>, and that the overall decomposition is dependent on methanogens (Kaiser and Hanselmann, 1982; Colberg, 1988). Additionally, lignin-derived monoaromatic compounds can also be utilized by nitrate- and sulfate reducers (Widdel, 1980; Colberg, 1988; Oshima, 2007; Philipp and Schink, 2012). Gut-associated lignin degradation appears to be dependent on specialized compartmented highly evolved gut ecosystems (e.g., from ruminants and termites) (Akin and Benner, 1988; Brune, 2013). In this regard, the primitive earthworm gut and the relative short gut passage time (Section 1.2.1) most likely complicate the microbial-derived degradation and utilization of earthworm-ingested lignin.

#### 1.3.4.2. Lipids

Lipids are hydrophobic, water insoluble and extremely diverse (Subramaniam *et al.*, 2011). Biopolymers are defined as organic macromolecules that are composed of repeating monomers and produced by living organisms (Stal, 2011). However, lipids are in general esters of fatty acids (Figure 10 C) and, per the aforementioned definition, not biopolymers (Fahy *et al.*, 2009; Subramaniam *et al.*, 2011). However, these macromolecules are important for energy storage as well as cell membrane structure and function (Subramaniam *et al.*, 2011). Lipids are present in all living cells and can constitute up to 10% of a microorganism (Neidhardt *et al.*, 1996; Delgado *et al.*, 2013; Valenzuela and Valenzuela, 2013). The degradation is ensured by lipases, a subclass of esterases (Jaeger *et al.*, 1994). The high amounts of ingested soil-derived microorganism (Torsvik *et al.*, 1990; Whitman *et al.*, 1998) and the detection of lipases in the earthworm gut (Laverack, 1963) suggest a lipid breakdown during the earthworm gut passage. The available components of the lipids, for example glycerol or glycerophospholipids, can be potentially used by the fermentative microbiota.

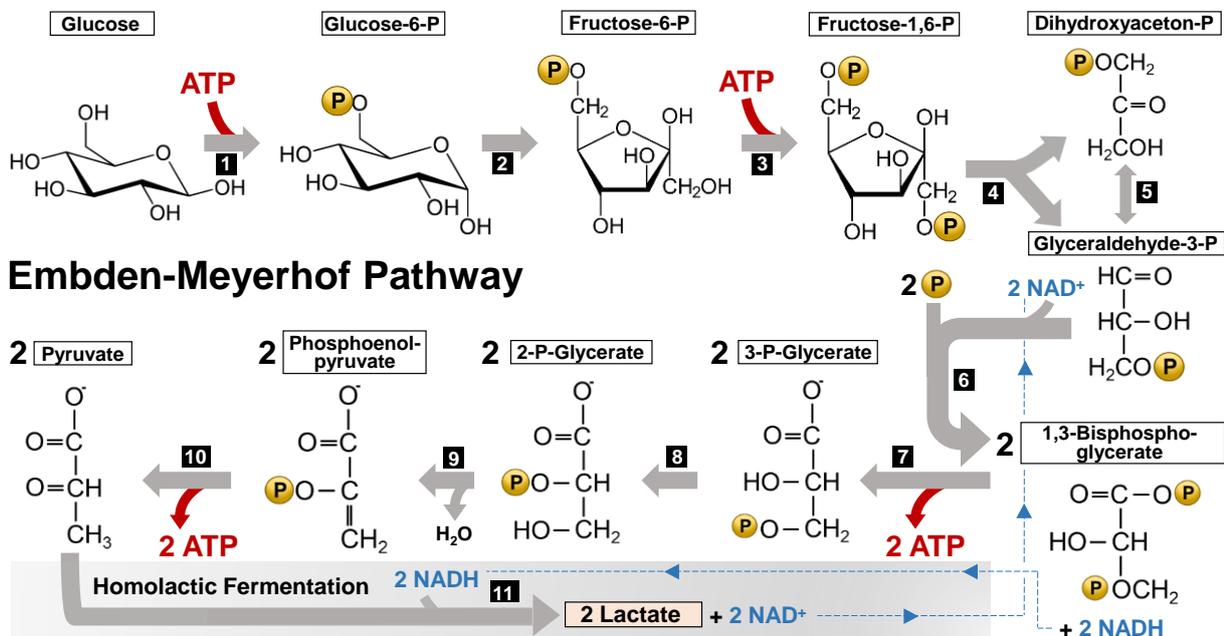
#### 1.3.4.3. Peptidoglycan

Peptidoglycan, also known as murein, forms the cell wall of almost all bacteria. Although the amount of peptidoglycan varies between gram negative (up to 10% of the total cell wall) and gram positive bacteria (up to 70% of the total cell wall) (Schleifer and Kandler, 1972), the structure, including a saccharide backbone with attached peptides, is identical. The peptidoglycan backbone consists of beta-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues and peptides that are attached via their *N* terminus and the carboxyl group of the muramic acid (Schleifer and Kandler, 1972). The linear chains of the two different saccharides and the attached peptides are cross-linked via inter peptide bridges that ensures the stability of peptidoglycan (Schleifer and Kandler, 1972; Meroueh *et al.*, 2006). During the bacterial growth and division, several peptidoglycan-degrading hydrolases are involved in the assembly and disassembly of the cell wall (Humann and Lenz, 2009). The ingestion of soil bacteria by the earthworm (Section 1.2.2) is linked to the grinding gizzard that introduces disrupted cell walls to the alimentary canal and the associated gut microbiota.

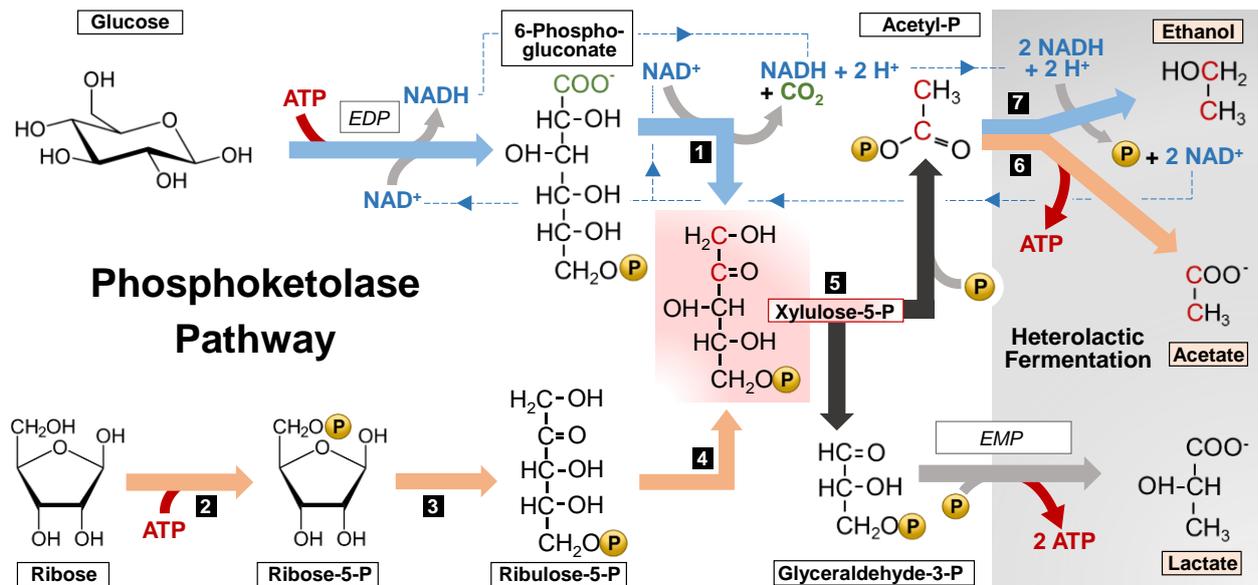


2000), a value that would yield approximately 100 mM glucose-equivalents in the immediate vicinity of a disrupted shoot (this approximation is based on a gram of shoot being equivalent to 1 ml).

The fermentation of saccharides involves activation and subsequent oxidation reactions. For these fermentative processes, microorganism can use (a) the Embden-Meyerhof-Parnas pathway also known as glycolysis (Figure 12), (b) the phosphoketolase pathway (Figure 13), (c) the Entner-Doudoroff pathway (Figure 14), or (d) the *Bifidobacterium bifidum* pathway (Figure 14) that is a combination of the first and the second pathway (Romano *et al.*, 1979; Buckel, 1999; Hogg, 2013; Prasanna *et al.*, 2014). All four pathways generate intermediates, including pyruvate, acetyl-phosphate or acetyl-CoA that subsequently function as terminal acceptor for electrons from the oxidation step (Figure 12, Figure 13, and Figure 14). The reduction process results in the production of one (e.g., homolactic acid fermentation [Figure 12 and Table 3]) or diverse fermentation products (e.g., mixed acid fermentation [Figure 15 and Table 3]). In this regard, the homolactic acid fermenters ensure energy conservation via the exclusive production of lactate, whereas mixed acid fermenters produce diverse products (e.g., formate, acetate and ethanol) (Figure 15 and Table 3) (Buckel, 1999; Moat *et al.*, 2002); both processes can start with the Embden-Meyerhof-Parnas pathway (Figure 12). The propionate fermentation is an example for another fermentation that is based on the sugar oxidation via the Embden-Meyerhof-Parnas pathway (Figure 16 and Table 3) (Buckel, 1999; Zhuge *et al.*, 2013).

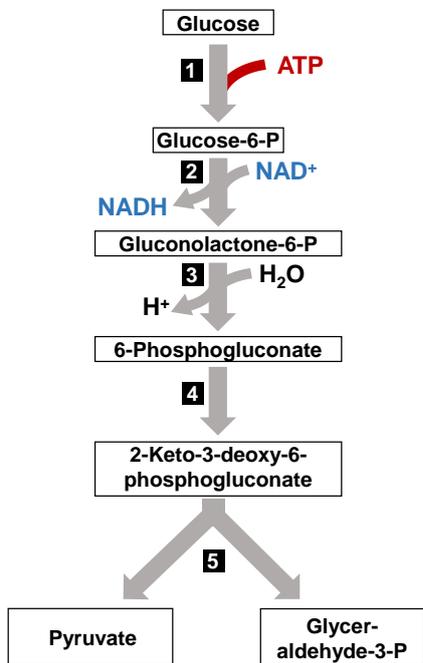


**Figure 12.** Embden-Meyerhof-Parnas pathway linked to the production of lactate by homolactic acid bacteria. Abbreviations: NAD<sup>+</sup>, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; P, phosphate. Enzymes: 1, Glucokinase; 2, Isomerase; 3, Phosphofructokinase; 4, Aldolase; 5, Triosephosphate isomerase; 6, Glyceraldehyde-3-dehydrogenase; 7, Phosphoglycerokinase; 8, Phosphoglyceromutase; 9, Enolase; 10, Pyruvate kinase; 11, Lactate dehydrogenase. Figure modified from Moat *et al.*, 2002; Engelkirk *et al.*, 2011; Hogg, 2013; Madigan *et al.*, 2015.

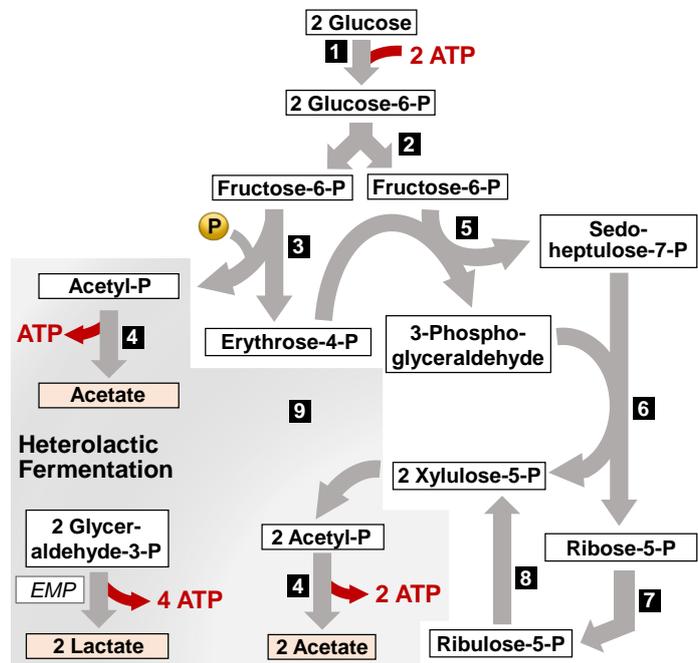


**Figure 13.** Phosphoketolase pathway linked to the production of ethanol, lactate, and acetate by heterolactic acid bacteria. Abbreviations: EDP, Entner-Doudoroff pathway; EMP, Embden-Meyerhof-Parnas pathway; NAD<sup>+</sup>, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; P, phosphate. Enzymes: 1, 6-Phosphogluconate dehydrogenase; 2, Ribokinase; 3, Ribose-5-phosphate isomerase; 4, Ribulose-5-phosphate epimerase; 5, Phosphoketolase-2; 6, Acetate kinase; 7, Phosphate acetyltransferase and alcohol dehydrogenase. Figure based on information obtained from Buckel, 1999; Årsköld *et al.*, 2008; Papagianni, 2012.

### Entner-Doudoroff Pathway



### *Bifidobacterium bifidum* Pathway



**Figure 14.** Enter-Doudoroff pathway and *B. bifidum* pathway. Abbreviations: EMP, Embden-Meyerhof-Parnas pathway; NAD<sup>+</sup>, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; P, phosphate. Panel A: Enzymes: 1, Glucokinase; 2, Glucose-6-phosphate dehydrogenase; 3, Lactonase; 4, 6-Phosphogluconate dehydrogenase; 5, 2-Dehydro-3-deoxy-6-phosphogluconate aldolase. Based on information obtained from Buckel, 1999; Hogg, 2013. Panel B: Enzymes: 1, Glucokinase; 2, Isomerase; 3, Phosphoketolase-1; 4, Acetate kinase; 5, Transaldolase; 6, Transketolase; 7, Ribose-5-phosphate isomerase; 8, Ribulose-5-phosphate-3 epimerase; 9, Phosphoketolase-2. Based on information obtained from Buckel, 1999; Prasanna *et al.*, 2014.

**Table 3.** Overall stoichiometries of potential saccharide fermentations in the gut of *L. terrestris*.

Fermentation	Equation	$\Delta G^{0'}$ (kJ/mol) <sup>a</sup>	Model Organism <sup>b</sup>	Refer- ence <sup>e</sup>
<b>Butyrate</b>	Glucose → Butyrate <sup>-</sup> + H <sup>+</sup> + 2 CO <sub>2</sub> + 2 H <sub>2</sub>	-255	<i>Clostridium pasteurianum</i>	1
<b>Ethanol</b>	Glucose → 2 Ethanol + 2 CO <sub>2</sub>	-239	<i>Zymomonas mobilis</i>	2, 3
<b>Lactate</b>	Glucose → 2 Lactate <sup>-</sup> + 2 H <sup>+</sup>	-198	<i>Lactococcus lactis</i>	1, 4
	Glucose → Lactate <sup>-</sup> + H <sup>+</sup> + Ethanol + CO <sub>2</sub>	-211	<i>L. mesenteroides</i>	1, 5
	Ribose → Lactate <sup>-</sup> + Acetate <sup>-</sup> + 2 H <sup>+</sup>	-210	<i>Lactobacillus pentosus</i>	1, 6
<b>Mixed acid</b>	Glucose → Lactate <sup>-</sup> + 0.4 Ethanol + 0.3 Acetate <sup>-</sup> + 0.02 Formate <sup>-</sup> + 0.2 Succinate <sup>2-</sup> + 0.5 H <sub>2</sub> + 0.5 CO <sub>2</sub> + 1.8 H <sup>+</sup>	-336 <sup>c</sup>	<i>Escherichia coli</i>	7
	Glucose → Acetate <sup>-</sup> + H <sup>+</sup> + Ethanol + H <sub>2</sub> + CO <sub>2</sub>	-255	<i>E. coli</i>	8
	Xylose + 0.9 H <sub>2</sub> O → 0.9 Acetate <sup>-</sup> + 0.8 Ethanol + 1.6 CO <sub>2</sub> + 1.72 H <sub>2</sub> + 0.1 Formate <sup>-</sup> + 0.96 H <sup>+</sup>	-181	<i>Bacteroides xyloxyticus</i>	9
<b>Propionate</b>	Glucose → 1.3 Propionate <sup>-</sup> + 0.6 Acetate <sup>-</sup> + 2 H <sup>+</sup> + 0.6 CO <sub>2</sub>	-311	<i>Clostridium propionicum</i>	1
<b>Acetogenesis</b>	Xylose → 2.5 Acetate <sup>-</sup> + 2.5 H <sup>+</sup>	-355 <sup>c</sup>	<i>Clostridium thermoaceticum</i>	10
	Glucose → 3 Acetate <sup>-</sup> + 3 H <sup>+</sup>	-427 <sup>c</sup>	<i>C. thermoaceticum</i>	10
	Cellulose + H <sub>2</sub> O → 6 Acetate <sup>-</sup> + 6 H <sup>+</sup>	-611 <sup>c,d</sup>	<i>Peptostreptococcus productus</i>	10

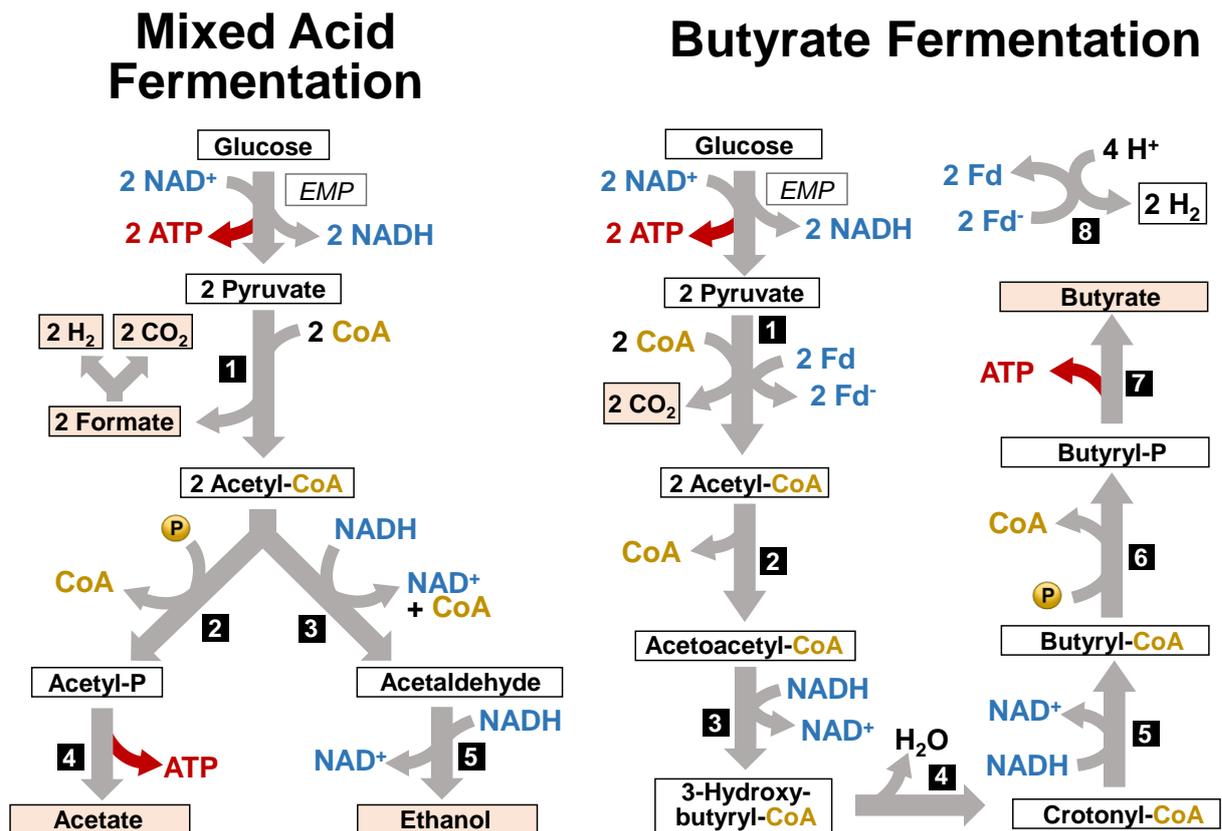
<sup>a</sup>Gibbs free energy (pH 7, 25 °C) yield per mol substrate.

<sup>b</sup>Representative model organisms for the respective fermentation.

<sup>c</sup>Calculated for this dissertation according to Thauer *et al.*, 1977.

<sup>d</sup>Gibbs free energy of formation for cellobiose was obtained by the sum of the gibbs free energy of formation of two glucose molecules and the gibbs free energy required for cellobiose hydrolysis (12.5 kJ/mol; Ha *et al.*, 2013).

<sup>e</sup>Table based on information obtained from: 1, Buckel, 1999; 2, Madigan *et al.*, 2015; 3, Ingram *et al.*, 1999; 4, Ishizaki and Ueda, 1995, 1959; 5, Gunsalus and Gibbs, 1951; 6, Bernstein, 1953; 7, Moat *et al.*, 2002; 8, Metzler and Metzler, 2003; 9, Biesterveld *et al.*, 1994; 10, Drake, 1994.

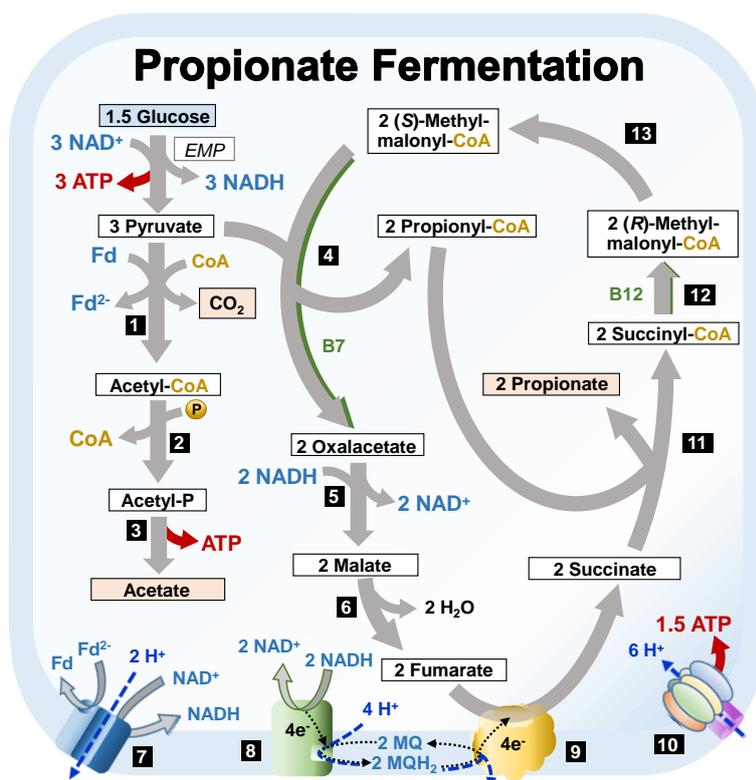


**Figure 15.** Mixed acid fermentation and butyrate fermentation. Panel A does not include the formation of all possible end products (e.g., succinate). Abbreviations: EMP, Embden-Meyerhof-Parnas pathway; CoA, coenzyme A; NAD<sup>+</sup>, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; P, phosphate. Panel A: Pathway observed in *Streptococcus*. Enzymes: 1, Pyruvate formate lyase; 2, Phosphoacetyl transferase; 3, Acetaldehyde dehydrogenase; 4, Acetate kinase; 5, Alcohol dehydrogenase. Based on information obtained from Buckel, 1999. Panel B: Pathway observed in *Clostridium butyricum*. Enzymes: 1, Pyruvate:ferredoxin oxidoreductase; 2, Thiolase; 3, 3-Hydroxybutyryl-CoA dehydrogenase; 4, Crotonase; 5, Butyryl-CoA dehydrogenase; 6, Phosphotransbutyrylase; 7, Butyrate kinase; 8, Ferredoxin-dependent hydrogenase. Based on information obtained from Buckel, 1999; Hackmann and Firkins, 2015.

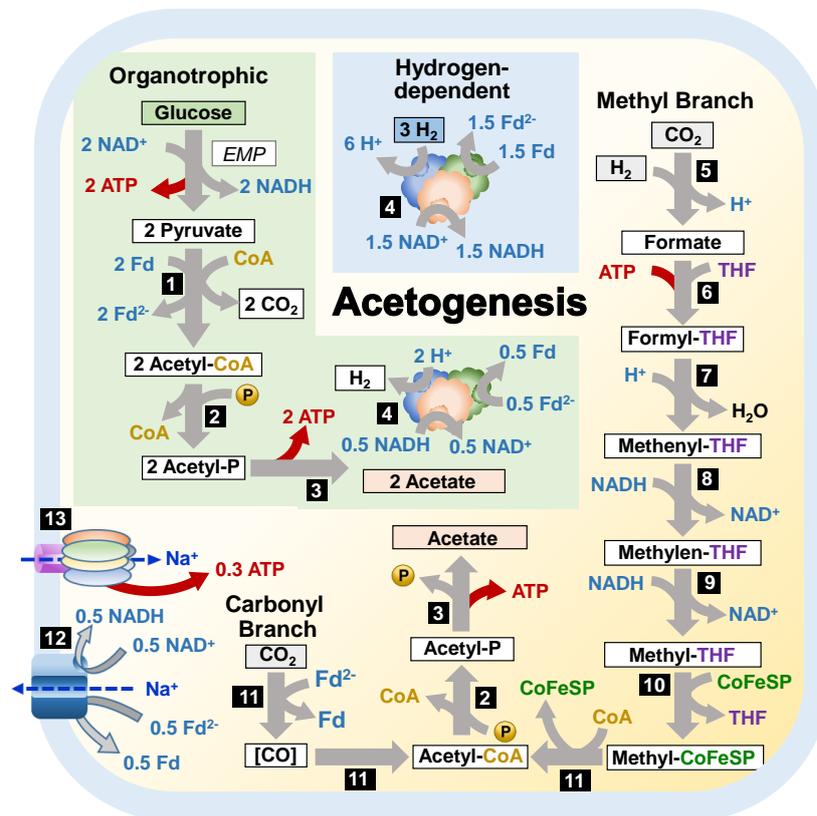
Whereas common saccharide fermentations start with hexoses, bacteria performing the phosphoketolase pathway or the pentose phosphate cycle are able to utilize pentoses as substrate molecule (Rosenberg, 1980; McMillan, 1993; Buckel, 1999; Årsköld *et al.*, 2008; Liu *et al.*, 2012). The phosphoketolase pathway (Figure 13 and Table 3) was observed, among others, for heterolactic acid bacteria, of which a specialized subgroup is also able to gain energy via the *B. bifidum* pathway by producing acetate and lactate (Figure 14; Buckel, 2001; Prasanna *et al.*, 2014).

Saccharides and fermentation-derived CO<sub>2</sub> and H<sub>2</sub> can potentially be consumed by acetogens, a physiologically defined group of anaerobic prokaryotes, that conserve energy via the Wood-Ljungdahl-Pathway, also known as reductive acetyl-CoA pathway, and produce acetate as sole product (Figure 17; Ljungdahl and Wood, 1969; Wood *et al.*, 1986; Drake, 1994; Drake *et al.*, 2008, 2013). The pathway consists of a carbonyl branch and a methyl branch, both ensure the re-oxidation of reduction equivalents that are obtained from the oxidation of either (a) organic carbon (e.g., glucose) by organotrophic acetogens, or (b) hydrogen by hydrogen-dependent acetogens (Figure 17; Drake, 1994; Drake *et al.*, 2013; Schuchmann and Müller, 2014). The electron

acceptor in both branches is the inorganic gas  $\text{CO}_2$ , and acetogenesis is therefore not a classic fermentation which uses organic intermediates as terminal electron acceptors. In addition to  $\text{H}_2$  and  $\text{CO}_2$  or glucose, acetogens can utilize other substrate like xylose, ethanol, formate, and lactate (Table 3 and Table 6; Drake, 1994; Weghoff *et al.*, 2015; Bertsch *et al.*, 2016). The key enzyme of the acetogenesis is the CO dehydrogenase/acetyl-CoA synthase. It catalyzes the reaction of enzyme-bound CO and a tetrahydrofolate (THF)-derived methyl group to acetyl-CoA (Figure 17; Ljungdahl and Wood, 1969; Wood *et al.*, 1986; Drake, 1994; Drake *et al.*, 2008, 2013; Schuchmann and Müller, 2014). The energy generation differs between the organotrophic and hydrogen dependent growth of acetogens. Thus, the organotrophic growth on glucose yield at least four ATP, whereas the hydrogen-dependent acetogenesis is fully conditional on the membrane-associated Rnf (*Rhodobacter* nitrogen fixation)- or Ech (energy converting hydrogenases)-complex (Figure 17; Schuchmann and Müller, 2014). Both enzyme complexes re-oxidize reduced ferredoxin and pump cations into the environment. The resulting proton motive force is coupled to membrane-bound ATPases which generate ATP by the relocalization of the external cations (Schuchmann and Müller, 2014). However, it is noteworthy that acetogens are capable of diverse dissimilatory processes including fermentation (Drake *et al.*, 2006, 2008).



**Figure 16.** Methylmalonyl pathway observed in *Propionibacterium*. Abbreviations: EMP, Embden-Meyerhof-Parnas pathway; CoA, coenzyme A; Fd, oxidized ferredoxin,  $\text{Fd}^{2-}$ , reduced ferredoxin;  $\text{NAD}^+$ , oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate;  $\text{H}^+$ ; hydrogen ion; MQ, menaquinone. Enzymes: 1, Pyruvate:ferredoxin oxidase; 2, Phosphoacetyl transferase; 3, Acetate kinase; 4, Transcarboxylase (contains biotin); 5, Malate dehydrogenase; 6, Fumarase; 7, Ferredoxin:  $\text{NAD}^+$  oxidoreductase (Rnf-complex); 8, NADH:quinone oxidoreductase; 9, Fumarate reductase; 10, ATPase; 11, Propionate CoA-transferase; 12, Methylmalonyl-CoA mutase (vitamin B12 as coenzyme); 13, Methylmalonyl-CoA epimerase. Figure based on information obtained from Buckel, 1999; Buckel and Thauer, 2012; Zhuge *et al.*, 2013; Guan *et al.*, 2014.



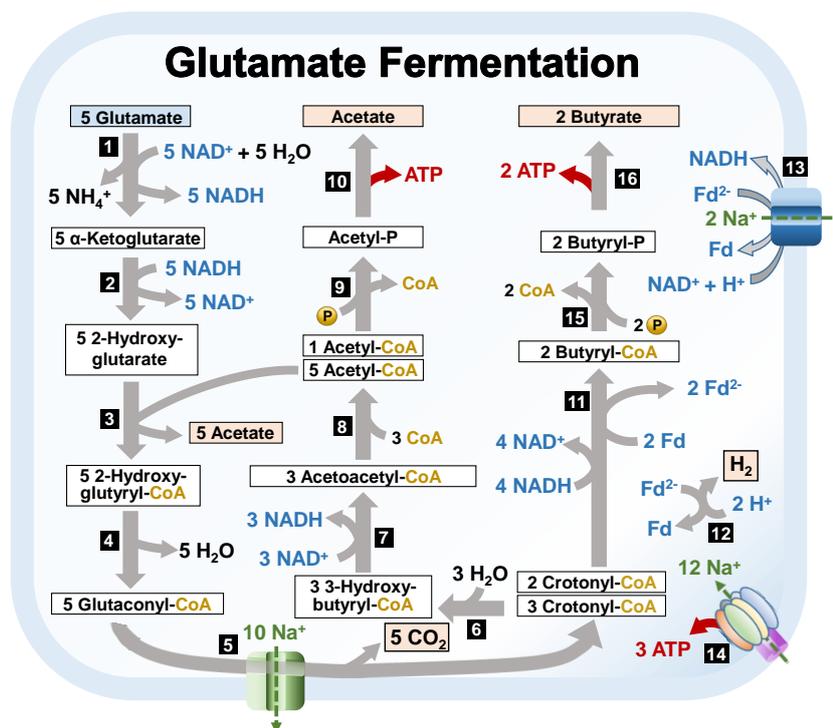
**Figure 17.** Acetogenesis observed in *Acetobacterium woodii*. Reactions in yellow zone are used by both organotrophic (green zone) and hydrogen-dependent (blue zone) acetogens. Abbreviations: EMP, Embden-Meyerhof-Parnas pathway; CoA, coenzyme A; CoFeSP, corrinoid-iron/sulfur-protein; THF, tetrahydrofolate; Fd, oxidized ferredoxin, Fd<sup>2-</sup>, reduced ferredoxin; NAD<sup>+</sup>, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; Na<sup>+</sup>, sodium ion. Enzymes: 1, Pyruvate:ferredoxin oxidase; 2, Phosphoacetyl transferase; 3, Acetate kinase; 4, Bifurcating hydrogenase; 5, Formate dehydrogenase; 6, Formyl-THF synthetase; 7, Formyl-THF cyclohydrolase; 8, Methylene-THF dehydrogenase; 9, Methylene-THF reductase; 10, Methyltransferase; 11, CO dehydrogenase/acetyl-CoA synthase; 12, Ferredoxin:NAD<sup>+</sup> oxidoreductase (Rnf-complex); 13, ATPase. Figure based on information obtained from Ljungdahl and Wood, 1969; Wood *et al.*, 1986; Drake *et al.*, 2008; Schuchmann and Müller, 2014.

### 1.4.2. Amino acid-derived fermentations

Approximately 2 mM amino acids may occur in the aqueous phase of the earthworm gut (Horn *et al.*, 2003; Section 1.3.2), indicating amino acids, with an average redox state similar to that of saccharides, as potential subjects to fermentation during the earthworm gut passage. In this regard, especially members of *Clostridiales* (e.g., *Paraclostridium bifermentans*) and *Fusobacteriales* (e.g., *Fusobacterium nucleatum*), occurring in soil, marine and freshwater, and intestines of animals including earthworms (Wiegel, 2009; James and Whitman, 2011; Wüst *et al.*, 2011; Meier *et al.*, 2018), are able to ferment amino acids (Barker, 1981).

Similar to the high diversity of possible saccharide fermentation pathways, the anaerobic utilization processes of amino acid are diverse and complex. Thus, at least five different pathways can be used for the microbial fermentation of glutamate (Buckel, 2001), whereby the methylaspartate pathway and the hydroxyglutarate pathway (Figure 18) are likely the most important processes for glutamate fermentation (Buckel, 2001). Both pathways lead to the production of ammonium, CO<sub>2</sub>, acetate, butyrate and H<sub>2</sub>, whereas the methylaspartate pathway

can be also used to generate propionate instead of butyrate and  $H_2$  (Figure 18 and Table 4; Buckel, 2001). *C. propionicum* is able to ferment alanine via the acrylate pathway, a pathway also involved in producing propionate from lactate (Schweiger and Buckel, 1984) (Table 6). Alanine is also a amino acids that can be utilized during the Stickland reaction, a process in which one amino acid serves as an electron donor and another amino acid serves as an electron acceptor (Figure 19 and Table 4) (Nisman, 1954; Buckel, 1999). In addition to the amino acid alanine, cysteine, glycine, leucine, serine, threonine, methionine, phenylalanine, tyrosine, and tryptophan can be used in both, the oxidative and reductive branch of the Stickland reaction, whereas isoleucine and valine or proline serve exclusively as electron donors or acceptor, respectively (Buckel, 1999). Similar to the parallel utilization of glycine and alanine (Figure 19), the exclusive fermentation of glycine terminates in the production of ammonium,  $CO_2$ , and acetate (Table 4; Buckel, 1999). Furthermore and dependent on the organism, threonine can be utilized by at least three different pathways, yielding different fermentation products. Thus, *C. propionicum* produce ammonium,  $CO_2$ , propionate and butyrate, whereas *C. pasteurianum* ferments threonine to ammonium and acetate (Table 4; Elsdén and Hilton, 1978; Buckel, 1999). The fermentation of the branched chain amino acids isoleucine and valine by *Spirochaeta isovalerica* lead to the formation of methylbutyrate and isobutyrate, respectively (McInerney, 1988).



**Figure 18.** Glutamate fermentation via hydroxyglutarate pathway. Abbreviations: CoA, coenzyme A; Fd, oxidized ferredoxin;  $Fd^{2-}$ , reduced ferredoxin;  $NAD^+$ , oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate;  $Na^+$ ; sodium ion;  $NH_4^+$ , ammonium. Enzymes: 1, Glutamate dehydrogenase; 2, 2-Hydroxyglutarat dehydrogenase; 3, CoA-transferase; 4, Hydroxyglutaryl-CoA fehydratase; 5, Glutaconyl decarboxylase; 6, Crotonyl-CoA hydratase; 7, 3-Hydroxybutyryl-CoA dehydrogenase; 8, Thiolase; 9, Phosphoacetyl transferase; 10, Acetate kinase; 11, Bcd (Butyryl-CoA dehydrogenase)/Etf-(Electron-transferring flavoprotein) complex; 12; Ferredoxin hydrogenase, 13, Ferredoxin: $NAD^+$  oxidoreductase (Rnf-complex); 14, ATPase; 15, Phosphotransbutyrylase; 16, Butyrate kinase. Figure based on information obtained from Buckel, 1999; Herrmann *et al.*, 2008.

**Table 4.** Overall stoichiometries of potential amino acid fermentations in the gut of *L. terrestris*.

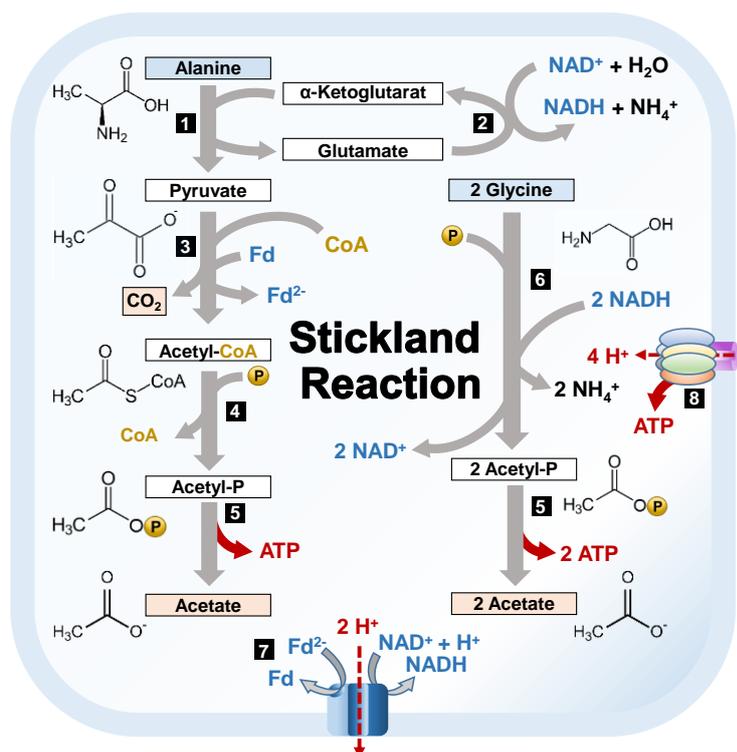
Amino Acid	Equation	$\Delta G^{0'}$ (kJ/mol) <sup>a</sup>	Model Organism <sup>b</sup>	Reference <sup>d</sup>
Glutamate	5 Glu <sup>-</sup> + 6 H <sub>2</sub> O + 2 H <sup>+</sup> → 6 Acetate <sup>-</sup> + 2 Butyrate <sup>-</sup> + H <sub>2</sub> + 5 CO <sub>2</sub> + 5 NH <sub>4</sub> <sup>+</sup>	-314	<i>Acidaminococcus fermentans</i>	1
	3 Glu <sup>-</sup> + 4 H <sub>2</sub> O → 5 Acetate <sup>-</sup> + Propionate <sup>-</sup> + 2 CO <sub>2</sub> + 3 NH <sub>4</sub> <sup>+</sup>	-187	<i>Selenomonas acidaminophila</i>	2
Aspartate	3 Asp <sup>-</sup> + 0.9 H <sup>+</sup> + 1.8 H <sub>2</sub> O → 2.4 Succinate <sup>2-</sup> + 0.3 Acetate <sup>-</sup> + 1.8 CO <sub>2</sub> + 3 NH <sub>4</sub> <sup>+</sup>	-250 <sup>c</sup>	<i>Camphylobacter</i> sp.	3
Threonine	3 Thr + H <sub>2</sub> O → 2 Propionate <sup>-</sup> + Butyrate <sup>-</sup> + 2 CO <sub>2</sub> + 3 NH <sub>4</sub> <sup>+</sup>	-321 <sup>c</sup>	<i>C. propionicum</i>	2
	Thr + H <sub>2</sub> O → 2 Acetate <sup>-</sup> + H <sup>+</sup> + NH <sub>4</sub> <sup>+</sup>	-146 <sup>c</sup>	<i>C. pasteurianum</i>	2, 4
Glycine	4 Gly + 2 H <sub>2</sub> O + H <sup>+</sup> → 3 Acetate <sup>-</sup> + 2 CO <sub>2</sub> + 4 NH <sub>4</sub> <sup>+</sup>	-217	<i>Eubacterium acidaminophilum</i>	2
Alanine	3 Ala + 2 H <sub>2</sub> O → Acetate <sup>-</sup> + 2 Propionate <sup>-</sup> + CO <sub>2</sub> + 3 NH <sub>4</sub> <sup>+</sup>	-135	<i>C. propionicum</i>	1
<b>Stickland Reaction</b>				5
Alanine and Glycine	Ala + 2 H <sub>2</sub> O → Acetate <sup>-</sup> + CO <sub>2</sub> + NH <sub>4</sub> <sup>+</sup> + 4 [H]		<i>Clostridium sticklandii</i> and <i>P. bifementans</i>	
	2 Gly + 4 [H] → 2 Acetate <sup>-</sup> + 2 NH <sub>4</sub> <sup>+</sup>			
Sum:	Ala + 2 Gly + 2 H <sub>2</sub> O → 3 Acetate <sup>-</sup> + 3 NH <sub>4</sub> <sup>+</sup> + CO <sub>2</sub>	-153 <sup>c</sup>		

<sup>a</sup>Gibbs free energy (pH 7, 25 °C) yield per equation.

<sup>b</sup>Representative model organisms for the respective fermentation.

<sup>c</sup>Calculated for this dissertation according to Thauer *et al.*, 1977.

<sup>d</sup>Table based on information obtained from: 1, Buckel and Thauer, 2012; 2, Buckel, 1999; 3, Laanbroek *et al.*, 1978; 4, Elsdén and Hilton, 1978; 5, McInerney, 1988.



**Figure 19.** Fermentation of alanine and glycine by Stickland reaction. Abbreviations: CoA, coenzyme A; Fd, oxidized ferredoxin;  $\text{Fd}^{2-}$ , reduced ferredoxin;  $\text{NAD}^+$ , oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ATP, adenosine triphosphate;  $\text{H}^+$ , hydrogen ion;  $\text{NH}_4^+$ , ammonium; P, phosphate. Enzymes: 1, Transaminase; 2, Glutamate dehydrogenase; 3, Pyruvate: ferredoxin oxidase; 4, Phospho-acetyl transferase; 5, Acetate kinase; 6, Glycerin reductase; 7, Ferredoxin: $\text{NAD}^+$  oxidoreductase (Rnf-complex). Figure based on information obtained from Andreesen, 1994; Madigan *et al.*, 2015.

### 1.4.3. Other fermentations

#### 1.4.3.1. Purines and pyrimidines

A efficient hydrolysis of RNA leads to the availability of the backbone saccharide ribose and attached purines (i.e., adenine and guanine) and pyrimidines (i.e., uracil and cytosine) (1.3.3; Figure 10 B). Only a specialized subgroup of anaerobic microorganisms (e.g., *Clostridium acidurici* and *Clostridium purinolyticum*) is able to ferment purines, a process yielding acetate,  $\text{CO}_2$ , and ammonium, whereby formate is only produced when adenine serves as substrate (Table 5; Gariboldi and Drake, 1984; Buckel, 1999). The ability to ferment RNA-derived pyrimidines is, until today, only described for *Clostridium uracilium*, a bacterium converting uracil and cytosine to alanine, ammonium, and  $\text{CO}_2$  (Table 5; Campbell, 1957; Vogels and Van der Drift, 1976).

#### 1.4.3.2. Glycerol and long chain fatty acids

In oxic and anoxic ecosystems, lipids are first hydrolyzed by lipases that derives the release of long chain fatty acids and other non-fatty acid compounds, like glycerol (Garton *et al.*, 1958, 1961; Dawson *et al.*, 1974). Non-fatty acid compounds are mainly fermented to short chain fatty acids (Bryant, 1979; McInerney *et al.*, 1979). In this regard, *Propionibacteria* utilize glycerol to gain energy by producing propionate (Table 5; Buckel, 1999). In oxygen-limited ecosystems (e.g.,

intestines), fermentative bacteria do not further degrade the remaining long chain fatty acids (McInerney, 1988). However, in ecosystems with higher retention times (e.g., anoxic sediments) long and short chain fatty acids are utilized by syntrophic bacteria (e.g., *Syntrophomonas wolfei*) to form acetate and H<sub>2</sub>, a process dependent on methanogens (Table 5; McInerney *et al.*, 1981; McInerney, 1988; Dong *et al.*, 2009). However, the relatively short gut passage time of *L. terrestris* (Section 1.2.1), and the absence of methanogenesis (Meier *et al.* 2018) suggesting that lipid-derived long chain fatty acids are not utilized by the gut microbiota. However, long chain fatty acids can be absorbed as energy source by the gut tissue of the earthworm (Sampedro *et al.*, 2006). In contrast, the fermentation of available glycerol is not dependent on the occurrence of methanogens and can be therefore a potential substrate for the earthworm gut microbes.

**Table 5.** Fermentation of purines, pyrimidines and lipids.<sup>a</sup>

Substrate and Equation	Model Organism <sup>b</sup>
<b>Purines</b>	
Adenine + 8 H <sub>2</sub> O + 3 H <sup>+</sup> → Acetate <sup>-</sup> + Formate <sup>-</sup> + 2 CO <sub>2</sub> + 5 NH <sub>4</sub> <sup>+</sup>	<i>C. acidurici</i>
Guanine + 7 H <sub>2</sub> O + 4 H <sup>+</sup> → Acetate <sup>-</sup> + 3 CO <sub>2</sub> + 5 NH <sub>4</sub> <sup>+</sup>	<i>P. asaccharolyticus</i>
<b>Pyrimidines</b>	
Uracil + 2 H <sub>2</sub> O + 3 H <sup>+</sup> + 2 e <sup>-</sup> → NH <sub>4</sub> <sup>+</sup> + CO <sub>2</sub> + beta-Alanine	<i>C. uracilium</i>
<b>Lipids</b>	
Glycerol → Propionate <sup>-</sup> + H <sup>+</sup> + H <sub>2</sub> O	<i>Propionibacterium</i>
<i>n</i> -LCFA → ( <i>n</i> - 2)-LCFA + 2 Acetate <sup>-</sup> + 2 H <sup>+</sup> + 2 H <sub>2</sub>	<i>S. wolfei</i>

<sup>a</sup>Table based on information obtained from McInerney *et al.*, 1981; Buckel, 1999; Dong *et al.*, 2009.

<sup>b</sup>Representative model organisms for the respective fermentation.

#### 1.4.4. Secondary processes in the earthworm gut

Products from primary fermentations can be subjects to anaerobic secondary processes. In this regard, formate can be converted to H<sub>2</sub> and CO<sub>2</sub> by an enzyme complex that (a) contains a formate dehydrogenases and a hydrogenase, (b) is common in many enteric bacteria, and (c) is most likely used for the maintenance of the pH (Table 6; Sawers, 1994; McDowall *et al.*, 2014). Another example for a secondary process is the decarboxylation of succinate to propionate by *Fusobacteriaceae*-affiliated species (Table 6; Schink and Pfennig, 1982). Fermentation-derived ethanol and lactate can be used to gain energy by propionate fermenters (e.g., *Pelobacter propionicus*) that produce via acrylate-pathway or methylmalonyl-pathway (Figure 16) propionate and acetate from ethanol, or propionate and CO<sub>2</sub> if lactate is the substrate molecule (Figure 16; Schink *et al.*, 1987; Tholozan *et al.*, 1992). Furthermore, ethanol and acetate can be converted by *Clostridium kluyveri* to butyrate (Table 6; Thauer *et al.*, 1968; Buckel, 1999). Fermentation products (e.g., H<sub>2</sub>, CO<sub>2</sub>, ethanol, lactate, and formate) can also be used by acetogens via the acetyl-CoA pathway (Table 3 and Table 6; Drake, 1994; Weghoff *et al.*, 2015; Bertsch *et al.*, 2016; Schuchmann and Müller, 2016).

**Table 6.** Overall stoichiometries of potential secondary processes in the gut of *L. terrestris*.

Equation	$\Delta G^{0'}$ (kJ/mol) <sup>a</sup>	Model Organism <sup>b</sup>	Reference <sup>d</sup>
Formate <sup>-</sup> + H <sup>+</sup> → H <sub>2</sub> + CO <sub>2</sub>	-3.0	<i>E. coli</i>	1, 2
Ethanol + Acetate <sup>-</sup> → Butyrate <sup>-</sup> + H <sub>2</sub> O	-39	<i>C. kluyveri</i>	3
3 Ethanol + 2 CO <sub>2</sub> → 2 Propionate <sup>-</sup> + Acetate <sup>-</sup> + 3 H <sup>+</sup> + 2 H <sub>2</sub> O	-126	<i>P. propionicus</i>	4
3 Lactate <sup>-</sup> → 2 Propionate <sup>-</sup> + Acetate <sup>-</sup> + CO <sub>2</sub> + H <sub>2</sub> O	-170	<i>P. propionicus</i>	4
Succinate <sup>2-</sup> + H <sup>+</sup> → Propionate <sup>-</sup> + CO <sub>2</sub> <sup>-</sup>	-25 <sup>c</sup>	<i>Propionigenium modestum</i>	5
<b>Acetogenesis</b>			
4 H <sub>2</sub> + 2 CO <sub>2</sub> → Acetate <sup>-</sup> + H <sup>+</sup> + 2 H <sub>2</sub> O	-95	<i>C. thermoaceticum</i>	6
2 Ethanol + 2 CO <sub>2</sub> → 3 Acetate <sup>-</sup> + 3 H <sup>+</sup>	-75	<i>Clostridium formicoaceticum</i>	6, 7
4 Formate <sup>-</sup> → Acetate <sup>-</sup> + 2 CO <sub>2</sub> + 2 H <sub>2</sub> O	-309 <sup>c</sup>	<i>C. thermoaceticum</i>	6
2 Lactate <sup>-</sup> → 3 Acetate <sup>-</sup>	-61	<i>Acetobacterium woodii</i>	8, 9

<sup>a</sup>Gibbs free energy (pH 7, 25 °C) yield per equation.

<sup>b</sup>Representative model organisms for the respective fermentation.

<sup>c</sup>Calculated for this dissertation according to Thauer *et al.*, 1977.

<sup>d</sup>Table based on information obtained from: 1, Lim *et al.*, 2012; 2, da Silva *et al.*, 2013; 3, Buckel, 1999; 4, Schink *et al.*, 1987; 5, Schink and Pfennig, 1982; 6, Drake, 1994; 7, Bertsch *et al.*, 2016; 8 Weghoff *et al.*, 2015; 9, Schuchmann and Müller, 2016.

## 1.5. Hypotheses and objectives

The lifestyle of earthworms in the underground make them an inconspicuous macrofauna in soil ecosystems. However, the tendency of these invertebrates to consume their home, i.e., soil, roots, litter, and associated microbes has sustainable effects on soil fertility, plant growth, and the cycling of elements (Section 1.1.2 and Section 1.1.3). The ingestion of diverse plant- and microbial-derived materials introduces diverse biopolymers (e.g., polysaccharides, protein, nucleic acids) to the alimentary canal (Section 1.3). Thus, the fermentative gut microbiota is (a) challenged with complex biomass and (b) potentially capable of degrading associated biopolymers. This process might (a) increase the earthworm-derived turnover dynamics of soil organic matter and (b) supply fermentation products as a source of nutrition for the earthworm (Section 1.2.2). Although several observations indicate that the earthworm gut is rich in anaerobic microbial activities (Section 1.2.2), how these activities are potentially linked to the utilization of diverse ingested biopolymers in the gut is, like the nature of the fermentative gut microorganisms, largely unresolved.

**Thus, the hypotheses of the present dissertation were:**

- I. The fermentative gut microbiota of *L. terrestris* can hydrolyze diverse plant- and microbial-derived polysaccharides and utilize saccharides from which they are composed. (discussed in Section 4.1)
- II. The fermentative gut microbiota of *L. terrestris* has the capacity to respond anaerobically to nutrient availability derived from disrupted microbial cells. (discussed in Section 4.2)
- III. Protein and RNA, as primary biopolymers of disrupted microbial biomass, trigger earthworm gut fermentations. (discussed in Section 4.2)
- IV. Amino acids (protein-derived) and ribose (RNA-derived) are subject to fermentation by contrasting earthworm gut taxa. (discussed in Section 4.2)
- V. The responsive and fermentative gut microbiota of *L. terrestris* is phylogenetically affiliated to common soil bacteria. (discussed in Section 4.3)
- VI. In contrast to the stimulatory effects of microbial- and plant-derived biopolymers, increasing water content has a minor impact on the gut fermentative microbiota. (discussed in Section 4.4)
- VII. The occurrence of the earthworm symbiont *Can. Lumbricincola* is effected by the ingested environmental substrate. (discussed in Section 4.5)

The objectives of this dissertation were to determine (a) if microbial- and plant-derived biopolymers, including monomers from which they are composed, enhance fermentation by gut-associated microbes of the model earthworm *L. terrestris*, and (b) which microbial taxa are responsible for the enhanced fermentations. To address these objectives, anoxic gut content or soil microcosms were supplemented with diverse dietary substrates (e.g., cell lysate, protein, RNA, cellulose, saccharides, amino acids) to quantitatively and qualitatively evaluate their fermentative capacities and responsive fermentative taxa. The experimental analysis required a 1:10 dilution of the extracted gut contents for obtaining adequate samples for chemical and molecular analyses. This potential disturbance of the fermentative gut content system was evaluated by a comparative analysis of the fermentative activities and associated taxa in diluted and undiluted gut contents. An additional objective of this dissertation was to examine the potential effect of different ingested materials on the earthworm symbiont *Can. Lumbricincola*. This objective was addressed by analyzing the relative 16S rRNA sequence abundances of *Can. Lumbricincola* in gut contents of earthworms maintained on different dietary substrates.

## 2. MATERIALS AND METHODS

### 2.1. Gut content and soil microcosms

#### 2.1.1. Earthworms and soil

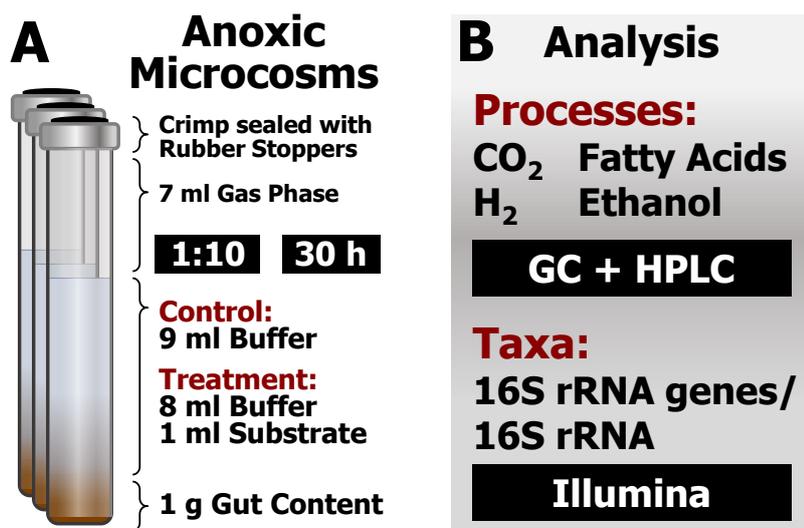
*L. terrestris* individuals from Fischerkönig Angelgeräte (Neustadt/Orla, Germany) were purchased from ANZO or Fisherman's World (Bayreuth, Germany). The earthworms were removed from the commercial worm bedding, washed with water, and maintained in a 50 l barrel filled with loamy soil from the meadow Trafo Wiese in Bayreuth (49°55'39"N, 11°31'46"E; Bayreuth, Germany). The worms were kept in the barrel at 20°C for approximately ten days prior to use. Turf at the top of the soil (which contained soil, roots, grass and leaves) served as feedstock. The effect of different environmental substrates on the earthworm symbiont *Can. Lumbicicola* was evaluated by using two 10 l buckets filled with soil or turf, whereby each bucket contained approximately 20 earthworms. The earthworms were stored in the buckets as mentioned above. The gut contents from earthworms maintained on worm bedding were extracted immediately after purchasing.

#### 2.1.2. Anoxic microcosms

A microcosm can be defined as "a simplified, physical model of an ecosystem that enables controlled experiments to be conducted in the laboratory" (Fath, 2019). The microcosms in this dissertation contained gut contents of *L. terrestris* or soil (Section 2.1.1). In more detail, earthworms from the barrel were washed with water and dried with paper towels. Afterwards, the earthworms were anesthetized on ice with CO<sub>2</sub> (100%) for 20 min. The extraction of gut content was conducted in an anoxic chamber (Mecaplex, Grenchen, Switzerland, gas phase: 100% dinitrogen) as described previously (Wüst *et al.*, 2011). In this regard, the squeeze-out procedure of the gut content was facilitated by cutting 2 mm of the posterior end of the worm by a scissor. The collected gut content (approximately 0.4 to 0.8 g gut content per worm) was then pooled, conscientious mixed, and divided into 27 ml sterile glass tubes. If not otherwise indicated, one gram fresh weight of gut content was supplemented with 1 ml substrate (Section 2.2), and anoxic sodium phosphate buffer (Section 2.3.3) was added to a total volume of 10 ml for each microcosm (Figure 20 A). Control treatments contained only gut content and sodium phosphate buffer. For undiluted gut content microcosms (Section 3.3), 15 ml glass tubes were carefully filled with 1 g gut content; no sodium phosphate buffer was added. All glass tubes were closed with sterile rubber stoppers (Glasgerätebau Ochs Laborfachhandel e.K., Bovenden, Germany), crimp sealed, and then flushed and pressurized (600 mbar) with sterile N<sub>2</sub> (100%).

The preparation of soil microcosms was identical to the preparation of gut content microcosms but instead of one gram gut content, one gram fresh weight of soil was used. The

incubation of the microcosms was in the dark at room temperature (approximately 21-24°C). Sampling of gas and liquid phases for gas chromatography (GC), high performance liquid chromatography (HPLC), and 16S rRNA gene and 16S rRNA analyses was under anoxic conditions with sterile syringes (BD, Heidelberg, Germany) (Figure 20 B and Section 2.5.2). All experiments were conducted with triplicated microcosms (three replicates per treatment). Destructive sampling was used for the analyses of undiluted gut content microcosms. In this regard, the incubation was stopped on ice and 9 ml ice-cold sodium phosphate buffer was added to ensure an adequate sampling for HPLC, 16S rRNA gene and 16S rRNA analyses.



**Figure 20.** Simplified overview of the experimental setup (A) and methods (B) that were used to evaluate the fermentations and associated taxa of earthworm gut contents. GC, gas chromatography; HPLC, high performance liquid chromatography.

## 2.2. Substrates

### 2.2.1. Plant- and microbial-derived lysates

#### 2.2.1.1. Leaf litter and root lysates

Leaf litter was collected in October at the bottom of beech and maple trees. Fine roots from turf (Section 2.1.1.) were washed with water and dried with paper towels. 20 g leaves and 30 g roots were mixed with 200 ml and 120 ml anoxic ice-cold sodium phosphate buffer (Section 2.3.3), respectively. In approximately 5 g steps and with cooling on ice, leaves and roots were separately cut and homogenized using a commercial blender (Model 32BLB0; Waring, Stamford, CT, USA). Resulting viscous slurries were centrifuged at 4 °C, for 30 min, at 10,000 rpm (J2-HS-centrifuge, JA20-rotor; Beckmann, IN, USA). Supernatant was sequentially filter-sterilized with 0.45 µm and 0.22 µm pore size cellulose-acetate filters (Sartorius, Göttingen, Germany), transferred into sterile anoxic 100 ml serum vials, and flushed 10 min with sterile argon (Ar, 100%).

#### 2.2.1.2. Yeast and bacterial cell lysates

*Saccharomyces cerevisiae* Sa-07140 (DSMZ, Braunschweig, Germany) and *E. coli* K12 (DSMZ) were cultivated at 30°C and 37°C in 4 x 500 ml sterile medium (pH 7; Section 2.3.1 and

Section 2.3.2), respectively. Cells were harvested after 3 days by centrifugation for 20 min at 7,500 rpm (approximately 10,000 × g [J2-HS-centrifuge, JA10-rotor, Beckmann]). Resulting cell pellets were washed three times with sodium phosphate buffer (Section 2.3.3). Afterwards, twenty grams fresh weight (FW) of pelleted cells were suspended in 20 ml sodium phosphate buffer; 400 µl DNase I (10,000 U/ml [Sigma-Aldrich, Taufkirchen, Germany]) was added to prevent the agglomeration of genomic DNA after cell lysis. The cell suspension was then inserted to three consecutive runs of a French press (95,000 to 110,000 kPa [FA-032-40K pressure cell, SLM Aminco, Urbana, IL, USA]). The ruptured cells were centrifuged for 20 min at 15,000 rpm (approximately 27,000 × g [J2-HS-centrifuge, JA20-rotor, Beckmann]) and the pellet containing the cell wall fragments, associated phospholipid membranes, and undrupted cells was discarded. The supernatant fluid was centrifuged again and approximately 27 ml of the supernatant fluid was diluted with 13 ml sodium phosphate buffer. The dilution was filter sterilized (0.2 µm pore size, cellulose-acetate membrane [Sartorius]), and transferred to sterile anoxic 100 ml serum vials that were crimp sealed with sterile rubber stoppers; the vials were then flushed with sterile Ar (100%).

## 2.2.2. Stock suspensions and solutions

### 2.2.2.1. Polysaccharides

For polysaccharide experiment A, microcrystalline cellulose (Merck, Darmstadt, Germany), chitin from shrimp shells (Sigma-Aldrich), pectin from citrus fruits (Sigma-Aldrich), maltodextrin from potato starch (Sigma-Aldrich), and xylan from birchwood (Sigma-Aldrich) were prepared as stock suspensions. Chitin was ground to powder with a mixer mill prior to use (MM400, Retsch, Haan, Germany). Dextran from *Leuconostoc* spp. with the relative molecular mass of approximately 70,000 (Sigma-Aldrich) was prepared as a stock solution. For polysaccharide experiment B, a stock solution of glycogen from bovine liver (Fluka, Schwerte, Germany) and a stock suspension of starch from wheat (Merck) were utilized. All polysaccharide stock suspensions or solutions containing 2 mmol/ml of carbon were prepared in 20 ml anoxic sodium phosphate buffer (Section 2.3.3) and transferred to sterile anoxic 100 ml serum vials that were crimp sealed with sterile rubber stoppers; the vials were then flushed 10 min with sterile Ar (100%). Sterility of non-soluble and non-filter-sterilized polysaccharides suspensions were checked by negative controls, containing 1 ml of suspension and 9 ml of sodium phosphate buffer.

### 2.2.2.2. Protein and RNA

The 10-fold concentrated stock solutions of protein consist of 22.5 mg/ml; bovine serum albumin (Merck) and sterile anoxic sodium phosphate buffer (Section 2.3.3) added to the amount of 10 ml. RNA (from the yeast *Cyberlindnera jadinii*; Sigma) was less soluble than protein, and thus less concentrated stock solutions (8.8 mg/ml) were prepared. The pH was adjusted to pH 7

with 1 M NaOH. Stock solutions of protein and RNA were filter sterilized (0.2 µm pore size, cellulose-acetate membrane [Sartorius]), transferred to sterile anoxic 100-ml serum vials, and crimp sealed with sterile rubber stoppers. The vials were then flushed with sterile Ar (100%). According to the manufacturer's specifications, the RNA contained 10% water, which was neglected for all calculations. The theoretical chemical formulas were used to calculate the amount of carbon provided in a given treatment: for protein  $[\text{CH}_{1.57}\text{N}_{0.27}\text{O}_{0.30}\text{S}_{0.013}]_n$  and for RNA  $[\text{C}_{9.5}\text{H}_{11.75}\text{N}_{3.75}\text{O}_7\text{P}]_n$  (based on 50% GC content and deprotonated phosphate).

#### 2.2.2.3. Yeast extract

Stock solutions of yeast extract were prepared by dissolving 0.52 g yeast extract (Carl Roth GmbH, Karlsruhe, Germany) in 10 ml anoxic sodium phosphate buffer (Section 2.3.3). The solution was filter sterilized and transferred to sterile 100 ml serum vials that were crimp sealed with sterile rubber stoppers; the vials were then flushed with sterile Ar (100%).

#### 2.2.2.4. Saccharides, amino acids, transient intermediates, and others

*N*-acetylglucosamine (AppliChem, Darmstadt, Germany), cellobiose (Sigma-Aldrich), glucose (AppliChem), ribose (Sigma-Aldrich), galacturonic acid monohydrate (Sigma-Aldrich), and xylose (Merck) were prepared as 50 mM stock solutions. All saccharides were dissolved in 20 ml anoxic sodium phosphate buffer, filter sterilized (0.22 µm pore size, cellulose-acetate membrane), and transferred to sterile anoxic 100 ml serum vials that were crimp sealed with sterile rubber stoppers; the vials were then flushed 10 min with sterile Ar (100%).

Stock solutions of casamino acids (Difco Laboratories, Detroit, MI), alanine (Merck), aspartate (Merck), glutamate (Merck), glycine (Sigma-Aldrich), leucine (AppliChem), threonine (Merck), tyrosine (Merck), valine (Merck), ribose (Sigma-Aldrich), ethanol (VWR Chemicals, Darmstadt, Germany), lactate (Sigma-Aldrich), formate (Sigma-Aldrich), succinate (Sigma-Aldrich), glucose (AppliChem), adenine (Carl Roth), uracil (Carl Roth), and glycerol (Grüssing, Filsum, Germany) were prepared with anoxic sodium phosphate buffer (Section 2.3.3 [pH was adjusted to pH 7 with NaOH]). Solutions were filter sterilized (0.22 µm pore size, cellulose-acetate membrane [Sartorius]) into sterile anoxic 100 ml serum vials that were crimp sealed with sterile rubber stoppers; the vials were then flushed 10 min with sterile Ar (100%).

### 2.3. Growth media, buffers, and solutions

The sterilization of growth media, buffers or solutions was ensured by autoclaving (Sanoclav, Wolf, Geislingen, Germany). Deionized double distilled water (ddH<sub>2</sub>O) was produced with a Seral Pro 90 CN ultrapure water purification system (Seral, Ransbach-Baumbach,

Germany). For nucleic acid extraction, ddH<sub>2</sub>O was filter sterilized (0.22 µm pore size, cellulose-acetate membrane [Sartorius]) and autoclaved.

### 2.3.1. Oxidic *S. cerevisiae* growth medium

Yeast extract ( <i>Carl Roth</i> )	7 g	
Tryptic soy broth ( <i>Fluka</i> )	7 g	
Glucose ( <i>AppliChem</i> )	10 g	
ddH <sub>2</sub> O	to 1 l	(pH 7)

### 2.3.2. Oxidic *E. coli* growth medium

Yeast extract ( <i>Carl Roth</i> )	8 g	
Tryptone ( <i>AppliChem</i> )	8 g	
Glucose ( <i>AppliChem</i> )	5 g	
NaCl ( <i>Carl Roth</i> )	5 g	
ddH <sub>2</sub> O	to 1 l	(pH 7)

### 2.3.3. Anoxic sodium phosphate buffer

NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O ( <i>Merck</i> )	1.9 g	
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O ( <i>Merck</i> )	3.4 g	
ddH <sub>2</sub> O	to 1 l	(pH 7)

The sodium phosphate buffer was boiled for 30 min in a Erlenmeyer flask and continuously flushed with N<sub>2</sub>. Afterwards, the buffer was transferred to a 1 l serum flask which was closed with a rubber stopper and a screw cap and flushed again for 10 min with sterile N<sub>2</sub> (100%).

### 2.3.4. Extraction buffer

The extraction buffer consisted of 200 ml potassium phosphate buffer and 400 ml CTAB/NaCl solution.

#### 2.3.4.1. Potassium phosphate buffer

K <sub>2</sub> HPO <sub>4</sub> ( <i>Merck</i> )	7.8 g	
KH <sub>2</sub> PO <sub>4</sub> ( <i>Merck</i> )	0.4 g	
ddH <sub>2</sub> O	to 200 ml	(pH 8)

#### 2.3.4.2. CTAB/ NaCl solution

CTAB ( <i>Carl Roth</i> )	40 g
NaCl ( <i>Carl Roth</i> )	20 g

ddH<sub>2</sub>O to 400 ml

### 2.3.5. Precipitation buffer

PEG (average  $M_n$  6.000; Sigma-Aldrich) 60 g  
NaCl (Carl Roth) 19 g  
ddH<sub>2</sub>O to 200 ml

## 2.4. Nucleic acid extraction

Time-dependent shifts in the microbial community were evaluated by 16S rRNA and 16S rRNA gene analysis (Figure 20 B). The nucleic acid extraction of ice-cooled gut content and soil samples was conducted as described (Griffiths *et al.*, 2000). In detail, 0.2 ml filter sterilized ddH<sub>2</sub>O (Section 2.3), 0.3 g of Ø 0.1 mm and 0.3 g of Ø 0.5 mm zirconia beads (Carl Roth) were added to approximately 0.3 g of gut content or soil in a 2 ml screw cup (A. Hartenstein GmbH, Würzburg, Germany). After the addition of 0.5 ml extraction buffer (Section 2.3.4) and 0.5 ml phenol chloroform:isoamyl alcohol (24:25:1; equilibrated and stabilized; AppliChem) the samples were inserted to two consecutive runs of a FastPrep FP120 bead beater (Thermo Savant, Holbrook, NY, USA) for 30 s at 5.5 m/s. The samples were centrifuged (5 min, 4°C, 15,000 × g; 1-15K microcentrifuge, Sartorius) and the supernatant was transferred into a new sterile vessel before chloroform:isoamyl alcohol (24:1, AppliChem, Darmstadt, Germany) was added. Sample were again centrifuged (5 min, 4°C, 15,000 × g) and the upper phase was transferred into a new sterile vessel. Twice as much precipitation buffer were added and the samples were mixed until a clear solution. After the incubation of the nucleic acids on ice for 2 h, the samples were centrifuged (10 min, 4°C, 18,000 × g) and the supernatant were discarded. The pellets were washed with 0.4 ml ice-cold RNase-free sterile ethanol (70%, VWR Chemicals) followed by another centrifugation step (5 min, 4°C, 15,000 × g). The ethanol was removed, pellets were dried at room temperature, and resuspended in 30 µl DNase/ RNase- free ddH<sub>2</sub>O (Gibco by Life Technologies, Darmstadt, Germany).

### 2.4.1. Enzymatic digestion of DNA or RNA

To obtain pure DNA or RNA, the nucleic acid samples were treated for 45 min at room temperature with 10 µg/µl RNase A (from bovine pancreas [Merck]) or for 45 min at 37°C with 1 U/µl DNase I (Thermo Fisher Scientific, Waltham, MA, USA), respectively. The enzymatic digestion of DNA or RNA was stopped by adding 0.7 volume of ice-cold isopropanol (100%, VWR Chemicals) and 0.1 volume of 5 M NaCl (Green and Sambrook, 2012). The precipitation of DNA or RNA was at -20°C for at least 12 h. After this incubation, the samples were centrifuged (60 min at 18000 × g, 4°C) and washed three times with 400 µl ice-cold ethanol (RNase free, 70%,

VWR Chemicals) and repeated centrifugation steps (5 min at 15,000 × g at 4°C). The resulting pellets were dried at room temperature and resuspended in 30 µl DNase/ RNase-free ddH<sub>2</sub>O.

## **2.4.2. Nucleic acid quantification**

### **2.4.2.1. Photospectrometrically analysis**

A 260/280 nm absorbance ratio was used to determine the concentration and purity of the extracted nucleic acids. A ratio of approximately 1.8 is generally accepted as pure DNA, whereas a ratio of approximately 2.0 reflects pure RNA (Wang and Fujii, 2011; Green and Sambrook, 2012). In this regard, ratios lower than 1.8 or 2.0 indicating contaminations with protein, phenol or huminic acid that absorbance is at or near to 280 nm (Wang and Fujii, 2011; Green and Sambrook, 2012).

### **2.4.2.2. Fluorescence-based analysis**

Additional to the photospectrometrically quantification a fluorescence-based method that is less sensitive to contaminations was used to determine the RNA concentrations. Therefore, the fluorescent reagent of Quant-iT-RiboGreen (Invitrogen, Carlsbad, CA, USA) was added, as described in the manufacturer's protocol, to 1 µl of the resuspended RNA samples. The fluorescence in the samples was quantified with a FLx800 microplate fluorimeter (BioTek, Bad Friedrichshall, Germany) and the software Gen5 (BioTek, Winooski, VT, USA).

## **2.4.3. Polymerase chain reaction**

Three different polymerase chain reaction (PCR) protocols were used to ensure reliable DNA and cDNA samples. For example, the 'control' PCR (Table 7A) was conducted to amplify DNA fragments and therefore to visualize (a) a successful nucleic acid extraction, (b) a sufficient enzymatic digestion of DNA in RNA samples, and (c) the efficient reverse transcription of RNA to complementary DNA (cDNA).

### **2.4.3.1. Reverse transcription PCR**

The SuperScript III RT kit (Invitrogen, Carlsbad, CA, USA) and the manufacturer's protocol were used for the reverse transcription of RNA into cDNA. 10 ng to 1 µg of RNA was added to 1 µl of 100 µM random hexamer primers (Microsynth, Balgach, Switzerland) and 1 µl of 10 mM dNTP mix (Invitrogen, Carlsbad, CA, USA). This reaction mixture was filled up to 14 µl with RNase/DNase-free ddH<sub>2</sub>O and incubated for 5 min at 65°C. The reverse transcription PCR was started after the addition of 4 µl 5 × First-Strand Buffer, 1 µl 0.1 M DTT and 1 µl SuperScript IV RT enzyme (200 U/µl). In the PCR cycler (SensoQuest GmbH, Göttingen, Germany) the PCR started with 5 min at 25°C, followed by 120 min at 50°C, and was stopped with 70 °C for 15 min.

**Table 7.** Reagents and cyclers protocols of the control PCR (A) and first strand bacterial 16S rRNA PCR (B).**(A) Control PCR**

Reaction Mix				Cycler protocol				
Reagent	Volume	Conc.	Final conc.	Step	Temp.	Duration	Description	Cycles
Master Mix <sup>a</sup>	10 µl	variable	variable	1	95 °C	5 min	Initial Denaturation	1
27F Primer	1 µl	10 µM	0.4 µM	2	95 °C	1 min	Denaturation	
907R Primer	1 µl	10 µM	0.4 µM	3	50 °C	30 s	Annealing	25
MgCl <sub>2</sub>	1 µl	25 mM	1 mM	4	72 °C	90 min	Extension	
Template	1 µl	as available	as available	5	72 °C	5 min	Final Extension	1
ddH <sub>2</sub> O	to 25 µl	-	-	6	4 °C	∞	Storage	-

**(B) First Step Bacterial 16S rRNA PCR**

Reaction Mix				Cycler protocol				
Reagent <sup>b</sup>	Volume	Conc.	Final conc.	Step	Temp.	Duration	Description	Cycles
KAPA Buffer	5 µl	5 x	1x	1	95 °C	3 min	Initial Denaturation	1
10 mM KAPA dNTP Mix	0.75 µl	10 µM	0.3 µM each	2	98 °C	20 s	Denaturation	
KAPA DNA Polymerase	0.5 µl	1 U/µL	1 U	3	65 °C	30 s	Annealing	20
Primer	0.75 µl	10 µM	0.3 µM	4	72 °C	30 s	Extension	
Primer	0.75 µl	10 µM	0.3 µM	5	72 °C	5 min	Final Extension	1
Template	-	-	0.5 ng/µl	6	4 °C	∞	Storage	-
ddH <sub>2</sub> O	to 25 µl	-	-					

<sup>a</sup>Two different master mixes were used over the years. The master mix purchased from 5PRIME (Hamburg, Germany) had a final concentration of 1.5 mM, whereas the master mix purchased from GenOn (Ludwigshafen am Rhein, Germany) had a final concentration of 1.8 mM.

### 2.4.3.2. Illumina sequencing: Bacterial 16S rRNA PCR

Bacterial 16S rRNA gene and 16S rRNA amplification were performed by Microsynth AG (Balgach, Switzerland). Some treatments were pooled and others were analyzed on a per replicate basis in order to evaluate reproducibility. In this regard, it is noteworthy that due to the limitations of Illumina sequencing, analyzing all replicates would have decreased the number of sequences obtained per sample, and obtaining a greater number of sequences for each of the treatments was therefore favored. First step PCR amplification of the 16S rRNA V3-V4 region from either cDNA (16S rRNA [RNA]) or genomic DNA (16S rRNA gene) was performed with the primers Bakt 341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt 805R (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann *et al.*, 2011) using a KAPA HiFi HotStart PCR Kit (KAPABiosystems, Wilmington, USA) per manufacturer's two-step PCR protocol (Table 7B). The same chemicals and thermoprotocols were used for the second step but 1 µl of purified PCR product (derived from the first step of the two-step PCR) per 50 µl reaction volume was used as template, primers Bakt 341F and Bakt 805R were extended with sample-specific multiplex identifiers, and the number of cycles was 12 instead of 20.

### 2.4.4. Agarose gel electrophoresis

The visualization of PCR-amplified DNA fragments was performed with agarose gel electrophoresis. 0.8% standard agarose (AppliChem) gels were prepared by heating up a mixture of agarose and TAE buffer (a mixture of tris base, acetic acid and EDTA; Millipore, Temecula, CA, USA) using a microwave. After the complete dissolution of the agarose in the buffer and a cooling down to approximately 50 °C, ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridium bromide; BioRad, Hercules, CA, USA) at a final concentration of 0.08 mg/ml was added. The solution was then transferred into a gel electrophoresis chamber (Mini- or Maxi-Sub cell, BioRad) filled with TAE buffer.

The samples for the gel wells consist of 5 µl PCR product plus 1 µl 6 × Blue Orange loading dye (Promega, Madison, WI, USA). For fragment size allocation a volume of 2 µl molecular-weight size marker (MWM 1, Bilatec, Viernheim, Germany) were transferred into at least one of remained gel wells. The electrophoresis ran for 50 min at 70 V (Power-Pak 3000, BioRad). The visualization of amplified DNA fragments were ensured with a UV light (302 nm; Transilluminator UVT-20M, Herolab GmbH, Wiesloch, Germany) and a self-made light-protecting chamber. The gel with the illuminating DNA bands was captured with a Canon PowerShot G5 camera (Canon, Krefeld, Germany).

## 2.5. Chemical analyses

### 2.5.1. Dry weight of gut content, dietary materials, and lysates

The dry weights (DW) of earthworm gut content, soil, turf, worm bedding, and cell lysates were determined by weighing before and after drying at 60°C for 7 days (Horn *et al.*, 2003; Wüst *et al.*, 2011).

**Table 8.** Dry weights of earthworm gut content, different dietary materials, and lysates.

Sample	Number of Replicates	Dry Weight (%) ± Standard Deviation	Section
Gut Content	10	45 ± 2.2	
Soil <sub>A</sub>	10	76 ± 4.8	2.1.1 and 3.3
Soil <sub>B</sub>	3	87 ± 1.2	2.1.1 and 3.5
Turf	3	87 ± 0.8	
Worm Bedding	3	37 ± 12	
<i>S. cerevisiae</i> Lysate <sub>A</sub>	3	5.1 ± 0.1	2.2.1.2 and 3.2
<i>E. coli</i> Lysate	3	5.3 ± 0.1	
<i>S. cerevisiae</i> Lysate <sub>B</sub>	3	5.3 ± 0.1	2.2.1.2 and 3.3.1
Root Lysate	3	1.3 ± 0.1	
Leaf Litter Lysate	3	0.9 ± 0.1	2.2.1.1 and 3.1.6

### 2.5.2. Gases, soluble organic compounds, and pH

Gases and soluble organic compounds were measured by GC or HPLC, respectively (Table 9). Calculated amounts of H<sub>2</sub> and CO<sub>2</sub> in the gas and liquid phases of microcosms are based on the ideal gas law and standard solubility tables (Blachnik, 1998; Equation 1-7). For CO<sub>2</sub>, amounts of bicarbonate, calculated from dissolved CO<sub>2</sub>, pH, and the dissociation constant, were taken into consideration. A WTW pH 323 pH-meter (Zeller, Hohenems, Austria) was used to measure pH. Final amounts of gases and organic compounds were normalized to the fresh weight or dry weight of gut content or soil. For converting amounts of a product from µmol per g fresh weight to mM or µmol per g dry weight, multiply by 0.1 (e.g., 100 µmol per g fresh weight equals 10 mM) or divide by 0.45 (e.g., 100 µmol per g fresh weight equals 222 µmol per g dry weight), respectively. Presented amounts of gases were cumulative and mostly in µmol/g<sub>FW</sub>. The theoretically production of gases in the removed liquid phase for sampling were considered and added to the cumulative amounts.

**Table 9.** Instrumentation utilized for analyses of soluble organic compounds and gases.

Parameter	Chromatograph			
	Hewlett Packard 1090 Series II	Agilent 1200 Series	Hp Hewlett 5890 Packard Series II	Schambeck SRI 8610C
<b>Detected compounds</b>	Organic acids	Organic acids	Hydrogen	Carbon dioxide
<b>Column</b>	Rezex ROA-Organic Acids (300 x 7,8 mm; Phenomenex, Torrance, CA, USA)	Rezex ROA-Organic Acids (300 x 7,8 mm; Phenomenex, Torrance, CA, USA)	Molecular sieve13X, 2 m x 1/8'' (Restek, Bellefonte, PA, USA)	Hayesep-D 2 m x 1/8'' (SRI Instruments, Earl St. Torrance, CA, USA)
<b>Oven temperature</b>	60°C	60°C	60°C	80°C
<b>Detector</b>	G1362A refractive index detector (RID)	G1362A refractive index detector (RID)	Thermal conductivity detector (TCD)	Thermal conductivity detector (TCD)
<b>Detector temperature</b>	40°C	40°C	175°C	175°C
<b>Flow rate</b>	0.8 ml/min	0.8 ml/min	20 ml/min	20 ml/min
<b>Injections volume</b>	20 µl	50 µl	0.1 ml	0.1 ml
<b>Software</b>	ChemStation (Agilent Technologies, Böblingen, Germany)	ChemStation (Agilent Technologies, Böblingen, Germany)	EuroChrom Software for Windows (Ver: Basic Edition V3. 05, Wissenschaftliche Gerätebau, Berlin, Germany)	Peak simple Software (Ver: 4.20, SRI Instruments)
<b>Mobile phase/ Carrier gas</b>	4 mM H <sub>3</sub> PO <sub>4</sub>	4 mM H <sub>3</sub> PO <sub>4</sub>	Argon	Helium

**Equation 1. Slope intercept form**

$$y = m * x + b; \quad x = \frac{y - b}{m}$$

$y$ , peak area of H<sub>2</sub> or CO<sub>2</sub> in the gas chromatogram;  $m$ , slope of the calibration curve;  $x$ , gas concentration (%);  $b$ , point of intersection with  $y$ -axis (was set to zero). For equation 2,  $x$  was divided by one hundred.

**Equation 2. Concentration of CO<sub>2</sub> or H<sub>2</sub> in the gas phase ( $C_g$  in  $mmol/ml$ )**

$$C_g = \frac{x * P_{amb} + P_{mc}}{R * T_{amb}}$$

$C_g$ , was calculated using the ideal gas law;  $P_{amb}$ , ambient pressure ( $mbar$ );  $P_{mc}$ , pressure in microcosm ( $mbar$ );  $R$ , universal gas constant ( $83.145 \frac{mbar * ml}{mmol * K}$ );  $T_{amb}$ , ambient temperature ( $kelvin; K$ ).

**Equation 3. Amount of CO<sub>2</sub> or H<sub>2</sub> in the gas phase ( $n_g$  in  $mmol$ )**

$$n_g = C_g * V_g$$

$V_g$ , volume of gas phase in microcosm ( $ml$ ).

**Equation 4. Amount of physically dissolved CO<sub>2</sub> or H<sub>2</sub> in the liquid phase ( $n_{lp}$  in  $mmol$ )**

$$n_{lp} = C_g * V_l * \alpha$$

$V_l$ , volume of liquid phase in microcosm ( $ml$ );  $\alpha$ , Bunsen solubility coefficients of CO<sub>2</sub> or H<sub>2</sub> in water (0.74 or 0.02 at 25°C; Blachnik, 1998).

**Equation 5. Amount of chemically dissolved CO<sub>2</sub> in the liquid phase ( $n_{lc}$  in  $mmol$ )**

$$n_{lp} = C_g * V_l * \alpha * 10^{-pKa+pH}$$

$n_{cl}$ , was considered for the total CO<sub>2</sub> that reacts at pH 7 chemically with the liquid phase mostly to hydrogen bicarbonate;  $pKa$ , the negative (base 10) logarithm of the acid dissociation constant of carbonic acid (6.37 at 25°C; Blachnik, 1998).

**Equation 6. Amount of CO<sub>2</sub> or H<sub>2</sub> in the liquid phase ( $n_l$  in  $mmol$ )**

$$n_l = n_{cl} + n_{pl}$$

**Equation 7. Total amount of CO<sub>2</sub> or H<sub>2</sub> in microcosm ( $n_t$  in  $mmol$ )**

$$n_t = n_l + n_g$$

### 2.5.3. Organic carbon quantification

Organic carbon content of leaf litter lysate, root lysate, turf, worm bedding, and soil were analyzed by the Keylab Experimental Biogeochemistry (Bayreuth Center of Ecological and Environmental Research, University of Bayreuth, Germany) (Table 10). Lysates, turf, worm bedding, and soil samples were dried for 7 days at 65 °C. Turf, worm bedding, and soil samples were then grounded to powder with a mixer mill (MM200, Retsch, Haan, Germany). 0.6 to 1.2 mg of dried lysate and 1 to 2 mg of dried turf, worm bedding, and soil were transferred in silver weighing boats (6 × 6 × 12 mm; Elemental Microanalysis, Okehampton, UK). Inorganic carbon (i.e., carbonates) was eliminated as CO<sub>2</sub> by the addition of 2 to 3 drops of 8 % (v/v) hydrochloric acid (Merck) and an overnight incubation, followed by another drying step for at least 2 h at 80°C. Silver boats with samples were placed in tin boats (6 × 6 × 12 mm; Lab Need, Nidderau, Germany), folded tightly, and combust with an O<sub>2</sub> inflow in a cobalt-chromium combustion column at 900 °C in a CHN element analyzer (Flash-EA 112; Thermo Fisher Scientific [formerly ThermoQuest]). With helium as carrier gas, free O<sub>2</sub> and H<sub>2</sub>O were eliminated from the resulting CO<sub>2</sub> by a copper reduction column and a water trap containing magnesium perchlorate (Mg[Cl<sub>4</sub>]<sub>2</sub>, Thermo Fisher Scientific), respectively. The amount of CO<sub>2</sub> was detected by a thermal conductivity detector (TDC).

**Table 10.** Organic carbon content of different dietary materials and plants lysates.

Substrate	Number of Replicates	Organic carbon content (%)	Sections
Root Lysate	8	30 ± 1.8	
Leaf Litter Lysate	8	24 ± 0.6	2.2.1.1 and 3.1.6
Soil	4	2.3 ± 0.0	
Turf	4	4.9 ± 0.7	2.1.1 and 3.5
Worm Bedding	4	48 ± 0.6	

### 2.5.4. Determination of ammonia

The production of ammonium in amino acids-supplemented microcosms was determined by using a modified protocol (Weatherburn, 1967). In this regard, 50 µl of 2% sodium phenolate (Merck), 25 µl of 0.005% sodium nitroprusside (Merck), and 25 µl of sodium hypochlorite working solution were mixed with 100 µl microcosms sample obtained from the respective microcosm in a 96-well multi test plate (neoLab, Heidelberg, Germany). The sodium hypochlorite working solution consisted of 25 ml sodium hypochlorite containing 12% Cl (Carl Roth) and 1.13 g NaOH (Carl Roth), and was filled up to 250 ml with ddH<sub>2</sub>O. The absorbance spectrum was measured at 630 nm with a µQuant spectrophotometer (BioTek Instruments GmbH, Bad Friedrichshall, Germany), after a 30 min incubation in the dark at 30 °C.

## 2.6. Sequence analyses

### 2.6.1. Data obtained by Illumina sequencing

Illumina sequencing, and clustering of sequences into operational taxonomic units (OTUs) was performed by Microsynth. In this regard, the Illumina MiSeq platform and a v2 500 cycles kit were used to sequence PCR libraries. The paired-end reads that passed Illumina's chastity filter were subject to de-multiplexing and trimming of adaptor residuals using Illumina's Real Time Analysis software. The quality of the reads was checked with FastQC 0.11.5 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The locus specific V3-V4 primers were trimmed from the reads with Cutadaptv1.14 (Martin, 2011). Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were stitched in-silico employing a minimum overlap of 15 bases with USEARCH 8.1.1861 (Edgar, 2010; Edgar and Flyvbjerg, 2015). Stitched sequences were quality filtered allowing a maximum of one expected error per stitched read; ambiguous bases were discarded (Edgar and Flyvbjerg, 2015). Remaining reads were clustered at a 97% similarity level (if not otherwise indicated) using USEARCH to form OTUs. Singletons and chimeras were discarded (Edgar, 2013). OTUs were aligned against the reference sequences of the Silva v128 database (Pruesse *et al.*, 2007), and taxonomies were predicted by employing a minimum confidence threshold of 0.6 using USEARCH.

### 2.6.2. Diversity analysis

#### 2.6.2.1. Rarefaction analysis

Rarefaction curves represent the diversity as a function of sequencing depth. These curves were generate to evaluate the richness of the different samples. The analysis based on the Hurlbert method (Hurlbert, 1971), and the calculations were conducted with aRarefact software (<http://www.uga.edu/strata/software/>). Flattening curves indicate a sufficient sampling and that most of the expected diversity was covered by the sampling effort. Weakly increasing curves reflect a lower diversity and are caused by either an insufficient sampling or a high prevalence of stimulated subgroups after the incubation.

#### 2.6.2.2. Alpha and beta diversity

Alpha diversity was analyzed by the calculation of Chao and Shannon indices. Chao1 indices are based on the ratio of expected phylotypes versus detected phylotypes (Equation 8) and reflect therefore the species richness. In contrast, the Shannon indices are based on both richness and evenness (Equation 9; Magurran, 2004; Lemos *et al.*, 2011). Typical values are generally between 1.5 and 3.5, and indices are rarely greater than 4. The Shannon index increases when both the richness and the evenness of the community increase.

**Equation 8. Chao1 index ( $S_{Chao1}$ )**

$$S_{Chao1} = P_{obs} + \left( \frac{(f_1)^2}{2f_2} \right)$$

$P_{obs}$ , overserved phylotypes per sample;  $f_1$ , overserved singletons per sample;  $f_2$ , overserved doubletons per sample.

**Equation 9. Shannon index ( $H'$ )**

$$H' = -\sum p_i \ln p_i ; p_i = \frac{n_i}{N}$$

$n_i$ , reads per phylotype;  $N$ , number of all reads per sample.

Two-dimensional non-metric multidimensional scaling (NMDS) was used to illustrate shifts in the microbial communities during incubation. This beta diversity analysis was based on the abundance of all detected phylotypes (clustered at a 97% similarity level) and the Bray-Curtis distance, a method not affected by the frequency of null values. The matrices was calculated and depicted with the Past 3 software (Hammer *et al.*, 2001). Proximity of points represent the degree of similarity between the different treatments.

**2.6.3. Phylogenetic trees**

Phylogenetic trees are based on the maximum parsimony, neighbor joining, or maximum likelihood algorithm and were generated using the ARB software (Ludwig, 2004). The trees consist of representative sequences of the most abundant OTUs (phylotypes; clustered at a 97% similarity level) and closely affiliated reference sequences. Branch length and bootstrap values (1,000 resamplings) are derived from the maximum parsimony tree, and *Thermotoga maritima* (AE000512) was used as outgroup. Accession numbers (Section 2.6.5.) occur at the end of each branch. Representative sequences of the most abundant or responsive phylotypes were aligned to public sequences of BLAST (Basic Local Alignment Search Tool; Zhang *et al.*, 2000) to determine the closest cultured microorganism and corresponding sequence identity.

**2.6.4. Sequence abundances**

The relative sequence abundances of detected phylotypes, including less abundant once not considered in the results section, are provided in the appendix (Table A1-A11).

**2.6.5. Accession numbers**

Representative sequences of phylotypes with  $\geq 0.1\%$  relative abundance were deposited at the European Nucleotide Archive (ENA; Table 11).

**Table 11.** Accession numbers of deposited sequences and associated experiments.

Experiment	Phylotype Descriptor	Study Number	Accession Numbers	Section
Yeast Cell Lysate	CL	PRJEB15377	LT626667-823	3.2.5
Protein and RNA	PR	PRJEB15410	LT626824-940	3.2.5
Yeast Extract	E	PRJEB25179	LT986012-173	3.3.3
Polysaccharide A	P <sub>A</sub>	PRJEB29296	LR027604-703	3.1.5
Saccharide	S	PRJEB29312	LR027704-803	3.1.5
Polysaccharide B	P <sub>B</sub>	PRJEB29747	LR129845-947	3.1.5
Amino Acid	A	PRJEB32428	LR588706-802	3.2.10
Transient Intermediate	T	PRJEB32429	LT588628-705	3.2.10
Ribose	R	PRJEB32430	LR588803-886	3.2.10
Dilution	D	PRJEB32458	LR589684-794	3.4.2
Symbiont	OC	PRJEB32464	LR589795-898	3.5

## 2.7. Further calculations and statistics

### 2.7.1. Theoretical carbon content of yeast extract and microbial- and plant-derived lysates

The amount of carbon per ml yeast cell lysate and commercial yeast extract was calculated based on the dry weight (Table 8) and a molar mass of 26.2 g/mol for *S. cerevisiae*-derived biomass; according to the chemical formula  $[\text{CH}_{1.613}\text{O}_{0.557}\text{N}_{0.158}\text{P}_{0.012}\text{S}_{0.003}\text{K}_{0.022}\text{Mg}_{0.003}\text{Ca}_{0.001}]_n$  (Von Stockar and Liu, 1999). The amount of carbon per ml *E. coli* lysate based also on the dry weight (Table 8) but on a different chemical formula  $([\text{CH}_{1.59}\text{O}_{0.374}\text{N}_{0.263}\text{P}_{0.0234}\text{S}_{0.006}]_n$ ; Von Stockar and Liu, 1999) that yielded a molar mass of 24.2 g/mol for *E. coli*-derived biomass. Both chemical formulas consider the total microbial biomass including cell walls and membranes rather than the pure cytoplasmic fraction which was used in this work. The amount of carbon per ml fresh plant lysate was calculated based on the dry weight (overnight at 80°C) and a molar mass of 30 g/mol; according to the chemical formula  $[\text{CH}_2\text{O}]_n$ . The amount of salt in the sodium phosphate buffer (4.3 mg/ml; used for preparations of the lysates [Section 2.2.1]) was taken into account.

### 2.7.2. Recoveries of carbon and reducing equivalents

For recoveries derived from different substrates, amounts of gases or organic compounds formed in unsupplemented controls were subtracted from those of supplemented treatments to obtain net amounts of a certain fermentation product X ( $n_{\text{net}}X$ ).  $n_{\text{net}}X$  was multiplied with the number of carbon atoms ( $n_c$ ) and the number of reducing equivalents ( $n_r$ ) to calculate the amount

of carbon ( $n_cX$ ; Equation 10) and the amount of reducing equivalents ( $n_rX$ , Equation 11) recovered in the fermentation product X, respectively (Table 12A).  $n_cX$  was divided by the total amount of carbon atoms supplemented as substrate ( $n_cS$ ) to obtain final carbon recoveries ( $R_cX$ ; Equation 12). Final recoveries of reducing equivalents ( $R_rX$ ) were calculated by dividing  $n_rX$  by the total amount of reducing equivalents supplemented as substrate ( $n_rS$ ) (Equation 13).  $n_rS$  was obtained by the multiplication of  $n_cS$  with the number of reducing equivalents per carbon atom of the substrate ( $n_rC$ , Table 12B) (Equation 14).

**Equation 10. Amount of carbon per fermentation product ( $n_cX$ )**

$$n_cX = n_{net}X * n_c$$

$n_{net}X$ , net amounts of a certain fermentation product X;  $n_cX$ , number of carbon atoms recovered in the fermentation product X (Table 12A).

**Equation 11. Amount of reducing equivalents per fermentation product ( $n_rX$ )**

$$n_rX = n_{net}X * n_r$$

$n_rX$ , number of electrons obtained by complete oxidation of fermentation product X to CO<sub>2</sub> (Table 12A).

**Equation 12. Carbon recovery per fermentation product ( $R_cX$  in %)**

$$R_cX = \frac{n_cX}{n_cS} * 100$$

$n_cS$ , total amount of substrate-derived carbon.

**Equation 13. Reducing equivalent recovery per fermentation product ( $R_rX$  in %)**

$$R_rX = \frac{n_rX}{n_rS} * 100$$

**Equation 14. Total amount of substrate-derived reducing equivalents ( $n_rS$ ):**

$$n_rS = n_rC * n_cS$$

$n_rC$ , reducing equivalents per substrate carbon atom.

**Table 12.** Reducing equivalents in fermentation products (A) and supplemented substrates (B).**(A) Fermentation Products<sup>a</sup>**

Product	Chemical Formula displaying n <sub>c</sub>	n <sub>r</sub> X
Hydrogen	H <sub>2</sub>	2
Carbon dioxide	CO <sub>2</sub>	-
Acetate	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> <sup>-</sup>	8
Butyrate	C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> <sup>-</sup>	20
Formate	CHO <sub>2</sub> <sup>-</sup>	2
Isobutyrate	C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> <sup>-</sup>	20
Lactate	C <sub>3</sub> H <sub>5</sub> O <sub>3</sub> <sup>-</sup>	12
Methylbutyrate	C <sub>5</sub> H <sub>9</sub> O <sub>2</sub> <sup>-</sup>	26
Propionate	C <sub>3</sub> H <sub>5</sub> O <sub>2</sub> <sup>-</sup>	14
Succinate	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> <sup>2-</sup>	14
Ethanol	C <sub>2</sub> H <sub>5</sub> OH	12

**(B) Supplemented Substrates<sup>b</sup>**

Substrate	Chemical Formula displaying n <sub>c</sub>	n <sub>r</sub> C
<b>Lysates and Extracts:</b>		
Leaf Litter Lysate	[CH <sub>2</sub> O] <sub>n</sub>	4.00
Root Lysate	[CH <sub>2</sub> O] <sub>n</sub>	4.00
<i>S. cerevisiae</i> Lysate <sup>c</sup>	[CH <sub>1.613</sub> O <sub>0.557</sub> N <sub>0.158</sub> P <sub>0.012</sub> S <sub>0.003</sub> K <sub>0.022</sub> Mg <sub>0.003</sub> Ca <sub>0.001</sub> ] <sub>n</sub>	4.02
<i>E. Coli</i> Lysate <sup>c</sup>	[CH <sub>1.59</sub> O <sub>0.374</sub> N <sub>0.263</sub> P <sub>0.0234</sub> S <sub>0.006</sub> ] <sub>n</sub>	4.02
Yeast Extract <sup>c</sup>	[CH <sub>1.613</sub> O <sub>0.557</sub> N <sub>0.158</sub> P <sub>0.012</sub> S <sub>0.003</sub> K <sub>0.022</sub> Mg <sub>0.003</sub> Ca <sub>0.001</sub> ] <sub>n</sub>	4.02
<b>Biopolymers:</b>		
Cellulose	[C <sub>12</sub> H <sub>20</sub> O <sub>10</sub> ] <sub>n</sub>	4.00
Chitin	[C <sub>8</sub> H <sub>13</sub> NO <sub>5</sub> ] <sub>n</sub>	4.00
Dextran	[C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ] <sub>n</sub>	4.00
Glycogen	[C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ] <sub>n</sub>	4.00
Maltodextrin	[C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ] <sub>n</sub>	4.00
Pectin	[C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> ] <sub>n</sub>	3.33
Protein (BSA)	[CH <sub>1.57</sub> N <sub>0.27</sub> O <sub>0.30</sub> S <sub>0.013</sub> ] <sub>n</sub>	4.15
RNA	[CH <sub>1.237</sub> N <sub>0.395</sub> O <sub>0.737</sub> ] <sub>n</sub>	3.10
Starch	[C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ] <sub>n</sub>	4.00
Xylan	[C <sub>5</sub> H <sub>8</sub> O <sub>4</sub> ] <sub>n</sub>	4.00
<b>Saccharides and Nucleobases:</b>		
Adenine	C <sub>5</sub> H <sub>5</sub> N <sub>5</sub>	2.00
Cellobiose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	4.00
Galacturonic Acid	C <sub>6</sub> H <sub>10</sub> O <sub>7</sub>	3.33
Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	4.00
Glycerol	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	4.67
<i>N</i> -acetylglucosamine	C <sub>8</sub> H <sub>15</sub> NO <sub>6</sub>	4.00
Ribose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	4.00

Substrate	Chemical Formula displaying $n_c$	$n_rC$
Uracil	$C_4H_4N_2O_2$	2.50
Xylose	$C_5H_{10}O_5$	4.00
<b>Amino Acids:</b>		
Alanine	$C_3H_7NO_2$	4.00
Aspartate	$C_4H_7NO_4$	3.00
Casamino Acids	$CH_{1.942}O_{0.481}N_{0.250}S_{0.005}$	4.20
Glutamate	$C_5H_9NO_4$	3.60
Glycine	$C_2H_5NO_2$	3.00
Threonine	$C_4H_9NO_3$	4.00
Valine	$C_5H_{11}NO_2$	4.80

<sup>a</sup> $n_rX$ , number of reduction equivalents recovered in a certain detected fermentation product. Numbers based on the redox states of the carbon atoms, and the assumption that the compound is completely oxidized to  $CO_2$ .

<sup>b</sup>Chemical formula of the biopolymers is based on the most abundant backbone subunit. Chemical formula of the microbial cell lysates obtained from Von Stockar and Liu, 1999.  $n_rC$ , reduction equivalents per carbon atom of substrate. Numbers based on the average redox state of the carbon atoms in a respective substrate, and the assumption that the compound is completely oxidized to  $CO_2$ .

### 2.7.3. Arithmetic average, standard deviation, and variance

The fermentation product profiles based on a three replicate analysis. Likewise, 16S rRNA sequence analysis was performed individually for the three replicates, if not otherwise indicated. Thus, arithmetic average (arithmetic mean) and standard deviation calculations based on the concentrations of fermentation products or the relative 16S rRNA gene or 16S rRNA abundances detected in the three replicates (Equation 15-17).

#### Equation 15. Arithmetic average ( $\bar{x}$ )

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

$n$ , number of replicates;  $i$ , variable number that starts at 1 and run to  $n$ ;  $x_i$ , concentration of a certain fermentation product or relative abundance of a certain taxa in a respective replicate.

#### Equation 16. Standard deviation ( $SD$ )

$$SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

#### Equation 17. Variance ( $SD^2$ )

$$SD^2 = \frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}$$

## 2.7.4. Statistical analyses

### 2.7.4.1. *t*-test

The unequal variance *t*-test was used to identify statistically significant differences in the replicated fermentation product profiles of either (a) supplemented treatments versus unsupplemented controls or (b) supplemented gut content treatments versus supplemented soil treatments. Unless otherwise stated, *P* values are based on the net amount of products at the end of the incubation. The unequal variance *t*-test was also used to evaluate (a) the response of abundant families or phylotypes and (b) the differences in the alpha diversities obtained from gut content or soil treatments (Section 2.6.2.2). The analysis were based on three replicates, and a *t*-test-derived *P* value of  $\leq 0.05$  indicates a statistically significant difference.

### 2.7.4.2. Linear discriminant analysis effect size analysis

Linear discriminant analysis (LDA) effect size (LEfSe) (Segata *et al.*, 2011) method was used to (a) evaluate the significant (Kruskal-Wallis test) response of abundant taxa (i.e., abundant families and phylotypes) derived from the 16S rRNA gene and 16S rRNA analysis, and (b) rank significant taxa according to the effect sizes. The LEfSe analysis was based on three replicates, and a Kruskal-Wallis test-derived alpha value of  $\leq 0.05$  indicates a statistically significant difference.

## 2.8. Contributions of coworkers

This dissertation was initiated based on preliminary work of my master study, and experiments were conceptualized by Prof. Dr. Harold L. Drake, Dr. Oliver Schmidt, and myself. Franziska Bär conducted preliminary experiments on polymers. Jennifer Guhl conducted practical work for the dilution experiment, microcosms supplemented with starch, glycogen, and ribose, and the RNA extraction of the symbiont experiment. Maraike Staeger conducted practical work for the amino acid and transient intermediate experiments. Ammonia quantification was conducted by Julia Schmidt.

## 3. RESULTS

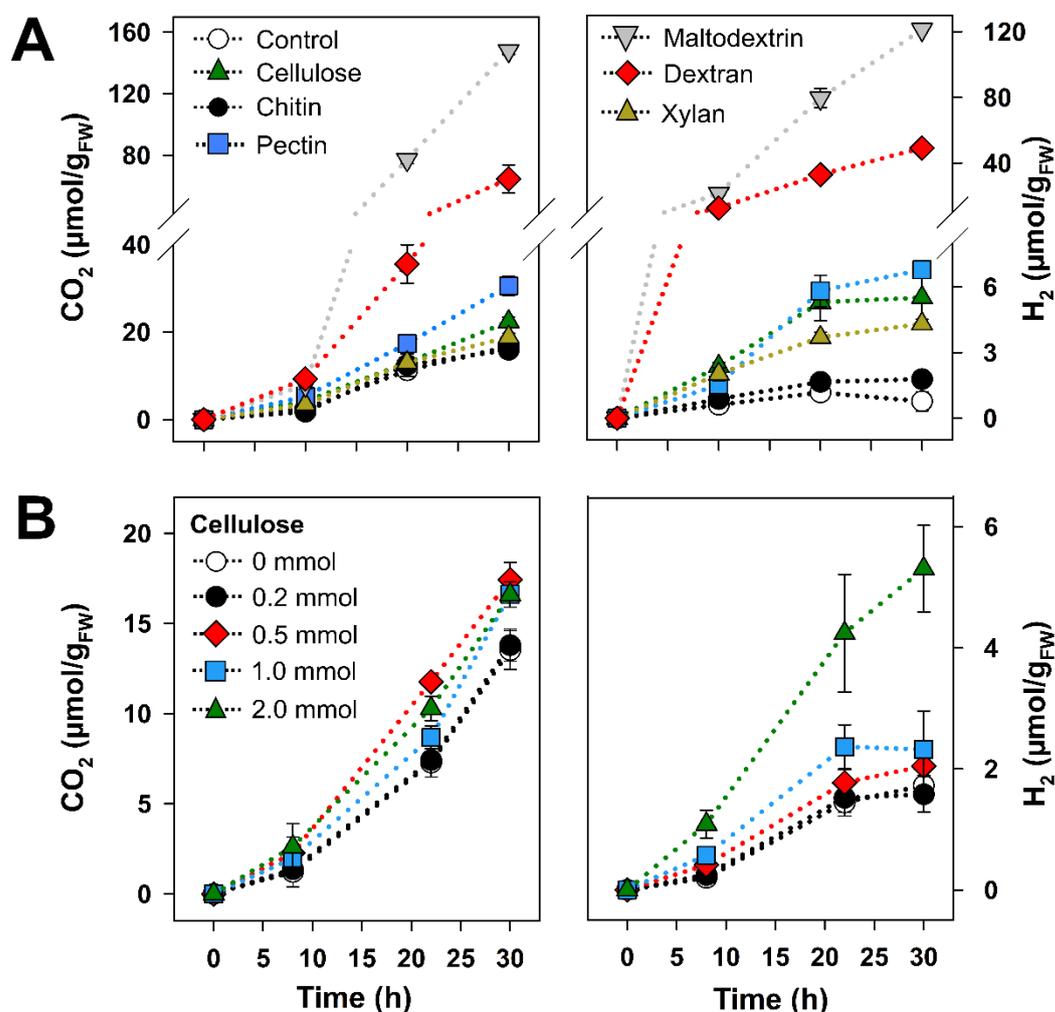
### 3.1. Impact of dietary polysaccharides and saccharides on the fermentative gut microbiota of *L. terrestris*

Earthworm-excreted gut mucus is subject to fermentative utilization by gut bacteria (Section 1.2.2). Although a reusage of mucus-derived organic carbon is advantageous for this invertebrate, a self-perpetuation is impossible. Therefore, the maintenance and growth of earthworms being ultimately dependent on the incorporation of nutrients from the environment. In this regard, the gizzard-linked disruption of ingested environmental materials introduces plant- and microbial-derived polysaccharides to the alimentary canal (Section 1.3), and fermentative gut microbiota capable of degrading polysaccharides might (a) enhance the earthworm-facilitated turnover dynamics of soil organic matter and (b) produce fermentation products as a source of nutrition for the earthworm (Section 1.2.2). However, relatively little is known about the capacity of fermentative microbes in the earthworm gut to utilize ingested polysaccharides. These considerations prompted the evaluation of the effects of model polysaccharides, as well as the saccharides from which they are constructed, on the gut content fermentation and associated microbiota of *L. terrestris*.

#### 3.1.1. Effect of polysaccharides on gut content fermentation

Diverse fermentations yield  $H_2$  and  $CO_2$ , and the simultaneous anaerobic production of these gases is an indicator of fermentation (Buckel, 1999). The formation of  $H_2$  and  $CO_2$  was slightly higher in anoxic cellulose-, pectin-, and xylan-supplemented treatments than in the unsupplemented control treatment (Figure 21 A and Table 13). Although the stimulation by cellulose was marginal, the production of  $CO_2$  and  $H_2$  was statistically significant (Table 14), and increasing amounts of cellulose triggered small increases in the production of these gases (Figure 21B and Figure 22), indicating a marginal usage of cellulose. The differences between products formed in the control treatment and structural polysaccharide treatments (e.g., chitin treatment) were relatively small (Figure 22 A and Table 13), and an apparent increase in a fermentation product was sometimes not significant (e.g., the apparent increase of acetate production in the cellulose treatment was not statistically significant [Table 14]). Likewise, no significant difference was observed between the collective amounts of fermentation products formed in these biopolymer treatments compared to the unsupplemented control (Figure 22), demonstrating that gut-associated fermenters were poised to respond weakly to these structural polysaccharides. In marked contrast to these results, the non-structural energy-storage polysaccharides maltodextrin and dextran yielded an enhanced production of  $H_2$  and  $CO_2$  (Figure 21), with a concomitant production of fatty acids (e.g., acetate and lactate) and ethanol (Figure 22 A). These observations

demonstrated that the fermentative gut microbiota of *L. terrestris* had a high capacity to use non-structural biopolymers, and this potential was consistent with the strongly stimulated fermentation in glycogen and starch treatments. In this regard, the production of glycogen- and starch-derived CO<sub>2</sub>, H<sub>2</sub>, acetate, lactate, and ethanol was significant compared to the unsupplemented control (Figure 22 B; Table 13 and Table 14). In contrast to the negligible amounts of carbon theoretically recovered in the detected fermentation products derived from the structural polysaccharide treatments (0.5 to 2.6%), approximately 45%, 15%, 42%, and 28% of maltodextrin-, dextran-, glycogen-, and starch-derived carbon, respectively, were theoretically recovered in these treatments (Table 15), indicating that the amount of these supplemental non-structural polysaccharides was adequate for the observed fermentations. The low recoveries of cellulose-, pectin-, xylan-, and chitin-derived carbon in fermentation products (Table 15), confirmed that these polysaccharides had only a minimal impact on fermentation.

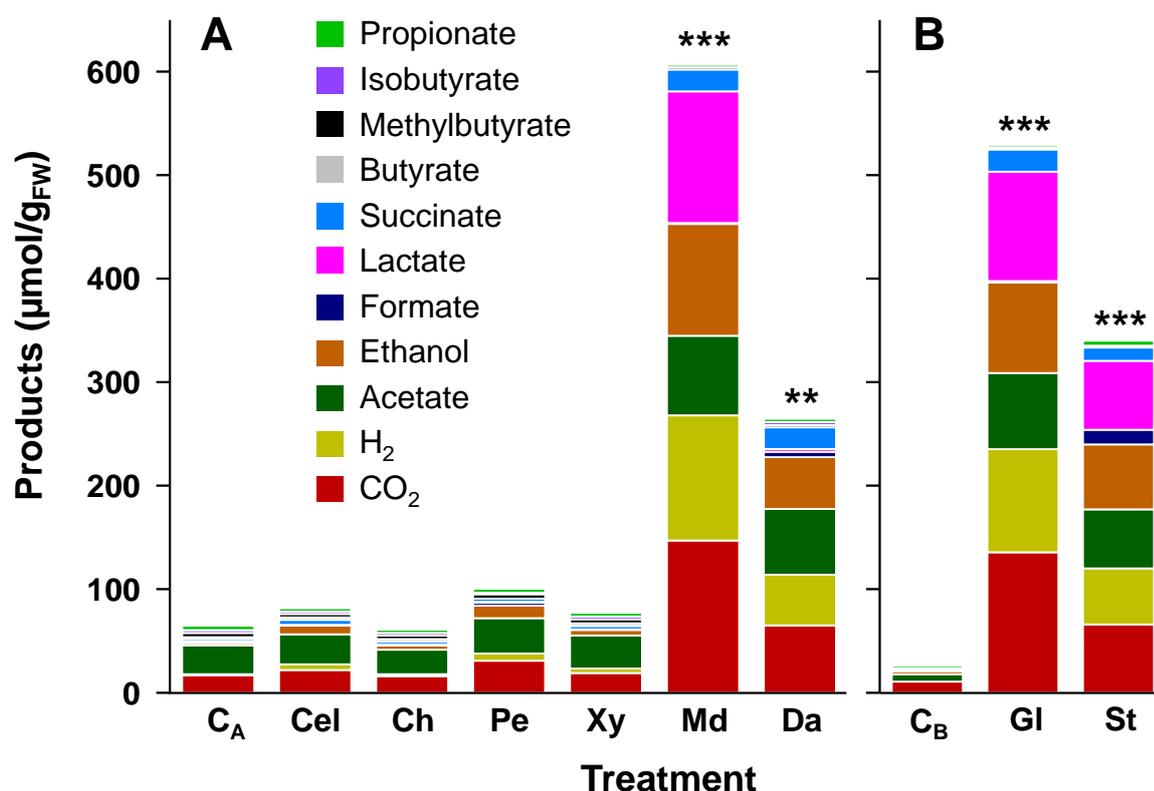


**Figure 21.** Effect of polysaccharides on the formation of H<sub>2</sub> and CO<sub>2</sub> in anoxic microcosms of *L. terrestris* gut contents. Controls lacked supplemental polysaccharides. Polysaccharides alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Panel A: The amount of polysaccharide-derived carbon added per microcosm approximated 2 mmol. Panel B: Effect of increasing amounts of cellulose on the formation of CO<sub>2</sub> or H<sub>2</sub>. The amount of carbon derived from cellulose added per microcosm approximated 0, 0.2, 0.5, 1.0, and 2.0 mmol. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 13.** Effect of polysaccharides on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

Treatment	Time (h)	Glucose ( $\mu\text{mol/g}_{\text{FW}}$ )	Products ( $\mu\text{mol/g}_{\text{FW}}$ )											pH
			CO <sub>2</sub>	H <sub>2</sub>	Acetate	Succinate	Formate	Propionate	Butyrate	Ethanol	Methylbutyrate	Iso-butyrate	Lactate	
Control <sub>A</sub>	0	0.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.1	1.2 ± 0.1	3.1 ± 1.8	0.0 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	7.0 ± 0.0
	30	0.0 ± 0.0	17 ± 1.7	0.8 ± 0.4	31 ± 11	3.6 ± 0.5	4.5 ± 4.6	4.2 ± 1.6	2.4 ± 0.5	2.9 ± 0.3	4.0 ± 1.2	3.7 ± 0.9	1.0 ± 0.1	7.1 ± 0.0
Cellulose	0	2.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.4	1.1 ± 0.1	3.2 ± 2.1	0.0 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	0.0 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	7.0 ± 0.0
	30	0.0 ± 0.0	22 ± 1.1	5.5 ± 1.0	32 ± 1.2	6.0 ± 0.3	3.4 ± 5.2	3.1 ± 0.1	2.8 ± 0.3	9.3 ± 0.8	3.4 ± 0.4	3.1 ± 0.1	1.1 ± 0.1	7.0 ± 0.0
Chitin	0	0.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.3	1.4 ± 0.3	3.4 ± 2.0	0.0 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	7.0 ± 0.0
	30	0.0 ± 0.0	16 ± 0.6	1.8 ± 0.3	27 ± 4.4	4.3 ± 0.4	3.5 ± 5.6	3.2 ± 0.5	2.9 ± 0.3	5.4 ± 0.4	3.3 ± 0.5	3.1 ± 0.3	1.1 ± 0.1	7.0 ± 0.0
Pectin	0	0.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	5.8 ± 1.0	0.7 ± 0.1	4.0 ± 1.6	0.0 ± 0.0	0.6 ± 0.1	0.9 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	6.8 ± 0.0
	30	0.0 ± 0.0	31 ± 2.1	6.8 ± 0.4	40 ± 3.2	3.6 ± 0.2	6.8 ± 5.7	3.8 ± 0.3	0.0 ± 0.0	13 ± 0.6	4.2 ± 0.1	1.8 ± 0.3	1.7 ± 0.1	6.8 ± 0.0
Xylan	0	2.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	6.6 ± 0.6	1.3 ± 0.2	4.5 ± 1.4	0.0 ± 0.0	0.6 ± 0.0	0.9 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	7.0 ± 0.0
	30	0.0 ± 0.0	19 ± 0.9	4.3 ± 0.1	39 ± 3.6	4.7 ± 0.3	4.2 ± 5.1	3.6 ± 0.3	3.1 ± 0.4	6.1 ± 1.4	3.9 ± 0.4	3.3 ± 0.3	1.2 ± 0.1	7.0 ± 0.0
Maltodextrin	0	173 ± 14	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.5	0.8 ± 0.0	3.2 ± 2.2	0.0 ± 0.0	0.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	7.0 ± 0.0
	30	77 ± 2.1	147 ± 2.4	121 ± 0.8	80 ± 4.3	22 ± 1.0	4.0 ± 0.6	2.4 ± 0.2	0.0 ± 0.0	108 ± 11	0.0 ± 0.0	2.6 ± 0.1	128 ± 5.6	5.3 ± 0.0
Dextran	0	38 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 0.2	1.2 ± 0.3	3.6 ± 2.4	0.0 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.6 ± 0.0	7.0 ± 0.0
	30	52 ± 0.6	65 ± 9.2	49 ± 1.6	67 ± 6.3	22 ± 1.6	8.8 ± 5.1	3.2 ± 0.4	0.0 ± 0.0	50 ± 8.0	2.2 ± 0.4	3.4 ± 0.1	3.4 ± 1.9	6.6 ± 0.0
Control <sub>B</sub>	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.1	1.1 ± 0.0	2.1 ± 0.1	0.7 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.9 ± 0.0	7.0 ± 0.0
	30	0.0 ± 0.0	11 ± 2.7	0.0 ± 0.3	14 ± 0.5	0.4 ± 0.1	3.6 ± 0.4	2.9 ± 0.1	1.1 ± 0.2	3.0 ± 0.8	0.9 ± 0.1	0.0 ± 0.0	1.0 ± 0.0	7.0 ± 0.0
Glycogen	0	105 ± 8.4	0.0 ± 0.0	0.0 ± 0.0	6.9 ± 0.2	1.1 ± 0.1	3.0 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	1.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.0	7.0 ± 0.0
	30	175 ± 19	136 ± 8.6	100 ± 1.8	80 ± 3.7	23 ± 0.9	4.6 ± 0.2	3.0 ± 0.5	2.6 ± 0.6	89 ± 8.0	0.2 ± 0.0	0.0 ± 0.0	107 ± 4.0	5.6 ± 0.0
Starch	0	18 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	7.5 ± 0.8	1.1 ± 0.1	3.4 ± 0.4	0.7 ± 0.0	0.5 ± 0.0	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1	7.0 ± 0.0
	30	15 ± 5.3	66 ± 0.9	54 ± 9.1	65 ± 3.1	14 ± 1.2	18 ± 0.5	5.4 ± 0.3	2.2 ± 0.3	64 ± 3.5	0.3 ± 0.2	0.0 ± 0.0	68 ± 4.9	6.1 ± 0.0

<sup>a</sup>The amount of polysaccharide-derived carbon added per microcosm approximated 2 mmol. Controls lacked supplemental polysaccharides. Polysaccharides alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses ( $\pm$  standard deviation). FW, fresh weight. Table modified and used with permission from Zeibich *et al.*, 2019a.



**Figure 22.** Collective amounts of fermentation products in polysaccharide-supplemented anoxic microcosms of *L. terrestris* gut contents. The amount of polysaccharide-derived carbon added per microcosm approximated 2 mmol. Polysaccharides alone did not display any fermentation activity. Abbreviations: C<sub>A</sub> and C<sub>B</sub>, unsupplemented controls of polysaccharide experiments A and B, respectively; Cel, cellulose; Ch, chitin; Pe, pectin; Xy, xylan; Md, maltodextrin; Da, dextran; Gl, glycogen; St; starch. Values are the average of triplicates and represent the net amounts of products at the end of the 30 h incubation (control values were subtracted). Data are provided in Table 13. The asterisks indicate significant differences between the collective amount of products formed in control and polysaccharide treatments (\*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; *t*-test with unequal variances). FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 14.** *P* values of fermentation products in polysaccharide-supplemented gut content microcosms.<sup>a</sup>

Product	CO <sub>2</sub>									
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	17	22	16	31	19	148	65	11	136	66
Variance	3.0	1.1	0.3	4.5	0.8	5.8	84	7.5	73	0.8
<i>P</i> value		0.013	0.542	0.001	0.147	0.000	0.000		0.000	0.009
Product	H <sub>2</sub>									
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	0.8	5.5	1.8	6.8	4.3	121	49	0.0	100	54
Variance	0.2	1.0	0.1	0.1	0.0	0.6	2.5	0.0	3.2	84
<i>P</i> value		0.006	0.036	0.000	0.004	0.000	0.000		0.000	0.009
Product	Acetate									
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	28	29	24	40	32	77	64	7.1	74	57
Variance	124	0.8	17	5.7	11	15	40	0.3	12	6.1
<i>P</i> value		0.881	0.569	0.471	0.616	0.010	0.015		0.001	0.001

Product		Succinate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	2.5	4.9	2.9	2.9	3.5	21	21	-0.7	21	13
Variance	0.2	0.1	0.1	0.0	0.2	1.0	2.1	0.0	0.9	1.3
P value		0.002	0.176	0.195	0.047	0.000	0.001		0.001	0.002
Product		Formate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	1.4	0.2	0.1	2.8	-0.3	0.8	5.1	1.5	1.5	14
Variance	7.9	9.7	13	25	14	8.2	43	0.3	0.0	0.8
P value		0.644	0.658	0.699	0.562	0.805	0.436		0.931	0.000
Product		Propionate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	4.2	3.1	3.2	3.8	3.6	2.4	3.2	2.3	2.2	4.7
Variance	2.7	0.0	0.2	0.1	0.1	0.0	0.1	0.0	0.2	0.1
P value		0.351	0.393	0.677	0.589	0.195	0.393		0.899	0.005
Product		Butyrate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	1.8	2.2	2.4	-0.6	2.5	-0.7	-0.6	0.6	2.0	1.7
Variance	0.4	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.3	0.1
P value		0.398	0.252	0.021	0.199	0.019	0.020		0.043	0.011
Product		Ethanol								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	2.1	8.4	4.5	12	5.1	108	50	3.0	88	63
Variance	0.1	0.5	0.2	0.4	1.8	110	64	0.6	62	12
P value		0.002	0.003	0.000	0.052	0.003	0.009		0.003	0.001
Product		Lactate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	0.3	0.4	0.5	1.1	0.5	127	2.8	0.1	106	67
Variance	0.0	0.0	0.0	0.0	0.0	30	3.7	0.0	16	24
P value		0.051	0.048	0.005	0.041	0.001	0.147		0.000	0.002
Product		Isobutyrate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	2.8	2.3	2.5	1.4	2.7	2.1	3.0	0.0	0.0	0.0
Variance	0.6	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
P value		0.375	0.515	0.083	0.760	0.238	0.809		-	-
Product		Methylbutyrate								
Treatment	C <sub>A</sub>	Cel	Ch	Pe	Xy	Md	Da	C <sub>B</sub>	Gl	St
Mean value <sup>b</sup>	4.0	3.4	3.3	4.2	3.9	0.0	2.2	0.9	0.1	0.2
Variance	1.4	0.2	0.3	0.0	0.2	0.0	0.1	0.0	0.0	0.0
P value		0.502	0.478	0.735	0.928	0.029	0.112		0.002	0.004

<sup>a</sup>P values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C<sub>A</sub>, C<sub>B</sub>) and cellulose (Cel), chitin (Ch), pectin (Pe), xylan (Xy), maltodextrin (Md), dextran (Da), glycogen (Gl) or starch (St) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation. See Table 13 for product profiles. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>Mean values (n = 3) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

**Table 15.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in structural (A) and non-structural (B) polysaccharide treatments.<sup>a</sup>**(A) Structural Polysaccharides**

Main Products	Recoveries (%)							
	Cellulose		Chitin		Pectin		Xylan	
	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE
CO <sub>2</sub>	0.3	na	-	na	0.7	na	0.1	na
H <sub>2</sub>	na	0.1	na	0.0	na	0.2	na	0.1
Ethanol	0.6	0.9	0.2	0.3	1.0	1.8	0.3	0.5
Succinate	0.5	0.4	0.1	0.1	0.1	0.1	0.2	0.2
Acetate	0.1	0.1	-	-	0.6	0.8	0.1	0.1
Lactate	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0
Butyrate	0.1	0.1	0.1	0.2	-	-	0.1	0.2
Methylbutyrate	-	-	-	-	0.1	0.1	-	-
<b>Total :</b>	1.6	1.8	0.5	0.7	2.6	3.1	0.9	1.0

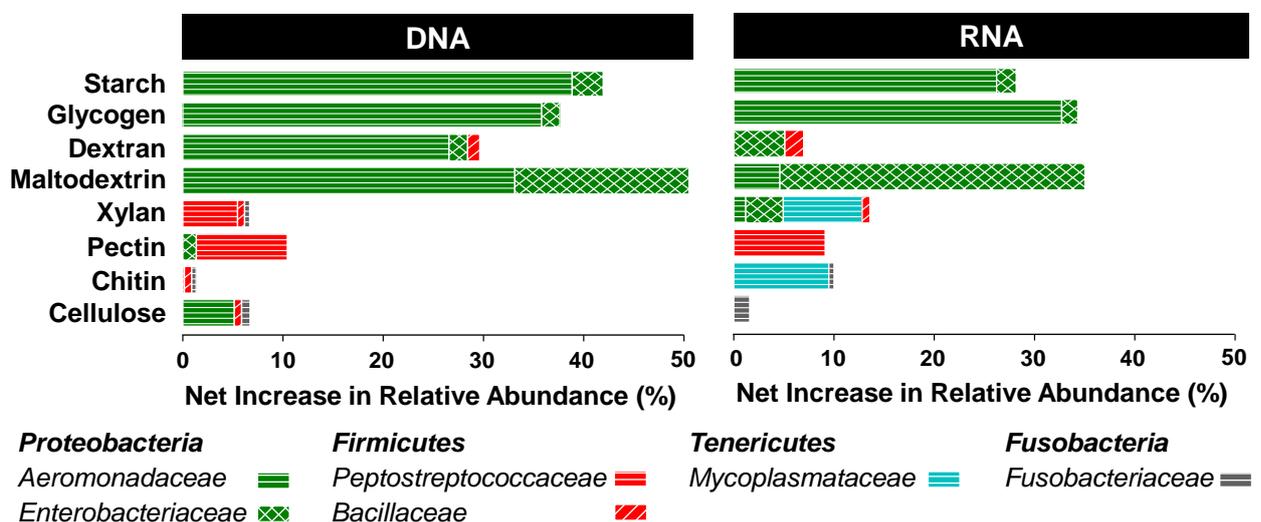
**(B) Non-structural Polysaccharides**

Main Products	Recoveries (%)							
	Maltodextrin		Dextran		Glycogen		Starch	
	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE
CO <sub>2</sub>	6.5	na	2.4	na	6.3	na	2.8	na
H <sub>2</sub>	na	3.0	na	1.2	na	2.5	na	1.3
Ethanol	11	16	4.8	7.2	8.4	13	6.0	9.0
Succinate	3.8	3.3	3.7	3.2	4.4	3.9	2.7	2.4
Acetate	4.9	4.9	3.6	3.6	6.6	6.6	5.0	5.0
Propionate	-	-	-	-	-	-	0.4	0.4
Formate	-	-	0.2	0.1	0.0	0.0	0.6	0.3
Lactate	19	19	0.4	0.4	16	16	10	10
Butyrate	-	-	-	-	0.3	0.4	0.2	0.3
<b>Total :</b>	45	46	15	16	42	42	28	29

<sup>a</sup>See Table 13 for product profiles. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. RE, reducing equivalents; -, no net increase of the product during the incubation in supplemented treatments relative to the control treatments; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019a.

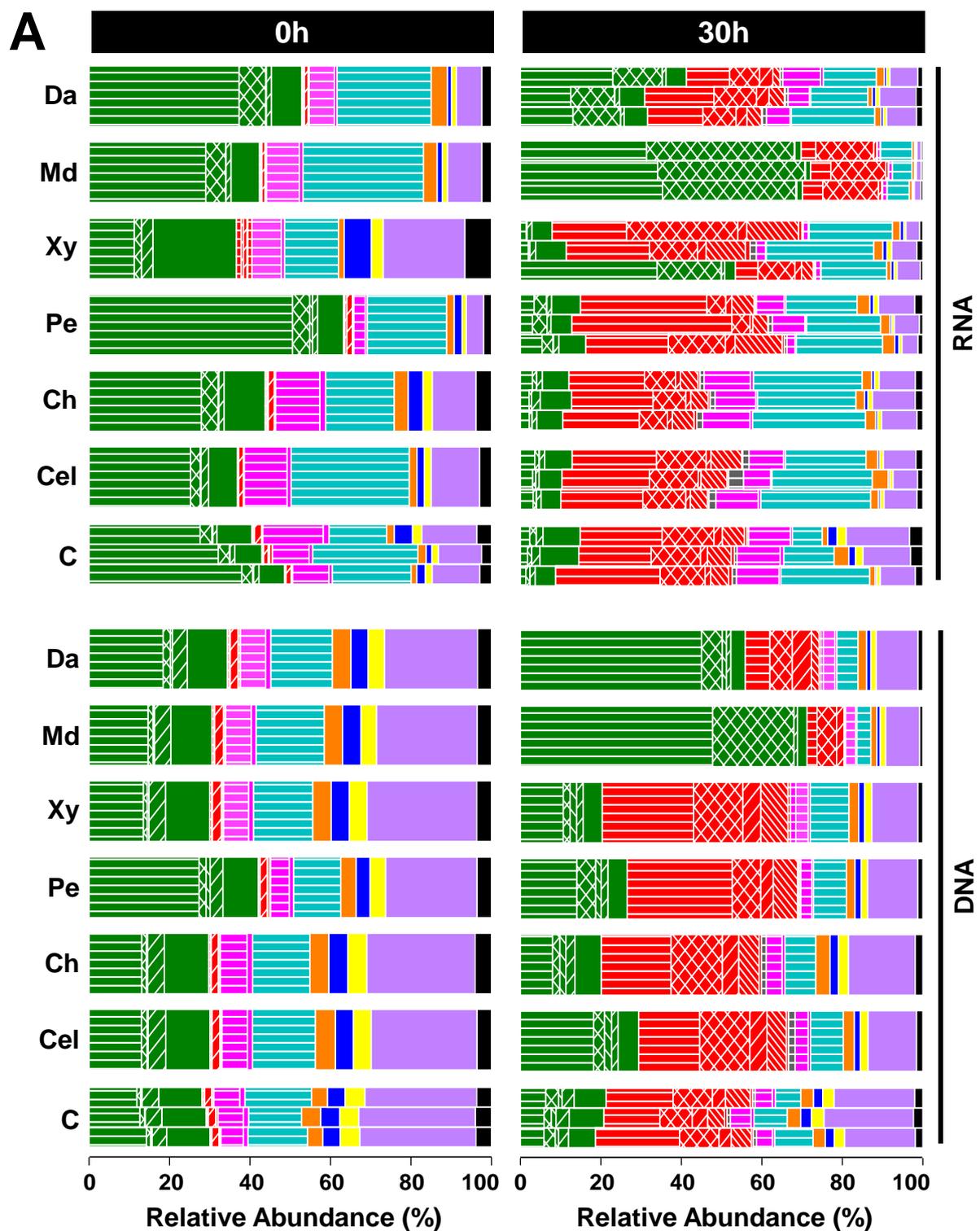
### 3.1.2. Effect of polysaccharides on gut fermentative bacterial families

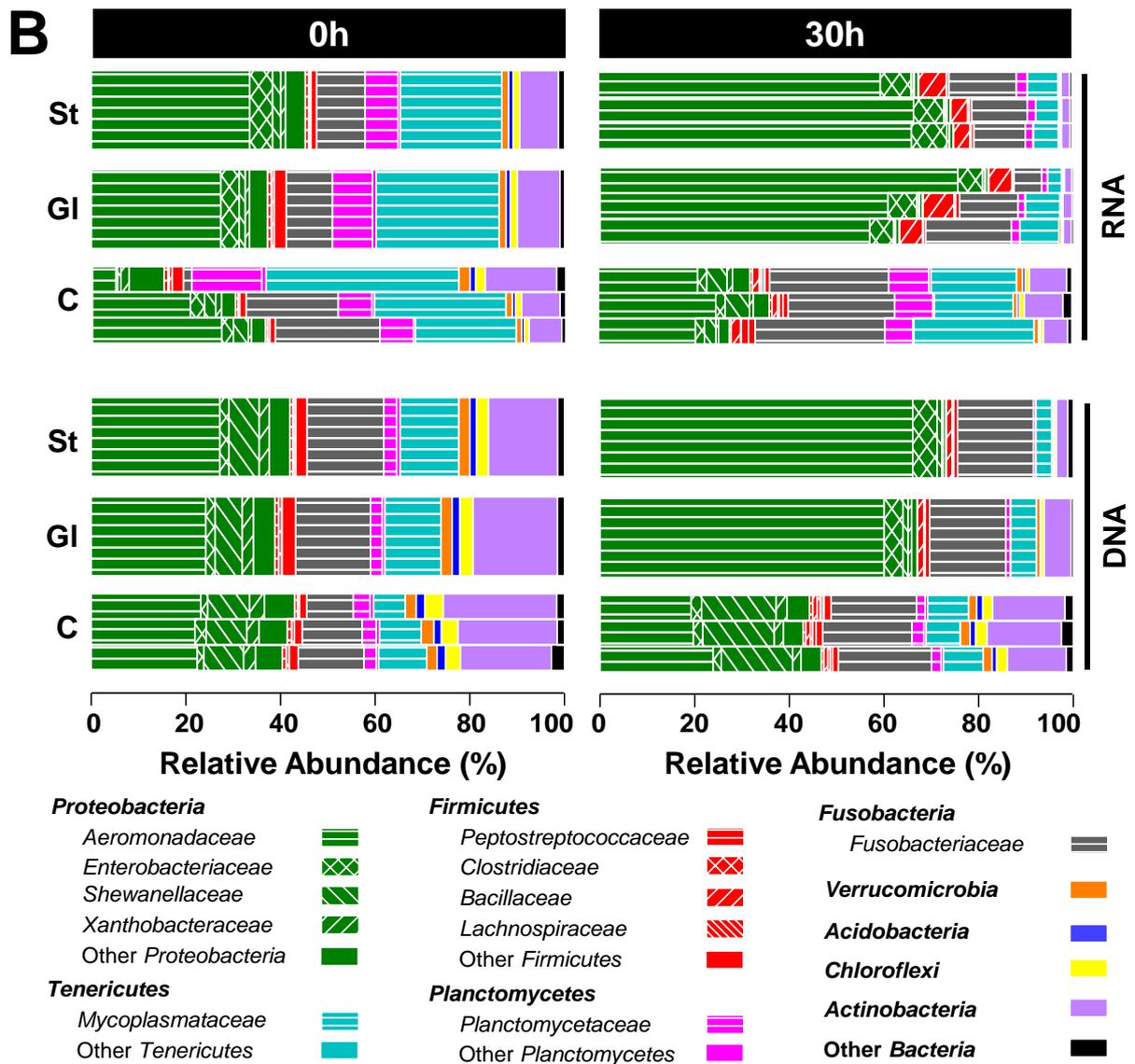
Time-dependent shifts in the microbial community composition were evaluated by 16S rRNA and 16S rRNA gene analyses. A total of 1,230,292 bacterial 16S rRNA gene and 16S rRNA sequences were obtained from the polysaccharide treatments, yielding 30 phyla (including candidate phyla). Based on the relative abundances of the detected 16S rRNA sequences in polysaccharide experiment A at the end of the incubation, the phylum *Proteobacteria* was stimulated by maltodextrin and dextran and the affiliated families *Aeromonadaceae* and *Enterobacteriaceae* displayed an increase in relative abundances in response to these two non-structural polysaccharides (Figure 23 and Figure 24 A). Indeed, at the end of the incubation, the relative 16S rRNA gene abundances of both families were significantly greater in maltodextrin and dextran treatments than in controls (Table 16). With another batch of earthworms maintained on a different soil, starch and glycogen also stimulated significantly the *Aeromonadaceae* and *Enterobacteriaceae* (Figure 23, Figure 24 B, and Table 16). Rarefaction analyses of both polysaccharide experiments indicated that the most abundant taxa were targeted (Figure 25). Furthermore, the number of detected phylotypes, the number of expected phylotypes (Chao1), and Shannon indices of the maltodextrin treatments at the end of the incubation period were lower than those of the controls (Figure 25 A and Table 17). This is consistent with the obvious stimulation of *Aeromonadaceae* and *Enterobacteriaceae* in maltodextrin treatment (Table 16).



**Figure 23.** Net increases in 16S rRNA gene (DNA) and 16S rRNA (RNA) relative abundances of bacterial families stimulated by supplemental polysaccharides in *L. terrestris* gut content microcosms. The graph is limited to families that displayed a net increase in relative abundance of  $\geq 4\%$  in at least one treatment and the families are color-coded to the respective phyla (see Figure 24 for the complete 16S rRNA gene and 16S rRNA analyses). Net increases of relative abundances were calculated as follows: (a) the calculation is based either on mean relative abundances when samples from the three replicates were analyzed separately (i.e., all RNA and DNA samples of control treatments and RNA samples at 30 h of supplemented treatments) or on single relative abundances when samples of the three replicates were pooled for sequence analyses (i.e., DNA samples at 0 h and 30 h and RNA samples at 0 h of supplemented treatments); (b) mean or single relative abundances at the beginning of incubation for control and supplemented treatments; (c) the resulting time-corrected relative abundances of control treatments were subtracted from those of supplemented treatments (negative time-corrected relative abundances of control treatments were ignored). Figure modified and used with permission from Zeibich *et al.*, 2019a.

The same trends of lower number of detected phylotypes, expected phylotypes (Chao1), and Shannon indices in maltodextrin treatment were also observed in starch and glycogen treatments (Figure 25 B and Table 17). The increase in the relative abundances of *Firmicutes*-affiliated families in the unsupplemented control treatment and in polysaccharide experiment A (Figure 24 A), suggesting that these taxa were stimulated by anoxia and involved in the fermentative usage of organic carbon endogenous to gut content. That *Firmicutes*-affiliated families were less responsive in polysaccharide experiment B in which the *Fusobacteria* were dominant (Figure 24 B), suggesting a species variability of the earthworm-ingested materials, including soil.





**Figure 24.** 16S rRNA gene (DNA) and 16S rRNA (RNA) analyses of polysaccharide experiments A (A) and B (B). The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Process data are shown in Table 13 and Figure 22. Information on all detected taxa is provided in Table A1 and Table A2. Abbreviations: 0 h and 30 h indicate the time of sampling in hours; C, unsupplemented control. Panel A: Cel, cellulose; Ch, chitin; Pe, pectin; Xy, xylan; Md, maltodextrin; Da, dextran. Panel B: Gl, glycogen; St, starch. Grouped bars indicate that the sequence analysis was performed individually for the three replicates and single bars indicate that DNA or RNA samples of the three replicates were pooled for the sequence analysis. Figure modified and used with permission from Zeibich *et al.*, 2019a.

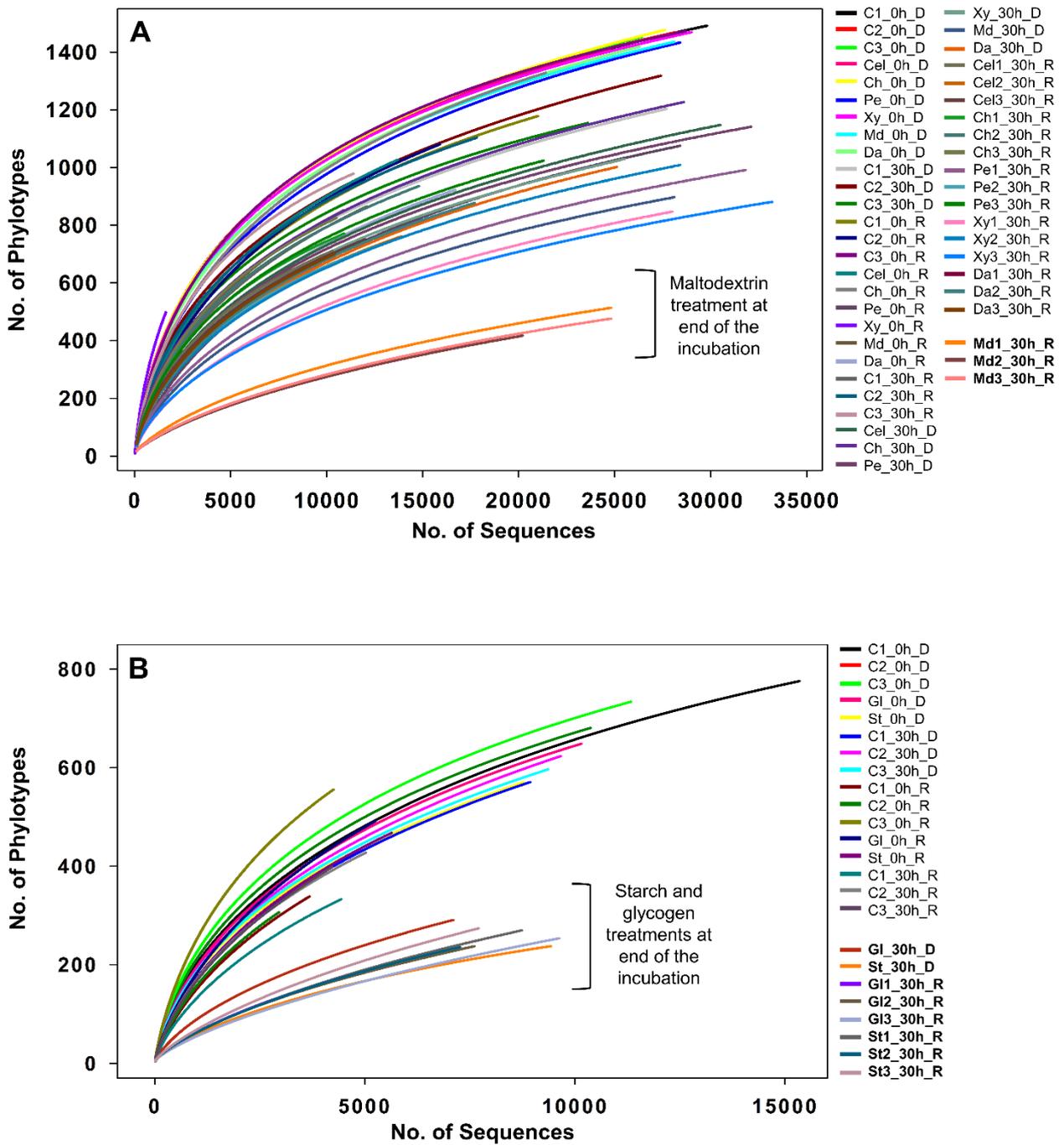
These aforementioned findings demonstrated bacterial shifts in the fermentative community during the anoxic gut content incubation, and NMDS analysis of all phylotypes (Section 2.6.2.2) confirmed the microbial gut community alterations in the control and supplemented treatments during the incubation (Figure 26 A and B). In this regard, the analysis illustrated great bacterial shifts in the microbial community of non-structural polysaccharides treatments (i.e., maltodextrin, dextran, glycogen, and starch) and marginal shifts in the microbial community of structural polysaccharide treatments (i.e., cellulose, chitin, pectin, xylan) compared to the microbial shifts in the control treatments (Figure 26).

**Table 16.** Statistical analyses of main stimulated families in polysaccharide treatments.<sup>a</sup>

Family	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
<b><i>Aeromonadaceae</i></b>	Control <sub>A</sub>	1.8	0.5	1.8	
	Cellulose	3.3	0.3	3.2	4.5 <sup>(1)</sup>
	Pectin	3.9	1.3	3.4	4.6 <sup>(1)</sup>
	Maltodextrin	34	2.0	34	5.5 <sup>(2)</sup>
	Dextran	16	5.9	13	5.2 <sup>(1)</sup>
	Control <sub>B</sub>	22	2.3	21	
	Glycogen	64	9.8	61	5.8 <sup>(1)</sup>
	Starch	64	3.9	66	5.8 <sup>(1)</sup>
<b><i>Enterobacteriaceae</i></b>	Control <sub>A</sub>	1.2	0.3	1.0	
	Pectin	3.2	0.2	0.8	4.5 <sup>(2)</sup>
	Maltodextrin	35	0.4	3.1	5.5 <sup>(1)</sup>
	Dextran	12	2.0	37	5.1 <sup>(2)</sup>
	Control <sub>B</sub>	2.0	0.7	12	
	Glycogen	5.5	0.1	1.9	4.7 <sup>(2)</sup>
	Starch	6.8	0.6	5.2	4.8 <sup>(2)</sup>
<b><i>Fusobacteriaceae</i></b>	Control <sub>A</sub>	0.7	0.3	0.7	
	Cellulose	2.3	1.2	1.7	4.4 <sup>(2)</sup>
<b><i>Mycoplasmataceae</i></b>	Control <sub>A</sub>	14	7.4	12	
	Chitin	27	1.8	27	5.4 <sup>(1)</sup>
<b><i>Clostridiaceae</i></b>	Control <sub>A</sub>	12	0.8	12	
	Maltodextrin	14	0.4	14	5.1 <sup>(3)</sup>
	Control <sub>B</sub>	1.5	0.4	1.5	
	Glycogen	5.4	1.0	4.9	4.7 <sup>(3)</sup>
	Starch	4.3	1.4	3.5	4.6 <sup>(3)</sup>
<b><i>Bacillaceae</i></b>	Control <sub>A</sub>	1.7	0.4	1.7	
	Dextran	3.1	0.3	3.2	4.5 <sup>(3)</sup>

<sup>a</sup>Families with the four highest ranks in the LEfSe analysis were considered. LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).



**Figure 25.** Rarefaction analyses of bacterial 16S rRNA gene and 16S rRNA sequences obtained from anoxic *L. terrestris* gut content microcosms supplemented with polysaccharides. Phylotypes were based on a 97% sequence similarity cutoff. Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Abbreviations: 0 h and 30 h indicate the time of sampling in hours; C, unsupplemented control; D, 16S rRNA genes; R, 16S rRNA. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Panel A: Polysaccharide experiment A. Cel, cellulose; Ch, chitin; Pe, pectin; Xy, xylan; Md, maltodextrin; Da, dextran. Panel B: Polysaccharide experiment B. Gl, glycogen; St, starch. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 17.** Alpha diversity of the microbial community in control and polysaccharide treatments.<sup>a</sup>

Sample (Sampling Time)	Treatment <sup>b</sup>	Number of sequences	Observed phylotypes <sup>c</sup> (normalized) <sup>d</sup>	Chao1 (normalized) <sup>d</sup>	Shannon (normalized) <sup>d</sup>
<b>DNA (0 h)</b>	Control <sub>A</sub> 1	29861	1492 (331)	1860 (331)	5.1 (4.4)
	Control <sub>A</sub> 2	28283	1470 (331)	1829 (331)	5.2 (4.5)
	Control <sub>A</sub> 3	26454	1453 (330)	1835 (330)	5.1 (4.5)
	Cellulose	24721	1407 (324)	1727 (324)	5.1 (4.4)
	Chitin	27694	1478 (331)	1967 (331)	5.2 (4.5)
	Pectin	28475	1434 (321)	1866 (321)	4.6 (4.0)
	Xylan	29009	1469 (329)	1864 (329)	5.1 (4.4)
	Maltodextrin	28127	1436 (326)	1840 (326)	5.0 (4.3)
	Dextran	26368	1420 (323)	1917 (323)	4.9 (4.2)
	Control <sub>B</sub> 1	15345	776 (198)	1046 (241)	4.0 (3.5)
	Control <sub>B</sub> 2	10377	681 (199)	984 (234)	4.1 (3.6)
	Control <sub>B</sub> 3	11331	734 (203)	1019 (236)	4.3 (3.7)
	Glycogen	10157	649 (189)	940 (227)	3.9 (3.4)
	Starch	8786	571 (177)	1037 (217)	3.7 (3.3)
	<b>DNA (30 h)</b>	Control <sub>A</sub> 1	27753	1204 (318)	1686 (318)
Control <sub>A</sub> 2		27415	1318 (332)	1814 (332)	5.0 (4.5)
Control <sub>A</sub> 3		23677	1155 (325)	1529 (325)	4.8 (4.4)
Cellulose		30509	1148 (294)	1575 (294)	4.4 (4.0)
Chitin		28612	1227 (317)	1645 (317)	4.7 (4.3)
Pectin		32152	1142 (296)	1526 (296)	4.2 (3.8)
Xylan		25600	1031 (291)	1536 (291)	4.3 (3.9)
Maltodextrin		28151	898 (239)	1291 (239)	2.9 (2.6)
Dextran		25158	1003 (268)	1408 (268)	3.5 (3.1)
Control <sub>B</sub> 1		8937	571 (179)	917 (223)	3.5 (3.1)
Control <sub>B</sub> 2		9669	624 (192)	995 (237)	3.7 (3.3)
Control <sub>B</sub> 3		9364	597 (190)	958 (232)	3.7 (3.3)
Glycogen		7102	291 (112)	519 (161)	2.2 (2.0)
Starch		9428	238 (77)	387 (126)	1.8 (1.7)
<b>RNA (0 h)</b>		Control <sub>A</sub> 1	21091	1180 (294)	1598 (294)
	Control <sub>A</sub> 2	15912	1081 (286)	1602 (286)	3.8 (3.2)
	Control <sub>A</sub> 3	28736	1473 (322)	1805 (322)	4.7 (3.9)
	Cellulose	13766	1029 (293)	1522 (293)	4.0 (3.4)
	Chitin	21406	1328 (312)	1764 (312)	4.4 (3.7)
	Pectin	28446	1076 (239)	1456 (239)	3.0 (2.5)
	Xylan <sup>e</sup>	1621	498 ( - )	938 ( - )	5.2 ( - )
	Maltodextrin	10991	855 (277)	1282 (277)	3.7 (3.1)
	Dextran	16795	925 (259)	1318 (259)	3.4 (2.9)
	Control <sub>B</sub> 1	3684	339 (144)	634 (175)	3.1 (2.7)
	Control <sub>B</sub> 2	2958	307 (152)	581 (183)	3.1 (2.9)
	Control <sub>B</sub> 3	4255	556 (213)	866 (242)	4.0 (3.4)
	Glycogen	5248	492 (182)	846 (223)	3.5 (3.0)
	Starch	5635	468 (180)	782 (229)	3.3 (3.0)

Sample (Sampling Time)	Treatment <sup>b</sup>	Number of sequences	Observed phylotypes <sup>c</sup> (normalized) <sup>d</sup>	Chao1 (normalized) <sup>d</sup>	Shannon (normalized) <sup>d</sup>
<b>RNA (30 h)</b>	Control <sub>A</sub> 1	12112	866 (279)	1396 (279)	4.1 (3.6)
	Control <sub>A</sub> 2	17846	1104 (320)	1520 (320)	4.8 (4.3)
	Control <sub>A</sub> 3	11443	980 (316)	1230 (316)	4.9 (4.3)
	Cellulose 1	10319	712 (271)	1162 (271)	4.0 (3.7)
	Cellulose 2	13955	763 (261)	1186 (261)	3.9 (3.6)
	Cellulose 3	12021	752 (271)	1228 (271)	4.2 (3.8)
	Chitin 1	10924	772 (276)	1101 (276)	4.1 (3.7)
	Chitin 2	14879	938 (294)	1492 (294)	4.2 (3.8)
	Chitin 3	10551	827 (286)	1227 (286)	4.2 (3.7)
	Pectin 1	31849	992 (231)	1373 (231)	3.9 (3.5)
	Pectin 2	12784	732 (257)	1082 (257)	3.7 (3.3)
	Pectin 3	21393	1026 (295)	1424 (295)	4.1 (3.6)
	Xylan 1	28056	848 (220)	1310 (220)	3.2 (2.9)
	Xylan 2	28402	1009 (251)	1480 (251)	4.0 (3.6)
	Xylan 3	33284	882 (209)	1335 (209)	3.6 (3.4)
	Maltodextrin 1	24839	514 (140)	926 (140)	2.4 (2.3)
	Maltodextrin 2	20220	417 (118)	767 (118)	2.3 (2.2)
	Maltodextrin 3	24893	477 (119)	831 (119)	2.3 (2.2)
	Dextran 1	17719	878 (268)	1371 (268)	4.0 (3.6)
	Dextran 2	22081	878 (283)	1330 (283)	4.2 (3.8)
	Dextran 3	10448	702 (251)	1110 (251)	4.0 (3.6)
	Control <sub>B</sub> 1	4433	333 (146)	634 (196)	2.9 (2.6)
	Control <sub>B</sub> 2	5029	428 (176)	743 (217)	3.4 (3.0)
	Control <sub>B</sub> 3	4226	399 (168)	813 (212)	3.3 (3.0)
	Glycogen 1	6579	219 (83)	395 (132)	2.0 (1.9)
	Glycogen 2	7610	238 (85)	659 (133)	2.1 (2.0)
	Glycogen 3	9627	254 (76)	555 (143)	1.7 (1.6)
	Starch 1	8739	270 (87)	543 (170)	1.9 (1.8)
	Starch 2	7268	236 (81)	459 (137)	1.9 (1.8)
	Starch 3	7704	274 (89)	541 (156)	2.1 (1.9)

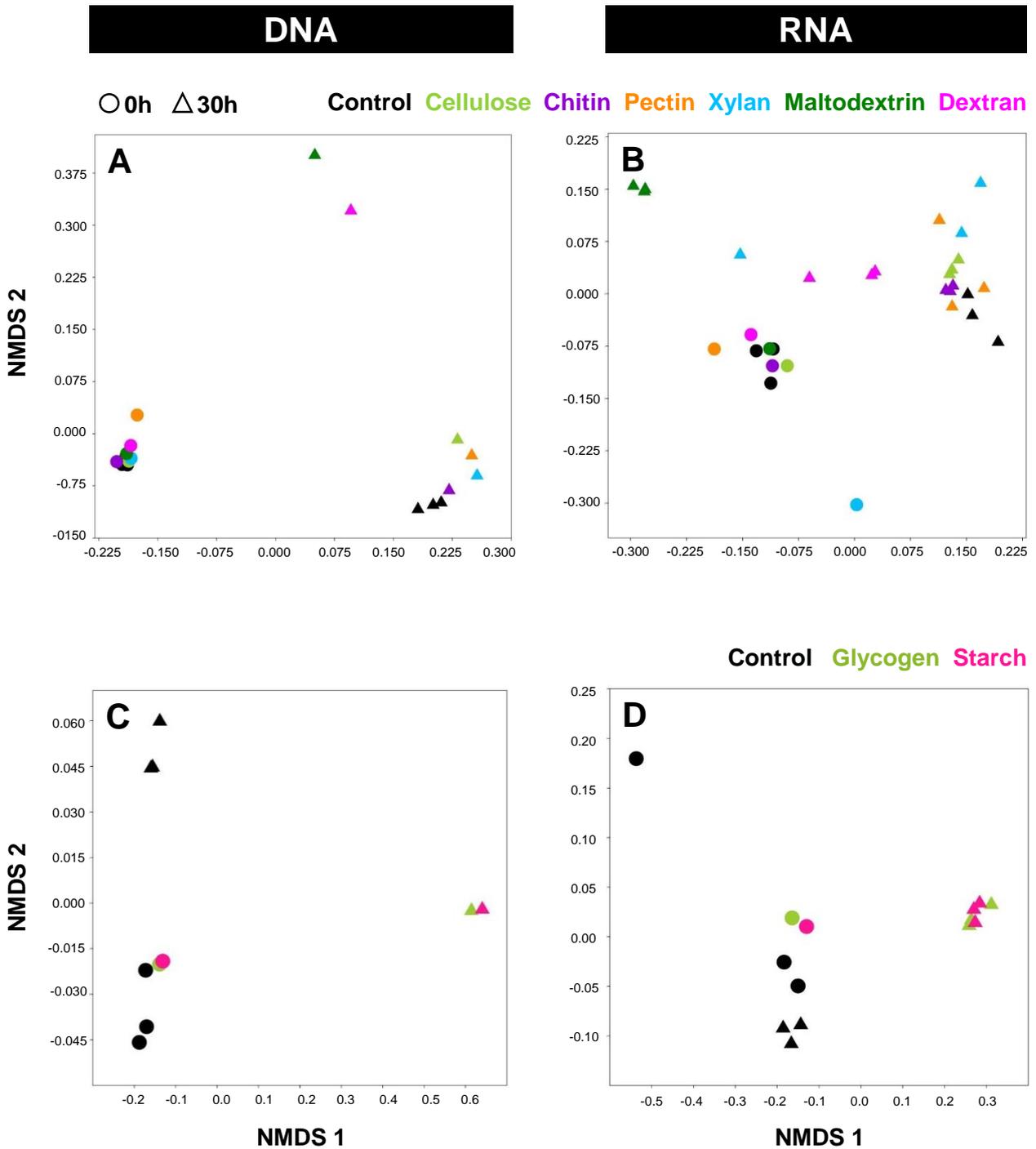
<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Numbers assigned to a treatment (e.g., Control<sub>A</sub> 1) indicate the respective replicate. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h.

<sup>c</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>d</sup>For comparison of amplicon libraries of different sizes, the polysaccharide data sets were normalized to 5,000 sequences (polysaccharide experiment A) or 2,500 sequences (polysaccharide experiment B).

<sup>e</sup>-, normalization was not possible because of the low number of sequences in this sample.



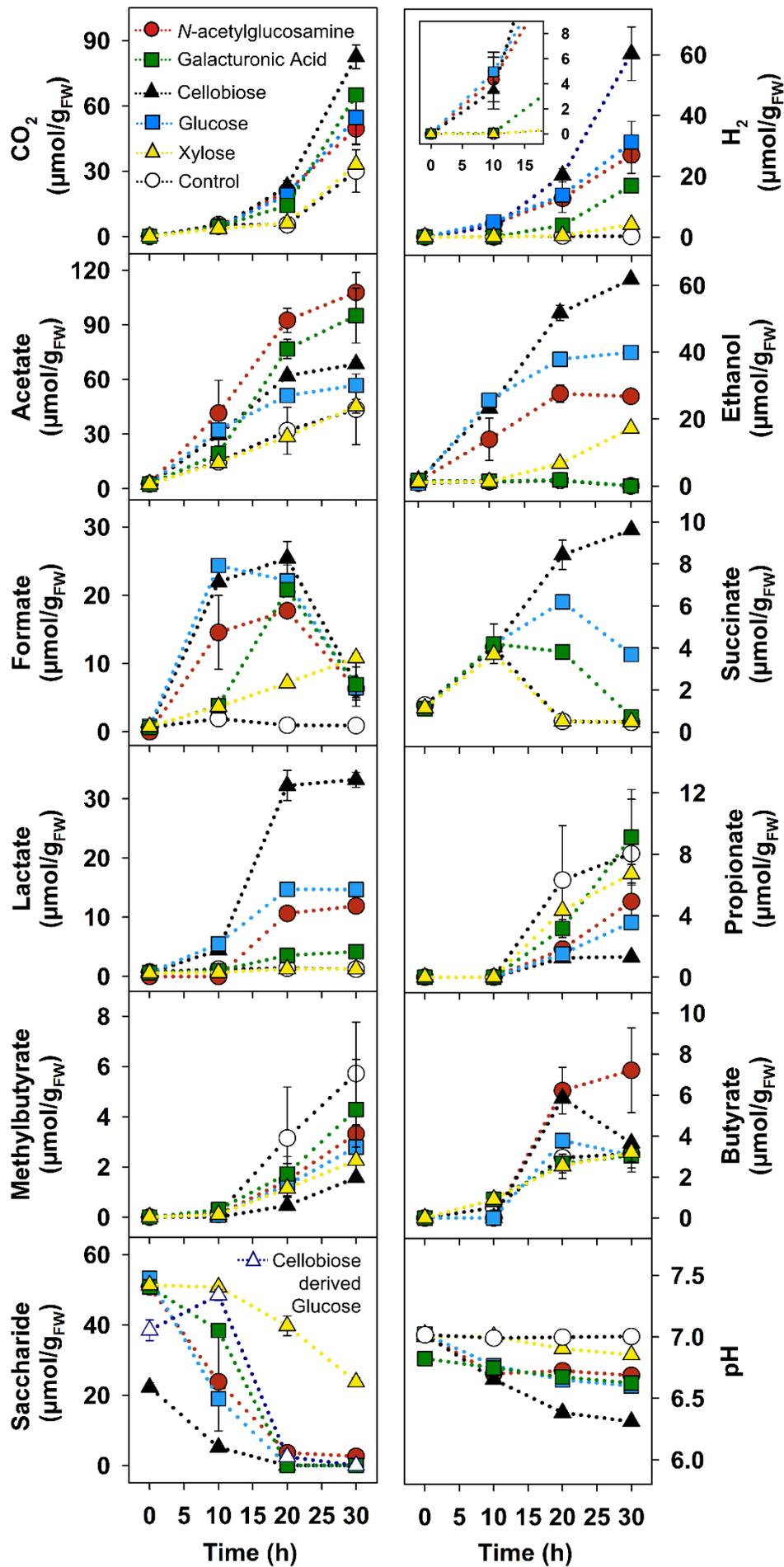
**Figure 26.** NMDS plot of the microbial community composition in polysaccharide treatments. Distance matrices (Bray-Curtis) are based on the relative abundances of all detected phylotypes in the different treatments (Table A1 and Table A2). Samples of the three replicates of the 16S rRNA gene (DNA) control treatment at 0 h and 30 h, 16S rRNA (RNA) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Proximity of symbols represent the degree of similarity between the different treatments. Figure modified and used with permission from Zeibich *et al.*, 2019a.

### 3.1.3. Effect of non-polymeric saccharides on gut content fermentation

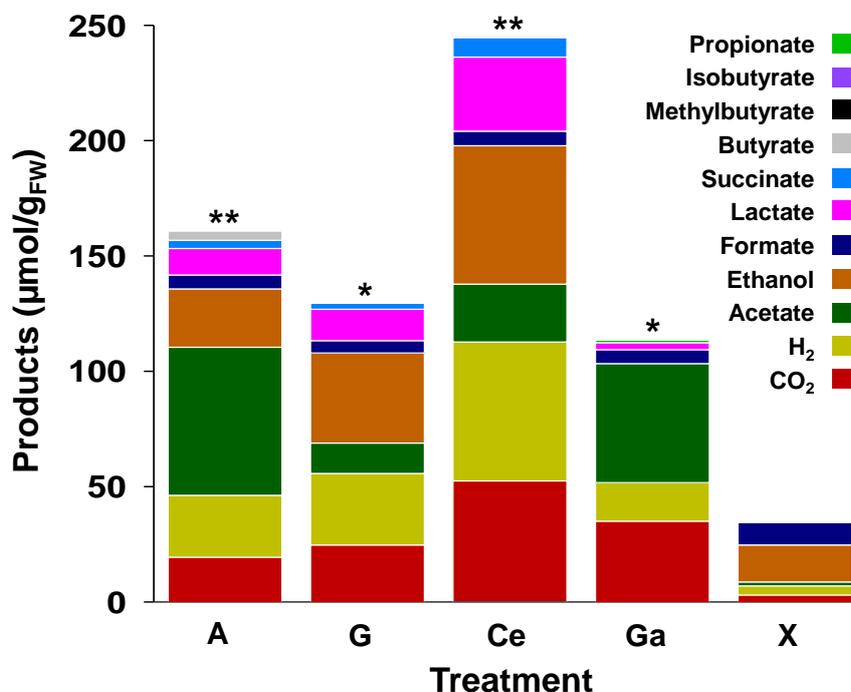
The aforementioned supplemented polysaccharides stimulated the gut content fermentations quantitatively different, suggesting the hydrolysis of the structural polysaccharides as fermentation limiting process. Indeed, saccharides from which most of these supplemented polysaccharides are composed (i.e., *N*-acetylglucosamine, cellobiose, glucose, and galacturonic acid) were consumed immediately and stimulated robust fermentations (Figure 27). The rapid transient increase of glucose from cellobiose indicated the presence of cellobiase activity, whereas the enhanced formation of certain products (e.g., H<sub>2</sub>, formate, and ethanol) without a notable delay in *N*-acetylglucosamine, cellobiose, and glucose treatments (Figure 27) demonstrated that the earthworm gut fermenters were readily to respond immediately to these glucose-based saccharides.

Formate and succinate were transient in certain treatments (Figure 27) and the relative amounts of saccharide-dependent products were not uniform. Thus, the fermentation activities varied quantitatively and qualitatively in the saccharide treatments (Figure 28), suggesting certain fermentative processes as saccharide-specific. For example, ethanol was an important end product in treatments with *N*-acetylglucosamine, cellobiose, glucose, and xylose, but not produced in the galacturonic acid treatment (Figure 28 and Table 18). Likewise, lactate accumulated in most of the hexose-based treatments but was less abundant in the xylose treatment, and H<sub>2</sub> was negligible in the control treatment but significantly produced in all supplemented treatments (Figure 28 and Table 18). The substantially higher acetate amounts in *N*-acetylglucosamine treatments than in glucose treatments (Figure 28 and Table 18) were most likely derived by the acetyl group of *N*-acetylglucosamine that was converted to acetate (Vincent *et al.*, 2004). Xylose was only slightly fermented, and the collective amount of products formed in this treatment was not significantly higher compared to that of the control treatment (Figure 28). This finding plus the weakly stimulation of fermentation by xylan (Figure 21 A) demonstrated that gut-associated fermenters had only a marginal capacity to hydrolyze xylan and ferment xylose.

The recoveries of carbon and reducing equivalents in fermentation products derived from supplemented saccharides ranged from 37 to 73% (Table 19), indicating that (a) dissimilation might have yielded additional undetected products (e.g., 2,3-butanediol from mixed-acid fermentation or acetone from solvent-producing clostridia [Buckel, 1999; Chen and Blaschek, 1999]) and/or (b) substrate/products were partially assimilated into biomass or chemically complexed in gut content. Furthermore, the recovery of carbon and reducing equivalents was nearly identical in a given treatment, indicating that anaerobic respirations that would cause higher relative amounts of CO<sub>2</sub> compared to the recovered reducing equivalents (e.g., denitrification [Drake and Horn, 2007], the reducing equivalents would be in inorganic nitrogen compounds that were not examined) were nearly inactive.



**Figure 27.** Effect of non-polymeric saccharides on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. The concentration of filter-sterilized non-polymeric saccharides approximated 5 mM. Controls lacked supplemental non-polymeric saccharide. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. Succinate and *N*-acetylglucosamine had nearly the same retention time, compromising the accurate measurement of succinate in that treatment; succinate was therefore not quantified in the *N*-acetylglucosamine treatment. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019a.



**Figure 28.** Collective amounts of fermentation products in non-polymeric saccharide-supplemented anoxic microcosms of *L. terrestris* gut contents. The concentration of filter-sterilized non-polymeric saccharides approximated 5 mM. Abbreviations: A, *N*-acetylglucosamine; G, glucose; Ce, cellobiose; Ga, galacturonic acid; X, xylose. Values are the average of triplicate analyses in Figure 27 and represent the net amounts of products at the end of the 30 h incubation (control values were subtracted). The asterisks indicate significant differences between the collective amount of products formed in control and non-polymeric saccharide treatments (\*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; *t*-test with unequal variances). FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 18.** *P* values of fermentation products in non-polymeric saccharide treatments.<sup>a</sup>

Product	CO <sub>2</sub>					
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	30	49	83	55	65	33
Variance	96	54	30	149	3.1	0.6
<i>P</i> value		0.052	0.004	0.052	0.026	0.648
Product	H <sub>2</sub>					
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	0.2	27	60	31	17	4.1
Variance	0.1	36	78	47	1.9	0.2
<i>P</i> value		0.016	0.007	0.016	0.002	0.001
Product	Acetate					
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	41	106	66	54	93	43
Variance	377	120	6.1	2.6	228	14
<i>P</i> value		0.015	0.153	0.358	0.022	0.894

Product		Succinate				
Treatment	C	A	Ce	G <sub>(20h)</sub>	Ga <sub>(20h)</sub>	X
Mean value <sup>b</sup>	0.0	nd <sup>c</sup>	8.5	5.1	2.7	0.0
Variance	0.0	-	0.0	0.0	0.0	0.0
P value		-	0.0	0.0	0.002	-
Product		Formate				
Treatment	C	A <sub>(20h)</sub>	Ce <sub>(20h)</sub>	G <sub>(10h)</sub>	Ga <sub>(20h)</sub>	X
Mean value <sup>b</sup>	0.3	18	25	23	20	10
Variance	0.1	0.9	5.9	0.5	0.3	0.4
P value		0.001	0.003	0.0	0.0	0.0
Product		Propionate				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	8.1	4.9	1.3	3.6	9.1	6.7
Variance	13	1.4	0.0	0.1	9.7	0.4
P value		0.284	0.081	0.16	0.715	0.591
Product		Butyrate				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	3.1	7.2	3.7	3.1	3.0	3.2
Variance	0.5	4.3	0.0	0.1	0.6	0.2
P value		0.084	0.341	0.871	0.888	0.909
Product		Methylbutyrate				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	5.7	3.3	1.6	2.8	4.3	2.3
Variance	4.2	1.4	0.0	0.0	4.0	0.3
P value		0.178	0.073	0.132	0.433	0.105
Product		Isobutyrate				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	1.0	1.1	0.3	0.3	0.8	0.1
Variance	0.3	0.3	0.0	0.0	0.1	0.0
P value		0.771	0.159	0.176	0.65	0.112
Product		Lactate				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	0.5	12	33	14	3.5	0.7
Variance	0.0	0.7	1.7	0.1	0.0	0.0
P value		0.002	0.001	0	0.003	0.068
Product		Ethanol				
Treatment	C	A	Ce	G	Ga	X
Mean value <sup>b</sup>	0.0	25	60	40	0.0	16
Variance	0.0	3.4	0.1	1.8	0.0	1.2
P value		0.002	0.0	0.0	-	0.002

<sup>a</sup>P values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C) and *N*-acetylglucosamine (A), cellobiose (Ce), glucose (G), galacturonic acid (Ga) or xylose (X) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation (unless otherwise indicated). For transient products (i.e., formate and succinate), the significance of differences of net amounts between control and supplemented treatments were tested for the time point of the highest concentration (shown in parentheses). See Figure 27 for product profiles. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

<sup>c</sup>nd, not determined. Succinate and *N*-acetylglucosamine had nearly the same retention time, compromising the accurate measurement of succinate in that treatment; succinate was therefore not quantified in the *N*-acetylglucosamine treatment.

**Table 19.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in non-polymeric saccharide treatments.<sup>a</sup>

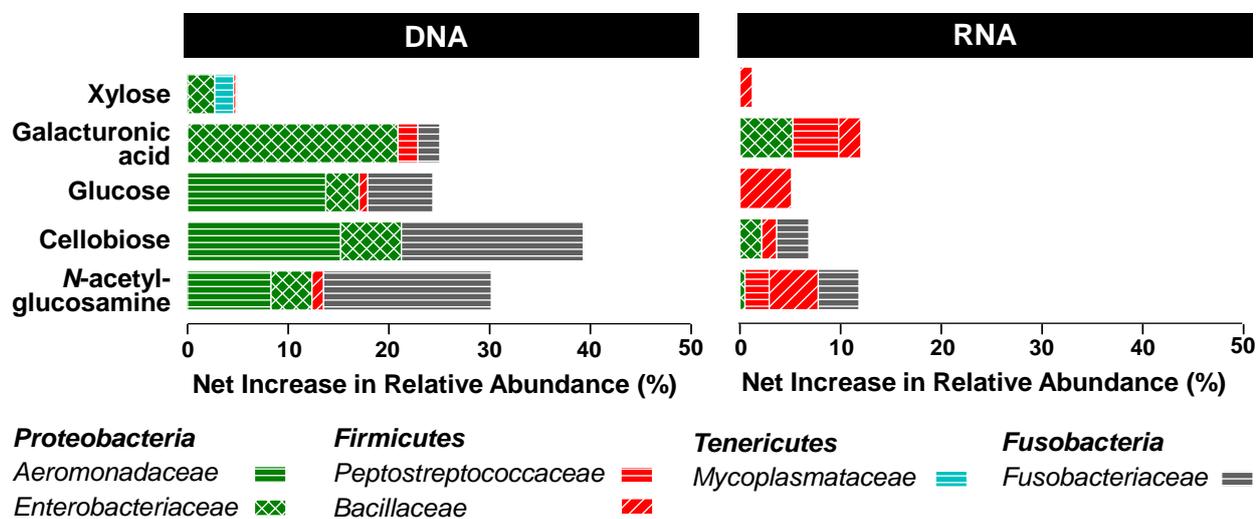
Main Products	Recoveries (%)									
	N-acetylglucosamine		Cellobiose		Glucose		Galacturonic acid		Xylose	
	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE
CO <sub>2</sub>	4.8	na	8.8	na	8.3	na	11	na	2.2	na
H <sub>2</sub>	na	3.3	na	5.1	na	5.2	na	3.1	na	1.4
Acetate	32	32	8.4	8.4	8.8	8.8	32	39	2.5	2.5
Ethanol	13	19	20	31	27	40	-	-	24	37
Lactate	8.4	8.4	16	16	14	14	2.9	3.4	0.6	0.6
Succinate	nd <sup>b</sup>	nd <sup>b</sup>	6.2	5.5	4.5	4.0	0.5	0.6	0.4	0.4
Formate	1.5	0.7	1.1	0.5	1.8	0.9	1.9	1.1	7.1	3.5
Butyrate	4.0	5.0	0.3	0.4	-	-	-	-	0.2	0.2
Propionate	-	-	-	-	-	-	1.0	1.4	-	-
Isobutyrate	0.1	0.2	-	-	-	-	-	-	-	-
<b>Total:</b>	<b>68</b>	<b>72</b>	<b>62</b>	<b>67</b>	<b>64</b>	<b>73</b>	<b>50</b>	<b>48</b>	<b>37</b>	<b>45</b>

<sup>a</sup>See Figure 27 for product profiles. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate consumed. Values are based on the arithmetic average of three replicate analyses. RE, reducing equivalents; -, no net increase of the product during the incubation relative to the control treatment; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019a.

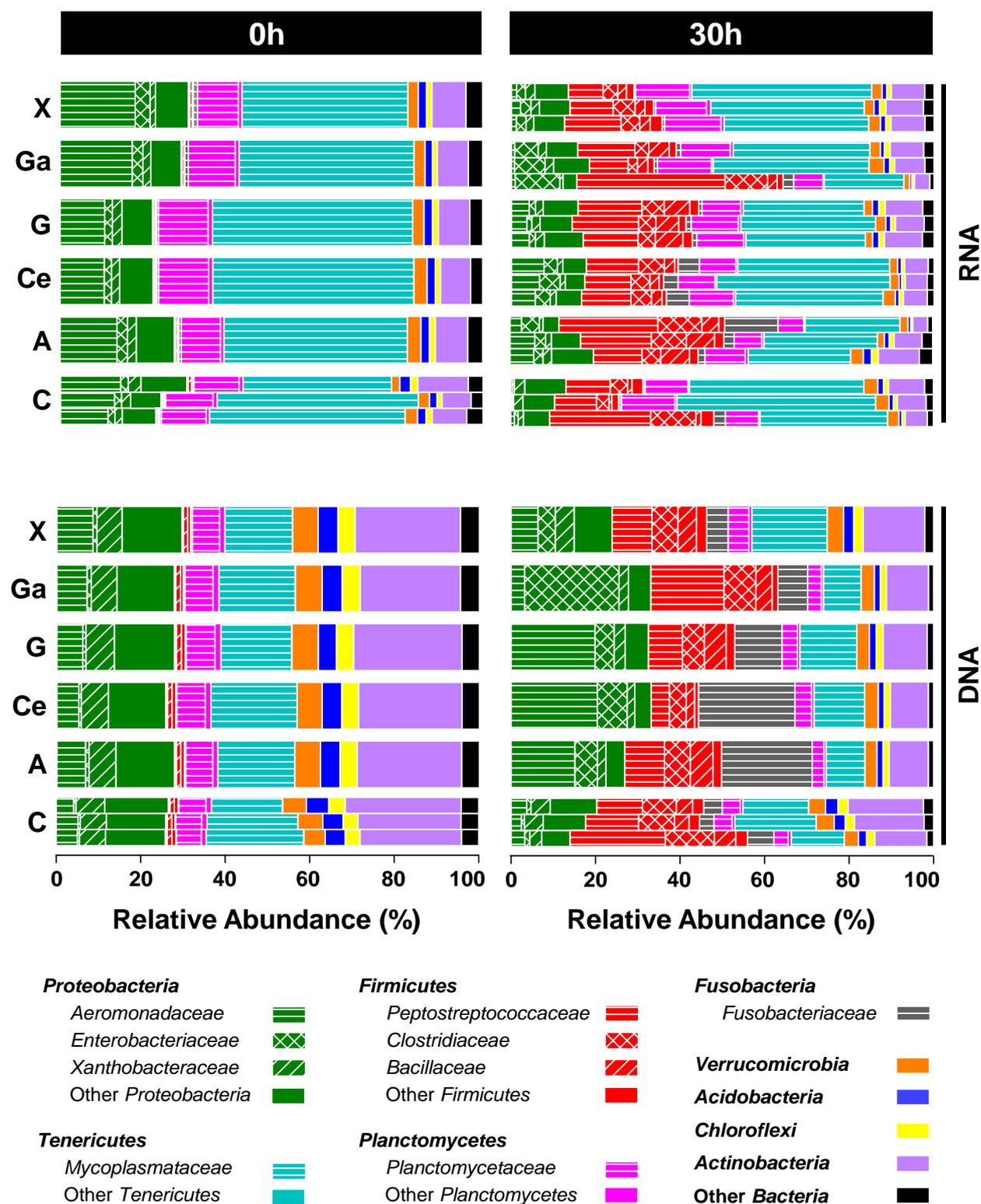
<sup>b</sup>nd, not determined. Succinate and *N*-acetylglucosamine had nearly the same retention time, compromising the accurate measurement of succinate in that treatment; succinate was therefore not quantified in the *N*-acetylglucosamine treatment.

### 3.1.4. Effect of non-polymeric saccharides on gut fermentative bacterial families

A total of 1,161,553 bacterial 16S rRNA gene and 16S rRNA sequences were obtained from the non-polymeric saccharide treatments, yielding 29 phyla (including candidate phyla). The relative abundances of 16S rRNA gene sequence analysis indicated that *Aeromonadaceae*, *Enterobacteriaceae*, and *Fusobacteriaceae* were the most stimulated families in glucose, *N*-acetylglucosamine, and cellobiose treatments. In contrast, *Enterobacteriaceae*-affiliated sequences were most abundant in the galacturonic acid treatment at the end of the anoxic incubation (Figure 29; Figure 30 and Table 20). Furthermore, microcosms supplemented with glucose or the glucose-based saccharides *N*-acetylglucosamine and cellobiose displayed the strongest increase in relative abundances of *Fusobacteriaceae*-affiliated 16S rRNA gene sequences at the end of the incubation (Figure 29, Figure 30 and Table 20). Xylose was less stimulatory compared to the other non-polymeric saccharides (Figure 29 and Figure 30), a finding consistent with the relatively low fermentation activity in the xylose treatment (Figure 27 and Figure 28). The differences in relative sequence abundances of the saccharide-responding taxa obtained from the 16S rRNA gene versus 16S rRNA-based analyses might have been due to temporal changes that occurred during the incubation.



**Figure 29.** Net increases in 16S rRNA gene (DNA) and 16S rRNA (RNA) relative abundances of bacterial families stimulated by supplemental non-polymeric saccharides in *L. terrestris* gut content microcosms. The graph is limited to families that displayed a net increase in relative abundance of  $\geq 4\%$  in at least one treatment and the families are color-coded to the respective phyla (see Figure 30 for the complete 16S gene rRNA and 16S rRNA analyses). Net increases of relative abundances were calculated as follows: (a) the calculation is based either on mean relative abundances when samples from the three replicates were analyzed separately (i.e., all RNA and DNA samples of control treatments and RNA samples at 30 h of supplemented treatments) or on single relative abundances when samples of the three replicates were pooled for sequence analyses (i.e., DNA samples at 0 h and 30 h and RNA samples at 0 h of supplemented treatments); (b) mean or single relative abundances at the beginning of incubation were subtracted from those at the end of incubation for control and supplemented treatments; (c) the resulting time-corrected relative abundances of control treatments were subtracted from those of supplemented treatments (negative time-corrected relative abundances of control treatments were ignored). Figure modified and used with permission from Zeibich *et al.*, 2019a.



**Figure 30.** 16S rRNA (RNA) and 16S rRNA gene (DNA) analyses of the non-polymeric saccharide experiment. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Process data are shown in Figure 27, and information on all detected taxa is provided in Table A3. Abbreviations: C, unsupplemented control; A, *N*-acetylglucosamine; Ce, cellobiose; G, glucose; Ga, galacturonic acid; X, xylose. Grouped bars indicate that the sequence analysis was performed individually for the three replicates and single bars indicate that DNA or RNA samples of the three replicates were pooled for the sequence analysis. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 20.** Statistical analyses of main stimulated families in non-polymeric saccharide treatments.<sup>a</sup>

Family	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
<b><i>Aeromonadaceae</i></b>	Control	0.5	0.3	0.4	
	<i>N</i> -acetylglucosamine	4.7	1.7	5.6	4.7 <sup>(2)</sup>
	Cellobiose	6.7	1.0	6.7	4.8 <sup>(1)</sup>
	Glucose	4.1	0.1	4.2	4.6 <sup>(2)</sup>
	Xylose	1.5	0.5	1.2	4.2 <sup>(3)</sup>
<b><i>Enterobacteriaceae</i></b>	Control	0.5	0.3	0.4	
	<i>N</i> -acetylglucosamine	3.0	1.1	2.5	4.5 <sup>(3)</sup>
	Cellobiose	3.8	0.9	3.5	4.6 <sup>(3)</sup>
	Glucose	1.4	0.2	1.5	4.4 <sup>(3)</sup>
	Galacturonic acid	7.8	2.5	7.2	4.9 <sup>(1)</sup>
	Xylose	2.3	0.2	2.3	4.4 <sup>(1)</sup>
<b><i>Fusobacteriaceae</i></b>	Control	1.3	1.2	0.7	
	Cellobiose	4.6	1.0	5.1	4.7 <sup>(2)</sup>
<b><i>Bacillaceae</i></b>	Control	1.0	0.3	1.0	
	<i>N</i> -acetylglucosamine	5.8	1.5	6.7	4.8 <sup>(1)</sup>
	Cellobiose	2.4	0.2	2.4	4.4 <sup>(4)</sup>
	Glucose	6.0	0.6	6.1	4.8 <sup>(1)</sup>
	Galacturonic acid	3.2	1.4	2.4	4.5 <sup>(2)</sup>
	Xylose	2.3	0.2	2.3	4.4 <sup>(2)</sup>

<sup>a</sup>Families with the four highest ranks in the LefSe analysis were considered. LefSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>LDA scores were calculated using LefSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).

The apparent increase of relative 16S rRNA gene and 16S rRNA sequences abundances of *Firmicutes* in control as well as the other treatments (Figure 30) corroborated previous findings that revealed a positive response of *Firmicutes*-affiliated species to anoxia and endogenous gut nutrients (Figure 24 A). The strong stimulation of certain *Firmicutes*-, *Proteobacteria*-, and *Fusobacteria*-affiliated families during the incubation in the non-polymeric saccharide treatments (Figure 30) was consistent with the lower numbers of detected phylotypes, expected phylotypes (Chao1), and Shannon indices at the end of the incubation compared to values obtained at the beginning of the incubation (Table 21). These findings suggested bacterial shifts in the gut content communities during the incubation. A presumption confirmed by the NMDS analysis (Section 2.6.2.2) of all phylotypes that displayed a different microbial community at the end of the incubation in the control treatment compared to non-polymeric saccharide treatments (Figure 31). The shifts were more pronounced for cellobiose, glucose, and *N*-acetylglucosamine treatments (Figure 31), an observation corroborating the potential of certain non-polymeric saccharides to stimulate fermentative gut content taxa. The rarefaction analyses of non-polymeric saccharide-supplemented treatments indicated that the most abundant taxa were targeted (Figure 32).

**Table 21.** Alpha diversity of the microbial community in control and non-polymeric saccharide treatments.<sup>a</sup>

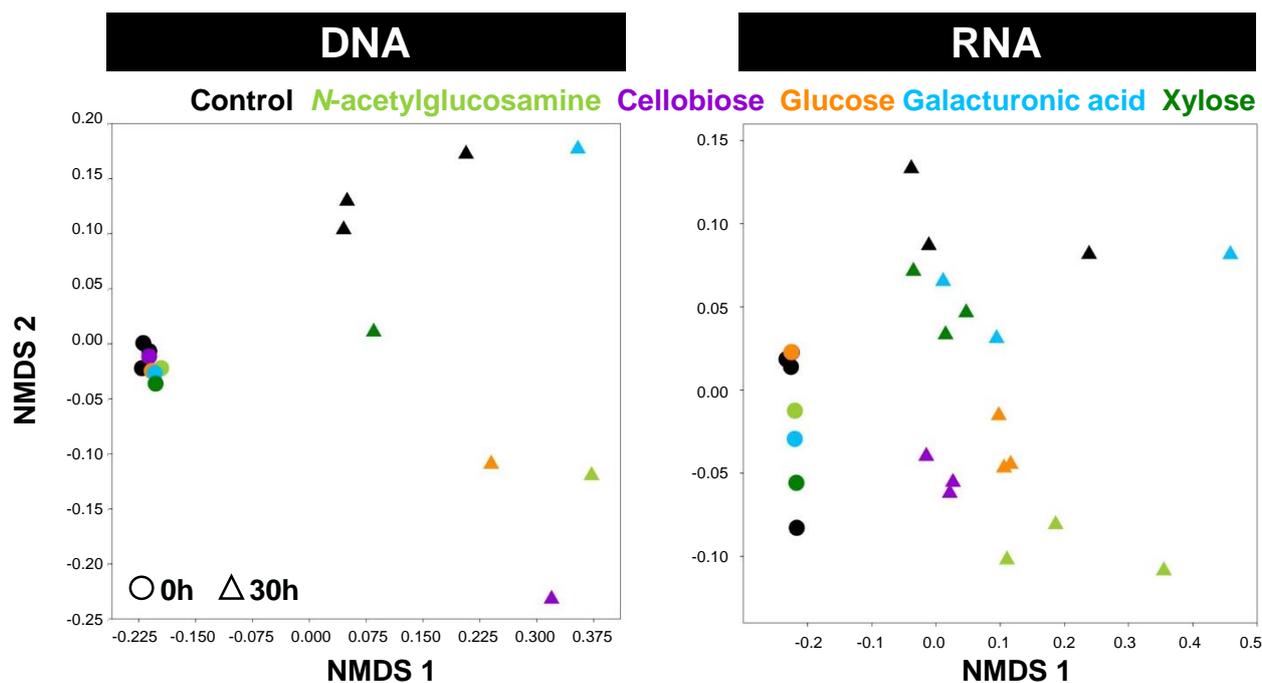
Sample (Sampling Time)	Treatment <sup>b</sup>	Number of sequences	Observed phylotypes <sup>c</sup> (normalized) <sup>d</sup>	Chao1 (normalized) <sup>d</sup>	Shannon (normalized) <sup>d</sup>
<b>DNA (0 h)</b>	Control 1	44009	1895 (894)	2375 (1094)	5.1 (4.9)
	Control 2	44620	1878 (894)	2432 (1083)	5.2 (5.0)
	Control 3	44383	1864 (906)	2247 (1101)	5.4 (5.2)
	<i>N</i> -acetylglucosamine	37023	1772 (889)	2271 (1095)	5.3 (5.1)
	Cellobiose	41497	1864 (896)	2321 (1098)	5.2 (5.0)
	Glucose	37614	1778 (901)	2210 (1081)	5.3 (5.1)
	Galacturonic acid	35812	1724 (892)	2135 (1093)	5.3 (5.1)
	Xylose	45239	1866 (903)	2340 (1112)	5.3 (5.1)
<b>DNA (30 h)</b>	Control 1	29368	1241 (661)	1814 (868)	4.3 (4.1)
	Control 2	32909	1443 (756)	1986 (979)	4.7 (4.6)
	Control 3	31551	1476 (775)	2012 (987)	4.9 (4.8)
	<i>N</i> -acetylglucosamine	33320	1176 (603)	1596 (832)	3.8 (3.7)
	Cellobiose	27626	1158 (614)	1720 (819)	3.7 (3.6)
	Glucose	32524	1265 (652)	1866 (883)	4.1 (4.0)
	Galacturonic acid	34699	1236 (627)	1717 (849)	4.0 (3.9)
	Xylose	29671	1331 (734)	1800 (931)	4.7 (4.6)
<b>RNA (0 h)</b>	Control 1	14910	1150 (746)	1746 (917)	3.8 (3.7)
	Control 2	16121	1126 (719)	1717 (908)	3.6 (3.5)
	Control 3	25041	1477 (850)	2125 (1062)	4.4 (4.2)
	<i>N</i> -acetylglucosamine	16279	1150 (744)	1620 (903)	3.8 (3.7)
	Cellobiose	17851	1205 (741)	1751 (917)	3.8 (3.6)
	Glucose	15421	1123 (735)	1607 (902)	3.8 (3.6)
	Galacturonic acid	18955	1186 (729)	1733 (916)	3.8 (3.7)
	Xylose	24808	1290 (742)	1779 (935)	3.9 (3.7)
<b>RNA (30 h)</b>	Control 1	19781	977 (598)	1480 (773)	3.7 (3.6)
	Control 2	16858	1024 (665)	1531 (820)	3.7 (3.6)
	Control 3	21921	1203 (730)	1660 (894)	4.0 (3.9)
	<i>N</i> -acetylglucosamine 1	27903	1367 (780)	1836 (978)	4.6 (4.5)
	<i>N</i> -acetylglucosamine 2	38425	1334 (679)	1779 (909)	4.0 (3.9)
	<i>N</i> -acetylglucosamine 3	21830	922 (530)	1551 (732)	3.5 (3.4)
	Cellobiose 1	18536	993 (634)	1430 (802)	3.9 (3.8)
	Cellobiose 2	20605	1010 (615)	1510 (823)	3.6 (3.5)
	Cellobiose 3	23897	1049 (623)	1453 (816)	3.8 (3.7)
	Glucose 1	32774	1380 (757)	1789 (972)	4.4 (4.3)
	Glucose 2	24656	1193 (707)	1662 (886)	4.2 (4.0)
	Glucose 3	28766	1358 (764)	1754 (988)	4.3 (4.2)
	Galacturonic acid 1	25452	1012 (550)	1586 (773)	3.4 (3.3)
	Galacturonic acid 2	19030	1131 (714)	1558 (903)	4.1 (4.0)
	Galacturonic acid 3	21253	1159 (708)	1658 (903)	4.2 (4.0)
	Xylose 1	23990	1275 (751)	1742 (960)	4.2 (4.1)
	Xylose 2	24084	1275 (747)	1653 (924)	4.2 (4.1)
	Xylose 3	20541	1136 (709)	1584 (881)	4.0 (3.9)

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2019a.

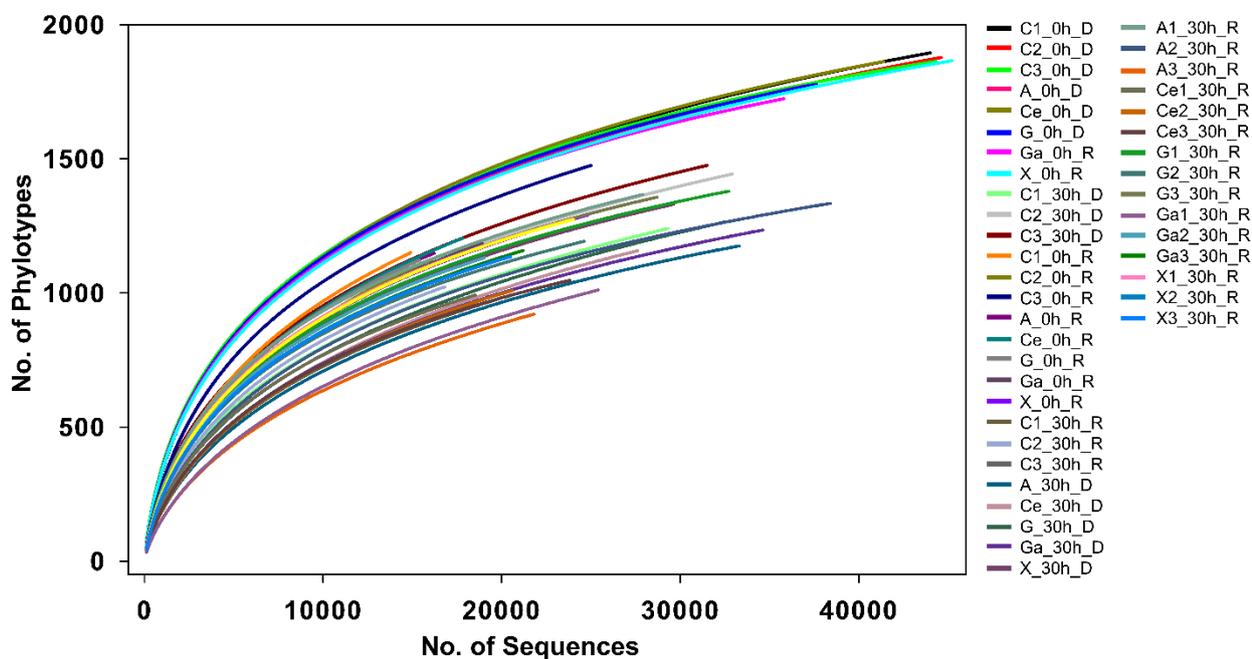
<sup>b</sup>Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Numbers assigned to a treatment (e.g., Control 1) indicate the respective replicate. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h.

<sup>c</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>d</sup>For comparison of amplicon libraries of different sizes, the data set were normalized to 10,000 sequences.



**Figure 31.** NMDS plot of the microbial community composition in non-polymeric saccharide treatments. Distance matrices (Bray-Curtis) are based on the relative abundances of all detected phylotypes in the different treatments (Table A3). Samples of the three replicates of the 16S rRNA gene (DNA) control treatment at 0 h and 30 h, 16S rRNA (RNA) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Proximity of symbols represent the degree of similarity between the different treatments. Figure modified and used with permission from Zeibich *et al.*, 2019a.

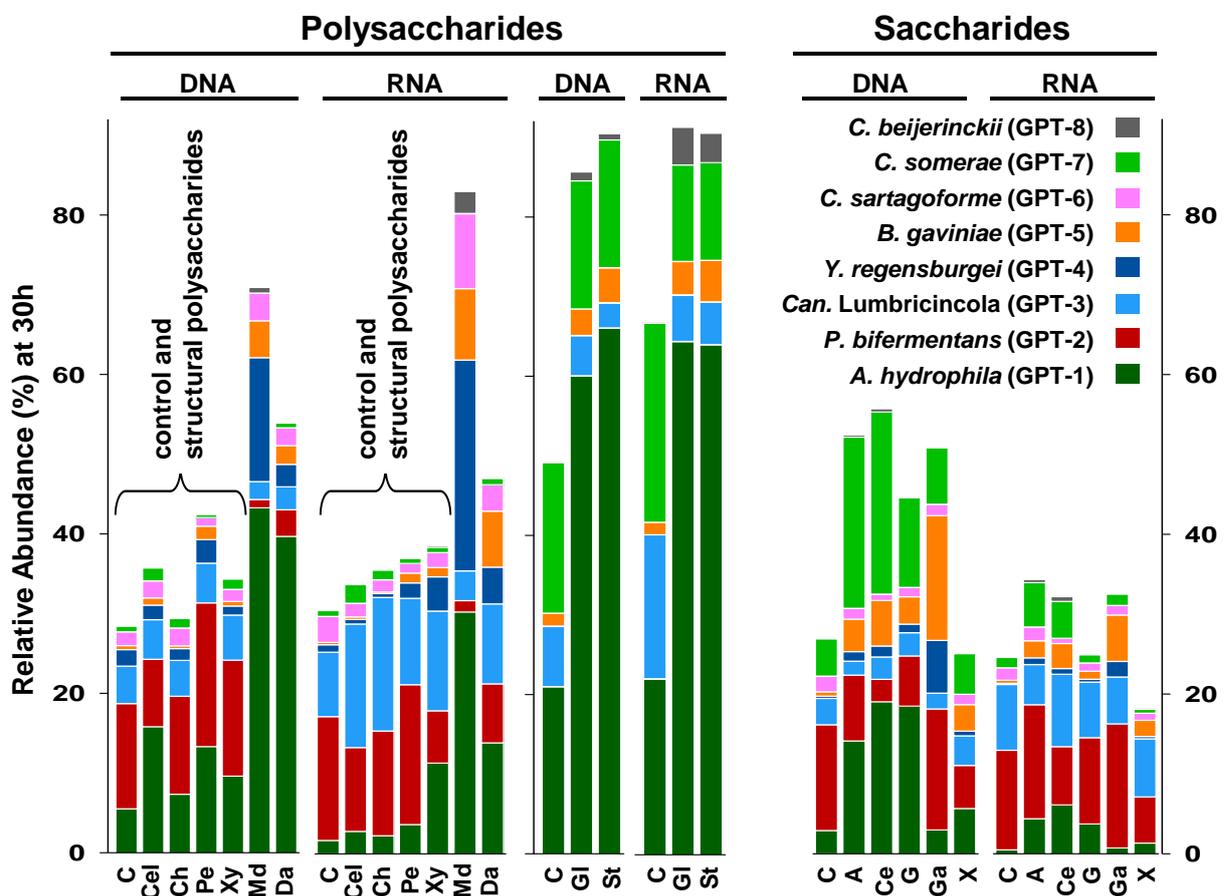


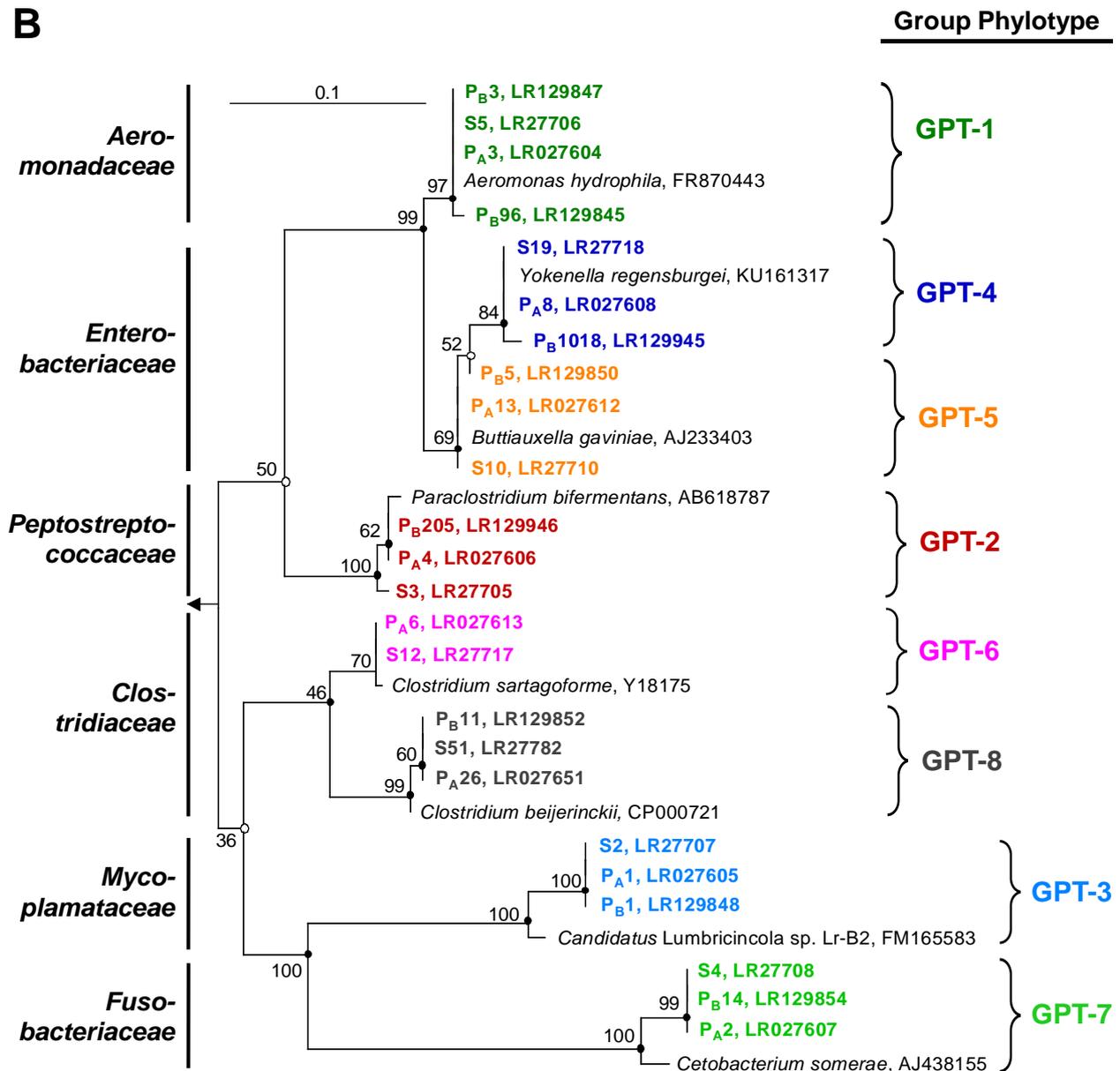
**Figure 32.** Rarefaction analyses of bacterial 16S rRNA gene and 16S rRNA sequences obtained from anoxic *L. terrestris* gut content microcosms supplemented with non-polymeric saccharides. Phylotypes were based on a 97% sequence similarity cutoff. Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Abbreviations: 0 h and 30 h indicate the time of sampling in hours; C, unsupplemented control; D, 16S rRNA genes; R, 16S rRNA. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. A, *N*-acetylglucosamine; Ce, cellobiose; G, glucose; Ga, galacturonic acid; X, xylose. Figure modified and used with permission from Zeibich *et al.*, 2019a.

### 3.1.5. Polysaccharide- and saccharide-responsive phylotypes

The *Proteobacteria*-affiliated families *Aeromonadaceae* and *Enterobacteriaceae* were significantly stimulated by almost all polysaccharides and saccharides (Table 16 and Table 20). Likewise, *Clostridiaceae* and *Fusobacteriaceae* were families significantly stimulated by several supplemental polysaccharides and saccharides (Table 16 and Table 20). These trends extended to eight group phylotypes (GPT; a group phylotype consists of identical or nearly identical phylotypes based on  $\geq 97\%$  nucleic sequence similarity) that displayed  $\geq 4\%$  higher relative abundance (at either the 16S rRNA gene or 16S rRNA level) in at least one of the treatments compared to the control treatment at the end of the incubation (Figure 33).

GPT-1 and GPT-5 (closely related to *Aeromonas hydrophila* and *Buttiauxella gaviniae*, respectively) were significantly stimulated at the end of incubation in all non-structural polysaccharide treatments and almost all saccharide treatments compared to the unsupplemented control (Figure 33 and Table 21). GPT-4 (closely related to *Yokenella regensburgeri*) displayed a significant positive response to maltodextrin, dextran, and galacturonic acid, whereas 16S rRNA gene abundances of GPT-7 (closely related to *Cetobacterium somerae*) were mostly enhanced in saccharide treatments (Figure 33). Furthermore, GPT-2 and GPT-3 (closely related to *P. bif fermentans* and *Can. Lumbricincola* respectively) as well as GPT-7 were consistently abundant in almost all treatments including the controls, suggesting that these taxa utilize non-saccharide-based endogenous gut nutrients.





**Figure 33.** 16S rRNA-based overview of the relative abundances of the main stimulated group phylotypes at the end of the incubation (A) and phylogenetic tree of these stimulated group phylotypes (B). Panel A: Each group phylotype (GPT) consists of identical or nearly identical phylotypes based on a  $\geq 97\%$  sequence similarity. Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one of the supplemented treatments displayed a  $\geq 4\%$  higher relative abundance than in the control treatment at the end of incubation. The group phylotypes are derived from the analyses of 16S rRNA genes (DNA) or 16S rRNA (RNA), and the bars display the relative abundances of each group phylotype at the end of the incubation. Abbreviations: C, unsupplemented control; Cel, cellulose; Ch, chitin; Pe, pectin; Xy, xylan; Md, maltodextrin; Da, dextran; A, *N*-acetylglucosamine; Ce, cellobiose; G, glucose; Ga, galacturonic acid; X, xylose. Panel B: The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees; empty circles, congruent nodes in maximum parsimony and maximum likelihood trees. Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch. Phylotype descriptors: P<sub>A</sub>, phylotypes derived from polysaccharide experiment A (Figure 22 A); P<sub>B</sub> phylotypes derived from polysaccharide experiment B (Figure 22 B); S, phylotypes derived from the non-polymeric saccharide experiment (Figure 27). Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 22.** Statistical analyses of main stimulated phylotypes displayed in Figure 33.<sup>a</sup>

Group Phylotype	Phylotype <sup>b</sup>	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>c</sup>	
<b>GPT-1</b>	P <sub>A3</sub>	Control	1.7	0.5	1.6		
		Cellulose	2.8	0.2	2.7	4.4 <sup>(3)</sup>	
		Pectin	3.7	1.2	3.2	4.1 <sup>(4)</sup>	
		Maltodextrin	30	1.7	31	5.5 <sup>(1)</sup>	
		Dextran	14	5.0	11	5.1 <sup>(1)</sup>	
	P <sub>B3</sub>	Control	9.0	1.8	8.5		
		Glycogen	16	3.1	16	5.2 <sup>(2)</sup>	
		Starch	14	1.2	14	5.1 <sup>(2)</sup>	
	P <sub>B96</sub>	Control	13	0.7	13		
		Glycogen	48	6.9	45	5.7 <sup>(1)</sup>	
		Starch	50	2.8	51	5.7 <sup>(1)</sup>	
	S5	Control	0.5	0.3	0.4		
		<i>N</i> -acetylglucosamine	4.4	1.6	5.2	4.6 <sup>(1)</sup>	
		Cellobiose	6.1	1.0	6.1	4.8 <sup>(1)</sup>	
		Glucose	3.7	0.2	3.8	4.6 <sup>(1)</sup>	
		Xylose	1.3	0.5	1.1	4.1 <sup>(4)</sup>	
	<b>GPT-3</b>	P <sub>A1</sub>	Control	8.1	4.7	6.6	
			Cellulose	15	1.9	16	5.2 <sup>(1)</sup>
			Chitin	17	1.4	17	5.2 <sup>(1)</sup>
<b>GPT-4</b>	P <sub>A8</sub>	Control	0.9	0.3	0.8		
		Pectin	1.9	0.3	2.0	4.3 <sup>(3)</sup>	
		Maltodextrin	26	1.7	27	5.4 <sup>(2)</sup>	
		Dextran	4.6	0.3	4.6	4.7 <sup>(3)</sup>	
	P <sub>B1018</sub>	Control	0.0 <sup>d</sup>	0.0	0.0		
		Starch	0.0 <sup>d</sup>	0.0	0.0	2.6 <sup>(9)</sup>	
	S19	Control	0.2	0.1	0.1		
		<i>N</i> -acetylglucosamine	0.9	0.3	0.7	3.9 <sup>(7)</sup>	
		Cellobiose	0.7	0.2	0.6	3.9 <sup>(7)</sup>	
		Glucose	0.4	0.0	0.4	3.6 <sup>(7)</sup>	
		Galacturonic Acid	1.9	0.8	1.7	4.3 <sup>(4)</sup>	
	<b>GPT-5</b>	P <sub>A13</sub>	Control	0.3	0.1	0.3	
			Maltodextrin	9.0	0.4	9.0	5.0 <sup>(4)</sup>
			Dextran	7.0	0.6	7.3	4.8 <sup>(2)</sup>
		P <sub>B5</sub>	Control	2.0	0.1	1.6	
Glycogen			4.2	0.4	4.3	4.6 <sup>(4)</sup>	
Starch			5.0	0.6	4.9	4.7 <sup>(3)</sup>	
S10		Control	0.4	0.1	0.3		
		<i>N</i> -acetylglucosamine	2.1	0.7	1.9	4.3 <sup>(4)</sup>	
		Cellobiose	3.1	0.7	2.9	4.5 <sup>(4)</sup>	
		Glucose	1.0	0.2	1.1	4.0 <sup>(5)</sup>	
		Galacturonic Acid	5.8	1.7	5.5	4.8 <sup>(1)</sup>	
Xylose		2.0	0.1	2.0	4.3 <sup>(2)</sup>		

Group Phylotype	Phylotype <sup>b</sup>	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>c</sup>
<b>GPT-6</b>	P <sub>A</sub> 6	Control	3.2	0.9	3.7	
		Maltodextrin	9.0	0.9	9.0	5.0 <sup>(3)</sup>
<b>GPT-7</b>	S4	Control	1.3	1.2	0.7	
		Cellobiose	4.6	1.0	5.1	4.7 <sup>(2)</sup>
<b>GPT-8</b>	P <sub>A</sub> 26	Control	0.0	0.0	0.1	
		Pectin	0.1	0.1	0.1	3.3 <sup>(5)</sup>
		Maltodextrin	2.7	0.1	2.7	4.4 <sup>(6)</sup>
		Dextran	0.1	0.0	0.1	3.1 <sup>(10)</sup>
	P <sub>B</sub> 11	Control	0.1	0.0	0.1	
		Glycogen	4.7	0.7	4.5	4.7 <sup>(3)</sup>
		Starch	3.7	1.3	3.0	4.5 <sup>(4)</sup>
	S51	Control	0.1	0.1	0.1	
		Cellobiose	0.6	0.1	0.6	3.8 <sup>(8)</sup>

<sup>a</sup>Only phylotypes that were significantly stimulated (based on LEfSe analysis) by a given supplement are shown. The LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019a.

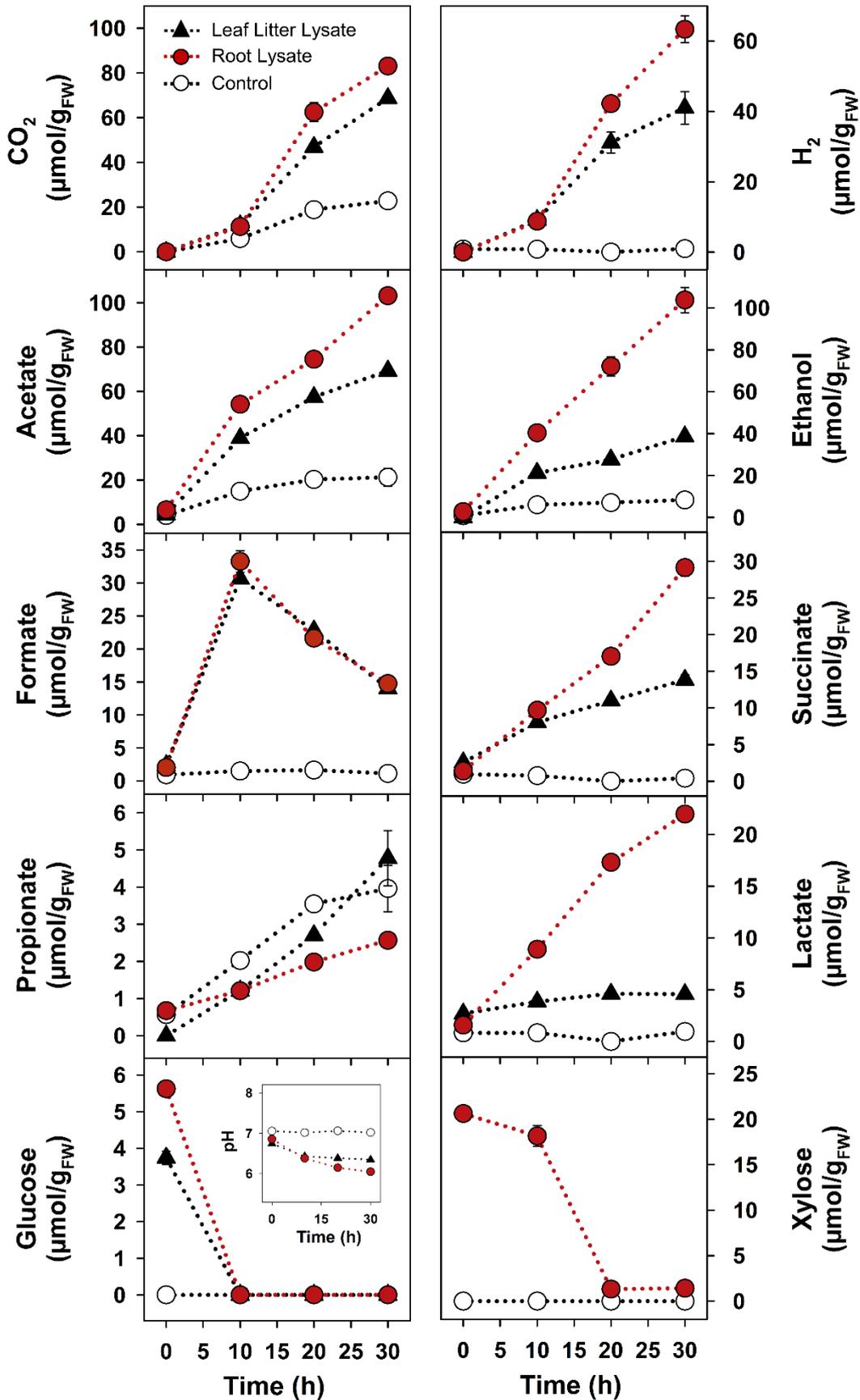
<sup>b</sup>P<sub>A</sub> and P<sub>B</sub>, phylotypes in polysaccharide treatments; S, phylotypes in non-polymeric saccharide treatments.

<sup>c</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking phylotypes exhibited a stronger response to supplement compared to lower ranking ones).

### 3.1.6. Effect of root and leaf litter lysates on gut content fermentation

The aforementioned findings demonstrated that structural plant polysaccharides stimulated the gut content fermentations noticeably weaker than the highly stimulatory energy-storage polysaccharides starch and maltodextrin as well as glucose, a saccharide common to roots and leaves; both dietary substrates for *L. terrestris* (Section 1.4.1). These contrasting findings prompted the evaluation of roots- and leaf litter-derived fermentations in earthworm gut contents.

Gut content fermentations were strongly increased by supplemented sterile lysates from disrupted roots or leaf litter (Figure 34 and Table 23), and the resulting fermentation profiles were similar to those obtained with diverse non-polymeric saccharides (Figure 27 and Figure 28). For example, formate was repeatedly transient (Figure 34) and ethanol, acetate, and succinate were the main electron sinks in these lysate treatments (Table 24). The detection of glucose and xylose, as well as unidentified compounds in the 'sugar region' of the high performance liquid chromatogram at the beginning of incubation, was consistent with the documented occurrence of saccharides in plant material. (Von Fircks and Sennerby-Forsse, 1998; Kromer and Gamian, 2000; Nadwodnik and Lohaus, 2008).



**Figure 34.** Effect of leaf litter lysate and root lysate on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. The amount of organic carbon derived from litter lysate and root lysate added per microcosm approximated 1.55 mmol and 1.08 mmol, respectively. Controls lacked supplemental lysate. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019a.

**Table 23.** *P* values of fermentation products in leaf litter lysate (A) and root lysate (B) treatments.<sup>a</sup>**(A) Leaf Litter Lysate**

	Products															
	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate <sub>(10h)</sub>		Acetate		Propionate		Ethanol	
	C	LL	C	LL	C	LL	C	LL	C	LL	C	LL	C	LL	C	LL
<b>Mean value<sup>b</sup></b>	23	69	1.9	41	0.0	11	0.1	1.9	0.6	28	17	65	3.4	4.8	7.5	39
<b>Variance</b>	1.2	2.1	0.3	22	0.0	0.5	0.5	0.0	0.1	0.0	19	1.0	0.4	0.6	0.3	2.8
<b><i>P</i> value</b>	0.000		0.005		0.001		0.003		0.000		0.003		0.069		0.001	

**(B) Root Lysate**

	Products															
	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate <sub>(10h)</sub>		Acetate		Propionate		Ethanol	
	C	RL	C	RL	C	RL	C	RL	C	RL	C	RL	C	RL	C	RL
<b>Mean value<sup>b</sup></b>	23	83	1.9	63	0.0	28	0.1	20	0.6	31	17	97	3.4	1.9	7.5	101
<b>Variance</b>	1.2	5.2	0.3	15	0.0	1.4	0.5	0.1	0.1	3.0	19	11	0.4	0.0	0.3	33
<b><i>P</i> value</b>	0.000		0.001		0.001		0.000		0.001		0.000		0.055		0.001	

<sup>a</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C) and leaf litter lysate (LL) or root lysate (RL) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation (unless otherwise indicated). For transient products (i.e., formate), the significance of differences of net amounts between control and supplemented treatments were tested for the time point of the highest concentration (shown in parentheses). See Figure 34 for product profile. Unsupplemented control treatments formed traces of butyrate, methylbutyrate, and isobutyrate, whereas supplemented treatments yielded less of these three products. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

That the amount of supplemented plant lysates was adequate for the observed fermentations, was corroborated by the approximately 55% and 18% carbon recovered in the detected fermentation products derived from root lysate and litter lysate, respectively (Table 24). The fermentation of lysate-derived glucose and xylose, and the rapid production of formate, acetate, ethanol, and lactate (Figure 34), demonstrated, as proof of principle that the fermentative earthworm gut microbiota were poised to respond immediately to utilizable plant-derived nutrients including saccharides.

**Table 24.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in leaf litter lysate and root lysate treatments.<sup>a</sup>

Main Products	Recoveries (%)			
	Leaf Litter Lysate		Root Lysate	
	Carbon	RE	Carbon	RE
CO <sub>2</sub>	3.0	na	3.9	na
H <sub>2</sub>	na	1.3	na	2.0
Acetate	6.1	6.1	10	10
Ethanol	4.0	6.0	12	18
Succinate	3.0	2.7	7.3	6.4
Propionate	0.3	0.3	-	-
Lactate	0.3	0.3	3.9	3.9
Formate	0.2	0.1	0.8	0.4
Isobutyrate	0.2	0.2	-	-
<b>Total:</b>	18	17	55	59

<sup>a</sup>See Figure 34 for product profiles. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. RE, reducing equivalents; -, no net increase of the product during the incubation in supplemented treatments relative to the control treatments; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019a.

### 3.2. Effect of microbial cell lysate, protein, and RNA on the fermentative microbiota of *L. terrestris*

The ingestion of dietary material coupled to the abrasive action of the gizzard introduces a wide range of nutrients into the alimentary canal. For example, protein a primary component of a disrupted microbial cell (Section 1.3.2). In this regard, approximately 2 mM amino acids can occur in the aqueous phase of the alimentary canal (Horn *et al.*, 2003). This and the decrease of protein in the gut from anterior to posterior corroborate the likelihood that protein hydrolysis in the gut yields fermentable amino acids. Thus, the availability of amino acids would be dependent on protein hydrolysis, and the secretion of earthworm-proteases into the anterior part of the alimentary canal indicates that the earthworm contributes to this process (Section 1.3.2). RNA is likewise a main component of a disrupted microbial cell (Section 1.3.3). These considerations indicate that microbes in the earthworm gut are challenged with protein and RNA derived from gizzard-disrupted cells, and prompted the evaluation of the effects of fresh cell lysate (used to simulate disrupted microbial cells), protein, and RNA on gut content fermentation and associated gut content microbiota.

#### 3.2.1. Effect of cell lysate on gut content fermentation

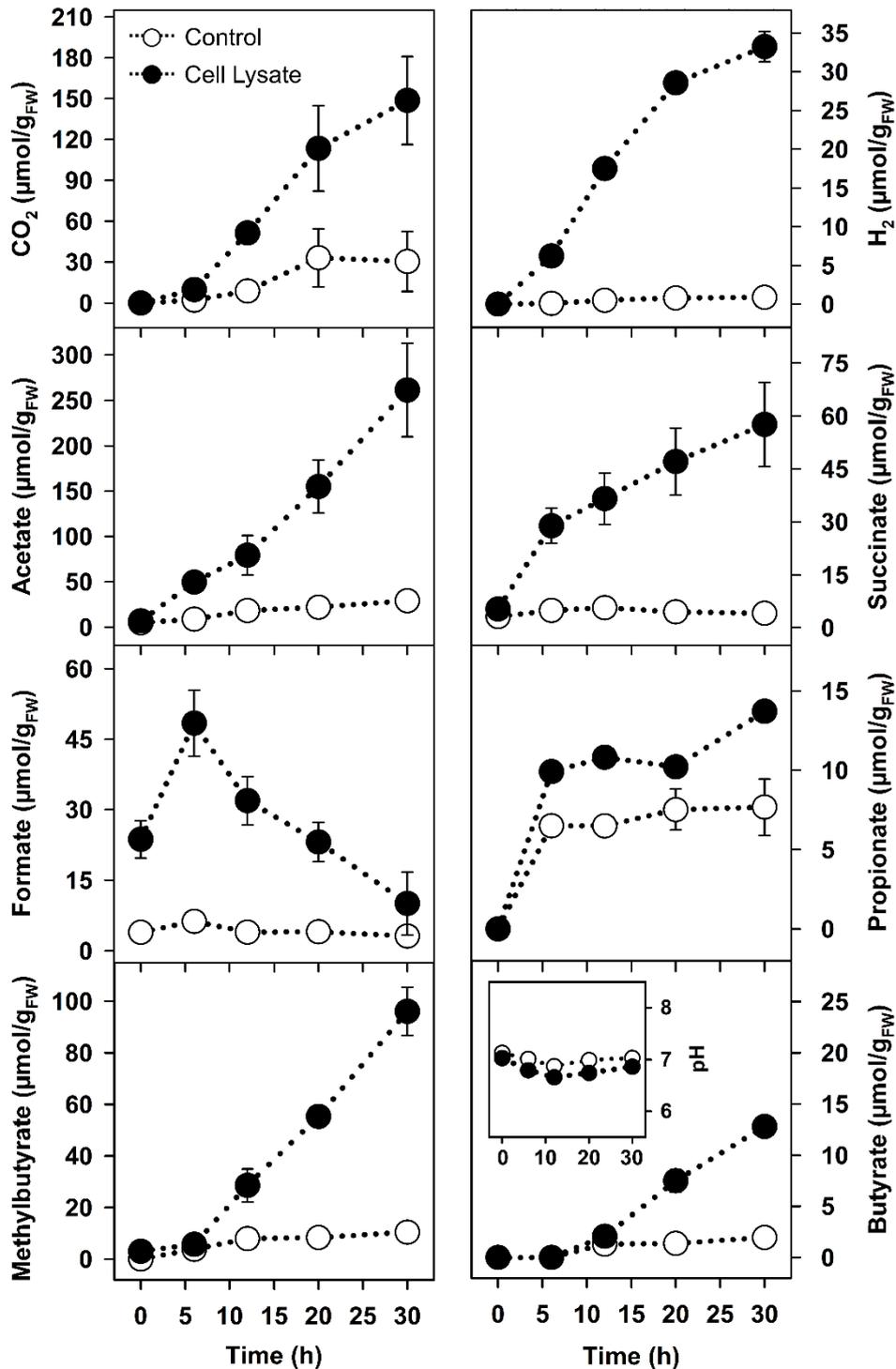
In a preliminary experiment, fresh cell-free lysates of either *S. cerevisiae* or *E. coli* enhanced the production of H<sub>2</sub> and CO<sub>2</sub> (Table 25), indicating that a fermentative response to lysate was independent of the source of lysate. Yeast-derived lysate was selected for more detailed experiments because (a) larger microbial cells such as fungal cells are assumed to be more susceptible to rupture by the gizzard than smaller microbial cells (Section 1.2.1) and (b) the analysis of prokaryotic 16S rRNA genes and 16S rRNA would not be impaired.

**Table 25.** Effect of cell lysates from *S. cerevisiae* and *E. coli* on the formation of CO<sub>2</sub> and H<sub>2</sub> in anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

Treatment	Replicate	pH		Gaseous products (μmol/g <sub>FW</sub> )			
		1	2	CO <sub>2</sub>		H <sub>2</sub>	
				1	2	1	2
Control		6.8	6.9	19	22	2.2	2.4
<i>E. coli</i>		6.6	6.6	60	55	5.9	9.7
<i>S. cerevisiae</i>		6.6	6.6	64	64	15	16

<sup>a</sup>The amount of carbon derived from filter sterilized *E. coli* lysate (5.3% dry weight) and *S. cerevisiae* lysate (5.1% dry weight) added per microcosm approximated 2.0 mmol and 2.2 mmol, respectively. Filter-sterilized lysate alone did not display any fermentation activity. Control lacked supplemental lysate. Earthworms were maintained at 15°C, and gut content microcosms were incubated at 15°C for 44 h. FW, fresh weight. Table modified and used with permission from Zeibich *et al.*, 2018.

The rapid anaerobic formation of  $H_2$ ,  $CO_2$ , and different fatty acids in yeast lysate treatments was significant compared to the unsupplemented control and indicated that available lysate was linked to diverse fermentations (Figure 35 and Table 26). In contrast to the other fermentation products that accumulated, the increase of formate was transient, an observation consistent with previous findings (Figure 27 and Figure 34).



**Figure 35.** Effect of yeast lysate on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. The amount of carbon derived from filter-sterilized lysate (6.0% dry weight) added per microcosm approximated 2.3 mmol. Controls lacked supplemental lysate. Lysate alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2018.

The initial pH approximated 7 and was relatively stable (Figure 35). The theoretical recoveries of carbon and reducing equivalents in fermentation products indicated that (a) approximately 55% of the lysate-derived organic carbon was fermented to the detectable products (Table 27) and (b) the amount of supplemented lysate was adequate for the observed enhanced fermentation.

**Table 26.** *P* values of fermentation products in yeast lysate treatments.<sup>a</sup>

	Products									
	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate	
	C	L	C	L	C	L	C	L	C	L
<b>Mean value<sup>b</sup></b>	30	149	0.9	33	4.1	58	1.3	1.5	6.2	48
<b>Variance</b>	479	1046	0.0	3.8	0.0	142	0.0	0.0	0.9	50
<b>P value</b>	0.001		0.006		0.016		0.103		0.009	
	Acetate		Propionate		Butyrate		Methylbutyrate		Total	
	C	L	C	L	C	L	C	L	C	L
	<b>Mean value<sup>b</sup></b>	29	262	7.7	14	2.0	13	10	96	92
<b>Variance</b>	31	2656	3.2	0.2	0.1	0.1	1.6	88	336	1441
<b>P value</b>	0.016		0.030		0.000		0.004		0.000	

<sup>a</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C) and yeast lysate (L) treatments at the end of incubation. See Figure 35 for product profile. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

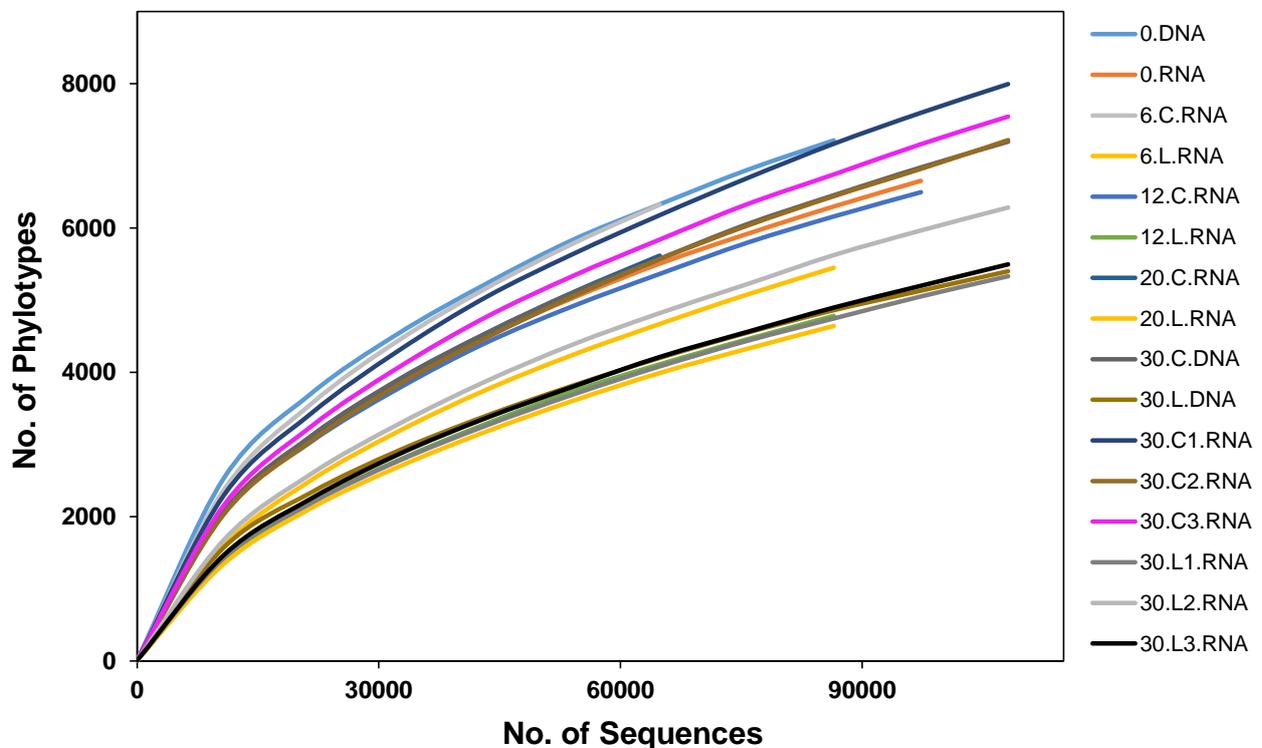
**Table 27.** Estimated recoveries of carbon and reducing equivalents (e.g., electrons) in yeast lysate treatments.<sup>a</sup>

Main Products	Recoveries (%)	
	Carbon	Reducing Equivalents
CO <sub>2</sub>	5.2	na
H <sub>2</sub>	na	0.7
Acetate	20	20
Methylbutyrate	18	23
Succinate	9.0	7.8
Propionate	0.8	0.9
Butyrate	1.9	2.4
Lactate	0.1	0.1
Total:	55	55

<sup>a</sup>See Figure 35 for product profile. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. Table modified and used with permission from Zeibich *et al.*, 2018.

### 3.2.2. Effect of cell lysate on gut fermentative bacterial families

A total of 1,715,804 bacterial 16S rRNA gene and 16S rRNA sequences were obtained, and rarefaction analyses indicated that the most abundant taxa were targeted (Figure 36). Based on the relative abundances of the detected sequences in control and lysate treatments at the end of the incubation, the phylum *Firmicutes* was notably stimulated by lysate and the affiliated families *Peptostreptococcaceae*, *Clostridiaceae*, and *Lachnospiraceae* displayed a strong increase of relative 16S rRNA abundances in lysate treatments compared to the unsupplemented control (Table 28 and Figure 37). The increases in relative abundances of *Peptostreptococcaceae*-, *Clostridiaceae*-, and *Lachnospiraceae*- affiliated 16S rRNA sequences were supported by statistical analyses of the comparative relative abundances of sequences in control and lysate treatments at the end of the incubation (Table 28).



**Figure 36.** Rarefaction analyses of bacterial 16S rRNA gene and 16S rRNA sequences obtained from control and yeast lysate treatments. Phylotypes were based on a 99% sequence similarity cutoff. Abbreviations: 0, 6, 10, 12, 20, 30 indicate the time of sampling in hours; C, unsupplemented control; L, lysate treatment. 16S rRNA gene (DNA) and 16S rRNA (RNA) samples of the three replicates were always pooled except for 16S rRNA samples at 30 hour. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Figure modified and used with permission from Zeibich *et al.*, 2018.

The phylum *Proteobacteria* was represented by the families *Aeromonadaceae* and *Enterobacteriaceae* (Figure 37). The relative abundances of these families varied during the incubation period. Thus, *Aeromonadaceae*-affiliated 16S rRNA sequences increased initially in the yeast lysate treatments but decreasing with time and were less abundant at the end of the incubation. In marked contrast, *Enterobacteriaceae*-affiliated 16S rRNA sequences increasing more gradually during the time period and dominated the *Proteobacteria*-affiliated sequences at

the end of the incubation. This increases in relative abundances of *Aeromonadaceae*- and *Enterobacteriaceae*- affiliated 16S rRNA sequences were confirmed by statistical analyses of the comparative relative abundances of sequences in control and lysate treatments at the end of the incubation (Table 28). The stability of the pH during the incubation (Figure 35) reinforced the likelihood that nutrient input rather than a change in pH was an important factor for the observed changes in the community composition of the lysate treatment. *Mycoplasmataceae* were represented by a phylotype with 99% similarity to *Can. Lumbricincola*, and 16S rRNA sequences of this phylotype had a high relative abundance in unsupplemented controls. Members of the genus *Can. Lumbricincola* occur in tissues, gut contents, and casts of earthworms (Nechitaylo, 2009), and affiliated 16S RNA gene and 16S rRNA sequences were also abundant in previous gut content treatments (Meier *et al.*, 2018; Figure 24 and Figure 30). Consistent with the strong stimulation of *Peptostreptococcaceae*, *Aeromonadaceae*, and *Enterobacteriaceae* in the lysate treatment, the number of detected and expected phylotypes as well as Shannon indices were lower at the end of the incubation period in this treatment compared to those of the unsupplemented control (Table 29).

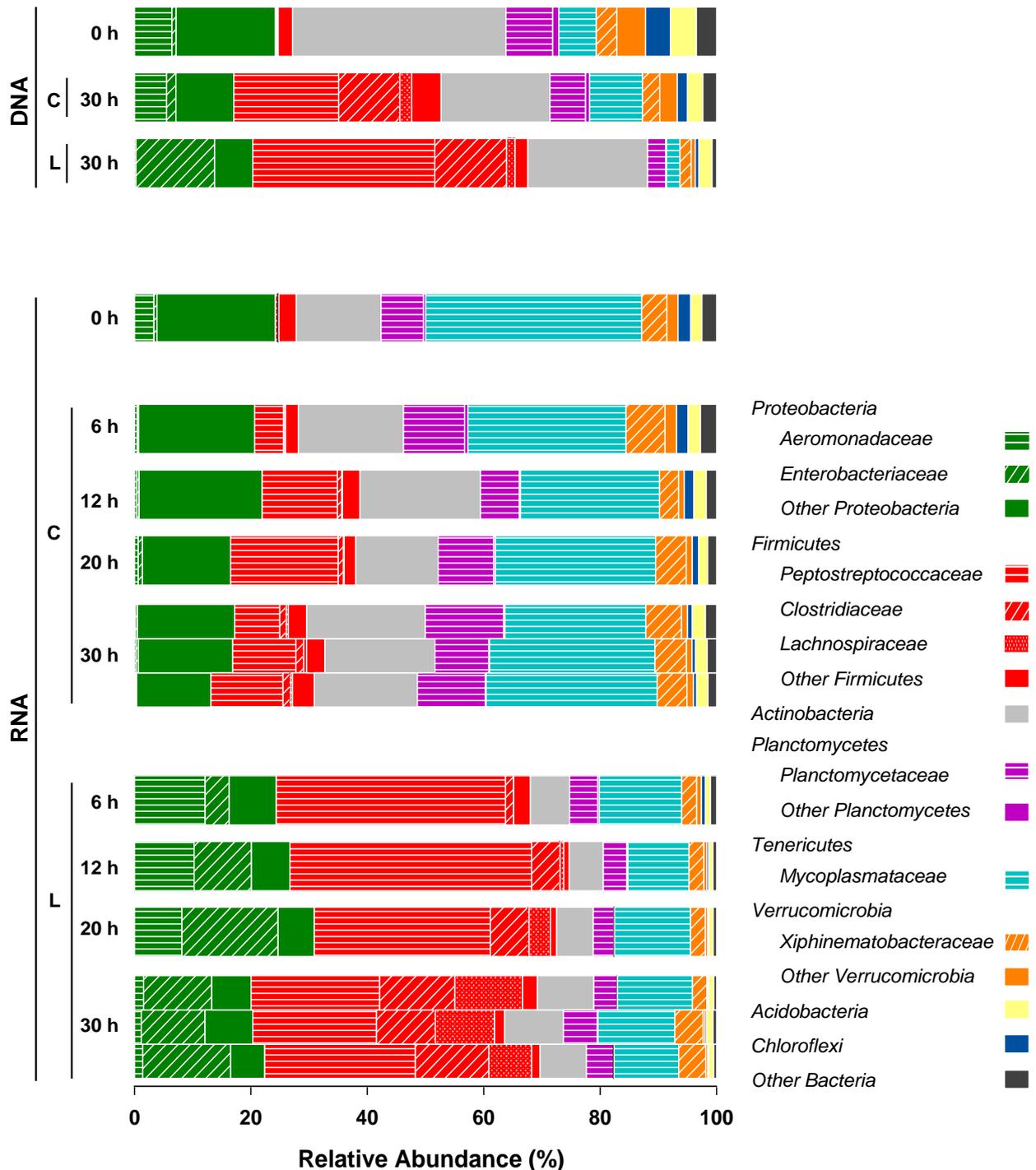
**Table 28.** Statistical analysis of main stimulated families in yeast lysate treatments.<sup>a</sup>

Family	Treatment	Mean	Variance	P value <sup>b</sup>	LDA Score (log10) <sup>c</sup>
<b><i>Aeromonadaceae</i></b>	Control	0.2	0.0	0.010	4.1 <sup>(5)</sup>
	Lysate	1.4	0.0		
<b><i>Clostridiaceae</i></b>	Control	1.3	0.0	0.007	5.1 <sup>(3)</sup>
	Lysate	12	2.4		
<b><i>Enterobacteriaceae</i></b>	Control	0.3	0.0	0.011	5.1 <sup>(2)</sup>
	Lysate	13	4.9		
<b><i>Lachnospiraceae</i></b>	Control	0.4	0.0	0.018	5.0 <sup>(4)</sup>
	Lysate	9.7	4.8		
<b><i>Peptostreptococcaceae</i></b>	Control	10	5.6	0.003	5.4 <sup>(1)</sup>
	Lysate	23	6.1		

<sup>a</sup>Families were designated (a) abundant when a family had a relative abundance of  $\geq 5\%$  in at least one sampling period and (b) stimulated when the increase in relative abundance over time was more pronounced in the lysate treatment compared to the respective unsupplemented control treatment. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>P values (significant at  $P \leq 0.05$ ) of control treatment vs. lysate treatment were calculated from relative abundances at the end of the 30 h incubation by *t*-test with unequal variances.

<sup>c</sup>LDA scores were calculated using LefSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).



**Figure 37.** Effect of yeast lysate on the temporal changes of the relative abundances of bacterial phyla in *L. terrestris* gut content microcosms based on the analyses of 16S rRNA (RNA) and 16S rRNA genes (DNA). The most abundant families (families with  $\geq 5\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Abbreviations: L, lysate treatment; C, unsupplemented control. Samples of the three replicates of a treatment were always pooled for each sampling time point, except for the 16S rRNA samples at the end of the 30 h incubation in which each bar represents one replicate (the high similarity of the three replicates illustrates the reproducibility of the analyses). Process data are shown in Figure 35 and information on all detected taxa is provided in Table A4. Figure modified and used with permission from Zeibich *et al.*, 2018.

**Table 29.** Alpha diversity of the microbial community in control and yeast lysate treatments.<sup>a</sup>

Sampling time:	0 h		6 h		12 h		20 h		
	Treatment: <sup>b</sup>	DNA	RNA	C.RNA	L.RNA	C.RNA	L.RNA	C.RNA	L.RNA
Number of sequences		90591	105034	70681	90615	104319	87844	70036	96059
Observed PTs <sup>c</sup> (normalized) <sup>d</sup>		7384 (6328)	6911 (5506)	6625 (6326)	5596 (4670)	6720 (5365)	4833 (4112)	5848 (5618)	4917 (3989)
Chao1 (normalized) <sup>d</sup>		11975 (10608)	11553 (9751)	11842 (11419)	10159 (8995)	11199 (9460)	8722 (7864)	10712 (10409)	9341 (7911)
Shannon (normalized) <sup>d</sup>		9.8 (9.7)	7.3 (7.3)	8.2 (8.1)	6.1 (6.0)	7.9 (7.9)	5.8 (5.8)	7.2 (7.2)	6.2 (6.2)
Sampling time:	30 h								
Treatment: <sup>b</sup>	C1.RNA	C2.RNA	C3.RNA	L1.RNA	L2.RNA	L3.RNA	C.DNA	L.DNA	
Number of sequences	109807	147651	126676	114515	126281	142222	111359	122114	
Observed PTs <sup>c</sup> (normalized) <sup>d</sup>	8086 (6179)	8411 (5566)	8150 (5841)	5509 (4083)	6825 (4822)	6332 (4214)	7323 (5573)	5745 (4200)	
Chao1 (normalized) <sup>d</sup>	14324 (11675)	14417 (10764)	13660 (10822)	10514 (8101)	12345 (9390)	11492 (8483)	12972 (10395)	9945 (7761)	
Shannon (normalized) <sup>d</sup>	8.3 (8.3)	7.6 (7.6)	7.7 (7.6)	6.6 (6.6)	7.2 (7.1)	6.7 (6.7)	8.3 (8.2)	7.2 (7.2)	

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>C and L corresponds to unsupplemented control and cell lysate treatments, respectively. 16S rRNA gene (DNA) or 16S rRNA (RNA) samples of the three replicates were always pooled except for RNA samples at 30 h. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate.

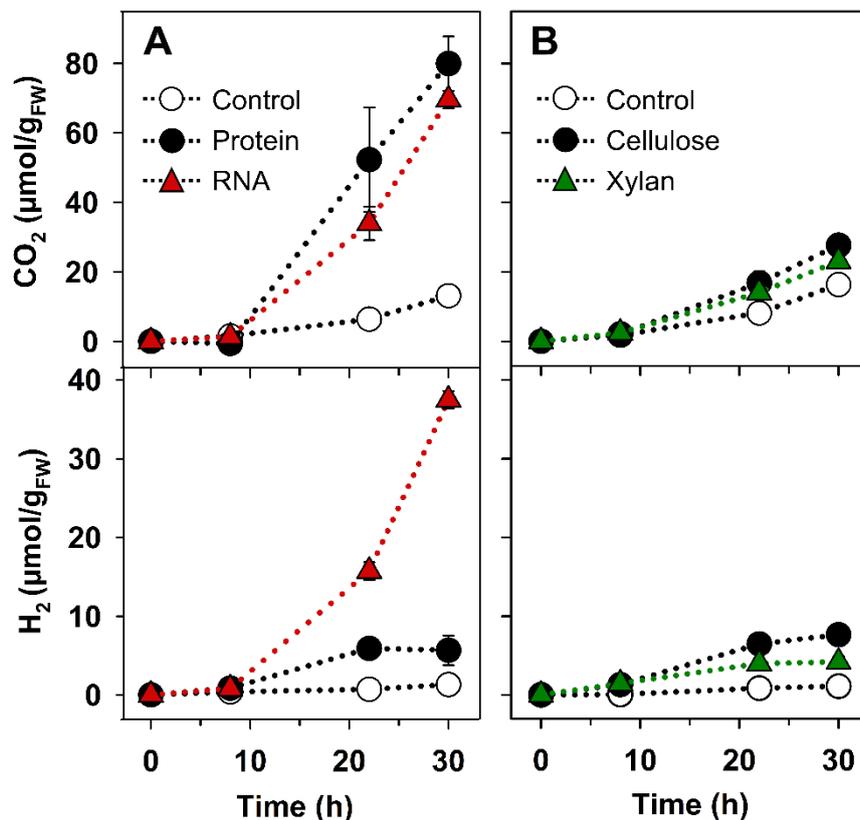
<sup>c</sup>Phylotypes (PTs) were clustered based on a sequence similarity cut-off of 99%.

<sup>d</sup>The data sets were normalized to 64,864 sequences for comparison of amplicon libraries of different sizes.

### 3.2.3. Effect of protein and RNA on gut content fermentation

Protein and RNA are the primary components of microbial cell lysate. Therefore, these two biopolymers were evaluated for their potential to stimulate fermentations and associated gut content microbes of earthworms. Gut contents were also challenged with cellulose and xylan to directly compare the potential specificity of protein- and RNA-linked stimulation.

The anaerobic production of H<sub>2</sub> and CO<sub>2</sub> in anoxic gut content was enhanced in all biopolymer treatments (Figure 38). Likewise, fatty acid production was augmented by all four biopolymers in gut content treatments (Table 30). However, protein and RNA were considerably more stimulatory than cellulose and xylan, and yielded dissimilar fermentation profiles. For example, RNA yielded high amounts of H<sub>2</sub> and succinate, whereas protein strongly enhanced the production of methylbutyrate and butyrate (Figure 38 and Table 30). That H<sub>2</sub> was only marginally produced in the protein treatment, suggesting the occurrence of Stickland reactions, a non-H<sub>2</sub>-producing process often engaged when the H<sub>2</sub> concentrations reach a certain level (Schink and Stams, 2013).



**Figure 38.** Effect of biopolymers on the formation of H<sub>2</sub> and CO<sub>2</sub> in anoxic microcosms of *L. terrestris* gut contents. The amount of biopolymer-derived carbon added per microcosm approximated 2.4 mmol. Controls lacked supplemental biopolymers. Biopolymers alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2018.

**Table 30.** Fatty acid profiles of anoxic microcosms of *L. terrestris* gut contents supplemented with different biopolymers.<sup>a</sup>

Treatment	Time (h)	Products (μmol/gFW) <sup>b</sup>						
		Acetate	Succinate	Formate	Propionate	Butyrate	Methylbutyrate	Lactate
<b>Protein and RNA</b>								
Control	0	2.2 ± 0.5	0.5 ± 0.0	3.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1
	30	23 ± 2.8	0.0 ± 0.0	2.9 ± 0.8	4.5 ± 0.3	1.9 ± 0.1	5.3 ± 0.1	0.5 ± 0.1
Protein	0	2.7 ± 0.1	0.4 ± 0.1	2.7 ± 0.3	0.0 ± 0.0	0.9 ± 0.2	0.0 ± 0.0	1.2 ± 0.2
	30	146 ± 5.6*	0.0 ± 0.0	13 ± 9.2	22 ± 1.7*	25 ± 2.6*	53 ± 1.3*	5.8 ± 2.4
RNA	0	18 ± 1.4	4.9 ± 0.3	2.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.9 ± 0.3
	30	87 ± 5.3*	19 ± 1.4*	39 ± 1.3*	8.0 ± 0.9*	3.2 ± 1.4	5.5 ± 1.2	12 ± 0.5*
<b>Cellulose and Xylan</b>								
Control	0	1.2 ± 0.1	0.5 ± 0.1	3.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.3	0.3 ± 0.0
	30	25 ± 1.4	1.9 ± 0.2	2.5 ± 0.8	3.7 ± 0.1	0.6 ± 0.0	6.8 ± 0.2	0.8 ± 0.1
Cellulose	0	1.8 ± 0.2	0.4 ± 0.1	0.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1
	30	33 ± 7.0	3.1 ± 0.1*	2.8 ± 2.9	4.0 ± 0.2	0.7 ± 0.0	7.1 ± 0.3	2.0 ± 0.2*
Xylan	0	12 ± 0.7	0.6 ± 0.0	1.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0
	30	45 ± 4.4*	2.7 ± 0.1*	3.0 ± 0.1	4.3 ± 0.1*	0.9 ± 0.0*	7.2 ± 0.3	1.6 ± 0.1*

<sup>a</sup>The amount of biopolymer-derived carbon added per microcosm approximated 2.4 mmol. Controls lacked supplemental biopolymers. Biopolymers alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses (± standard derivation). FW, fresh weight. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>The asterisk (\*) indicates significant *P* values (*P* ≤ 0.05) of control vs. protein, RNA, cellulose, or xylan treatments at the end of incubation. *P* values were calculated by *t*-test with unequal variances.

The rapid increase of fermentation activity in protein and RNA treatments indicated that the facultative aerobes and anaerobes in gut content were not nutrient saturated and poised to respond to these biopolymers. Indeed, increasing amounts of protein and RNA yielded increasing amounts of CO<sub>2</sub> and H<sub>2</sub>, respectively (Table 31), indicating a cause-and-effect relation between the availability of protein and RNA and the anaerobic production of these gases.

Time-resolved fermentation analysis and statistical analysis of protein treatments displayed a strongly enhanced and significant production of CO<sub>2</sub>, acetate, propionate, butyrate, and methylbutyrate compared to the unsupplemented control treatments (Figure 39 and Table 32). Furthermore, casamino acids stimulated fermentation similarly to that obtained in the protein treatment (Table 33). In marked contrast to the fermentation profile of protein treatments, RNA treatments and associated statistical analysis displayed a significantly enhanced production of H<sub>2</sub>, CO<sub>2</sub>, formate, acetate, and succinate compared to the unsupplemented control treatments (Figure 39 and Table 32). Acetate and formate were the dominant initial products detected. The initial pH approximated 7 and was relatively stable (Figure 39), corroborated the likelihood that nutrient input rather than a change in pH was an important factor for the observed enhanced fermentations in protein and RNA treatments. Formate was transient in both protein and RNA treatments. An observation consistent with previous studies and treatments supplemented with yeast lysate (Figure 27, Figure 34, and Figure 35). The transient accumulation of formate in protein and RNA treatments, and the transient accumulation of succinate and lactate in protein treatments, suggest that these products were metabolic intermediates and most likely consumed by secondary processes.

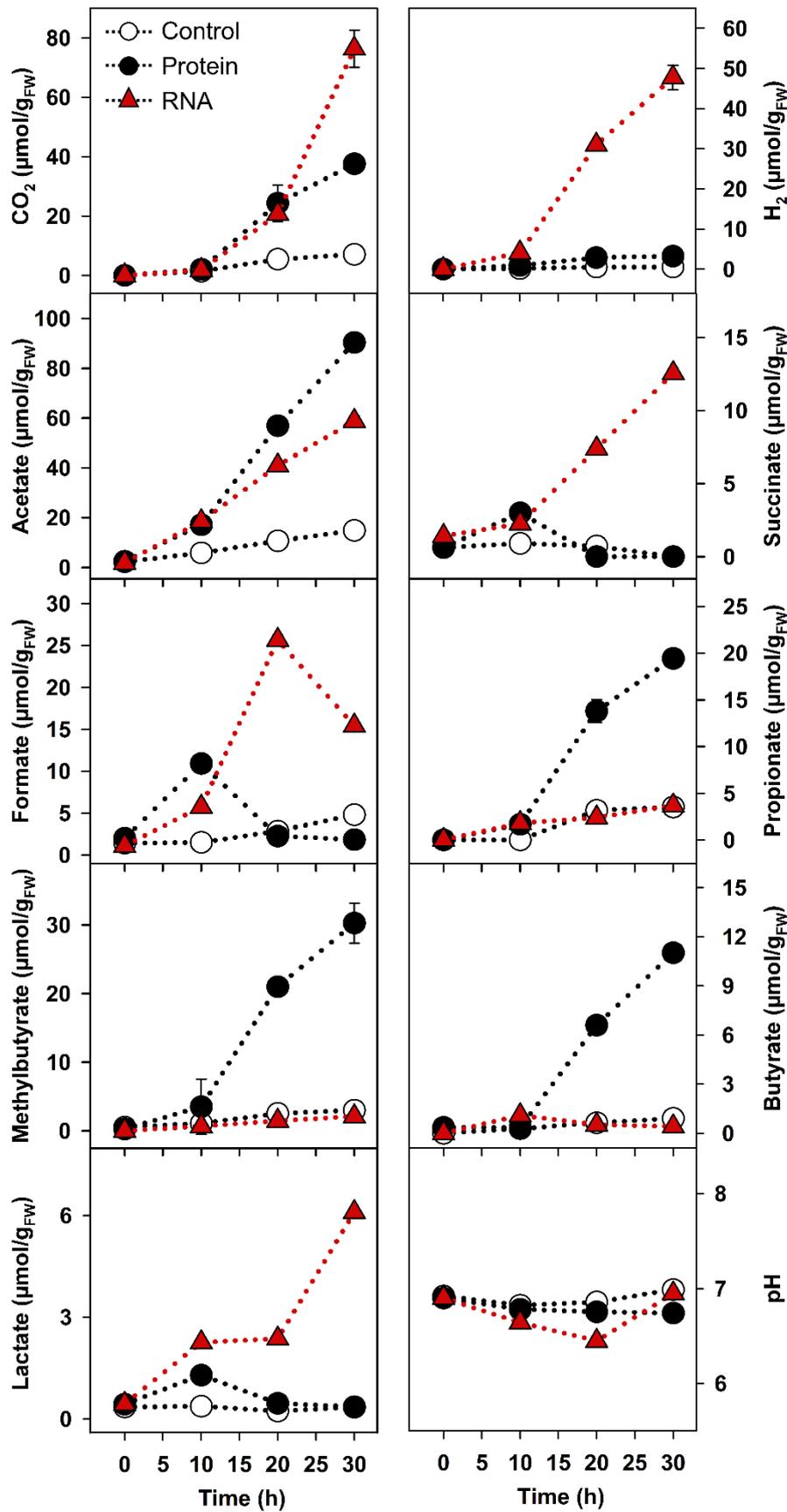
**Table 31.** Effect of different amounts of protein and RNA on the formation of CO<sub>2</sub> or H<sub>2</sub>, respectively, in anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

Treatment	Carbon (mmol)	μmol/g <sub>FW</sub> <sup>b</sup>	P Value <sup>c</sup>
<b>Protein</b>		CO <sub>2</sub>	
	0.0	10 ± 2.8	
	0.5	25 ± 2.0	0.002
	2.0	56 ± 5.1	0.000
<b>RNA</b>		H <sub>2</sub>	
	0.0	0.8 ± 0.1	
	0.5	8.7 ± 1.8	0.002
	2.0	47 ± 9.3	0.001

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Amounts of CO<sub>2</sub> and H<sub>2</sub> at the end of incubation (30 h). Values are the arithmetic average of three replicate analyses (± standard deviation). FW, fresh weight.

<sup>c</sup>P values were calculated by *t*-test with different variances and are based on the difference between the unsupplemented control and the supplemented treatment. Values are significant at *P* ≤ 0.05.



**Figure 39.** Effect of protein or RNA on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. The amount of protein- and RNA-derived carbon approximated 1 mmol per microcosm. Controls lacked supplemental protein or RNA. Protein or RNA alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2018.

**Table 32.** *P* values of the fermentation products in protein (A) and RNA (B) treatments.<sup>a</sup>

**(A) Protein treatment**

	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate		Acetate		Propionate		Butyrate		Methylbutyrate	
	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P
<b>Mean value<sup>b</sup></b>	7.1	38	0.5	3.2	0.0	0.0	0.3	0.4	1.5	11	15	90	3.5	19	0.9	11	3.0	30
<b>Variance</b>	0.2	3.5	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.7	0.2	7.4	0.1	0.5	0.0	0.0	0.0	8.6
<b><i>P</i> value</b>	0.001		0.001		-		0.502		0.016		0.010		0.000		0.009		0.004	

**(B) RNA treatment**

	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate		Acetate		Propionate		Butyrate		Methylbutyrate	
	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R
<b>Mean value<sup>b</sup></b>	7.1	76	0.5	48	0.0	13	0.3	6.1	2.8	26	15	59	3.5	3.7	0.9	0.4	3.0	2.1
<b>Variance</b>	0.2	39	0.0	9.6	0.0	0.1	0.0	0.0	0.1	0.4	0.2	4.8	0.1	0.0	0.0	0.0	0.0	0.4
<b><i>P</i> value</b>	0.001		0.003		0.000		0.000		0.000		0.001		0.775		0.118		0.158	

<sup>a</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C) and protein (P) or RNA (R) treatments at the end of incubation. See Figure 39 for product profile. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

**Table 33.** Fermentation profiles (A) and estimated recoveries of carbon and reducing equivalents (e.g., electrons) (B) in casamino acids, ribose, adenine, uracil, or glycerol treatments.<sup>a</sup>**(A) Fermentation Profile<sup>b</sup>**

Treatment	Time (h)	pH	Products ( $\mu\text{mol/g}_{\text{FW}}$ )									
			CO <sub>2</sub>	H <sub>2</sub>	Acetate	Ethanol	Succinate	Lactate	Formate	Propionate	Butyrate	Methylbutyrate
<b>Control<sub>A</sub></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.2	0.0 ± 0.0	0.7 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	7.0 ± 0.0	7.5 ± 1.0	0.7 ± 0.1	16 ± 0.4	0.0 ± 0.0	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	3.0 ± 0.1	1.3 ± 0.1	1.7 ± 0.1
<b>Casamino acids<sup>c</sup></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.1	0.0 ± 0.0	0.6 ± 0.0	1.3 ± 0.0	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	6.9 ± 0.0	33 ± 2.8*	3.8 ± 0.2*	90 ± 3.4*	0.0 ± 0.0	2.6 ± 0.7*	1.4 ± 0.3	3.2 ± 0.9*	16 ± 1.1*	8.7 ± 0.4*	14 ± 1.5*
<b>Ribose<sup>d</sup></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.3	0.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	6.8 ± 0.0	12 ± 1.0*	3.9 ± 0.8*	32 ± 3.1*	19 ± 2.3*	1.9 ± 0.4*	1.3 ± 0.1*	7.8 ± 0.4*	3.1 ± 0.2	1.5 ± 0.2	2.0 ± 0.2
<b>Adenine<sup>d</sup></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.2	0.0 ± 0.0	0.7 ± 0.1	0.9 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	7.0 ± 0.0	5.1 ± 0.4*	0.5 ± 0.2	11 ± 2.4	0.9 ± 0.1*	1.7 ± 0.3*	0.7 ± 0.0	0.6 ± 0.1	1.2 ± 0.2*	0.6 ± 0.4	1.5 ± 0.3
<b>Uracil<sup>d</sup></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.4	0.0 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	7.0 ± 0.0	10 ± 1.5	0.8 ± 0.1	17 ± 1.2	-	1.2 ± 0.1*	1.2 ± 0.2	1.2 ± 0.5	3.0 ± 0.3	1.6 ± 0.1*	2.7 ± 0.2*
<b>Control<sub>B</sub></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.1	0.0 ± 0.0	1.1 ± 0.0	0.9 ± 0.0	2.1 ± 0.1	0.7 ± 0.0	0.5 ± 0.0	0.0 ± 0.0
	30	7.0 ± 0.0	11 ± 2.7	0.1 ± 0.3	14 ± 0.5	3.0 ± 0.8	0.4 ± 0.1	1.0 ± 0.0	-	2.9 ± 0.1	1.1 ± 0.2	0.9 ± 0.1
<b>Glycerol<sup>d</sup></b>	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.6 ± 0.4	0.8 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	2.3 ± 0.1	0.7 ± 0.0	0.6 ± 0.2	0.0 ± 0.0
	30	7.0 ± 0.0	18 ± 3.7	1.0 ± 0.8	15 ± 1.6	4.3 ± 0.5	0.4 ± 0.0	1.0 ± 0.0	-	6.6 ± 0.8*	1.2 ± 0.2	0.4 ± 0.2

**(B) Recoveries (%)<sup>e</sup>**

Treatment		CO <sub>2</sub>	H <sub>2</sub>	Acetate	Ethanol	Succinate	Lactate	Formate	Prop- ionate	Butyrate	Methyl- butyrate	Total
<b>Casamino acids<sup>c</sup></b>	Carbon	2.1	na	12	-	0.8	0.0	0.2	3.2	2.5	5.0	<b>26</b>
	Reducing Equivalents	na	0.1	12	-	0.6	0.0	0.1	3.6	2.9	6.2	<b>25</b>
<b>Ribose<sup>d</sup></b>	Carbon	3.5	na	24	27	2.3	2.0	5.2	0.1	0.6	1.2	<b>66</b>
	Reducing Equivalents	na	1.2	24	40	2.0	2.0	2.6	0.2	0.8	1.5	<b>75</b>
<b>Adenine<sup>d</sup></b>	Carbon	0.0	na	0.0	0.9	2.7	0.0	0.1	0.0	0.0	0.0	<b>3.7</b>
	Reducing Equivalents	na	0.0	0.0	2.7	4.8	0.0	0.1	0.0	0.0	0.0	<b>7.6</b>
<b>Uracil<sup>d</sup></b>	Carbon	1.2	na	2.2	0.0	1.6	0.6	0.5	0.0	0.6	2.5	<b>9.1</b>
	Reducing Equivalents	na	0.1	3.6	0.0	2.2	1.0	0.4	0.0	1.1	5.2	<b>13</b>
<b>Glycerol<sup>d</sup></b>	Carbon	25	na	16	3.9	0.1	1.5	-	38	0.5	-	<b>85</b>
	Reducing Equivalents	na	1.6	14	4.9	0.1	1.2	-	38	0.6	-	<b>60</b>

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Controls lacked supplemental substrates. Values are the arithmetic average of three replicate analyses ( $\pm$  standard derivation). FW, fresh weight. The asterisk (\*) indicates significant *P* values (significant at  $P \leq 0.05$ ) of control vs. casamino acids, ribose, adenine, uracil, or glycerol treatments at the end of incubation. *P* were calculated by *t*-test with unequal variances.

<sup>c</sup>The amount of casamino acid-derived carbon added per microcosm approximated 1.2 mmol,

<sup>d</sup>The amount of ribose, adenine, uracil, and glycerol per microcosm approximated 5 mM. 3 mM of ribose were consumed. The consumption of uracil and glycerol were not determinable due to overlapping retention times with ethanol and formate, respectively. Adenine was not detectable.

<sup>e</sup>See A for product profile. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Recoveries in ribose treatments are based on the amount of substrate consumed. Values are based on the arithmetic average of three replicate analyses. -, no net increase of the product during the 30 h incubation. na, not applicable.

40% and 24% of protein- and RNA-derived carbon, respectively, and 24% and 23% of protein- and RNA-derived reduction equivalents, respectively, were theoretically recovered in the detected fermentation products (Table 34). These theoretical recoveries (a) corroborate the likelihood that protein and RNA were responsible for the observed diverse fermentations and (b) indicated that the supplemental amounts of these biopolymers were adequate for the observed fermentation products. Furthermore, the recovery of carbon and reducing equivalents tended to be identical in both treatments, indicating that anaerobic respirations were nearly inactive. The marked production of propionate and methylbutyrate in protein treatments and casamino acid treatments is consistent with amino acid-derived fermentations (Barker, 1981; Nanninga, 1985; McInerny, 1988; Smith and Macfarlane, 1997) (Figure 39 and Table 33). These considerations reinforcing the likelihood that protein fermentation was due to the hydrolysis of this biopolymer and subsequently fermentative utilization of available amino acids.

**Table 34.** Estimated recoveries of carbon and reducing equivalents (e.g., electrons) in protein and RNA treatments.<sup>a</sup>

Main Products	Recoveries (%)			
	Protein		RNA	
	Carbon	Reducing Equivalents	Carbon	Reducing Equivalents
CO <sub>2</sub>	3.1	na	6.9	na
H <sub>2</sub>	na	0.1	na	3.1
Acetate	15	15	8.9	12
Methylbutyrate	14	17	-	-
Succinate	-	-	4.7	5.3
Propionate	4.8	5.4	0.3	0.4
Butyrate	4.0	4.9	-	-
Formate	-	-	1.1	0.7
Lactate	-	-	1.7	2.2
Total:	40	42	24 (45) <sup>b</sup>	23 (34) <sup>b</sup>

<sup>a</sup>See Figure 39 for product profiles of protein and RNA treatments. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Parenthetical values are the estimated recoveries based on RNA-derived ribose as sole source of carbon and reducing equivalents.

The hydrolysis of RNA ensure the release of its subunits ribose, purines, and pyrimidines. Ribose-supplemented treatments displayed enhanced amounts of diverse fermentation products (Table 33), and the theoretical amounts of recovered carbon and reducing equivalents from supplemental RNA did not exceed the amounts available from RNA-derived ribose (Table 34

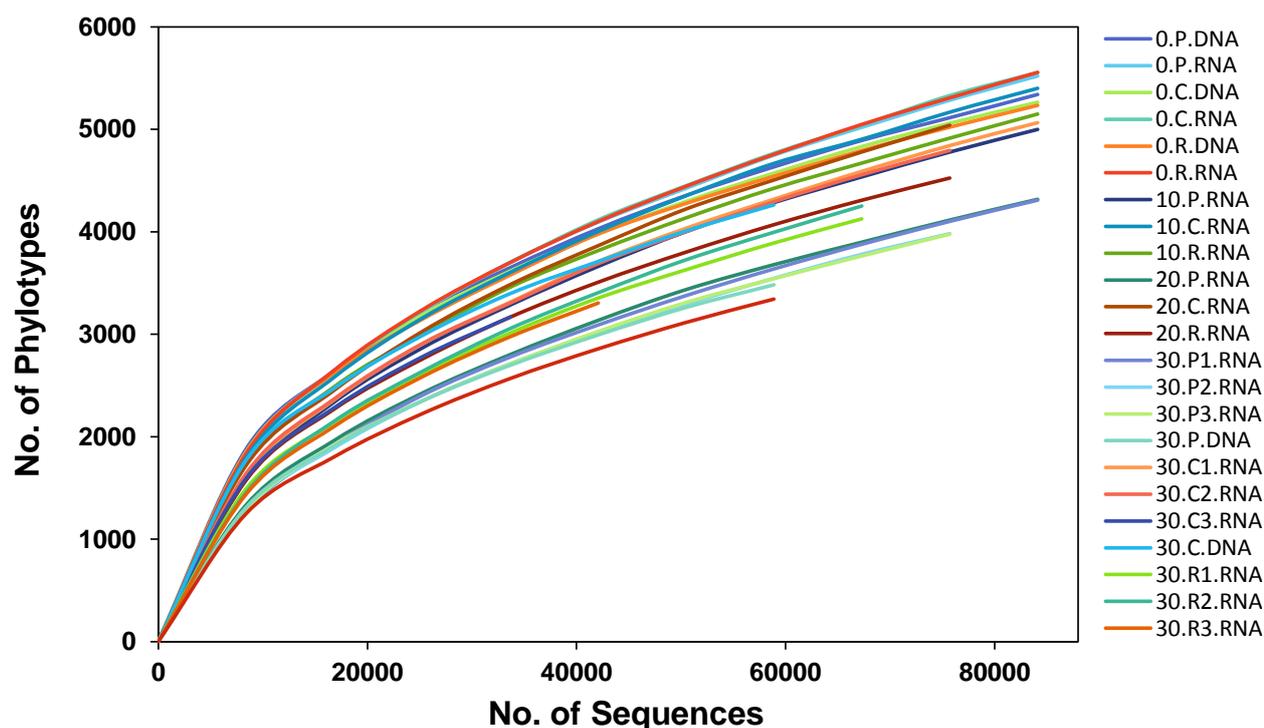
[parenthetical values]). Furthermore, the RNA-derived production of succinate and formate is consistent with ribose-linked fermentations (Stanier and Adams, 1944; Altermatt *et al.*, 1955; Rosenberg, 1980). These findings suggest that the fermentative utilization of RNA-derived ribose was likely the important driver of the observed and enhanced fermentation in RNA treatments. Although supplemented adenine (a purine) and uracil (a pyrimidine) yielded no enhanced gut content fermentation as single substrates (Table 33), it cannot be excluded that purines and pyrimidines were utilized during RNA fermentation (e.g., assimilation for cell biosynthesis), and thereby indirectly enhanced ribose-based fermentation.

Ethanol was a major product in ribose treatments, constituting approximately 40% of the recovered reducing equivalents (Table 33B). In marked contrast, ethanol was not detected in protein and casamino acid treatments. Ethanol and uracil had overlapping retention times on the high performance liquid chromatograph column which did not allow accurate determination of ethanol in the RNA treatment. However, that ethanol was a major product in the ribose treatment and indicated that ethanol was most likely formed during RNA-based fermentation. Ethanol is also produced during the earthworm gut content-fermentation of xylose (Figure 25; Meier *et al.*, 2018) confirming that ethanol is one of the main products formed during pentose fermentations. Lipids can constitute up to 10% of a microbial cell, and the hydrolysis of glycerophospholipids would increase the availability of glycerol that can be fermented to propionate (Section 1.3.4.2 and Section 1.4.3.2; Buckel, 1999; Chen *et al.*, 2016). Indeed, glycerol-supplemented treatments yielded a significant production of propionate compared to the unsupplemented control (Table 33A).

### **3.2.4. Effect of protein and RNA on gut fermentative bacterial families**

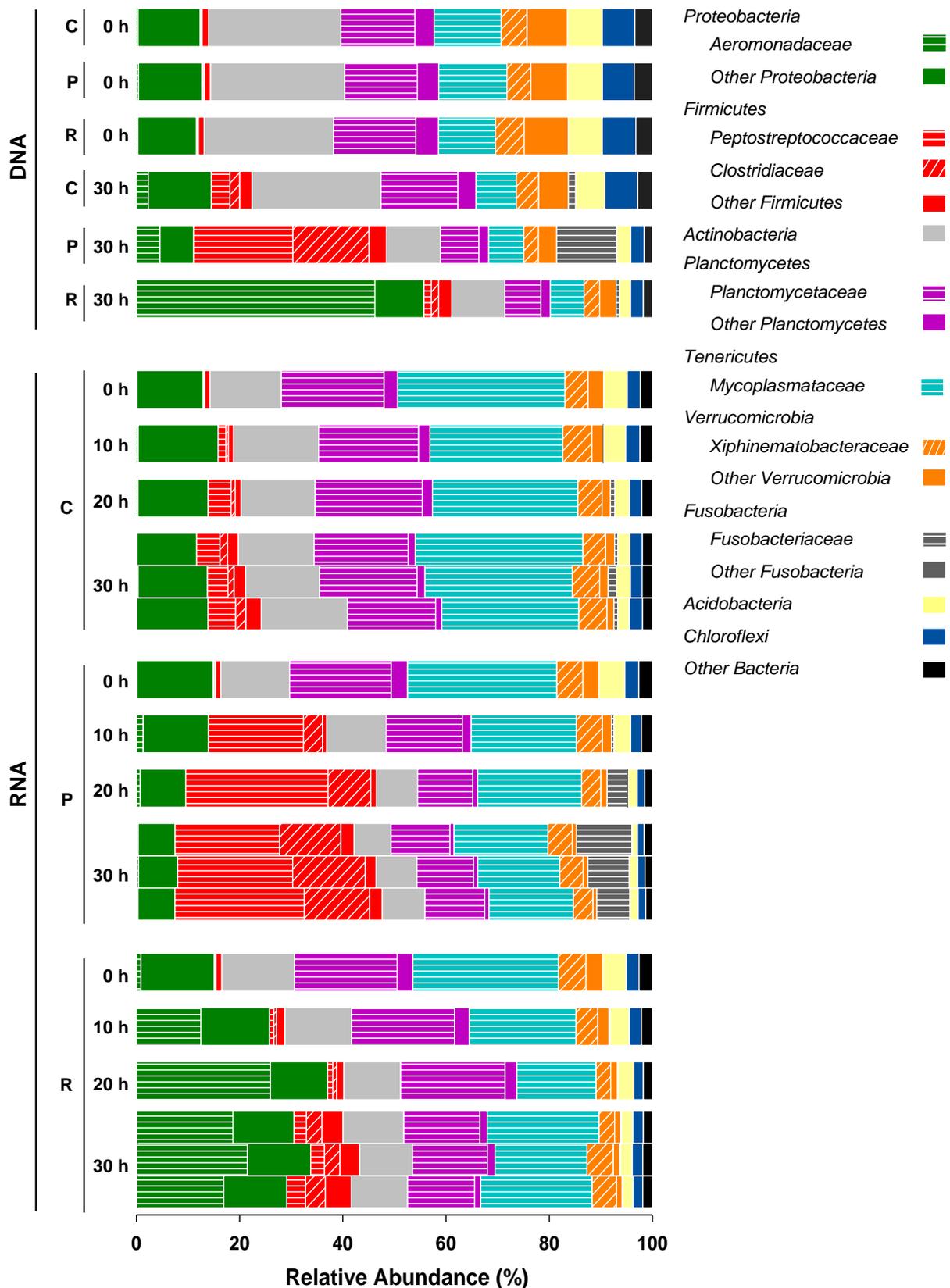
A total of 2,019,822 bacterial 16S rRNA and 16S rRNA gene sequences were obtained, yielding 26 phyla (including candidate phyla). The rarefaction analyses indicated that the most abundant taxa of the unsupplemented control treatments, the protein treatments, and the RNA treatments were effectively targeted (Figure 40). Furthermore, the analysis illustrated, that the relative abundances of 16S rRNA gene and 16S rRNA sequences were almost identical in all treatments prior to incubation (Figure 41 [sequences at 0 h]).

Based on the analyzed relative 16S rRNA and 16S rRNA gene sequence abundances, *Actinobacteria*, *Proteobacteria*, *Planctomycetes*, *Tenericutes*, and *Verrucomicrobia* were abundant in gut content at the beginning of incubation, and the relative abundances of 16S rRNA sequences demonstrated that these taxa were active throughout the incubation period in unsupplemented controls (Figure 41). Consistent with previous findings (Figure 28), *Firmicutes*-affiliated families displayed a increase in relative abundance in unsupplemented controls (Figure 24).



**Figure 40.** Rarefaction analyses of bacterial 16S rRNA (RNA) and 16S rRNA gene (DNA) sequences obtained from protein and RNA treatments. Phylotypes were based on a 97% sequence similarity cutoff. Abbreviations: 0, 6, 10, 12, 20, 30 indicate the time of sampling in hours; C, unsupplemented control; P, protein treatment; R, RNA treatment. For both panels, 16S rRNA gene or 16S rRNA samples of the three replicates were always pooled except for 16S rRNA samples at 30 hour. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Figure modified and used with permission from Zeibich *et al.*, 2018.

The marked change in relative abundances of 16S rRNA-based families in the first 10 h of incubation demonstrated that the bacterial community responded quickly to the availability of protein and RNA (Figure 41). The molecular analyses indicated that the community-response to protein was dissimilar to the community-response to RNA. Thus, the *Firmicutes* were mostly stimulated by protein, whereas the *Proteobacteria* were mainly stimulated by RNA (Figure 41). In this regard, *Peptostreptococcaceae*-, *Clostridiaceae*-, and *Fusobacteriaceae*-affiliated phylotypes were stimulated by protein, whereas *Aeromonadaceae*-affiliated phylotypes were stimulated by RNA (Figure 41). The increases in relative abundances of *Aeromonadaceae*-, *Clostridiaceae*-, *Fusobacteriaceae*-, and *Peptostreptococcaceae*-affiliated 16S rRNA sequences were supported by statistical analyses, based on the quantitative differences of the relative abundances of sequences in unsupplemented control and protein or RNA treatments at the end of the incubation (Table 35). Members of the *Mycoplasmataceae*, *Planctomycetaceae*, and *Xiphinematobacteraceae* displayed a relatively stable abundance in all treatments, suggesting that the constant high abundance of these families was not dependent on supplemental protein or RNA. However, the increase in the relative abundances of these families corresponded to a decrease in the relative abundances of the *Mycoplasmataceae* and *Planctomycetaceae*.



**Figure 41.** Effect of protein or RNA on the temporal changes of the relative abundances of bacterial phyla in *L. terrestris* gut content microcosms based on the analyses of 16S rRNA (RNA) and 16S rRNA genes (DNA). The most abundant families (i.e., families with  $\geq 5\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Abbreviations: C, unsupplemented control; P, protein treatment; R, RNA treatment. Samples of the three replicates of a treatment were always pooled for each sampling time point, except for the 16S rRNA samples at the end of the 30 h incubation in which each bar represents one replicate. Process data are shown in Figure 39, and information on all detected taxa is provided in Table A5. Figure modified and used with permission from Zeibich *et al.*, 2018.

**Table 35.** Statistical analyses of main stimulated families in protein or RNA treatments.<sup>a</sup>

Family	Treatment	Mean	Variance	P Value <sup>b</sup>	LDA Score (log10) <sup>c</sup>
<i>Aeromonadaceae</i>	Control	0.2	0.0		
	RNA	19	5.5	0.005	5.3 <sup>(1)</sup>
	Protein	0.4	0.0	0.119 (0.005)	
<i>Clostridiaceae</i>	Control	1.5	0.2		
	Protein	13	1.3	0.000	5.1 <sup>(2)</sup>
	RNA	3.3	0.2	0.009 (0.001)	
<i>Fusobacteriaceae</i>	Control	1.0	0.3		
	Protein	8.4	4.7	0.029	4.9 <sup>(3)</sup>
	RNA	0.2	0.0	0.120 (0.023)	
<i>Peptostreptococcaceae</i>	Control	4.6	0.5		
	Protein	23	5.8	0.006	5.4 <sup>(1)</sup>
	RNA	2.9	0.4	0.035 (0.005)	

<sup>a</sup>Families were designated (a) abundant when a family had a relative abundance of  $\geq 5\%$  in at least one sampling period and (b) stimulated when the increase in relative abundance over time was more pronounced in at least one treatment (protein or RNA) compared to the respective unsupplemented control. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>P values (significant at  $P \leq 0.05$ ) of control vs. lysate, protein, or RNA treatments were calculated from relative abundances at the end of the 30 h incubation by *t*-test with unequal variances (parenthetical values indicate P values of protein vs. RNA treatments).

<sup>c</sup>LDA scores were calculated using LEfSe (protein vs. RNA treatments). Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).

The number of detected and expected phylotypes decreased during the incubation (Table 36). A trend more pronounced for the protein and RNA treatments compared to the unsupplemented controls. Furthermore, the Shannon indices decreased in the protein and RNA treatments whereas those in the controls remained relatively constant with time (Table 36). These findings reinforced the aforementioned stimulation of subgroups (e.g., *Aeromonadaceae* in RNA treatments and *Peptostreptococcaceae* in protein treatments) of the microbial gut content community in protein and RNA treatments.

The collective relative abundance of the most responsive taxa of the lysate treatment (a) constituted approximately 60% of the total abundance of the detected taxa and (b) was greater than that of either the protein or RNA treatments (Figure 42). However, there was an approximately three-fourths overlap between the responsive families in lysate treatments and the responsive families in the protein and RNA treatments (Figure 42). This overlap consist of the the dominant responsive families in protein and RNA treatments. Thus, *Peptostreptococcaceae* (protein), *Clostridiaceae* (protein), and *Aeromonadaceae* (RNA) collectively constituted approximately three-fourths of the responsive families in the lysate treatment (Figure 42).

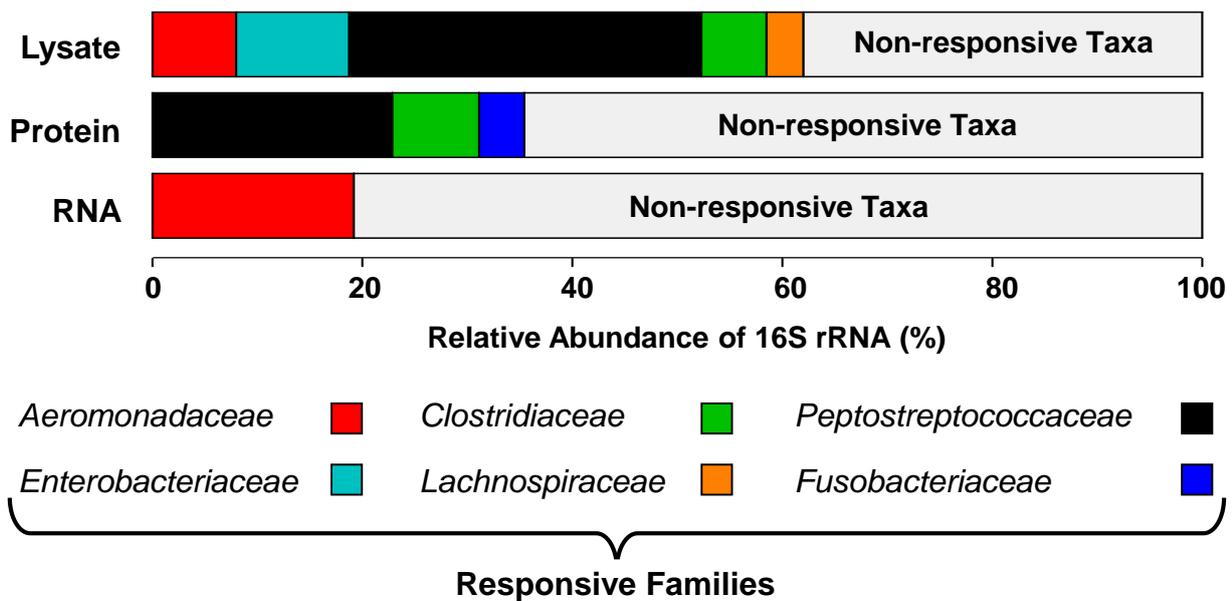
**Table 36.** Alpha diversity of the microbial community in control, protein and RNA treatments.<sup>a</sup>

<b>Sampling Time</b>	<b>0 h</b>						<b>10 h</b>			<b>20 h</b>		
<b>Treatment:</b>	<b>C.DNA</b>	<b>P.DNA</b>	<b>R.DNA</b>	<b>C.RNA</b>	<b>P.RNA</b>	<b>R.RNA</b>	<b>C.RNA</b>	<b>P.RNA</b>	<b>R.RNA</b>	<b>C.RNA</b>	<b>P.RNA</b>	<b>R.RNA</b>
Number of sequences	95270	98173	93122	96621	98821	101284	108667	118733	103052	80188	90359	82138
Observed PTs <sup>b</sup> (normalized) <sup>c</sup>	5527 (3478)	5660 (3572)	5444 (3404)	5886 (3463)	5898 (3517)	6004 (3504)	6008 (3572)	5780 (3392)	5578 (3353)	5175 (2910)	4452 (2522)	4689 (2720)
Chao1 (normalized) <sup>c</sup>	7925 (5967)	8092 (5999)	7908 (5797)	9270 (6431)	9151 (6383)	9723 (6339)	9382 (6094)	9177 (5734)	8510 (5744)	8668 (6121)	7092 (4988)	7542 (5481)
Shannon (normalized) <sup>c</sup>	9.3 (9.2)	9.3 (9.2)	9.3 (9.2)	8.0 (7.9)	8.3 (8.2)	8.3 (8.2)	8.4 (8.3)	7.6 (7.5)	8.2 (8.1)	8.0 (7.9)	6.6 (6.5)	7.6 (7.5)
<b>Sampling Time</b>	<b>30 h</b>											
<b>Treatment:</b>	<b>C1.RNA</b>	<b>C2.RNA</b>	<b>C3.RNA</b>	<b>P1.RNA</b>	<b>P2.RNA</b>	<b>P3.RNA</b>	<b>R1.RNA</b>	<b>R2.RNA</b>	<b>R3.RNA</b>	<b>C.DNA</b>	<b>P.DNA</b>	<b>R.DNA</b>
Number of sequences	84481	83599	35563	114393	80012	78166	73608	69987	48891	59211	61820	63663
Observed PTs <sup>b</sup> (normalized) <sup>c</sup>	5077 (2866)	5011 (2855)	3247 (1788)	4944 (2769)	4082 (2237)	4033 (2241)	4299 (2421)	4329 (2355)	3544 (1911)	4274 (2583)	3558 (1997)	3465 (1909)
Chao1 (normalized) <sup>c</sup>	8266 (5806)	8117 (5742)	5378 (5302)	8014 (5075)	6927 (4930)	6730 (4713)	7128 (5208)	7330 (5539)	6063 (5242)	6557 (5439)	5838 (4707)	5739 (4589)
Shannon (normalized) <sup>c</sup>	7.7 (7.6)	7.9 (7.8)	7.9 (7.9)	6.7 (6.6)	6.7 (6.6)	6.7 (6.6)	7.5 (7.4)	7.4 (7.3)	7.4 (7.3)	9.3 (9.3)	7.1 (7.0)	6.2 (6.2)

<sup>a</sup>C, P, and R corresponds to unsupplemented control, protein, and RNA treatments, respectively. 16S rRNA gene or 16S rRNA samples of the three replicates were always pooled except for 16S rRNA samples at 30 h. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. DNA, 16S rRNA genes; RNA, 16S rRNA. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Phylotypes (PTs) were clustered based on a sequence similarity cut-off of 97%.

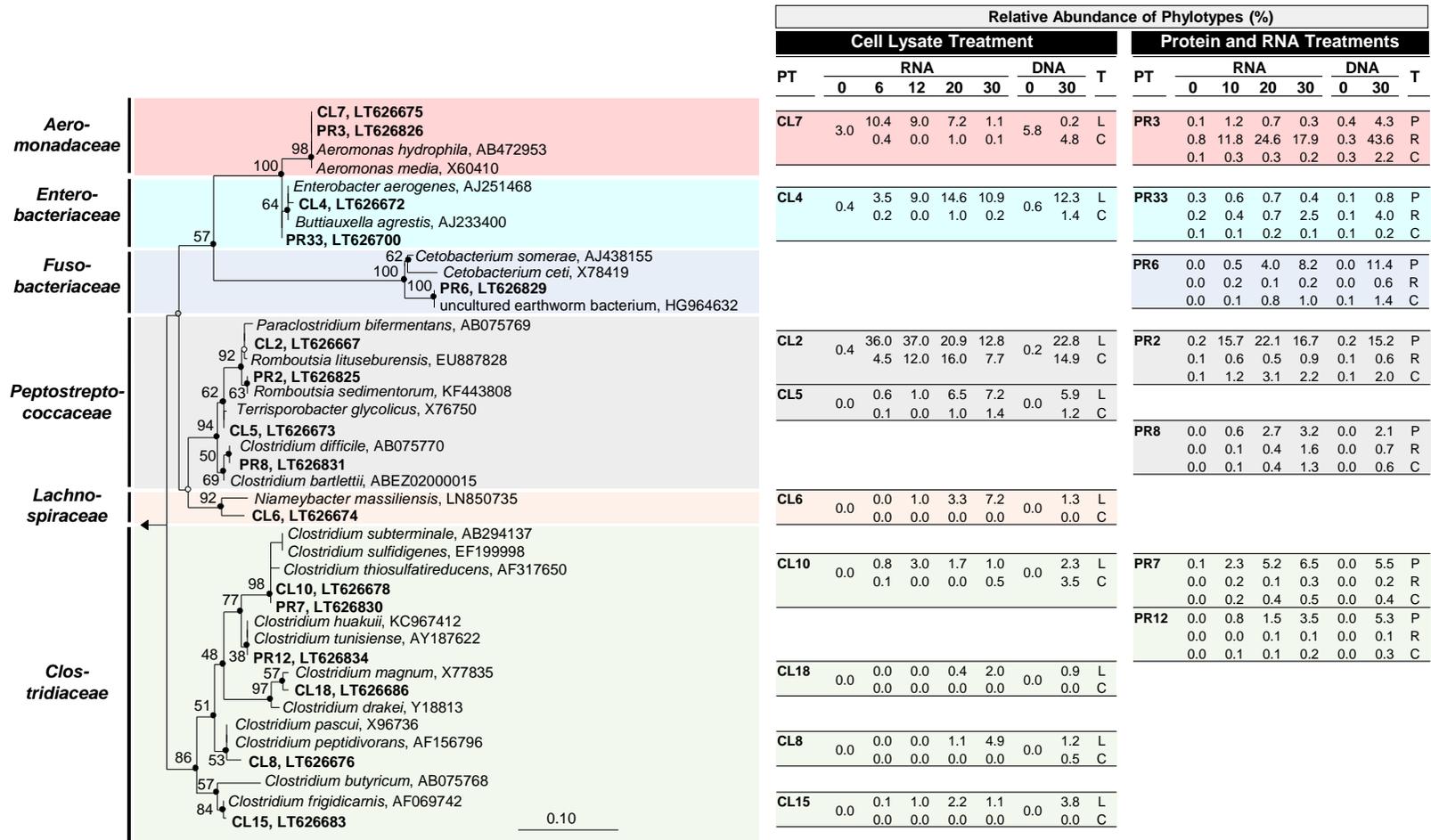
<sup>c</sup>The data sets were normalized to 33,658 sequences for comparison of amplicon libraries of different sizes.



**Figure 42.** Average relative abundances of 16S rRNA sequences of the most responsive families of lysate, protein, and RNA treatments. Families were considered to be responsive when a family in a given treatment displayed a minimum increase in relative abundance of 5% above control values in at least one of the sampling periods. The values for each family are based on the arithmetic average of all abundances detected at 6, 12, 20 and 30 h for the yeast lysate treatment and at 10, 20, and 30 h for the protein or RNA treatments. Figure modified and used with permission from Zeibich *et al.*, 2018.

### 3.2.5. Lysate-, protein-, and RNA-responsive phylotypes

The contrasting trends of stimulated fermentative families in lysate, protein, or RNA treatments extended to several phylotypes (Figure 43). For example, the phylotypes PR2, PR6, PR7, and PR12 displayed the strongest response to protein and the relative 16S rRNA abundances of these four phylotypes were significantly higher in protein treatments than in the RNA treatments at the end of the incubation (Table 37). Of these four phylotypes, phylotype PR2 (closely related to *Romboutsia lituseburensis*), was most responsive at both transcript- and gene-levels (Figure 43). Phylotype PR6 (related to *C. somerae*) responded late in the protein treatment (Figure 43). Phylotypes PR7, PR8, and PR12 (closely related to *Clostridium thiosulfatireducens*, *Clostridium difficile*, and *Clostridium tunisiense*, respectively) displayed a statistically significant response to protein, whereas phylotype PR3 (closely related to *Aeromonas media* and *A. hydrophila*) was the dominant phylotype that responded significantly to RNA (Figure 43 and Table 37). The phylotype CL5 (closely related to *Terrisporobacter glycolicus*) and phylotype CL18 (closely related to *Clostridium magnum*) responded only to the lysate treatment. Phylotype CL2 (closely related to *P. bifermentans*) responded rapidly to yeast lysate during the first 6 h of incubation but subsequently decreased in relative abundance, whereas phylotypes CL8 (closely related to *Clostridium peptidivorans*) and CL6 (closely related to the *Niameybacter massiliensis*) had a more sustained response to yeast lysate, with maximum relative abundances of 16S rRNA at the end of the 30 hour incubation (Figure 43).



**Figure 43.** 16S rRNA-based phylogenetic tree of responsive phylotypes and affiliated reference sequences. Phylotypes (PT) are based on a sequence similarity cut-off of 97% and were designated responsive when a phylotype in a given treatment displayed a minimum increase in relative abundance of 2% above control values in at least one of the sampling periods. The phylotypes are derived from the analysis of either 16S rRNA (RNA) and 16S rRNA genes (DNA). The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees. Empty and grey circles at nodes, congruent nodes in two trees (neighbor-joining congruent with maximum parsimony or maximum parsimony congruent with maximum likelihood). Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch. Relative abundances (in %) of phylotypes in the table are shown for each sampling period (i.e., 0, 6, 12, 20 and 30 h for the yeast lysate treatment, and 0, 10, 20, and 30 h for the protein or RNA treatments). Closely related phylotypes (i.e., >97% sequence similarity) that increased in the yeast lysate (L) treatment and protein (P) or RNA (R) treatments were placed on the same horizontal level. C, unsupplemented control, T, treatment. Figure modified and used with permission from Zeibich *et al.*, 2018.

**Table 37.** Statistical analyses of main stimulated phylotypes displayed in Figure 43.<sup>a</sup>**(A) Lysate**

Phylotype	Treatment	Mean	Standard Deviation	Median	LDA Score (log <sub>10</sub> ) <sup>b</sup>
CL4	Control	0.1	0.0	0.1	4.9 <sup>(2)</sup>
	Lysate	6.7	1.0	6.2	
CL5	Control	1.1	0.1	1.1	4.9 <sup>(3)</sup>
	Lysate	6.5	4.3	6.6	
CL6	Control	0.0	0.0	0.0	5.0 <sup>(1)</sup>
	Lysate	7.2	2.1	7.4	
CL7	Control	0.1	0.0	0.1	4.0 <sup>(10)</sup>
	Lysate	0.8	0.1	0.8	
CL8	Control	0.0	0.0	0.0	4.8 <sup>(4)</sup>
	Lysate	4.9	0.7	4.8	
CL10	Control	0.4	0.1	0.3	4.1 <sup>(8)</sup>
	Lysate	0.9	0.1	0.9	
CL15	Control	0.0	0.0	0.0	4.2 <sup>(7)</sup>
	Lysate	1.1	0.2	1.2	
CL18	Control	0.0	0.0	0.0	4.4 <sup>(6)</sup>
	Lysate	2.0	1.1	1.6	

**(B) Protein and RNA**

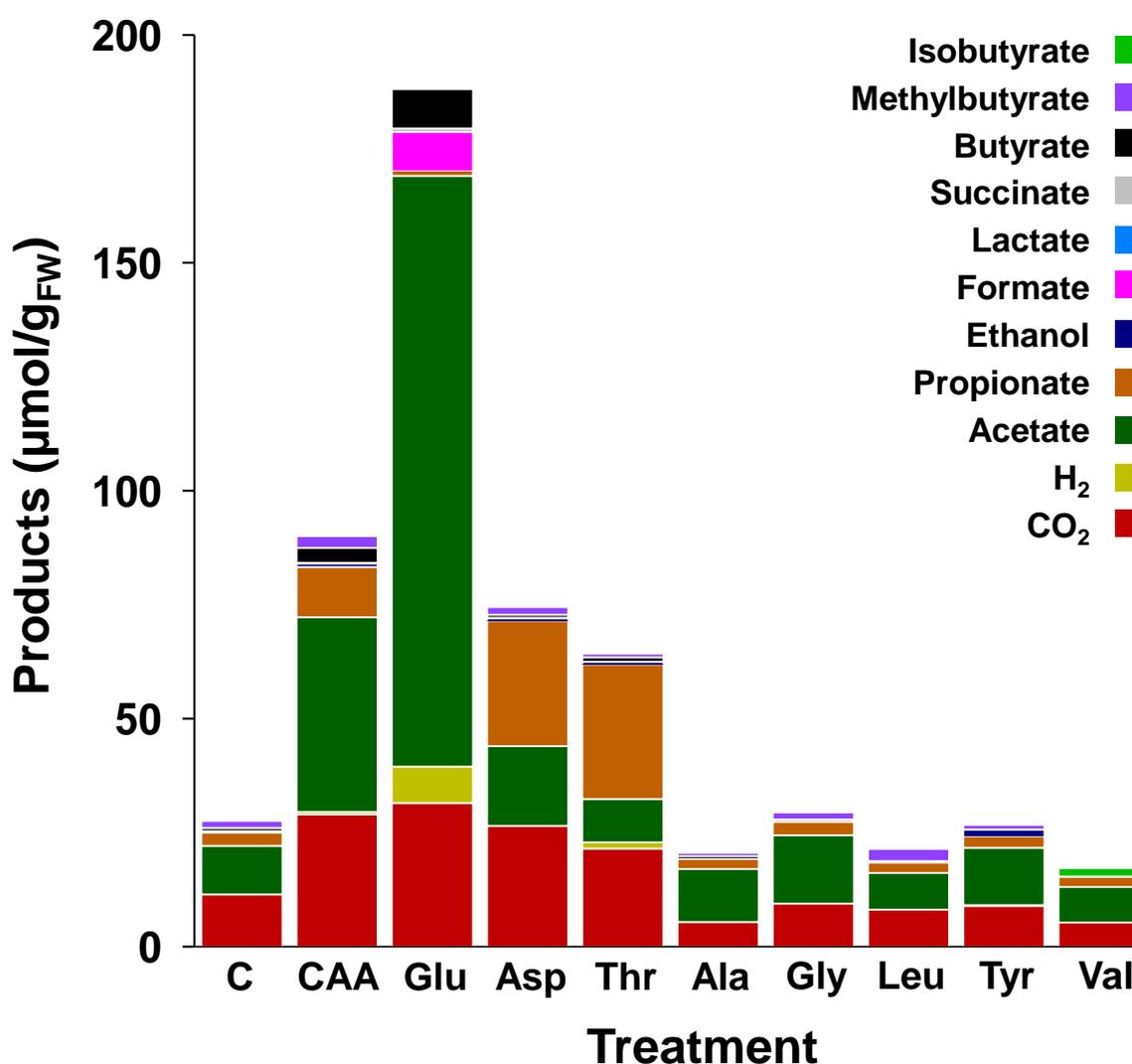
Phylotype	Treatment	Mean	Standard Deviation	Median	LDA Score (log <sub>10</sub> ) <sup>b</sup>
PR2	Protein	17	1.9	17	5.3 <sup>(1)</sup>
	RNA	0.9	0.1	0.9	
PR6	Protein	8.2	2.1	7.9	5.0 <sup>(2)</sup>
	RNA	0.2	0.0	0.2	
PR7	Protein	6.5	0.7	6.3	4.9 <sup>(3)</sup>
	RNA	0.3	0.1	0.3	
PR8	Protein	3.2	0.5	3.1	4.7 <sup>(4)</sup>
	RNA	1.6	0.4	1.4	
PR12	Protein	3.5	0.2	3.4	4.6 <sup>(5)</sup>
	RNA	0.1	0.0	0.1	
PR3	Protein	0.3	0.0	0.3	5.3 <sup>(2)</sup>
	RNA	15	1.9	14	
PR33	Protein	0.1	0.0	0.1	4.2 <sup>(6)</sup>
	RNA	1.2	0.1	1.2	

<sup>a</sup>Only phylotypes that were significantly stimulated (based on LEfSe analyses) by a given supplement are shown. The LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking phylotypes exhibited a stronger response to supplement compared to lower ranking ones).

### 3.2.6. Amino acid-derived fermentation in gut content of *L. terrestris*

The fermentation of protein in the gut is dependent on diverse proteases that hydrolyze peptide bonds, yielding monomeric amino acids (Section 1.3.2 and Section 1.4.2), and the marked stimulation of gut fermentations by protein warranted a more detailed analysis. In a preliminary study, eight fermentable amino acids (Buckel, 1999) were evaluated for their potential to stimulate gut content fermentation (Figure 44). In this regard, only glutamate, aspartate, and threonine yielded an obvious stimulation of fermentation (Figure 44 and Table 38). Based on an equal amount of available amino acids, casamino acids (a mixture of amino acids) also enhanced the gut content fermentation but was less stimulatory than glutamate.



**Figure 44.** Collective amounts of fermentation products in amino acid treatments of the preliminary study. Initial amino acid concentrations approximated 10 mM. Control lacked supplemental amino acids. Values are the average of duplicate analyses shown in Table 38 and represent the net amounts of products at the end of the 30 h incubation. Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Leu, leucine; Tyr, tyrosine; Val, valine; FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019b.

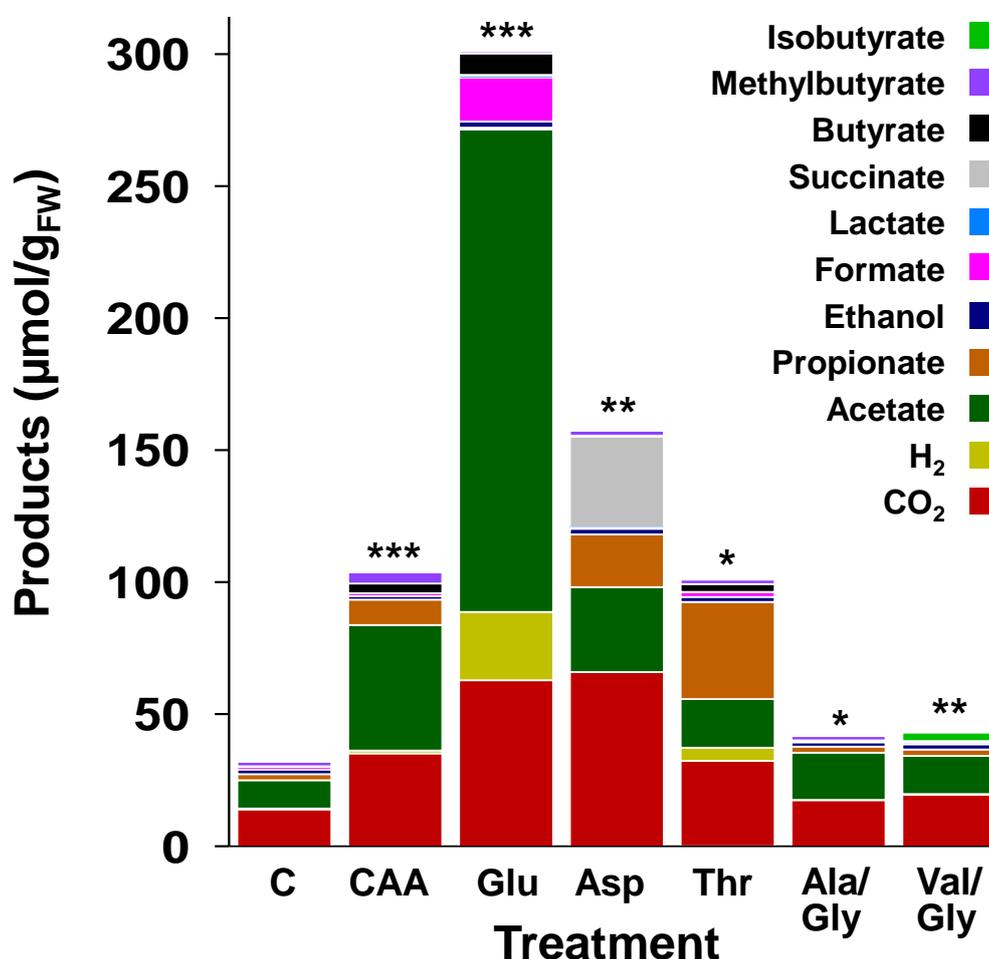
**Table 38.** Effect of amino acids on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

Treatment	Time (h)	Products ( $\mu\text{mol/g}_{\text{FW}}$ )											pH
		CO <sub>2</sub>	H <sub>2</sub>	Acetate	Succinate	Propionate	Formate	Lactate	Ethanol	Butyrate	Iso-butyrate	Methyl-butyrate	
Control	0	0.0/0.0	0.0/0.0	6.1/6.6	1.4/1.5	0.5/0.6	2.4/2.4	0.8/0.8	1.2/1.1	0.7/0.6	0.0/0.1	0.0/0.0	7.0/7.0
	30	13/9.9	0.0/0.0	17/17	0.0/0.0	3.5/3.4	0.6/0.6	0.5/0.5	1.5/1.5	1.4/1.3	0.1/0.1	1.4/1.7	7.1/7.1
Casamino Acids	0	0.0/0.0	0.0/0.0	9.3/9.3	2.0/1.9	0.0/0.0	2.9/3.1	1.2/1.2	0.7/0.7	0.6/0.6	0.1/0.1	0.0/0.0	7.0/7.0
	30	28/30	0.5/0.6	52/52	1.3/1.1	11/11	0.4/0.3	1.3/1.4	1.5/1.5	3.8/3.9	0.3/0.3	2.6/2.5	7.0/7.0
Alanine	0	0.0/0.0	0.0/0.0	5.7/6.0	1.3/1.3	0.6/0.6	2.5/2.7	1.0/0.8	1.0/1.1	0.7/0.7	0.0/0.1	0.0/0.0	7.1/7.1
	30	5.4/5.4	0.0/0.0	18/17	0.4/0.5	3.0/2.6	1.1/0.5	0.6/0.5	1.0/1.0	1.3/1.3	0.1/0.1	0.7/0.8	7.1/7.1
Aspartate	0	0.0/0.0	0.0/0.0	5.4/5.7	1.3/1.4	0.5/0.5	2.5/4.6	0.9/0.9	1.1/1.0	0.6/0.6	0.1/0.0	0.0/0.0	6.8/6.8
	30	25/28	0.0/0.0	22/24	1.5/1.4	28/28	0.2/0.2	0.9/0.9	1.7/1.6	1.2/1.3	0.3/0.3	1.7/1.7	6.9/6.9
Glutamate	0	0.0/0.0	0.0/0.0	5.5/5.3	1.2/1.1	0.5/0.5	2.6/2.5	0.9/0.9	0.8/0.8	0.6/0.7	0.1/0.1	0.0/0.0	6.7/6.7
	30	25/38	5.0/11	130/140	1.9/2.0	1.6/1.7	11/11	0.7/0.5	1.8/1.5	9.0/9.6	0.0/0.0	0.3/0.4	6.6/6.6
Glycine	0	0.0/0.0	0.0/0.0	5.7/5.3	1.2/1.2	0.5/0.5	2.3/2.3	0.9/0.9	0.9/0.9	0.6/0.6	0.0/0.1	0.0/0.0	6.7/6.7
	30	7.9/11	0.0/0.0	21/20	0.2/0.2	3.4/3.4	0.8/0.2	0.5/0.5	0.9/1.1	1.1/1.0	0.1/0.1	1.6/1.6	6.7/6.7
Leucine	0	0.0/0.0	0.0/0.0	5.8/8.1	1.3/1.9	0.5/0.6	1.7/2.4	0.8/1.1	0.9/1.3	0.6/0.9	0.1/0.1	0.0/0.0	7.1/7.0
	30	7.6/8.6	0.0/0.0	15/15	0.2/0.2	2.8/2.8	0.8/0.9	0.5/0.5	1.3/1.2	1.0/1.0	0.1/0.1	2.6/2.6	7.1/7.1
Threonine	0	0.0/0.0	0.0/0.0	8.0/9.0	1.8/2.0	0.8/1.1	3.0/3.0	1.0/1.0	0.9/0.9	0.9/1.0	0.1/0.0	0.0/0.0	7.0/7.1
	30	26/17	1.2/1.5	18/18	1.1/1.3	32/29	4.1/1.1	1.0/1.0	1.5/1.5	1.9/1.9	0.3/0.2	0.9/0.8	7.0/7.0
Tyrosine	0	0.0/0.0	0.0/0.0	6.5/6.3	1.6/1.5	0.6/0.5	2.6/2.7	1.0/0.9	1.0/1.0	0.9/0.9	0.1/0.1	0.0/0.0	7.1/7.1
	30	9.0/8.8	0.2/0.2	19/19	0.0/0.0	3.0/3.1	0.3/0.3	0.5/0.5	2.3/2.6	0.9/1.0	0.1/0.0	1.0/1.0	7.1/7.1
Valine	0	0.0/0.0	0.0/0.0	7.9/7.6	1.8/1.6	0.6/0.6	2.5/2.5	0.8/0.8	1.3/1.0	0.9/0.8	0.0/0.1	0.0/0.0	7.1/7.1
	30	7.1/3.5	0.0/0.0	16/15	0.0/0.0	2.9/2.9	0.3/0.3	0.5/0.5	0.8/0.9	0.9/0.8	1.9/1.8	0.0/0.1	7.1/7.1

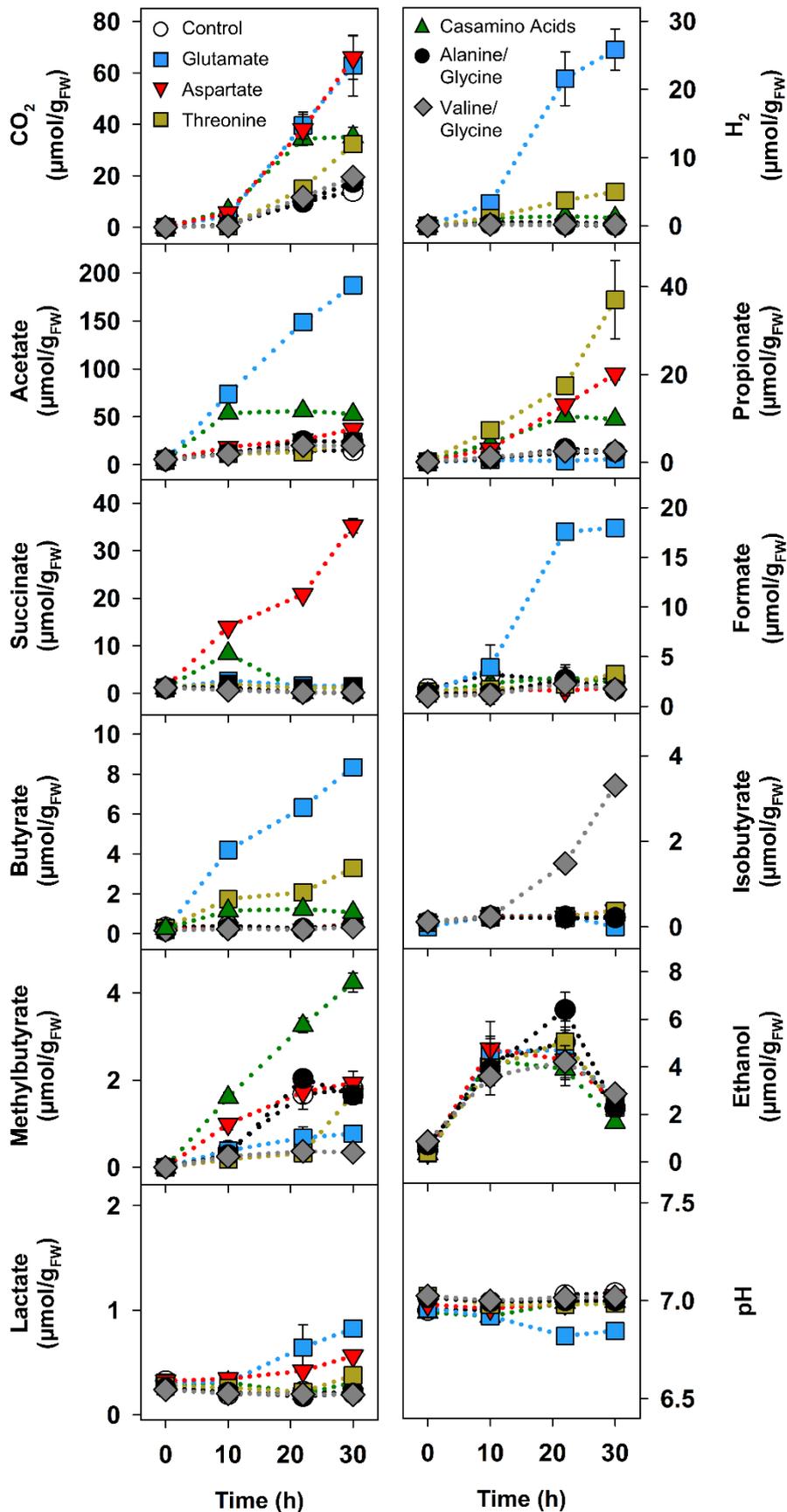
<sup>a</sup>The amount of amino acids per microcosm approximated 10 mM. Controls lacked supplemental amino acids. Amounts of products formed in the duplicates are separated by a slash. FW, fresh weight. Table modified and used with permission from Zeibich *et al.*, 2019b.

These preliminary findings demonstrated that the stimulation of fermentation was limited to specific amino acids. Therefore, only the stimulatory amino acids glutamate, aspartate, threonine, and casamino acids were selected for more detailed studies including taxa analysis. The potential for Stickland reactions (simultaneous fermentation of two amino acids; Section 1.4.2) in gut contents was assessed by supplementing glycine and either alanine or valine.

As in the preliminary experiment, the glutamate treatment displayed the strongest response (Figure 45) and the formation of diverse products without an apparent delay (Figure 46), illustrating the high capacity of a single amino acid to stimulate gut fermenters. Several pathways can be utilized for glutamate fermentation, and the associated fermenters produce acetate, CO<sub>2</sub>, H<sub>2</sub>, formate, and butyrate (Stams and Hansen, 1984; Buckel, 2001; Section 1.4.2), products that significantly increased in the glutamate treatment (Figure 45, Figure 46, and Table 39).



**Figure 45.** Collective amounts of fermentation products in amino acid-supplemented anoxic microcosms of *L. terrestris* gut contents. Initial concentrations approximated 10 mM for casamino acids, glutamate, aspartate, threonine, and glycine, and 5 mM for alanine and valine. Control lacked supplemental amino acids. Values are the average of triplicate analyses in Figure 46 and represent the net amounts of products at the end of the 30 h incubation. The asterisks indicate significant differences between the collective amount of products formed in control and amino acid treatments (\*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; *t*-test with unequal variances; see Table 39 for *P* values, mean values, and variances). Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Val, valine; FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019b.



**Figure 46.** Effect of amino acids on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. Initial concentrations approximated 10 mM for casamino acids, glutamate, aspartate, threonine, and glycine, and 5 mM for alanine and valine. Control lacked supplemental amino acids. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019b.

**Table 39.** *P* values of fermentation products in amino acid treatments.<sup>a</sup>

Products		CO <sub>2</sub>					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	14	35	63	66	32	17	20
Variance	4.2	14	142	70	6.6	9.0	5.5
<i>P</i> value		0.003	0.02	0.009	0.001	0.168	0.034
Products		H <sub>2</sub>					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	0.4	1.1	26	0.0	0	-1.1	-1.0
Variance	0.0	0.0	9.3	0.0	0.2	0.0	0.0
<i>P</i> value		0.023	0.005	0.000	0.264	0.000	0.002
Products		Acetate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	11	48	183	32	18	18	14
Variance	0.0	2.4	2.5	1.7	27	1.3	0.4
<i>P</i> value		0.001	0.000	0.001	0.127	0.009	0.010
Products		Succinate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	-0.9	-0.9	0.5	34	0.0	-1.1	-1.0
Variance	0.0	0.0	0.0	2.1	0.2	0.0	0.0
<i>P</i> value		0.650	0.004	0.001	0.095	0.002	0.130
Products		Formate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	1.1	1.1	17	0.7	1.8	0.4	0.7
Variance	1.0	0.2	0.3	0.8	0.8	0.2	0.1
<i>P</i> value		0.999	0.000	0.640	0.397	0.388	0.596
Products		Propionate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	2.3	9.6	0.6	20	37	2.3	2.4
Variance	0.0	0.1	0.0	1.7	78	0.1	0.0
<i>P</i> value		0.000	0.000	0.002	0.021	0.760	0.186
Products		Butyrate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	0.1	3.7	8.2	0.3	3.0	0.3	0.2
Variance	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>P</i> value		0.001	0.000	0.237	0.000	0.234	0.902
Products		Methylbutyrate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	1.8	4.2	0.8	1.9	1.7	1.7	0.3
Variance	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>P</i> value		0.000	0.000	0.493	0.385	0.165	0.000
Products		Isobutyrate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	0.1	0.2	0.0	0.3	0.3	0.1	3.2
Variance	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>P</i> value		0.067	0.057	0.078	0.036	0.549	0.000

Products		Lactate					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	-0.1	0.0	0.5	0.2	0.1	0.0	0.0
Variance	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P value		0.040	0.000	0.025	0.133	0.052	0.023
Products		Ethanol					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	1.8	1.3	2.3	2.2	1.8	1.6	2.0
Variance	0.3	0.0	0.0	0.1	0.0	0.0	0.0
P value		0.317	0.243	0.403	0.774	0.627	0.563
Products		Total					
Treatment	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Mean value <sup>b</sup>	31	103	301	158	101	41	42
Variance	2.9	9.6	219	55	146	13	3.4
P value		0.000	0.001	0.001	0.010	0.027	0.002

<sup>a</sup>P values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C) and casamino acid (CAA), glutamate (Glu), aspartate (Asp), threonine (Thr), alanine and glycine (Ala/Gly), and valine and glycine (Val/Gly) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation. See Figure 46 for product profile. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

90% and 92% of glutamate-derived carbon and reducing equivalents, respectively, were theoretically recovered in the detected fermentation products (Table 40). These recoveries and the formation of almost the same amount of ammonium compared to the supplemented glutamate (Table 41) illustrated that nearly all of the supplemented glutamate was utilized. In contrast to the glutamate treatment, the aspartate treatment yielded high amounts of propionate and succinate, whereas threonine significantly stimulated the production of propionate and CO<sub>2</sub> (Figure 45, Figure 46, and Table 39). Propionate is also one of the main product of threonine fermentation in the human gut (Smith and Macfarlane, 1997). The comparative amounts of detected ammonium at the end of the incubation and the theoretical recoveries of carbon and reducing equivalents (Table 40 and Table 41) demonstrated (a) that the amount of supplemented amino acid was adequate for the detected products, and (b) that the fermentative gut microbiota is more efficient to utilize glutamate than aspartate or threonine. The increased amounts of ammonium in the amino acid treatments (Table 41) suggests that detected *in situ* amounts of ammonium in the alimentary canal and cast of earthworms (Parle, 1963a; Drake and Horn, 2007) might at least be partially caused by the deamination and fermentation of amino acids in the gut.

The co-amino acid treatments (alanine plus glycine or valine plus glycine) displayed only a marginally stimulation of fermentations (Figure 45 and Figure 46). Furthermore, only 5 to 6% of the amino acids-derived carbon and reducing equivalent were recovered in the detected fermentation products (Table 40). However, the collective amounts of fermentation products formed in the co-amino acid treatments were significantly greater than in the unsupplemented

**Table 40.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in amino acid treatments.<sup>a</sup>

Main Products	Recoveries (%)											
	Casamino Acids		Glutamate		Aspartate		Threonine		Alanine/ Glycine		Valine/ Glycine	
	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE
CO <sub>2</sub>	4.0	na	10	na	13	na	4.6	na	1.0	na	1.3	na
H <sub>2</sub>	na	0.1	na	2.8	na	-	na	0.6	na	-	na	-
Acetate	14	13	69	76	11	14	3.8	3.8	4.1	5.5	1.7	1.5
Ethanol	-	-	0.2	0.4	0.2	0.4	0.1	0.1	-	-	0.1	0.1
Lactate	0.1	0.1	0.4	0.4	0.3	0.4	0.2	0.2	0.1	0.1	0.1	0.0
Succinate	-	-	1.1	1.0	35	41	0.8	0.7	-	-	-	-
Formate	-	-	3.1	1.7	-	-	0.2	0.1	-	-	-	-
Butyrate	2.7	3.1	6.4	8.8	0.2	0.3	2.9	3.6	0.2	0.3	-	-
Propionate	4.2	4.7	-	-	13	21	26	30	0.0	0.1	0.1	0.1
Isobutyrate	0.1	0.1	-	-	0.1	0.2	0.1	0.2	-	-	2.7	3.1
Methylbutyrate	2.3	2.9	-	-	0.2	0.3	-	-	-	-	-	-
Total	28	25	90	92	73	77	39	39	5.4	6.0	5.9	5.0

<sup>a</sup>See Figure 46 for product profiles. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. RE, reducing equivalents; -, no net increase of the product during the incubation in supplemented treatments relative to the control treatments; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019b.

control, suggesting that gut content microbes had at least a low potential for conducting Stickland reactions (Figure 45, Figure 46, and Table 39). Furthermore, isobutyrate was significantly produced in valine/glycine treatments but was only detected at trace levels in all other amino acid and control treatments (Figure 45 and Figure 46), a finding confirmed by the production of isobutyrate when valine is utilized (McInerney, 1988). Treatments supplemented with casamino acids yielded CO<sub>2</sub>, acetate, propionate, and methylbutyrate as main fermentation products (Figure 45 and Figure 46). Except of the leucine treatment in the preliminary experiment, methylbutyrate was much less produced in all other amino acid treatment (Table 38, Figure 45, and Figure 46), suggesting that the fermentation of leucine may have been at least partially contributed to the production of these product in the casamino acid treatments (Table 38, Figure 45, and Figure 46).

73% and 77% of aspartate-derived carbon and reducing equivalents, respectively, were theoretically recovered in the detected fermentation products, and the theoretically recoveries of threonine-derived carbon and reducing equivalents in the detected products approximated 39% and 39%, respectively (Table 40). In comparison, only 28% and 25% of casamino acid-derived carbon and reducing equivalents, respectively, were recovered in the detected fermentation products (Table 40). The collective findings indicated the fermenters of gut content were not capable of fermenting all amino acids equally, a trend consistent with certain amino acids being less easily fermented by the microbial community of the human colon (Smith and Macfarlane, 1997). However, the enhanced formation of fermentation products in certain treatments (Figure 45 and Figure 46) illustrated that gut fermenters were poised to respond to specific amino acids.

**Table 41.** Production of ammonium in amino acid-supplemented anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

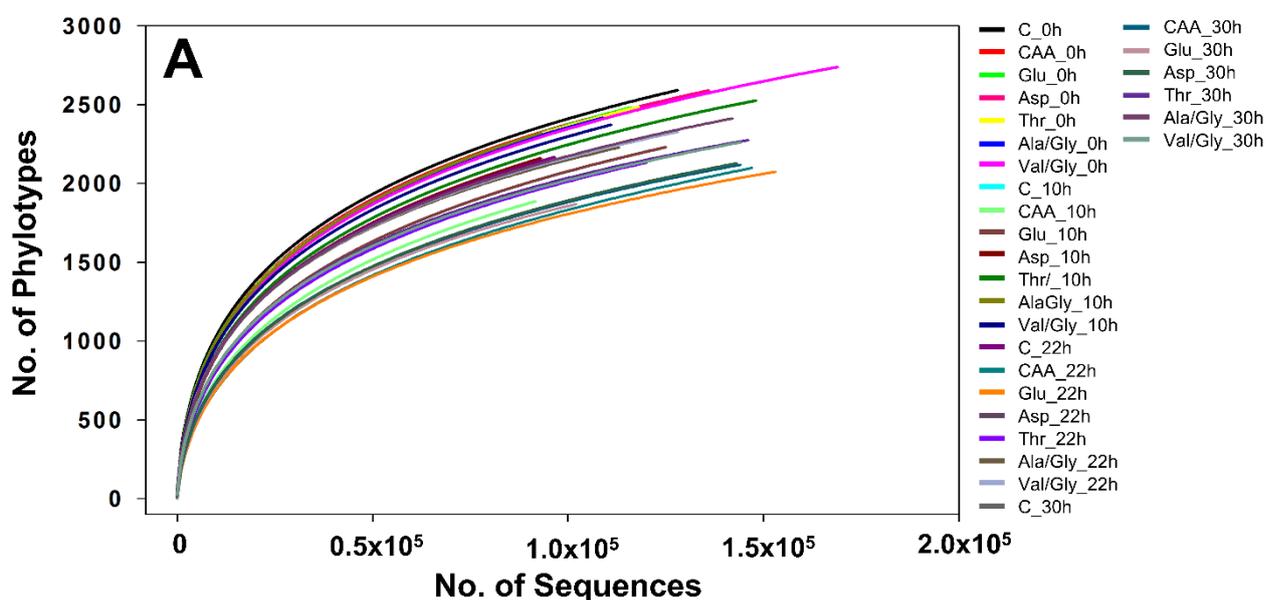
Treatment	Time (h)	NH <sub>4</sub> <sup>+</sup> (mM)
<b>Control</b>	0	0.6 ± 0.5
	30	0.0 ± 0.0
<b>Casamino Acids</b>	0	0.8 ± 0.3
	30	4.7 ± 0.6
<b>Glutamate</b>	0	0.9 ± 0.1
	30	9.5 ± 1.0
<b>Aspartate</b>	0	1.0 ± 0.0
	30	7.8 ± 0.7
<b>Threonine</b>	0	0.6 ± 0.2
	30	2.7 ± 0.2
<b>Alanine/Glycine</b>	0	0.6 ± 0.0
	30	1.3 ± 0.3
<b>Valine/Glycine</b>	0	0.4 ± 0.1
	30	1.2 ± 0.3

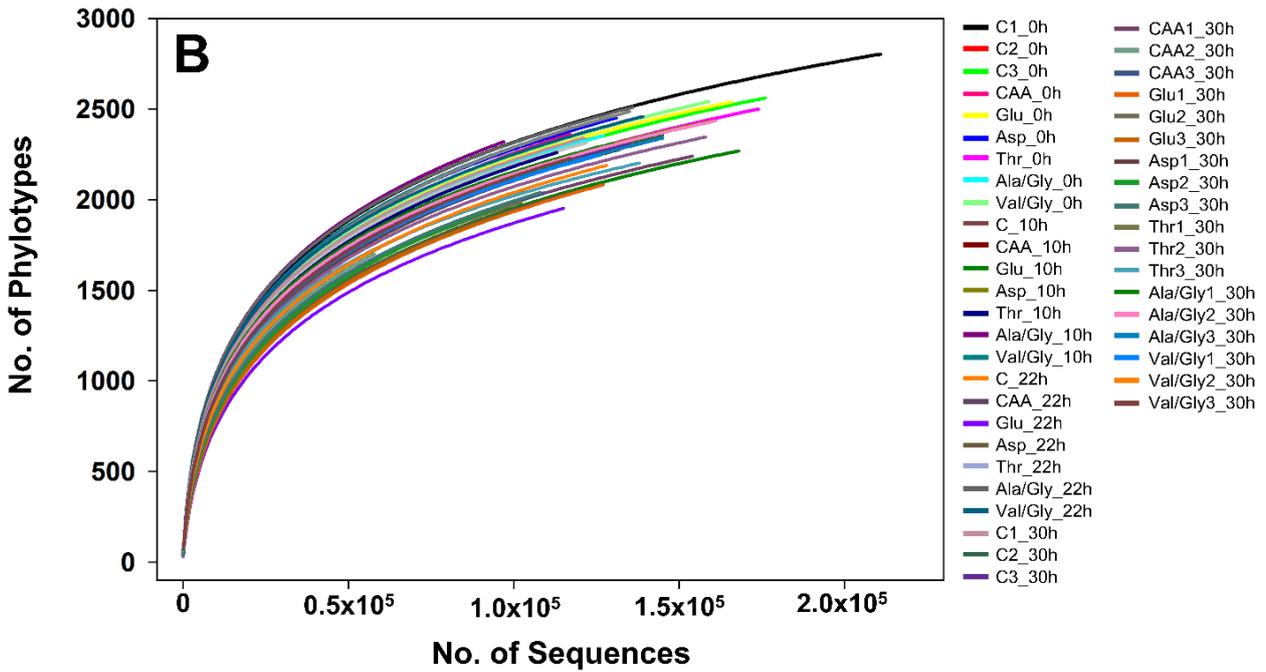
<sup>a</sup>The amount of amino acids per microcosm approximated 10 mM. Controls lacked supplemental amino acids. Values are the arithmetic average of three replicate analyses (± standard deviation). See Figure 46 for product profile. Table modified and used with permission from Zeibich *et al.*, 2019b.

### 3.2.7. Effect of amino acids on gut fermentative bacterial families

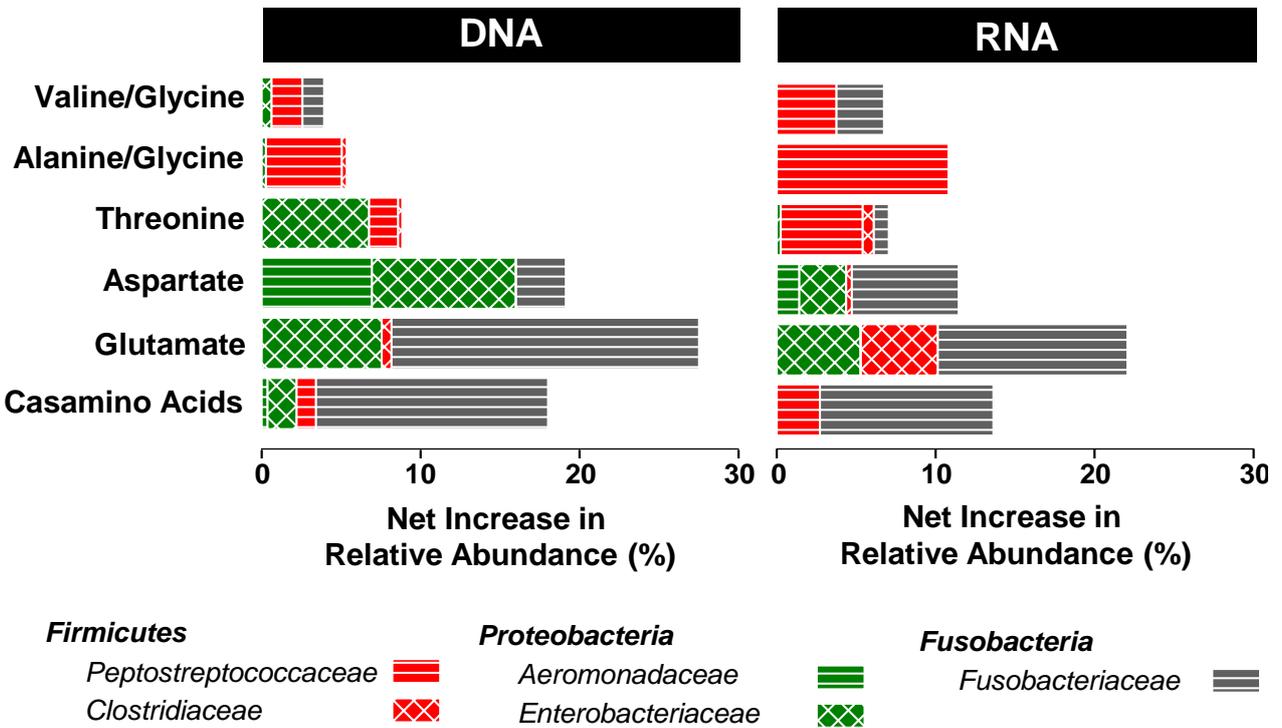
A total of 9,169,869 bacterial 16S rRNA gene and 16S rRNA sequences, associated to 32 phyla (including candidate phyla), were obtained from the amino acid treatments, and the rarefaction analyses indicated that the most abundant taxa were targeted (Figure 47). Based on the net increases of the relative 16S rRNA gene and 16S rRNA sequence abundances, *Fusobacteriaceae* were mostly stimulated by glutamate, aspartate, and casamino acids. Furthermore, *Aeromonadaceae* were stimulated in aspartate treatment, and the relative abundance of *Peptostreptococcaceae*-affiliated sequences increased mainly in casamino acid, threonine, and co-amino acid treatments. Apart from that, *Clostridiaceae* and *Enterobacteriaceae* displayed a significant increase in glutamate treatments compared to the unsupplemented control (Figure 48, Figure 49, and Table 42). *Enterobacteriaceae* was also significantly stimulated by aspartate and threonine (Figure 48, Figure 49, and Table 42). Both co-amino acid treatments displayed a significant stimulation of *Lachnospiraceae* compared to the unsupplemented control, suggesting this family as responsible for the marginal enhanced Stickland reaction (Table 42).

Consistent with the strong stimulation of *Enterobacteriaceae*, *Clostridiaceae*, and *Fusobacteriaceae* in the glutamate treatment (Figure 48), the number of detected phylotypes, the number of expected phylotypes (Chao1), and Shannon indices of this treatment were lower than those of unsupplemented controls (Figure 49). The apparent shift in the fermentative community during the incubation was (a) corroborated by the NMDS analysis (Section 2.6.2.2) of all the detected phylotypes (Figure 50), and (b) more obvious in amino acid than in unsupplemented control treatments. The similarity of the bacterial community composition in the amino acid treatments at the beginning of incubation (Figure 49 A) and in the triplicate analysis at the end of incubation (Figure 49 B) demonstrate the reproducibility of the phylogenetic analyses that is further reinforced by the groupings in the NMDS plot (Figure 50).





**Figure 47.** Rarefaction analyses of bacterial 16S rRNA genes (A) and 16S rRNA (B) sequences obtained from anoxic *L. terrestris* gut content microcosms supplemented with amino acids. Phylotypes were based on a 97% sequence similarity cutoff. Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22 h or 30 h. Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Val, valine. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Figure modified and used with permission from Zeibich *et al.*, 2019b.



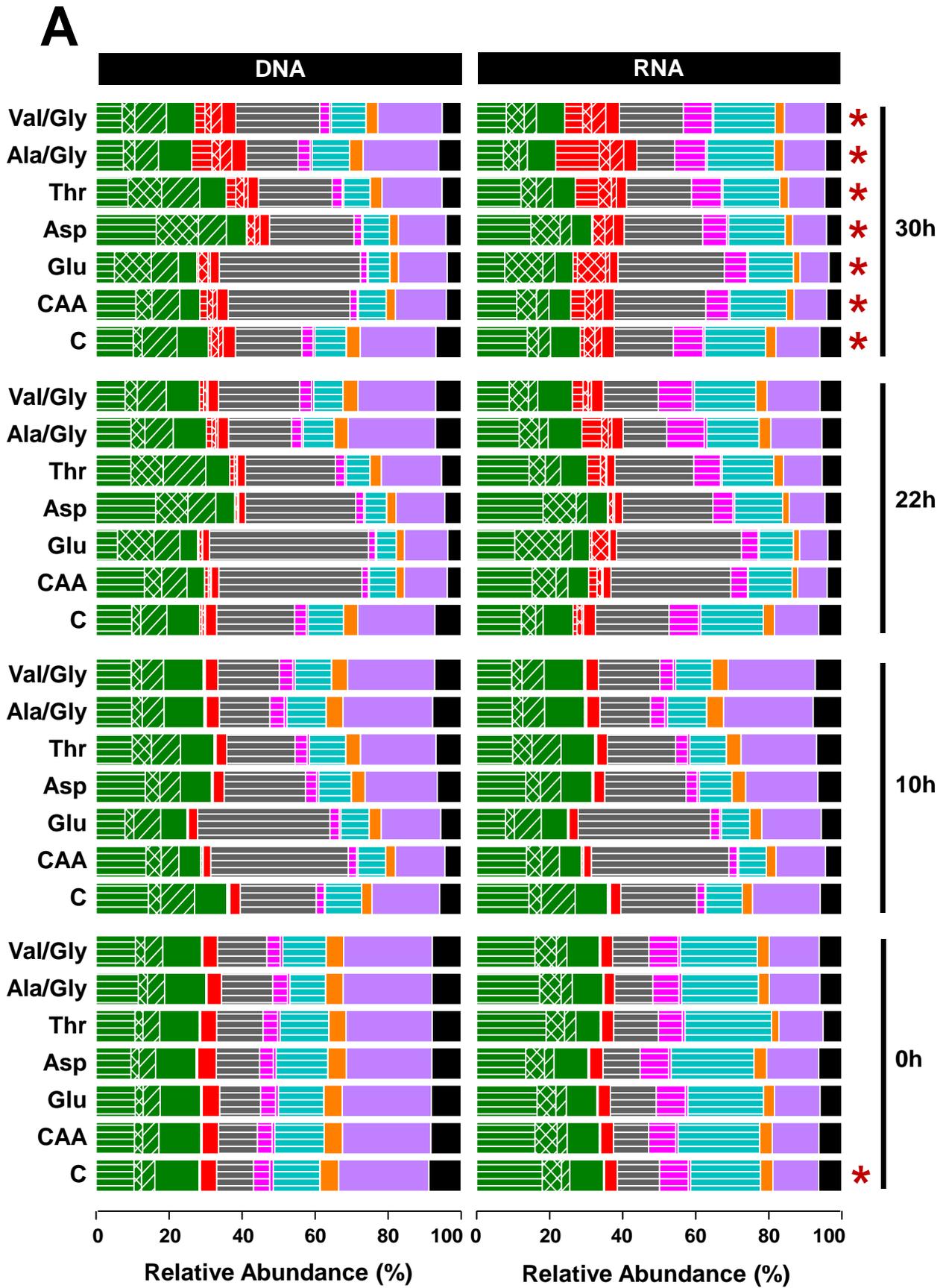
**Figure 48.** Net increases in 16S rRNA gene (DNA) and 16S rRNA (RNA) relative abundances of bacterial families stimulated by supplemental amino acids in *L. terrestris* gut content microcosms. The graph is limited to families that displayed a net increase in relative abundance of  $\geq 4\%$  in at least one treatment and the families are color-coded to the respective phyla (see Figure 49 for the complete 16S rRNA and 16S rRNA gene analyses). Net increases of relative abundances were calculated as follows: (a) the calculation is based either on mean relative abundances when samples from the three replicates were analyzed separately (i.e., all RNA and DNA samples of control treatments and RNA samples at 30 h of supplemented treatments) or on single relative abundances when samples of the three replicates were pooled for sequence analyses (i.e., DNA samples at 0 h and 30 h and RNA samples at 0 h of supplemented treatments); (b) mean or single relative abundances at the beginning of incubation were subtracted from those at the end of incubation for control and supplemented treatments; (c) the resulting time-corrected relative abundances of control treatments were subtracted from those of supplemented treatments (negative time-corrected relative abundances of control treatments were ignored). Figure modified and used with permission from Zeibich *et al.*, 2019b.

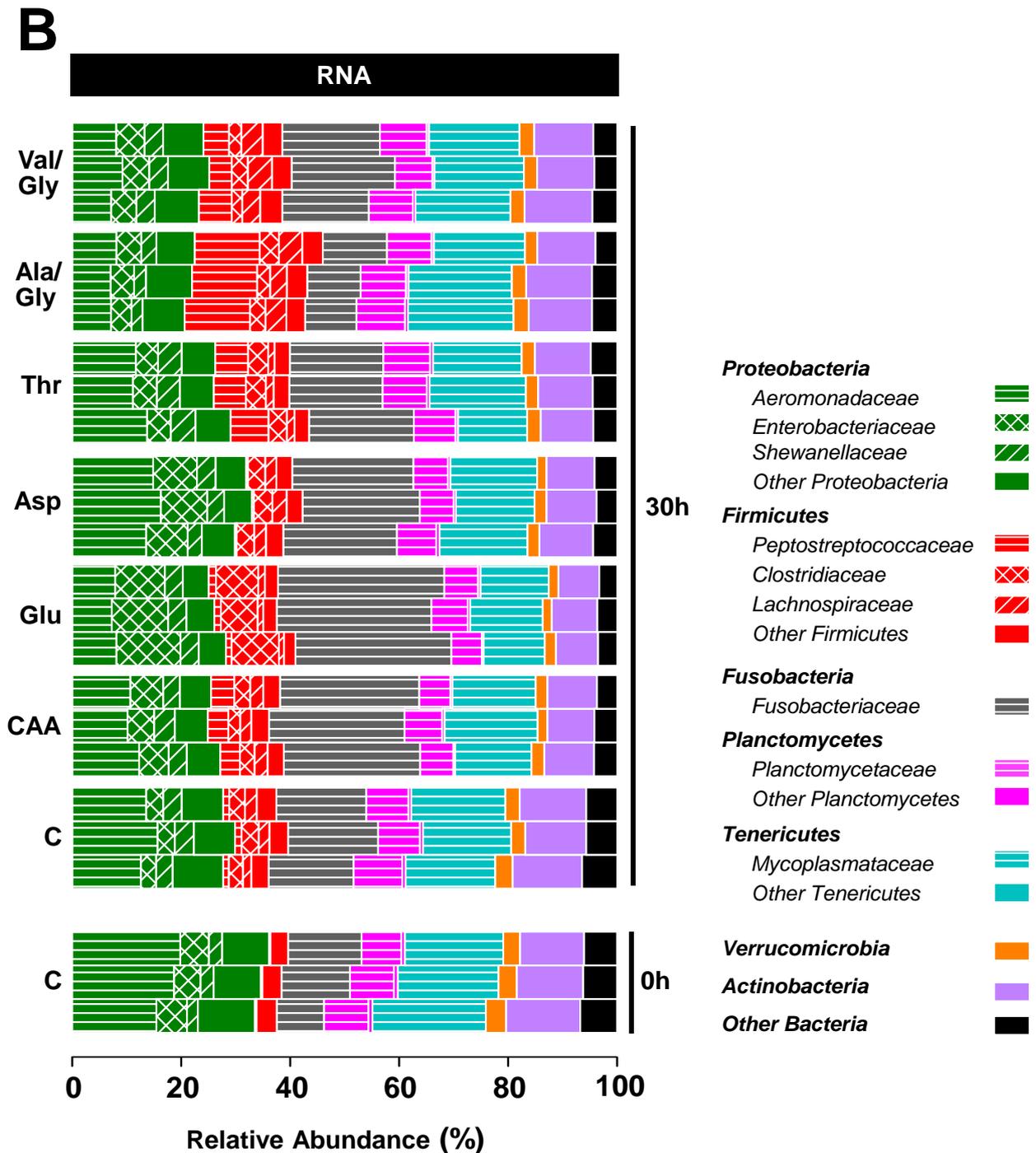
**Table 42.** Statistical analyses of stimulated families in amino acid treatments.<sup>a</sup>

Family	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
<b><i>Clostridiaceae</i></b>	Control	2.9	0.3	2.9	
	Glutamate	7.7	1.0	7.8	4.9 <sup>(3)</sup>
	Threonine	3.6	0.2	3.6	4.5 <sup>(3)</sup>
<b><i>Enterobacteriaceae</i></b>	Control	3.0	0.2	3.1	
	Casamino Acids	5.4	0.6	5.4	4.7 <sup>(2)</sup>
	Glutamate	10	1.3	10	5.0 <sup>(2)</sup>
	Aspartate	8.1	0.4	8.1	4.9 <sup>(2)</sup>
	Threonine	4.3	0.2	4.3	4.6 <sup>(4)</sup>
	Alanine/Glycine	4.2	0.4	4.3	4.6 <sup>(2)</sup>
	Valine/Glycine	4.9	0.3	4.9	4.7 <sup>(2)</sup>
<b><i>Fusobacteriaceae</i></b>	Control	16	0.5	16	
	Casamino Acids	25	0.3	25	5.4 <sup>(1)</sup>
	Glutamate	29	2.3	29	5.5 <sup>(1)</sup>
	Aspartate	22	0.7	22	5.3 <sup>(1)</sup>
	Threonine	18	1.2	17	5.3 <sup>(1)</sup>
<b><i>Lachnospiraceae</i></b>	Control	1.9	0.3	2.0	
	Alanine/Glycine	3.7	0.6	3.8	4.6 <sup>(3)</sup>
	Valine/Glycine	3.9	0.6	3.9	4.6 <sup>(3)</sup>
<b><i>Peptostreptococcaceae</i></b>	Control	1.1	0.1	1.1	
	Casamino Acids	3.9	0.3	3.8	4.6 <sup>(3)</sup>
	Threonine	6.3	0.6	6.0	4.8 <sup>(2)</sup>
	Alanine/Glycine	12	0.1	12	5.1 <sup>(1)</sup>
	Valine/Glycine	4.9	1.0	4.7	4.7 <sup>(1)</sup>
<b><i>Shewanellaceae</i></b>	Control	3.3	0.3	3.4	
	Threonine	4.4	0.1	4.3	4.6 <sup>(3)</sup>

<sup>a</sup>Families reaching a LDA score  $\geq 4.0$  were considered. LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).





**Figure 49.** 16S rRNA gene and 16S rRNA analyses of control and amino acid treatments. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Process data are shown in Figure 46, and information on all detected taxa is provided in Table A6. Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Val, valine. Panel A: Single bars without asterisk indicate that 16S rRNA gene (DNA) or 16S rRNA (RNA) samples of the three replicates were pooled for the sequence analysis. Asterisk indicates analysis was performed individually for the three replicates (see grouped bars Panel B). Figure modified and used with permission from Zeibich *et al.*, 2019b.

**Table 43.** Alpha diversity of the microbial community in control and amino acid treatments.<sup>a</sup>

Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
<b>DNA (0 h)</b>	Control	128322	2594 (477)	3418 (480)	5.0 (4.5)
	Casamino acids	108230	2431 (476)	3114 (479)	4.9 (4.4)
	Glutamate	116420	2485 (476)	3342 (481)	4.9 (4.4)
	Aspartate	136612	2594 (476)	3445 (484)	4.8 (4.3)
	Threonine	118672	2491 (475)	3290 (477)	4.8 (4.3)
	Alanine/Glycine	109665	2422 (475)	3202 (479)	4.8 (4.3)
	Valine/Glycine	169345	2741 (475)	3534 (479)	4.8 (4.3)
<b>DNA (10 h)</b>	Control <sup>d</sup>	6431	646 ( - )	1084 ( - )	4.1 ( - )
	Casamino acids	91618	1887 (465)	2652 (473)	3.5 (3.2)
	Glutamate	125262	2232 (471)	3011 (476)	3.8 (3.4)
	Aspartate	93662	2165 (475)	3036 (480)	4.3 (3.9)
	Threonine	148875	2531 (476)	3274 (479)	4.5 (4.0)
	Alanine/Glycine	93869	2328 (478)	3156 (484)	4.8 (4.3)
	Valine/Glycine	111828	2378 (477)	3185 (480)	4.7 (4.2)
<b>DNA (22 h)</b>	Control	96516	2167 (480)	2940 (484)	4.5 (4.0)
	Casamino acids	147534	2101 (472)	3155 (482)	3.3 (3.0)
	Glutamate	153193	2076 (474)	2897 (483)	3.3 (3.0)
	Aspartate	120118	2000 (475)	2723 (482)	3.6 (3.3)
	Threonine	120781	2137 (479)	2855 (483)	4.1 (3.7)
	Alanine/Glycine	113657	2233 (478)	2949 (481)	4.7 (4.2)
	Valine/Glycine	128504	2330 (480)	3050 (484)	4.5 (4.0)
<b>DNA (30 h)</b>	Control	119569	2294 (480)	3027 (483)	4.5 (4.1)
	Casamino acids	144816	2123 (473)	2870 (481)	3.7 (3.4)
	Glutamate	102791	1874 (470)	2484 (477)	3.5 (3.2)
	Aspartate	143408	2130 (473)	2894 (483)	3.9 (3.5)
	Threonine	146505	2278 (480)	2916 (483)	4.3 (3.9)
	Alanine/Glycine	142761	2417 (480)	3218 (483)	4.7 (4.3)
	Valine/Glycine	144847	2265 (479)	3006 (484)	4.3 (3.9)
<b>RNA (0 h)</b>	Control 1	211207	2803 (477)	3406 (482)	4.6 (4.0)
	Control 2	124938	2345 (476)	2969 (478)	4.4 (3.9)
	Control 3	176174	2561 (477)	3309 (480)	4.3 (3.8)
	Casamino acids	117131	2362 (476)	3143 (478)	4.4 (3.9)
	Glutamate	166463	2540 (477)	3164 (479)	4.4 (3.9)
	Aspartate	131510	2454 (477)	3232 (479)	4.5 (4.0)
	Threonine	174976	2503 (476)	3180 (479)	4.1 (3.7)
	Alanine/Glycine	127750	2356 (478)	3013 (484)	4.4 (3.9)
	Valine/Glycine	159830	2544 (478)	3302 (480)	4.5 (4.0)
<b>RNA (10 h)</b>	Control	122862	2318 (482)	2958 (486)	4.3 (3.8)
	Casamino acids	168746	2271 (480)	2887 (482)	3.6 (3.2)
	Glutamate	126412	2260 (480)	3121 (482)	3.9 (3.5)

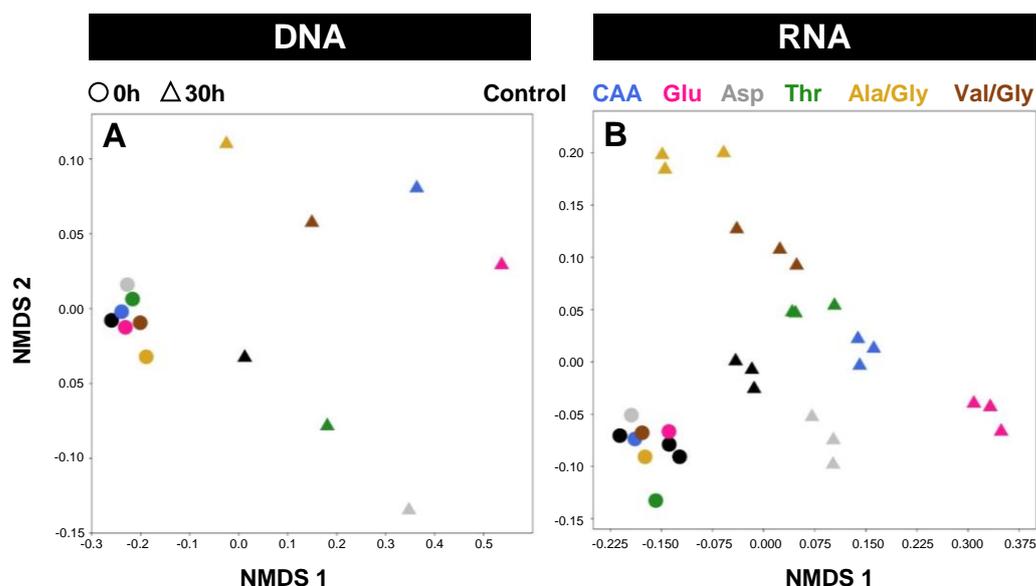
Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
<b>RNA (10 h)</b>	Aspartate	160540	2435 (481)	3019 (483)	4.2 (3.7)
	Threonine	113043	2260 (481)	3026 (484)	4.3 (3.8)
	Alanine/Glycine	97295	2321 (481)	3012 (482)	4.6 (4.1)
	Valine/Glycine	120964	2414 (482)	3167 (486)	4.6 (4.1)
<b>RNA (22 h)</b>	Control	135915	2490 (486)	3131 (486)	4.4 (3.9)
	Casamino acids	94755	1862 (483)	2533 (485)	3.6 (3.2)
	Glutamate	115249	1953 (481)	2632 (485)	3.6 (3.2)
	Aspartate	120291	2053 (483)	2723 (485)	3.8 (3.5)
	Threonine	140737	2333 (484)	2963 (485)	4.2 (3.8)
	Alanine/Glycine	136979	2514 (485)	3179 (486)	4.8 (4.3)
	Valine/Glycine	139028	2459 (485)	3186 (485)	4.7 (4.2)
<b>RNA (30 h)</b>	Control 1	121953	2345 (485)	3160 (486)	4.6 (4.1)
	Control 2	145730	2359 (485)	3011 (486)	4.4 (3.9)
	Control 3	135750	2316 (486)	2954 (486)	4.4 (4.0)
	Casamino acids 1	154449	2241 (484)	2914 (485)	4.0 (3.7)
	Casamino acids 2	57895	1700 (482)	2362 (484)	4.0 (3.6)
	Casamino acids 3	109922	2041 (482)	2660 (485)	4.0 (3.6)
	Glutamate 1	127517	2084 (484)	2784 (486)	3.8 (3.4)
	Glutamate 2	102218	1985 (482)	2647 (485)	3.9 (3.5)
	Glutamate 3	98379	1925 (482)	2524 (485)	3.8 (3.4)
	Aspartate 1	132724	2280 (483)	2914 (485)	4.2 (3.7)
	Aspartate 2	98432	1980 (484)	2806 (485)	4.0 (3.6)
	Aspartate 3	108184	2039 (484)	2690 (485)	4.0 (3.6)
	Threonine 1	103154	2048 (484)	2595 (487)	4.3 (3.9)
	Threonine 2	158797	2348 (485)	3010 (486)	4.4 (3.9)
	Threonine 3	138039	2202 (486)	2808 (487)	4.4 (4.0)
	Alanine/Glycine 1	130264	2312 (486)	2997 (486)	4.6 (4.1)
	Alanine/Glycine 2	161641	2437 (484)	3129 (486)	4.6 (4.1)
	Alanine/Glycine 3	145579	2341 (484)	3080 (486)	4.5 (4.1)
	Valine/Glycine 1	126821	2248 (484)	2895 (486)	4.5 (4.1)
	Valine/Glycine 2	128701	2192 (484)	2898 (487)	4.3 (3.9)
Valine/Glycine 3	117114	2228 (483)	2948 (484)	4.5 (4.0)	

<sup>a</sup>Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22 h or 30 h. Numbers assigned to a treatment (e.g., Control 1) indicate the respective replicate. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>c</sup>For comparison of amplicon libraries of different sizes, the data sets were normalized to 50,000 sequences.

<sup>d</sup>-, normalization was not possible because of the low number of sequences in this sample.



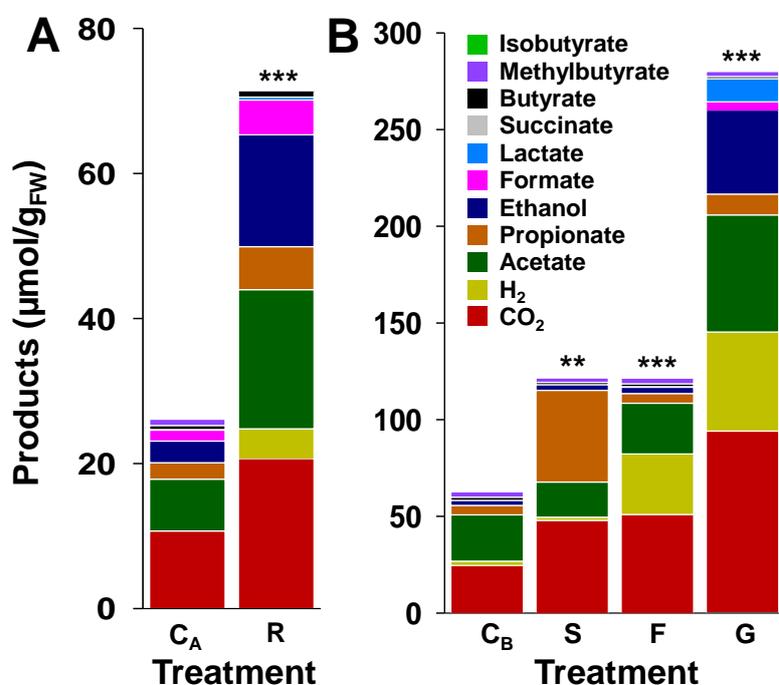
**Figure 50.** NMDS plot of the microbial community composition in amino acid treatments. Distance matrices (Bray-Curtis) are based on the relative abundances of all detected phylotypes in the different treatments (Table A6). Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22 h or 30 h. Proximity of symbols represent the degree of similarity between the different treatments. Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Val, valine; DNA, 16S rRNA genes; RNA, 16S rRNA. Figure modified and used with permission from Zeibich *et al.*, 2019b.

### 3.2.8. Fermentation of ribose and effects of transient intermediates

The fermentation of RNA is dependent on its degradation by RNases that in the end yield ribose, purines, and pyrimidines (Section 1.3.3 and Section 1.4.1). In marked contrast to ribose-supplemented treatments microcosms supplemented with adenine (a purine) and uracil (a pyrimidine) displayed only a slightly enhancement of fermentation activity (Table 33), suggesting ribose as the primary fermentable component of RNA. Indeed, the fermentation profile of the ribose treatment was similar to that of the RNA treatment (Figure 39 and Figure 51 A), and the collective amount of products in the ribose treatment were strongly enhanced and significant compared to the controls (Figure 51 A). Comparable to certain amino acid treatments, propionate and H<sub>2</sub> were significantly produced in the ribose treatment (Table 39 and Table 45). Previous studies demonstrated that the production of propionate and H<sub>2</sub> was coincident with the transient accumulation of succinate and formate, respectively (Figure 27, Figure 34, and Figure 35), suggesting the decarboxylation of succinate to propionate and CO<sub>2</sub> (Schink and Pfennig, 1982) and the conversions of formate by formate-hydrogen lyase to H<sub>2</sub> and CO<sub>2</sub> (Sawers, 1994; McDowall *et al.*, 2014) (Section 1.4.4). The consumptions of potential transient intermediates like succinate, formate, ethanol, and lactate in earthworm gut contents were not investigated before, and therefore evaluated more closely.

Gut content treatments supplemented with succinate and formate indicated a consumption of these primary fermentation products (Figure 52) that significantly enhanced the collective fermentation profile (Figure 51 B and Table 45). The decrease of succinate was concomitant with the enhanced production of propionate and CO<sub>2</sub>, whereas the decrease of formate was

concomitant with the enhanced production of H<sub>2</sub> and CO<sub>2</sub> (Figure 52). That the control treatment displayed also a transient occurrence of succinate with a concomitant accumulation of propionate, reinforces the likelihood that the production of propionate in the earthworm gut is at least partially dependent on the production of succinate. Supplemented lactate and ethanol were not used during incubation and therefore not further evaluated (Figure 52).

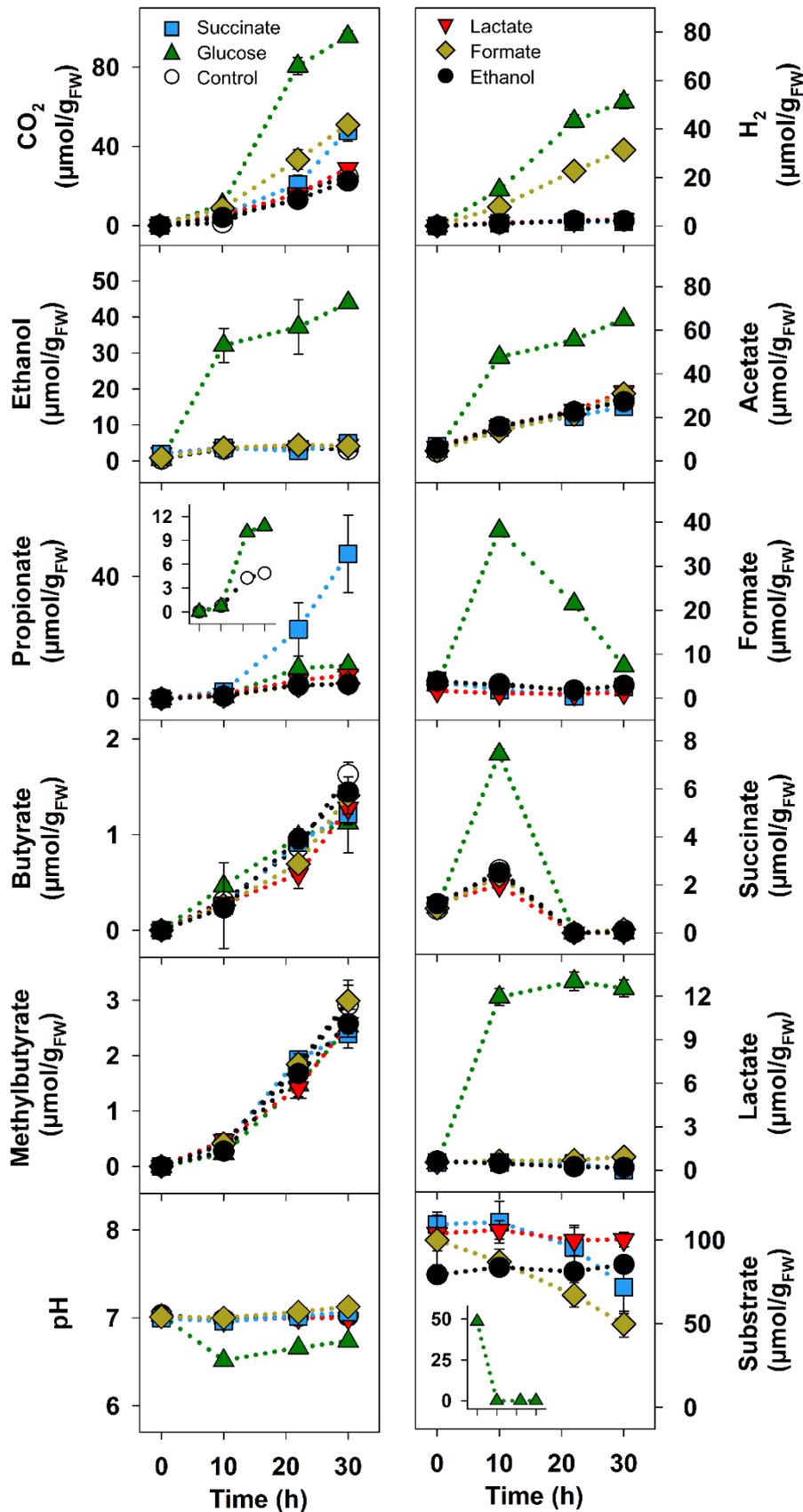


**Figure 51.** Collective amounts of fermentation products in ribose (A), transient intermediate, and glucose (B) treatments. Values are the average of triplicate analyses shown in Table 44 (ribose) and Figure 52 (transient intermediates) and represent the net amounts of products at the end of the 30 h incubation. The asterisks indicate significant differences between the collective amount of products formed in unsupplemented control and supplemented treatments (\*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ;  $t$ -test with unequal variances; see Table 45 for  $P$  values, mean values, and variances). Abbreviations: C<sub>A</sub> and C<sub>B</sub>, unsupplemented control; R, ribose; S, succinate, F, formate; G, glucose; FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019b.

**Table 44.** Effect of ribose on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents.<sup>a</sup>

Treatment	Time (h)	Ribose	Products (μmol/g <sub>FW</sub> )			
			CO <sub>2</sub>	H <sub>2</sub>	Acetate	Succinate
Control	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.1	1.1 ± 0.0
	30	0.0 ± 0.0	11 ± 2.7	0.1 ± 0.3	14 ± 0.5	0.4 ± 0.1
Ribose	0	56 ± 23	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 2.0	1.1 ± 0.3
	30	39 ± 3.2	21 ± 2.5	4.1 ± 0.8	26 ± 1.7	1.0 ± 0.3
		pH	Propionate	Formate	Butyrate	Ethanol
Control	0	7.0 ± 0.0	0.7 ± 0.0	2.1 ± 0.1	0.5 ± 0.0	0.0 ± 0.0
	30	7.0 ± 0.0	2.9 ± 0.1	3.6 ± 0.4	1.1 ± 0.2	3.0 ± 0.8
Ribose	0	7.0 ± 0.0	0.7 ± 0.1	2.7 ± 0.7	0.5 ± 0.0	0.9 ± 0.1
	30	6.9 ± 0.0	6.6 ± 0.4	7.5 ± 0.6	1.3 ± 0.1	17 ± 0.8

<sup>a</sup>The amount of ribose supplemented per microcosm approximated 5 mM. Controls lacked supplemental ribose. Values are the arithmetic average of three replicate analyses (± standard derivation). FW, fresh weight. Table modified and used with permission from Zeibich *et al.*, 2019b.



**Figure 52.** Effect of transient intermediates and glucose on the fermentation product profiles of anoxic microcosms of *L. terrestris* gut contents. Initial concentrations approximated 10 mM for succinate, formate, lactate, and ethanol, and 5 mM for glucose. The control lacked supplement. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight. Figure modified and used with permission from Zeibich *et al.*, 2019b.

Gut content treatments supplemented with glucose, a saccharide detected in the earthworm gut (Wüst *et al.*, 2009b), also displayed a transient accumulation of succinate and formate and the simultaneous formation of propionate and H<sub>2</sub>, respectively (Figure 52). 82 to 98% of glucose- or ribose-derived carbon and reducing equivalents were recovered in the detected fermentation products (Table 46), indicating that the amount of supplemented saccharides were adequate for the enhanced fermentation product profile. The collective findings demonstrated that the secondary utilization of succinate and formate might contribute to the production of propionate and H<sub>2</sub>, respectively, during the earthworm gut fermentation.

**Table 45.** *P* values of fermentation products in ribose, succinate, formate, and glucose treatments.<sup>a</sup>

Product		CO <sub>2</sub>					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	11	21	25	48	51	94	
Variance	7.5	6.3	16	29	4.6	1.2	
<i>P</i> value		0.010		0.004	0.002	0.001	
Product		H <sub>2</sub>					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	0.1	4.1	2.3	1.7	31	51	
Variance	0.1	0.7	0.0	0.0	5.3	7.9	
<i>P</i> value		0.004		0.030	0.002	0.001	
Product		Acetate					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	7.1	19	24	18	26	61	
Variance	0.3	2.4	4.8	2.1	7.8	2.2	
<i>P</i> value		0.006		0.031	0.328	0.000	
Product		Succinate					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	-0.7	0.1	-0.9	-	-0.9	-1.1	
Variance	0.0	0.1	0.0	-	0.0	0.0	
<i>P</i> value		0.105		-	0.821	0.075	
Product		Formate					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	1.5	4.8	0	-1.4	-	4.2	
Variance	0.3	0.3	0.1	0.3	-	0.1	
<i>P</i> value		0.002		0.040	-	0.000	
Product		Propionate					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	2.3	5.9	4.9	47	5.0	11	
Variance	0.0	0.2	0.2	161	0.2	0.0	
<i>P</i> value		0.005		0.028	0.727	0.002	
Product		Butyrate					
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G	
Mean value <sup>b</sup>	0.0	0.0	1.6	1.2	1.4	1.1	
Variance	0.0	0.0	0.0	0.0	0.0	0.1	
<i>P</i> value		-		0.013	0.185	0.082	

Product		Methylbutyrate				
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G
Mean value <sup>b</sup>	0.0	0.0	2.9	2.4	3	2.5
Variance	0.0	0.0	0.2	0.1	0.1	0.0
P value		-		0.178	0.822	0.282
Product		Isobutyrate				
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G
Mean value <sup>b</sup>	0.0	0.0	0.2	0.2	0.3	0.0
Variance	0.0	0.0	0.0	0.0	0.0	0.0
P value		-		0.525	0.281	0.006
Product		Ethanol				
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G
Mean value <sup>b</sup>	3.0	15	2.6	2.9	3.2	43
Variance	0.6	0.7	0.3	0.8	0.5	2.1
P value		0.003		0.666	0.332	0.000
Products		Total				
Treatment	C <sub>A</sub>	R	C <sub>B</sub>	S	F	G
Mean value <sup>b</sup>	25	66	77	231	172	299
Variance	8.5	18	103	460	431	197
P value		0.000		0.002	0.006	0.000

<sup>a</sup>P values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the net amount of products in control (C<sub>A</sub> and C<sub>B</sub>), ribose (R), succinate (S), formate (F), or glucose (G) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation (unless otherwise indicated). See Figure 51 for product profiles. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

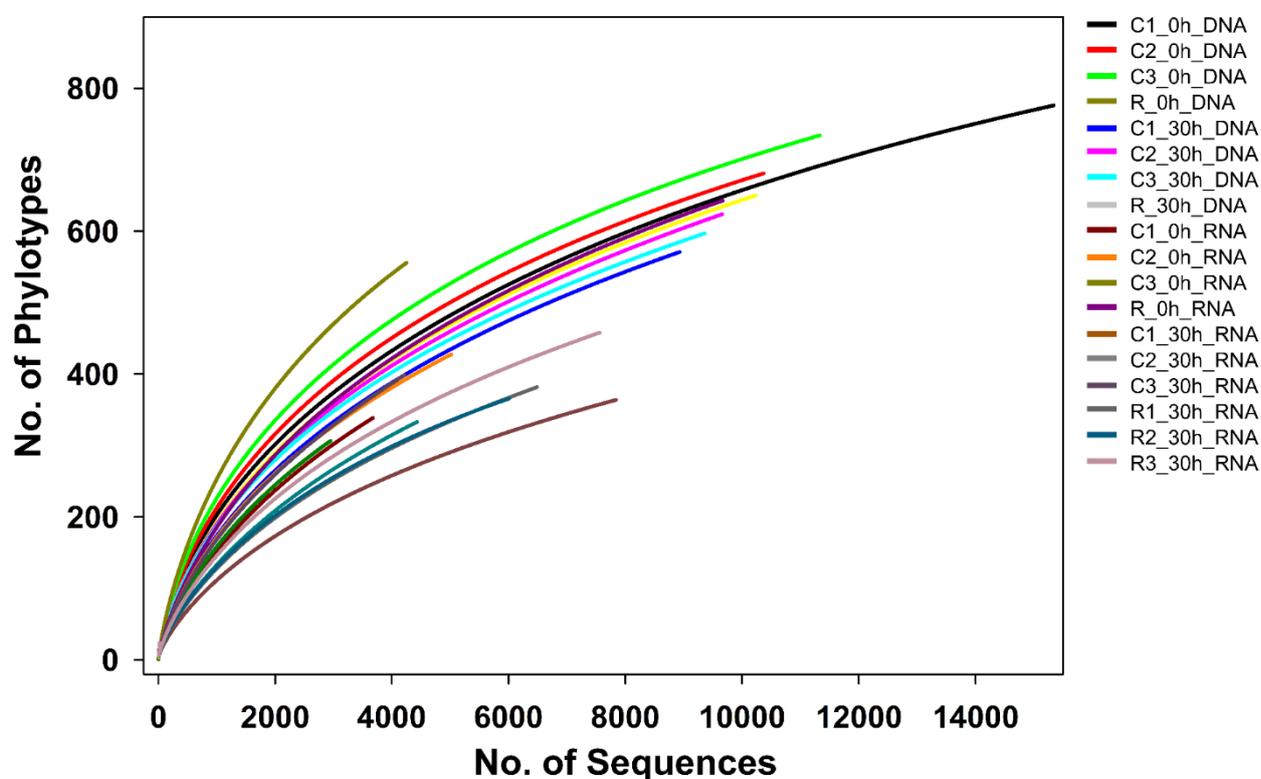
**Table 46.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in ribose, succinate, formate, and glucose treatments.<sup>a</sup>

Main Products	Recoveries (%)							
	Ribose		Succinate		Formate		Glucose	
	Carbon	RE	Carbon	RE	Carbon	RE	Carbon	RE
CO <sub>2</sub>	10	na	16	na	52	na	24	na
H <sub>2</sub>	na	2.1	na	-	na	58	na	8.4
Acetate	25	25	-	-	9	18	25	25
Ethanol	26	40	0.4	0.7	2.3	6.8	28	42
Succinate	2.5	2.2	-	-	-	-	-	-
Lactate	1.0	1.0	-	-	4.6	9.2	13	13
Formate	3.4	1.7	-	-			1.5	0.7
Propionate	11	13	86	114	0.8	2.0	6.1	7.2
Isobutyrate	-	-	-	-	0.7	1.7	-	-
Methylbutyrate	-	-	-	-	0.7	1.9	-	-
<b>Total</b>	<b>82</b>	<b>87</b>	<b>102</b>	<b>115</b>	<b>71</b>	<b>98</b>	<b>98</b>	<b>96</b>

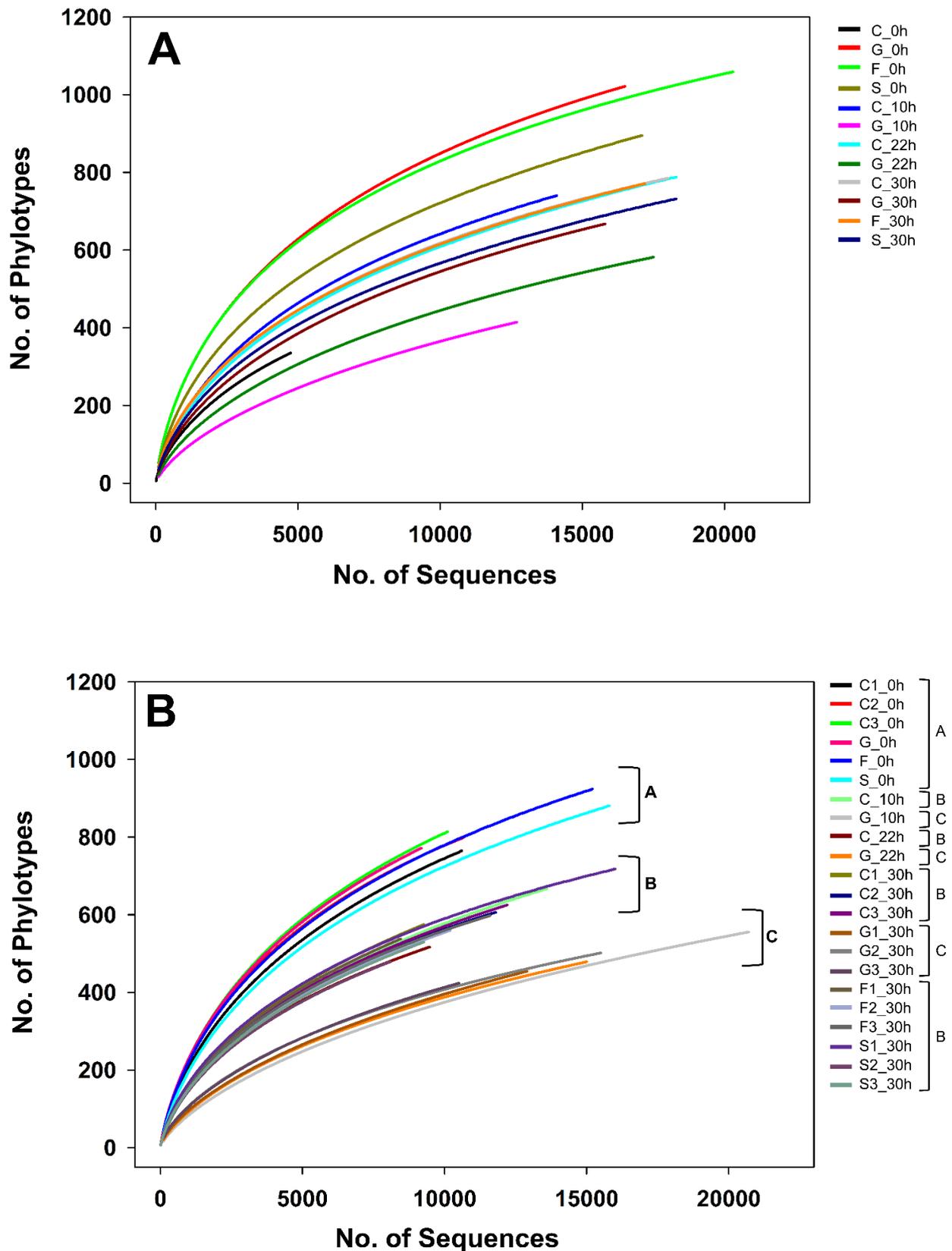
<sup>a</sup>See Figure 51 for product profiles. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate consumed. Values are based on the arithmetic average of three replicate analyses. RE, reducing equivalents; -, no net increase of the product during the incubation in supplemented treatments relative to the control treatments; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019b.

### 3.2.9. Effect of ribose and transient intermediates on gut fermentative bacterial families

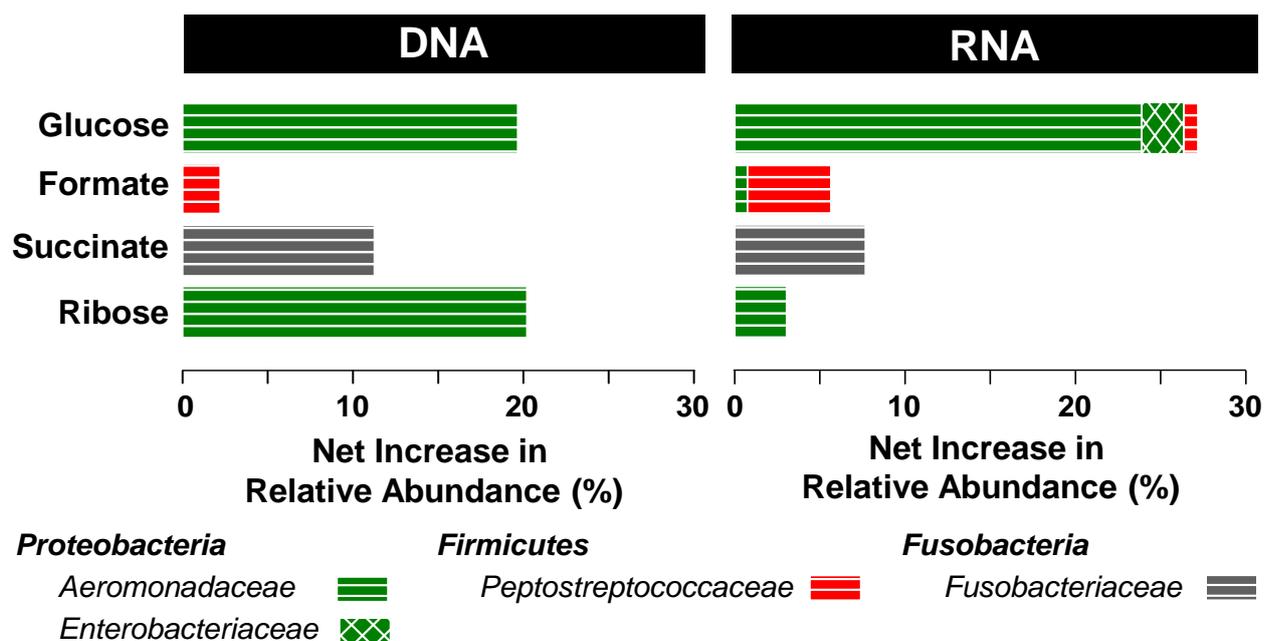
A total of 606,090 bacterial 16S rRNA gene and 16S rRNA sequences, associated to 25 phyla (including candidate phyla), were obtained from the saccharide and transient intermediate treatments, and the rarefaction analyses indicated that the most abundant taxa were targeted (Figure 53 and Figure 54). The analysis of the relative sequence abundances demonstrated that *Aeromonadaceae* were stimulated by ribose and glucose (Figure 55, Figure 56, Figure 57, and Table 47). *Fusobacteriaceae*-affiliated sequences displayed a net increase of affiliated sequences in succinate treatments, whereas formate mostly stimulated *Peptrostreptococcaceae* (Figure 55). The associated relative sequence abundances of these families were significantly greater in the respective supplemented treatment than in the control at the end of incubation (Table 47). Consistent with the strong fermentative response of *Aeromonadaceae* to glucose (Figure 51 and Figure 55), the number of detected phylotypes, the number of expected phylotypes (Chao1), and Shannon indices of the glucose treatment were lower than those of unsupplemented control (Figure 54 B and Table 48). These findings indicated that shifts in the community occurred during the incubation, and NMDS analysis (Section 2.6.2.2) of all detected phylotypes confirmed that the microbial communities changed during the incubation (Figure 58).



**Figure 53.** Rarefaction analyses of bacterial 16S rRNA gene (DNA) and 16S rRNA (RNA) sequences obtained from anoxic *L. terrestris* gut content microcosms supplemented with ribose. Phylotypes were based on a 97% sequence similarity cutoff. Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Abbreviations: 0 h and 30 h indicate the time of sampling in hours; C, unsupplemented control; R, ribose. Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Figure modified and used with permission from Zeibich *et al.*, 2019b.



**Figure 54.** Rarefaction analyses of bacterial 16S rRNA gene (A) and 16S rRNA (B) sequences obtained from anoxic *L. terrestris* gut content microcosms supplemented with glucose and transient intermediates. Phylotypes were based on a 97% sequence similarity cutoff. Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22 h or 30 h. Abbreviations: C, unsupplemented control; S, succinate; F, formate; G, glucose. Identification numbers (e.g., C1) indicate the respective replicates. Figure modified and used with permission from Zeibich *et al.*, 2019b.



**Figure 55.** Net increases in 16S rRNA gene (DNA) and 16S rRNA (RNA) relative abundances of bacterial families stimulated by supplemental saccharides, succinate, and formate in *L. terrestris* gut content microcosms. The graph is limited to families that displayed a net increase in relative abundance of  $\geq 4\%$  in at least one treatment and the families are color-coded to the respective phyla (see Figure 56 and Figure 57 for the complete 16S rRNA and 16S rRNA gene analyses). Net increases of relative abundances were calculated as follows: (a) the calculation is based either on mean relative abundances when samples from the three replicates were analyzed separately (i.e., all RNA and DNA samples of control treatments and RNA samples at 30 h of supplemented treatments) or on single relative abundances when samples of the three replicates were pooled for sequence analyses (i.e., DNA samples at 0 h and 30 h and RNA samples at 0 h of supplemented treatments); (b) mean or single relative abundances at the beginning of incubation were subtracted from those at the end of incubation for control and supplemented treatments; (c) the resulting time-corrected relative abundances of control treatments were subtracted from those of supplemented treatments (negative time-corrected relative abundances of control treatments were ignored). Figure modified and used with permission from Zeibich *et al.*, 2019b.

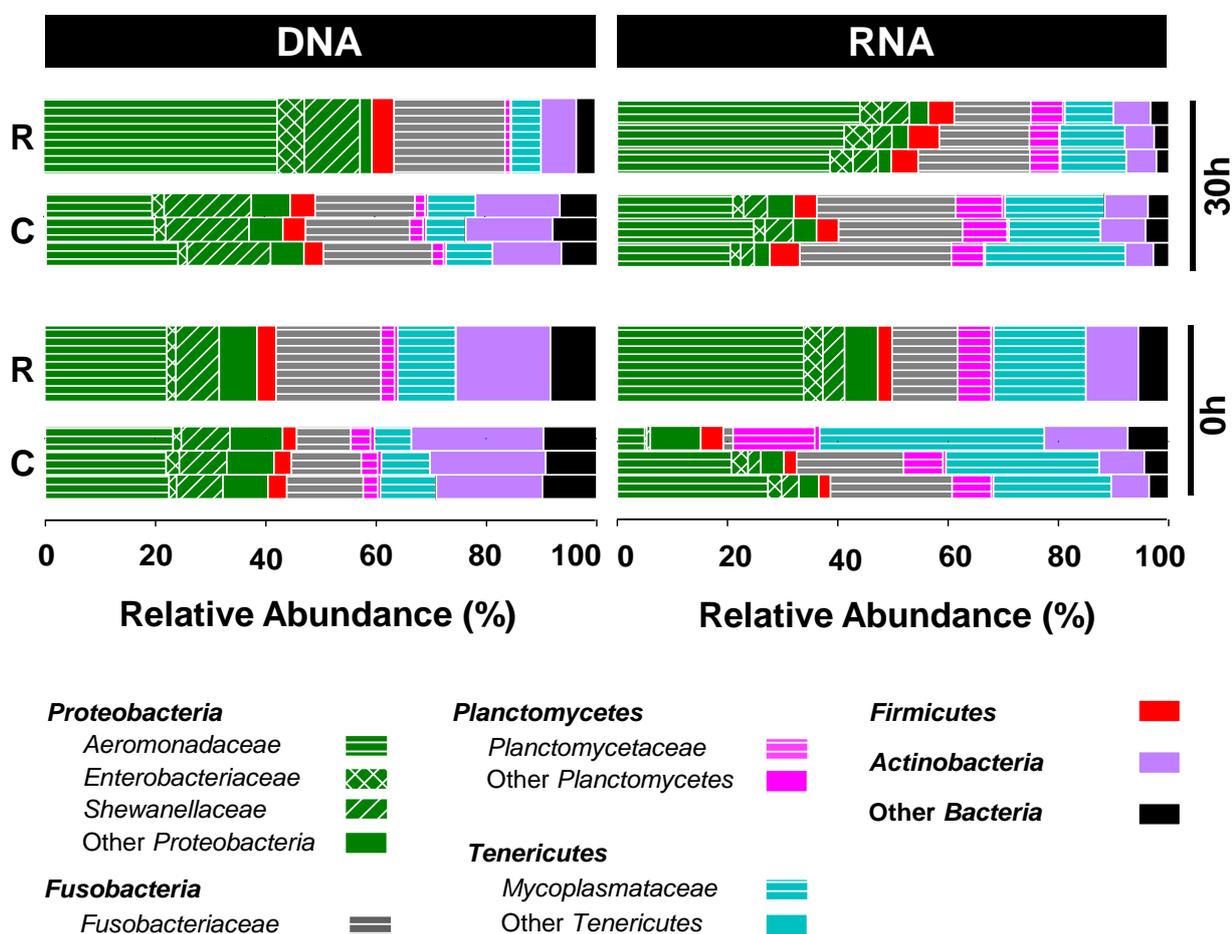
**Table 47.** Statistical analyses of stimulated families in ribose, succinate, formate, and glucose treatments.<sup>a</sup>

Family	Treatment	Mean	Standard Deviation	Median	LDA Score (log <sub>10</sub> ) <sup>b</sup>
<i>Aeromonadaceae</i>	Control <sub>A</sub>	22	2.3	21	
	Ribose	41	2.7	41	5.6 <sup>(1)</sup>
	Control <sub>B</sub>	21	1.9	20	
	Glucose	42	3.7	41	5.6 <sup>(1)</sup>
<i>Clostridiaceae</i>	Control <sub>B</sub>	2.1	0.2	2.1	
	Formate	3.3	0.2	3.2	4.5 <sup>(3)</sup>
	Glucose	3.7	0.4	3.6	4.6 <sup>(4)</sup>
<i>Enterobacteriaceae</i>	Control <sub>A</sub>	2.0	0.1	1.9	
	Ribose	4.4	0.5	4.1	4.6 <sup>(2)</sup>
	Control <sub>B</sub>	3.3	3.0	3.5	
	Formate	4.3	0.4	4.2	4.6 <sup>(2)</sup>
	Glucose	4.4	0.4	4.6	4.6 <sup>(2)</sup>

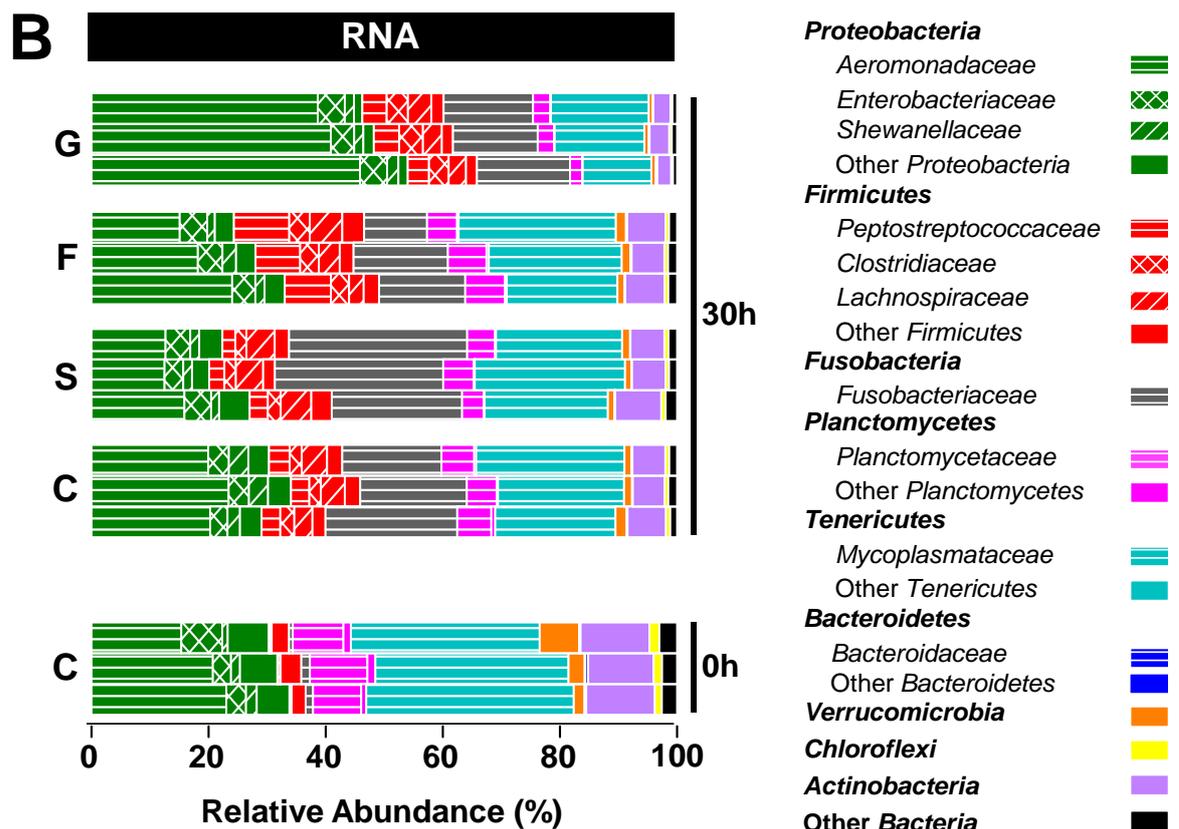
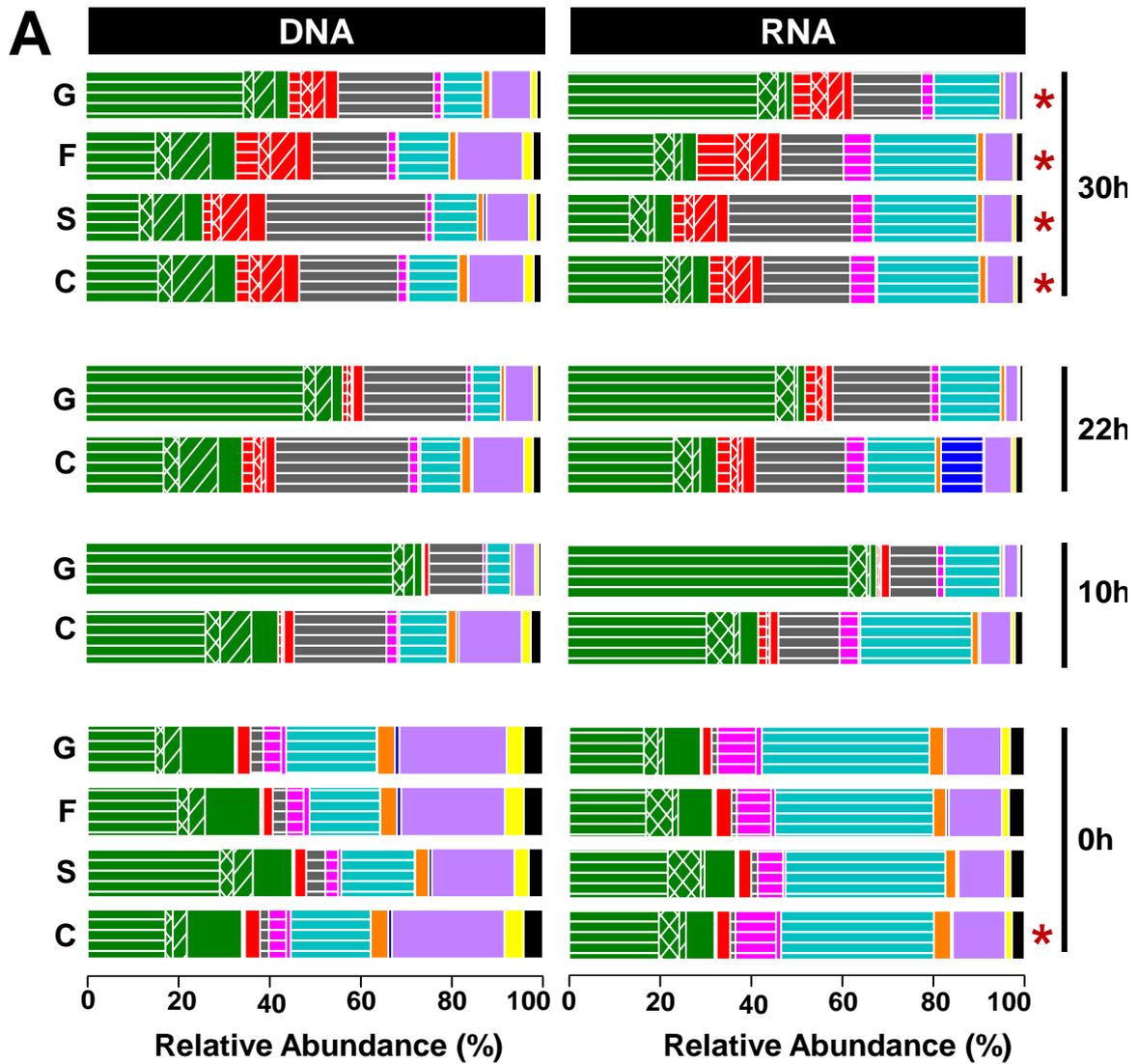
Family	Treatment	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
<i>Lachnospiraceae</i>	Control <sub>B</sub>	3.8	0.6	4.1	4.7 <sup>(1)</sup>
	Succinate	4.9	0.3	4.8	
<i>Peptostreptococcaceae</i>	Control <sub>B</sub>	3.3	0.2	3.2	5.0 <sup>(1)</sup>
	Formate	8.3	1.0	7.9	
	Glucose	4.0	0.4	4.2	

<sup>a</sup>Families reaching a LDA score  $\geq 4.0$  were considered. LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at 30 h of incubation. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to supplement compared to lower ranking ones).



**Figure 56.** 16S rRNA gene (DNA) and 16S rRNA (RNA) analyses of control and ribose treatments. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Process data are shown Figure 51 and Table 44, and information on all detected taxa is provided in Table A7. Abbreviations: C, unsupplemented control; R, ribose treatment. Single bars indicate that 16S rRNA or 16S rRNA gene samples of the three replicates were pooled for the sequence analysis and grouped bars indicate that the sequence analysis was performed individually for the three replicates. Figure modified and used with permission from Zeibich *et al.*, 2019b.



**Figure 57.** 16S rRNA (RNA) and 16S rRNA gene (DNA) analyses of control, succinate, formate, and glucose treatments. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Process data are shown in Figure 52, and information on all detected taxa is provided in Table A8. Abbreviations: C, unsupplemented control; S, succinate; F, formate; G, glucose. Panel A: Single bars without asterisk indicate that DNA or RNA samples of the three replicates were pooled for the sequence analysis. Asterisk indicates analysis was performed individually for the three replicates (see grouped bars Panel B). Figure modified and used with permission from Zeibich *et al.*, 2019b.

**Table 48.** Alpha diversity of the microbial community in control, ribose, glucose and transient intermediate treatments.<sup>a</sup>

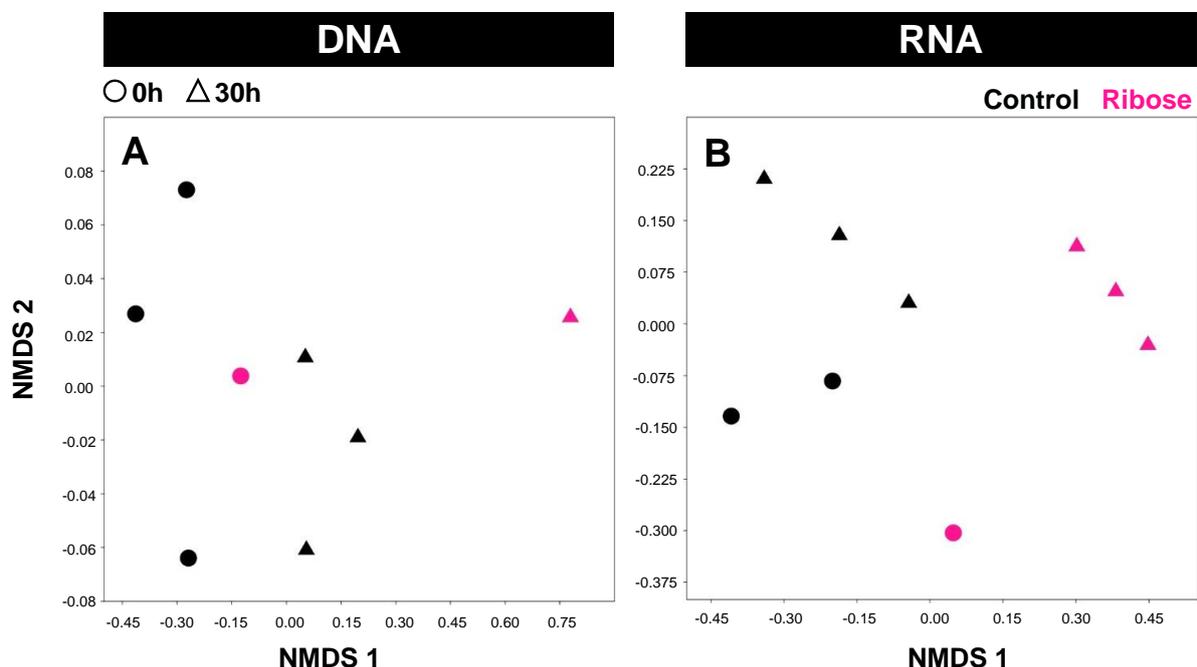
Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>	
<b>DNA (0 h)</b>	Control <sub>A</sub> 1	15345	776 (198)	1046 (241)	4.0 (3.5)	
	Control <sub>A</sub> 2	10377	681 (199)	984 (234)	4.1 (3.6)	
	Control <sub>A</sub> 3	11331	734 (203)	1019 (236)	4.3 (3.7)	
	Ribose	10234	650 (188)	974 (227)	3.8 (3.4)	
	Control <sub>B</sub>	15359	965 (235)	1315 (253)	4.6 (3.9)	
	Succinate	17131	896 (221)	1311 (239)	3.9 (3.3)	
	Formate	20373	1060 (232)	1380 (245)	4.5 (3.8)	
	Glucose	16530	1022 (232)	1337 (247)	4.6 (3.8)	
<b>DNA (10 h)</b>	Control	14156	742 (216)	1109 (243)	3.5 (3.0)	
	Glucose	12798	416 (139)	720 (185)	1.7 (1.5)	
<b>DNA (22 h)</b>	Control	18327	789 (216)	1193 (244)	3.4 (3.0)	
	Glucose	17591	583 (175)	912 (223)	2.3 (2.1)	
<b>DNA (30 h)</b>	Control <sub>A</sub> 1	8937	571 (179)	317 (223)	3.5 (3.1)	
	Control <sub>A</sub> 2	9669	624 (192)	995 (237)	3.7 (3.3)	
	Control <sub>A</sub> 3	9364	597 (190)	958 (232)	3.7 (3.3)	
	Ribose	7845	364 (136)	611 (196)	2.6 (2.4)	
	Control	18096	787 (223)	1105 (255)	3.7 (3.3)	
	Succinate	18342	733 (209)	1071 (237)	3.3 (2.9)	
	Formate	17229	772 (220)	1105 (249)	3.9 (3.3)	
	Glucose	15892	669 (204)	998 (236)	3.1 (2.4)	
<b>RNA (0 h)</b>	Control <sub>A</sub> 1	3684	339 (144)	634 (175)	3.1 (2.7)	
	Control <sub>A</sub> 2	2958	307 (152)	581 (183)	3.1 (2.9)	
	Control <sub>A</sub> 3	4255	556 (213)	866 (242)	4.0 (3.4)	
	Ribose	9677	643 (188)	10110 (234)	3.5 (3.1)	
	Control <sub>B</sub> 1	10695	768 (216)	1178 (237)	3.5 (3.0)	
	Control <sub>B</sub> 2	10734	802 (225)	1185 (240)	3.8 (3.2)	
	Control <sub>B</sub> 3	10158	816 (227)	1242 (246)	3.9 (3.3)	
	Succinate	15874	883 (218)	1325 (240)	3.5 (2.9)	
	Formate	15238	925 (232)	1332 (248)	3.8 (3.2)	
	Glucose	9185	772 (224)	1163 (240)	3.8 (3.2)	
	<b>RNA (10 h)</b>	Control	13621	668 (197)	1074 (229)	3.0 (2.7)
		Glucose	20734	556 (142)	1019 (199)	1.9 (1.7)
<b>RNA (22 h)</b>	Control	9473	517 (202)	891 (228)	3.2 (2.9)	
	Glucose	15076	480 (157)	865 (211)	2.2 (2.0)	

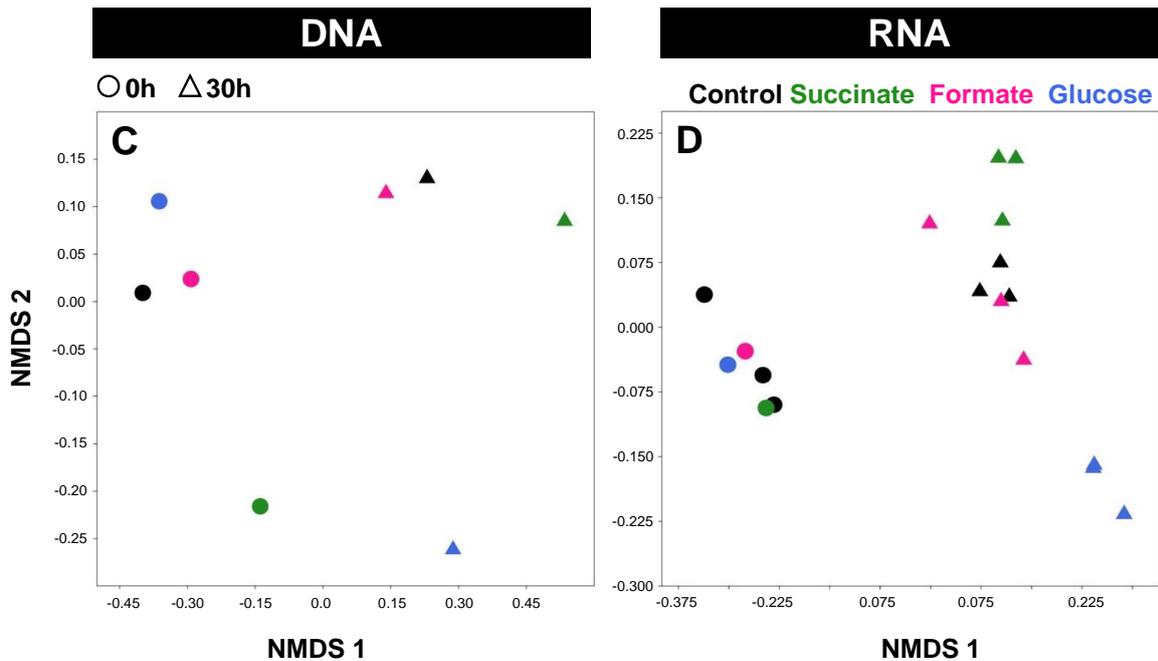
Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
RNA (30 h)	Control <sub>A</sub> 1	4433	333 (146)	634 (196)	2.9 (2.6)
	Control <sub>A</sub> 2	5019	428 (176)	743 (217)	3.4 (3.0)
	Control <sub>A</sub> 3	4226	399 (168)	813 (212)	3.3 (3.0)
	Ribose 1	6497	382 (149)	675 (206)	2.9 (2.6)
	Ribose 2	6020	366 (146)	554 (198)	3.0 (2.7)
	Ribose 3	7561	458 (160)	773 (215)	3.1 (2.8)
	Control <sub>B</sub> 1	9280	575 (202)	986 (229)	3.3 (3.0)
	Control <sub>B</sub> 2	11820	607 (204)	987 (242)	3.3 (2.9)
	Control <sub>B</sub> 3	12244	626 (214)	1004 (252)	3.3 (3.0)
	Succinate 1	16056	719 (220)	1012 (250)	3.5 (3.1)
	Succinate 2	8100	479 (192)	793 (224)	3.1 (2.8)
	Succinate 3	9278	530 (200)	913 (234)	3.2 (2.8)
	Formate 1	8459	538 (208)	951 (237)	3.4 (3.1)
	Formate 2	10208	560 (197)	979 (223)	3.4 (3.1)
	Formate 3	11610	598 (207)	897 (237)	3.5 (3.2)
	Glucose 1	12941	455 (151)	851 (195)	2.5 (2.3)
	Glucose 2	15522	502 (167)	808 (210)	2.7 (2.5)
	Glucose 3	10518	424 (162)	744 (206)	2.7 (2.5)

<sup>a</sup>For the ribose experiment: Samples of the three replicates of the 16S rRNA gene (DNA) control treatment at 0 h and 30 h, 16S rRNA (RNA) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. For the transient intermediate experiment: Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22h, or 30 h. Identification numbers (e.g., Control<sub>A</sub>1) indicate the respective replicates. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>c</sup>For comparison of amplicon libraries of different sizes, the transient data sets were normalized to 5,000 and the ribose data set were normalized to 2,500 sequences.

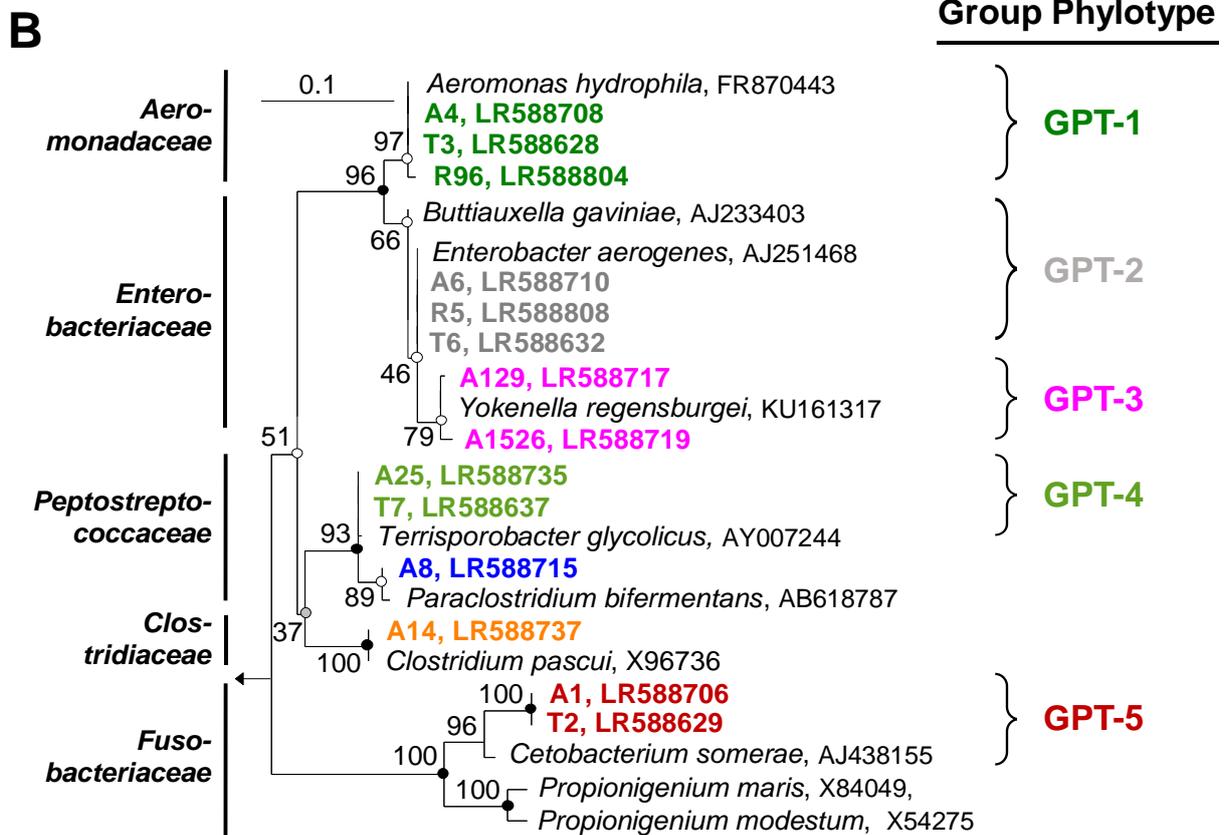
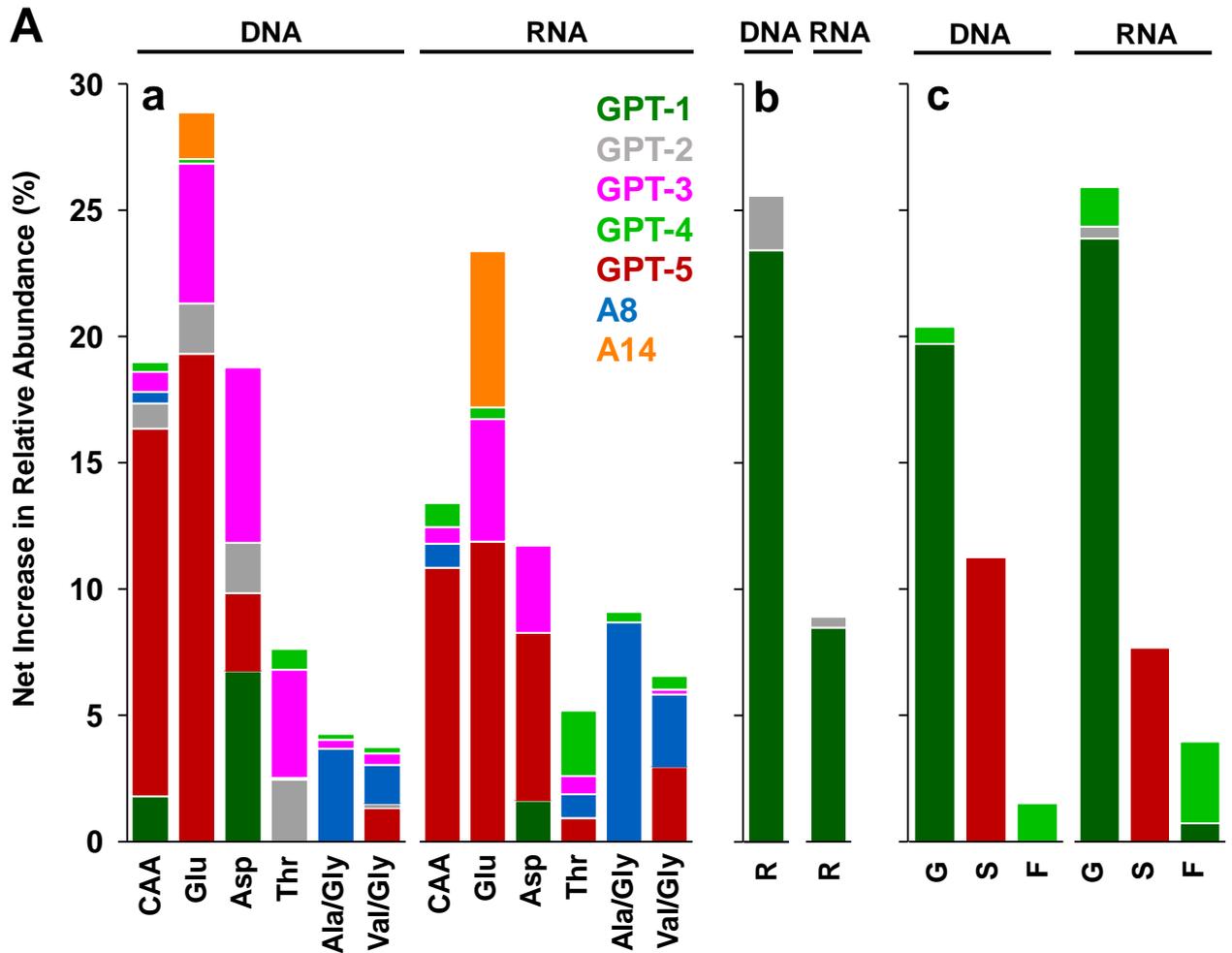




**Figure 58.** NMDS plot of the microbial community composition in ribose (A and B), and succinate, formate, and glucose (C and D) treatments. Distance matrices (Bray-Curtis) are based on the relative abundances of all detected phylotypes in the different treatments (Table A8). Proximity of symbols represent the degree of similarity between the different treatments. Panel A and B: Samples of the three replicates of the 16S rRNA gene (DNA) control treatment at 0 h and 30 h, 16S rRNA (RNA) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Panel C and D: Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Figure modified and used with permission from Zeibich *et al.*, 2019b.

### 3.2.10. Amino acid- and ribose-responsive phylotypes

The aforementioned trends of simulated families in amino acid, saccharide, succinate, and formate treatments (Figure 48 and Figure 55) extended to five group phylotypes and two single phylotypes (Table 49 and Figure 59). GPT-1, closely related to *A. hydrophila*, was significantly stimulated by supplemented ribose and glucose. The the relative 16S rRNA abundances associated to GPT-1 display also a net increase in casamino acid and aspartate treatments (Figure 59). The family *Enterobacteriaceae* was represented by GPT-2 (closely related to *Enterobacteria aerogenes*), and GPT-3 (closely related to *Y. regensburgeri*). GPT-2 was moderate stimulated in all treatments expect of succinate and formate treatments, GPT-3 responded significantly to all amino acid treatments expect of the co-amino acid treatments (Figure 59 and Table 49). Furthermore, GPT-4, closely related to the acetogen *T. glycolicus*, displayed the strongest net increase in relative 16S rRNA abundance in threonine and formate treatments (Figure 59). The two single phylotypes A8 (closely related to *P. bifermantans*) and A14 (closely related to *Clostridium pasculi*) were significantly stimulated in co-amino acid treatments and glutamate treatments, respectively (Figure 59 and Table 49). The family *Fusobacteriaceae* was represented by GPT-5, a group phylotype related to *C. somerae* and mostly stimulated in treatments supplemented with casamino acid, glutamate, aspartate, and succinate (Figure 59).



**Figure 59.** Net increase in 16S rRNA gene (DNA) and 16S rRNA (RNA) relative abundances of the main stimulated group phylotypes (A) and phylogenetic tree of these stimulated group phylotypes (B). Panel A: Each group phylotype (GPT) consists of identical or nearly identical phylotypes based on a  $\geq 97\%$  sequence similarity. Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one of the supplemented treatments displayed a  $\geq 2\%$  net increase in relative abundance. Net increases of relative abundances were calculated as follows (8): (a) the calculation is based either on mean relative abundances when samples from the three replicates were analyzed separately (i.e., all RNA and DNA samples of control treatments and RNA samples at 30 h of supplemented treatments) or on single relative abundances when samples of the three replicates were pooled for sequence analyses (i.e., DNA samples at 0 h and 30 h and RNA samples at 0 h of supplemented treatments); (b) mean or single relative abundances at the beginning of incubation were subtracted from those at the end of incubation for control and supplemented treatments; (c) the resulting time-corrected relative abundances of control treatments were subtracted from those of supplemented treatments (negative time-corrected relative abundances of control treatments were ignored). Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine; Gly, glycine; Val, valine; S, succinate; F, formate; G, glucose. Panel B: The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees; empty circles, congruent nodes in maximum parsimony and maximum likelihood trees; gray circles, congruent nodes in maximum parsimony and neighbor-joining trees. Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch. Phylotype descriptors: A, phylotypes derived from amino acid experiment (Figure 46); R, phylotypes derived from ribose experiment (Figure 51 A); T, phylotypes derived from transient intermediate experiment (Figure 51 B). Figure modified and used with permission from Zeibich *et al.*, 2019b.

**Table 49.** Statistical analyses of main stimulated phylotypes displayed in Figure 59.<sup>a</sup>

Group Phylotype	Phylotype <sup>b</sup>	Treatment	Mean	Standard Deviation	Median	LDA Score (log <sub>10</sub> ) <sup>c</sup>
<b>GPT-1</b>	T3	Control	21	1.9	20	
		Glucose	42	3.7	41	5.8 <sup>(1)</sup>
	R96	Control	13	0.7	13	
		Ribose	30	1.5	29	5.5 <sup>(1)</sup>
<b>GPT-2</b>	A6	Control	2.1	0.2	2.2	
		Casamino Acids	3.3	0.3	3.3	4.5 <sup>(2)</sup>
		Glutamate	4.3	0.6	4.2	4.6 <sup>(3)</sup>
		Aspartate	3.2	0.2	3.1	4.5 <sup>(2)</sup>
		Alanine/Glycine	2.9	0.3	3.0	4.5 <sup>(3)</sup>
		Valine/Glycine	3.4	0.1	3.4	4.5 <sup>(2)</sup>
	T6	Control	2.9	0.2	2.9	
		Glucose	3.7	0.3	3.6	4.6 <sup>(2)</sup>
		Formate	3.9	0.4	3.8	4.6 <sup>(2)</sup>
	R5	Control	1.6	0.1	1.6	
		Ribose	3.3	0.5	3.1	4.5 <sup>(2)</sup>
	<b>GPT-3</b>	A129	Control	0.4	0.0	0.4
Casamino Acids			1.1	0.1	1.0	4.0 <sup>(5)</sup>
Glutamate			3.2	0.3	3.2	4.5 <sup>(4)</sup>
Aspartate			2.4	0.1	2.4	4.4 <sup>(3)</sup>
Threonine			1.0	0.1	1.0	4.0 <sup>(6)</sup>
Alanine/Glycine			0.6	0.1	0.7	3.8 <sup>(7)</sup>
Valine/Glycine			0.7	0.1	0.8	3.9 <sup>(4)</sup>

Group Phylotype	Phylotype <sup>b</sup>	Treatment	Mean	Standard Deviation	Median	LDA Score (log <sub>10</sub> ) <sup>c</sup>
	A1526	Control	0.3	0.0	0.3	
		Glutamate	2.6	0.4	2.6	4.4 <sup>(5)</sup>
		Aspartate	2.0	0.1	2.1	4.3 <sup>(4)</sup>
		Threonine	0.8	0.0	0.8	3.9 <sup>(7)</sup>
		Alanine/Glycine	0.5	0.1	0.5	3.7 <sup>(10)</sup>
		Valine/Glycine	0.6	0.1	0.6	3.8 <sup>(9)</sup>
<b>GPT-4</b>	A25	Control	0.1	0.0	0.2	
		Casamino Acids	1.1	0.3	1.0	4.0 <sup>(4)</sup>
		Glutamate				3.8 <sup>(7)</sup>
		Threonine	2.7	0.2	2.6	4.4 <sup>(3)</sup>
		Alanine/Glycine	0.6	0.3	0.4	3.8 <sup>(8)</sup>
		Valine/Glycine	0.7	0.2	0.8	3.8 <sup>(5)</sup>
	T7	Control	1.0	0.1	0.9	
		Glucose	2.5	0.5	2.8	4.4 <sup>(3)</sup>
		Formate	4.2	0.8	4.2	4.6 <sup>(1)</sup>
<b>GPT-5</b>	A1	Control	16	0.5	16	
		Casamino Acids	25	0.3	25	5.4 <sup>(1)</sup>
		Glutamate	29	1.1	29	5.5 <sup>(1)</sup>
		Aspartate	21	0.7	21	5.3 <sup>(1)</sup>
		Threonine	18	1.2	17	5.2 <sup>(1)</sup>
	A8	Control	0.6	0.0	0.6	
Casamino Acids		1.6	0.1	1.6	4.2 <sup>(3)</sup>	
Threonine		1.6	0.3	1.4	4.2 <sup>(4)</sup>	
Alanine/Glycine		9.3	1.0	9.7	5.0 <sup>(1)</sup>	
Valine/Glycine		3.5	1.2	3.2	4.5 <sup>(1)</sup>	
	A14	Control	0.0	0.0	0.0	
		Glutamate	6.2	0.9	6.2	4.8 <sup>(2)</sup>

<sup>a</sup>Only phylotypes that were significantly stimulated (based on LEfSe analyses) by a given supplement are shown. The LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA sequences of the three replicates per treatment at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>A, phylotypes derived from amino acid experiment (Figure 46); R, phylotypes derived from ribose experiment (Figure 51 A); T, phylotypes derived from transient intermediate experiment (Figure 51 B).

<sup>c</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking phylotypes exhibited a stronger response to supplement compared to lower ranking ones).

### 3.3. The nature of the earthworm gut microbiota

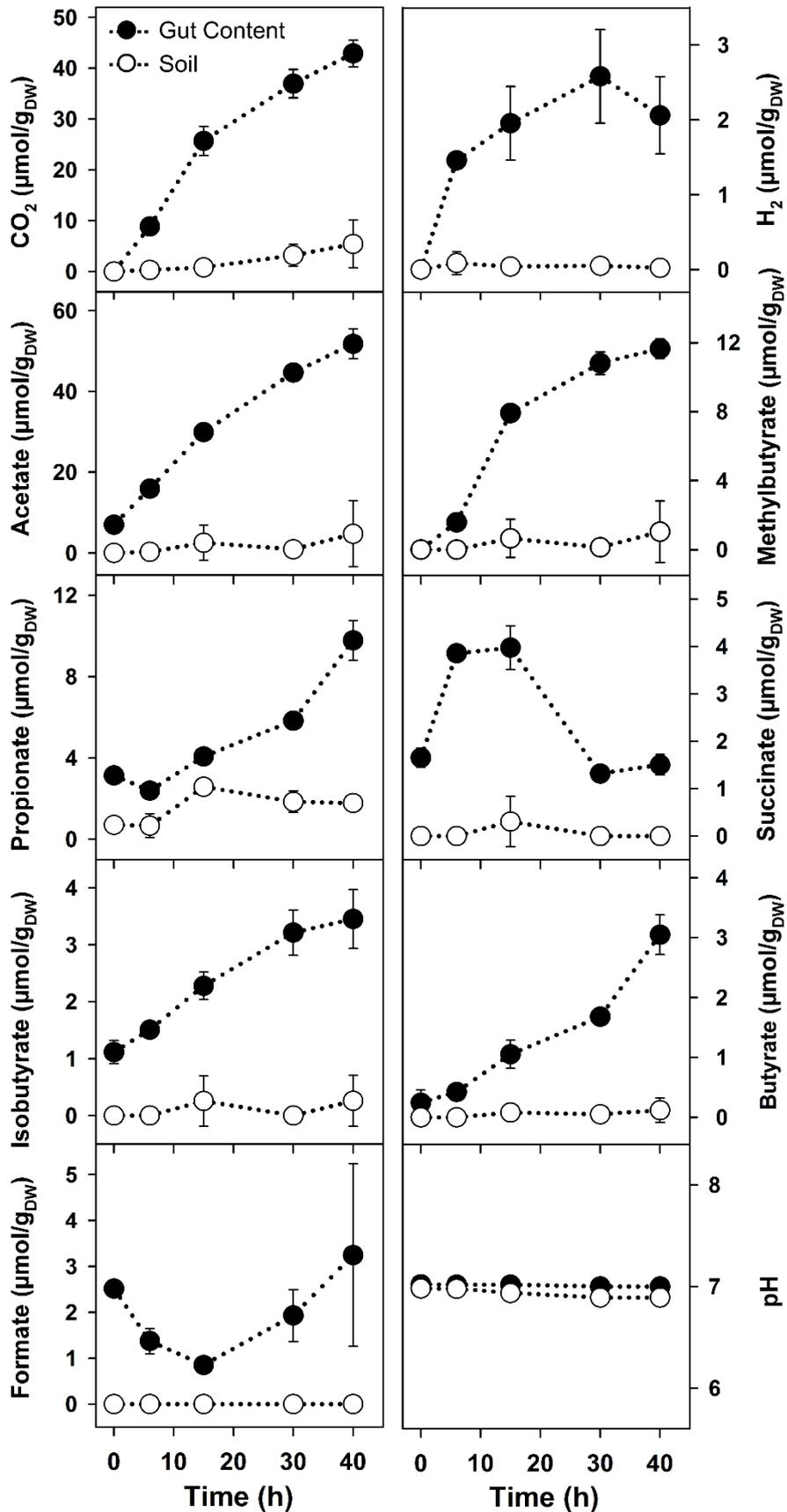
The nature of the microorganisms contributing to the observed earthworm gut fermentations is less well understood. However, cultivation and molecular methods demonstrated similar bacteria in soil, earthworm gut, and earthworm casts (Bassalik, 1913; Furlong *et al.*, 2002; Egert *et al.*, 2004). Such findings reinforces the presumption that most bacteria in the earthworm gut are ingested and transient (Section 1.2.2).

Based on the consideration that the detection of a taxon is at least partially dependent on its metabolic activity, it could be possible to experimentally bring both soil and gut communities to a similar metabolic status, and thereby minimize such potential detection bias. If gut fermenters that might originate from soil are mainly inactive prior ingestion, the nutrient richness of fresh yeast cell lysate might stimulate these fermenters in soil similarly to that observed in gut contents. This theoretical potential of complex substrates to stimulate gut-like fermentations in soil prompted the comparison of the activities and associated microbiota in soil and gut contents of *L. terrestris*.

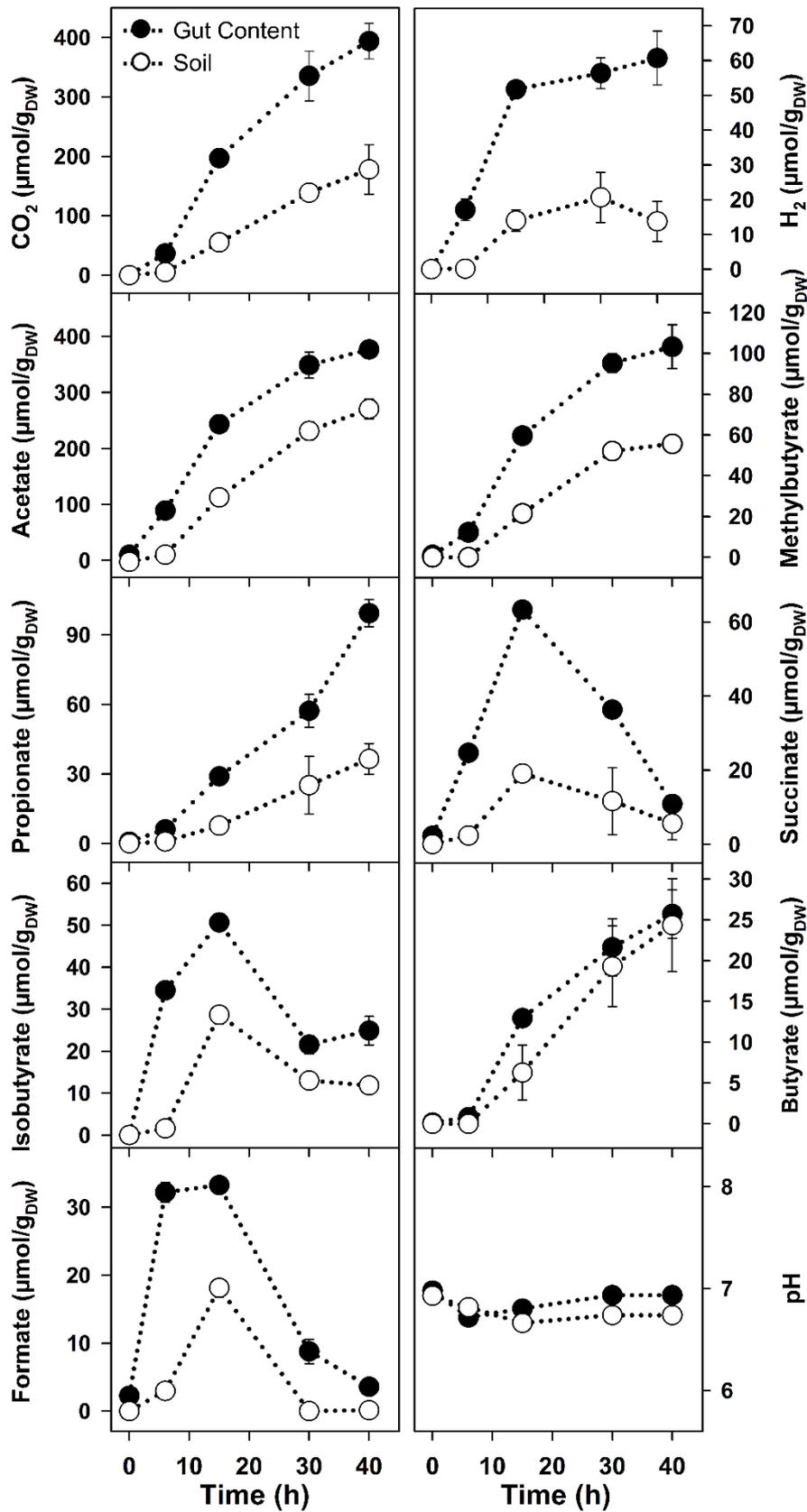
#### 3.3.1. Fermentative responses of gut contents and soil

In unsupplemented microcosms, soil displayed marginal activity whereas gut contents produced diverse fermentation products such as H<sub>2</sub>, CO<sub>2</sub>, acetate, methylbutyrate, propionate, and succinate (Figure 60). In gut content treatments, the rapid formation of fermentation products appeared to occur without appreciable delay, suggesting that gut-associated fermenters were poised to respond to endogenous nutrients in gut contents. These findings illustrated qualitatively and quantitatively dissimilar fermentative activities in soil and gut content.

Fresh microbial cell lysate (a) simulates gizzard-disrupted biomass and (b) strongly enhanced fermentative activities of gut content-associated microbes (Section 3.2.1). Although unsupplemented soil treatments displayed only a negligible fermentative activity, soil and gut contents displayed nearly identical fermentative responses when both were challenged with the complex nutrients of cell lysate (Figure 61). Time-resolved analysis indicated that the initial fermentative response to supplemented cell lysate was more rapid in gut content treatments than in soil treatments (Figure 61). The formation of the dominant fermentation products in response to lysate was statistically significant (Table 50) and characteristic of those formed by fermentative facultative aerobes and fermentative obligate anaerobes (Buckel, 1999). Protein and RNA were evaluated for their potential to (a) stimulate fermentation in soil and thus (b) mimic the capacity of cell lysate to enhance fermentation. Although these biopolymers greatly enhanced gut content fermentations (Section 3.2.3), the fermentative response of soil to either protein or RNA was negligible compared to the strong response of soil to cell lysate (Table 51).



**Figure 60.** Fermentation product profiles of unsupplemented anoxic *L. terrestris* gut content and soil microcosms. Traces of lactate are not shown. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. DW, dry weight. Figure modified and used with permission from Zeibich *et al.*, 2019c.



**Figure 61.** Fermentation product profiles of anoxic *L. terrestris* gut content and soil microcosms supplemented with cell lysate. Traces of lactate are not shown. The amount of carbon derived from filter-sterilized lysate (5.0% dry weight) added per microcosm approximated 2 mmol. Filter sterilized lysate alone did not display any fermentation activity. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. DW, dry weight. Figure modified and used with permission from Zeibich *et al.*, 2019c.

**Table 50.** *P* values of fermentation products in cell lysate-supplemented gut content (A) and soil (B) treatments, and *P* values of gut content versus soil treatments (C).<sup>a</sup>

**(A) Gut Content<sup>b</sup>**

	<b>Products</b>																			
	CO <sub>2</sub>		H <sub>2</sub>		Succinate (15h)		Lactate (6h)		Formate (15h)		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L
<b>Mean value<sup>c</sup></b>	43	394	2.1	61	4.0	63	2.4	14	0.9	33	52	377	9.8	99	3.0	26	12	103	3.5	25
<b>Variance</b>	6.8	886	0.3	60	0.2	1.4	0.1	1.5	0.0	0.2	14	125	1.0	34	0.1	8.7	0.3	116	0.3	12
<b><i>P</i> value</b>	0.002		0.006		0.000		0.004		0.000		0.000		0.001		0.006		0.005		0.008	

**(B) Soil<sup>b</sup>**

	<b>Products</b>																			
	CO <sub>2</sub>		H <sub>2</sub>		Succinate (15h)		Lactate (6h)		Formate (15h)		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L	C	L
<b>Mean value<sup>c</sup></b>	5.4	178	0.0	14	0.3	19	0.0	2.8	0.0	18	4.7	271	1.8	36	0.1	24	1.0	56	0.3	12
<b>Variance</b>	22	1747	0.0	33	0.3	2.3	0.0	0.3	0.0	1.8	67	314	0.1	44	0.0	33	3.2	16	0.2	0.0
<b><i>P</i> value</b>	0.019		0.054		0.002		0.014		0.002		0.000		0.012		0.018		0.000		0.000	

**(C) Gut Content versus Soil<sup>d</sup>**

	Products																			
	CO <sub>2</sub>		H <sub>2</sub>		Succinate (15h)		Lactate (6h)		Formate (15h)		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
<b>Mean value<sup>c</sup></b>	394	178	61	14	63	19	14	2.8	33	18	337	271	99	36	26	24	103	56	25	12
<b>Variance</b>	886	1747	60	33	1.4	2.3	1.5	0.3	0.2	1.8	125	314	34	44	8.7	33	116	16	12	0.0
<b>P value</b>	0.002		0.001		0.000		0.001		0.003		0.003		0.000		0.737		0.006		0.003 <sub>(15h)</sub>	

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2019c.

<sup>b</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the amount of products in control (C) and cell lysate (L) treatments. For transient products (i.e., formate, succinate, isobutyrate and lactate), the significance of differences of net amounts between control and supplemented treatments were tested for the time point of the highest concentration (shown in parentheses). See Figure 60 and Figure 61 for product profiles.

<sup>c</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{DW}}$ . DW, dry weight.

<sup>d</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the amount of products in lysate-supplemented gut content (G) and lysate-supplemented soil (S) treatments. Unless otherwise indicated, the values are based on the amount of products at the end of the incubation.

**Table 51.** Effect of protein, RNA, and cell lysate on fermentation product profiles of anoxic soil treatments.<sup>a</sup>

Supplement	Time (h)	pH	Products ( $\mu\text{mol/g}_{\text{DW}}$ )									
			Total	CO <sub>2</sub>	H <sub>2</sub>	Acetate	Succinate	Formate	Propionate	Butyrate	Methylbutyrate	Iso-butyrate
Protein	0	6.9 ± 0.0	<b>1</b>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.4	0.9 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
	30	6.8 ± 0.0	<b>18</b>	4.0 ± 0.8	0.5 ± 0.1	5.0 ± 0.7	1.9 ± 1.2	6.2 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.0 ± 0.0
RNA	0	6.9 ± 0.0	<b>5</b>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.3	2.5 ± 4.3	0.5 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	6.7 ± 0.0	<b>23</b>	6.3 ± 1.1	3.0 ± 0.8	5.3 ± 1.1	3.3 ± 3.6	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 5.5	0.0 ± 0.0	0.0 ± 0.0
Lysate	0	6.9 ± 0.0	<b>1</b>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.4	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	6.7 ± 0.0	<b>507</b>	136 ± 11	21 ± 7.2	230 ± 16	12 ± 9.0	0.0 ± 0.0	23 ± 12	20 ± 5.0	52 ± 1.8	13 ± 1.9

<sup>a</sup>The amount of protein-, RNA-, and lysate-derived carbon added per microcosm approximated 2.4, 2.4, and 2.0 mmol, respectively. Values are the arithmetic average of three replicate analyses ( $\pm$  standard derivation). Lysate values obtained from Figure 61. DW, dry weight. Table modified and used with permission from Zeibich *et al.*, 2019c.

Likewise, soil microcosms displays essentially no fermentative activity when they were challenged with glucose and other gut-associated saccharides that rapidly augment fermentation in gut contents (Meier *et al.*, 2018). These findings indicates that the marked response of soil to complex nutrients, available in cell lysate, did not occur when saccharides, protein, or RNA were provided as 'high quality' substrates.

The pH approximated 7 and did not vary much in both soil and gut content microcosms during the incubation (Figure 61), reinforcing the likelihood that the complex nutrient input rather than a change in pH was an important factor for the observed fermentation activities. Based on the theoretical recoveries of lysate-derived carbon and reducing equivalents, approximately half of the lysate-derived organic carbon was recovered in the fermentation products of soil and gut content treatments (Table 52), demonstrating that the amount of available supplemental organic carbon was adequate for the observed fermentative responses to lysate. Formate, succinate, and isobutyrate were transient in both treatments (Figure 61). Previous findings indicated that succinate can be decarboxylated to CO<sub>2</sub> and propionate, whereas formate can be converted to CO<sub>2</sub> and H<sub>2</sub> (Section 3.2.8). The continuous and strong production of CO<sub>2</sub>, H<sub>2</sub>, and propionate in soil and gut content treatments (a) reinforced the occurrence of these secondary processes and (b) illustrated that such processes were not exclusive for gut contents.

**Table 52.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in yeast lysate-supplemented gut content or soil treatments.<sup>a</sup>

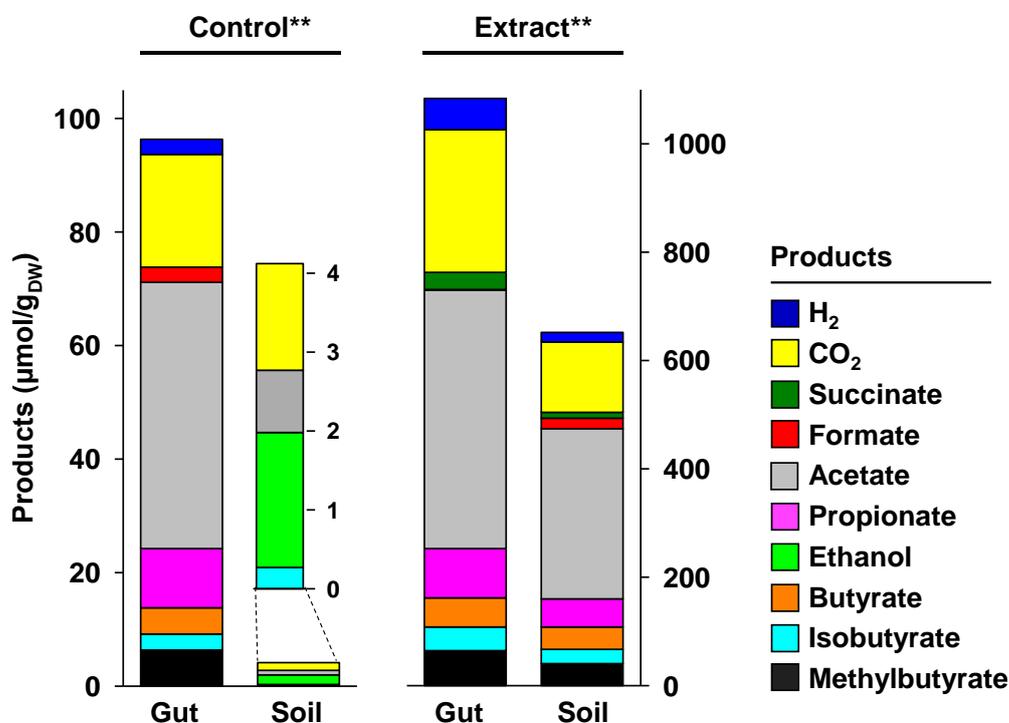
Main Products	Gut Content			Soil		
	Net amt. ( $\mu$ mol)	Recoveries (%)		Net amt. ( $\mu$ mol)	Recoveries (%)	
		Carbon	Reducing equivalents		Carbon	Reducing equivalents
CO <sub>2</sub>	158	7.7	na	131	6.4	na
H <sub>2</sub>	26	na	0.6	10	na	0.3
Acetate	145	14	14	204	20	20
Methylbutyrate	41	10	13	41	10	13
Propionate	41	6.1	7.0	27	4.0	4.6
Butyrate	10	2.0	2.5	18	3.6	4.5
Isobutyrate	11	2.2	2.7	9.8	1.9	2.4
Succinate	3.9	0.8	0.7	4.2	0.8	0.7
Formate	0.3	-	-	0.1	-	-
Total:	437	43	41	447	47	45

<sup>a</sup>See Figure 60 and Figure 61 for product profiles of lysate treatments. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. Recoveries were calculated from the net amounts of products. amt., amount; -, no net increase of the product during the incubation; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019c.

### 3.3.2. Effect of yeast extract on fermentative taxa in gut content and soil

The findings derived from fresh lysate treatments demonstrated that a complex source of nutrients rather than single high quality substrates yielded a similar stimulation of fermentative microbes in both gut contents and soil. Commercially yeast extract was used as substrate to evaluate (a) if this finding was reproducible with an alternative source of complex nutrients and (b) which bacterial taxa were associated with the observed fermentations.

Although supplemented gut content produced higher amounts of fermentation products than supplemented soil, the yeast extract was highly stimulatory for both and yielded nearly identical fermentation profiles in gut content and soil treatments (Figure 62, Table 53, and Table 54). The dominant end products of the yeast extract treatments were similar to those of the fresh lysate treatments, and approximately half of the yeast extract-derived carbon and reducing equivalents were recovered in the detected fermentation products (Table 55). Similar to cell lysate treatments, (a) the pH approximated 7 and did not vary (Table 53), and (b) the formation of the main fermentation products in response to yeast extract was statistically significant for both treatments (Figure 62). Thus, the stimulatory effect of fresh lysate was reproduced with yeast extract, confirming that the availability of complex nutrients stimulated similar fermentations in both gut contents and soil.



**Figure 62.** Effect of yeast extract on the fermentation product profiles of anoxic gut content and soil microcosms. The amount of extract-derived carbon added per microcosm approximated 2 mmol. Filter sterilized extract alone did not display any fermentation activity. Values are the average of three replicate analyses and represent the net production of products at the end of the 40 h incubation. DW, dry weight. The two asterisks (\*\*) indicate significant differences between the collective amount of products formed in gut and soil treatments ( $P \leq 0.01$ ,  $t$ -test with unequal variances). Figure modified and used with permission from Zeibich *et al.*, 2019c.

**Table 53.** Effect of yeast extract on the fermentation product profiles of gut content (A) and soil (B) treatments.<sup>a</sup>

Treatment	Time (h)	pH	Products ( $\mu\text{mol/g}_{\text{DW}}$ )									
			CO <sub>2</sub>	H <sub>2</sub>	Acetate	Succinate	Formate	Propionate	Butyrate	Methylbutyrate	Iso-butyrates	Lactate
<b>(A) Gut Content</b>												
Control	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 1.1	2.1 ± 0.2	4.7 ± 6.7	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.1	1.3 ± 0.1
	40	6.9 ± 0.0	20 ± 5.7	2.7 ± 0.1	50 ± 6.6	1.7 ± 0.2	7.4 ± 8.2	11 ± 0.6	5.7 ± 0.7	6.4 ± 0.3	5.0 ± 0.9	2.4 ± 0.3
Extract	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 0.9	5.6 ± 0.7	4.1 ± 5.3	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
	40	6.8 ± 0.0	263 ± 5.0	58 ± 13	480 ± 38	37 ± 2.6	5.4 ± 16	54 ± 2.8	54 ± 11	65 ± 5.3	44 ± 4.9	3.6 ± 0.9
<b>(B) Soil</b>												
Control	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	0.3 ± 0.0	1.7 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.2	0.7 ± 0.0
	40	6.9 ± 0.0	1.4 ± 0.2	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	1.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.0	0.7 ± 0.1
Extract	0	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.4	1.9 ± 0.3	3.4 ± 2.9	0.0 ± 0.1	1.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
	40	6.6 ± 0.0	130 ± 3.2	18 ± 2.7	315 ± 18	13 ± 1.8	23 ± 23	52 ± 3.4	42 ± 1.9	41 ± 2.1	27 ± 1.0	0.3 ± 0.2

<sup>a</sup>The amount of yeast extract-derived carbon added per microcosm approximated 2 mmol. Controls lacked supplemental yeast extract. Values are the arithmetic average of three replicate analyses ( $\pm$  standard deviation). DW, dry weight. Table modified and used with permission from Zeibich *et al.*, 2019c.

**Table 54.** *P* values of fermentation products in yeast extract-supplemented gut content (A) and soil (B) treatments, and *P* values of gut content versus soil treatments (C).<sup>a</sup>**(A) Gut Content<sup>b</sup>**

Products	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Mean value <sup>c</sup>	19	263	2.7	58	1.7	37	2.4	3.6	7.4	7.9	50	480	11	91	5.7	54	6.4	65	5.0	44
Variance	32	26	0.0	158	0.0	6.9	0.1	0.9	67	188	43	1441	0.4	8.1	0.5	130	0.1	28	0.9	24
<i>P</i> value	0.000		0.017		0.002		0.156		0.960		0.003		0.000		0.018		0.003		0.005	

**(B) Soil<sup>b</sup>**

Products	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Mean value <sup>c</sup>	1.4	130	0.0	18	0.0	13	0.7	0.3	1.0	22	1.5	315	0.0	52	0.0	42	0.0	41	1.5	27
Variance	0.0	10	0.0	7.4	0.0	3.1	0.0	0.0	0.2	538	2.2	322	0.0	11	0.0	3.6	0.0	4.5	0.0	1.1
<i>P</i> value	0.000		0.008		0.006		0.026		0.250		0.001		0.001		0.001		0.001		0.001	

**(C) Gut Content versus Soil<sup>d</sup>**

Products	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate		Acetate		Propionate		Butyrate		Methylbutyrate		Isobutyrate	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
Mean value <sup>c</sup>	263	130	58	18	37	13	3.6	0.3	7.9	22	480	315	91	52	54	42	65	41	44	27
Variance	26	10	158	7.4	6.9	3.1	0.9	0.0	188	538	1411	322	8.1	11	130	3.6	28	4.5	24	1.1
<i>P</i> value	0.000		0.033		0.001		0.026		0.417		0.006		0.000		0.204		0.005		0.026	

<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2019c.

<sup>b</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the amount of products at the end of the incubation in control (C) and yeast extract (E) treatments. See Table 53 for product profiles.

<sup>c</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{DW}}$  (DW, dry weight).

<sup>d</sup>*P* values are based on the difference between the amount of products at the end of the incubation in extract-supplemented gut content (G) and extract-supplemented soil (S) treatments.

**Table 55.** Estimated recoveries of carbon and reducing equivalents (i.e., electrons) in yeast extract-supplemented gut content and soil treatments.<sup>a</sup>

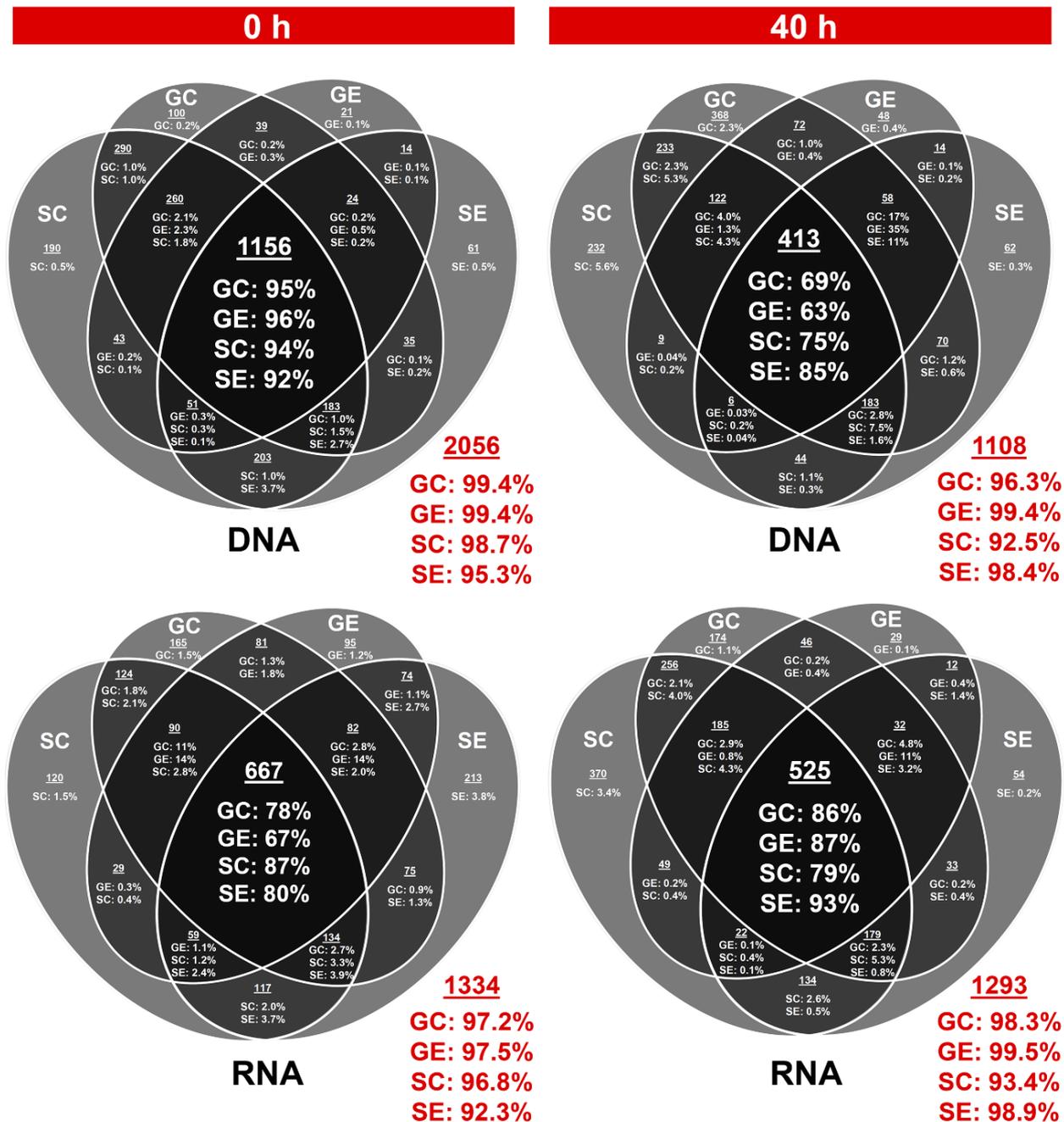
Main Products	Gut Content			Soil		
	Net amt. ( $\mu\text{mol}$ )	Recoveries (%)		Net amt. ( $\mu\text{mol}$ )	Recoveries (%)	
		Carbon	Reducing equivalents		Carbon	Reducing equivalents
CO <sub>2</sub>	110	5.5	na	89	4.4	na
H <sub>2</sub>	25	na	0.6	14	na	0.3
Acetate	194	19	19	238	24	24
Methylbutyrate	26	6.5	8.5	31	7.7	10
Propionate	36	5.4	6.3	39	5.9	6.9
Butyrate	22	4.4	5.5	32	6.3	7.8
Isobutyrate	18	3.7	4.6	20	4.0	4.9
Succinate	14	2.9	2.5	8.4	1.7	1.5
Formate	-	-	-	15	0.8	0.4
Total:	445	48	47	485	55	55

<sup>a</sup>See Table 53 for product profiles of extract treatments. Net amounts of products formed in the unsupplemented control were subtracted from those of supplemented treatments; recoveries are based on the amount of substrate provided. Values are based on the arithmetic average of three replicate analyses. amt., amount; -, no net increase of the product during the incubation; na, not applicable. Table modified and used with permission from Zeibich *et al.*, 2019c.

A total of 439,704 bacterial 16S rRNA gene and 16S rRNA sequences were obtained, yielding 2,804 phylotypes associated to 27 phyla (including candidate phyla). Most of the bacterial phylotypes detected in gut content treatments were also detected in soil treatments. Thus, 92.3% to 99.5% of the collective relative sequence abundances of the phylotypes were similar to gut and soil treatments (Figure 63). In addition, as shown in the core values of the Venn diagrams, the relative abundances of the phylotypes common to all four treatments constituted the majority of the detected sequences (Figure 63).

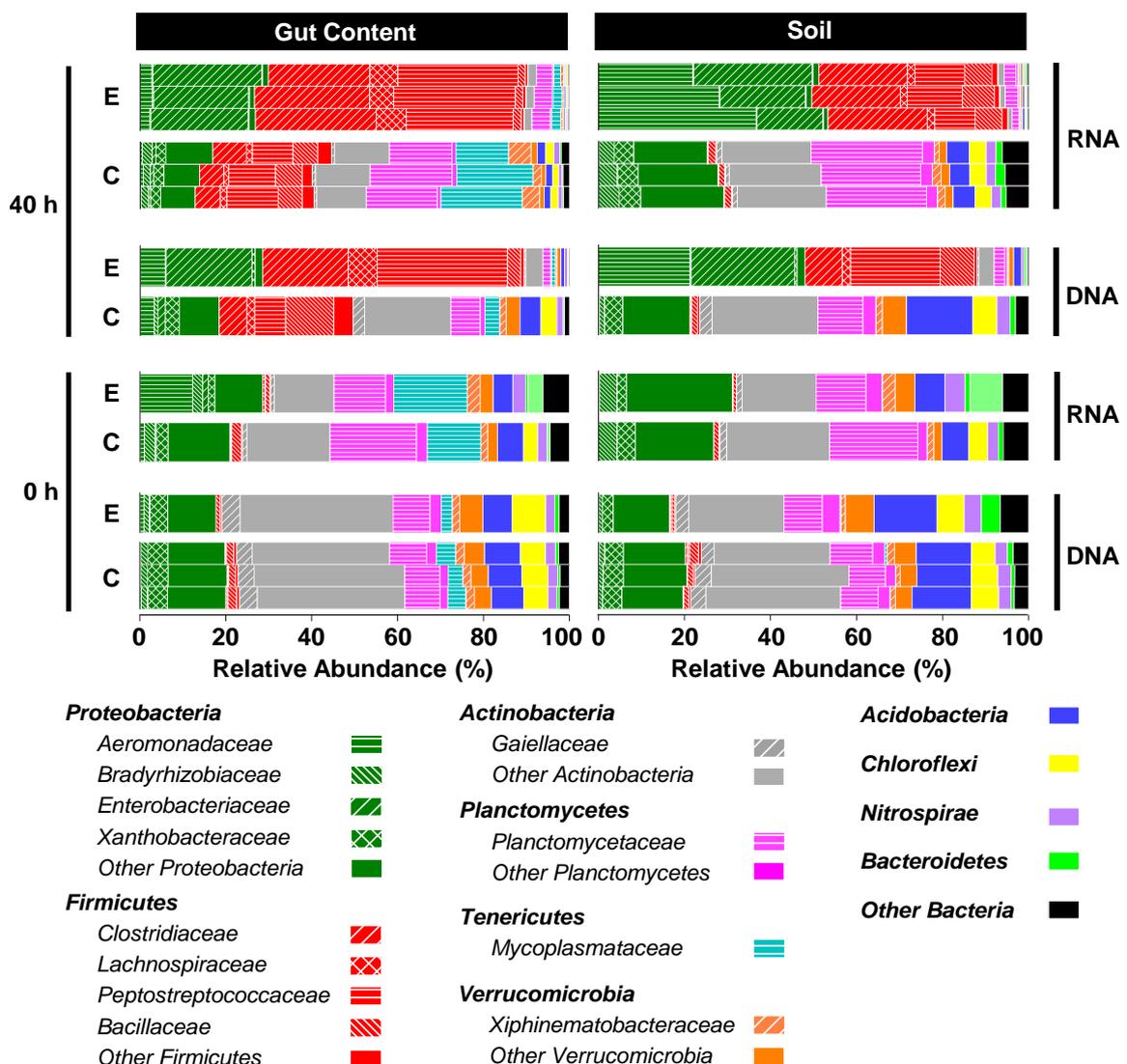
The 16S rRNA gene and 16S rRNA sequence-pool in unsupplemented soil treatments was nearly identical at the beginning and ending of the incubation (Figure 64), a finding that is consistent with the negligible fermentative activity in these treatments (Figure 62). Based on the increase in relative abundance of 16S rRNA gene or 16S rRNA sequences, *Clostridiaceae*, *Peptostreptococcaceae*, *Bacillaceae*, and *Aeromonadaceae* were responsive families in unsupplemented gut content treatments (Figure 64). Of these four families, the *Clostridiaceae*, *Peptostreptococcaceae*, and *Aeromonadaceae* were low abundant in unsupplemented soil treatments (Figure 64). In marked contrast to the detected differences between unsupplemented soil and gut content treatments, a large overlap occurred in the responsive taxa of yeast extract-supplemented gut content and soil microcosms. In this regard, the relative 16S rRNA gene and 16S rRNA sequence abundances of *Proteobacteria*- and *Firmicutes* displayed a strong increased in both extract-supplemented treatments (Figure 64). At the end of the incubation, the *Aeromonadaceae*, *Bacillaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Lachnospiraceae*, and *Peptostreptococcaceae* were the dominant families similar to gut content and soil treatments

supplemented with yeast extract (Figure 64). Although both treatments displayed different relative abundances of these six families, they collectively constituted approximately 87% and 91% of the 16S rRNA relative sequence abundances from supplemented gut content and soil treatments, respectively, at the end of the 40 h incubation. This observation indicated that the majority of the responsive taxa in lysate gut content treatments were affiliated to soil-based taxa. The apparent changes in the relative abundances of 16S rRNA sequences affiliated with these families were supported by statistical analyses (Table 56).



**Figure 63.** Venn diagrams of all detected phylotypes (97% similarity cut-off) in gut content and soil treatments at the beginning (0 h) and end (40 h) of the incubation. Underlined values are the number of phylotypes, with the collective relative abundance of these phylotypes in a given treatment shown in percent (%). Red values pertain to the phylotypes common to all treatments. Abbreviations: GC, gut content control; GE, gut content yeast extract; SC, soil control; SE, soil yeast extract; DNA, 16S rRNA gene; RNA, 16S rRNA. Figure modified and used with permission from Zeibich *et al.*, 2019c.

The trend of strongly stimulated subgroups in the fermentative communities of supplemented soil and gut content was corroborated by alpha diversity analysis. In this regard, the number of detected phylotypes, the number of expected phylotypes (Chao1), and Shannon indices at the end of the incubation were lower than those of unsupplemented controls (Figure 66 and Table 57). In addition, the collective relative abundances of the phylotypes similar to each of the replicated sequence analyses (a) were nearly identical and (b) constituted the vast majority (i.e., 90 to 97%) of the sequences obtained (Figure 65). These findings illustrated the reproducible detection of the most abundant phylotypes (Figure 65), and the associated rarefaction analyses indicated that the majority of the affiliated sequences in soil and gut content treatments were targeted (Figure 66).



**Figure 64.** Effect of yeast extract on the relative abundances of the most abundant fermentative families in *L. terrestris* gut content and soil microcosms. Abundances are based on the analyses of 16S rRNA genes (DNA) or 16S rRNA (RNA). The families represent taxa that had a  $\geq 4\%$  relative abundance in at least one sampling and are color-coded to the respective phylum. Information on all detected taxa is provided in Table A9. Abbreviations: C, unsupplemented control; E, yeast extract treatment. Samples of the three replicates of a treatment were pooled for each sampling, except for the 16S rRNA gene samples at the beginning of the incubation and 16S rRNA samples at the end of the incubation in which each bar represents one replicate. Figure modified and used with permission from Zeibich *et al.*, 2019c.

**Table 56.** Statistical analyses of abundant responsive families in yeast extract-supplemented gut content (A) and soil (B) treatments.<sup>a</sup>**(A) Gut Content**

Family	Treatment	Mean	Variance	<i>P</i> value <sup>b</sup>	LDA Score (log <sub>10</sub> ) <sup>c</sup>
<i>Aeromonadaceae</i>	Control	0.5	0.1	0.001	4.4 <sup>(5)</sup>
	Extract	2.6	0.1		
<i>Bacillaceae</i> <sup>d</sup>	Control	5.8	0.1	0.000	-4.8 <sup>(5)</sup>
	Extract	1.8	0.0		
<i>Clostridiaceae</i>	Control	6.5	1.5	0.001	5.4 <sup>(2)</sup>
	Extract	26	5.3		
<i>Enterobacteriaceae</i>	Control	0.7	0.0	0.002	5.4 <sup>(3)</sup>
	Extract	23	3.0		
<i>Lachnospiraceae</i>	Control	1.3	0.1	0.007	4.8 <sup>(4)</sup>
	Extract	6.4	0.5		
<i>Peptostreptococcaceae</i>	Control	11	1.6	0.001	5.4 <sup>(1)</sup>
	Extract	27	3.6		

**(B) Soil**

Family	Treatment	Mean	Variance	<i>P</i> value <sup>b</sup>	LDA Score (log <sub>10</sub> ) <sup>c</sup>
<i>Aeromonadaceae</i>	Control	0.0	0.0	0.021	5.5 <sup>(1)</sup>
	Extract	29	55		
<i>Bacillaceae</i>	Control	1.6	0.0	0.006	4.8 <sup>(5)</sup>
	Extract	6.7	0.4		
<i>Clostridiaceae</i>	Control	0.1	0.0	0.002	5.3 <sup>(2)</sup>
	Extract	21	2.1		
<i>Enterobacteriaceae</i>	Control	0.0	0.0	0.028	5.3 <sup>(3)</sup>
	Extract	21	38		
<i>Lachnospiraceae</i>	Control	0.0	0.0	0.001	4.2 <sup>(6)</sup>
	Extract	1.7	0.0		
<i>Peptostreptococcaceae</i>	Control	0.1	0.0	0.008	5.1 <sup>(4)</sup>
	Extract	11	3.0		

<sup>a</sup>A family was considered to be responsive when the mean relative abundance of 16S rRNA sequences in at least one yeast extract treatment (gut content or soil) was at least 4% greater than that of the unsupplemented control at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019c.

<sup>b</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the relative abundance of 16S rRNA sequences in control and yeast extract treatments at the end of the incubation.

<sup>c</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to extract compared to lower ranking ones).

<sup>d</sup>The negative LDA score reflects the higher relative abundance of *Bacillaceae*-affiliated 16S rRNA sequences in control compared to extract treatments.

**Table 57.** Alpha diversity of the microbial community in control and yeast extract-supplemented gut content (A) and soil (B) treatments.<sup>a</sup>**(A) Gut Content**

Sample (Sampling Time)	Treatment <sup>b</sup>	Number of sequences	Observed phylotypes <sup>c</sup> (normalized) <sup>d</sup>	Chao1 (normalized) <sup>d</sup>	Shannon (normalized) <sup>d</sup>
DNA (0 h)	Control 1	21172	1533 (791)	1993 (1048)	5.9 (5.7)
	Control 2	22232	1583 (794)	2018 (1059)	5.9 (5.7)
	Control 3	24907	1659 (796)	2053 (1040)	6.0 (5.7)
	Extract	25394	1608 (786)	2126 (1045)	5.9 (5.7)
RNA (0 h)	Control	13925	1418 (817)	1872 (1046)	5.9 (5.6)
	Extract	11244	1177 (690)	1653 (920)	5.1 (4.9)
DNA (40 h)	Control	30246	1519 (672)	2068 (951)	5.4 (5.2)
	Extract	22629	742 (295)	1338 (496)	3.3 (3.2)
RNA (40 h)	Control 1	7840	872 (580)	1293 (741)	4.8 (4.7)
	Control 2	7197	878 (590)	1353 (752)	5.0 (4.8)
	Control 3	12629	1138 (647)	1538 (858)	5.2 (5.0)
	Extract 1	12511	564 (276)	954 (441)	3.2 (3.2)
	Extract 2	12853	530 (262)	995 (417)	3.2 (3.1)
	Extract 3	11362	492 (256)	1001 (394)	3.1 (3.1)
<b>P value<sup>e</sup></b>			0.041 (0.004)	0.032 (0.003)	0 (0.003)

**(B) Soil**

Sample (Sampling Time)	Treatment <sup>b</sup>	Number of sequences	Observed phylotypes <sup>c</sup> (normalized) <sup>d</sup>	Chao1 (normalized) <sup>d</sup>	Shannon (normalized) <sup>d</sup>
DNA (0 h)	Control 1	25672	1794 (849)	2243 (1144)	6.1 (5.9)
	Control 2	24505	1800 (855)	2274 (1133)	6.1 (5.9)
	Control 3	21094	1787 (883)	2313 (1163)	6.2 (5.9)
	Extract	19222	1727 (863)	2189 (1092)	6.3 (6.0)
RNA (0 h)	Control	10311	1340 (830)	1792 (1031)	6.1 (5.8)
	Extract	10063	1421 (841)	1940 (1032)	6.2 (5.9)
DNA (40 h)	Control	7777	1242 (809)	1816 (994)	6.1 (5.9)
	Extract	19844	850 (365)	1428 (619)	3.1 (3.0)
RNA (40 h)	Control 1	6544	1079 (755)	1519 (918)	5.8 (5.6)
	Control 2	7438	1165 (775)	1682 (951)	5.9 (5.7)
	Control 3	9787	1286 (811)	1752 (1000)	6.0 (5.8)
	Extract 1	15477	529 (219)	1173 (400)	2.7 (2.6)
	Extract 2	13072	558 (260)	1105 (438)	2.9 (2.8)
	Extract 3	12757	578 (273)	1048 (441)	2.9 (2.9)
<b>P value<sup>e</sup></b>			0.010 (0.000)	0.006 (0.000)	0.000 (0.000)

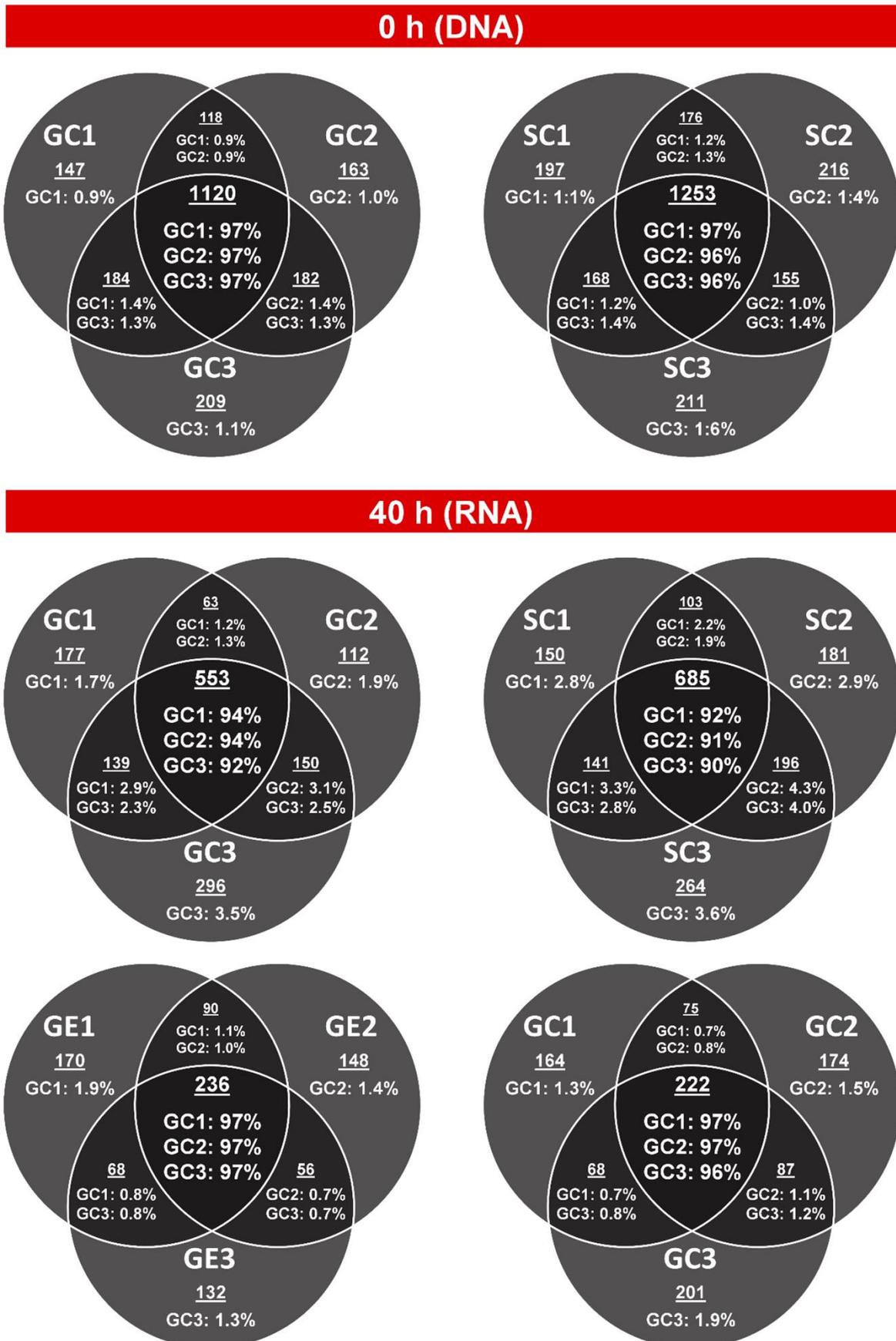
<sup>a</sup>Table modified and used with permission from Zeibich *et al.*, 2019c.

<sup>b</sup>Samples of the three replicates of a treatment were pooled except for 16S rRNA gene (DNA) samples at 0 h and 16S rRNA (RNA) samples at 40 h. Numbers assigned to a treatment (e.g., Control 1) indicate the respective replicate. C, control treatment; E, yeast extract treatment.

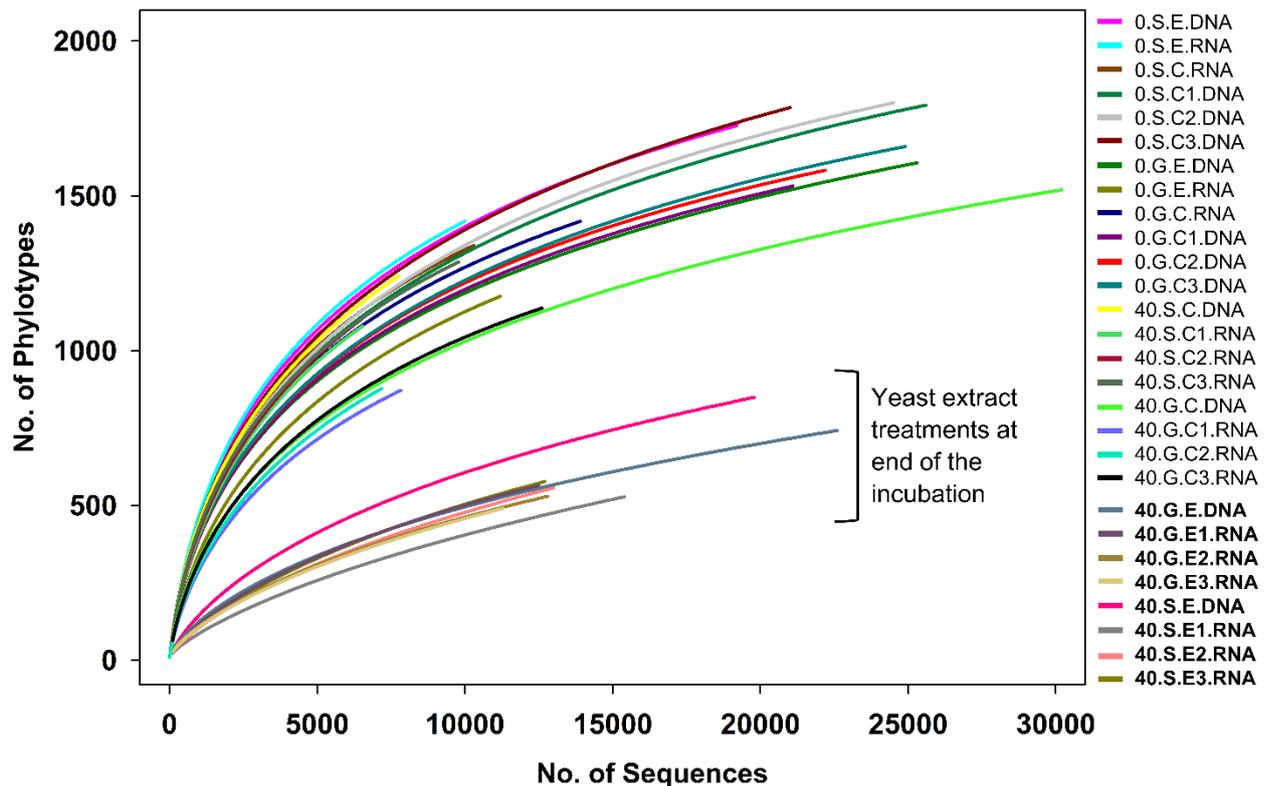
<sup>c</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>d</sup>The data sets were normalized to 5,000 sequences for comparison of amplicon libraries of different sizes.

<sup>e</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the 16S rRNA analysis and the difference between the values of observed phylotypes, Chao1, and Shannon at the end of the incubation in control and yeast extract treatments.



**Figure 65.** Venn diagrams of all detected phylotypes (97% similarity cut-off) in the three replicates of gut content and soil treatments at the beginning (0 h) and end (40 h) of the incubation. Underlined values are the number of phylotypes, with the collective relative abundance of these phylotypes in a given replicate shown in percent (%). Numbers assigned to a treatment (e.g., GC1) indicate the respective replicate. Abbreviations: GC, gut content control; SC, soil control; GE, gut content yeast extract; SE, soil yeast extract; DNA, 16S rRNA gene; RNA, 16S rRNA. Figure modified and used with permission from Zeibich *et al.*, 2019c.



**Figure 66.** Rarefaction analyses of bacterial 16S rRNA (RNA) and 16S rRNA gene (DNA) sequences obtained from control (C) and yeast extract (E) treatments. Phylotypes were based on a 97% sequence similarity cut-off. Samples of the three replicates of a treatment were pooled except for 16S rRNA gene samples at the beginning of the incubation (0 h) and 16S rRNA samples at the end of incubation (40 h). Numbers assigned to a treatment (e.g., C1) indicate the respective replicate. Additional abbreviations: 0 and 40, time of sampling in hours; G, gut content; S, soil. Figure modified and used with permission from Zeibich *et al.*, 2019c.

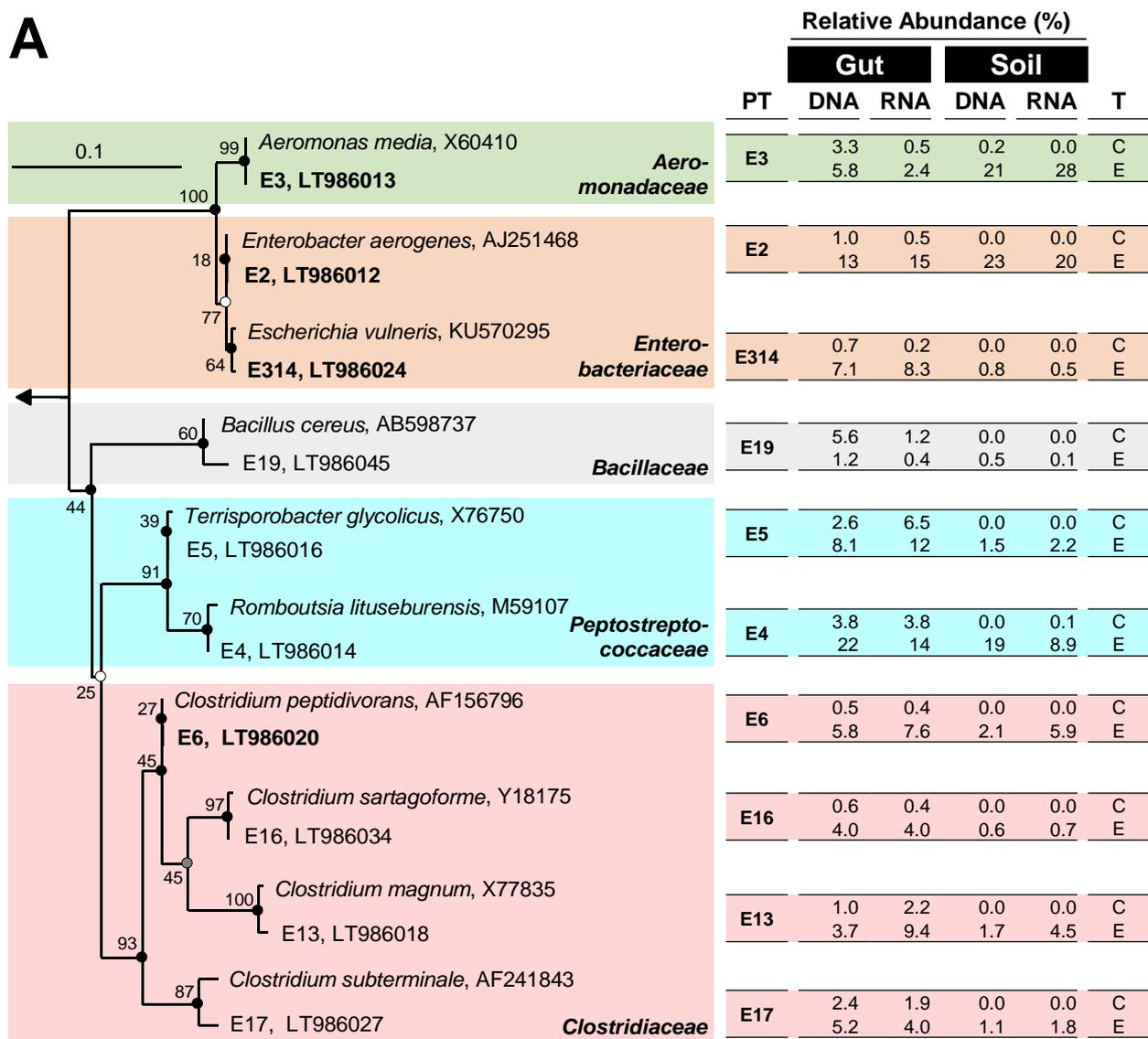
### 3.3.3. Responsive soil- and gut content-phylotypes

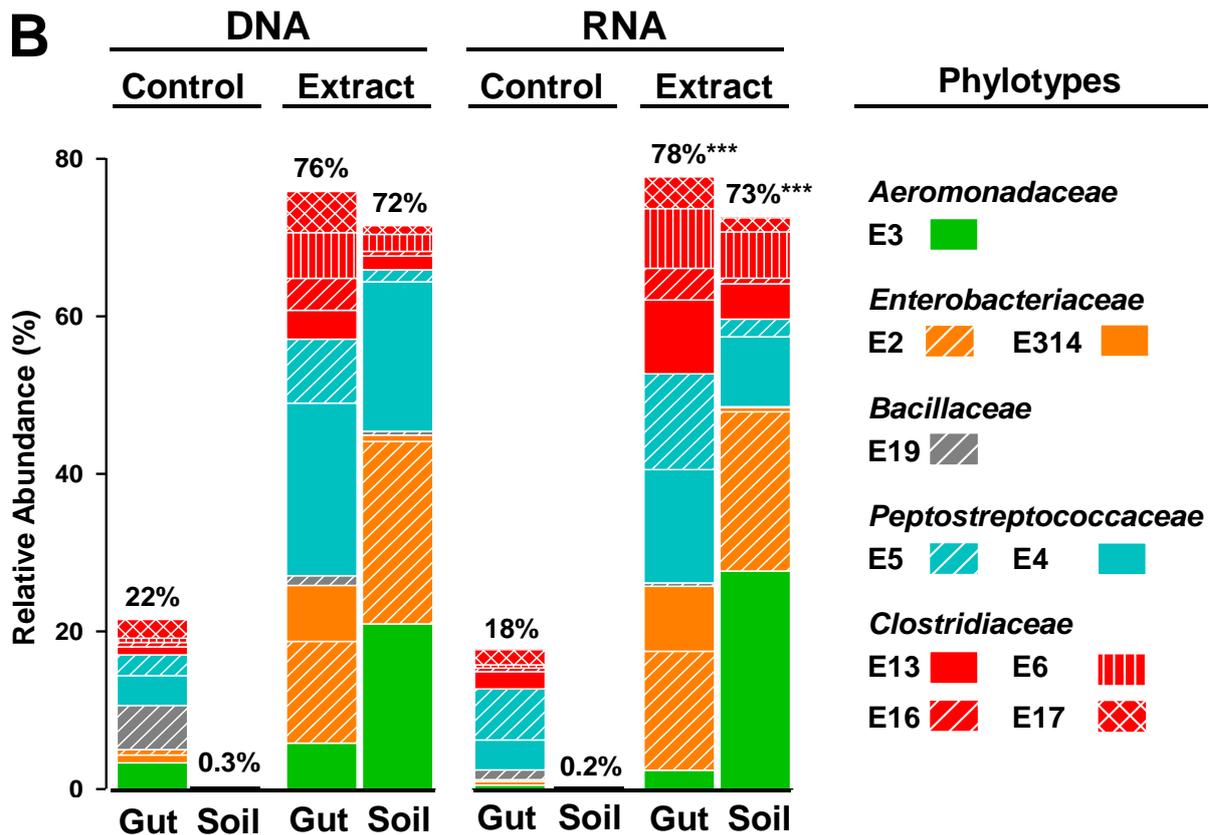
Based on the relative sequence abundances of 16S rRNA sequences, the aforementioned trend of extract-stimulated families extended to ten abundant phylotypes (Figure 67). For example, the relative sequence abundance of phylotype E2 (closely related to *E. aerogenes*), phylotype E17 (closely related to *Clostridium subterminale*), and phylotype E6 (closely related to *C. peptidivorans*) significantly increased in extract-supplemented treatments of both gut contents and soil (Figure 67). Phylotype E5 (closely related to *T. glycolicus*) and phylotype E13 (closely related to *C. magnum*) were additional phylotypes that were responsive in both extract-supplemented treatments. Furthermore, sequences affiliated to phylotype E4 (closely related to *R. lituseburensis*) and phylotype E314 (closely related to *Escherichia vulneris*) were abundant in gut content treatments but less abundant in soil treatments at the end of the incubation (Figure 67).

The relative abundance of a phylotype was in some cases different between gut content and soil treatments, or control and supplemented treatments. Thus, phylotype E3 (closely related to *A. hydrophila*) was abundant in yeast extract-supplemented soil but less abundant in extract-supplemented gut content at the end of incubation (Figure 67 B). Furthermore, phylotype E19

(related to *Bacillus cereus*) was abundant in control gut content treatments but less abundant in extract-supplemented treatments (Figure 67 B). However, the ten phylotypes were hardly detectable in unsupplemented soil treatments at the end of the incubation, and collectively constituting only 0.3% and 0.2% of the relative 16S rRNA gene and 16S rRNA abundances, respectively. In marked contrast, these ten phylotypes collectively constituted 22% and 18% of the relative 16S rRNA gene and 16S rRNA abundances, respectively, in unsupplemented gut content treatments (Figure 67 B). This finding illustrates that these phylotypes responded to endogenous gut nutrients but were only marginally able to respond to soil nutrients. However, the same phylotypes were distinctly abundant in gut content and soil when these treatments were supplemented with a complex substrate. They collectively constituting 76% and 78% of the relative 16S rRNA gene and 16S rRNA abundances, respectively, in supplemented gut content treatments, and 72% and 73% of the relative 16S rRNA gene and 16S rRNA abundances, respectively, in supplemented soil treatments (Figure 67 A).

**A**





**Figure 67.** 16S rRNA-based phylogenetic tree of stimulated phylotypes in gut content and soil treatments (A) and comparative overview of the relative abundances of these ten phylotypes (B) at the end of the incubation. Panel A: The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees; empty circles, congruent nodes in neighbor-joining and maximum parsimony trees; grey circles, congruent nodes in maximum parsimony and maximum likelihood trees. Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch. Phylotypes (E) are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one gut content or soil treatment displayed a minimum increase in relative abundance of 4% during the incubation. The phylotypes are derived from the analyses of 16S rRNA genes (DNA) or 16S rRNA (RNA), and the table displays the relative abundances of each phylotype at the end of the incubation. Abbreviations: T, treatment; C, unsupplemented control; E, yeast extract. Panel B: Combined relative abundance of the most responsive phylotypes in each treatment at the end of the incubation. The three asterisks (\*\*\*) beside the collective total 16S rRNA relative abundances (%) of the phylotypes indicate significant differences between the control and yeast extract treatments ( $P \leq 0.001$ , *t*-test with unequal variances). Figure modified and used with permission from Zeibich *et al.*, 2019c.

**Table 58.** Statistical analyses of phylotypes displayed in Figure 67.<sup>a</sup>

**(A) Gut Content**

Phylotype	Treatment	Mean	Variance	<i>P</i> value	LDA Score (log10) <sup>b</sup>
E2	Control	0.5	0.0	0.002	5.2 <sup>(1)</sup>
	Extract	15	1.4		
E3	Control	0.5	0.1	0.002	4.4 <sup>(10)</sup>
	Extract	2.4	0.1		
E4	Control	3.8	0.3	0.001	5.2 <sup>(2)</sup>
	Extract	14	1.0		
E5	Control	6.5	0.6	0.021	5.1 <sup>(3)</sup>
	Extract	12	4.2		

Phylotype	Treatment	Mean	Variance	<i>P</i> value	LDA Score (log <sub>10</sub> ) <sup>b</sup>
E13	Control	2.2	0.5	0.003	5.0 <sup>(4)</sup>
	Extract	9.4	1.3		
E6	Control	0.4	0.1	0.006	4.9 <sup>(6)</sup>
	Extract	7.6	0.8		
E314	Control	0.2	0.0	0.004	4.9 <sup>(5)</sup>
	Extract	8.3	0.7		
E17	Control	1.9	0.3	0.012	4.6 <sup>(8)</sup>
	Extract	4.0	0.1		
E16	Control	0.4	0.0	0.174	4.6 <sup>(7)</sup>
	Extract	4.0	9.0		
E19 <sup>c</sup>	Control	1.2	0.0	0.001	- 4.1 <sup>(7)</sup>
	Extract	0.4	0.0		

**(B) Soil**

Phylotype	Treatment	Mean	Variance	<i>P</i> value	LDA Score (log <sub>10</sub> ) <sup>b</sup>
E2	Control	0.0	0.0	0.031	5.3 <sup>(2)</sup>
	Extract	20	39		
E3	Control	0.0	0.0	0.022	5.4 <sup>(1)</sup>
	Extract	28	54		
E4	Control	0.1	0.0	0.008	4.9 <sup>(3)</sup>
	Extract	8.9	1.9		
E5	Control	0.0	0.0	0.008	4.4 <sup>(10)</sup>
	Extract	2.2	0.1		
E13	Control	0.0	0.0	0.006	4.7 <sup>(5)</sup>
	Extract	4.5	0.4		
E6	Control	0.0	0.0	0.002	4.8 <sup>(4)</sup>
	Extract	5.9	0.2		
E314	Control	0.0	0.0	0.035	3.7 <sup>(15)</sup>
	Extract	0.5	0.0		
E17	Control	0.0	0.0	0.003	4.2 <sup>(11)</sup>
	Extract	1.8	0.0		
E16	Control	0.0	0.0	0.017	3.9 <sup>(14)</sup>
	Extract	0.7	0.0		
E19	Control	0.0	0.0	0.027	3.1 <sup>(26)</sup>
	Extract	0.1	0.0		

<sup>a</sup>Mean values are based on the relative abundance of 16S rRNA sequences of the three replicates at the end of the incubation. *P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances and are based on the difference between the relative abundance of 16S rRNA sequences in control and yeast extract treatments at the end of the incubation. Table modified and used with permission from Zeibich *et al.*, 2019c.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking phylotypes exhibited a stronger response to extract compared to lower ranking ones).

<sup>c</sup>The negative LDA score reflects the higher relative abundance of E19-affiliated 16S rRNA sequences in control compared to extract treatments.

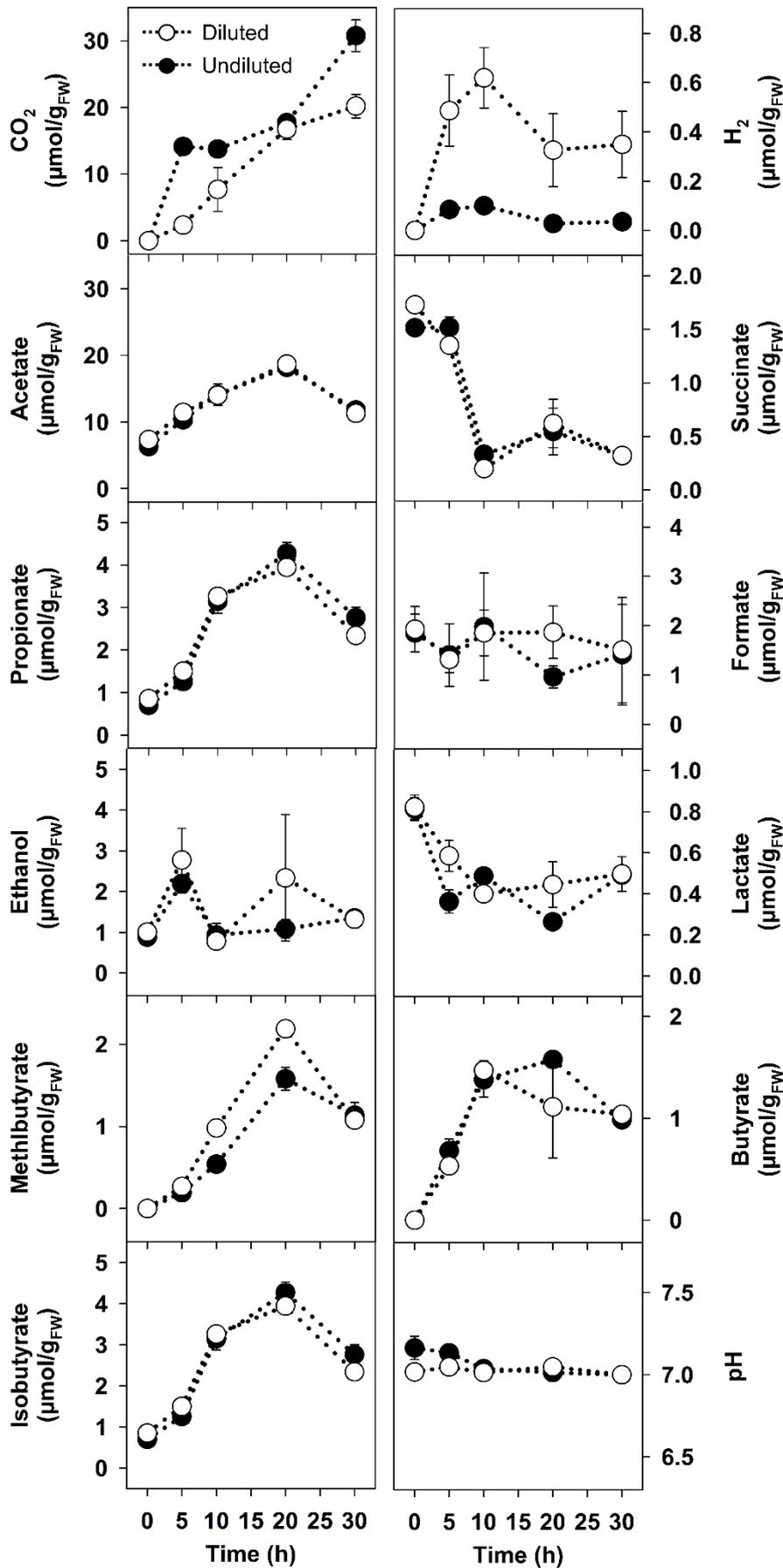
### 3.4. Impact of increased water content on the fermentative gut community of *L. terrestris*

Large experimental set ups required a 1:10 dilution of the extracted gut contents for obtaining adequate samples for chemical and molecular analyses (Wüst *et al.*, 2011; Section 2.1.2). In addition to the experimentally necessary dilution of the gut associated microbes, the earthworm ingested soil bacteria experience also *in situ* high fluctuations in water content during gut passage (Section 1.2; Horn *et al.*, 2003). These considerations prompted the comparison of the fermentation in diluted and undiluted gut contents by (a) evaluating the fermentative activities and (b) analyzing the microbial shifts during anoxic incubations.

#### 3.4.1. Effect of increased water content on gut fermentative taxa

The differences between fermentation products formed during the incubation in diluted and undiluted gut content treatments were small and, except of CO<sub>2</sub>, not significant (Figure 68 and Table 59), indicating only a marginal impact of the dilution on the fermentative activity of gut-associated microbes. However, the collective net amount of products was significantly higher in diluted gut contents than in the undiluted gut contents, an observation mainly caused by the enhanced CO<sub>2</sub> production in undiluted treatments (Figure 69 A). As observed in previous studies, accumulated succinate was in both diluted and undiluted treatments consumed during the incubation and the concomitant production of propionate and CO<sub>2</sub>, suggesting the decarboxylation of succinate. This assumption was reinforced by the lack of propionate production at the end of incubation when succinate was hardly detectable.

Based on the 16S rRNA gene and 16S rRNA analyses, a total of 360,508 bacterial sequences were obtained from the diluted and undiluted treatments, yielding 29 phyla (including candidate phyla). Consistent with the marginal differences in fermentation activity, the analyses indicated that most of the abundant responsive families of the diluted and undiluted gut content communities displayed nearly identical shifts during the anoxic incubation (Figure 69 B and Figure 70). In this regard, the relative sequence abundances (either 16S rRNA or 16S rRNA) of *Shewanellaceae*, *Peptostreptococcaceae*, *Lachnospiraceae* and *Fucobacteriaceae* were significantly greater in both treatments at the end of incubation than at the beginning of incubation (Figure 69 B, Figure 70, and Table 60). The stimulation of a family was in some cases quantitatively or qualitatively different between diluted and undiluted gut contents. For example, the *Firmicutes*-affiliated family *Peptostreptococcaceae* responded positively in both treatments, but was apparently more stimulated in the undiluted gut content than in the diluted gut content (Figure 69 B). The diluted treatment displayed in addition to the weakly stimulated *Peptostreptococcaceae* a significant increase of relative sequence abundances affiliated to *Lachnospiraceae* and *Clostridiaceae* (Figure 69, Figure 70, and Table 60).



**Figure 68.** Effect of increased water content on the fermentation product profiles of anoxic microcosms of *L. terrestris*. Values are the arithmetic average of three replicate analyses, and error bars indicate the standard deviations. Some standard deviations are smaller than the size of the symbol and therefore not apparent. FW, fresh weight.

**Table 59.** *P* values of fermentation products in undiluted (U) and diluted (D) gut contents of *L. terrestris*.<sup>a</sup>

Products	CO <sub>2</sub>		H <sub>2</sub>		Succinate		Lactate		Formate	
	U	D	U	D	U	D	U	D	U	D
<b>Treatment</b>	U	D	U	D	U	D	U	D	U	D
<b>Mean value<sup>b</sup></b>	31	20	0.0	0.3	-1.2	-1.4	-0.3	-0.3	-0.4	-0.4
<b>Variance</b>	5.6	3.1	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.7
<b><i>P</i> value</b>	0.003		0.057		0.032		0.970		0.987	

Products	Acetate		Propionate		Ethanol		Butyrate		Methylbutyrate	
	U	D	U	D	U	D	U	D	U	D
<b>Treatment</b>	U	D	U	D	U	D	U	D	U	D
<b>Mean value<sup>b</sup></b>	5.5	3.9	2.1	1.5	0.5	0.3	1.0	1.0	1.1	1.1
<b>Variance</b>	0.1	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>P</i> value</b>	0.072		0.075		0.257		0.432		0.624	

<sup>a</sup>*P* values (significant at  $P \leq 0.05$ ) were calculated by *t*-test with unequal variances, and are based on the difference between the net amount of products at the end of the incubation in undiluted (U) and diluted (D) treatments. To calculate net amounts, amounts of products at the beginning of incubation were subtracted from those at the end of incubation. See Figure 68 for product profiles.

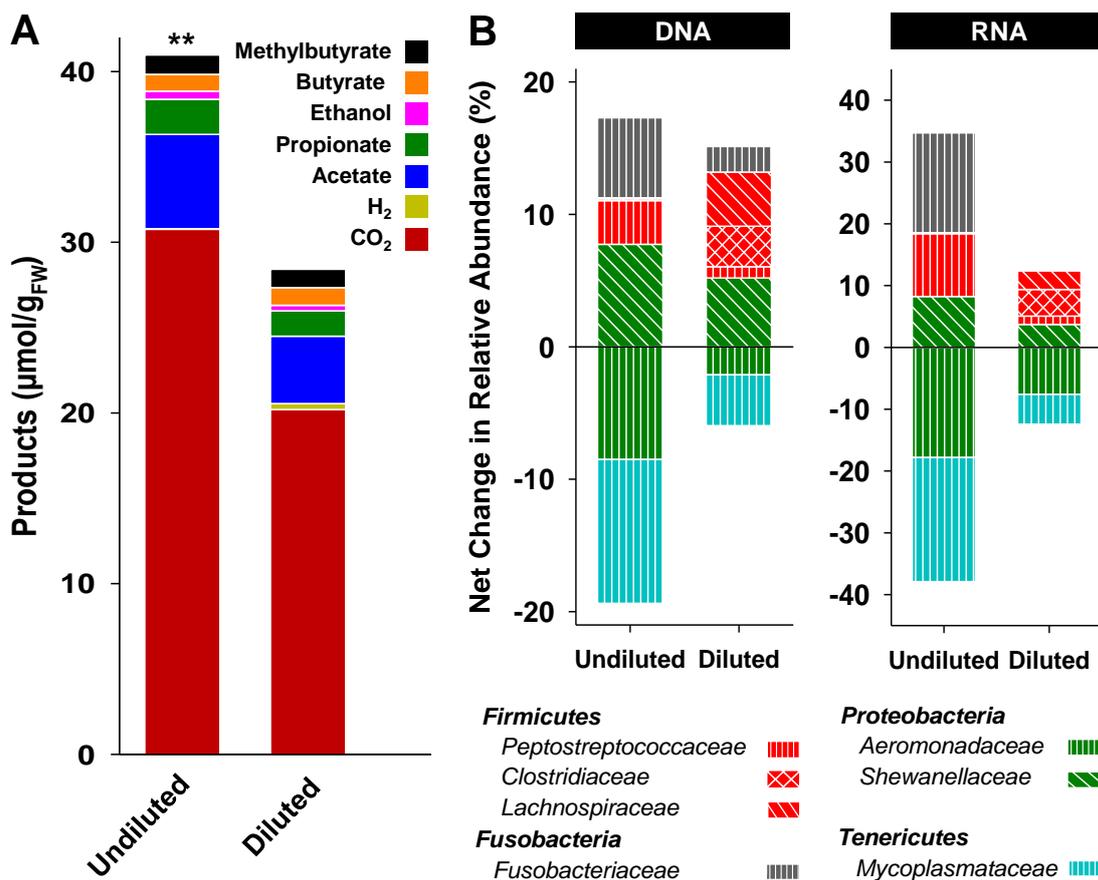
<sup>b</sup>Mean values ( $n = 3$ ) are in  $\mu\text{mol/g}_{\text{FW}}$ . FW, fresh weight.

In marked contrast to the stimulation of aforementioned families, *Mycoplasmataceae* and *Aeromonadaceae* displayed a significant decrease in both treatments during incubation (Figure 69 B, Figure 70, and Table 60). However, the detectable shifts of the families in undiluted and diluted communities during the incubation were more similar than dissimilar. Furthermore, these shifts were more pronounced in undiluted than diluted gut contents and therefore at least in part responsible for the higher fermentative activity in undiluted treatments. Consistent with the stimulation of *Firmicutes*-, *Proteobacteria*-, and *Fusobacteria*-affiliated families, the numbers of detected phylotypes, the number of expected phylotypes (Chao1), and the Shannon indices were slightly lower at the end of the incubation than at the beginning of incubation (Table 61), and the rarefaction analyses indicated that the most abundant taxa were targeted (Figure 71).

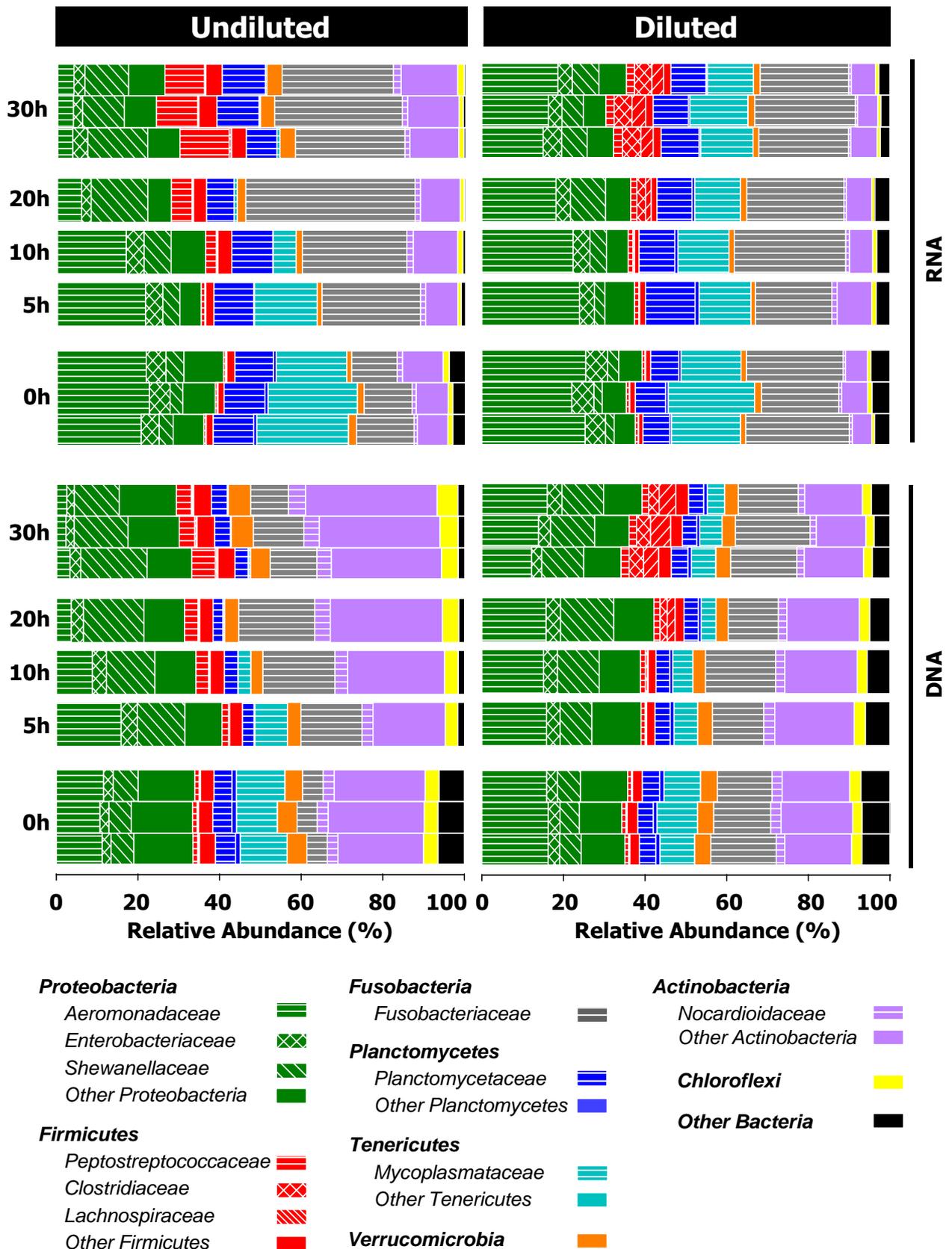
### 3.4.2. Responsive phylotypes

The aforementioned findings demonstrated that especially *Peptostreptococcaceae*, *Shewanellaceae* and *Fusobacteriaceae* were stimulated in both treatments during the incubation, whereas *Mycoplasmataceae* and *Aeromonadaceae* responded negatively during the incubation (Figure 69). These trends extended to six most responsive phylotypes (Figure 72 A). For example, *Fusobacteriaceae*-affiliated phylotype D1 (related to *C. somerae*), *Shewanellaceae*-affiliated phylotype D2 (closely related to *Shewanella putrefaciens*), and *Peptostreptococcaceae*-affiliated phylotype D5 (closely related to *P. bifermentans*) displayed a  $\geq 4\%$  net increase (at either the 16S rRNA or 16S rRNA gene level) in at least one of the treatments during the incubation (Figure 72). The stimulation of phylotype D2 and phylotype D5 was supported by the results of statistical analyses (Table 62). *Aeromonadaceae*-affiliated phylotype D4 (closely related to *A.*

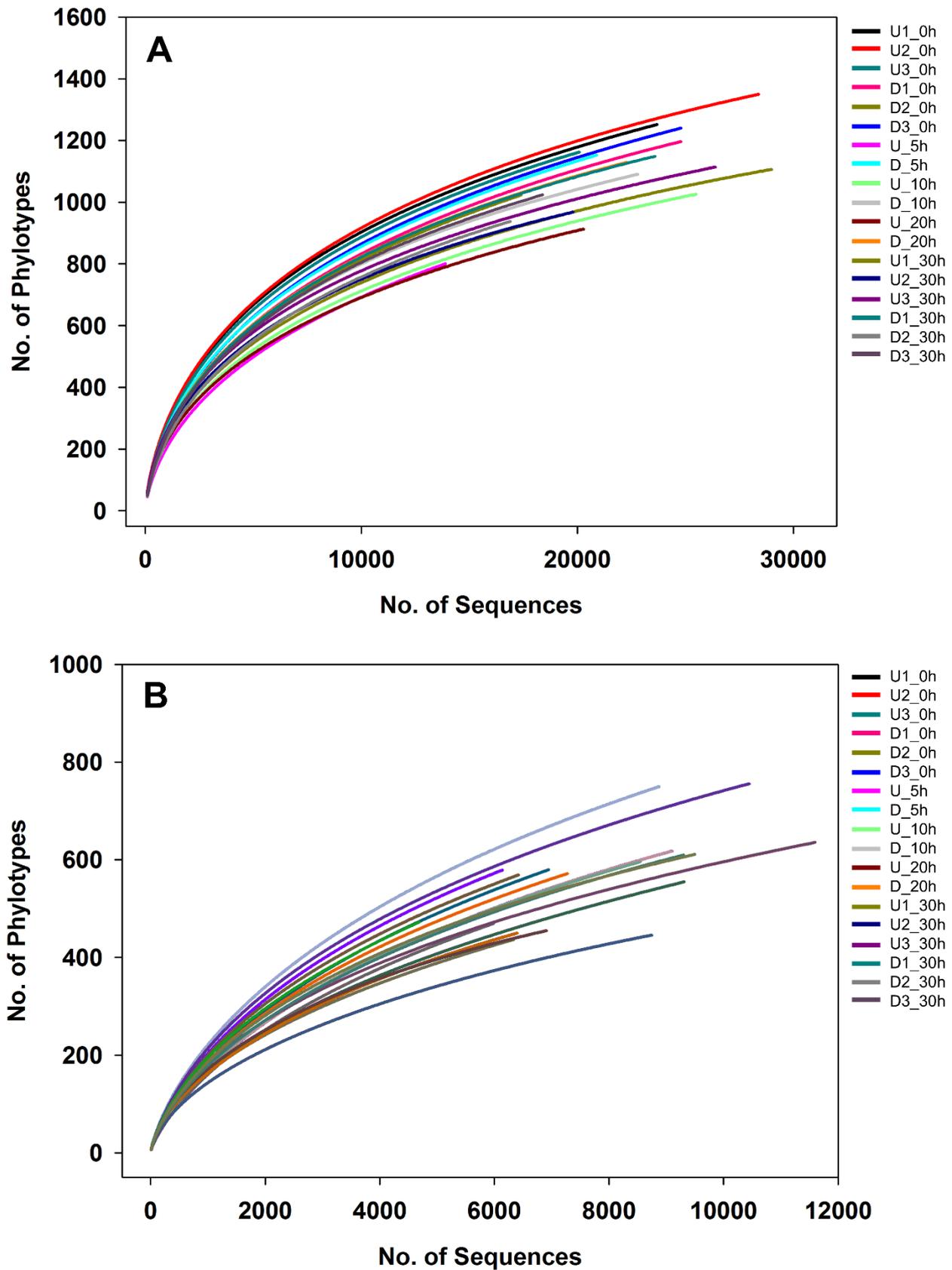
*hydrophila* and *A. media*) displayed a significant decrease at 16S rRNA gene and 16S rRNA level in both treatments during the incubation (Figure 72 and Table 62). Phylotype D179 (also closely related to *A. hydrophila* and *A. media* [Figure 72 C]) displayed a contrasting net change in relative abundance based on 16S rRNA gene and 16S rRNA analysis (Figure 72 B). Thus, the relative 16S rRNA gene abundance of this phylotype increased during incubation in diluted treatments, whereas the relative 16S rRNA abundance displayed a net decrease during incubation in this treatment (Figure 72 B). This observation is consistent with previous findings, demonstrating an initial increase and subsequent decrease of *A. hydrophila*-affiliated relative 16S rRNA abundances during incubation, whereby the affiliated relative 16S rRNA gene abundances at the end of incubation were apparently higher than the decreased 16S rRNA abundances (Figure 43). The family *Mycoplasmataceae* was represented by phylotype D3 (closely related to *Can. Lumbricincola*), a taxon that responded negatively in both treatments (Figure 72). Consistent with the aforementioned responses of fermentative families, the shifts of the most responsive phylotypes were more pronounced in undiluted treatments than diluted treatments.



**Figure 69.** Collective amounts of fermentation products (A) and most responsive families (B) in undiluted and diluted gut contents of *L. terrestris*. Panel A: Values are the average of triplicate analyses in Figure 68 and represent the net amounts of products at the end of the 30 h incubation. The asterisks indicate a significant difference between the collective amount of products formed in the undiluted treatment and diluted treatment (\*\*,  $P \leq 0.01$ ). FW, fresh weight. Panel B: Families were considered to be responsive when a family in at least one of the undiluted or diluted treatments displayed a  $\geq 4\%$  higher or lower relative 16S rRNA gene (DNA) or 16S rRNA (RNA) sequence abundance at the end of incubation than at the beginning of incubation. All 16S rRNA gene and 16S rRNA samples at 0 h and 30 h were analyzed separately.



**Figure 70.** Effect of a increased water content on the temporal changes of the relative abundances of bacterial phyla in *L. terrestris* gut content microcosms based on the analyses of 16S rRNA genes and 16S rRNA. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Information on all detected taxa is provided in Table A10. All 16S rRNA gene (DNA) and 16S rRNA (RNA) samples at 0 h and 30 h were analyzed separately, and samples of the three replicates were pooled for each of the other treatments at 5 h, 10 h, or 20 h. Process data are shown in Figure 68.



**Figure 71.** Rarefaction analyses of bacterial 16S rRNA gene (A) and 16S rRNA (B) sequences obtained from undiluted (U) and diluted (D) gut contents of *L. terrestris*. Phylotypes were based on a 97% sequence similarity cut-off. All 16S rRNA gene and 16S rRNA samples at 0 h and 30 h were analyzed separately, and samples of the three replicates were pooled for each of the other treatments at 5 h, 10 h, or 20 h. Identification numbers (e.g., D1) indicate the respective replicates.

**Table 60.** Statistical analyses of the most responsive families displayed in Figure 69 based on 16S rRNA gene (A) and 16S rRNA (B) analysis.<sup>a</sup>**(A) 16S rRNA gene**

Treatment	Family	Sampling Time (h)	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
Undiluted	<i>Clostridiaceae</i>	0	0.2	0.0	0.2	
		30	0.3	0.0	0.3	3.5 <sup>(7)</sup>
	<i>Fusobacteriaceae</i>	0	5.0	0.0	5.0	
		30	11	1.6	11	5.0 <sup>(2)</sup>
	<i>Peptostreptococcaceae</i>	0	1.1	0.0	1.1	
		30	4.4	1.2	3.8	4.6 <sup>(5)</sup>
	<i>Shewanellaceae</i>	0	5.7	0.3	5.6	
		30	14	2.7	13	5.1 <sup>(1)</sup>
	<i>Aeromonadaceae</i>	0	11	1.0	12	
		30	0.3	0.2	0.2	5.0 <sup>(1)</sup>
	<i>Mycoplasmataceae</i>	0	11	0.4	11	
		30	2.7	0.5	2.5	5.0 <sup>(2)</sup>
Diluted	<i>Clostridiaceae</i>	0	0.1	0.0	0.1	
		30	3.2	0.5	3.5	4.5 <sup>(3)</sup>
	<i>Lachnospiraceae</i>	0	0.1	0.0	0.1	
		30	4.2	0.6	4.0	4.6 <sup>(2)</sup>
	<i>Peptostreptococcaceae</i>	0	1.0	0.1	0.9	
		30	1.8	0.2	1.8	4.3 <sup>(4)</sup>
	<i>Shewanellaceae</i>	0	5.2	0.4	5.3	
		30	10	0.3	10	5.0 <sup>(1)</sup>
	<i>Mycoplasmataceae</i>	0	9.1	0.7	8.9	
		30	5.2	0.9	5.5	5.0 <sup>(1)</sup>

**(B) 16S rRNA**

Treatment	Family	Sampling Time (h)	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
Undiluted	<i>Fusobacteriaceae</i>	0 h	12	1.6	12	
		30 h	29	2.5	28	5.4 <sup>(1)</sup>
	<i>Lachnospiraceae</i>	0 h	0.1	0.1	0.1	
		30 h	0.2	0.0	0.2	3.3 <sup>(5)</sup>
	<i>Peptostreptococcaceae</i>	0 h	0.5	0.0	0.5	
		30 h	11	1.3	10	5.0 <sup>(3)</sup>
	<i>Shewanellaceae</i>	0 h	3.6	0.7	3.4	
		30 h	12	2.4	11	5.1 <sup>(2)</sup>
	<i>Aeromonadaceae</i>	0 h	22	1.0	22	
		30 h	3.8	0.1	3.8	5.3 <sup>(1)</sup>
	<i>Mycoplasmataceae</i>	0 h	21	2.8	22	
		30 h	0.5	0.2	0.3	5.3 <sup>(2)</sup>

Treatment	Family	Sampling Time	Mean	Standard Deviation	Median	LDA Score (log10) <sup>b</sup>
Diluted	<i>Clostridiaceae</i>	0 h	0.1	0.0	0.1	
		30 h	4.4	0.1	4.4	4.6 <sup>(3)</sup>
	<i>Lachnospiraceae</i>	0 h	0.0	0.0	0.0	
		30 h	3.1	0.3	3.0	4.5 <sup>(4)</sup>
	<i>Peptostreptococcaceae</i>	0 h	0.7	0.0	0.7	
		30 h	2.0	0.1	2.0	4.3 <sup>(5)</sup>
	<i>Shewanellaceae</i>	0 h	2.3	0.4	2.1	
		30 h	6.0	0.7	6.2	4.8 <sup>(2)</sup>
	<i>Aeromonadaceae</i>	0 h	24	1.9	25	
		30 h	17	1.8	16	5.4 <sup>(1)</sup>
	<i>Mycoplasmataceae</i>	0 h	18	3.3	17	
		30 h	13	1.5	13	5.2 <sup>(2)</sup>

<sup>a</sup>LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA gene and 16S rRNA sequences of the three replicates per treatment at the beginning (0 h) and the end (30 h) of incubation. Green-colored families displayed a significantly positive response during incubation. Red-colored families displayed a significantly negative response during incubation.

<sup>b</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to a treatment than lower ranking ones).

**Table 61.** Alpha diversity of the microbial community in undiluted and diluted gut contents of *L. terrestris*.<sup>a</sup>

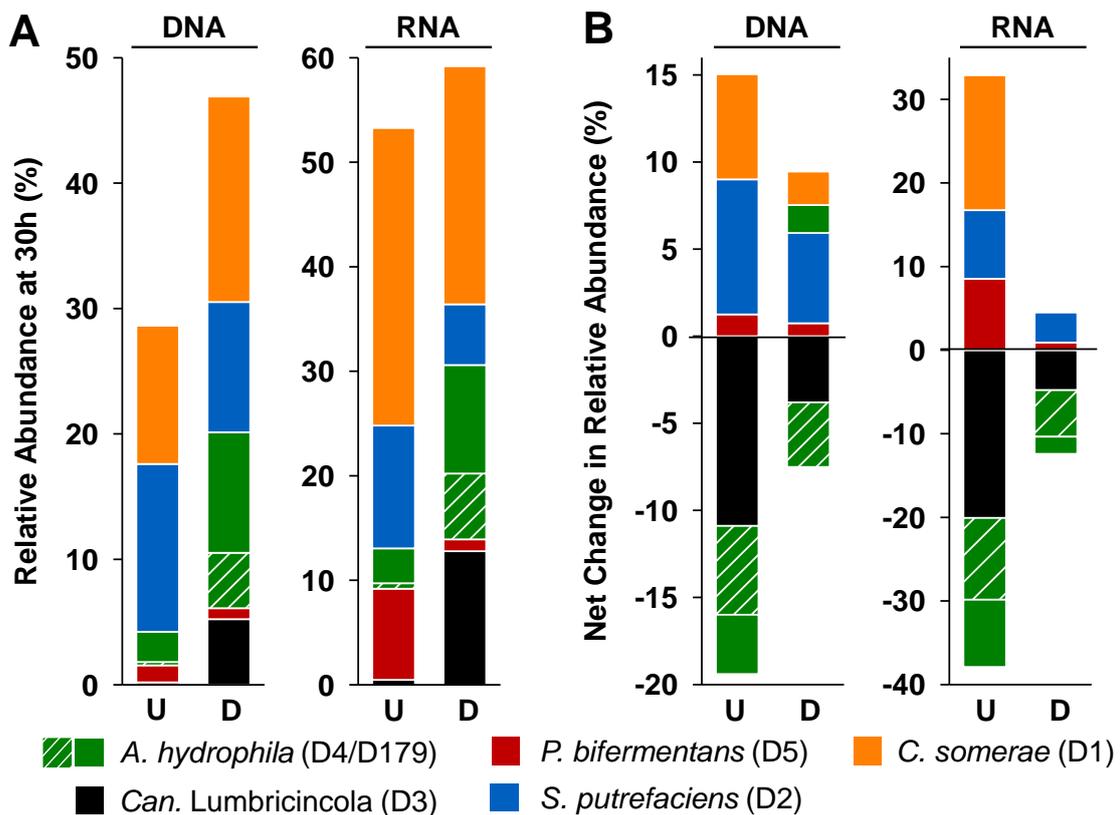
Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
<b>DNA (0 h)</b>	Undiluted 1	23712	1252 (287)	1793 (355)	5.0 (4.4)
	Undiluted 2	28421	1350 (290)	1776 (347)	5.1 (4.5)
	Undiluted 3	20125	1163 (281)	1651 (343)	4.9 (4.4)
	Diluted 1	24894	1198 (269)	1632 (345)	4.5 (3.9)
	Diluted 2	17425	1024 (265)	1603 (333)	4.5 (4.0)
	Diluted 3	24801	1240 (270)	1801 (329)	4.6 (4.0)
<b>DNA (5 h)</b>	Undiluted	13968	803 (233)	1257 (298)	4.1 (3.8)
	Diluted	20919	1153 (278)	1737 (340)	4.6 (4.1)
<b>DNA (10 h)</b>	Undiluted	25551	1027 (247)	1451 (306)	4.4 (4.0)
	Diluted	22833	1091 (270)	1552 (345)	4.4 (3.9)
<b>DNA (20 h)</b>	Undiluted	20390	915 (232)	1289 (282)	4.3 (3.9)
	Diluted	22392	1131 (280)	1589 (342)	4.5 (4.1)
<b>DNA (30 h)</b>	Undiluted 1	29093	1108 (244)	1493 (297)	4.6 (4.1)
	Undiluted 2	19850	969 (249)	1342 (297)	4.7 (4.2)
	Undiluted 3	26441	1115 (253)	1572 (302)	4.9 (4.4)
	Diluted 1	23661	1150 (273)	1582 (347)	4.5 (4.0)
	Diluted 2	16928	939 (259)	1352 (331)	4.3 (3.8)
	Diluted 3	18474	1026 (268)	1494 (339)	4.5 (4.0)
<b>RNA (0 h)</b>	Undiluted 1	6142	579 (218)	930 (270)	3.8 (3.4)
	Undiluted 2	6422	569 (211)	979 (275)	3.8 (3.4)

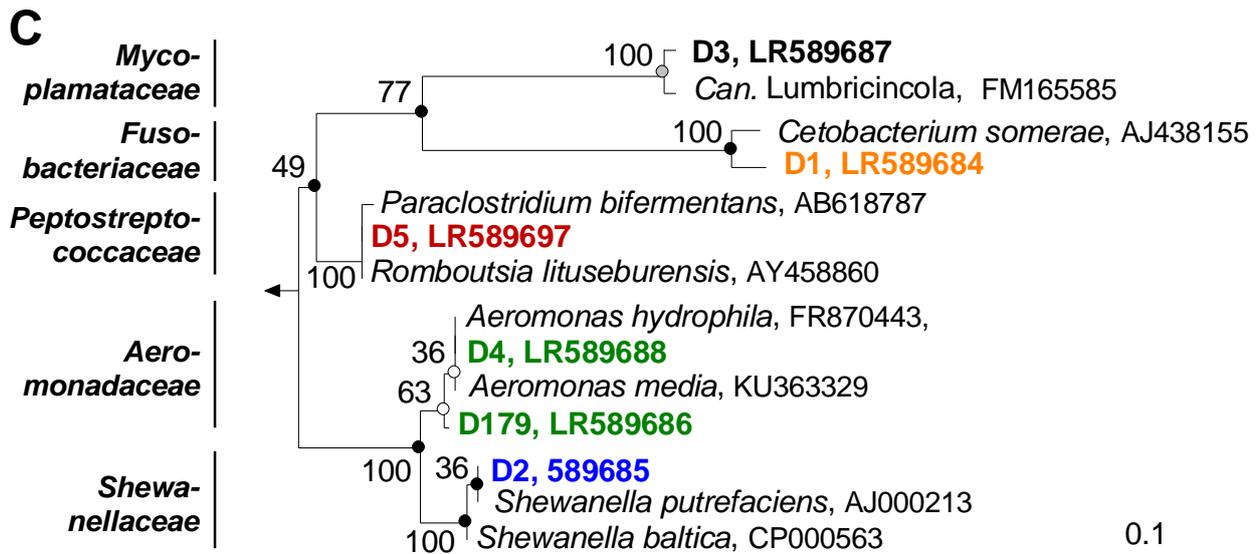
Sample (Sampling Time)	Treatment	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
RNA (0 h)	Undiluted 3	8883	750 (237)	1198 (297)	4.1 (3.7)
	Diluted 1	5996	470 (175)	858 (229)	3.2 (2.9)
	Diluted 2	6957	580 (200)	1026 (273)	3.6 (3.2)
	Diluted 3	9108	618 (194)	964 (274)	3.4 (3.1)
RNA (5 h)	Undiluted	9320	555 (191)	1076 (263)	3.5 (3.2)
	Diluted	10455	756 (236)	1168 (304)	3.9 (3.5)
RNA (10 h)	Undiluted	11653	637 (207)	1016 (274)	3.8 (3.5)
	Diluted	8551	596 (197)	970 (262)	3.5 (3.1)
RNA (20 h)	Undiluted	8760	446 (168)	717 (232)	3.0 (2.8)
	Diluted	7284	572 (211)	985 (275)	3.7 (3.3)
RNA (30 h)	Undiluted 1	6345	437 (188)	819 (241)	3.5 (3.3)
	Undiluted 2	6402	450 (176)	758 (227)	3.5 (3.2)
	Undiluted 3	6918	455 (178)	652 (220)	3.7 (3.4)
	Diluted 1	4699	473 (208)	883 (257)	3.8 (3.5)
	Diluted 2	9310	610 (202)	963 (273)	3.7 (3.3)
	Diluted 3	9501	611 (213)	894 (283)	3.8 (3.4)

<sup>a</sup>All 16S rRNA gene and 16S rRNA samples at 0 h and 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 5 h, 10 h, or 20 h. Identification numbers (e.g., Diluted1) indicate the respective replicates.

<sup>b</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>c</sup>The data sets were normalized to 2,500 sequences for comparison of amplicon libraries of different sizes.





**Figure 72.** 16S rRNA gene (DNA)- and 16S rRNA (RNA)-based overview of the most abundant phylotypes in undiluted (U) and diluted (D) treatments at the end of incubation (A), the net change in DNA and RNA relative sequence abundances affiliated to these phylotypes (B), and phylogenetic tree (C). Panel A: Phylotypes were considered to be abundant when a phylotype in at least one of the treatments displayed a  $\geq 4\%$  relative abundance at the end of the 30 h incubation. Panel B: Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be responsive when a phylotype in at least one of the undiluted or diluted treatment displayed a  $\geq 4\%$  higher or lower relative 16S rRNA or 16S rRNA gene abundances at the end of incubation than at the beginning of incubation. Panel C: The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees; grey circles, congruent nodes in neighbor-joining and maximum parsimony trees; empty circles, congruent nodes in maximum parsimony and maximum likelihood trees. Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch.

**Table 62.** Statistical analyses of the most responsive phylotypes displayed in Figure 72 based on 16S rRNA gene (A) and 16S rRNA (B) analysis.<sup>a</sup>

**(A) 16S rRNA genes**

Treatment	Phylotype <sup>b</sup>	Sampling Time	Mean	Standard Deviation	Median	LDA Score (log10) <sup>c</sup>
Undiluted	<b>D3</b>	0 h	11	1.0	11	5.0 <sup>(1)</sup>
		30 h	0.2	0.1	0.1	
	<b>D4</b>	0 h	5.4	0.2	5.4	4.7 <sup>(3)</sup>
		30 h	0.3	0.1	0.3	
	<b>D179</b>	0 h	5.8	0.3	5.7	4.8 <sup>(2)</sup>
		30 h	2.4	0.5	2.3	
	<b>D1</b>	0 h	5.0	0.0	5.0	5.0 <sup>(5)</sup>
		30 h	11	1.6	11	
	<b>D2</b>	0 h	5.6	0.3	5.5	5.1 <sup>(1)</sup>
		30 h	13	2.7	13	
	<b>D5</b>	0 h	0.1	0.0	0.1	4.1 <sup>(10)</sup>
		30 h	1.4	1.3	0.7	

Treatment	Phylotype <sup>b</sup>	Sampling Time	Mean	Standard Deviation	Median	LDA Score (log10) <sup>c</sup>
Diluted	D3	0 h	9.0	0.7	8.9	5.0 <sup>(1)</sup>
		30 h	5.2	0.9	5.5	
	D4	0 h	8.1	0.2	8.2	4.9 <sup>(2)</sup>
		30 h	4.4	0.6	4.5	
	D2	0 h	5.2	0.4	5.3	5.0 <sup>(1)</sup>
		30 h	10	0.3	10	
	D5	0 h	0.1	0.0	0.1	3.9 <sup>(7)</sup>
		30 h	0.9	0.0	0.9	
	D179	0 h	8.0	0.0	8.0	5.0 <sup>(2)</sup>
		30 h	9.6	1.3	9.4	

**(B) 16S rRNA**

Treatment	Phylotype <sup>b</sup>	Sampling Time	Mean	Standard Deviation	Median	LDA Score (log10) <sup>c</sup>
Undiluted	D3	0 h	21	2.8	22	5.3 <sup>(1)</sup>
		30 h	0.5	0.3	0.3	
	D4	0 h	10	0.2	10	5.0 <sup>(3)</sup>
		30 h	0.5	0.1	0.6	
	D179	0 h	11	1.2	12	5.1 <sup>(2)</sup>
		30 h	3.3	0.2	3.2	
	D1	0 h	12	1.6	12	5.5 <sup>(1)</sup>
		30 h	29	2.5	27	
	D2	0 h	3.6	0.7	3.3	5.1 <sup>(2)</sup>
		30 h	12	2.4	11	
	D5	0 h	0.2	0.0	0.2	4.9 <sup>(3)</sup>
		30 h	8.7	1.4	8.0	
Diluted	D3	0 h	18	3.3	17	5.2 <sup>(1)</sup>
		30 h	13	1.5	13	
	D4	0 h	12	1.0	12	5.1 <sup>(2)</sup>
		30 h	6.3	0.5	6.2	
	D2	0 h	2.2	0.4	2.0	4.8 <sup>(1)</sup>
		30 h	5.8	0.7	6.0	
	D5	0 h	0.2	0.1	0.2	4.1 <sup>(4)</sup>
		30 h	1.1	0.1	1.1	

<sup>a</sup>LEfSe analysis, mean value, standard deviation, and median are based on the relative abundance of 16S rRNA gene and 16S rRNA sequences of the three replicates per treatment at the beginning (0 h) and the end (30 h) of incubation.

<sup>b</sup>Green-colored phylotypes displayed a significantly positive response during incubation. Red-colored phylotypes displayed a significantly negative response during incubation.

<sup>c</sup>LDA scores were calculated using LEfSe. Numbers in parentheses display the rank in the LDA analysis (i.e., higher ranking families exhibited a stronger response to a treatment than lower ranking ones).

### 3.5. Effect of ingested material on *Can. Lumbricincola*

The anecic model earthworm *L. terrestris* ingest a wide range of differential material (e.g., plant material, soil, and associated microorganisms [Section 1.3]). In this regard, the feeding behavior of *L. terrestris* introduces diverse organic matter to the alimentary canal that potentially causes high fluctuations of organic carbon in the earthworm gut. Previous studies demonstrated the emission of gut fermentation-derived H<sub>2</sub> by *L. terrestris* (Wüst *et al.*, 2009b), and further evaluations, including different earthworm diets, indicated a positive correlation between the emitted H<sub>2</sub> and the organic carbon content of ingested material (Feustel, Oppermann, Schmidt, and Drake; data not published). Furthermore, the findings of this study also illustrates a potential impact of the dietary material on abundant families of the gut content microbiota, including *Mycoplasmataceae*. In this regard, this earthworm-associated bacterial family displayed a positive response, when *L. terrestris* was maintained on organic carbon rich dietary substrates (Feustel, Oppermann, Schmidt, Drake; data not published).

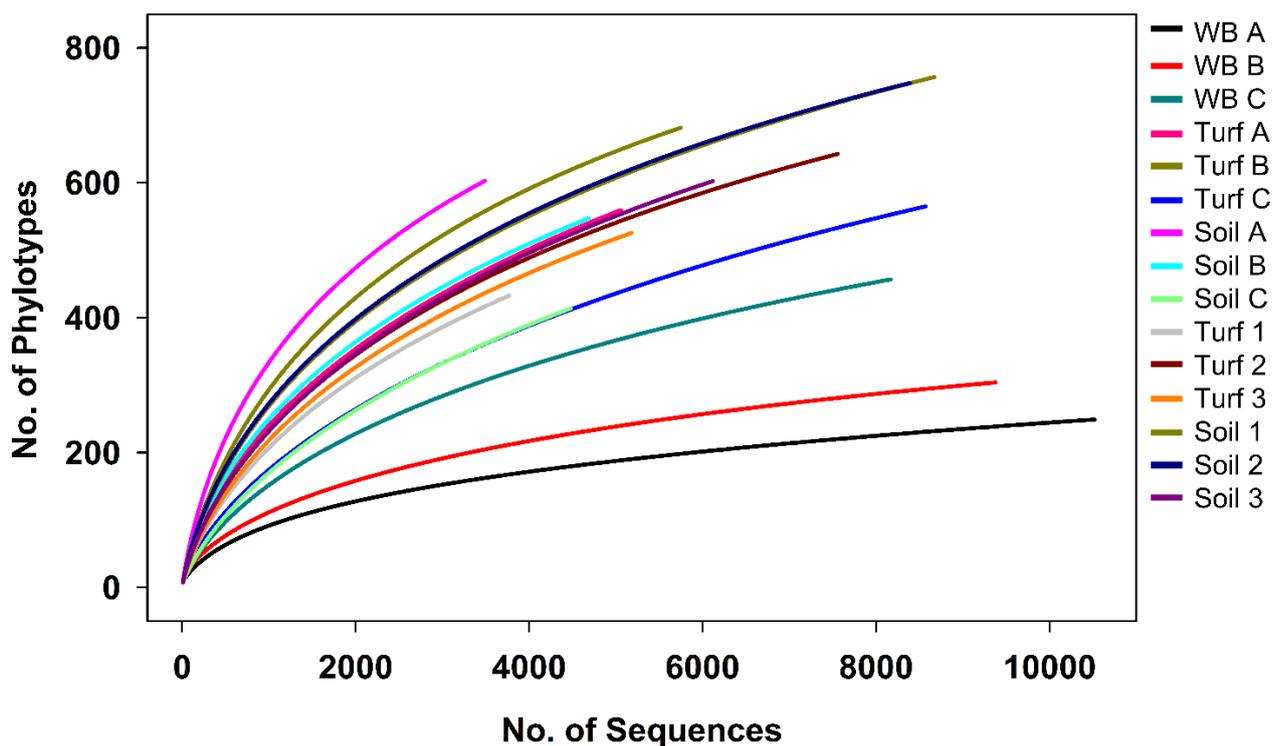
Interestingly, *Mycoplasmataceae*-affiliated phylotypes closely related to *Can. Lumbricincola* displayed essentially no positive response to any supplemental nutrient (e.g., amino acids, saccharides, microbial- and plant-derived lysates, protein, RNA) evaluated in this dissertation. Although these phylotypes are strongly associated with *L. terrestris* (Nechitaylo *et al.*, 2009), their metabolism and function in this invertebrate is largely unresolved. These considerations prompted the evaluation of the response of *Can. Lumbricincola*-affiliated phylotypes in gut contents of earthworms maintained on dietary substrates, containing different amounts of organic carbon (worm bedding, turf, or soil [Section 2.1.1 and Section 2.5.3]).

A total of 99,886 bacterial 16S rRNA sequences were obtained from the different gut content samples, yielding 25 phyla (including candidate phyla), and rarefaction analyses indicated that the most abundant taxa were targeted (Figure 73). Based on the 16S rRNA abundance analyses at family-level, only the family *Pseudomonadaceae* was significantly associated with one of the three different gut contents. Thus, this family displayed a significantly higher 16S rRNA abundance in gut contents extracted from earthworms maintained on worm bedding than in gut contents of earthworms kept on soil or turf (Figure 74; Statistical analyses using LEfSe revealed a LDA score [log<sub>10</sub>] of 5.2 [Section 2.7.4.2]). The *Tenericutes*-affiliated family *Mycoplasmataceae* displayed no significant response to any of the three dietary substrates.

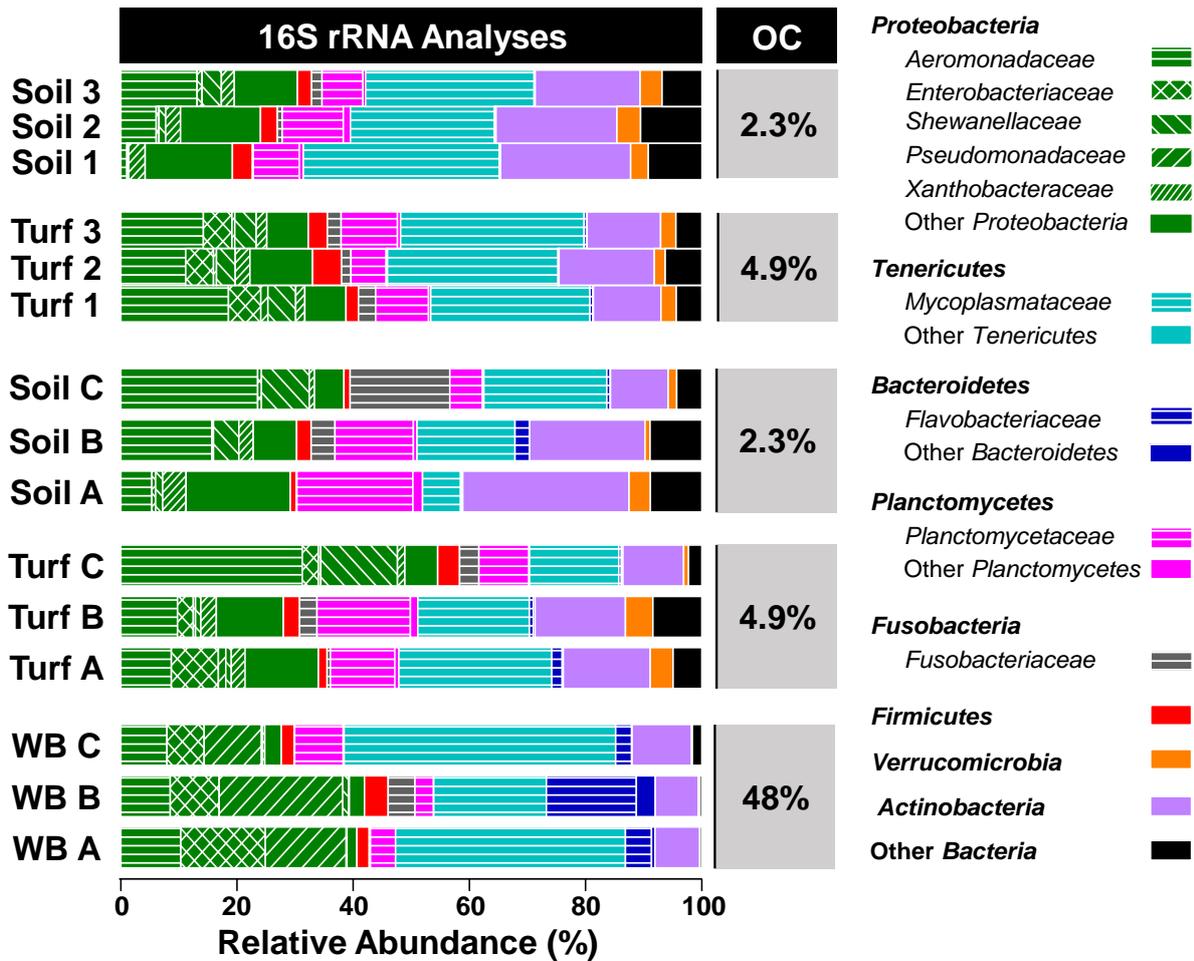
However, based on the relative 16S rRNA abundance analyses at phylotype-level, three *Can. Lumbricincola*-affiliated taxa were abundant and responded differentially to the three earthworm-ingested dietary substrates (Figure 75). Thus, phylotype OC8 (closely related to an uncultured earthworm bacterium and distantly related to *Can. Lumbricincola*) displayed a significant higher relative 16S rRNA abundance in gut contents extracted from worms maintained on worm bedding (Figure 75; Statistical analyses using LEfSe revealed a LDA score [log<sub>10</sub>] of 5.3 [Section 2.7.4.2]). Furthermore, this phylotype was hardly detectable in earthworm gut

contents derived from dietary substrates with limited organic carbon contents (Figure 75 A and B). Likewise, phylotype OC6 (closely related to *Can. Lumbricincola*) displayed a higher 16S rRNA abundance in earthworm gut contents derived from worm bedding than in the gut contents derived from turf and soil (Figure 75). In marked contrast, phylotype OC1 (also closely related to *Can. Lumbricincola*) displayed a positive response in gut contents of earthworm that ingested turf and soil (Figure 75), an observation more pronounced in gut contents pooled from approximately 20 worm individuals maintained on turf or soil (Figure 75 B).

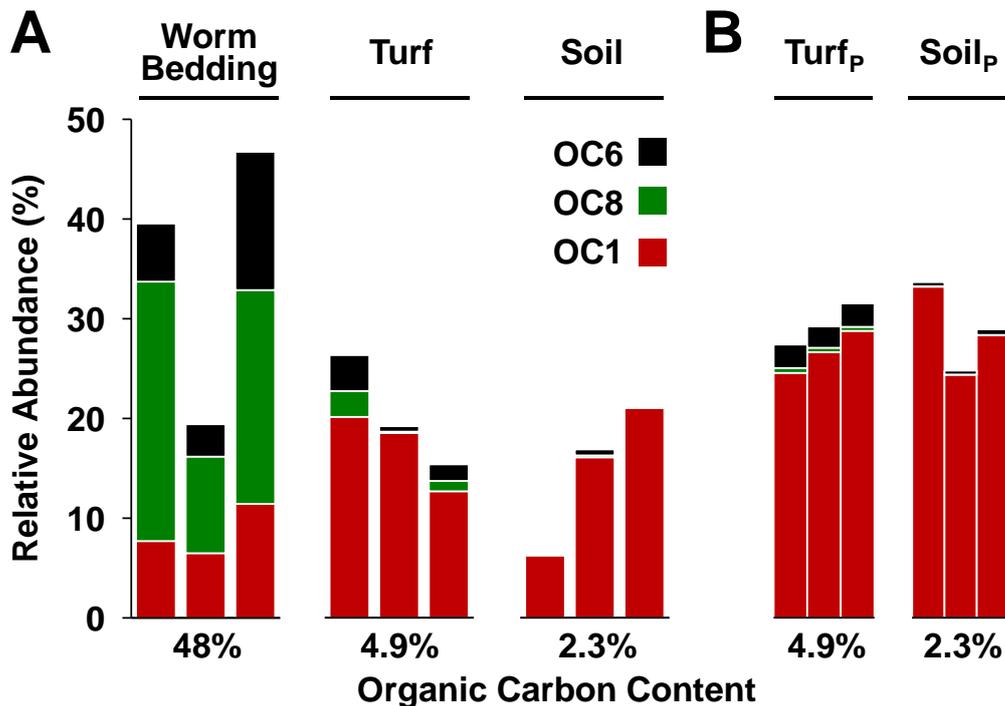
Based on the mean values, the numbers of detected phylotypes, the number of expected phylotypes (Chao1), and Shannon indices were lowest in gut contents extracted from earthworms maintained on the worm bedding (Table 63), indicating a lower bacterial diversity in alimentary canals of earthworms that are finding on organic carbon rich dietary substrates. That the gut content diversity decreases when the availability of organic carbon increases is corroborated by previous microcosm experiments (Figure 25, Figure 36, and Figure 66).

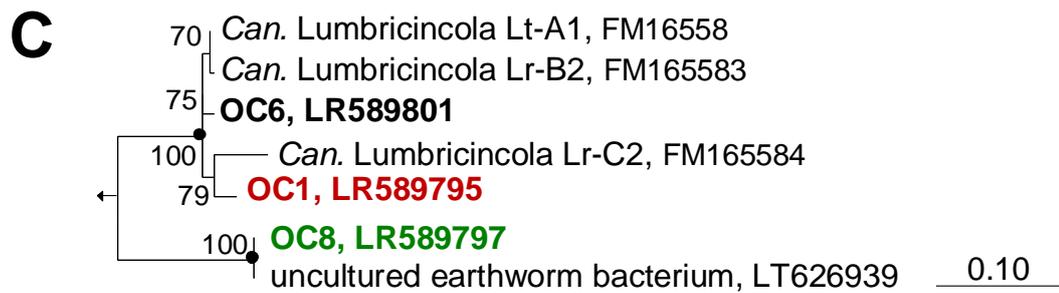


**Figure 73.** Rarefaction analyses of bacterial 16S rRNA sequences obtained from gut contents of earthworms maintained on different dietary substrates. Phylotypes were based on a 97% sequence similarity cut-off. Capital letter assigned to a substrate [e.g., Turf A] indicate the respective individual. Number assigned to a substrate [e.g., Turf 1] indicate the respective replicate of the three replicate analyses of pooled gut content from approximately 20 individuals per substrate. WB, worm bedding.



**Figure 74.** Effect of dietary substrates on the relative 16S rRNA sequence abundances of gut-associated families. The most abundant families (i.e., families with  $\geq 4\%$  relative abundance in at least one sampling period) are displayed in the color of the respective phylum. Information on all detected taxa is provided in Table A11. Capital letter assigned to a substrate [e.g., Turf A] indicate the respective individual. Number assigned to a substrate [e.g., Turf 1] indicate the respective replicate of the three replicate analyses of pooled gut content from approximately 20 individuals per substrate. Abbreviations: OC, organic carbon content; WB, worm bedding.





**Figure 75.** 16S rRNA-based overview of the most abundant and responsive *Can. Lumbricincola*-affiliated phylotypes in gut contents of earthworms maintained on different dietary substrates (A and B), and phylogenetic tree of these phylotypes (C). Phylotypes (OC) are based on a sequence similarity cut-off of 97% and were considered to be abundant when a phylotype displayed a  $\geq 4\%$  relative 16S rRNA abundances in at least one of the different gut contents. Panel A: 16S rRNA analyses of three worm individuals per substrate. Panel B: the 16S rRNA analyses of pooled ( $\rho$ ) gut content from approximately 20 individuals per substrate. Panel C: The phylogenetic tree was calculated using the neighbor-joining, maximum parsimony, and maximum likelihood methods. Solid circles, congruent nodes in three trees. Branch length and bootstrap values (1,000 resamplings) are from the maximum parsimony tree. The bar indicates 0.1 change per nucleotide. *T. maritima* (AE000512) was used as outgroup. Accession numbers occur at the end of each branch.

**Table 63.** Alpha diversity of the microbial community in gut contents of earthworms maintained on different dietary substrates.<sup>a</sup>

Dietary Substrate	Number of sequences	Observed phylotypes <sup>b</sup> (normalized) <sup>c</sup>	Chao1 (normalized) <sup>c</sup>	Shannon (normalized) <sup>c</sup>
Worm Bedding A	10525	249 (60)	381 (75)	2.9 (2.4)
Worm Bedding B	9385	304 (78)	412 (102)	3.4 (2.9)
Worm Bedding C	8180	457 (122)	681 (167)	3.4 (2.8)
Turf A	5065	560 (210)	899 (233)	4.4 (3.9)
Turf B	5757	682 (239)	928 (264)	4.8 (4.1)
Turf C	8572	565 (171)	866 (216)	3.6 (3.2)
Soil A	3493	603 (228)	841 (244)	5.4 (4.6)
Soil B	4696	548 (212)	882 (245)	4.5 (3.9)
Soil C	4484	414 (175)	814 (221)	3.3 (2.9)
Turf 1	3776	433 (200)	711 (240)	3.9 (3.5)
Turf 2	7565	643 (216)	919 (250)	4.2 (3.7)
Turf 3	5187	526 (208)	848 (246)	3.9 (3.5)
Soil 1	8678	757 (229)	1075 (257)	4.3 (3.7)
Soil 2	8394	748 (225)	1001 (254)	4.6 (4.0)
Soil 3	6119	595 (216)	902 (255)	4.1 (3.6)

<sup>a</sup>Analyses based on 16S rRNA sequences. Capital letter assigned to a substrate [e.g., Turf A] indicate the respective individual. Number assigned to a substrate [e.g., Turf 1] indicate the respective replicate of the three replicate analyses of pooled gut content from approximately 20 individuals per substrate.

<sup>b</sup>Phylotypes were clustered based on a sequence similarity cut-off of 97%.

<sup>c</sup>The data sets were normalized to 2,500 sequences for comparison of amplicon libraries of different sizes.

## 4. DISCUSSION

Evolutionarily, earthworms and worm-like animals are among the oldest known animals (Seilacher, 1998; Morris and Peel, 2008), and their gut ecosystems are therefore representative of primitive gut ecosystems. Although it is assumed that dietary biopolymers are important to the maintenance of earthworms, how these complex nutrients are transformed and utilized in the alimentary canal is not resolved. As outlined in the Introduction, the main objectives of the work described in this dissertation were to evaluate the potentials of the fermentative gut microbiota of *L. terrestris* to utilize dietary biopolymers and convert them to products that can be of nutritional value for these invertebrates.

### 4.1. Dietary polysaccharides: fermentative capacities of a primitive gut ecosystem (Hypothesis I)

It can be presumed that ancient gut ecosystems were at least partially dependent on microbes capable of utilizing the 'easiest substrates' first. For example, structural polysaccharides (e.g., cellulose, xylan, chitin) are designed for stability and must therefore be more difficult to decompose than polysaccharides (e.g., energy storage polymers) whose importance is based on rapid utilization in response to the energy needs of a cell (Ebert and Schenk, 1968). In this regard, more easily hydrolysable energy storage polysaccharides such as starch (activation energy approximates 26 kJ/mol [Prajapati *et al.*, 2014]) were preferential candidates for polysaccharide-based gut fermentations (i.e., best to use the easiest first). In marked contrast, the use of difficult to degrade structural polysaccharides such as cellulose, a polymer with extensive hydrogen bonding to ensure stability (activation energy approximates 92 kJ/mol [Kunov-Kruse *et al.*, 2013; Sørensen *et al.*, 2015]) was most likely not strategically prioritized in the oxygen-limited ancient gut ecosystem of the earthworm during evolution. The utilization of structural polysaccharides might require more highly evolved gut communities that are specialized in the anaerobic breakdown of such hardly degradable polysaccharides like cellulose and lignin (Dietrich *et al.*, 2014; Xue *et al.*, 2018). Interestingly, although ruminants harbor these highly specialized cellulose-degrading gut microbes that greatly enhance the breakdown and utilization of cellulolytic fibers, the microbiota is unable to completely digest the ingested plant-derived structural polysaccharides (Russell *et al.*, 2009), demonstrating the challenging hydrolysis of these durable polymers.

The gut passage of earthworms can be up to 24 h and is dependent in part on the feeding status of the earthworm (Parle, 1963a; Satchell, 1967; Wüst *et al.*, 2011). The findings indicate that structural polysaccharides (e.g., cellulose, chitin, pectin, and xylan) were poorly utilized in this time period. Nonetheless, it is noteworthy that they marginally enhanced the formation of fermentation products. For example, cellulose, chitin, pectin, and xylan treatments displayed

statistically significant amounts of H<sub>2</sub> (Table 14). These observations indicated that earthworm gut-associated microbes have the capacity to utilize at least marginally certain structural polysaccharides, a process most likely facilitated by digestive enzymes in the alimentary canal of earthworms (Laverack, 1963; Edwards and Fletcher, 1988; Nozaki *et al.*, 2009). Indeed, worm-derived digestive enzymes present in extracted gut contents might contribute to the slightly enhanced fermentation observed in the structural polysaccharide microcosms. The hardly detectable stimulation of fermentation in chitin treatments (Figure 22 A) illustrates that the availability of chitin-derived *N*-acetylglucosamine would likely be limited during the earthworm gut passage. However, this monosaccharide is also a major component of peptidoglycan of the bacterial cell wall, and cellular disruption of bacterial cell walls would yield *N*-acetylglucosamine and other potentially fermentable cell wall-associated monomers (Silhavy *et al.*, 2010).

#### 4.1.1. Fermentative phylotypes responsive to polymeric and non-polymeric saccharides

The *Proteobacteria*-affiliated families *Aeromonadaceae* and *Enterobacteriaceae* were significantly stimulated by almost all polymeric and non-polymeric saccharides (Table 16 and Table 20). Furthermore, at least one of the supplemented polymeric or non-polymeric saccharides significantly stimulated *Clostridiaceae* and/or *Fusobacteriaceae* (Table 16 and Table 20). These trend extended to several phylotypes that displayed a  $\geq 4\%$  higher relative abundance (at either the 16S rRNA gene or 16S rRNA level) in at least one of the treatments compared to the control treatment at the end of the incubation (Table 64).

GPT-1 (P<sub>A</sub>3/P<sub>B</sub>96/P<sub>B</sub>3/S5, 99 to 100% identity to *A. hydrophila*) was significantly stimulated by all supplemental polymeric and non-polymeric saccharides except of chitin, xylan, and galacturonic acid (Table 64). The facultative aerobe *A. hydrophila* is known to produce extracellular amylases that can hydrolyze alpha-1,4-glycosidic bonds in polysaccharides such as starch, maltodextrin, and glycogen (Gobius and Pemberton, 1988; Emele, 2001). Furthermore, *A. hydrophila* utilizes glucose and forms acetate, ethanol, lactate, succinate, formate, CO<sub>2</sub>, and H<sub>2</sub> (Stanier and Adams, 1944; Lee *et al.*, 2008). *A. hydrophila*-associated phylotypes responded also positively in gut contents of *L. terrestris* in other studies and experiments (Meier *et al.*, 2018; Section 3.2.4). That the *A. hydrophila*-associated GPT-1 was also stimulated in cellobiose, xylose, and *N*-acetylglucosamine treatments (Table 64) suggests that this phylotype might be able to hydrolyze, in addition to alpha-1,4-glycosidic bonds, beta-1,4-glycosidic bonds. Furthermore, the stimulation in these treatments is consistent with the ability of some *A. hydrophila* strains to ferment cellobiose and xylose (Stanier and Adams, 1944; Popoff and Véron, 1976). The stimulation of *A. hydrophila*-affiliated phylotypes in dextran treatments is consistent with the detection of pullulanase-excretion genes that are required for the extracellular activity of the respective enzyme (hydrolyzes alpha-1,6-glycosidic bonds) (Howard *et al.*, 1993).

*Enterobacteriaceae*-affiliated GPT-5 (P<sub>A</sub>13/P<sub>B</sub>5/S10, 99 to 100% identity to *B. gaviniae*) was significantly stimulated by starch, maltodextrin, glycogen, dextran, and all supplemental non-polymeric saccharides (Table 64). The genus *Buttiauxella* is abundant in intestinal ecosystems (e.g., snails, slugs, and other molluscs), and the fermentative facultative aerobe *B. gaviniae* is able to ferment starch, *N*-acetylglucosamine, cellobiose, glucose, galacturonic acid, and xylose (Müller *et al.*, 1996).

GPT-4 (P<sub>A</sub>8/P<sub>B</sub>1018/S19, 99 to 100% identity to *Y. regensburgeri*) responded most strongly to maltodextrin, but was also responsive to dextran, pectin, and most of the non-polymeric saccharides (Table 64). *Y. regensburgeri* ferments diverse saccharides, for example glucose, cellobiose, and xylose (Kosako *et al.*, 1984). Although GPT-4 was stimulated by galacturonic acid, the utilization of galacturonic acid by *Yokenella* is unknown. However, certain species of the *Enterobacteriaceae* utilize galacturonic acid and produce ethanol, acetate, lactate, succinate, formate, and CO<sub>2</sub> (Kraght and Starr, 1952; Grohmann *et al.*, 1994; Hata *et al.*, 2016).

The obligate anaerobic family *Fusobacteriaceae* was represented by GPT-7 (P<sub>A</sub>14/P<sub>B</sub>2/S4, 96% identity to *C. somerae*), a group phylotype stimulated by all saccharides except of xylose (Table 64). Although a 96% sequence identity is relatively low in terms of species-level classification, it is of interest to note that this microaerotolerant anaerobe can be saccharolytic and ferments saccharides to acetate, propionate, butyrate, and succinate (Finegold *et al.*, 2003; James and Whitman, 2011). *Clostridiaceae*-affiliated GPT-6 (P<sub>A</sub>6/S12, 100% identity to *Clostridium sartagoforme*) responded most strongly to maltodextrin (Table 64). *C. sartagoforme* is able to hydrolyze starch and ferments glucose to formate, acetate, and lactate (Šimůnek *et al.*, 2001). *Clostridiaceae*-affiliated GPT-8 (P<sub>A</sub>26/P<sub>B</sub>11/S51, 99 to 100% identity to *Clostridium beijerinckii*) was stimulated by glycogen, starch, and maltodextrin (Table 64). *C. beijerinckii* ferments saccharides to solvents, like acetone and butanol (Chen and Blaschek, 1999). *Peptostreptococaceae*-affiliated GPT-2 (P<sub>A</sub>4/P<sub>B</sub>205/S3, 99% identity to *P. bifementans*) was abundant in several treatments, including the unsupplemented controls (Table 64), suggesting that *P. bifementans* utilize supplemented or gut content-endogenous saccharides, and produces ethanol, formate, lactate, acetate, CO<sub>2</sub>, and H<sub>2</sub> (Chamkha *et al.*, 2001a).

Likewise, the *Mycoplasmataceae*, represented by group GPT-3 (P<sub>A</sub>1/P<sub>B</sub>1/S2, 99% similarity to *Can. Lumbricincola* sp. LR-C2), were abundant in all gut content treatments, including the unsupplemented controls. Although this GPT was significantly stimulated in cellulose and chitin treatments (Table 22), the extremely low levels of fermentation activity in these structural polysaccharide treatments (Figure 22) suggest that this taxon utilized these structural polysaccharides tenuously. It is of interest to note that this phylotype did not increase in relative abundance in controls treatments whereas other taxa did (Figure 24 and Figure 30), suggesting that it was less capable of responding to gut content nutrients than were other taxa that were most likely ingested (Section 3.3). This finding is consistent with other studies and experiments that

**Table 64.** Summary of the most abundant and stimulated phylotypes in control, polysaccharide and non-polymeric saccharide treatments (Figure 33).<sup>a</sup>

GPT	Phylotype	Phyla	Family	Closest cultured microorganism	Sequence Identity	Stimulated by	Aerobe/ Anaerobe <sup>b</sup>	References <sup>b</sup>
GPT-1	P <sub>A</sub> 3/P <sub>B</sub> 96/ P <sub>B</sub> 3/S5	Proteobacteria	Aero- monadaceae	<i>A. hydrophila</i>	99-100%	Cellulose, pectin, dextran, maltodextrin, glycogen, starch, <i>N</i> -acetylglucosamine, cellobiose, glucose, xylose	Facultative aerobe	1
GPT-2	P <sub>A</sub> 4/P <sub>B</sub> 205/ S3	Firmicutes	Pepto- streptococcaceae	<i>P. bifementans</i>	99%	-	Obligate anaerobe	2
GPT-3	P <sub>A</sub> 1/P <sub>B</sub> 1/S2	Tenericutes	Myco- plasmataceae	<i>Can. Lumbricincola</i>	99%	-	Facultative aerobe	3
GPT-4	P <sub>A</sub> 8/P <sub>B</sub> 1018 /S19	Proteobacteria	Enter- obacteriaceae	<i>Y. regensburgi</i>	99-100%	Pectin, maltodextrin, dextran, starch, <i>N</i> -acetylglucosamine, cellobiose, glycose, galacturonic acid	Facultative aerobe	4
GPT-5	P <sub>A</sub> 13/P <sub>B</sub> 5/ S10	Proteobacteria	Enter- bacteriaceae	<i>B. gaviniae</i>	99-100%	Maltodextrin, dextran, glycogen, starch, <i>N</i> -acetylglucosamine, glucose, cellobiose, galacturonic acid, xylose	Facultative aerobe	5
GPT-6	P <sub>A</sub> 6/S12	Firmicutes	Clostridiaceae	<i>C. sartagoforme</i>	100%	Maltodextrin	Obligate anaerobe	6
GPT-7	P <sub>A</sub> 14/P <sub>B</sub> 2/ S4	Fusobacteria	Fuso- bacteriaceae	<i>C. comerae</i>	96%	Cellobiose, glucose, <i>N</i> -acetylglucosamine, galacturonic acid	Obligate anaerobe	7
GPT-8	P <sub>A</sub> 26/P <sub>B</sub> 11/ S51	Firmicutes	Clostridiaceae	<i>C. beijerinckii</i>	99-100%	Glycogen, starch, maltodextrin, pectin, cellobiose	Obligate anaerobe	6

<sup>a</sup>Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one of the supplemented treatments displayed a  $\geq 4\%$  higher relative abundance (at either the 16S rRNA gene or 16S rRNA level) than in the control treatment at the end of incubation. The group phylotypes are derived from the analyses of 16S rRNA genes or 16S rRNA. P<sub>A</sub> and P<sub>B</sub>, phylotypes in polysaccharide treatments; S, phylotypes in non-polymeric saccharide treatments. -, no polysaccharide- or saccharide-derived stimulation.

<sup>b</sup>Information about the closest cultured microorganism obtained from: 1, Martin-Carnahan and Joseph, 2005; 2, Sasi Jyothsna *et al.*, 2016; 3, Brown *et al.*, 2011; 4, Farmer and Brenner, 2005; 5, Kämpfer, 2005; 6, Wiegand, 2009; 7, Tsuchiya *et al.*, 2007.

have indicated that this taxon is not responsive during gut content fermentation (Meier *et al.*, 2018; Section 3.2, and Section 3.3). The *Mycoplasmataceae* belong to the *Mollicutes*, a class that can be parasitic/pathogenic in plants and animals including invertebrates (Brown *et al.*, 2011), and *Mycoplasmataceae*-affiliated species have been detected in other annelids (Murakami *et al.*, 2015).

The diversity of responsive group phylotypes (Table 64) demonstrates that the limitation of O<sub>2</sub> after ingestion and the availability of various saccharides can have broad impact on the fermentative gut microbiota of earthworms. Although anaerobes were represented in the group phylotypes (e.g., GPT-7), facultative aerobes sometimes displayed a strong response (e.g., GPT-1, GPT-4, and GPT-5), indicating the potential importance of ingested facultative aerobes to gut fermentation of ingested saccharides. Independent of the quantity of responsiveness of a given group phylotype, the collective responsiveness of the group phylotypes was similar in control and structural polysaccharide treatments (Figure 33). In marked contrast, polysaccharide treatments that yielded strongly enhances fermentations (e.g., maltodextrin and glycogen [Figure 22]) displayed a greater collective responsiveness of the group phylotypes compared to that of control treatments (Figure 33).

#### 4.1.2. Polysaccharide-based fermentation network

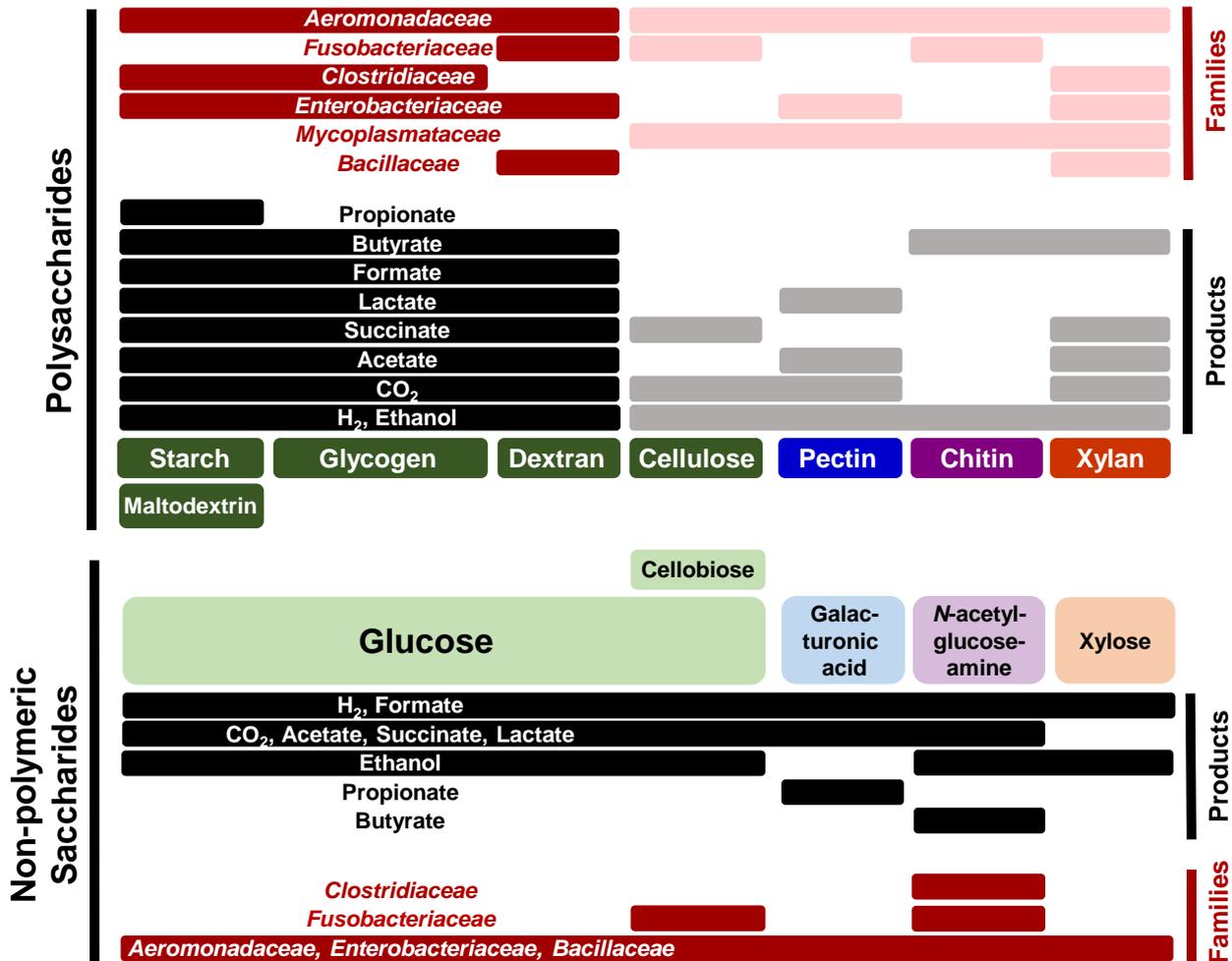
The collective findings indicates that diverse plant- and microbial-derived saccharides, have a contrasting impact on earthworm gut-associated fermentative bacteria (Figure 76). Thus, the hypothetical model illustrates the facultative aerobic and obligate anaerobic families that were potential responsible for the fermentations detected in the different treatments (Table 64). Furthermore, it summarize the diverse fermentation products that accumulated in response to supplemented polymeric and non-polymeric saccharides. These detected products are consistent with those detected in the alimentary canal (Wüst *et al.*, 2009b), indicating their potential *in situ* relevance. The experimental design did not simulate all *in situ* parameters of the gut, and the model (Figure 76) is therefore not intended to display all fermentations that could occur in this ecosystem (e.g., protein-driven fermentation is not integrated into the model [Section 3.2]). Rather, it emphasize potential fermentations in the earthworm gut that could occur in response to a given ingested polymeric- or non-polymeric saccharide. Nonetheless and consistent with theoretical considerations (Ebert and Schenk, 1968), the findings illustrate that fermentative gut bacteria would respond more rapidly to non-structural polysaccharides (e.g., glycogen and starch) than to structural polysaccharides (e.g., cellulose and chitin) during gut passage. The formation of glucose in (a) the cellobiose treatment (Figure 27) and (b) certain non-structural polysaccharide treatments (e.g., maltodextrin and glycogen [Table 13]) indicates the hydrolytic potentials of gut content. These observations are consistent with the utilization of supplemented polysaccharides and concomitant formation of fermentation products in the gut ecosystem of earthworms (Horn *et al.*, 2003; Wüst *et al.*, 2009b). In this regard, certain

fermentation products that were produced in marginal amounts achieved potential *in situ* relevance in other studies. For example, butyrate was a minor product but has been shown in previous studies to (a) approximate 5 mM in the aqueous phase of the midgut and hindgut (Wüst *et al.*, 2009b) and (b) be produced in gut content fermentations (Wüst *et al.*, 2011; Section 3.2). Gut contents supplemented with protein yielded butyrate as main fermentation product (Section 3.2.3), indicating that the butyrate detected in the midgut and hindgut (up to 6 mM in the aqueous phase) was most likely derived by the utilization of available amino acids. That *Fusobacteria* were hardly detectable in the polysaccharide experiment A (Figure 23) but abundant in the polysaccharide experiment B and the non-polymeric saccharide experiment (Figure 23 and Figure 29) reflects the differences in the microbiota of the dietary substrate ingested by the earthworm. Indeed, these experiments based on separate batches of earthworms that were maintained on soils collected at different times. Furthermore, the competitive potential of the earthworm to assimilate non-polymeric saccharides from the gut must also be taken into consideration. However, the concentration of fatty acids in the aqueous phase of the midgut can exceed 30 mM (Wüst *et al.*, 2009b), indicating the high potential of gut fermenters to utilize available gut endogenous nutrients prior to the absorption by the earthworm.

As illustrated in Figure 76 and Table 64, facultative aerobes displayed a wide range of response to the diverse supplemented saccharides. This trend was particularly apparent in the strong response of the *Proteobacteria*-affiliated families to maltodextrin, dextran, glycogen, and starch (Figure 23). However, obligatory anaerobic taxa affiliated to the *Firmicutes* were responsive to endogenous nutrients of gut contents and increased in most treatments, including the unsupplemented control (Figure 24 and Figure 30), and it can be therefore not excluded that this phylum were also involved in saccharide-stimulated fermentations. The apparent enrichment of *Fusobacteriaceae* (James and Whitman, 2011) in control and non-polymeric saccharide treatments (Figure 29 and Figure 30) reinforces the likelihood that anaerobes were at least in part responsible for the observed enhanced fermentations. Furthermore, changes in relative abundances during the incubations were not evaluated, so transient phylotypes (i.e., phylotypes that might have increased in abundance initially but returned to low abundance at the end of incubation [Section 3.2]) would have not been detected and therefore worthy of further studies.

As noted above, the experimental design did not simulate all *in situ* gut conditions. For example, the gut contents was subjected to a 1:10 dilution and might have therefore altered the efficiency of the gut community to utilize structural polysaccharides. Furthermore, the supplemented polysaccharides are only representative of the large diversity of naturally occurring polysaccharides. For example, poly-beta-hydroxybutyrate, a polyester produced by most *Bacillus* species, represent another bacterial energy storage polymer (Wilkinson, 1959, 1963) that was not tested in this study but warrants further evaluations. Although O<sub>2</sub> is not detectable in the gut (Horn *et al.*, 2003; Wüst *et al.*, 2009b), it cannot be excluded that traces of O<sub>2</sub> diffuse into the alimentary canal at the inner-surface of the gut wall and facilitate a negligible amount of aerobic

polymer degradation at the transient oxic microzones proximal to the gut wall. In addition, the occurrence of anaerobic respirations in the digestive tract (e.g., denitrification and iron reduction [Karsten and Drake, 1997; Matthies *et al.*, 1999; Horn *et al.*, 2006a; Wüst *et al.*, 2009a]) might contribute to the breakdown of polysaccharides. These considerations indicate a potentially more efficient degradation and utilization of structural polysaccharides during the earthworm gut passage than overserved in the anoxic microcosms.



**Figure 76.** Hypothetical model of contrasting gut content fermentations and associated families stimulated by polymeric and non-polymeric saccharides. Fermentation products and associated families were only displayed if the average of triplicates ( $\mu\text{mol/g}_{\text{FW}}$  for products and relative abundance of 16S rRNA for families) was at least 20% higher than the respective control at the end of incubation. Bars with lighter shading indicate that the potential to utilize structural polysaccharides would be marginal compared to the more robust potential to utilize non-structural polysaccharides. Figure modified and used with permission from Zeibich *et al.*, 2019a.

## 4.2. Protein- and RNA-enhanced gut fermentation (Hypothesis II-IV)

### 4.2.1. Protein and RNA as main fermentable cell constituents

The anecic earthworm *L. terrestris* consumes soil (Palm *et al.*, 2013), a dietary material that can harbor  $10^9$  or more microbial cells per gram dry weight (Torsvik *et al.*, 1990; Whitman *et al.*, 1998). Thus, ingested material provides an enormous number of microorganisms to the  $O_2$ -limited alimentary canal of the earthworm. The ingestion is linked to the abrasive action of the gizzard that (a) ensure the disruption of larger cells and thus (b) the release of diverse microbial-derived biopolymers into the gut. In this regard, protein and RNA, the primary polymers of such cells, constitute up to 50% and 20%, respectively, on a dry weight basis (Babel and Müller, 1985; Lange and Heijnen, 2001; Delgado *et al.*, 2013). The findings demonstrated that fermentative gut microbes were poised to respond rapidly to these two biopolymers (Figure 39 and Figure 41)

#### 4.2.1.1. Fermentative phylotypes responsive to microbial cell lysate, protein and RNA

The degree of the protein and RNA-based enhanced fermentative activity in anoxic gut content treatments was greater than that observed with structural polysaccharides (Figure 38 and Figure 22 A). This finding is consistent with the more intractable nature of plant cell wall-derived structural polysaccharides compared to the more soluble cytoplasmic biopolymers that can be easier attacked by hydrolytic enzymes. Furthermore, it reflects the diverse capacities of gut-associated microbes to hydrolyze these different biopolymers under  $O_2$ -limited conditions. The product profiles of treatments supplemented with microbial cell lysate, protein, and RNA indicated that these substrates were fermented by facultative aerobes and obligate anaerobes (Figure 35, Figure 39, and Table 65). Interestingly,  $H_2$  accumulated in RNA treatments but did not accumulate in protein treatments, a phenomenon most likely caused by amino acid fermenters that switch to non- $H_2$ -producing Stickland reaction when  $H_2$  concentrations reach a certain level (Schink and Stams, 2013).

16S rRNA analyses displayed an approximately three-fourths overlap between the responsive family-level taxa in protein and RNA treatments and the responsive family-level taxa in the cell lysate treatments (Figure 42), suggesting that many of the stimulated taxa in the lysate treatment were stimulated by lysate-derived protein and RNA. In contrast, the families *Enterobacteriaceae* and *Lachnospiraceae* responded to cell lysate but appeared to be non-responsive in protein or RNA treatments (Figure 42), illustrating that nutrients other than protein and RNA in cell lysate stimulated additional taxa and associated processes not linked to either one of these biopolymers. In this regard, cell lysate contains many components in addition to protein and RNA, including diverse saccharides (Babel and Müller, 1985; Delgado *et al.*, 2013) that can be fermented by *Enterobacteriaceae*-affiliated taxa (Meier *et al.*, 2018; Figure 29). This

illustrates that the strong response of *Enterobacteriaceae*-affiliated phylotype CL4 (99% identity to *E. aerogenes*) to cell lysate (Table 65 and Figure 43) might have been due to lysate-derived saccharides. Consistent with saccharides (ribose from RNA) being potentially utilized by these *Enterobacteriaceae*-affiliated taxa, phylotype PR33 was not stimulated by supplemental protein but displayed a modest stimulation in RNA treatments.

*Clostridia* are known for their consumption of diverse saccharides as well as amino acids, and several clostridial phylotypes (Table 65) were responsive to supplemental microbial cell lysate and protein. Phylotypes CL5 and CL18 were closely related to potential acetogens (100% identity to *T. glycolicus* and *C. magnum*, respectively [Schink, 1984; Küsel *et al.*, 2001]) that were also detected in gut contents of the CH<sub>4</sub>-emitting earthworm *Eudrilus eugeniae* (Schulz *et al.*, 2015). However, gut contents of *L. terrestris* displayed no methanogenic potential (Meier *et al.*, 2018). Although the observed consumption of formate might have been associated with acetogenesis, further experiments indicated that non-acetogenic formate-hydrogen lyase-containing taxa are most likely responsible for the formate conversion (Section 3.2.9). A finding corroborated by the concomitant accumulation of H<sub>2</sub> that exceeded the H<sub>2</sub>-consuming capacity of acetogens. However, that acetogens can at least minimally profit from the conversion of formate to CO<sub>2</sub> and H<sub>2</sub> was reinforced by the increase of acetogen-affiliated sequences in formate-supplemented gut content treatments (Figure 51B and Figure 59).

The phylotypes PR2, PR6, PR7, and PR12 were most strongly stimulated in protein treatments. Of these four phylotypes, phylotype PR2 (99% identity to *R. lituseburensis*), displayed an increase at both 16S rRNA genes and 16S rRNA relative abundances-levels, (Figure 43). Obligate anaerobes of *Rombutsia* are common in soil, humus, lake sediments, and intestinal tracts of mammals (Wiegel, 2009; Gerritsen *et al.*, 2014; Wang *et al.*, 2015; Ricaboni *et al.*, 2016). *Rombutsia*-affiliated species ferment amino acids and carbohydrates to acetate, formate, ethanol, propionate, butyrate, isobutyrate, and methylbutyrate, and *R. lituseburensis* utilizes gelatin, chopped meat, and casein, indicating it produces proteases (Wiegel, 2009; Gerritsen *et al.*, 2014; Wang *et al.*, 2015). Phylotype PR6 represented the members of the family *Fusobacteriaceae* (Table 65). This family responded late in the protein treatment and the phylotype PR6 had a 96% sequence identity to its closest cultured relative *C. somerae* (Table 65). As mentioned before, a 96% sequence identity is relatively low in terms of species-level classification, but it is noteworthy that *C. somerae*, a species occurring in gastrointestinal systems, is not able to hydrolyze complex proteins but ferment amino acids and peptides to acetate, propionate, and butyrate (Finegold *et al.*, 2003; Tsuchiya *et al.*, 2007). *Fusobacteriaceae*-affiliated sequences with identities up to 99% to phylotype PR6 (HG964632; Figure 43) were also present in gut content of the epigeic earthworm *E. eugeniae* (Schulz *et al.*, 2015). This finding and the positive response of *Fusobacteriaceae*-affiliated phylotype PR6 in gut contents of the anecic earthworm *L. terrestris* supplemented with protein, indicates that this family may contribute to the degradation of amino acids in earthworms of contrasting feeding guilds.

**Table 65.** Summary of the most stimulated phylotypes in lysate, protein and RNA treatments (Figure 43).<sup>a</sup>

Phylotype	Phyla	Family	Closest cultured microorganism	Sequence Identity	Stimulated by	Aerobe/ Anaerobe <sup>b</sup>	References <sup>b</sup>
CL2/PR2	Firmicutes	Peptostreptococcaceae	<i>R. lituseburensis</i>	99%	Lysate and protein	Obligate anaerobe	1, 2
CL4/PR33	Proteobacteria	Enterobacteriaceae	<i>E. aerogenes</i>	99-100%	Lysate and RNA	Facultative aerobe	3, 4
CL7/PR3	Proteobacteria	Aeromonadaceae	<i>A. hydrophila</i>	99-100%	Lysate and RNA	Facultative aerobe	5
CL10/PR7	Firmicutes	Clostridiaceae	<i>C. thiosulfatireducens</i>	100%	Lysate and protein	Obligate anaerobe	2
CL5	Firmicutes	Peptostreptococcaceae	<i>T. glycolicus</i>	100%	Lysate	Obligate anaerobe	6
CL6	Firmicutes	Lachnospiraceae	<i>N. massiliensis</i>	96%	Lysate	Obligate anaerobe	7
PR6	Fusobacteria	Fusobacteriaceae	<i>C. comerae</i>	95%	Protein	Obligate anaerobe	8
CL8	Firmicutes	Clostridiaceae	<i>C. peptidivorans</i>	99%	Lysate	Obligate anaerobe	2
PR8	Firmicutes	Peptostreptococcaceae	<i>C. difficile</i>	99%	Protein	Obligate anaerobe	2
PR12	Firmicutes	Clostridiaceae	<i>C. tunisiense</i>	100%	Protein	Obligate anaerobe	2
CL15	Firmicutes	Clostridiaceae	<i>C. fridigicarnis</i>	100%	Lysate	Obligate anaerobe	2
CL18	Firmicutes	Clostridiaceae	<i>C. magnum</i>	100%	Lysate	Obligate anaerobe	2

<sup>a</sup>Phylotypes are based on a sequence similarity cut-off of 97% and were designated responsive when a phylotype in a given treatment displayed a minimum increase in relative abundance of 2% above control values in at least one of the sampling periods. The phylotypes are derived from the analysis of 16S rRNA and 16S rRNA genes. CL, phylotypes in cell lysate treatments; PR, phylotypes in protein and RNA treatments.

<sup>b</sup>Information about the closest cultured microorganism obtained from: 1, Gerritsen *et al.*, 2014; 2, Wiegel, 2009; 3, Yokoi *et al.*, 1995; 4, Grimont and Grimont, 2005; 5, Martin-Carnahan and Joseph, 2005; 6, Chamkha *et al.*, 2001b; 7, Rainey, 2009; 8, Tsuchiya *et al.*, 2007.

A more moderate response to protein was displayed by phylotypes most closely related to proteolytic anaerobes, including *C. thiosulfatireducens* (100% identity to phylotype PR7), *C. difficile* (99% identity to phylotype PR8), and *C. tunisiense* (100% identity to phylotype PR12) (Table 65 and Figure 43) (Seddon and Borriello, 1992; Hernández-Eugenio, 2002; Thabet *et al.*, 2004). The fermentation products acetate, methylbutyrate, propionate, and butyrate are common products of amino acid fermentations (McInerney, 1988; Buckel, 1999) and accumulated in the protein treatment. Furthermore, the most abundant phylotypes detected in these treatments were closely related to species that produce acetate, methylbutyrate, propionate, and butyrate while fermenting amino acids (Suen *et al.*, 1988; Chamkha *et al.*, 2001b; Finegold *et al.*, 2003; Tsuchiya *et al.*, 2007; Wiegel, 2009; Ruan *et al.*, 2014; Wang *et al.*, 2015). Thus, several phylotypes that responded positively to protein were associated with proteolytic taxa.

The dominant phylotype, phylotype PR3, that responded to RNA (Table 65 and Figure 43) represented the genus *Aeromonas* (100% identity to *A. media* and *A. hydrophila*), that was continuously detected in gut contents of *L. terrestris* (Wüst *et al.*, 2011; Meier *et al.*, 2018; Section 3.1.2 and 3.1.4) and casts of *Lumbricus rubellus* (Furlong *et al.*, 2002). *Aeromonas*-affiliated facultative aerobes can hydrolyze RNA and ferment pentoses (e.g., via pentose phosphate cycle [McMillan, 1993]) to acetate, succinate, and formate (Stanier and Adams, 1944; Allen *et al.*, 1983; Abbott *et al.*, 2003; Martin-Carnahan and Joseph, 2005; Li *et al.*, 2017). The stimulation of this phylotype by the supplemented pentose ribose (Section 3.2.8) is consistent with the stimulation of *Aeromonadaceae*-affiliated taxa in gut contents of *L. terrestris* supplemented with xylose (Meier *et al.*, 2018; Figure 33 A). Thus, the enhanced fermentation in pentose treatments and the negligible fermentative response to supplemented purines and pyrimidines reinforce the likelihood that ribose was the main driver of the enhanced fermentation in RNA treatments (Figure 41). The production of extracellular RNases by ingested soil microbes (Hankin and Anagnostakis, 1975; Mishra *et al.*, 2017) can contribute to the degradation and utilization of RNA in the anoxic alimentary canal and suggest that the hydrolysis of RNA can be independent of ribose fermenting taxa.

Phylotype CL2 (99% identity to the amino acid and carbohydrate fermenter *P. bif fermentans* [Wiegel, 2009]) was rapidly stimulated by supplemented microbial cell lysate during the first 6 h of incubation but subsequently decreased in relative abundance. In contrast, phylotypes CL8 (99% identity to the proteolytic fermenter *C. peptidivorans* [Whitman *et al.*, 1998]) and CL6 (95% identity to the potentially proteolytic *Lachnospiraceae*-affiliated fermenter *N. massiliensis* [Tidjani Alou *et al.*, 2017]) had a more sustained response to cell lysate, yielding a maximum relative abundance of 16S rRNA at the end of incubation (Figure 43). This observation reflects the capacity of fermenters with broad substrate spectra to be initially more competitive for the diversity of substrates derived from microbial cell lysate. However, the closely related phylotypes CL2 and PR2 displayed a differential response in unsupplemented controls, with the response of CL2 being more pronounced. Such observation might be due in part to a different nutrient status of gut

content at the time of gut content-extraction. Furthermore, these two experiments (lysate and protein/RNA) based on separate batches of earthworms that were maintained on soils collected at different times and such observed differences between the controls visualize the varying microbiota of the dietary substrate ingested by the earthworm.

#### 4.2.1.2. Protein- and RNA-based fermentation network

The findings illustrate that the primary biopolymers of disrupted microbial biomass, protein and RNA, display a high potential to stimulate a subsets of the fermentative earthworm gut bacteria. The hypothetical model (Figure 77) is a summarizing abstraction of the main findings and therefore restricted to the most responsive families. As such, the model emphasizes that protein and RNA may contribute to the overall fermentation profile of the earthworm alimentary canal.

The hydrolysis and fermentation of protein in the earthworm gut is consistent with the decreasing amount of protein during the gut passage (Tillinghast *et al.*, 2001). Such protein-based fermentations occurs also in other gut ecosystems. For example, the fermentation of protein in the gastrointestinal tract of higher animals, including humans, can affect the functional status of gut microbiota and the health status of the animal (Windey *et al.*, 2012; Pieper *et al.*, 2016; Yao *et al.*, 2016). Until today, it seems that there is no other study that has evaluated microbial taxa that facilitate RNA-based fermentation in a gut ecosystem.

The experimental design of the experiments did not simulate all of the *in situ* conditions of the gut, and the quantitative differences observed for the contrasting phylotypes can therefore not be extended to *in vivo* conditions. As such, the model does not include the possibility that less responsive taxa also participated in the protein- and RNA-based fermentation. Likewise, it does not address what taxa might respond to low nutrient input. However, the findings qualitatively illustrate the potential competitiveness of subgroups of the fermentative gut microbes that could respond to protein- and RNA-derived organic carbon in the alimentary canal and thus contribute to gut-associated fermentations. In this regard, the rapid stimulation of the phylotypes CL2, CL7, PR2, and PR3 (Figure 43) illustrate the remarkable anaerobic abilities of phylotypes that are related to bacterial species with phenotypes that are consistent with the fermentation profiles obtained. The accumulation of fermentation-derived H<sub>2</sub> in the treatments is consistent with the occurrence of H<sub>2</sub> in the gut and concomitant emission of this gas by *L. terrestris* (Wüst *et al.*, 2009b). This process can be linked to secondary H<sub>2</sub>-consuming processes in soil (Osborne *et al.*, 2010; Khdhiri *et al.*, 2017). The production of fermentation-derived CO<sub>2</sub> is less certain and would in part be influenced by the formation of carbonates in the pH neutral alimentary canal (Horn *et al.*, 2003).

The protein-, RNA-, and cell lysate-dependent stimulation of both fermentation and associated taxa were rapid and occurred within the initial 6 to 10 h of incubation. This

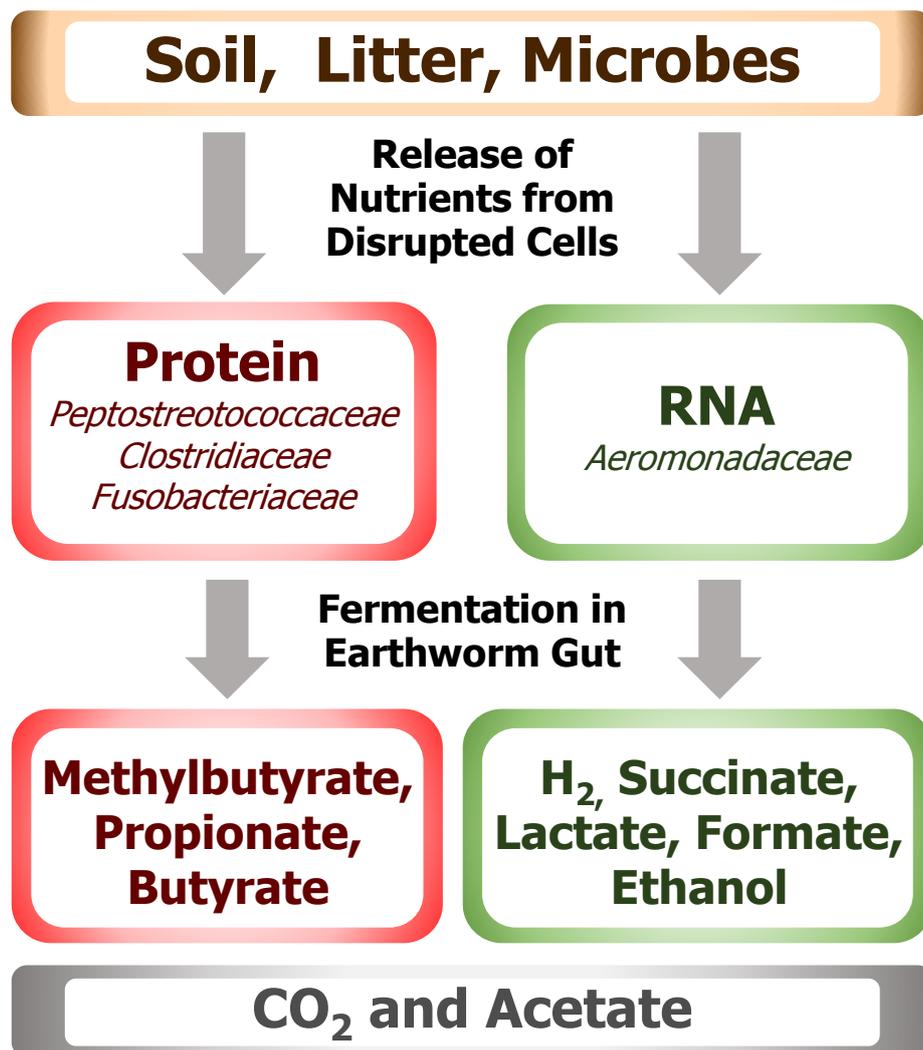
demonstrates that such substrates have the potential to stimulate microbes during gut passage. DNA is another nucleic acid released from gizzard-disrupted microbial cells and can constitute up to 3% of the dry weight (Babel and Müller, 1985; Delgado *et al.*, 2013). Given the marked potential of RNA to stimulate fermentation, it seems likely that the hydrolysis of DNA could also contribute to fermentation in the gut, and therefore worthy of further studies. In addition to gizzard-derived protein, this biopolymer is also a component of gut mucus and reinforces the likelihood that protein is available in the alimentary canal and therefore subject to utilization during gut passage (Rahemtulla and Løvtrup, 1975; Martin *et al.*, 1987; Brown *et al.*, 2000).

The *in situ* availability of protein and RNA in the gut can be additionally enhanced by other lytic events than those facilitated by the gizzard. In this regard, viruses represent one of the most abundant biological agents in the biosphere, and bacteriophages can be ten-fold more abundant than their bacterial hosts (Ashelford *et al.*, 2003; Clokie *et al.*, 2011). Indeed, soil is an excellent matrix for bacteria-phage interaction and the lysis of the bacterial host cell (Ashelford *et al.*, 2003; Armon, 2011; Clokie *et al.*, 2011) in the alimentary canal might therefore increase the amount of available polymers. Bacterial lysis by earthworm-derived lysozyme (Ville *et al.*, 1995; Schuch *et al.*, 2010), and the lysis of fungal cells by bacteria (Horikoshi and Iida, 1958; Mitchell and Alexander, 1963) are additional examples for lytic events that can occur in the gut system. For example, *B. cereus*, a species affiliated to phylotypes detected in gut content microcosms (Section 3.3.3), produces chitinase to lyse the hyphae of several fungal species (Mitchell and Alexander, 1963). These multiple mechanisms that potentially occur in the earthworm ingested material would increase the amount of fermentable biopolymers and thus enhance the formation of fermentation product that can be a source of nutrients for the earthworm (Bergman, 1990; Sampedro *et al.*, 2006).

The evaluated transformations of protein and RNA are obviously not restricted to the anoxic earthworms gut and therefore most likely highly stimulatory to fermentative microbes in all other O<sub>2</sub>-limited environments. However, the impact of these fermentative transformations on ecosystem- and microbiome-level have not been as profoundly evaluated as those of plant-derived polysaccharides such as cellulose (Weimer, 1992; Leschine, 1995). At the global level and based on the productivity of both autotrophic and heterotrophic microbes, the capacity of prokaryotes to synthesize protein- and RNA-rich biomass might be similar to the capacity of plants to produce polysaccharides (Gasol *et al.*, 1997; Whitman *et al.*, 1998; Kuzyakov and Larionova, 2005; Müren *et al.*, 2005; Calvo-Díaz *et al.*, 2011; Aytan *et al.*, 2018), illustrating the tremendous global potential of microbes to synthesize protein and RNA.

The evolution of life is presumed to have started approximately 4 billion years ago under O<sub>2</sub>-free conditions, and the production of O<sub>2</sub> by cyanobacteria and the occurrence of plants that finally became the major producers of polysaccharides is believed to have developed approximately 2.5 and 1 billion years ago, respectively (Shih, 2015). In this regard and on the assumption that protein and RNA were also the major biopolymers of primordial microbial cells,

it is most likely that these biopolymers were the main drivers of early fermentation and other redox processes conducted by obligate anaerobes. These reflections and the strong stimulation of fermentations by protein and RNA demonstrated at the primitive gut ecosystem of *L. terrestris* illustrates the huge biological capacity to profit from the fermentative transformation of these biopolymers. Further evaluations of these transformations in other anoxic environments would increase our understanding of how they contribute to the anaerobic turnover of organic carbon in today's biosphere.



**Figure 77.** Hypothetical model illustrating the ingested soil microorganisms that are able of fermenting protein and RNA derived from gizzard-disrupted microbial and plant cells.

#### 4.2.2. Amino acids and ribose as main drivers of protein and RNA fermentations

Assuming protein and RNA constitute 50% and 20%, respectively, of microbial cytoplasm on a dry weight basis (Babel and Müller, 1985; Lange and Heijnen, 2001; Delgado *et al.*, 2013), and cytoplasm has an 80% water content, the cytoplasm would contain up to 1 M polymeric amino acids and 100 mM polymeric ribose. This estimate is based on the assumption that the average amino acid has a molecular weight of 100 and that RNA consist of approximately 40% ribose. An intact bacterial cell in the direct proximity of a gizzard-ruptured cell would therefore experience an extraordinarily high amount of protein/amino acids and RNA/ribose. As noted above, additional sources of protein/amino acids and RNA/ribose that might enhance the available amount of these compounds in the gut ecosystem can be other lytic events, ingested plant biomass, and earthworm-excreted gut mucus (note: mucus does not contain RNA) (Needham, 1957; Martin *et al.*, 1987; Section 4.2.1.2). Independent of its origin, the amount of protein in the alimentary canal decreases rapidly from anterior to posterior, whereas the amount of ammonium increases from anterior to posterior (Tillinghast *et al.*, 2001). Furthermore, the amounts of ammonium in the gut and cast of earthworms are relatively high in comparison to the negligible amounts detected in pre-ingested soil (Parle, 1963b; Drake and Horn, 2007). These reflections suggest that gut fermentation of protein and concomitant deamination of amino acids lead to the accumulation of ammonium in the alimentary canal. In this regard, the significant stimulation of gut bacteria by certain supplemented amino acids is consistent with the availability of these biopolymer and the products of its hydrolysis in the alimentary canal.

##### 4.2.2.1. Fermentative phylotypes responsive to amino acids, ribose, and transient intermediates

The contrasting product profiles of amino acid, ribose, succinate, and formate treatments suggesting that these substrates were fermented or converted by contrasting obligate anaerobes and facultative aerobes. In this regard, *Firmicutes*- and *Fusobacteria*-affiliated obligate anaerobes were responsive during the fermentation of protein, whereas the fermentation of RNA was linked to responsive *Proteobacteria*-affiliated facultative aerobes (Section 3.2.4). The numerous phylotypes including five main GPT that were responsive in the amino acid and ribose treatments were also affiliated to these families (Table 66 and Figure 59).

In this regard, group phylotype GPT-1 (A4/R96/T3, 99 to 100% identity to *A. hydrophila*) was significantly stimulated by aspartate, ribose, and glucose (Table 66 and Figure 59). This phylotype responded additionally to RNA and saccharides (Section 3.2.4 and Section 3.1.4; Meier *et al.*, 2018). As mentioned before, the facultative aerobe *A. hydrophila* is able to ferment simple saccharides to acetate, ethanol, lactate, succinate, formate, CO<sub>2</sub>, and H<sub>2</sub> (Stanier and Adams, 1944; Allen *et al.*, 1983; Abbott *et al.*, 2003; Martin-Carnahan and Joseph, 2005; Li *et al.*, 2017).

Although *A. hydrophila* is not known to ferment aspartate, this species and closely related *A. media* harbor aspartate ammonia-lyase that could transform aspartate into the electron acceptor fumarate which reductively forms succinate (Knight and Blakemore, 1998; Parmeggiani *et al.*, 2018). In addition, *A. hydrophila* synthesizes aspartate transcarbamoylase that is required in the formation of pyrimidine precursors (Wild and Wales, 1990).

*Enterobacteriaceae*-affiliated group phylotype GPT-2 (A6/R5/T6, 99 to 100% identity to the facultative aerobes *B. gaviniae* and *E. aerogenes*) displayed a positive response in glutamate, aspartate, threonine, casamino acid, and ribose treatments (Table 66 and Figure 59). The *Buttiauxella*- and *Enterobacter*-affiliated phylotypes were also stimulated in gut contents supplemented with RNA or cell lysate (Section 3.2). *B. gaviniae* produces fatty acids and gases when fermenting sugars such as ribose, and several *Buttiauxella*-associated species can utilize amino acids including glutamate, aspartate, and threonine as sole carbon and energy sources (Müller *et al.*, 1996).

Sequences of the *Yokenella*-affiliated group phylotype GPT-3 (A129/A1526, 97 to 99% identity to the facultative aerobe *Y. regensburgensis*) displayed an apparent net increase in relative abundance in glutamate, aspartate, and threonine treatments. Although it is unknown that *Y. regensburgensis* can utilize amino acids, its presence in human wounds and infections is consistent with the potential ability to utilize amino acids (Abbott and Janda, 1994; Jain *et al.*, 2013).

Threonine and formate stimulated the group phylotype GPT-4 (A25/T7) that was closely related to the potential acetogen *T. glycolicus* (Table 66 and Figure 59). The stimulation in threonine and formate treatments (a) reflects the ability of *T. glycolicus* to convert threonine to propionate (Chamkha *et al.*, 2001b), and (b) is congruent with the potential for this acetogen to form acetate from formate (Küsel *et al.*, 2001). Acetogen-affiliated phylotypes were also stimulated in yeast lysate-supplemented treatments that displayed high amounts of transient formate (Section 3.2.1). However, acetogens are capable of diverse anaerobic processes including fermentation (Drake *et al.*, 2006, 2008), and the response of a potential acetogen in a certain treatment is therefore not strictly dependent on acetogenesis.

*Fusobacteriaceae*-affiliated phylotypes, continuously detected in gut contents of *L. terrestris*, were strongly stimulated by supplemental protein (Section 3.2.5). The associated group phylotype GPT-5 (A1/T2, 96% identity to *C. somerae*) was stimulated by glutamate, aspartate, valine/glycine, and succinate treatments (Table 66 and Figure 59). *C. somerae* occurs in gastrointestinal systems and ferments amino acids and peptides to acetate, propionate, and butyrate (Finegold *et al.*, 2003; Tsuchiya *et al.*, 2007). For example, threonine can be converted to propionate (James and Whitman, 2011), an observation consistent with the significant production of propionate in the threonine treatment (Table 39). Group phylotype GPT-5 was more distantly related to species of the strictly anaerobic genus *Propionigenium* (93% sequence identity).

**Table 66.** Summary of the most stimulated phylotypes in amino acid, ribose, succinate and formate treatments (Figure 59).<sup>a</sup>

GPT	Phylotype	Family	Closest cultured microorganism	Sequence Identity	Stimulated by	Aerobe/Anaerobe <sup>b</sup>	References <sup>b</sup>
GPT-1	A4/R96/T3	<i>Aeromonadaceae</i>	<i>A. hydrophila</i>	99-100%	Casamino acids, aspartate, glucose, ribose	Facultative aerobe	1
GPT-2	A6/R5/T6	<i>Enterobacteriaceae</i>	<i>B. gaviniae</i>	99%	Casamino acids, glutamate, aspartate, threonine, ribose	Facultative aerobe	2
GPT-3	A129/A1526	<i>Enterobacteriaceae</i>	<i>Y. regensburgeri</i>	97-99%	Casamino acids, glutamate, aspartate, threonine	Facultative aerobe	3
GPT-4	A25/T7	<i>Peptostreptococcaceae</i>	<i>T. glycolicus</i>	99-100%	Casamino acids, threonine, formate	Obligate anaerobe	4
GPT-5	A1/T2	<i>Fusobacteriaceae</i>	<i>C. somerae</i>	96%	Casamino acids, glutamate, aspartate, valine and glycine, succinate	Obligate anaerobe	5
	A8	<i>Peptostreptococcaceae</i>	<i>P. bifermantans</i>	99%	Casamino acids, alanine and glycine, valine and glycine	Obligate anaerobe	6
	A14	<i>Clostridiaceae</i>	<i>C. pascui</i>	100%	Glutamate	Obligate anaerobe	7

<sup>a</sup>Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one of the supplemented treatments displayed a  $\geq 2\%$  net increase in relative abundance. The phylotypes are derived from the analysis of 16S rRNA and 16S rRNA genes. A, phylotypes derived from amino acid experiment; R, phylotypes derived from ribose experiment; T, phylotypes derived from transient intermediate experiment.

<sup>b</sup>Information about the closest cultured microorganism obtained from: 1, Martin-Carnahan and Joseph, 2005; 2, Kämpfer, 2005; 3, Farmer and Brenner, 2005; 4, Chamkha *et al.*, 2001b; 5, Tsuchiya *et al.*, 2007; 6, Sasi Jyothsna *et al.*, 2016; 7, Wiegel, 2009.

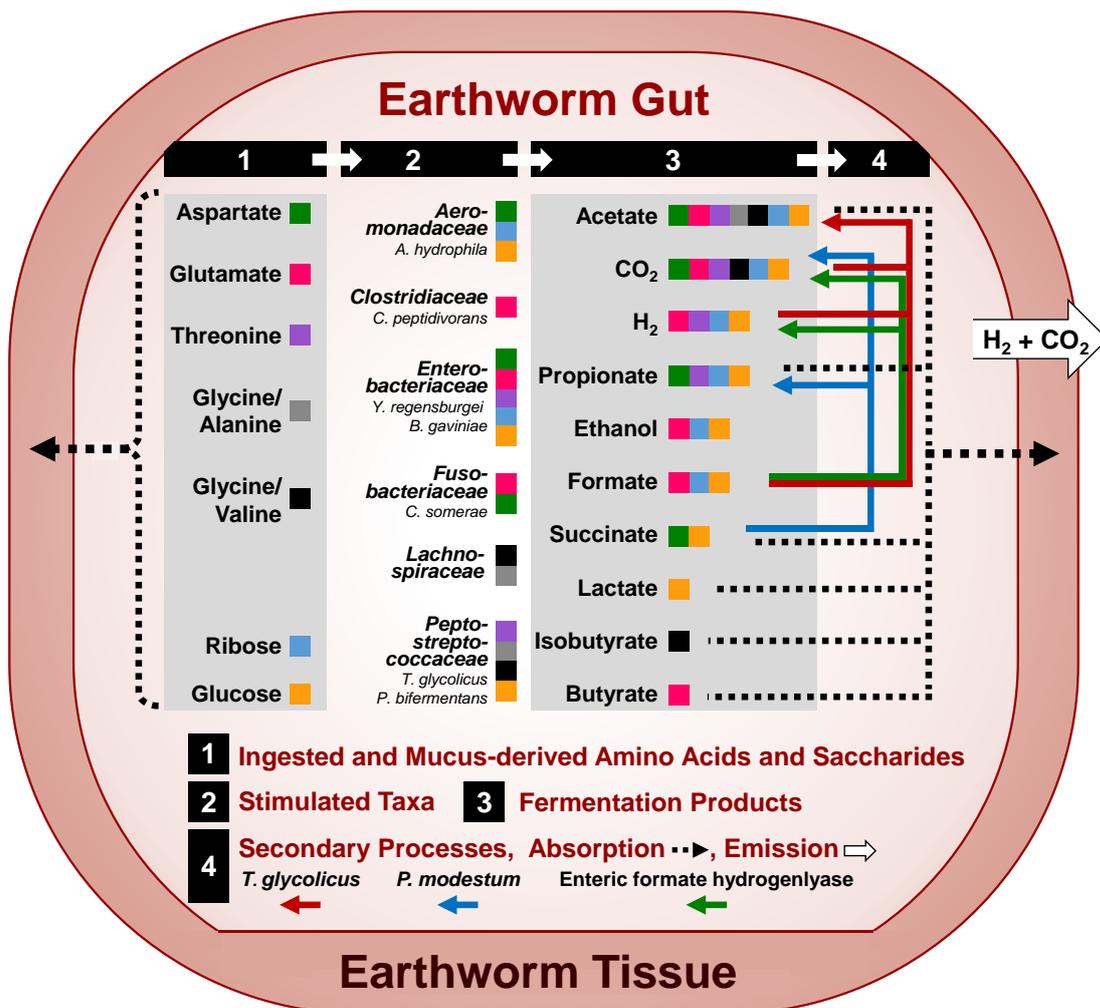
Members of this genus are able to utilize succinate for growth and produce propionate (Schink and Pfennig, 1982; Janssen and Liesack, 1995), a process consistent with the product profile of the succinate treatment (Figure 51 and Figure 52) that displayed a positive response of this group phylotype (Table 66 and Figure 59). Glutamate-stimulated phylotype A14 (Table 66 and Figure 59) is closely related to *C. pascui* (100% identity) and was also stimulated by protein rich cell lysate (Section 3.2.5). *C. pascui* is a proteolytic spore-forming anaerobe that is known to ferment glutamate (Wilde *et al.*, 1997). Phylotype A8 (99% identity to the amino acid fermenter *P. bifermentans*) responded in the co-amino acid treatments (Table 66 and Figure 59), reflecting the potential of this phylotype to conduct Stickland reaction (McInerny, 1988). That *P. bifermentans*, isolated from the human gut, can be cultivated on co-amino acids such as alanine plus glycine (Smith and Macfarlane, 1998) is consistent with the stimulation of phylotype A8 in co-amino acid treatments. This phylotype was also slightly stimulated in the casamino acid treatment (Table 66 and Figure 59) and strongly stimulated by protein and cell lysate (Section 3.2.5), findings consistent with the ability to ferment a mixture of amino acids. In addition to the catabolic fermentative processes associated to the aforementioned main stimulated phylotypes, it is noteworthy, that the supplemented amino acids could have also stimulated phylotypes via assimilatory processes. Indeed, given the availability of diverse amino acids in the alimentary canal (Drake and Horn, 2007), it is likely that amino acids would be utilized both catabolically and anabolically by ingested microbes during gut passage.

#### 4.2.2.2. Potential interactions of amino acid and ribose fermenters in the earthworm gut

The findings illustrates that certain amino acids stimulated fermentative subgroups of contrasting gut-associated affiliated taxa (Figure 78). Furthermore, the findings indicate that ribose lead to a stimulation of fermentative *Proteobacteria*-affiliated taxa. The fermentation-derived metabolic intermediates succinate and formate were converted by secondary processes associated with the positive response of *Firmicutes*- and *Fusobacteria*-affiliated taxa (Figure 78).

The model (Figure 78) is restricted to the main findings and therefore does not depict all potential fermentations in the alimentary canal of the earthworm. For example, several of the common amino acids that occur in the gut (Drake and Horn, 2007) were not evaluated but would be subject to *in situ* fermentations. In this regard, isoleucine can represent approximately 10% of the amino acids in the earthworm gut (Drake and Horn, 2007) and might be fermented to methylbutyrate (McInerny, 1988). This is consistent with (a) the formation of methylbutyrate in the casamino acid treatment (Figure 45), and (b) the *in situ*-occurrence of methylbutyrate in the earthworm gut (Wüst *et al.*, 2009b). Thus, the evaluation of other amino acids, including other stickland reactions, would warrant further studies. As displayed in the hypothetical model the glycoprotein-rich gut mucus (Laverack, 1963; Martin *et al.*, 1987) provides additional fermentable amino acids and saccharides for ingested microbes. Although it is advantageous for the

earthworm to utilize the fatty acids derived from fermentation of gut mucus (Figure 78), such recycling of worm-derived organic carbon cannot explain how the earthworm perpetuates. In this regard, ingested nutrients including biopolymers must be utilized. Both microbial- and plant-derived organic matter rapidly stimulate fermentation by gut-associated bacteria (Section 3.1 and Section 3.2), and dietary polymers that are more easily hydrolyzed are primary sources of fermentable organic carbon. The resulting animal-microbe interactions have been more extensively characterized in higher developed and compartmentalize gut ecosystems with specific host-associated syntrophs that optimize the breakdown of ingested polymers, including structural polysaccharides (e.g., termites and ruminants [Dietrich *et al.*, 2014; Xue *et al.*, 2018]). However, the relatively simple, more primitive gut of the earthworm exemplary illustrates the competitive and beneficial interactions that can occur between the animal host and hosted fermenters (Figure 78). Thus, available hydrolysis-derived amino acids can be either directly absorbed by the earthworm or fermented by the gut microbiota, whereby the latter case lead to products that potentially also serve as nutrition source for the earthworm (Figure 78; Adibi *et al.*, 1967; Bergman, 1990; Sampedro *et al.*, 2006).



**Figure 78.** Hypothetical model of fermentative transformations of amino acids and saccharides in the earthworm gut. The model depicts events that are interfaced to (a) the *in situ* hydrolysis of dietary protein, dietary RNA, and glycoprotein-rich mucus, and (b) the earthworm’s utilization of biopolymer constituents and fermentation-derived products. Figure modified and used with permission from Zeibich *et al.*, 2019b.

### 4.3. Fermenters in the earthworm gut: just on a visit (Hypothesis V)

An endemic and fermentative intestinal microbiota is a typical characteristic of many well studied higher (e.g., ruminants and humans) and lower (e.g., termites and cockroaches) animals (Brune and Friedrich, 2000; Lozupone *et al.*, 2012; Espey, 2013; Dietrich *et al.*, 2014). However, the nature and origin of the microorganisms contributing to the fermentations in the earthworm gut is not well understood. Nonetheless, the ingestion of soil introduces an enormous metabolic (i.e., microbial) potential to the anoxic alimentary canal (Section 1.2.2). For example, the activity of denitrifiers in the gut lead to the emission of  $N_2O$  by the earthworm (Karsten and Drake, 1997; Matthies *et al.*, 1999; Horn *et al.*, 2006a). Based on genetically analysis, these gut denitrifiers are phylogenetical affiliated to those of soil denitrifiers, suggesting that the denitrifiers in the gut are derived from the ingested matter rather than endemic to the host (Ihssen *et al.*, 2003; Horn *et al.*, 2006b; Wüst *et al.*, 2009a; Depkat-Jakob *et al.*, 2010, 2012). However, on the basis of recovered carbon and reductants in the products, fermentation rather than denitrification or iron reduction is the most dominant anaerobic process in the alimentary canal of the earthworm (Horn *et al.*, 2006a; Wüst *et al.*, 2009b). Independent of such processes the aforementioned considerations reinforce the likelihood that most of the microbes in the earthworm gut are ingested and transient (Drake and Horn, 2007). In comparison to the nutrient-poor soil, the gut of earthworms is a nutrient-rich microzone in soils (Section 1.2.2) and the ingestion of fermentative dormant soil taxa into the gut might therefore change their metabolic status and thus detectability (at both the phenotypic and genotypic levels).

The inability of soil to respond fermentatively to anoxia was independent of supplemented saccharides, protein, or RNA (Meier *et al.*, 2018; Table 51), and in marked contrast to the gut content that responded rapidly to these 'high quality' substrates. These findings might be interpreted to mean that the fermentative microbiota of gut content and soil differ. However, the supplement of a substrate with high nutrient complexity finally brought both soil and gut communities experimentally to a similar metabolic status, what reduce this potential detection bias. If gut fermenters originate from soil but are mostly dormant pre-ingestion, the nutrient richness of cell lysate and commercial yeast extract might stimulate soil microbes similarly to that observed with gut content. In this regard, cell lysate, simulating complex gizzard-disrupted biomass, greatly enhanced the fermentative activity of both soil and gut microbiota (Kristůfek *et al.*, 1994; Schönholzer *et al.*, 1999; Section 3.2), yielding nearly identical fermentation profiles.

#### 4.3.1. Fermentative soil taxa responsive to simulated gut conditions

The comparative evaluation of fermentative soil and gut microbes indicated that *Aeromonadaceae*, *Bacillaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Lachnospiraceae* and *Peptostreptococcaceae* were the main families detected in both gut content and soil treatments.

The occurrence of these families is consistent with previous findings, demonstrating a saccharide-derived response of *Aeromonadaceae*- and *Enterobacteriaceae*-affiliated phylotypes and a protein-derived response of *Clostridiaceae*- and *Peptostreptococcaceae*-affiliated phylotypes in anoxic gut content treatments (Meier *et al.*, 2018; Section 3.1.5).

**Table 67.** Summary of the most extract-stimulated phylotypes in *L. terrestris* gut content and soil treatments (Figure 61).<sup>a</sup>

Phylo-type	Phyla	Family	Closest cultured microorganism	Sequence Identity	Aerobe/ Anaerobe <sup>b</sup>	Refer-ences <sup>b</sup>
E2	Proteo-bacteria	Entero-bacteriaceae	<i>E. aerogenes</i>	99%	Facultative aerobe	1, 2
E3	Proteo-bacteria	Aero-monadaceae	<i>A. media</i>	100%	Facultative aerobe	3
E4	Fimicutes	Peptostrepto-coccaceae	<i>R. lituseburensis</i>	99%	Obligate anaerobe	4, 5
E5	Fimicutes	Peptostrepto-coccaceae	<i>T. glycolicus</i>	99%	Obligate anaerobe	6
E6	Fimicutes	Clostridiaceae	<i>C. peptidivorans</i>	100%	Obligate anaerobe	5
E13	Fimicutes	Clostridiaceae	<i>C. magnum</i>	100%	Obligate anaerobe	5
E16	Fimicutes	Clostridiaceae	<i>C. sartagoforme</i>	100%	Obligate anaerobe	5
E17	Fimicutes	Clostridiaceae	<i>C. subterminale</i>	100%	Obligate anaerobe	5
E19	Fimicutes	Bacillaceae	<i>B. cereus</i>	96%	Facultative aerobe	7
E314	Proteo-bacteria	Entero-bacteriaceae	<i>E. vulneris</i>	99%	Facultative aerobe	8

<sup>a</sup>Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be stimulated when a phylotype in at least one gut content or soil treatment displayed a minimum increase in relative abundance of 4% during the incubation. The phylotypes are derived from 16S rRNA genes or 16S rRNA analyses.

<sup>b</sup>Information about the closest cultured microorganism obtained from: 1, Yokoi *et al.*, 1995; 2, Grimont and Grimont, 2005; 3, Martin-Carnahan and Joseph, 2005; 4, Gerritsen *et al.*, 2014; 5, Wiegel, 2009; 6, Chamkha *et al.*, 2001b; 7, Logan and De Vos, 2009; 8, Scheutz and Strockbine, 2005.

Independent of the quantitative dissimilarities between the treatments, the ten most abundant phylotypes were detected in both gut content and soil. Thus, *Proteobacteria*-affiliated phylotype E2 (99% identity to *E. aerogenes*) and *Clostridiaceae*-affiliated phylotype E6 (100% identity to *C. peptidivorans*) were abundant in both extract-supplemented soil and gut content treatments (Table 67). *E. aerogenes* is a facultative soil aerobe and ferments carbohydrates and polypeptone to diverse products including hydrogen, acetate, succinate, and lactate (Yokoi *et al.*, 1995; Deepa *et al.*, 2010; Section 4.2.1.1). *Firmicutes*-affiliated *C. peptidivorans* ferments amino acids (Mechichi *et al.*, 2000; Wiegel, 2009) and was also stimulated in protein and amino acid treatments (Section 3.2.5 and Section 3.2.10). *T. glycolicus*-affiliated phylotype E5 (99% identity) and *C. magnum*-affiliated phylotype E13 (100% identity), both related to acetogens that are detected in soils and sediments (Gaston and Stadtman, 1963; Schink, 1984; Küsel *et al.*, 2001;

Meyer *et al.*, 2007), were additional phylotypes that responded in both treatments.

As indicated before, acetogens utilize diverse substrates by either anaerobic respiration or fermentation (Drake *et al.*, 2008). The *Aeromonadaceae*-affiliated phylotype E3 (100% identity to *A. hydrophila* [Table 67]) was more abundant in supplemented soil treatments than in gut content treatments. Species of the genus *Aeromonas*, were continuously detected in earthworm gut contents and casts (Furlong *et al.*, 2002; Wüst *et al.*, 2011; Meier *et al.*, 2018; Section 3.1.5, Section 3.2.5, and Section 3.3.3). *A. hydrophila* occur in soil and aquatic habitats and ferment carbohydrates to ethanol and divers fatty acids (Brandi *et al.*, 1996; Martin-Carnahan and Joseph, 2005; Lee *et al.*, 2008). Although the affiliated phylotype was more responsive in soil treatments, it is known from previous experiments that this phylotype can (a) respond early in supplemented gut content treatments and (b) diminish to marginal levels at the end of incubations (Section 3.2.5). In contrast, other phylotypes (e.g., closely related to *E. aerogenes*) become more abundant in the latter stages of incubation (Section 3.2.5). In this regard, the relative abundance of a phylotype at the end of the incubation does not reflect potential shifts that might occur during the incubation. Furthermore, even with additional complex nutrients, it is most likely that fermentative phylotypes in soil would respond slower under experimentally anoxic conditions than would the same phylotypes in gut contents since the latter are primed to respond anaerobically in the anoxic gut. Indeed, at the beginning of incubation the fermentation was marginally delayed in soil microcosms compared to the more rapid fermentative response of gut content (Figure 61). The death of certain microbes in the gut content and the antimicrobial characteristics of the earthworm digestive fluids (Kristufek *et al.*, 1994; Schönholzer *et al.*, 1999; Khomyakov *et al.*, 2007) are additional factors that could have led to observed differences between the relative abundance of a given phylotype in soil and gut content treatments.

*Enterobacteriaceae*-affiliated phylotype E314 (99% identity to *E. vulneris*) and *Peptostreptococcaceae*-affiliated phylotype E4 (99% identity to *R. lituseburensis* [Table 67]) were abundant in gut content treatments but less abundant in soil treatments at the end of the incubation (Figure 67). Members of the genus *Escherichia* are facultative aerobes occurring in terrestrial and aquatic environments and form diverse products including lactate, acetate, and formate when fermenting carbohydrates. This genus consist of enteric bacteria that potentially convert formate to H<sub>2</sub> and CO<sub>2</sub> (Scheutz and Strockbine, 2005; Walk *et al.*, 2009). *R. lituseburensis* is a proteolytic anaerobe that produces butyrate, acetate, and isobutyrate (Wiegel, 2009; Gerritsen *et al.*, 2014; Wang *et al.*, 2015). Soil is known to harbor such proteolytic bacteria (Fuka *et al.*, 2009), and phylotype E17 was another responsive phylotype closely related to a proteolytic fermenter common to soil (100% identity to *C. subterminale*; Smith, 1975; Suen *et al.*, 1988). That the relative abundances of the phylotypes detected in all four treatments (soil and gut content in each case supplemented or unsupplemented) constituted the majority of the detected sequences is consistent with previous studies that have indicated that bacteria in the earthworm gut are present in soil (Bassalik, 1913; Brown, 1995; Furlong *et al.*, 2002; Egert *et al.*, 2004). The stimulation of several proteolytic amino acid-fermenting phylotypes (e.g., E2, E4, E6,

E17) in yeast extract treatments is consistent with the availability of proteins and associated amino acids in yeast extract. That a *Bacillaceae*-affiliated phylotype (phylotype E19, 96% identity to *B. cereus*) was abundant in control gut content treatments, but not significantly stimulated in yeast extract treatments of either gut content or soil (Figure 61), suggesting that this phylotype was less competitive when nutrient availability increased and therefore of minimal importance to the fermentation profiles of extract-supplemented treatments. However, the detection of the ten most abundant gut-associated phylotypes in soil was greatly enhanced by the stimulation of soil-based taxa with supplemented complex nutrients.

#### 4.3.2. Perspectives on a transient dominated gut

The findings indicate that responsive fermentative gut phylotypes in the earthworm gut originate from soil. A conclusion confirmed by earlier studies that revealed similar bacteria in soil and the gut of the earthworm (Bassalik, 1913; Furlong *et al.*, 2002; Egert *et al.*, 2004). Furthermore, the study reinforces the likelihood that fermentative microorganisms that may be dormant or difficult to detect pre-ingestion might become active post-ingestion and contribute to the collective microbial metabolic potentials of the gut ecosystem. However, it cannot be excluded that resident microbes also participate in the microbiology of the earthworm gut. In this regard, although the abrasion properties of the passage of soil through the alimentary canal of anecic earthworms might theoretically prevent a resistant gut wall-associated distribution of ingested microbes, microscopic studies illustrate that the gut wall is at least minimally colonized (Jolly *et al.*, 1993; Mendez *et al.*, 2003). For example, *Mycoplasmataceae*-affiliated *Can. Lumbricincola* species are associated with the gut and other anatomical regions of the earthworm (Nechitaylo *et al.*, 2009). *Can. Lumbricincola*-affiliated phylotypes did not increase in response to the complex nutrient input (Figure 64), an observation confirmed by earlier findings that indicated *Mycoplasmataceae*-affiliated phylotypes were not stimulated by any supplemented saccharides, protein, or RNA (Meier *et al.*, 2018; Section 3.1.5 and Section 3.2.5). Nutrient limitation and extended incubation times can improve the cultivability and culture-based detection of rare taxa in soil, reflecting the nutrient poorness of this habitat (Joseph *et al.*, 2003; Stevenson *et al.*, 2004; Davis *et al.*, 2005; Stott *et al.*, 2008). In marked contrast to the nutrient-poor conditions in soil, the alimentary canal of the earthworm represents a nutrient-rich anoxic microzone that introduces ingested soil microbes to a temporary large amount of nutrients. However, the alimentary canal of the earthworm is O<sub>2</sub>-limited and therefore of primary value to ingested facultative aerobes and anaerobes. In this regard, the relatively high number of cultivable microorganisms in soil that are capable of anaerobic growth (10<sup>7</sup> to 10<sup>9</sup> per gram dry weight soil [Karsten and Drake, 1997; Küsel *et al.*, 1999]) are provided with ideal conditions for anaerobic activity in the anoxic gut. The O<sub>2</sub>-limited conditions in the gut are also important for the earthworm since anoxia optimizes the fermentative production of fatty acids that can be utilized by the earthworm (Bergman, 1990; Sampedro *et al.*, 2006).

#### 4.4. Impact of increased water content on the microbial gut community of *L. terrestris* (Hypothesis VI)

Large experimental set ups, including three replicate analysis and treatments supplemented with different substrates, require a 1:10 dilution of the extracted gut contents for obtaining adequate samples for chemical and molecular analyses (Wüst *et al.*, 2011; Section 2.1.2). In addition to the experimentally necessary dilution of the gut content-associated microbes, previous studies illustrated that the earthworm ingested soil bacteria experience also *in situ* high fluctuations in water content during the gut passage (Horn *et al.*, 2003). Indeed, soil exhibit a water content of approximately 20%, whereas the anterior part of the earthworm gut ecosystem contain a water content of up to 80% (Horn *et al.*, 2003), indicating a potential four-fold dilution of the ingested biomass and associated microorganisms in this region of the alimentary canal. Furthermore, it is of interest to note, that the water content in the alimentary canal decreases by approximately 20% from the anterior region to the posterior end, demonstrating that ingested bacteria are subject to further water fluctuations.

##### 4.4.1. Fermentative microbes responsive to increased water contents

Despite the 1:10 dilution of material, the findings demonstrated that the undiluted and diluted fermentative gut microbiota responded nearly identical during anoxic incubation. However, the fermentative activity and the increase of sequence abundances of certain families, like *Shewanellaceae*, *Peptostreptococcaceae* and *Fusobacteriaceae*, were more pronounced in undiluted gut contents than in diluted gut contents. The stronger stimulation of these families in undiluted gut contents was concomitant with a higher amount of collective fermentation products (Figure 69A), indicating that these families were most likely the main drivers for the observed fermentation profiles, including acetate, CO<sub>2</sub>, and propionate as main products. In marked contrast, the facultative aerobic *Aeromonadaceae* and *Mycoplasmataceae* responded negatively in both treatments during incubation (Figure 69), suggesting that these families were less competitive than *Shewanellaceae*, *Peptostreptococcaceae* and *Fusobacteriaceae*. Nonetheless, the trends observed on family-level were extended to several abundant phylotypes. Thus, phylotype D2, D5, and D1, most closely related to *S. putrefaciens* (100% identity, *Shewanellaceae*), *P. bifermentans* (99% identity, *Peptostreptococcaceae*), and *C. somerae* (96% identity, *Fusobacteriaceae*) (Table 68), respectively, displayed a positive response during anoxic incubation (Figure 72), suggesting a fermentative stimulation of these phylotypes by endogenous gut nutrients, including saccharides and amino acids (Horn *et al.*, 2003; Wüst *et al.*, 2009b). As noted above, *C. somerae* is able to ferment amino acids and peptides to acetate, propionate, and butyrate and occurs in other gastrointestinal systems (Finegold *et al.*, 2003; Tsuchiya *et al.*, 2007). *P. bifermentans* utilize carbohydrates and amino acids and form diverse fermentation products,

including H<sub>2</sub>, acetate, formate, butyrate isobutyrate and propionate (Wiegel, 2009). Members of the genus *Shewanella* use inorganic or organic electron acceptors (e.g., NO<sub>3</sub><sup>-</sup>, Fe<sup>3+</sup>) that are common to soil and detected in the earthworm gut (Drake and Horn, 2007; Zhou *et al.*, 2019; Wüst *et al.*, 2009a) to oxidize carbon compounds or H<sub>2</sub> (Bowman, 2005). In addition to nitrate or iron reduction, members of these genus can also be fermentative and produce acids from carbohydrate such as glucose or *N*-acetylglucosamine (Bowman, 2005). In this regard, these saccharides can be derived from earthworm-ingested and -disrupted microbial cell walls (Schleifer and Kandler, 1972).

**Table 68.** Summary of the most responsive phylotypes in diluted and undiluted treatments (Figure 72).<sup>a</sup>

Phylo-type	Phyla	Family	Closest cultured microorganism	Sequence Identity	Response	Aerobe/ Anaerobe <sup>b</sup>	Refer-ences <sup>b</sup>
D1	<i>Fuso-bacteria</i>	<i>Fuso-bacteriaceae</i>	<i>C. somerae</i>	96%	positive	Obligate anaerobe	1
D2	<i>Firmi-cutes</i>	<i>Shewa-nellaceae</i>	<i>S. putrefaciens</i>	100%	positive	Obligate anaerobe	2
D3	<i>Teneri-cutes</i>	<i>Mycoplasma-taceae</i>	<i>Can. Lumbricincola</i>	99%	negative	Facultative aerobe	3
D4	<i>Proteo-bacteria</i>	<i>Aeromona-daceae</i>	<i>A. hydrophila</i>	99%	negative	Facultative aerobe	4
D5	<i>Firmi-cutes</i>	<i>Peptostrepto-coccaceae</i>	<i>P. bif fermentans</i>	99%	positive	Obligate anerobe	5
D179	<i>Proteo-bacteria</i>	<i>Aeromona-daceae</i>	<i>A. hydrophila</i>	99%	negative	Facultative aerobe	4

<sup>a</sup>Phylotypes are based on a sequence similarity cut-off of 97% and were considered to be responsive when a phylotype in at least one of the undiluted or diluted treatment displayed a  $\geq 4\%$  higher or lower relative 16S rRNA or 16S rRNA gene abundances at the end of incubation than at the beginning of incubation.

<sup>b</sup>Information about the closest cultured microorganism obtained from: 1, Tsuchiya *et al.*, 2007; 2, Bowman, 2005; 3, Brown *et al.*, 2011; 4, Martin-Carnahan and Joseph, 2005; 5, Sasi Jyothsna *et al.*, 2016.

Consistent with the negative response of *Aeromonadaceae* and *Mycoplasmataceae*, the phylotypes D4/D179 (both 99% identity to *A. hydrophila*) and phylotype D3 (99% identity to *Can. Lumbricincola*) displayed a decrease in both diluted and undiluted treatments during incubation. These findings suggest that these taxa were less competitive to respond positively to gut content nutrients than were other taxa, and therefore of lower importance to the observed fermentation profiles (Figure 69A). The decrease of sequence abundances affiliated to phylotypes of *Aeromonadaceae* is consistent with previous findings that indicated no positive response of these taxa in unsupplemented control treatments (Section 3.1.2, Section 3.1.4, Section 3.2.4, Section 3.2.7, and Section 3.2.9). That *Can. Lumbricincola*-affiliated phylotypes displayed a negative response in the treatments, was also consistent with other studies and previous findings that have demonstrated that this taxon was, independent of a supplemented substrate, not stimulated during gut content incubations (Meier *et al.*, 2018; Figure 24, Figure 30, Figure 37, Figure 41,

Figure 49, Figure 56, Figure 64). The negative response of D4, D179, and D3 and their affiliated-families was more pronounced in undiluted gut contents, suggesting a stronger competition in this treatment. This enhanced competition was most likely caused by the enhanced stimulation of *Shewanellaceae*, *Peptostreptococcaceae*, and *Fusobacteriaceae* in undiluted gut contents compared to diluted gut contents.

#### 4.4.2. The minor effect of increasing water content

Water has in biological systems two main functions. Thus, the availability of water (a) provides dissolved nutrients, metabolic wastes, and metabolic products, and (b) ensures the stability and function of biopolymers (e.g., protein and RNA) (Gervais et al., 1996; Chaplin, 2001). In this regard, if water becomes inadequate cell metabolism slows or stop, and the structure and function of membranes and biopolymers can no longer be maintained (Gervais et al., 1996).

Although the experimentally necessary dilution of the gut contents and the associated microbiota is a much higher dilution than may occur *in situ*, the magnitude of the physiological response of the 1:10 diluted gut content was only slightly less compared to the undiluted gut content. Consistent with that, the undiluted treatment yielded a stronger community response than the diluted treatments. Although *Firmicutes*-affiliated families displayed a different response in both treatments (*Clostridiaceae* and *Lachnospiraceae* increased in diluted treatment but did not in undiluted treatments), the fermentation profiles of the two treatments were nearly identical (Figure 68). Thus, the communities in undiluted and diluted gut contents, that slightly differ, catalyzed identical fermentative processes, illustrating the functional redundancy of both communities (Miki et al., 2013; Langer et al., 2015). Nonetheless, the fermentative activity and community of both treatments were qualitatively more similar than dissimilar, demonstrating that the diluted gut content reflects the response of the system without a major disturbance of the dilution.

In contrast to the stimulatory effects of microbial- and plant-derived biopolymers, the *in situ* dilution of ingested material and associated microorganisms in the anterior part of the gut ecosystem has most likely a minor effect on the fermentative gut microbiota. Thus, the earthworm gut community composition and the associated fermentations are potentially more effected by the varying physiological properties of soil-derived microorganisms and the high complexity of ingested dietary materials (e.g., structural and non-structural polysaccharides, protein, RNA). This assumption is reinforced by the marginally fermentation activity of microcosms containing diluted soil that was only enhanced when soil was additionally supplemented with a complex substrate (Section 3.3). The dilution of ingested soil microorganisms in the anterior region of the earthworm is concomitant with an increase of available nutrients derived from gizzard-disrupted cells and gut mucus. Thus, evaluating the potential effect of increased water content on the fermentative gut microbiota that is simultaneously challenged with complex substrates warrants further studies.

## 4.5. Potential impact of dietary substrates on the earthworm symbiont *Can. Lumbricincola* (Hypothesis VII)

Earthworms feed on diverse materials, including plant matter, soil, and associated microbes (Section 1.3). Thus, dependent on the current feeding status of the earthworm, the ingested material varies in composition, including the amount of organic carbon, an important nutrition source for the fermentative gut community of the earthworm. Indeed, earlier studies demonstrated, that the carbon content of ingested material effected the earthworm gut community, including the family *Mycoplasmataceae* (Feustel, Oppermann, Schmidt, and Drake; data not published). In this regard, the *Tenericutes*-affiliated *Mycoplasmataceae* responded negatively to dietary substrates with limited organic carbon content. To further resolve these observations, additional experiments were conducted to highlight the potential impact of different environmental substrates on *Can. Lumbricincola* in gut contents of *L. terrestris*.

The findings indicated that on family-level the gut communities of the earthworms kept on different dietary substrates were qualitatively similar but displayed quantitative differences that were more pronounced on phylotype-level. The detectable differences in the gut bacterial community is confirmed by the assumption that the earthworm gut is dominated by ingested bacteria that are further influenced by available nutrients derived from ingested materials (Section 3.3). Thus, the absence or presence of a bacterial taxon in this gut ecosystem is dependent on the nature of the ingested material at time of feeding. In contrast to taxa common to both soil and earthworms, the *Mycoplasmataceae*-affiliated taxon *Can. Lumbricincola* was exclusively and continuously detected in earthworm gut contents and not in the dietary material (Section 3.3; Nechitaylo *et al.*, 2009). Furthermore, other studies confirm that this group of *Mollicutes* were strongly associated with earthworms but not detectable in their native substrates, a finding (a) consistent with the inability of *Mycoplasmataceae*-affiliated taxa to withstand outside the hosts (McAuliffe, 2006), and (b) corroborating that this taxon is a potential resistant bacterial symbiont in the earthworms.

### 4.5.1. Differential response of *Can. Lumbricincola*-affiliated taxa

16S rRNA based analysis on phylotype-level revealed phylotype OC1 and OC6 as abundant phlotypes closely related to *Can. Lumbricincola* (100% identity). Furthermore, OC8 was an abundant phylotype distantly related to *Can. Lumbricincola* (87% identity). Interestingly, phylotype OC1 was more abundant in gut contents obtained from earthworms kept on organic carbon limited substrates (i.e., turf and soil), whereas phylotype OC6 and OC8, hardly detectable in this substrates, were more abundant in worm bedding (Figure 75). These finding indicate that *Can. Lumbricincola*-affiliated phlotypes were differently effected by the contrasting environmental substrates. Chemoorganotrophic *Mycoplasmataceae* occur in coelom, gut tissues, gut contents and casts of several earthworm species and are characterizes by the lack of a cell wall (Nechitaylo *et al.*, 2009; Brown *et al.*, 2011). Members of this family ferment glucose and/or

hydrolyze arginine via the arginine deaminase pathway to ammonia, CO<sub>2</sub>, and ornithine (Schimke *et al.*, 1966; Razin *et al.*, 1980; Buckel, 1999; Brown *et al.*, 2011). Furthermore, they are known for their symbiotic lifestyle with eukaryotes (e.g., annelids, fishes, and crustacean) as mutualist, commensals or parasites (Holben *et al.*, 2002; Johansson and Pettersson, 2002; Maniloff, 2002; Nechitaylo *et al.*, 2009; Chen *et al.*, 2015; Murakami *et al.*, 2015; Llewellyn *et al.*, 2016). The type of symbiosis (i.e., mutualistic, commensalistic, or parasitic) of this taxon that accounts for its association with the earthworm is still unknown and warrants further studies. Interestingly, some members of the family have a requirement for urea as sole energy source and/or sterols (e.g., cholesterol) for growth (Brown *et al.*, 2011). Urea is a metabolic waste product in the coelomic fluid, intestine, and gut tissue of earthworms (Cohen and Lewis, 1949; Laverack, 1963; Bishop and Campbell, 1965), whereas cholesterol is an important membrane component in earthworms (Cerbulis and Wight Taylor, 1969; McLaughlin, 1971; Okamura *et al.*, 1985; Petersen and Holmstrup, 2000). These considerations illustrate and reinforce the dependence of *Can. Lumbricincola* on host-specific factors, a conclusion consistent with the studies of this dissertation that demonstrated no positive response of this taxon to any of the supplemented substrates (e.g., protein, RNA, saccharides, cell lysates, amino acids). In addition to *Can. Lumbricincola*, the earthworm can harbor other microbes that are not detectable in the dietary material and thus potential earthworm symbionts. In this regard, *Verminephrobacter eiseniae* (*Comamonadaceae*-affiliated *beta*-*proteobacterium*), associated with the nephridia (Pinel *et al.*, 2008), and an uncultured *Verrucomicrobium* (Nechitaylo *et al.*, 2010) was only detected in earthworm-derived sources.

#### **4.5.2. Hypothetical links between *Can. Lumbricincola* and dietary substrates**

That two of the three abundant *Can. Lumbricincola*-affiliated phylotypes were negatively influenced by organic carbon limited environmental substrates is confirm with previous studies that demonstrated a decrease of *Mycoplasmataceae* when earthworms were maintained on such substrates (Feustel, Oppermann, Schmidt, and Drake; data not published). Independent of the supplemented substrates, *Can. Lumbricincola* was also not competitive in gut content microcosms of other studies, an observation confirm with the assumption that this taxon is strongly dependent on host-specific factors (e.g., urea). That the three *Can. Lumbricincola*-affiliated phylotypes responded differently to the dietary substrates indicated that ingested environmental material can affect the occurrence of these phylotypes. In this regard, diverse potential factors could influence the colonization of different anatomical regions in earthworms by these phylotypes (Figure 79).

The highly variable direct and indirect links between the composition of ingested substrate and *Can. Lumbricincola* are most likely primary effected by the fermentative activities of the gut microbiota and the nitrogen metabolism of the earthworm (Figure 79). For example, the stimulation of a urea-dependent *Can. Lumbricincola*-affiliated phylotype can be influenced by (a)



## 4.6. General conclusions and limitations

The collective findings of this dissertation indicated that (a) the earthworm gut microbiota is poised to respond rapidly to nutrient input, (b) nonstructural polysaccharides, protein, and RNA of disrupted cells are subject to fermentation by earthworm gut microbes, (c) certain amino acids and ribose are the main drivers for intestinal protein and RNA fermentation, and (d) the hydrolysis of certain ingested structural polysaccharides may be the limiting factor in the ability of gut fermenters to utilize them. Furthermore, the findings illustrate that (a) responsive fermentative taxa may originate from soil, and (b) the detectability of the fermentative taxa is conditional on their nutrient-dependent metabolic status.

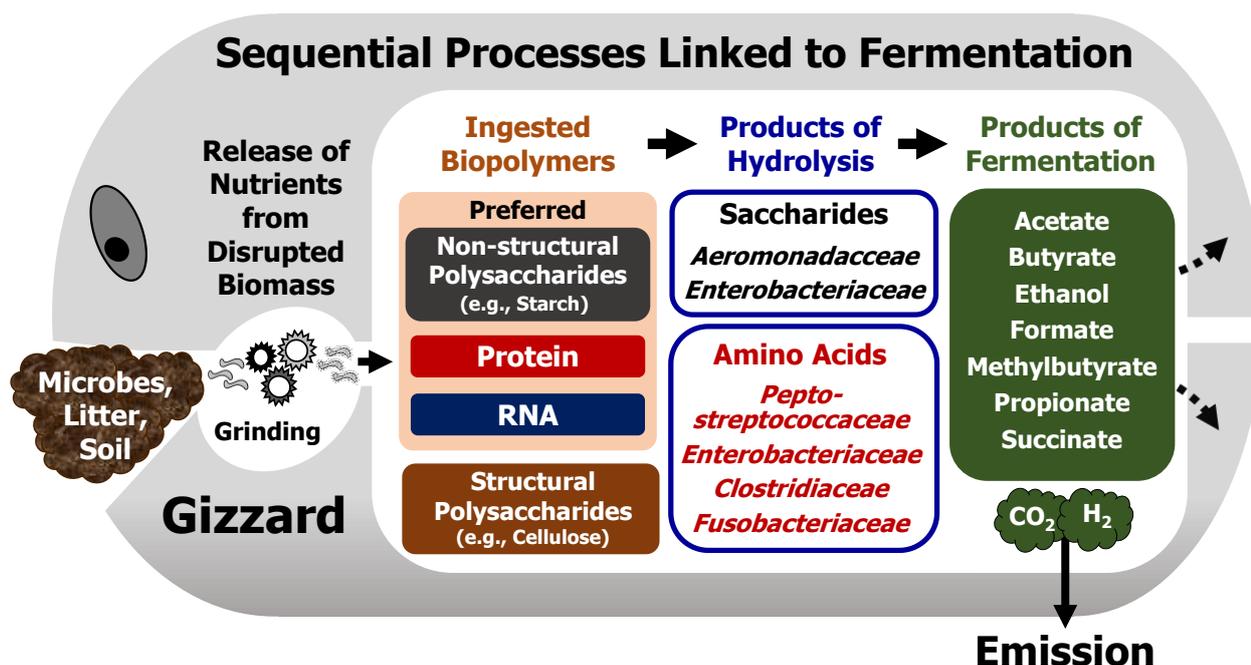
Figure 80 summarizes the main findings and illustrates the potential trophic interactions between the earthworm and ingested fermentative taxa in the alimentary canal. This hypothetical model is a theoretical abstraction and does not depict all anaerobic processes in the alimentary canal. For example, gut denitrification that leads to the earthworm's emission of  $N_2O$  (Ihsen *et al.*, 2003; Horn *et al.*, 2006a; Drake and Horn, 2007; Wüst *et al.*, 2009a) is not included. As such, the model emphasize the potential that non-structural polysaccharides, protein, and RNA contribute to the overall fermentation profile of the alimentary canal.

The trophic interactions between the earthworm and the ingested microbial community (Figure 80) is in part responsible for the environmental impact of the earthworm in the terrestrial biosphere and the survival of the earthworm throughout its evolution. In this regard and like in other animals, the earthworm can utilize the fatty acids produced by the fermentative gut microbiota (Bergman, 1990; Sampedro *et al.*, 2006) that is most likely important to the overall strategies by which the earthworm obtains dietary nutrients. However, the earthworm, like other animals, would also benefit from assimilating initial products of biopolymer hydrolysis (e.g., protein-derived amino acids or polysaccharide-derived saccharides [Figure 80]) prior to microbial fermentation (Adibi and Gray, 1967; Robinson and Alvarado, 1971; Kiela and Ghishan, 2016). This illustrates a potentially *in situ* competition between the earthworm and gut fermenters for the initial products of biopolymer hydrolysis. In this regard, earthworm salivary glands secrete proteases and amylases into the alimentary canal (Laverack, 1963), indicating that the earthworm contributes to the breakdown of ingested biopolymers during gut passage. As such, certain non-proteolytic amino acid fermenters and non-saccharolytic sugar fermenters likely benefit from the protease rich gut.

The experimental protocol did not simulate all *in situ* conditions of the gut, but was adequate for the evaluation of the responsiveness of specific fermentative taxa to a supplemental substrate. Thus, the strong quantitative enhancement of a given phylotype is not proposed to occur *in situ*. However, the gut content microcosms reflected the response of the system without a major disturbance of the experimental necessary dilution, and the observed fermentations are consistent with the diverse *in situ* fermentative activities that can occur in the alimentary canal

(Wüst *et al.*, 2009b). Likewise, that the fermentative potentials of the closest cultured microorganism associated to the most abundant phylotypes were consistent with the observed fermentations in the treatments, reinforce the likelihood that the detected organisms could account for the observed activities.

Illumina sequencing generates relatively short read lengths that can compromise the accurate taxonomic assignment of sequences to a known species (Yarza *et al.*, 2014; Singer *et al.*, 2016), and phylotype assessments should be qualified accordingly. Furthermore, the detection of a phylotype based on the 16S rRNA genes and 16S rRNA analysis is dependent on the primer-dependent detection of a given target sequence, and a greater number of targets increases the likelihood of detection. In this regard, the number of 16S rRNA genes per genome varies and is dependent on the species (Větrovský and Baldrian, 2013). Thus, an organism with a highly active metabolism and a low gene copy number might therefore be more detectable with 16S rRNA-based analysis since the number of ribosomes can exceed  $10^4$  per cell (Xie *et al.*, 2008). Nonetheless, the fermentative activities of the detected phylotypes were consistent with the phenotypic properties of the species most closely related to a phylotype.



**Figure 80.** Summarizing model illustrating the potential trophic interactions between the earthworm *L. terrestris* and ingested soil microorganisms capable of fermenting biopolymer-derived organic carbon, a source of which can be gizzard-disrupted cells. Broken arrows symbolize the utilization of fermentation products by the earthworm. Figure modified and used with permission from Zeibich *et al.*, 2018.

The taxonomic analysis was simplified by constraining the evaluation to the most abundant phylotypes. However, less abundant phylotypes could have also contribute to gut fermentations. For example, 4,471 phylotypes were detected at the beginning of incubation in the amino acid experiment, illustrating the large *in situ* potential of the ingested microbial community to respond to the nutrient-rich earthworm gut which includes diverse biopolymers and millimolar concentrations of saccharides, amino acids, fatty acids, and diverse inorganic compounds (Drake

and Horn, 2007; Wüst *et al.*, 2009b; Section 3.1 and Section 3.2). The fermentative response to such endogenous nutrients is dependent on the occurrence of a given phylotype in pre-ingested soil. For example, the occurrence of *Fusobacteriaceae* is variable in gut content, being in some cases relatively abundant and responsive, and in other cases hardly detectable and non-responsive.

The high number of phylotypes associated to less abundant bacterial families (Table A1-A11), raising questions as to their potential *in situ* activity. The nutrient availability along the alimentary canal is highly variable (Wüst *et al.*, 2009b), and the *in situ* activity of many detected phylotypes might be therefore (a) temporary during the gut passage and constrained to specific zones in the gut, a matter that warrant further studies. Indeed, detected fluctuating fatty acids in the gut illustrating different types of fermentation that appear in different regions of the alimentary canal (Wüst *et al.*, 2009b). Furthermore, given the large functional redundancy of fermentative taxa (Miki *et al.*, 2013; Langer *et al.*, 2015), *in situ* fermentation could be implemented by alternative taxa to those that were abundant and stimulated in a given treatment. In this regard, responsive fermenters in gut content appear to be largely ingested and thus fermentative taxa in the gut can vary with the substrate (i.e., soil and plant debris) on which the earthworm is maintained (Section 3.3 and Section 4.3).

The community analysis was constrained to bacteria and the associated 16S rRNA genes and 16S rRNA. However, considering the occurrence of facultative aerobic fungi in soil with the capacity of fermentation (Collins *et al.*, 1995; Reith *et al.*, 2002; Kurakov *et al.*, 2008; Tonouchi, 2010) and the assumption that the earthworm gut is dominated by ingested microbes (Section 4.3), it cannot be excluded that such fungi (a) facilitate biopolymer degradation in the alimentary canal of earthworms, and (b) contributed to the observed fermentation profiles. Indeed, the production of cellulases, glucosidases, chitinases, and xylanases by facultative aerobic fungi (Durrant, 1996; Reguera and Leschine, 2001) would enhance the degradation of ingested biopolymers in the gut of earthworms. Thus, evaluating the potential effects of earthworm-ingested fungi on the overall gut fermentation profile of the earthworm warrants further studies. Likewise, the potential involvement of lytic phages in the microbial dynamics of the gut is under explored and worthy of investigation.

## 5. SUMMARY

Gut microbial communities are of interest because of their importance to animal development and health. The alimentary canal of the earthworm is representative of primitive gut ecosystems, and gut fermenters capable of degrading ingested biomass-derived biopolymers might contribute to the environmental impact and survival of this important terrestrial invertebrate. However, relatively little is known about the capacity of fermentative microbes in the earthworm gut to utilize such biopolymers. Thus, the work described in this dissertation evaluated the hypothesis that the gut microbiota of the model earthworm *L. terrestris* hydrolyze and ferment diverse dietary plant- and microbial-derived biopolymers.

Structural polysaccharides had a marginal impact on the fermentation in anoxic gut content treatments. In marked contrast, nonstructural polysaccharides greatly enhanced the formation of diverse fermentation products and stimulated *Aeromonadaceae* and *Enterobacteriaceae*. Although the experimental design required a 1:10 dilution of the gut contents, comparative analysis of the fermentative community in diluted and undiluted gut contents indicated that the dilution did not cause a major disturbance of the system.

The disruption of ingested plant and microbial cells by the earthworm gizzard and other lytic events introduces protein and RNA in the anoxic alimentary canal of earthworms. Yeast cell lysate, as proof-of principle, augmented the production of H<sub>2</sub>, CO<sub>2</sub>, and diverse fatty acids in anoxic gut content microcosms, indicating that the cell lysate triggered diverse fermentations. Likewise, protein and RNA enhanced fermentations in gut contents and yielded contrasting product profile. The combined product profile of protein and RNA treatments was similar to that of cell lysate treatments, and 16S rRNA gene- and 16S rRNA-based analyses indicated that many taxa that responded to cell lysate were similar to taxa that responded to protein or RNA. To further resolve protein- and RNA-derived fermentations, amino acids and ribose were evaluated as potential drivers of fermentation in gut content of the model earthworm *L. terrestris*. Of eight amino acids tested, glutamate, aspartate, and threonine were most stimulatory and yielded dissimilar fermentations facilitated by contrasting taxa. Ribose yielded a complex fermentation profile primarily produced by the *Aeromonadaceae*.

Although theoretical considerations suggest that most microbes in the earthworm gut are likely ingested and transient, the non-responsiveness of soil microbes to a specific 'high quality' gut substrate and anoxia has made it difficult to demonstrate that responsive gut fermenters are derived from soil. Therefore, soil and gut content of *L. terrestris* were further examined for their fermentative capabilities. In unsupplemented anoxic treatments, fermentation was negligible with soil but rapid with gut content. However, both soil and gut content facilitated similar fermentations when challenged with complex nutrients, and the responsive fermentative taxa in these treatments displayed marked similarities, indicating that (a) most of the fermentative taxa in the gut originate from ingested soil, and (b) detectable differences between the fermentative taxa in soil and gut

contents were in part caused by the nutrient-dependent metabolic status of the community members. *Mycoplasmataceae*-affiliated phylotypes that might be symbionts of the earthworm displayed essentially no positive response to any supplemental nutrient, suggesting their occurrence in the earthworm is dependent on host specific factors. In this regard, the occurrence of these phylotypes shifted in response to the dietary substrate of the earthworm.

In conclusion, the findings in this dissertation demonstrate that ingested fermentative gut microbes of the earthworm are poised to respond rapidly to nonstructural polysaccharides, protein, and RNA, biopolymers that can be derived from disrupted biomass. The marked ability of gut fermenters to utilize constituents derived from the hydrolysis of these biopolymers suggest that they compete with the earthworm for these biopolymer constituents (a negative symbiosis) and also produce fatty acids that can be utilized by the earthworm (a positive symbiosis). These complex microbe-host interactions illustrate that biopolymer-driven gut fermentations are likely functionally linked to the development and health of this invertebrate.

## 6. ZUSAMMENFASSUNG

Mikrobielle Darmgemeinschaften haben in den letzten Jahrzehnten aufgrund ihrer tragenden Rolle in der Entwicklung und Gesundheit von Mensch und Tier großes wissenschaftliches Interesse erlangt. Der Verdauungstrakt des Regenwurms repräsentiert ein archaisches Modeldarmökosystem und die assoziierten fermentativen Bakterien, welche theoretisch in der Lage sind, diverse Biopolymere der inkorporierten Biomasse abzubauen, tragen höchstwahrscheinlich zum Umwelteinfluss und Überleben dieses wichtigen terrestrischen Invertebraten bei. Obwohl die Aktivität der Regenwürmer im Boden von besonderer Bedeutung ist, ist nur wenig bekannt über die fermentative Fähigkeit der Darmmikrobiota, vom Regenwurm aufgenommene, mikrobielle und pflanzliche Biopolymere zu hydrolysieren und zu fermentieren. Daher wurden im Rahmen zahlreicher Studien die Fähigkeiten der Darmmikrobiota untersucht, diät-relevante Biopolymere zu nutzen.

Strukturelle Polysaccharide hatten nur einen geringen Einfluss auf die Fermentation in den anoxischen Darminhalt-Mikrokosmen. Im Gegensatz dazu stimulierten nicht-strukturelle Polysaccharide (a) die Bildung verschiedener Fermentationsprodukte und (b) die fakultativ-aeroben Familien *Aeromonadaceae* sowie *Enterobacteriaceae* erheblich. Obwohl das experimentelle Design eine 1:10 Verdünnung erforderte, konnte durch den Vergleich der fermentativen Mikroorganismen und deren Aktivitäten in verdünnten und unverdünnten Darminhalten gezeigt werden, dass die Verdünnung keine nennenswerte Störung des Systems verursachte.

Die Zerkleinerung von aufgenommenen größeren Pflanzen- und Microben-Zellen durch den Regenwurm-gizzard und anderen lytischen Ereignissen führt zur Freisetzung verschiedenster Nährstoffe im Verdauungstrakt, unter anderem großer Mengen an Protein und RNA. Als „*Proof of Principle*“ wurde Hefezelllysate zu anoxischen Darminhalt-Mikrokosmen gegeben. Das Lysat führte zu einer starken Stimulation der fermentativen Prozesse, was an der erhöhten Produktion von H<sub>2</sub>, CO<sub>2</sub> und diversen Fettsäuren zu erkennen war. Ebenso steigerten Protein und RNA die Darmfermentation und führten zu unterschiedlichen Fermentationsprofilen. Das kombinierte Produktprofil von den Mikrokosmen mit Protein oder RNA war ähnlich dem Fermentationsprofil der Mikrokosmen mit Hefezelllysate. Des Weiteren zeigte die 16S rRNA Gen und 16S rRNA Analyse, dass viele Taxa, die vom Zelllysate stimuliert wurden, auch positiv auf Protein oder RNA reagierten. Für die weitere Aufklärung der Protein- und RNA-stimulierten Fermentationen im anoxischen Regenwurmdarm wurden verschiedene Aminosäuren und Ribose als potenziell-stimulierende Treiber der Fermentationen evaluiert. Von acht getesteten Aminosäuren stimulierten vor allem Glutamat, Aspartat und Threonin die Fermentation am meisten/stärksten und führten zu unterschiedlichen Fermentationsprofilen assoziiert mit unterschiedlichen Taxa. Mikrokosmen mit Ribose zeigten ebenfalls ein komplexes Fermentationsprofil, welches hauptsächlich von den *Aeromonadaceae* gebildet wurde.

Obwohl theoretische Überlegungen darauf hindeuten, dass sich die Mehrheit der aufgenommenen Bodenmikroben nur vorübergehend im Regenwurmdarm befinden und somit nicht regenwurm-spezifisch sind, war der Nachweis aufgrund einer zu geringen metabolischen Reaktion der Bodenmikroorganismen zu Anoxia und hochwertigen endogenen Nährstoffen bisher jedoch schwierig. Um die fermentativen Fähigkeiten von Mikroben in Boden und Darminhalt von *L. terrestris* untersuchen zu können, wurden beide mit einem nährstoffreichen komplexen Substrat versetzt. Die Fermentation in den Boden-Mikrokosmen war ohne zusätzliches komplexes Substrat - im Gegensatz zu den Darminhalt-Mikrokosmen - erwartungsgemäß sehr schwach. Wurde jedoch Hefeextrakt zu Boden und Darminhalt gegeben, zeigten beide Mikrokosmen starke und ähnliche fermentative Reaktionen und die stimulierten dominanten Phylotypen waren in beiden Mikrokosmen nahezu identisch. Daran wurde deutlich, dass die Mehrheit der fermentativen Darmbakterien im Regenwurm vom inkorporiertem Boden abstammt und die detektierten Unterschiede zwischen den Mikroben im Boden und im Darminhalt teilweise durch den metabolischen Status der einzelnen Taxa entstanden sind. *Mycoplasmataceae*-verwandte Phylotypen, welche hochwahrscheinlich Symbionten des Regenwurms sind, wurden von keinem der verschiedenen zugegebenen Substrate stimuliert. Dies deutete darauf hin, dass deren Vorkommen im Regenwurm von Host spezifischen Faktoren abhängt. Dementsprechend führten verschiedene Substrate, auf dem die Regenwürmer gehalten wurden, zu unterschiedlichen Reaktionen dieser Phylotypen.

Die Hauptergebnisse dieser Dissertation verdeutlichen, dass die vom Regenwurm aufgenommene fermentative Darmmikroben bereit sind, schnell auf nichtstrukturelle Polysaccharide, Protein und RNA von zerkleinerter Biomasse zu reagieren. Die ausgesprochene Fähigkeit der Darmfermentierer, die von der Hydrolyse stammenden Bestandteile dieser Biopolymere zu verwerten, deutet darauf hin, dass sie mit dem Regenwurm um die Produkte der Hydrolyse konkurrieren (negative Symbiose) und gleichzeitig Fermentationsprodukte bilden, welche vom Regenwurm genutzt werden können (positive Symbiose). Diese komplexen Mikrob-Wirt-Interaktionen illustrieren, dass die biopolymer-getriebenen Darmfermentationen höchstwahrscheinlich funktionell mit der Entwicklung und Gesundheit dieses Invertebraten zusammenhängen.

## 7. REFERENCES

- Aam**, B.B., Heggset, E.B., Norberg, A.L., Sørli, M., Vårum, K.M., and Eijsink, V.G. (2010) Production of chitooligosaccharides and their potential applications in medicine. *Mar Drugs* **8**: 1482–1517.
- Abbott**, S.L., Cheung, W.K.W., and Janda, J.M. (2003) The Genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J Clin Microbiol* **41**: 2348–2357.
- Abul-Soud**, M., Hassanein, M.K., Ablmaaty, S.M., Medany, M., and Abu-Hadid, A.F. (2009) Vermiculture and vermicomposting technologies use in sustainable agriculture in Egypt. *J Agric Res* **87**: 1–16.
- Abbott**, S.L. and Janda, J.M. (1994) Isolation of *Yokenella regensburgei* (“*Koserella trabulsii*”) from a patient with transient bacteremia and from a patient with a septic knee. *J Clin Microbiol* **32**: 2854–2855.
- Addison**, J.A. (2009) Distribution and impacts of invasive earthworms in Canadian forest ecosystems. *Biol Invasions* **11**: 59–79.
- Adibi**, S.A. and Gray, S.J. (1967) Intestinal absorption of essential amino acids in man. *Gastroenterology* **52**: 837–845.
- Akin**, D.E. and Benner, R. (1988) Degradation of polysaccharides and lignin by ruminal bacteria and fungi. *Appl Environ Microbiol* **54**: 1117–1125.
- Allen**, D.A., Austin, B., and Colwell, R.R. (1983) *Aeromonas media*, a new species isolated from river water. *Int J Syst Evol Microbiol* **33**: 599–604.
- Altermatt**, H.A., Simpson, F.J., and Neish, A.C. (1955) The anaerobic dissimilation of D-ribose-1-C<sup>14</sup>, D-xylose-1-C<sup>14</sup>, D-xylose-2-C<sup>14</sup>, and D-xylose-5-C<sup>14</sup> by *Aerobacter aerogenes*. *Can J Biochem Physiol* **33**: 615–621.
- Ander**, P. and Eriksson, K.-E. (1976) The importance of phenol oxidase activity in lignin degradation by the white-rot fungus *Sporotrichum pulverulentum*. *Arch Microbiol* **109**: 1–8.
- Anderson**, O.R. and Bohlen, P.J. (1998) Abundances and diversity of gymnamoebae associated with earthworm (*Lumbricus terrestris*) middens in a northeastern U.S. forest. *Soil Biol Biochem* **30**: 1213–1216.
- Andresen**, J.R. (1994) Glycine metabolism in anaerobes. *Antonie Van Leeuwenhoek* **66**: 223–237.
- Andrews**, M., Raven, J.A., Lea, P.J., and Sprent, J.I. (2006) A Role for shoot protein in shoot–root dry matter allocation in higher plants. *Ann Bot* **97**: 3–10.
- Armon**, R. (2011) Soil Bacteria and Bacteriophages. In, Witzany, G. (ed), *Biocommunication in soil microorganisms*. Heidelberg, DE: Springer Press, 67–112.
- Arora**, D. and Gill, P. K. (2000) Laccase production by some white rot fungi under different nutritional conditions. *Bioresour Technol* **73**: 283–285.
- Årsköld**, E., Lohmeier-Vogel, E., Cao, R., Roos, S., Radstrom, P., and van Niel, E.W.J. (2008) Phosphoketolase pathway dominates in *Lactobacillus reuteri* ATCC 55730 containing dual pathways for glycolysis. *J Bacteriol* **190**: 206–212.
- Ashelford**, K.E., Day, M.J., and Fry, J.C. (2003) Elevated abundance of bacteriophage infecting bacteria in soil. *Appl Env Microbiol* **69**: 285–289.
- Aytan**, U., Feyzioglu, A.M., Valente, A., Agirbas, E., and Fileman, E.S. (2018) Microbial plankton communities in the coastal southeastern Black Sea: biomass, composition and trophic interactions. *Oceanologia* **60**: 139–152.

- Babel**, W. and Müller, R.H. (1985) Correlation between cell composition and carbon conversion efficiency in microbial growth: a theoretical study. *Appl Microbiol Biotechnol* **22**: 201–207.
- Bal**, L. (1977) The formation of carbonate nodules and intercalary crystals in the soil by the earthworm *Lumbricus rubellus*. *Pedobiologia* **17**: 102–106.
- Barker**, H.A. (1981) Amino acid degradation by anaerobic bacteria. *Annu Rev Biochem* **50**: 23–40.
- Barnosky**, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., et al. (2011) Has the earth's sixth mass extinction already arrived? *Nature* **471**: 51–57.
- Barois**, I., Lavelle, P., Brossard, M., Tondoh, J., Martinez, M.A., Rossi, J.P., et al. (1999) Ecology of Earthworm Species with Large Environmental Tolerance and/or Extended Distributions. In, Lavelle P., Brussaard L., Hedrix P.F. (eds), *Earthworm Management in tropical Agroecosystems*. Wallingford, UK: CAB International, pp. 57–85.
- Barrion**, A.T. and Litsinger, J.A. (1997) *Dichogaster* nr. *curgensis* Michaelsen (Annelida: Octochaetidae): An earthworm pest of terraced rice in the Philippine Cordilleras. *Crop Prot* **16**: 89–93.
- Bartnicki-Garcia**, S. (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annu Rev Microbiol* **22**: 87–108.
- Bassalik**, K. (1913) Über Silikatzersetzung durch Bodenbakterien. *Z. Gärungphysio* **2**: 1–32.
- Bastardie**, F., Capowiez, Y., and Cluzeau, D. (2005) 3D characterisation of earthworm burrow systems in natural soil cores collected from a 12-year-old pasture. *Appl Soil Ecol* **30**: 34–46.
- Bastardie**, F., Capowiez, Y., de Dreuzy, J.-R., and Cluzeau, D. (2003) X-ray tomographic and hydraulic characterization of burrowing by three earthworm species in repacked soil cores. *Appl Soil Ecol* **24**: 3–16.
- Bayer**, E.A., Kenig, R., and Lamed, R. (1983) Adherence of *Clostridium thermocellum* to cellulose. *J Bacteriol* **156**: 818–827.
- Bayer**, E.A., Shoham, Y., and Lamed, R. (2013) Lignocellulose-Decomposing Bacteria and Their Enzyme Systems. In, Rosenberg E., DeLong E. F., Lory S., Stackebrandt E. (eds), *The Prokaryotes*. 4th edn, Vol 3. Berlin, DE: Springer Press, pp. 215–266.
- Baylis**, J.P., Cherrett, J.M., and J. B. Ford (1986) A survey of the invertebrates feeding on living clover roots (*Trifolium repens* L.) using <sup>32</sup>P as a radiotracer. *Pedobiologia* **29**: 201–208.
- Beloqui**, A., Nechitaylo, T.Y., Lopez-Cortes, N., Ghazi, A., Guazzaroni, M.-E., Polaina, J., et al. (2010) Diversity of glycosyl hydrolases from cellulose-depleting communities enriched from casts of two earthworm species. *Appl Environ Microbiol* **76**: 5934–5946.
- Bergman**, E.N. (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* **70**: 567–590.
- Bernier**, N. and Ponge, J.F. (1994) Humus form dynamics during the sylvogenetic cycle in a mountain spruce forest. *Soil Biol Biochem* **26**: 183–220.
- Bernstein**, I.A. (1953) Fermentation of ribose-C<sup>14</sup> by *Lactobacillus pentosus*. *J Biol Chem* **205**: 309–316.
- Bertoft**, E. (2017) Understanding Starch Structure: Recent Progress. *Agronomy* **7**: 56.
- Bertora**, C., van Vliet, P.C.J., Hummelink, E.W.J., and van Groenigen, J.W. (2007) Do earthworms increase N<sub>2</sub>O emissions in ploughed grassland? *Soil Biol Biochem* **39**: 632–640.
- Bertsch**, J., Siemund, A.L., Kremp, F., and Müller, V. (2016) A novel route for ethanol oxidation in the acetogenic bacterium *Acetobacterium woodii*: the acetaldehyde/ethanol dehydrogenase pathway. *Environ Microbiol* **18**: 2913–2922.
- Biesterveld**, S., Kok, M.D., Dijkema, C., Zehnder, A.J., and Stams, A.J. (1994) D-xylose catabolism in *Bacteroides xyloxyticus* X5-1. *Arch Microbiol* **161**: 521–527.

- Bishop**, S.H. and Campbell, J.W. (1965) Arginine and urea biosynthesis in the earthworm *Lumbricus terrestris*. *Comp Biochem Physiol* **15**: 51–71.
- Blachnik**, R. (1998) *Taschenbuch für Chemiker und Physiker*. Band III. Berlin, DE: Springer Press.
- Blake**, A.B. and Suchodolski, J.S. (2016) Importance of gut microbiota for the health and disease of dogs and cats. *Anim Front* **6**: 37–42.
- Blanchart**, E., Lavelle, P., Braudeau, E., Le Bissonnais, Y., and Valentin, C. (1997) Regulation of soil structure by geophagous earthworm activities in humid savannas of Cote d'Ivoire. *Soil Biol Biochem* **29**: 431–439.
- Blanksby**, S.J. and Ellison, G.B. (2003) Bond dissociation energies of organic molecules. *Acc Chem Res* **36**: 255–263.
- Bonkowski**, M., Griffiths, B.S., and Ritz, K. (2000) Food preferences of earthworms for soil fungi. *Pedobiologia* **44**: 666–676.
- Borken**, W., Gründel, S., and Beese, F. (2000) Potential contribution of *Lumbricus terrestris* L. to carbon dioxide, methane and nitrous oxide fluxes from a forest soil. *Biol Fertil Soils* **32**: 142–148.
- Boruff**, C.S. and Buswell, A.M. (1934) The anaerobic fermentation of lignin. *J Am Chem Soc* **56**: 886–888.
- Bouché**, M.B. (1977) Strategies lombriciennes. *Ecol Bull* **25**: 122–132.
- Bowman**, J.P. (2005) Genus XIII. *Shewanella*. In, Brenner, D.J., Krieg, N.R., Staley J T, and Garrity, G. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol. 2. New York, NY: Springer Press, pp. 480–491.
- Bowman**, S.M. and Free, S.J. (2006) The structure and synthesis of the fungal cell wall. *BioEssays* **28**: 799–808.
- Brandi**, G., Sisti, M., Schiavano, G.F., Salvaggio, L., and Albano, A. (1996) Survival of *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* in soil. *J Appl Bacteriol* **81**: 439–444.
- Breidenbach**, J. (2002) Normalanatomie und -histologie des Lumbriciden *Lumbricus terrestris* L. (Annelida, Oligochaeta). University of Köln, DE: Doctoral thesis.
- Brown**, D.R., May, M., Bradbury, J.M., Johansson, K.E., and Neimark, H. (2011) Family I. *Mycoplasmataceae*. In, Krieg, N.R., Staley, J.T., Brown, D.R., and Heldlund, B.P. *et al.* (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol. 4. New York, NY: Springer Press, pp. 575–639.
- Brown**, G. (1999) Comment les Vers de Terre Influencent la Croissance des Plantes : Études en Serre sur les Interactions avec le Système Racinaire. University of Paris, FR: Diploma thesis.
- Brown**, G.G. (1995) How do earthworms affect microfloral and faunal community diversity? *Plant Soil* **170**: 209–231.
- Brown**, G.G., Barois, I., and Lavelle, P. (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *Eur J Soil Biol* **36**: 177–198.
- Brown**, G.G., Feller, C., Blanchart, E., Deleporte, P., and Chernyanskii, S.S. (2004) With Darwin, earthworms turn intelligent and become human friends. *Pedobiologia* **47**: 924–933.
- Brune**, A. (2013) Symbiotic Associations Between Termites and Prokaryotes. In, Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. (eds), *The Prokaryotes*. 4th edn, Vol 1. Berlin, DE: Springer Press, pp. 545–577.
- Brune**, A. and Friedrich, M. (2000) Microecology of the termite gut: structure and function on a microscale. *Curr Opin Microbiol* **3**: 263–269.

- Brussaard, G.L.** (1999) On the mechanism of interactions between earthworms and plants. *Pedobiologia* **43**: 880–885.
- Bryant, M.P.** (1979) Microbial methane production-theoretical aspects. *J Anim Sci* **48**: 193–201.
- Buckel, W.** (1999) Fermentation is an Anaerobic Redox Process. In, Lengeler, J.W., Drews, G., and Schlegel, H.S. (eds), *Biology of the Prokaryotes*. Stuttgart, DE: Thieme Press, pp. 269–324.
- Buckel, W.** (2001) Unusual enzymes involved in five pathways of glutamate fermentation. *Appl Microbiol Biotechnol* **57**: 263–273.
- Buckel, W.** and Thauer, R.K. (2012) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na<sup>+</sup> translocating ferredoxin oxidation. *Biochim Biophys Acta BBA-Bioenerg* **1827**: 94–113.
- Calvo-Díaz, A., Díaz-Pérez, L., Suárez, L.A., Morán, X.A.G., Teira, E., and Maranón, E.** (2011) Decrease in the autotrophic-to-heterotrophic biomass ratio of picoplankton in oligotrophic marine waters due to bottle enclosure. *Appl Env Microbiol* **77**: 5739–5746.
- Campbell, L.L.** (1957) Reductive degradation of pyrimidines. I. The isolation and characterization of a uracil fermenting bacterium, *Clostridium uracilicum* nov. spec. *J Bacteriol* **73**: 220–224.
- Campbell, M.M.** and Sederoff, R.R. (1996) Variation in Lignin Content and Composition: Mechanisms of control and implications for the genetic improvement of plants. *Plant Physiol* **110**: 3–13.
- Capowiez, Y., Pierret, A., and Moran, C.J.** (2003) Characterisation of the three-dimensional structure of earthworm burrow systems using image analysis and mathematical morphology. *Biol Fertil Soils* **38**: 301–310.
- Cave, G., Tolley, L.C., and Strain, B.R.** (1981) Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. *Physiol Plant* **51**: 171–174.
- Cerbulis, J.** and Wight Taylor, M. (1969) Neutral lipid and fatty acid composition of earthworms (*Lumbricus terrestris*). *Lipids* **4**: 363–368.
- Chaplin, M.F.** (2001) Water: its importance to life. *Biochem Mol Biol Educ* **29**: 54–59.
- Chamkha, M., Patel, B.K.C., Garcia, J.-L., and Labat, M.** (2001a) Isolation of *Clostridium bifermentans* from oil mill wastewaters converting cinnamic acid to 3-phenylpropionic acid and emendation of the species. *Anaerobe* **7**: 189–197.
- Chamkha, M., Labat, M., Patel, B.K., and Garcia, J.-L.** (2001b) Isolation of a cinnamic acid-metabolizing *Clostridium glycolicum* strain from oil mill wastewaters and emendation of the species description. *Int J Syst Evol Microbiol* **51**: 2049–2054.
- Channarayappa and Biradar, D.P.** (2019) *Soil Basics, Management and Rhizosphere Engineering for Sustainable Agriculture*. Boca Raton, FL: CRC Press.
- Chen, C.-K.** and Blaschek, H.P. (1999) Effect of acetate on molecular and physiological aspects of *Clostridium beijerinckii* NCIMB 8052 solvent production and strain degeneration. *Appl Environ Microbiol* **65**: 499–505.
- Chen, C.M.** and Lui, C.L. (1963) Dynamics of the populations and communities of rice insect pests in the bank of Rung-Ting Lake region, Hunan. *Acta Entomol Sin* **12**: 649–657.
- Chen, X., Di, P., Wang, H., Li, B., Pan, Y., Yan, S., and Wang, Y.** (2015) Bacterial community associated with the intestinal tract of Chinese mitten crab (*Eriocheir sinensis*) farmed in Lake Tai, China. *PLOS ONE* **10**: e0123990.
- Chen, Y., Wang, T., Shen, N., Zhang, F., and Zeng, R.J.** (2016) High-purity propionate production from glycerol in mixed culture fermentation. *Bioresour Technol* **219**: 659–667.

- Childress**, J.J. and Nygaard, M. (1974) Chemical composition and buoyancy of midwater crustaceans as function of depth of occurrence off Southern California. *Mar Biol* **27**: 225–238.
- Clokic**, M.R., Millard, A.D., Letarov, A.V., and Heaphy, S. (2011) Phages in nature. *Bacteriophage* **1**: 31–45.
- Cohen**, S. and Lewis, H.B. (1949) The nitrogenous metabolism of the earthworm (*Lumbricus terrestris*). *J Biol Chem* **180**: 79–91.
- Colberg**, P.J. (1988) Anaerobic Microbial Degradation of Cellulose, Lignin, Oligolignols, and Monoaromatic Lignin Derivatives. In, Zehnder, A.J.B. (ed), *Biology of anaerobic microorganisms*. New York, NY: Wiley, pp. 333–372.
- Collins**, H.P., Robertson, G.P., and Klug, M.J. (1995) *The significance and regulation of soil biodiversity*. Dordrecht, NL: Kluwer Academic Press.
- Cooke**, A. and Luxton, M. (1980) Effect of microbes on food selection by *Lumbricus terrestris*. *Rev Ecol Biol Sol* **17**: 365–370.
- Cortez**, J. and Hameed, R.H. (2001) Simultaneous effects of plants and earthworms on mineralisation of <sup>15</sup>N-labelled organic compounds adsorbed onto soil size fractions. *Biol Fertil Soils* **33**: 218–225.
- Crang**, R.E., Holsen, R.C., and Hitt, J.B. (1968) Calcite production in mitochondria of earthworm calciferous glands. *Bioscience* **18**: 299–301.
- Curry**, J.P. and Schmidt, O. (2007) The feeding ecology of earthworms - A review. *Pedobiologia* **50**: 463–477.
- Darwin**, C. (1881) *The Formation of Vegetable Mould Through the Action of Worms with Observations on Their Habits*. London, UK: John Murray Press.
- Dash**, H.K., Beura, B.N., and Dash, M.C. (1986) Gut load, transit time, gut microflora and turnover of soil, plant and fungal material by some tropical earthworms. *Pedobiologia* **29**: 13–20.
- Dash**, M.C., Satpathy, B., Behera, N., and Charulata, D. (1984) Gut load and turnover of soil, plant and fungal material by *Drawida calebii*, a tropical earthworm. *Rev Ecol Biol* **21**: 387–393.
- Dash**, M.C., Senapati, B.K., and Mishra, C.C. (1980) Nematode feeding by tropical earthworms. *Oikos* **34**: 322–325.
- Davis**, K.E.R., Joseph, S.J., and Janssen, P.H. (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Appl Environ Microbiol* **71**: 826–834.
- Dawson**, R.M., Hemington, N., Grime, D., Lander, D., and Kemp, P. (1974) Lipolysis and hydrogenation of galactolipids and the accumulation of phytanic acid in the rumen. *Biochem J* **144**: 169–171.
- Decaëns**, T. and Rossi, J.P. (2001) Spatio-temporal structure of earthworm community and soil heterogeneity in a tropical pasture. *Ecography* **24**: 671–682.
- Decker**, K., Jungermann, K., and Thauer, R.K. (1970) Energy production in anaerobic organisms. *Angew Chem Int Ed Engl* **9**: 138–158.
- Deepa**, C.K., Dastager, S.G., and Pandey, A. (2010) Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. *World J Microbiol Biotechnol* **26**: 1233–1240.
- Delgado**, F.F., Cermak, N., Hecht, V.C., Son, S., Li, Y., Knudsen, S.M., et al. (2013) Intracellular water exchange for measuring the dry mass, water mass and changes in chemical composition of living cells. *PLoS ONE* **8**: doi: 10.1371/journal.pone.0067590.
- de Nobel**, H., van den Ende, H., and Klis, F.M. (2000) Cell wall maintenance in fungi. *Trends Microbiol* **8**: 344–345.

- Depkat-Jakob**, P.S., Hilgarth, M., Horn, M.A., and Drake, H.L. (2010) Effect of earthworm feeding guilds on ingested dissimilatory nitrate reducers and denitrifiers in the alimentary canal of the earthworm. *Appl Environ Microbiol* **76**: 6205–6214.
- Depkat-Jakob**, P.S., Hunger, S., Schulz, K., Brown, G.G., Tsai, S.M., and Drake, H.L. (2012) Emission of methane by *Eudrilus eugeniae* and other earthworms from Brazil. *Appl Environ Microbiol* **78**: 3014–3019.
- Desmond**, E. and Gribaldo, S. (2009) Phylogenomics of sterol synthesis: insights into the origin, evolution, and diversity of a key eukaryotic feature. *Genome Biol Evol* **1**: 364–381.
- Deutscher**, M.P. (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. *Nucleic Acids Res* **34**: 659–666.
- Dewick**, P.M. (2006) *Essentials of Organic Chemistry: For students of pharmacy, medicinal Chemistry and Biological Chemistry*. Chichester, UK: Wiley Press.
- Dietrich**, C., Kohler, T., and Brune, A. (2014) The Cockroach origin of the termite gut microbiota: patterns in bacterial community structure reflect major evolutionary events. *Appl Environ Microbiol* **80**: 2261–2269.
- Djekrif**, D.S., Gillmann, L., Bennamoun, L., Ait-Kaki, A., Labbani, K., Nouadri, T., and Meraihi, Z.V. (2016) Amylolytic yeasts: Producers of  $\alpha$ -amylase and pullulanase. *Int J Life-Sci Sci Res* **2**: 339–354.
- Dolgonosov**, B.M. and Gubernatorova, T.N. (2010) Modeling the biodegradation of multicomponent organic matter in an aquatic environment: 2. Analysis of the structural organization of lignin. *Water Resour* **37**: 320–331.
- Domínguez**, J., Aira, M., and Gómez-Brandón, M. (2010) Vermicomposting: Earthworms Enhance the Work of Microbes. In, Insam, H., Franke-Whittle, I., and Goberna, M. (eds), *Microbes at Work*. Berlin, DE: Springer Press, pp. 93–114.
- Domsch**, K.H. and Banse, H.-J. (1972) Mykologische Untersuchungen an Regenwurmexkrementen. *Soil Biol Biochem* **4**: 31–38.
- Dong**, L., Zhenhong, Y., Yongming, S., Xiaoying, K., and Yu, Z. (2009) Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. *Int J Hydrog Energy* **34**: 812–820.
- Dotterweich**, H. and Franke, H. (1936) Die Ausscheidung von Kalziumkarbonat, Strontiumkarbonat und Kalziumphosphat in den Kalkdrüsen von *Lumbricus terrestris* L. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **23**: 42–50.
- Doube**, B. and Brown, G. (2004) Functional Interactions between Earthworms, Microorganisms, Organic Matter, and Plants. In, Edwards, C. (ed), *Earthworm Ecology*. Boca Raton, FL: CRC Press, pp. 213–239.
- Drake**, H.L. (1994) *Acetogenesis*. Boston, MA: Springer Press.
- Drake**, H.L., Gößner, A.S., and Daniel, S.L. (2008) Old acetogens, new light. *Ann N Y Acad Sci* **1125**: 100–128.
- Drake**, H.L. and Horn, M.A. (2007) As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. *Annu Rev Microbiol* **61**: 169–189.
- Drake**, H.L., Schramm, A., and Horn, M.A. (2006) Earthworm Gut Microbial Biomes: Their Importance to Soil Microorganisms, Denitrification, and the Terrestrial Production of the Greenhouse Gas  $N_2O$ . In, H König, H., Varma, A. (eds), *Intestinal Microorganisms of Termites and Other Invertebrates*. New York, NY: Springer Press, pp. 65–87.
- Drake**, H.L., Küsel, K., and Matthies, C. (2013) Acetogenic Prokaryotes. In, Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F.L. (eds), *The Prokaryotes*. 4th edn, Vol 3. Berlin, DE: Springer Press, pp. 3–58.

- Durrant**, L.R. (1996) Biodegradation of lignocellulosic materials by soil fungi isolated under anaerobic conditions. *Int Biodeterior Biodegrad* **37**: 189–195.
- Ebert**, K.H. and Schenk, G. (1968) Mechanisms of biopolymer growth: the formation of dextran and levan. *Adv Enzymol Relat Areas Mol Biol* **30**: 179–221.
- Edgar**, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.
- Edgar**, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* **10**: 996–998.
- Edgar**, R.C. and Flyvbjerg, H. (2015) Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **31**: 3476–3482.
- Edwards**, C. A. (2004) *Earthworm Ecology*. 2nd edn. New York, NY: CRC Press.
- Edwards**, C.A. and Bohlen P.J. (1996) *Biology and Ecology of Earthworms*. 3rd edn. London, UK: Chapman and Hall Press.
- Edwards**, C.A. and Fletcher, K.E. (1988) Interactions between earthworms and microorganisms in organic-matter breakdown. *Agric Ecosyst Environ* **24**: 235–247.
- Egert**, M., Marhan, S., Wagner, B., Scheu, S., and Friedrich, M.W. (2004) Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae). *FEMS Microbiol Ecol* **48**: 187–197.
- Eggert**, C., Temp, U., and Eriksson, K.-E.L. (1997) Laccase is essential for lignin degradation by the white-rot fungus *Pycnoporus cinnabarinus*. *FEBS Lett* **407**: 89–92.
- Einbu**, A. (2007) Characterisation of chitin and a study of its acid-catalysed hydrolysis. Norwegian University of Science and Technology, NO: Doctoral thesis.
- Elsden**, S.R. and Hilton, M.G. (1978) Volatile acid production from threonine, valine, leucine and isoleucine by clostridia. *Arch Microbiol* **117**: 165–172.
- Emele**, F.E. (2001) Rapid iodometric detection of *Aeromonas* amylase and its diagnostic significance. *Diagn Microbiol Infect Dis* **40**: 91–94.
- Engelkirk**, P.G. and Duben-Engelkirk, J.L. (2011) *Burton's microbiology for the health sciences*. 9th edn. Philadelphia, PA: Lippincott Williams & Wilkins Press.
- Espey**, M.G. (2013) Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. *Free Radic Biol Med* **55**: 130–140.
- Evans**, W.C. (1963) The microbiological degradation of aromatic compounds. *J Gen Microbiol* **32**: 177–184.
- Fahy**, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R., Shimizu, T., et al. (2009) Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* **50**: 9–14.
- Farmer**, J.J. and Brenner, F.W. (2005) Genus XLII. *Yokenella*. In, Brenner, D.J., Krieg, N.R., Staley, J.T., and Garrity, G. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 2. New York, NY: Springer Press, pp. 848–850.
- Fath**, B. (2019) *Encyclopedia of ecology*. 2nd edn. Oxford, UK: Elsevier.
- Ferrière**, G. (1980) Fonctions des lombriciens. VII. Une méthode d'analyse de la matière organique végétale ingérée. *Pedobiologia* **20**: 263–273.
- Fesel**, P.H. and Zuccaro, A. (2016) Beta-glucan: crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genet Biol* **90**: 53–60.
- Finogold**, S.M., Vaisanen, M.-L., Molitoris, D.R., Tomzynski, T.J., Song, Y., Liu, C., et al. (2003) *Cetobacterium somerae* sp. nov. from human feces and emended description of the genus *Cetobacterium*. *Syst Appl Microbiol* **26**: 177–181.

- Flint**, H.J., Bayer, E.A., Rincon, M.T., Lamed, R., and White, B.A. (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol* **6**: 121–131.
- Fouhse**, J.M., Zijlstra, R.T., and Willing, B.P. (2016) The role of gut microbiota in the health and disease of pigs. *Anim Front* **6**: 30–36.
- Fraser**, P.M. and Boag, B. (1998) The distribution of lumbricid earthworm communities in relation of flatworms: a comparison between New Zealand and Europe. *Pedobiologia* **42**: 542–553.
- Fraser-Reid**, B.O., Tatsuta, K., and Thiem, J. (2008) *Glycoscience: Chemistry and Chemical Biology*. 2nd edn. Berlin, DE: Springer Press.
- Fry**, S.C. (1988) *The Growing Plant Cell Wall: Chemical and Metabolic Analysis*. New York, NY: John Wiley Press.
- Fuka**, M.M., Engel, M., Hagn, A., Munch, J.C., Sommer, M., and Schlöter, M. (2009) Changes of diversity pattern of proteolytic bacteria over time and space in an agricultural soil. *Microb Ecol* **57**: 391–401.
- Furlong**, M.A., Singleton, D.R., Coleman, D.C., and Whitman, W.B. (2002) Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and casts of the earthworm *Lumbricus rubellus*. *Appl Environ Microbiol* **68**: 1265–1279.
- Garbeva**, P., van Veen, J.A., and van Elsas, J.D. (2003) Predominant *Bacillus* spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microb Ecol* **45**: 302–316.
- Gariboldi**, R.T. and Drake, H.L. (1984) Glycine synthase of the purinolytic bacterium, *Clostridium acidurici*. Purification of the glycine-CO<sub>2</sub> exchange system. *J Biol Chem* **259**: 6085–6089.
- Garnier**, C., Axelos, M.A., and Thibault, J.F. (1994) Selectivity and cooperativity in the binding of calcium ions by pectins. *Carbohydr Res* **256**: 71–81.
- Garton**, G.A., Hobson, P.N., and Lough, A.K. (1958) Lipolysis in the rumen. *Nature* **182**: 1511–1512.
- Garton**, G.A., Lough, A.K., and Vioque, E. (1961) Glyceride hydrolysis and glycerol fermentation by sheep rumen contents. *J Gen Microbiol* **25**: 215–225.
- Gasol**, J.M., del Giorgio, P.A., and Duarte, C.M. (1997) Biomass distribution in marine planktonic communities. *Limnol Oceanogr* **42**: 1353–1363.
- Gaston**, L.W. and Stadtman, E.R. (1963) Fermentation of ethylene glycol by *Clostridium glycolicum*, sp. n. *J Bacteriol* **85**: 356–362.
- Gavrilov**, K. (1963) Earthworms, producers of biologically active substances. *Zh Obshch Biol* **24**: 149–154.
- Gee**, M., Reeve, R.M., and McCready, R.M. (1959) Measurement of plant pectic substances, reaction of hydroxylamine with pectinic acids. Chemical studies and histochemical estimation of the degree of esterification of pectic substances in fruit. *J Agric Food Chem* **7**: 34–38.
- Gerritsen**, J., Fuentes, S., Grievink, W., van Niftrik, L., Tindall, B.J., Timmerman, H.M., et al. (2014) Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastrointestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus *Clostridium* into the genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov. *Int J Syst Evol Microbiol* **64**: 1600–1616.
- Gervais** P., Maréchal P.A., and Molin P. (1996) Water relations of solid-state fermentation. *J Sci Ind Res* **55**: 343–357.
- Gobius**, K.S. and Pemberton, J.M. (1988) Molecular cloning, characterization, and nucleotide sequence of an extracellular amylase gene from *Aeromonas hydrophila*. *J Bacteriol* **170**: 1325–1332.

- Gold**, M.H., Wariishi, H., and Valli, K. (1989) Extracellular peroxidases involved in lignin degradation by the white rot basidiomycete *Phanerochaete chrysosporium*. In, Whitaker, J.R. and Sonnet, P.E. (eds), *Biocatalysis in Agricultural Biotechnology*. Washington, DC: American Chemical Society, pp. 127–140.
- Gonzalez-Ruiz**, V., I., A., Antonia, M., Ribelles, P., Teresa, M., and Carlos, J. (2011) An overview of analytical techniques employed to evidence drug-DNA interactions. Applications to the design of genosensors. In, Olsztynska, S. and Komorowska, M. (eds), *Biomedical Engineering, Trends, Research and Technologies*. Rijeka, HR: InTech.
- Gravatt**, D.A. and Kirby, C.J. (1998) Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. *Tree Physiol* **18**: 411–417.
- Greaves**, M.P. and Wilson, M.J. (1970) The degradation of nucleic acids and montmorillonite-nucleic-acid complexes by soil microorganisms. *Soil Biol Biochem* **2**: 257–268.
- Green III**, F. and Highley, T.L. (1997) Mechanism of brown-rot decay: paradigm or paradox. *Int Biodeterior Biodegrad* **39**: 113–124.
- Green**, M.R. and Sambrook, J. (2012) *Molecular Cloning: a Laboratory Manual*. 4th edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Griffiths**, R.I., Whiteley, A.S., O'Donnell, A.G., and Bailey, M.J. (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA-and rRNA-based microbial community composition. *Appl Environ Microbiol* **66**: 5488–5491.
- Grimont**, P.A. and Grimont, F. (2005) Genus XII. *Enterobacter*. In, Brenner, D.J., Krieg, N.R., Staley J T, and Garrity, G. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol. 2. New York, NY: Springer Press, pp. 661–669.
- Grohmann**, K., Baldwin, E.A., Buslig, B.S., and Ingram, L.O. (1994) Fermentation of galacturonic acid and other sugars in orange peel hydrolysates by the ethanologenic strain of *Escherichia coli*. *Biotechnol Lett* **16**: 281–286.
- Guan**, N., Shin, H., Chen, R.R., Li, J., Liu, L., Du, G., and Chen, J. (2014) Understanding of how *Propionibacterium acidipropionici* respond to propionic acid stress at the level of proteomics. *Sci Rep* **4**: doi: 10.1038/srep06951.
- Gunn**, A. and Cherrett, J.M. (1993) The exploitation of food resources by soil meso- and macro-invertebrates. *Pedobiologia* **37**: 303–320.
- Gunsalus**, I.C. and Gibbs, M. (1951) The heterolactic fermentation. II. Position of C<sup>14</sup> in the products of glucose dissimilation by *Leuconostoc mesenteroides*. *J Biol Chem* **194**: 871–875.
- Ha**, S.-J., Galazka, J.M., Joong Oh, E., Kordić, V., Kim, H., Jin, Y.-S., and Cate, J.H.D. (2013) Energetic benefits and rapid cellobiose fermentation by *Saccharomyces cerevisiae* expressing cellobiose phosphorylase and mutant cellodextrin transporters. *Metab Eng* **15**: 134–143.
- Hackmann**, T.J. and Firkins, J.L. (2015) Electron transport phosphorylation in rumen butyrovibrios: unprecedented ATP yield for glucose fermentation to butyrate. *Front Microbiol* **6**: doi: 10.3389/fmicb.2015.00622.
- Hameed**, R. and Bouchè, M.B. (1993) Influence de la qualité de la litière apportée à *Lumbricus terrestris* L. sur la dynamique de l'azote et de la production végétale. *Pedobiologia* **37**: 178–192.
- Hammer**, Ø., Harper, D.A.T., and Ryan, P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* **4**: 1–9.
- Hankin**, L. and Anagnostakis, S.L. (1975) The use of solid media for detection of enzyme production by fungi. *Mycologia* **67**: 597–607.
- Harti**, A.E., Saghi, M., Molina, J.-A.E., and Teller, G. (2001a) Production de composés indoliques rhizogènes par le ver de terre *Lumbricus terrestris*. *Can J Zool* **79**: 1921–1932.

- Harti**, A.E., Saghi, M., Molina, J.-A.E., and Teller, G. (2001b) Production d'une substance rhizogène à effet similaire à celui de l'acide indole acétique par le ver de terre *Lumbricus terrestris*. *Can J Zool* **79**: 1911–1920.
- Hartl**, L., Zach, S., and Seidl-Seiboth, V. (2012) Fungal chitinases: diversity, mechanistic properties and biotechnological potential. *Appl Microbiol Biotechnol* **93**: 533–543.
- Hata**, H., Natori, T., Mizuno, T., Kanazawa, I., Eldesouky, I., Hayashi, M., et al. (2016) Phylogenetics of family *Enterobacteriaceae* and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov.: *Atlantibacter hermannii* gen. nov., comb. nov. *Microbiol Immunol* **60**: 303–311.
- Herlemann**, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* **5**: 1571–1579.
- Hernández-Eugenio**, G. (2002) *Clostridium thiosulfatireducens* sp. nov., a proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**: 1461–1468.
- Herrmann**, G., Jayamani, E., Mai, G., and Buckel, W. (2008) Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. *J Bacteriol* **190**: 784–791.
- Hill**, G.E. (2014) Cellular Respiration: The nexus of stress, condition, and ornamentation. *Integr Comp Biol* **54**: 645–657.
- Hirth**, J.R., McKenzie, B.M., and Tisdall, J.M. (1997) Do the roots of perennial ryegrass elongate to biopores filled with the casts of endogeic earthworms? *Soil Biol Biochem* **29**: 529–531.
- Hogg**, S. (2013) *Essential microbiology*. 2nd edn. Chichester, UK: Wiley-Blackwell.
- Holben**, W.E., Williams, P., Gilbert, M.A., Saarinen, M., Särkilahti, L.K., and Apajalahti, J.H.A. (2002) Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma* phylotype in farmed and wild salmon. *Microb Ecol* **44**: 175–185.
- Holme**, T., Palmstierna, H., Aurivillius, B., Eliasson, N.A., and Thorell, B. (1956) Changes in glycogen and nitrogen-containing compounds in *Escherichia coli* B during Growth in Deficient Media. II. Phosphorus and Sulphur Starvation. *Acta Chem Scand* **10**: 1553–1556.
- Horikoshi**, K. and Iida, S. (1958) Lysis of fungal mycelia by bacterial enzymes. *Nature* **181**: 917–918.
- Horn**, M.A., Mertel, R., Gehre, M., Kastner, M., and Drake, H.L. (2006a) *In Vivo* emission of dinitrogen by earthworms via denitrifying bacteria in the gut. *Appl Environ Microbiol* **72**: 1013–1018.
- Horn**, M.A., Drake, H.L., and Schramm, A. (2006) Nitrous oxide reductase genes (*nosZ*) of denitrifying microbial populations in soil and the earthworm gut are phylogenetically similar. *Appl Environ Microbiol* **72**: 1019–1026.
- Horn**, M.A., Schramm, A., and Drake, H.L. (2003) The earthworm gut: an ideal habitat for ingested N<sub>2</sub>O-producing microorganisms. *Appl Environ Microbiol* **69**: 1662–1669.
- Horstmann**, S., Lynch, K., and Arendt, E. (2017) Starch characteristics linked to gluten-free products. *Foods* **6**: doi: 10.3390/foods6040029
- Howard**, S.P., Critch, J., and Bedi, A. (1993) Isolation and analysis of eight *exe* genes and their involvement in extracellular protein secretion and outer membrane assembly in *Aeromonas hydrophila*. *J Bacteriol* **175**: 6695–6703.
- Huang**, K. and Xia, H. (2018) Role of earthworms' mucus in vermicomposting system: biodegradation tests based on humification and microbial activity. *Sci Total Environ* **610–611**: 703–708.

- Humann, J.** and Lenz, L.L. (2009) Bacterial peptidoglycan-degrading enzymes and their impact on host muropeptide detection. *J Innate Immun* **1**: 88–97.
- Hurlbert, S.H.** (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**: 577–586.
- Iglesias, A.A.** and Preiss, J. (1992) Bacterial glycogen and plant starch biosynthesis. *Biochem Educ* **20**: 196–203.
- Ihssen, J.,** Horn, M.A., Matthies, C., Gossner, A., Schramm, A., and Drake, H.L. (2003) N<sub>2</sub>O-producing microorganisms in the gut of the earthworm *Aporrectodea caliginosa* are indicative of ingested soil bacteria. *Appl Environ Microbiol* **69**: 1655–1661.
- Ingram, L.O.,** Aldrich, H.C., Borges, A.C.C., Causey, T.B., Martinez, A., Morales, F., et al. (1999) Enteric bacterial catalysts for fuel ethanol production. *Biotechnol Prog* **15**: 855–866.
- Ishizaki, A.** and Ueda, T. (1995) Growth kinetics and product inhibition of *Lactococcus lactis* IO-1 culture in xylose medium. *J Ferment Bioeng* **80**: 287–290.
- Jaeger, K.-E.,** Ransac, S., Dijkstra, B.W., Colson, C., Heuvel, M., and Misset, O. (1994) Bacterial lipases. *FEMS Microbiol Rev* **15**: 29–63.
- Jain, S.,** Gaiind, R., Gupta, K.B., Dawar, R., Kumar, D., Paul, P., et al. (2013) *Yokenella regensburgeri* infection in India mimicking enteric fever. *J Med Microbiol* **62**: 935–939.
- James, J.T.,** and Whitman, W.B. (2011) Family I. *Fusobacteriaceae*. In Whitman, W.B., Krieg, N.R., Staley, J.T., Brown, D.R., Hedlund, B.P., Paster, B.J., et al. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol. 4. New York, NY: Springer Press, pp. 748–765.
- Janssen, P.H.** and Liesack, W. (1995) Succinate decarboxylation by *Propionigenium maris* sp. nov., a new anaerobic bacterium from an estuarine sediment. *Arch Microbiol* **164**: 29–35.
- Jayasinghe, B.A.T.D.** and Parkinson, D. (2009) Earthworms as the vectors of actinomycetes antagonistic to litter decomposer fungi. *Appl Soil Ecol* **43**: 1–10.
- Jeanes, A.,** Haynes, W.C., Wilham, C.A., Rankin, J.C., Melvin, E.H., Austin, M.J., et al. (1954) Characterization and classification of dextrans from ninety-six strains of bacteria<sup>1b</sup>. *J Am Chem Soc* **76**: 5041–5052.
- Jiménez, J.J.** (1999) Estructura de las comunidades y dinámica de las poblaciones de lombrices de tierra en las sabanas naturales y perturbadas de Carimagua (Colombia). Complutense University of Madrid, ES: Doctoral thesis.
- Johansson, K.-E.** and Pettersson, B. (2002) Taxonomy of Mollicutes. In, Razin, S. and Herrmann, R. (eds), *Molecular Biology and Pathogenicity of Mycoplasmas*. Boston, MA: Springer Press, pp. 1–29.
- Jolly, J.M.,** Lappin-Scott, H.M., Anderson, J.M., and Clegg, C.D. (1993) Scanning electron microscopy of the gut microflora of two earthworms: *Lumbricus terrestris* and *Octolasion cyaneum*. *Microb Ecol* **26**: 235–245.
- Jones, C.G.,** Lawton, J.H., and Shachak, M. (1994) Organisms as ecosystem engineers. *Oikos* **69**: 373–386.
- Joseph, S.J.,** Hugenholtz, P., Sangwan, P., Osborne, C.A., and Janssen, P.H. (2003) Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol* **69**: 7210–7215.
- Judas, M.** (1992) Gut content analysis of earthworms (*Lumbricidae*) in a beechwood. *Soil Biol Biochem* **24**: 1413–1417.
- Kaiser, J.-P.** and Hanselmann, K.W. (1982) Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. *Arch Microbiol* **133**: 185–194.

- Kämpfer**, P. (2005) Genus VII. *Buttiauxella*. In, Garrity, G., Brenner, D.J., Krieg, N.R., and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 2. New York, NY: Springer, pp. 641–645.
- Kapaun**, E. and Reisser, W. (1995) A chitin-like glycan in the cell wall of a *Chlorella* sp. (*Chlorococcales*, *Chlorophyceae*). *Planta* **197**: 577–582.
- Karsten**, G.R. and Drake, H.L. (1997) Denitrifying bacteria in the earthworm gastrointestinal tract and *in vivo* emission of nitrous oxide (N<sub>2</sub>O) by earthworms. *Appl Environ Microbiol* **63**: 1878–1882.
- Kauppinen**, S., Christgau, S., Kofod, L.V., Halkier, T., Dörreich, K., and Dalbøge, H. (1995) Molecular cloning and characterization of a rhamnogalacturonan acetylerase from *Aspergillus aculeatus*. Synergism between rhamnogalacturonan degrading enzymes. *J Biol Chem* **270**: 27172–27178.
- Khalikova**, E., Susi, P., and Korpela, T. (2005) Microbial dextran-hydrolyzing enzymes: fundamentals and applications. *Microbiol Mol Biol Rev* **69**: 306–325.
- Khdhiri**, M., Piché-Choquette, S., Tremblay, J., Tringe, S.G., and Constant, P. (2017) The tale of a neglected energy source: elevated hydrogen exposure affects both microbial diversity and function in soil. *Appl Environ Microbiol* **83**: doi: 10.1128/AEM.00275-17.
- Khomyakov**, N.V., Kharin, S.A., Nechitailo, T.Y., Golyshin, P.N., Kurakov, A.V., Byzov, B.A., and Zvyagintsev, D.G. (2007) Reaction of microorganisms to the digestive fluid of earthworms. *Microbiology* **76**: 45–54.
- Kiela**, P.R. and Ghishan, F.K. (2016) Physiology of intestinal absorption and secretion. *Best Pract Res Clin Gastroenterol* **30**: 145–159.
- Kim**, D., Kim, J.-S., Park, I.-Y., Kwak, H.-J., Lee, D.H., Cho, S.-J., and Park, S.C. (2016) A novel chitinase from the earthworm, *Eisenia andrei*. *Anim Cells Syst* **20**: 48–51.
- Kizilkaya**, R., Karaca, A., Turgay, O.C., and Cetin, S.C. (2011) Earthworm Interactions with Soil Enzymes. In, Karaca, A. (ed), *Biology of Earthworms*. Berlin, DE: Springer Press, pp. 141–158.
- Klemm**, D., Heublein, B., Fink, H.-P., and Bohn, A. (2005) Cellulose: fascinating biopolymer and sustainable raw material. *Angew Chem Int Ed* **44**: 3358–3393.
- Knight**, V. and Blakemore, R. (1998) Reduction of diverse electron acceptors by *Aeromonas hydrophila*. *Arch Microbiol* **169**: 239–248.
- Knollenberg**, W.G., Merritt, R.W., and Lawson, D.L. (1985) Consumption of leaf litter by *Lumbricus terrestris* (*Oligochaeta*) on a Michigan woodland floodplain. *Am Midl Nat* **113**: 1–6.
- Kosako**, Y., Sakazaki, R., and Yoshizaki, E. (1984) *Yokenella regensburgei* gen. nov., sp. nov.: a new genus and species in the family *Enterobacteriaceae*. *Jpn J Med Sci Biol* **37**: 117–124.
- Kraght**, A.J. and Starr, M.P. (1952) Fermentation of galacturonic acid and glucose by a strain of *Erwinia carotovora*. *J Bacteriol* **64**: 259–264.
- Kretzschmar**, A. (1998) Earthworm Interaction with Soil Organization. In, Edwards, C.A. (ed) *Earthworm Ecology*. Boca Raton, FL: St. Lucie Press, pp. 163–176.
- Kristófek**, V., Tajovský, K., and Pizl, V. (1994) Ultrastructural analysis of the intestinal content of earthworm *Lumbricus rubellus* Hoffm. (Annelida, *Lumbricidae*). *Acta Microbiol Immunol Hung* **41**: 283–290.
- Kromer**, K. and Gamian, A. (2000) Analysis of soluble carbohydrates, proteins and lipids in shoots of M 7 apple rootstock cultured *in vitro* during regeneration of adventitious roots. *J Plant Physiol* **156**: 775–782.
- Kunov-Kruse**, A.J., Riisager, A., Saravanamurugan, S., Berg, R.W., Kristensen, S.B., and Fehrmann, R. (2013) Revisiting the Brønsted acid catalysed hydrolysis kinetics of polymeric carbohydrates in ionic liquids by *in situ* ATR-FTIR spectroscopy. *Green Chem* **15**: 2843–2848.

- Kurakov**, A.V., Lavrent'Ev, R.B., Nechitailo, T.Y., Golyshin, P.N., and Zvyagintsev, D.G. (2008) Diversity of facultatively anaerobic microscopic mycelial fungi in soils. *Microbiology* **77**: 90–98.
- Küsel**, K., Karnholz, A., Trinkwalter, T., Devereux, R., Acker, G., and Drake, H.L. (2001) Physiological ecology of *Clostridium glycolicum* RD-1, an aerotolerant acetogen isolated from sea grass roots. *Appl Environ Microbiol* **67**: 4734–4741.
- Küsel**, K., Wagner, C., and Drake, H.L. (1999) Enumeration and metabolic product profiles of the anaerobic microflora in the mineral soil and litter of a beech forest. *FEMS Microbiol Ecol* **29**: 91–103.
- Kuzyakov**, Y. and Larionova, A.A. (2005) Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *J Plant Nutr Soil Sci* **168**: 503–520.
- Laanbroek**, H.J., Lambers, J.T., De Vos, W.M., and Veldkamp, H. (1978) L-aspartate fermentation by a free-living *Campylobacter* species. *Arch Microbiol* **117**: 109–114.
- Langan**, P., Sangha, A.K., Wymore, T., Parks, J.M., Yang, Z.K., Hanson, B.L., et al. (2014) L-sorbinose binding, isomerization, and epimerization by D-xylose isomerase: X-ray/neutron crystallographic and molecular simulation study. *Structure* **22**: 1287–1300.
- Lange**, H.C. and Heijnen, J.J. (2001) Statistical reconciliation of the elemental and molecular biomass composition of *Saccharomyces cerevisiae*. *Biotechnol Bioeng* **75**: 334–344.
- Langer**, S.G., Ahmed, S., Einfalt, D., Bengelsdorf, F.R., and Kazda, M. (2015) Functionally redundant but dissimilar microbial communities within biogas reactors treating maize silage in co-fermentation with sugar beet silage: Biogas-producing microbial communities. *Microb Biotechnol* **8**: 828–836.
- Lattaud**, C., Locati, S., Mora, P., Rouland, C., and Lavelle, P. (1998) The diversity of digestive systems in tropical geophagous earthworms. *Appl Soil Ecol* **9**: 189–195.
- Lattaud**, C., Mora, P., Garvìn, M., Locati, S., and Poulard, C. (1999) Enzymatic digestive capabilities in geophagous earthworms - origin and activities of cellulolytic enzymes. *Pedobiologia* **43**: 842–850.
- Lattaud**, C., Zhang, B.G., Locati, S., Rouland, C., and Lavelle, P. (1997) Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (Megascolecidae). *Soil Biol Biochem* **29**: 335–339.
- Lavelle**, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Heal, O.W., and Dhillon, S. (1998) Soil function in a changing world: the role of invertebrate ecosystem engineers. *Eur J Soil Biol* **33**: 159–193.
- Lavelle**, P. and Martin, A. (1992) Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soils of the humid tropics. *Soil Biol Biochem* **24**: 1491–1498.
- Laverack**, M.S. (1963) The Physiology of Earthworms. In, Kerkut, G.A. (ed) *International Series of Monographs on Pure and Applied Biology*. Vol. 15. New York, NY: Pergamon Press.
- Lee**, S., Kim, J., Shin, S.G., and Hwang, S. (2008) Biokinetic parameters and behavior of *Aeromonas hydrophila* during anaerobic growth. *Biotechnol Lett* **30**: 1011–1016.
- Lehninger**, A.L., Nelson, D.L., and Cox, M.M. (2008) *Lehninger principles of biochemistry*. 5th edn. New York, NY: W.H. Freeman & Company.
- Lemos**, L.N., Fulthorpe, R.R., Triplett, E.W., and Roesch, L.F.W. (2011) Rethinking microbial diversity analysis in the high throughput sequencing era. *J Microbiol Methods* **86**: 42–51.
- Leschine**, S.B. (1995) Cellulose degradation in anaerobic environments. *Annu Rev Microbiol* **49**: 399–426.
- Lewis**, R. (1992) *Life*. Dubuque, IA: Wm. C. Brown Publishers.

- Li, S.-W., He, H., Zeng, R.J., and Sheng, G.-P. (2017) Chitin degradation and electricity generation by *Aeromonas hydrophila* in microbial fuel cells. *Chemosphere* **168**: 293–299.
- Liang, D., Leung, R.K.-K., Guan, W., and Au, W.W. (2018) Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathog* **10**: doi: 10.1186/s13099-018-0230-4.
- Lim, J.K., Bae, S.S., Kim, T.W., Lee, J.-H., Lee, H.S., and Kang, S.G. (2012) Thermodynamics of formate-oxidizing metabolism and implications for H<sub>2</sub> production. *Appl Environ Microbiol* **78**: 7393–7397.
- Lin, W., Mathys, V., Ang, E.L.Y., Koh, V.H.Q., Martínez Gómez, J.M., Ang, M.L.T., et al. (2012) Urease activity represents an alternative pathway for *Mycobacterium tuberculosis* nitrogen metabolism. *Infect Immun* **80**: 2771–2779.
- Liu, L., Zhang, L., Tang, W., Gu, Y., Hua, Q., Yang, S., et al. (2012) Phosphoketolase pathway for xylose catabolism in *Clostridium acetobutylicum* revealed by <sup>13</sup>C metabolic flux analysis. *J Bacteriol* **194**: 5413–5422.
- Ljungdahl, L.G. and Wood, H.G. (1969) Total synthesis of acetate from CO<sub>2</sub> by heterotrophic bacteria. *Annu Rev Microbiol* **23**: 515–538.
- Llewellyn, M.S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G.R., et al. (2016) The biogeography of the Atlantic salmon (*Salmo salar*) gut microbiome. *ISME J* **10**: 1280–1284.
- Logan, N.A. and De Vos, P. (2009) Genus I. *Bacillus*. In, De Vos, P., Garrity, G., Jones, D., Krieg, N.R., and Ludwig, W. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 3. New York, NY: Springer, pp. 21–128.
- Loquet, M. and Vincelas, M. (1987) Activité cellulolytique liée au tube digestif d'*Eisenia fetida andrei*. *Rev Écol Biol Sol* **24**: 559–571.
- Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., and Knight, R. (2012) Diversity, stability and resilience of the human gut microbiota. *Nature* **489**: 220–230.
- Lubbers, I.M., Brussaard, L., Otten, W., and Van Groenigen, J.W. (2011) Earthworm-induced N mineralization in fertilized grassland increases both N<sub>2</sub>O emission and crop-N uptake. *Eur J Soil Sci* **62**: 152–161.
- Ludwig, W. (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* **32**: 1363–1371.
- Machuca, A. and Ferraz, A. (2001) Hydrolytic and oxidative enzymes produced by white- and brown-rot fungi during *Eucalyptus grandis* decay in solid medium. *Enzyme Microb Technol* **29**: 386–391.
- Madigan, M.T., Martinko, J.M., Bender, K.S., Buckley, D.H., and Stahl, D.A. (2015) Brock, T.D. (ed) *Brock biology of microorganisms*. 14th edn. Boston Columbus Indianapolis London, UK: Pearson.
- Magurran, A.E. (2004) *Measuring Biological Diversity*. Malden, Ma: Blackwell Pub.
- Maniloff, J. (2002) Phylogeny and Evolution. In, Herrmann, R. and Razin, S. (eds), *Molecular Biology and Pathogenicity of Mycoplasmas*. Boston, MA: Springer Press, pp. 31–34.
- Maraun, M., Alpehi, J., Bonkowski, M., and Buryr, R. (1999) Middens of the earthworm *Lumbricus terrestris* (*Lumbricidae*): Microhabitats for micro- and mesofauna in forest soil. *Pedobiologia* **43**: 276–287.
- Martin, A. (1991) Short- and long-term effects of the endogeic earthworm *Millsonia anomala* (*Omodeo*) (*Megascolecidae*, *Oligochaeta*) of tropical savannas, on soil organic matter. *Biol Fertil Soils* **11**: 234–238.

- Martin, A.**, Cortez, J., Barois, I., and Lavelle, P. (1987) Les mucus intestinaux de ver de terre moteur de leurs interactions avec la microflore. *Rev Ecol Biol Sol* **24**: 549–58.
- Martin, M.** (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17**: 10–12.
- Martin-Carnahan, A.** and Joseph, S.W. (2005) Family I. *Aeromonadaceae*. In, Garrity, G., Brenner, D.J., Krieg, N.R., and Staley J.T. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 2. New York, NY: Springer Press, pp. 556–578.
- Matthies, C.**, Griebshammer, A., Schmittroth, M., and Drake, H.L. (1999) Evidence for involvement of gut-associated denitrifying bacteria in emission of nitrous oxide (N<sub>2</sub>O) by earthworms obtained from garden and forest soils. *Appl Environ Microbiol* **65**: 3599–3604.
- McAuliffe, L.** (2006) Biofilm formation by mycoplasma species and its role in environmental persistence and survival. *Microbiology* **152**: 913–922.
- McDowall, J.S.**, Murphy, B.J., Haumann, M., Palmer, T., Armstrong, F.A., and Sargent, F. (2014) Bacterial formate hydrogenlyase complex. *Proc Natl Acad Sci* **111**: E3948–E3956.
- McInerney, M.J.**, Bryant, M.P., Hespell, R.B., and Costerton, J.W. (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl Environ Microbiol* **41**: 1029–1039.
- McInerney, M.J.**, Bryant, M.P., and Pfennig, N. (1979) Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. *Arch Microbiol* **122**: 129–135.
- McInerney, M.J.** (1988) Anaerobic Hydrolysis and Fermentation of Fats and Proteins. In, *Biology of Anaerobic Microorganisms*. New York, NY: Wiley and Sons Press, pp. 373–416.
- McLaughlin, J.** (1971) Biochemical studies on *Eisenia foetida* (savigny, 1826), the brandling worm-III. Tissue lipids and sterols. *Comp Biochem Physiol Part B Comp Biochem* **38**: 147–163.
- McMillan, J. D.** (1993) Xylose Fermentation to Ethanol: A Review. Golden, CO: National Renewable Energy Laboratory.
- Mechichi, T.**, Fardeau, M.-L., Labat, M., Garcia, J.-L., Verhe, F., and Patel, B.K. (2000) *Clostridium peptidivorans* sp. nov., a peptide-fermenting bacterium from an olive mill wastewater treatment digester. *Int J Syst Evol Microbiol* **50**: 1259–1264.
- Meier, A.B.**, Hunger, S., and Drake, H.L. (2018) Differential engagement of fermentative taxa in gut contents of the earthworm *Lumbricus terrestris*. *Appl Environ Microbiol* **84**: doi: 10.1128/AEM.01851-17.
- Mendez, R.**, Borges, S., and Betancourt, C. (2003) A microscopical view of the intestine of *Onychochaeta borincana* (*Oligochaeta: Glossoscolecidae*). *Pedobiologia* **47**: 900–903.
- Meroueh, S.O.**, Bencze, K.Z., Heseck, D., Lee, M., Fisher, J.F., Stemmler, T.L., and Mobashery, S. (2006) Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proc Natl Acad Sci* **103**: 4404–4409.
- Metzler, D.E.** and Metzler, C.M. (2003) *Biochemistry: The Chemical Reactions of Living Cells*. 2nd edn. San Diego, CA: Harcourt/Academic Press.
- Meyer, J.**, Schmidt, A., Michalke, K., and Hensel, R. (2007) Volatilisation of metals and metalloids by the microbial population of an alluvial soil. *Syst Appl Microbiol* **30**: 229–238.
- Migge-Kleian, S.**, McLean, M.A., Maerz, J.C., and Heneghan, L. (2006) The influence of invasive earthworms on indigenous fauna in ecosystems previously uninhabited by earthworms. *Biol Invasions* **8**: 1275–1285.
- Miki, T.**, Yokokawa, T., and Matsui, K. (2013) Biodiversity and multifunctionality in a microbial community: a novel theoretical approach to quantify functional redundancy. *Proc R Soc B Biol Sci* **281**: doi: 10.1098/rspb.2013.2498.

- Mishra**, P., Jarial, R., and Kanwar, S.S. (2017) Process optimization for the production and purification of an extracellular ribonuclease from a soil bacterial isolate RNS3 using one-factor-at-a-time (OFAT) approach. *J Adv Microbiol* **3**: 16–26.
- Mishra**, P.C. and Dash, M.C. (1980) Digestive enzymes of some earthworms. *Experientia* **36**: 1156–1157.
- Mitchell**, R. and Alexander, M. (1963) Lysis of soil fungi by bacteria. *Can J Microbiol* **9**: 169–177.
- Moat**, A.G., Foster, J.W., and Spector, M.P. (2002) *Microbial Physiology*. 4th edn. New York, NY: Wiley-Liss.
- Mohnen**, D. (2008) Pectin structure and biosynthesis. *Curr Opin Plant Biol* **11**: 266–277.
- Moon**, R.J., Martini, A., Nairn, J., Simonsen, J., and Youngblood, J. (2011) Cellulose nanomaterials review: structure, properties and nanocomposites. *Chem Soc Rev* **40**: 3941.
- Morris**, S.C. and Peel, J.S. (2008) The earliest annelids: Lower cambrian polychaetes from the Sirius Passet Lagerstätte, Peary Land, North Greenland. *Acta Palaeontol Pol* **53**: 137–148.
- Mort**, A.J., Qiu, F., and Maness, N.O. (1993) Determination of the pattern of methyl esterification in pectin. Distribution of contiguous nonesterified residues. *Carbohydr Res* **247**: 21–35.
- Müller**, H.E., Brenner, D.J., Fanning, G.R., Grimont, P.A., and Kämpfer, P. (1996) Emended description of *Buttiauxella agrestis* with recognition of six new species of *Buttiauxella* and two new species of *Kluyvera*: *Buttiauxella ferragutiae* sp. nov., *Buttiauxella gaviniae* sp. nov., *Buttiauxella brennerae* sp. nov., *Buttiauxella izardii* sp. nov., *Buttiauxella noackiae* sp. nov., *Buttiauxella warmboldiae* sp. nov., *Kluyvera cochleae* sp. nov., and *Kluyvera georgiana* sp. nov. *Int J Syst Evol Microbiol* **46**: 50–63.
- Müller**, V. (2008) Bacterial Fermentation. In, John Wiley & Sons, Ltd (eds), *Encyclopedia of Life Sciences*. Chichester, UK: John Wiley & Sons, doi: 10.1002/9780470015902a.
- Murakami**, T., Segawa, T., Bodington, D., Dial, R., Takeuchi, N., Kohshima, S., and Hongoh, Y. (2015) Census of bacterial microbiota associated with the glacier ice worm *Mesenchytraeus solifugus*. *FEMS Microbiol Ecol* **91**: doi: 10.1264/jsme2.ME16158.
- Müren**, U., Berglund, J., Samuelsson, K., and Andersson, A. (2005) Potential effects of elevated sea-water temperature on pelagic food webs. *Hydrobiologia* **545**: 153–166.
- Nadwodnik**, J. and Lohaus, G. (2008) Subcellular concentrations of sugar alcohols and sugars in relation to phloem translocation in *Plantago major*, *Plantago maritima*, *Prunus persica*, and *Apium graveolens*. *Planta* **227**: 1079–1089.
- Naessens**, M., Cerdobbel, A., Soetaert, W., and Vandamme, E.J. (2005) *Leuconostoc dextransucrase* and dextran: production, properties and applications. *J Chem Technol Biotechnol* **80**: 845–860.
- Nakamura**, Y., Itakura, J., and Matsuzaki, I. (1995) Influence of the earthworm *Pheretima hilgendorfi* (Megascolecidae) on *Plasmodiophora brassicae* clubroot galls of cabbage seedlings in pots. *Edaphologia* **54**: 39–41.
- Nanninga**, H. (1985) Amino acid fermentation and hydrogen transfer in mixed cultures. *FEMS Microbiol Lett* **31**: 261–269.
- Nechitaylo**, T.Y., Timmis, K.N., and Golyshin, P.N. (2009) “*Candidatus Lumbricincola*”, a novel lineage of uncultured *Mollicutes* from earthworms of family *Lumbricidae*. *Environ Microbiol* **11**: 1016–1026.
- Nechitaylo**, T.Y., Yakimov, M.M., Godinho, M., Timmis, K.N., Belogolova, E., Byzov, B.A., et al. (2010) Effect of the earthworms *Lumbricus terrestris* and *Aporrectodea caliginosa* on bacterial diversity in soil. *Microb Ecol* **59**: 574–587.
- Needham**, A.E. (1957) Components of nitrogenous excreta in the earthworms *Lumbricus terrestris*, L. and *Eisenia foetida* (Savigny). *J Exp Biol* **34**: 425–446.

- Neidhardt, F.C., Ingraham, J.L., and Schaechter, M. (1996)** *Physiology of the Bacterial Cell: A Molecular Approach*, Sunderland, MA: Sinauer Associates.
- Nes, W.D. (2011)** Biosynthesis of cholesterol and other sterols. *Chem Rev* **111**: 6423–6451.
- Neville, A.C. and Luke, B.M. (1969)** A two-system model for chitin-protein complexes in insect cuticles. *Tissue Cell* **1**: 689–707.
- Nielson, R.L. (1965)** Presence of plant growth substances in earthworms demonstrated by paper chromatography and the went pea test. *Nature* **208**: 1113–1114.
- Nisman, B. (1954)** The Stickland reaction. *Bacteriol Rev* **18**: 16–42.
- Nozaki, M., Miura, C., Tozawa, Y., and Miura, T. (2009)** The contribution of endogenous cellulase to the cellulose digestion in the gut of earthworm (*Pheretima hilgendorfi*: Megascolecidae). *Soil Biol Biochem* **41**: 762–769.
- Ochoa-Villarreal, M., Aispuro-Hernández, E., Vargas-Arispuro, I., and Ángel, M. (2012)** Plant Cell Wall Polymers: Function, Structure and Biological Activity of Their Derivatives. In, De Souza Gomes, A. (ed), *Polymerization*. Rijeka, HR: In Tech. pp. 63–86
- Okamura, N., Stoskopf, M., Yamaguchi, H., and Kishimoto, Y. (1985)** Lipid composition of the nervous system of earthworms (*Lumbricus terrestris*). *J Neurochem* **45**: 1875–1879.
- Ornston, L.N. and Stanier, R.Y. (1964)** Mechanism of beta-ketoadipate formation by bacteria. *Nature* **204**: 1279–1283.
- Osborne, C.A., Peoples, M.B., and Janssen, P.H. (2010)** Detection of a reproducible, single-member shift in soil bacterial communities exposed to low levels of hydrogen. *Appl Environ Microbiol* **76**: 1471–1479.
- Oshima, T. (2007)** On the anaerobic metabolism of aromatic compounds in the presence of nitrate by soil microorganisms. *Z Allg Mikrobiol* **5**: 386–394.
- Ovreås, L. and Torsvik, V. (1998)** Microbial diversity and community structure in two different agricultural soil communities. *Microb Ecol* **36**: 303–315.
- Palm, J., van Schaik, N.L.M.B., and Schröder, B. (2013)** Modelling distribution patterns of anecic, epigeic and endogeic earthworms at catchment-scale in agro-ecosystems. *Pedobiologia* **56**: 23–31.
- Papagianni, M. (2012)** Metabolic engineering of lactic acid bacteria for the production of industrially important compounds. *Comput Struct Biotechnol J* **3**: doi: 10.5936/csbj.201210003.
- Pařenicová, L., Benen, J.A., Kester, H.C., and Visser, J. (2000)** pgaA and pgaB encode two constitutively expressed endopolygalacturonases of *Aspergillus niger*. *Biochem J* **345**: 637–644.
- Parle, J.N. (1963a)** Micro-organisms in the intestines of earthworms. *Microbiology* **31**: 1–11.
- Parle, J.N. (1963b)** A microbiological study of earthworm casts. *Microbiology* **31**: 13–22.
- Parmeggiani, F., Weise, N.J., Ahmed, S.T., and Turner, N.J. (2018)** Synthetic and therapeutic applications of ammonia-lyases and aminomutases. *Chem Rev* **118**: 73–118.
- Parmelee, R.W., Beare, M.H., Cheng, W., Hendrix, P.-F., Rider, S.J., Crossley, D.A., and Coleman, D.C. (1990)** Earthworms and enchytraeids in conventional and no-tillage agroecosystems: A biocide approach to assess their role in organic matter breakdown. *Biol Fertil Soils* **10**: 1–10.
- Perel, T.S. (1977)** Differences in lumbricid organization connected with ecological properties. *Oikos* **25**: 56–63.
- Périé, F.H. and Gold, M.H. (1991)** Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. *Appl Env Microbiol* **57**: 2240–2245.

- Peters, W.** (1972) Occurrence of chitin in Mollusca. *Comp Biochem Physiol Part B Comp Biochem* **41**: 541–550.
- Petersen, S.O.** and Holmstrup, M. (2000) Temperature effects on lipid composition of the earthworms *Lumbricus rubellus* and *Eisenia nordenskiöldi*. *Soil Biol Biochem* **32**: 1787–1791.
- Philipp, B.** and Schink, B. (2012) Different strategies in anaerobic biodegradation of aromatic compounds: nitrate reducers versus strict anaerobes. *Environ Microbiol Rep* **4**: 469–478.
- Pearce, T.G.** (1972) The calcium relations of selected *Lumbricidae*. *J Anim Ecol* **41**: 167–188.
- Pieper, R.,** Villodre Tudela, C., Taciak, M., Bindelle, J., Pérez, J.F., and Zentek, J. (2016) Health relevance of intestinal protein fermentation in young pigs. *Anim Health Res Rev* **17**: 137–147.
- Pinel, N.,** Davidson, S.K., and Stahl, D.A. (2008) *Verminephrobacter eiseniae* gen. nov., sp. nov., a nephridial symbiont of the earthworm *Eisenia foetida* (Savigny). *Int J Syst Evol Microbiol* **58**: 2147–2157.
- Pinkert, A.,** Marsh, K.N., Pang, S., and Staiger, M.P. (2009) Ionic liquids and their interaction with cellulose. *Chem Rev* **109**: 6712–6728.
- Popoff, M.** and Véron, M. (1976) A taxonomic study of the *Aeromonas hydrophila*-*Aeromonas punctata* group. *Microbiology* **94**: 11–22.
- Prajapati, V.S.,** Trivedi, U.B., and Patel, K.C. (2014) Kinetic and thermodynamic characterization of glucoamylase from *Colletotrichum* sp. KCP1. *Indian J Microbiol* **54**: 87–93.
- Prasanna, P.H.P.,** Grandison, A.S., and Charalampopoulos, D. (2014) *Bifidobacteria* in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. *Food Res Int* **55**: 247–262.
- Pruesse, E.,** Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glockner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**: 7188–7196.
- Rahemtulla, F.** and Løvtrup, S. (1975) The comparative biochemistry of invertebrate mucopolysaccharides III. Oligochaeta and hirudinea. *Comp Biochem Physiol* **50**: 627–629.
- Rainey, Fred A.** (2009) Family V. *Lachnospiraceae*. In, Vos, W.M.D., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., and Rainey, F. A. (eds), *Bergey's Manual of Systematic Bacteriology*. 2 nd, Vol 3. New York, NY: Springer, pp. 921–968.
- Rangel, F.,** Thomas, R.J., Jiménez, J.J., and Decäens, T. (1999) Nitrogen dynamics associated with earthworm casts of *Martiodrilus carimaguensis* Jiménez and Moreno in a Colombian savanna Oxisol. *Pedobiologia* **43**: 557–560.
- Rao, M.B.,** Tanksale, A.M., Ghatge, M.S., and Deshpande, V.V. (1998) Molecular and biotechnological aspects of microbial proteases. *Microbiol Mol Biol Rev MMBR* **62**: 597–635.
- Rautiainen, S.,** Lehtinen, P., Chen, J., Vehkamäki, M., Niemelä, K., Leskelä, M., and Repo, T. (2015) Selective oxidation of uronic acids into aldaric acids over gold catalyst. *RSC Adv* **5**: 19502–19507.
- Raw, F.** (1962) Studies of earthworm populations in orchards. I. Leaf burial in apple orchards. *Ann Appl Biol* **50**: 389–404.
- Rizhiya, E.,** Bertora, C., van Vliet, P.C.J., Kuikman, P.J., Faber, J.H., and van Groenigen, J.W. (2007) Earthworm activity as a determinant for N<sub>2</sub>O emission from crop residue. *Soil Biol Biochem* **39**: 2058–2069.
- Razin, S.,** Kutner, S., Efrati, H., and Rottem, S. (1980) Phospholipid and cholesterol uptake by *Mycoplasma* cells and membranes. *Biochim Biophys Acta* **598**: 628–640.
- Razin, S.** and Tully, J.G. (1970) Cholesterol requirement of mycoplasmas. *J Bacteriol* **102**: 306–310.

- Reguera**, G. and Leschine, S.B. (2001) Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments. *FEMS Microbiol Lett* **204**: 367–374.
- Reith**, F., Drake, H.L., and Küsel, K. (2002) Anaerobic activities of bacteria and fungi in moderately acidic conifer and deciduous leaf litter. *FEMS Microbiol Ecol* **41**: 27–35.
- Ricaboni**, D., Mailhe, M., Khelaifia, S., Raoult, D., and Million, M. (2016) *Romboutsia timonensis*, a new species isolated from human gut. *New Microbes New Infect* **12**: 6–7.
- Roach**, P.J., Depaoli-Roach, A.A., Hurley, T.D., and Tagliabracci, V.S. (2012) Glycogen and its metabolism: some new developments and old themes. *Biochem J* **441**: 763–787.
- Robertson**, J.D. (1936) The function of the calciferous glands of earthworms. *J Exp Biol* **13**: 279–297.
- Robinson**, J.W.L. and Alvarado, F. (1971) Interaction between the sugar and amino-acid transport systems at the small intestinal brush border: A comparative study. *Pflügers Arch Eur J Physiol* **326**: 48–75.
- Robyt**, J.F. (1995) Mechanisms in the Glucansucrase Synthesis of Polysaccharides and Oligosaccharides from Sucrose. In, *Advances in Carbohydrate Chemistry and Biochemistry*. Vol 51. San Diego, CA: Elsevier Academic Press, pp. 133–168.
- Romano**, A.H., Trifone, J.D., and Brustolon, M. (1979) Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in fermentative bacteria. *J Bacteriol* **139**: 93–97.
- Rosenberg**, S.L. (1980) Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. *Enzyme Microb Technol* **2**: 185–193.
- Ruan**, Z., Wang, Y., Zhang, C., Song, J., Zhai, Y., Zhuang, Y., et al. (2014) *Clostridium huakuui* sp. nov., an anaerobic, acetogenic bacterium isolated from methanogenic consortia. *Int J Syst Evol Microbiol* **64**: 4027–4032.
- Russell**, J.B., Muck, R.E., and Weimer, P.J. (2009) Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiol Ecol* **67**: 183–197.
- Saha**, B.C. (2003) Hemicellulose bioconversion. *J Ind Microbiol Biotechnol* **30**: 279–291.
- Sampedro**, L., Jeannotte, R., and Whalen, J.K. (2006) Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. *Soil Biol Biochem* **38**: 2188–2198.
- Sasi Jyothsna**, T.S., Tushar, L., Sasikala, C., and Ramana, C.V. (2016) *Paraclostridium benzoelyticum* gen. nov., sp. nov., isolated from marine sediment and reclassification of *Clostridium bifermentans* as *Paraclostridium bifermentans* comb. nov. Proposal of a new genus *Paeniclostridium* gen. nov. to accommodate *Clostridium sordellii* and *Clostridium ghonii*. *Int J Syst Evol Microbiol* **66**: 1268–1274.
- Satchell**, J.E. (1967) Lumbricidae. In, Burgess, A., and Raw, F. (eds), *Soil Biology*. London, UK: Academic Press, pp. 259–322.
- Sawers**, G. (1994) The hydrogenases and formate dehydrogenases of *Escherichia coli*. *Antonie Van Leeuwenhoek* **66**: 57–88.
- Schelfhout**, S., Mertens, J., Verheyen, K., Vesterdal, L., Baeten, L., Muys, B., and De Schrijver, A. (2017) Tree species identity shapes earthworm communities. *Forests* **8**: 85.
- Scheutz**, F. and Strockbine, N.A. (2005) Genus I. *Escherichia*. In, Garrity, G., Brenner, D.J., Krieg, N.R., and Staley J.T. (eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 2. New York, NY: Springer Press, pp. 607–624.
- Schimke**, R.T., Berlin, C.M., Sweeney, E.W., and Carroll, W.R. (1966) The generation of energy by the arginine dihydrolase pathway in *Mycoplasma hominis* 07. *J Biol Chem* **241**: 2228–2236.
- Schink**, B. (1984) *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch Microbiol* **137**: 250–255.

- Schink, B.** (1999) Ecophysiology and Ecological Niches of Prokaryotes. In, Lengeler, J.W., Drews, G., and Schlegel, H.G. (eds), *Biology of the Prokaryotes*. Stuttgart, DE: Thieme Press, pp. 723–762.
- Schink, B., Kremer, D.R., and Hansen, T.A.** (1987) Pathway of propionate formation from ethanol in *Pelobacter propionicus*. *Arch Microbiol* **147**: 321–327.
- Schink, B. and Pfennig, N.** (1982) *Propionigenium modestum* gen. nov. sp. nov. a new strictly anaerobic, nonsporulating bacterium growing on succinate. *Arch Microbiol* **133**: 209–216.
- Schink, B. and Stams, A.J.M.** (2013) Syntrophism among Prokaryotes. In, Rosenberg E., DeLong E. F., Lory S., Stackebrandt E. (ed), *The Prokaryotes*. 4th edn, Vol. 2. Berlin, DE: Springer Press, pp. 471–493.
- Schinner, F. and Sonnleitner, R.** (1996) *Bodenökologie: Mikrobiologie und Bodenenzymatik: Grundlagen, Klima, Vegetation und Bodentyp*. Vol 1. Berlin, DE: Springer Press.
- Schleifer, K.H. and Kandler, O.** (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**: 407–477.
- Schönholzer, F., Hahn, D., and Zeyer, J.** (1999) Origins and fate of fungi and bacteria in the gut of *Lumbricus terrestris* L. studied by image analysis. *FEMS Microbiol Ecol* **28**: 235–248.
- Schuch, R., Pelzek, A.J., Kan, S., and Fischetti, V.A.** (2010) Prevalence of *Bacillus anthracis*-like organisms and bacteriophages in the intestinal tract of the earthworm *Eisenia fetida*. *Appl Environ Microbiol* **76**: 2286–2294.
- Schuchmann, K. and Müller, V.** (2014) Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. *Nat Rev Microbiol* **12**: 809–821.
- Schuchmann, K. and Müller, V.** (2016) Energetics and application of heterotrophy in acetogenic bacteria. *Appl Env Microbiol* **82**: 4056–4069.
- Schulz, K., Hunger, S., Brown, G.G., Tsai, S.M., Cerri, C.C., Conrad, R., and Drake, H.L.** (2015) Methanogenic food web in the gut contents of methane-emitting earthworm *Eudrilus eugeniae* from Brazil. *ISME J* **9**: 1778–92.
- Schweiger, G. and Buckel, W.** (1984) On the dehydration of (*R*)-lactate in the fermentation of alanine to propionate by *Clostridium propionicum*. *FEBS Lett* **171**: 79–84.
- Seddon, S.V. and Borriello, S.P.** (1992) Proteolytic activity of *Clostridium difficile*. *J Med Microbiol* **36**: 307–311.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C.** (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* **12**: doi: 10.1186/gb-2011-12-6-r60.
- Seilacher, A.** (1998) Triploblastic animals more than 1 billion years ago: Trace fossil evidence from India. *Science* **282**: 80–83.
- Shaw, C. and Pawluk, S.** (1986a) Fecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils. *Pedobiologia* **29**: 377–389.
- Shaw, C. and Pawluk, S.** (1986b) The development of soil structure by *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* in patent materials belonging to different texture classes. *Pedobiologia* **29**: 327–239.
- Shih, P.M.** (2015) Cyanobacterial evolution: fresh insight into ancient questions. *Curr Biol* **25**: 192–193.
- Shreiner, A.B., Kao, J.Y., and Young, V.B.** (2015) The gut microbiome in health and in disease: *Curr Opin Gastroenterol* **31**: 69–75.
- Silhavy, T.J., Kahne, D., and Walker, S.** (2010) The Bacterial Cell Envelope. *Cold Spring Harb Perspect Biol* **2**: doi: 10.1101/cshperspect.a000414.

- da Silva**, K.R., Salles, J.F., Seldin, L., and van Elsas, J.D. (2003) Application of a novel *Paenibacillus*-specific PCR-DGGE method and sequence analysis to assess the diversity of *Paenibacillus* spp. in the maize rhizosphere. *J Microbiol Methods* **54**: 213–231.
- da Silva**, S.M., Voordouw, J., Leitao, C., Martins, M., Voordouw, G., and Pereira, I.A. (2013) Function of formate dehydrogenases in *Desulfovibrio vulgaris* Hildenborough energy metabolism. *Microbiology* **159**: 1760–1769.
- Šimůnek**, J., Hodrová, B., Bartoňová, H., and Kopečný, J. (2001) Chitinolytic bacteria of the mammal digestive tract. *Folia Microbiol (Praha)* **46**: 76–78.
- Singer**, E., Bushnell, B., Coleman-Derr, D., Bowman, B., Bowers, R.M., Levy, A., et al. (2016) High-resolution phylogenetic microbial community profiling. *ISME J* **10**: 2020–2032.
- Sjörström**, E. (1993) *Wood Chemistry*. 2nd edn. San Diego, CA: Academic Press.
- Slepecky**, R.A. and Leadbetter, E.R. (1984) On the Prevalence and Role of Spore-Forming Bacteria and their Spores in Nature. In, Hurst, A. and Gould, G. (eds), *The Bacterial Spore*. New York, NY: Academic Press, pp. 79–99.
- Smith**, E.A. and Macfarlane, G.T. (1997) Dissimilatory amino acid metabolism in human colonic bacteria. *Anaerobe* **3**: 327–337.
- Smith**, E.A. and Macfarlane, G.T. (1998) Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS Microbiol Ecol* **25**: 355–368.
- Smith**, L.D. (1975) Common mesophilic anaerobes, including *Clostridium botulinum* and *Clostridium tetani*, in 21 soil specimens. *Appl Microbiol* **29**: 590–594.
- Smith**, P.J., Wang, H.-T., York, W.S., Peña, M.J., and Urbanowicz, B.R. (2017) Designer biomass for next-generation biorefineries: Leveraging recent insights into xylan structure and biosynthesis. *Biotechnol Biofuels* **10**: doi: 10.1186/s13068-017-0973-z.
- Smole**, M.S., Kreže, T., Strnad, S., Kleinschek, K.S., and Hribernik, S. (2005) Characterization of grass fibres. *J Mater Sci* **40**: 5349–5353.
- Sørensen**, T.H., Cruys-Bagger, N., Borch, K., and Westh, P. (2015) Free energy diagram for the heterogeneous enzymatic Hydrolysis of glycosidic bonds in cellulose. *J Biol Chem* **290**: 22203–22211.
- Stal**, L.J. (2011) Biopolymer. In, Gargaud, M., Amils, R., Quintanilla, J.C., Cleaves, H.J., Irvine, W.M., Pinti, D.L., and Viso, M. (eds), *Encyclopedia of Astrobiology*. Berlin, DE: Springer Press, pp. 199–200.
- Stams**, A.J.M. and Hansen, T.A. (1984) Fermentation of glutamate and other compounds by *Acidaminobacter hydrogenoformans* gen. nov. sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate-reducing and methanogenic bacteria. *Arch Microbiol* **137**: 329–337.
- Stanier**, R.Y. and Adams, G.A. (1944) The nature of the *Aeromonas* fermentation. *Biochem J* **38**: 168–171.
- Stevenson**, B.S., Eichorst, S.A., Wertz, J.T., Schmidt, T.M., and Breznak, J.A. (2004) New strategies for cultivation and detection of previously uncultured microbes. *Appl Environ Microbiol* **70**: 4748–4755.
- Stockdale**, E.A., Shepherd M.A.\*, Fortune, S., and Cuttle, S.P. (2002) Soil fertility in organic farming systems - fundamentally different? *Soil Use Manag* **18**: 301–308.
- Storch**, V., Welsch, U., and Kükenthal, W. (2009) *Kükenthal Zoologisches Praktikum*, 26th edn. Heidelberg, DE: Springer Press.

- Stott**, M.B., Crowe, M.A., Mountain, B.W., Smirnova, A.V., Hou, S., Alam, M., and Dunfield, P.F. (2008) Isolation of novel bacteria, including a candidate division, from geothermal soils in New Zealand. *Environ Microbiol* **10**: 2030–2041.
- Subramaniam**, S., Fahy, E., Gupta, S., Sud, M., Byrnes, R.W., Cotter, D., et al. (2011) Bioinformatics and systems biology of the lipidome. *Chem Rev* **111**: 6452–6490.
- Suen**, J.C., Hatheway, C.L., Steigerwalt, A.G., and Brenner, D.J. (1988) *Clostridium argentinense* sp. nov.: a genetically homogeneous group composed of all strains of *Clostridium botulinum* toxin type G and some nontoxigenic strains previously identified as *Clostridium subterminale* or *Clostridium hastiforme*. *Int J Syst Evol Microbiol* **38**: 375–381.
- Sugumaran**, M. and Vaidyanathan, C.S. (1978) Metabolism of aromatic compounds. *J Indian Inst Sci* **60**: 57–123.
- Sundar Raj AA**, Rubila S, Jayabalan R, Ranganathan TV (2012) A review on pectin: Chemistry due to general properties of pectin and its pharmaceutical uses. *Sci Rep* **1**: doi: 10.4172/scientificreports.550.
- Suthar**, S. and Singh, S. (2008) Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). *Int J Environ Sci Technol* **5**: 99–106.
- Thabet**, O.B.D., Fardeau, M.-L., Jouliau, C., Thomas, P., Hamdi, M., Garcia, J.-L., and Olivier, B. (2004) *Clostridium tunisiense* sp. nov., a new proteolytic, sulfur-reducing bacterium isolated from an olive mill wastewater contaminated by phosphogypse. *Anaerobe* **10**: 185–190.
- Thauer**, R.K., Jungermann, K., and Decker, K. (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* **41**: 100–180.
- Thauer**, R.K., Jungermann, K., Henninger, H., Wenning, J., and Decker, K. (1968) The energy metabolism of *Clostridium kluyveri*. *Eur J Biochem* **4**: 173–180.
- Tholozan**, J.L., Touzel, J.P., Samain, E., Grivet, J.P., Prensier, G., and Albagnac, G. (1992) *Clostridium neopropionicum* sp. nov., a strict anaerobic bacterium fermenting ethanol to propionate through acrylate pathway. *Arch Microbiol* **157**: 249–257.
- Tidjani Alou**, M., Cadoret, F., Brah, S., Diallo, A., Sokhna, C., Mehrej, V., et al. (2017) ‘*Khelaiifiella massiliensis*’, ‘*Niameybacter massiliensis*’, ‘*Brachybacterium massiliense*’, ‘*Enterobacter timonensis*’, ‘*Massilibacillus massiliensis*’, new bacterial species and genera isolated from the gut microbiota of healthy infants. *New Microbes New Infect* **19**: doi:10.1016/j.nmni.2017.02.002.
- Tillinghast**, E.K. (1967) Excretory pathways of ammonia and urea in the earthworm *Lumbricus terrestris* L. *J Exp Zool* **166**: 295–300.
- Tillinghast**, E.K., O'Donnell, R., Eves, D., Calvert, E., and Taylor, J. (2001) Water-soluble luminal contents of the gut of the earthworm *Lumbricus terrestris* L. and their physiological significance. *Comp Biochem Physiol A Mol Integr Physiol* **129**: 345–353.
- Timberlake**, K. (2003) *Chemistry: An Introduction to General, Organic and Biological Chemistry*. San Francisco, CA: Benjamin Cummings.
- Timell**, T.E. (1967) Recent progress in the chemistry of wood hemicelluloses. *Wood Sci Technol* **1**: 45–70.
- Tiwari**, S.C., Tiwari, B.K., and Mishra, R.R. (1990) Microfungal species associated with the gut content and casts of *Drawida assamensis* gates. *Plant Sci* **100**: 379–382.
- Tomati**, U., Grappelli, A., and Galli, E. (1988) The hormone-like effect of earthworm casts on plant growth. *Biol Fertil Soils* **5**: 288–294.
- Tonouchi**, A. (2010) Isolation and characterization of a novel facultative anaerobic filamentous fungus from Japanese rice field soil. *Int J Microbiol* **2009**: doi: 10.1155/2009/571383.

- Torsvik**, V., Goksøyr, J., and Daae, F.L. (1990) High diversity in DNA of soil bacteria. *Appl Environ Microbiol* **56**: 782–787.
- Tracey**, M.V. (1951) Cellulase and chitinase of earthworms. *Nature* **167**: 776–777.
- Trigo**, D., Barois, I., Garvin, M.H., Huerta, E., Irisson, S., and Lavelle, P. (1999) Mutualism between earthworms and soil microflora. *Pedobiologia* **43**: 866–873.
- Tsuchiya**, C., Sakata, T., and Sugita, H. (2007) Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett Appl Microbiol* **46**: 43–48.
- Urbášek**, F. and Pilž, V. (1991) Activity of digestive enzymes in the gut of five earthworm species (*Oligochaeta: Lumbricidae*). *Rev Écol Biol Sol* **26**: 461–468.
- Valenzuela**, R. and Valenzuela, A. (2013) Overview About Lipid Structure. In, *Lipid Metabolism*. Intech Open. doi: 10.5772/52306.
- Větrovský**, T. and Baldrian, P. (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS ONE* **8**: doi: 10.1371/journal.pone.0057923
- Ville**, P., Roch, P., Cooper, E.L., Masson, P., and Narbonne, J.-F. (1995) PCBs increase molecular-related activities (lysozyme, antibacterial, hemolysis, proteases) but inhibit macrophage-related functions (phagocytosis, wound healing) in earthworms. *J Invertebr Pathol* **65**: 217–224.
- Vincent**, F., Yates, D., Garman, E., Davies, G.J., and Brannigan, J.A. (2004) The three-dimensional structure of the *N*-acetylglucosamine-6-phosphate deacetylase, NagA, from *Bacillus subtilis*: A member of the urease superfamily. *J Biol Chem* **279**: 2809–2816.
- Vogels**, G. van der and Van der Drift, C. (1976) Degradation of purines and pyrimidines by microorganisms. *Bacteriol Rev* **40**: 403–468.
- Von Fircks**, Y. and Sennerby-Forsse, L. (1998) Seasonal fluctuations of starch in root and stem tissues of coppiced *Salix viminalis* plants grown under two nitrogen regimes. *Tree Physiol* **18**: 243–249.
- Von Stockar**, U. and Liu, J.-S. (1999) Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. *Biochim Biophys Acta BBA-Bioenerg* **1412**: 191–211.
- Voragen**, A.G.J., Coenen, G.-J., Verhoef, R.P., and Schols, H.A. (2009) Pectin, a versatile polysaccharide present in plant cell walls. *Struct Chem* **20**: 263–275.
- Walk**, S.T., Alm, E.W., Gordon, D.M., Ram, J.L., Toranzos, G.A., Tiedje, J.M., and Whittam, T.S. (2009) Cryptic lineages of the genus *Escherichia*. *Appl Environ Microbiol* **75**: 6534–6544.
- Wang**, Y.-J. and Wang, L. (2000) Structures and properties of commercial maltodextrins from corn, potato, and rice starches. *Starch-Stärke* **52**: 296–304.
- Wang**, Y. and Fujii, T. (2011) Evaluation of methods of determining humic acids in nucleic acid samples for molecular biological analysis. *Biosci Biotechnol Biochem* **75**: 355–357.
- Wang**, Y., Song, J., Zhai, Y., Zhang, C., Gerritsen, J., Wang, H., et al. (2015) *Romboutsia sedimentorum* sp. nov., isolated from an alkaline-saline lake sediment and emended description of the genus *Romboutsia*. *Int J Syst Evol Microbiol* **65**: 1193–1198.
- Washburn**, L.R. and Somerson, N.L. (1977) *Mycoplasma* growth inhibition by arginine. *J Clin Microbiol* **5**: 378–380.
- Weatherburn**, M.W. (1967) Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* **39**: 971–974.
- Weete**, J.D., Abril, M., and Blackwell, M. (2010) Phylogenetic distribution of fungal sterols. *PLoS ONE* **5**: doi: 10.1371/journal.pone.0010899.

- Weghoff**, M.C., Bertsch, J., and Müller, V. (2015) A novel mode of lactate metabolism in strictly anaerobic bacteria. *Environ Microbiol* **17**: 670–677.
- Wei**, J.H., Yin, X., and Welander, P.V. (2016) Sterol synthesis in diverse bacteria. *Front Microbiol* **7**: doi: 10.3389/fmicb.2016.00990.
- Weimer**, P.J. (1992) Cellulose degradation by ruminal microorganisms. *Crit Rev Biotechnol* **12**: 189–223.
- White**, G.F., Russell, N.J., and Tidswell, E.C. (1996) Bacterial scission of ether bonds. *Microbiol Rev* **60**: 216–232.
- Whitman**, W.B., Coleman, D.C., and Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc Natl Acad Sci* **95**: 6578–6583.
- Widdel**, F. (1980) Anaerober Abbau von Fettsäuren und Benzoessäure durch neu isolierte Arten Sulfat-reduzierender Bakterien. University of Göttingen, DE: Doctoral thesis.
- Wieczorek**, A.S., Hetz, S.A., and Kolb, S. (2014) Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries. *Biogeosciences* **11**: 3339–3352.
- Wiegel** J. (2009) Family I. *Clostridiaceae*. In, De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, E.A., Schleifer, K.H., Whitman, W.B.(eds), *Bergey's Manual of Systematic Bacteriology*. 2nd edn, Vol 3. New York, NY: Springer Press, pp. 736–864.
- Wild**, J.R. and Wales, M.E. (1990) Molecular evolution and genetic engineering of protein domains involving aspartate transcarbamoylase. *Annu Rev Microbiol* **44**: 193–218.
- Wilde**, E., Collins, M.D., and Hippe, H. (1997) *Clostridium pasculi* sp. nov., a new glutamate-fermenting sporeformer from a pasture in Pakistan. *Int J Syst Evol Microbiol* **47**: 164–170.
- Wilkinson**, J. (1959) The problem of energy-storage compounds in bacteria. *Exp Cell Res* **7**: 111–130.
- Wilkinson**, J.F. (1963) Carbon and energy storage in bacteria. *Microbiology* **32**: 171–176.
- Windey**, K., De Preter, V., and Verbeke, K. (2012) Relevance of protein fermentation to gut health. *Mol Nutr Food Res* **56**: 184–196.
- Wolter**, C. and Scheu, S. (1999) Changes in bacterial numbers and hyphal lengths during the gut passage through *Lumbricus terrestris* (*Lumbricidae*, *Oligochaeta*). **43**: 891–900.
- Wood**, H.G., Ragsdale, S.W., and Pezacka, E. (1986) The acetyl-CoA pathway of autotrophic growth. *FEMS Microbiol Lett* **39**: 345–362.
- Wu**, G. (2009) Amino acids: metabolism, functions, and nutrition. *Amino Acids* **37**: 1–17.
- Wüst**, P.K., Horn, M.A., and Drake, H.L. (2011) *Clostridiaceae* and *Enterobacteriaceae* as active fermenters in earthworm gut content. *ISME J* **5**: 92–106.
- Wüst**, P.K., Horn, M.A., Henderson, G., Janssen, P.H., Rehm, B.H.A., and Drake, H.L. (2009a) Gut-associated denitrification and *in vivo* emission of nitrous oxide by the earthworm families *Megascolecidae* and *Lumbricidae* in New Zealand. *Appl Environ Microbiol* **75**: 3430–3436.
- Wüst**, P.K., Horn, M.A., and Drake, H.L. (2009b) *In situ* hydrogen and nitrous oxide as indicators of concomitant fermentation and denitrification in the alimentary canal of the earthworm *Lumbricus terrestris*. *Appl Environ Microbiol* **75**: 1852–1859.
- Xie**, X.S., Choi, P.J., Li, G.-W., Lee, N.K., and Lia, G. (2008) Single-molecule approach to molecular biology in living bacterial cells. *Annu Rev Biophys* **37**: 417–444.
- Xing**, Y., Jones, P., Bosch, M., Donnison, I., Spear, M., and Ormondroyd, G. (2018) Exploring design principles of biological and living building envelopes: what can we learn from plant cell walls? *Intell Build Int* **10**: 78–102.

- Xue**, M., Sun, H., Wu, X., Guan, L.L., and Liu, J. (2018) Assessment of rumen microbiota from a large dairy cattle cohort reveals the pan and core bacteriomes contributing to varied phenotypes. *Appl Environ Microbiol* **84**: doi: 10.1128/AEM.00970-18.
- Yadav**, S. (2017) Contribution of Earthworm to Bioremediation as a Living Machine: Bioremediation. In, Bhakta, J. (ed), *Handbook of Research on Inventive Bioremediation Techniques*. Hershey, PA: IGI Global, pp. 324–340.
- Yan**, Q. and Fong, S.S. (2015) Bacterial chitinase: nature and perspectives for sustainable bioproduction. *Bioresour Bioprocess* **2**: doi: 10.1186/s4064.
- Yao**, C.K., Muir, J.G., and Gibson, P.R. (2016) Review article: insights into colonic protein fermentation, its modulation and potential health implications. *Aliment Pharmacol Ther* **43**: 181–196.
- Yarza**, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., et al. (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* **12**: 635–645.
- Yokoi**, H., Ohkawara, T., Hirose, J., Hayashi, S., and Takasaki, Y. (1995) Characteristics of hydrogen production by aciduric *Enterobacter aerogenes* strain HO-39. *J Ferment Bioeng* **80**: 571–574.
- Yoon**, J.-J. and Kim, Y.-K. (2005) Degradation of crystalline cellulose by the brown-rot basidiomycete *Fomitopsis palustris*. *J Microbiol Seoul Korea* **43**: 487–492.
- Yuzwa**, S.A. and Vocadlo, D.J. (2014) O-GlcNAc and neurodegeneration: biochemical mechanisms and potential roles in Alzheimer's disease and beyond. *Chem Soc Rev* **43**: 6839–6858.
- Zhang** Z, Schwartz S, Wagner L, Miller W. (2000). A greedy algorithm for aligning DNA sequences. *J Comput Biol* **7**: 203-214.
- Zargar**, V., Asghari, M., and Dashti, A. (2015) A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. *ChemBioEng Rev* **2**: 204–226.
- Zeibich**, L., Schmidt, O., and Drake, H.L. (2018) Protein- and RNA-enhanced fermentation by gut microbiota of the earthworm *Lumbricus terrestris*. *Appl Environ Microbiol* **84**: doi: 10.1128/AEM.00657-18.
- Zeibich**, L., Schmidt, O., and Drake, H.L. (2019a) Dietary polysaccharides: fermentation potentials of a primitive gut ecosystem. *Environ Microbiol*.**21**: doi: 10.1111/1462-2920.14556.
- Zeibich**, L., Schmidt, O., and Drake, H.L. (2019b) Amino acid and ribose: drivers of protein and RNA fermentation by ingested bacteria of a primitive gut ecosystem. *Appl. Environ. Microbiol.* **85**: doi:10.1128/AEM.01297-19.
- Zeibich**, Schmidt, and Drake (2019c) Fermentation in the earthworm gut: do transients matter? *FEMS Microbiol Ecol* **95**: doi: 10.1093/femsec/fiy221.
- Zhou**, G.-W., Yang, X.-R., Sun, A.-Q., Li, H., Lassen, S.B., Zheng, B.-X., and Zhu, Y.-G. (2019) mobile incubator for iron(III) reduction in the gut of the soil-feeding earthworm *Pheretima guillelmi* and interaction with denitrification. *Environ Sci Technol* **53**: 4215–4223.
- Zhuge**, X., Liu, L., Shin, H., Chen, R.R., Li, J., Du, G., and Chen, J. (2013) Development of a *Propionibacterium-Escherichia coli* shuttle vector for metabolic engineering of *Propionibacterium jensenii*, an efficient producer of propionic acid. *Appl Environ Microbiol* **79**: 4595–4602.

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Phyla, Class, Family <sup>a</sup>	Sampling Time:										Relative Abundance (%)									
	Treatment:	0h					30h					C1	C2	C3	Ce	Ch	Pe	Xy	Md	Da
<i>Micromonosporaceae</i> (29)	2.1	2.1	2.2	2.2	2.1	1.6	2.1	2.1	1.8	1.8	2.1	1.7	1.2	1.4	1.2	1.1	0.8	1.0		
<i>Nocardioideaceae</i> (23)	2.3	2.2	2.2	1.9	2.0	1.9	2.1	1.9	1.8	1.3	1.6	1.3	0.9	1.1	0.8	1.0	0.6	0.7		
<i>Propionibacteriaceae</i> (5)	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1		
<i>Pseudonocardioaceae</i> (10)	0.4	0.4	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1		
<i>Streptomycetaceae</i> (2)	0.9	0.9	1.0	0.9	1.0	0.8	1.0	0.9	0.9	0.6	0.7	0.6	0.4	0.5	0.5	0.5	0.2	0.3		
<i>Streptosporangiaceae</i> (3)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0		
<i>Thermomonosporaceae</i> (6)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0		
Unassigned <i>Actinobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Coriobacteria</i> ,																				
<i>Coriobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Coriobacteria</i> (16)	2.4	2.5	2.5	2.4	2.7	2.4	2.6	2.5	2.4	1.6	2.1	2.0	1.2	1.7	1.1	1.2	0.8	1.1		
<i>Rubrobacteria</i> ,																				
<i>Rubrobacteriaceae</i> (5)	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.4	0.4	0.1	0.2	0.1	0.1	0.1	0.1		
<i>Thermoleophilia</i> ,																				
<i>Gaiellaceae</i> (9)	2.2	2.4	2.1	2.2	2.1	1.9	2.0	2.1	1.9	1.3	1.7	1.5	1.0	1.4	1.0	1.0	0.6	1.0		
Unassigned <i>Gaiellales</i> (35)	4.6	4.7	4.7	4.9	4.5	3.9	4.7	4.3	4.0	3.1	3.8	3.3	2.2	2.9	2.3	1.9	1.5	1.9		
<i>Conexibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Patulibacteraceae</i> (4)	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Solirubrobacteraceae</i> (4)	0.7	0.6	0.7	0.4	0.5	0.4	0.5	0.5	0.4	0.3	0.4	0.4	0.2	0.3	0.2	0.1	0.2	0.2		
Unassigned <i>Solirubrobacterales</i> (46)	3.3	3.5	3.1	2.9	3.0	2.5	3.2	2.8	2.3	1.9	2.4	2.3	1.3	1.7	1.4	1.1	1.0	1.1		
Unassigned <i>Actinobacteria</i> (6)	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1		
<b>Armatimonadetes,</b>																				
<i>Armatimonadia</i> ,																				
Unassigned <i>Armatimonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Chthonomonadetes</i> ,																				
<i>Chthonomonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Chthonomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Armatimonadetes</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Bacteroidetes,</b>																				
<i>Bacteroidia</i> ,																				
<i>Prevotellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Rikenellaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Bacteroidales</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Cytophagia</i> ,																				
<i>Cyclobacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Cytophagaceae</i> (20)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Flavobacteriia</i> ,																				
<i>Flavobacteriaceae</i> (9)	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Flavobacteriales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Sphingobacteria</i> ,																				
<i>Chitinophagaceae</i> (24)	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0		
<i>Saprospiraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Sphingobacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Sphingobacteriales</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>BRC1,</b>																				
Unassigned <i>BRC1</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Chlamydiae,</b>																				
<i>Chlamydiae</i> ,																				
<i>Chlamydiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Parachlamydiaceae</i> (42)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0		
<i>Simkaniaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Chlorobi,</b>																				
<i>Chlorobia</i> ,																				
Unassigned <i>Chlorobiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Chloroflexi,</b>																				
<i>Anaerolineae</i> ,																				
<i>Anaerolineaceae</i> (12)	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Ardenticateria</i> ,																				
Unassigned <i>Ardenticateria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

Phyla, Class, Family <sup>p</sup>	Sampling Time:		0h								30h									
	Treatment:		C1	C2	C3	Cel	Ch	Pe	Xy	Md	Da	C1	C2	C3	Ce	Ch	Pe	Xy	Md	Da
	Relative Abundance (%)																			
<i>Caldilineae</i> ,																				
<i>Caldilineaceae</i> (10)			0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chloroflexia</i> ,																				
<i>Roseiflexaceae</i> (8)			0.2	0.3	0.4	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Unassigned <i>Kallotenuales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gitt-GS-136</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>JG30-KF-CM66</i> (7)			0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>KD4-96</i> (11)			2.7	2.5	2.5	2.4	2.3	2.0	2.0	1.8	1.9	1.3	1.5	1.5	0.9	1.1	0.7	0.8	0.6	0.6
<i>Ktedonobacteria</i> ,																				
<i>Thermosporotrichaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacterales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteria</i> (9)			0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
<i>S085</i> (11)			0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.0	0.0	0.1	0.1	0.1
<i>SBR2076</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>SHA-26</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermomicrobia</i> ,																				
Unassigned <i>Thermomicrobia</i> (35)			0.6	0.5	0.7	0.5	0.6	0.5	0.7	0.6	0.6	0.3	0.4	0.4	0.2	0.4	0.2	0.2	0.2	0.2
<i>TK10</i> (15)			0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0
Unassigned <i>Chloroflexi</i> (5)			0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1
<b>Cyanobacteria</b> ,																				
<i>Chloroplast</i> ,																				
Unassigned <i>Chloroplast</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyanobacteria</i> ,																				
Unassigned <i>Cyanobacteria</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Melainabacteria</i> ,																				
Unassigned <i>Obscuribacterales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>ML635J-2</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Deferribacteres</b> ,																				
Unassigned <i>Deferribacteres</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Deinococcus-Thermus</b> ,																				
Unassigned <i>Deinococci</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Elusimicrobia</b> ,																				
<i>Elusimicrobia</i>																				
Unassigned <i>Elusimicrobia</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fibrobacteres</b> ,																				
<i>Fibrobacteria</i> ,																				
<i>Fibrobacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Fibrobacterales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Firmicutes</b> ,																				
<i>Bacilli</i> ,																				
<i>Alicyclobacillaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bacillaceae</i> (16)			1.8	1.8	1.8	2.0	1.8	1.7	2.2	2.2	2.0	3.0	3.8	3.2	4.3	4.1	3.1	4.5	1.9	4.8
<i>Lactobacillaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Paenibacillaceae</i> (28)			0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.9	1.0	1.1	2.5	1.5	2.8	2.3	0.1	1.1
<i>Planococcaceae</i> (8)			0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Staphylococcaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermoactinomyces</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacilli</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Clostridia</i> ,																				
<i>Christensenellaceae</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Clostridiaceae</i> (24) [ <b>GPT-6</b> , <b>GPT-8</b> ]			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	9.8	8.1	9.8	12	13	7.1	12	4.9	5.5
<i>Defluviitaleaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Eubacteriaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gracilibacteraceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Heliobacteriaceae</i> (8)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lachnospiraceae</i> (38)			0.1	0.1	0.1	0.0	0.1	0.2	0.1	0.1	0.1	5.0	4.2	6.2	4.8	5.0	6.1	6.8	0.0	1.9

Phyla, Class, Family <sup>p</sup>	Sampling Time:		0h							30h									
	Treatment:	C1	C2	C3	Cel	Ch	Pe	Xy	Md	Da	C1	C2	C3	Ce	Ch	Pe	Xy	Md	Da
Relative Abundance (%)																			
<i>Peptococcaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Peptostreptococcaceae</i> (6) [GPT-2]	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	20	13	16	13	16	23	20	2.4	5.1	
<i>Ruminococcaceae</i> (39)	0.1	0.1	0.0	0.1	0.1	0.3	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.2	
<i>Thermoanaerobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Clostridia</i> (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.3	0.2	0.3	0.1	0.3	0.0	0.0	
<i>Erysipelotrichia</i> ,																			
<i>Erysipelotrichaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Limnochordia</i> ,																			
<i>Limnochordaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Negativicutes</i> ,																			
<i>Acidaminococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Veillonellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Fusobacteria</b> ,																			
<i>Fusobacteria</i> ,																			
<i>Fusobacteriaceae</i> (1) [GPT-7]	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.8	0.8	0.6	1.6	1.2	0.4	1.3	0.0	0.6	
<b>Gemmatimonadetes</b> ,																			
<i>Gemmatimonadetes</i>																			
<i>Gemmatimonadaceae</i> (22)	0.7	0.8	0.8	0.6	0.8	0.7	0.8	0.6	0.7	0.4	0.5	0.5	0.3	0.4	0.3	0.2	0.2	0.3	
Unassigned <i>Gemmatimonadetes</i> (5)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Latescibacteria</b> ,																			
<i>Lentsphaeria</i> ,																			
Unassigned <i>Victivallales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Latescibacteria</i> (19)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Nitrospirae</b> ,																			
<i>Nitrospira</i> ,																			
<i>Nitrospiraceae</i> (9)	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	
Unassigned <i>Nitrospirales</i> (14)	1.7	1.9	1.6	1.6	1.7	1.4	1.5	1.6	1.5	0.8	1.1	0.9	0.7	0.8	0.6	0.6	0.3	0.5	
<b>Parcubacteria</b> ,																			
Unassigned <i>Parcubacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Planctomycetes</b> ,																			
<i>OM190</i> (39)	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	
<i>Phycisphaerae</i> ,																			
<i>Phycisphaeraceae</i> (15)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tepidisphaeraceae</i> (48)	0.9	1.0	0.9	0.8	1.1	0.8	0.9	0.9	1.0	0.5	0.6	0.6	0.5	0.5	0.3	0.4	0.3	0.4	
Unassigned <i>Phycisphaerae</i> (9)	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Planctomycetacia</i> ,																			
<i>Planctomycetaceae</i> (488)	5.6	6.3	6.5	6.3	6.7	4.6	6.2	6.4	6.2	4.0	5.1	4.3	3.2	4.0	2.8	3.1	2.5	2.9	
Unassigned <i>Planctomycetes</i> (2)	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Proteobacteria</b> ,																			
<i>Alphaproteobacteria</i> ,																			
<i>Caulobacteraceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Hyphomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bradyrhizobiaceae</i> (6)	1.3	1.2	1.2	1.4	1.4	1.0	1.3	1.1	1.2	0.8	1.1	1.0	0.7	0.9	0.5	0.6	0.2	0.5	
<i>Brucellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Hyphomicrobiaceae</i> (6)	0.7	0.8	0.7	0.7	0.8	0.6	0.7	0.7	0.6	0.5	0.6	0.7	0.4	0.5	0.3	0.3	0.2	0.3	
<i>Methylobacteriaceae</i> (4)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	
<i>Phyllobacteriaceae</i> (3)	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.1	0.1	
<i>Rhizobiaceae</i> (2)	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Rhodobiaceae</i> (2)	1.4	1.5	1.6	1.7	1.9	1.3	1.8	1.7	1.5	1.3	1.6	1.5	0.8	1.2	0.8	0.9	0.4	0.6	
<i>Roseiariaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Xanthobacteraceae</i> (5)	3.6	4.1	4.2	4.4	4.1	3.2	4.1	4.1	3.7	2.5	3.3	3.0	1.6	2.2	1.7	1.6	0.9	1.1	
Unassigned <i>Rhizobiales</i> (15)	0.4	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1	
<i>Rhodobacteraceae</i> (4)	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Acetobacteraceae</i> (8)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Rhodospirillaceae</i> (13)	0.6	0.7	0.7	0.6	0.5	0.5	0.6	0.6	0.7	0.3	0.4	0.4	0.3	0.4	0.2	0.2	0.1	0.2	
Unassigned <i>Rhodospirillales</i> (33)	1.0	1.0	1.0	0.7	0.9	0.7	0.8	0.8	0.8	0.6	0.8	0.6	0.4	0.5	0.3	0.4	0.2	0.3	
<i>Holosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	



Phyla, Class, Family <sup>p</sup>	Sampling Time: 0h										Sampling Time: 30h									
	Treatment:	C1	C2	C3	Ce	Ch	Pe	Xy	Md	Da	C1	C2	C3	Ce	Ch	Pe	Xy	Md	Da	
Unassigned SR1_Absconditabacteria (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia,</b>																				
Unassigned Tectomicrobia (10)	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	
<b>Tenericutes,</b>																				
<i>Mollicutes,</i>																				
<i>Anaeroplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Entomoplasmatales (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mycoplasmataceae</i> (4) [GPT-3]	15	13	17	16	14	12	15	17	15	9.5	8.3	6.3	8.1	7.7	8.2	9.6	3.5	5.2		
<b>TM6_Dependentiae,</b>																				
Unassigned TM6-Dependentiae (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Verrucomicrobia,</b>																				
OPB35 soil group (45)	0.3	0.4	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	
<i>Opitutae,</i>																				
<i>Opitutaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Opitutae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Spartobacteria,</i>																				
<i>Chthoniobacteraceae</i> (28)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	
<i>Xiphinematobacteraceae</i> (4)	1.4	1.5	1.2	1.4	1.4	1.2	1.4	1.2	1.5	1.2	1.3	1.3	1.2	1.3	0.9	1.1	0.5	0.9		
Unassigned Chthoniobacterales (24)	1.7	2.5	2.2	2.8	2.7	2.1	2.5	2.6	2.4	1.5	1.7	1.6	1.2	1.8	1.0	1.1	0.9	1.1		
<i>Verrucomicrobiae,</i>																				
<i>Verrucomicrobiaceae</i> (14)	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	
Unassigned Verrucomicrobiales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Verrucomicrobia (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Archeae</b>																				
<b>Euryarchaeota,</b>																				
<i>Methanobacteria,</i>																				
<i>Methanobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Thermoplasmatales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Thaumarchaeota,</b>																				
Unassigned Thaumarchaeota (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

**(B) 16S rRNA**

Phyla, Class, Family <sup>p</sup>	Sampling Time: 0h										Sampling Time: 30h																					
	Treatment:	C 1	C 2	C 3	Ce	Ch	Pe	Xy	Md	Da	C 1	C 2	C 3	Ce 1	Ce 2	Ce 3	Ch 1	Ch 2	Ch 3	Pe 1	Pe 2	Pe 3	Xy 1	Xy 2	Xy 3	Md 1	Md 2	Md 3	Da 1	Da 2	Da 3	
<b>Acidobacteria,</b>																																
<i>Acidobacteria,</i>																																
<i>Acidobacteriaceae</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Acidobacteriales (3)	0.1	0.0	0.2	0.1	0.1	0.1	0.3	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	
<i>Blastocatellia,</i>																																
<i>Blastocatellaceae</i> (15)	0.0	0.0	0.1	0.1	0.1	0.1	0.6	0.1	0.1	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1		
<i>Holophagae,</i>																																
Unassigned <i>Holophagae</i> (6)	0.0	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Solibacteres,</i>																																
<i>Solibacteraceae</i> (19)	0.2	0.3	0.3	0.2	0.2	0.1	0.6	0.3	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.2	
Subgroup_2 (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Subgroup_5 (6)	0.0	0.0	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	
Subgroup_6 (78)	1.4	0.7	3.1	1.2	2.6	1.5	4.1	0.7	0.6	0.2	0.8	1.5	0.3	0.2	0.2	0.3	0.5	0.3	0.6	0.2	0.5	0.5	0.6	0.5	0.1	0.1	0.1	0.1	0.3	0.4	0.2	
Subgroup_11 (3)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Subgroup_17 (15)	0.1	0.1	0.2	0.1	0.2	0.1	0.5	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Subgroup_18 (1)	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

Phyla, Class, Family <sup>p</sup>	Sampling Time:										30h																					
	Treatment:	0h									30h																					
		C 1	C 2	C 3	Ce	Ch	Pe	Xy	Md	Da	C 1	C 2	C 3	Cel 1	Cel 2	Cel 3	Ch 1	Ch 2	Ch 3	Pe 1	Pe 2	Pe 3	Xy 1	Xy 2	Xy 3	Md 1	Md 2	Md 3	Da 1	Da 2	Da 3	
											Relative Abundance (%)																					
Subgroup_22 (10)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Actinobacteria,</b>																																
<b>Acidimicrobia,</b>																																
Acidimicrobiaceae (20)	0.8	1.0	0.9	0.9	0.7	0.3	1.3	0.7	0.5	0.6	0.7	0.7	0.8	0.7	0.7	0.8	0.8	0.6	0.3	0.7	0.5	0.3	0.5	0.3	0.1	0.1	0.1	0.5	0.6	0.8		
Iamaceae (5)	0.1	0.1	0.1	0.1	0.1	0.0	0.3	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	
Unassigned Acidimicrobiales (45)	1.5	1.7	1.7	2.0	1.5	0.5	1.9	1.3	0.9	1.3	1.6	2.2	1.0	0.8	1.3	1.0	1.5	1.5	0.6	0.8	1.5	0.7	0.7	0.5	0.2	0.1	0.1	1.2	1.3	1.3		
<b>Actinobacteria,</b>																																
Bifidobacteriaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Catenulisporaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mycobacteriaceae (7)	0.3	0.3	0.2	0.3	0.2	0.1	0.7	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.0	0.0	0.0	0.2	0.2	0.1		
Nocardiaceae (9)	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	
Acidothermaceae (6)	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.0	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.2	0.1		
Cryptosporangiaceae (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Frankiaceae (3)	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.0	0.0	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	
Geodermatophilaceae (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Nakamurellaceae (3)	0.1	0.1	0.1	0.0	0.1	0.0	0.5	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	
Sporichthyaceae (4)	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned																																
Frankiales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Glycomycetaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Kineosporiaceae (1)	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Brevibacteriaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Cellulomonadaceae (1)	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.4	0.2	0.1	0.0	0.3	0.3	0.0	0.0	0.0	0.1	0.2	0.1		
Demequinaceae (1)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Intrasporangiaceae (3)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.1		
Microbacteriaceae (6)	0.1	0.1	0.2	0.1	0.2	0.0	0.2	0.1	0.1	0.1	0.3	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0		
Micrococcaceae (2)	0.2	0.1	0.1	0.1	0.2	0.1	0.7	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0		
Promicromonosporaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Micromonosporaceae (29)	1.5	1.3	1.2	1.5	1.2	0.2	2.2	1.0	0.7	1.3	1.8	1.9	1.3	0.9	1.3	1.4	1.7	1.3	0.1	1.1	1.4	0.5	0.1	0.1	0.1	0.1	0.1	1.0	1.0	0.8		
Nocardioideae (23)	1.2	1.0	1.2	1.4	0.8	0.5	1.9	0.8	0.6	0.8	1.1	1.5	0.9	0.7	0.8	1.1	0.9	0.9	0.5	0.6	0.8	0.4	0.6	0.3	0.1	0.1	0.1	0.6	1.0	0.7		
Propionibacteriaceae (5)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1			
Pseudonocardaceae (10)	0.2	0.2	0.2	0.3	0.3	0.2	0.4	0.1	0.1	0.2	0.3	0.4	0.3	0.3	0.2	0.2	0.3	0.2	0.1	0.2	0.3	0.1	0.2	0.1	0.0	0.0	0.3	0.2	0.1			
Streptomycetaceae (2)	0.7	0.6	0.8	0.6	0.7	0.2	1.2	0.5	0.3	0.6	0.7	1.0	0.6	0.2	0.3	0.6	0.6	0.3	0.3	0.3	0.5	0.1	0.2	0.1	0.1	0.1	0.1	0.3	0.5	0.3		
Streptosporangiaceae (3)	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Thermomonosporaceae (6)	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned																																
Actinobacteria (1)	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
<b>Coriobacteria,</b>																																
Coriobacteriaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Coriobacteria (16)	0.5	0.5	0.7	0.7	0.6	0.1	0.6	0.5	0.3	0.4	0.4	0.8	0.4	0.3	0.4	0.7	0.5	0.0	0.3	0.3	0.4	0.4	0.1	0.0	0.1	0.0	0.0	0.3	0.4	0.3		
<b>Rubrobacteria,</b>																																
Rubrobacteriaceae (5)	0.5	0.3	0.3	0.3	0.3	0.1	0.4	0.3	0.2	0.3	0.5	0.4	0.3	0.2	0.4	0.3	0.3	0.4	0.1	0.2	0.											





Phyla, Class, Family <sup>P</sup>	Sampling Time:			0h						30h																					
	Treatment:	C 1	C 2	C 3	Ce	Ch	Pe	Xy	Md	Da	C 1	C 2	C 3	Cel 1	Cel 2	Cel 3	Ch 1	Ch 2	Ch 3	Pe 1	Pe 2	Pe 3	Xy 1	Xy 2	Xy 3	Md 1	Md 2	Md 3	Da 1	Da 2	Da 3
	Relative Abundance (%)																														
Unassigned Clostridia (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.5	0.5	0.3	0.2	0.2	0.5	0.4	0.4	0.3	0.1	0.2	0.2	0.8	0.6	0.0	0.0	0.0	0.1	0.1	0.0	
<i>Erysipelotrichia</i> , <i>Erysipelotrichaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Limnochordia</i> , <i>Limnochordaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Negativicutes</i> , <i>Acidaminococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Veillonellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Fusobacteria</b> , <i>Fusobacteria</i> , <i>Fusobacteriaceae</i> (1) [GPT-7]	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.1	1.0	0.7	0.5	1.7	3.7	1.6	1.5	1.2	1.0	0.6	1.0	0.3	0.2	1.5	0.3	0.0	0.0	0.0	1.1	0.7	0.6	
<b>Gemmatimonadetes</b> , <i>Gemmatimonadetes</i> , <i>Gemmatimonadaceae</i> (22)	0.3	0.2	0.5	0.2	0.2	0.1	1.2	0.1	0.2	0.2	0.3	0.5	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.1	
Unassigned <i>Gemmatimonadetes</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Latescibacteria</b> , <i>Lentisphaeria</i> , Unassigned <i>Victivallales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Latescibacteria</i> (19)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Nitrospirae</b> , <i>Nitrospira</i> , <i>Nitrospiraceae</i> (9)	0.1	0.2	0.3	0.1	0.3	0.1	0.4	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1
Unassigned <i>Nitrospirales</i> (14)	1.1	0.7	1.5	1.1	1.4	0.5	1.8	0.9	0.8	0.6	1.5	1.2	0.5	0.5	0.4	0.4	0.6	0.5	0.3	0.3	0.7	0.2	0.3	0.2	0.2	0.1	0.1	0.5	0.6	0.3	
<b>Parcubacteria</b> , Unassigned <i>Parcubacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Planctomycetes</b> , OM190 (39)	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Phycisphaerae</i> , <i>Phycisphaeraceae</i> (15)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tepidisphaeraceae</i> (48)	0.6	0.7	1.1	0.8	1.1	0.4	0.6	0.7	0.5	0.4	0.8	0.4	0.5	0.3	0.4	0.5	0.4	0.7	0.3	0.4	0.4	0.1	0.2	0.1	0.0	0.0	0.0	0.3	0.3	0.6	
Unassigned <i>Phycisphaerae</i> (9)	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Planctomycetacia</i> , <i>Planctomycetaceae</i> (488)	9.0	9.1	15	11	11	2.8	7.3	8.2	6.2	11	11	10	11	6.8	8.6	12	10	12	2.0	8.1	6.9	1.2	2.3	1.3	1.1	0.8	0.7	5.8	5.3	9.4	
Unassigned <i>Planctomycetes</i> (7)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Proteobacteria</b> , <i>Alphaproteobacteria</i> , <i>Caulobacteraceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Hyphomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bradyrhizobiaceae</i> (6)	1.3	1.7	1.1	1.8	2.1	1.0	2.2	1.6	2.1	1.0	2.1	1.4	1.1	1.3	1.5	1.4	1.8	1.5	1.3	1.2	0.9	0.3	1.0	0.8	0.3	0.3	0.4	1.3	1.4	1.5	
<i>Brucellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Hyphomicrobiaceae</i> (6)	0.4	0.5	0.5	0.5	0.5	0.4	0.9	0.5	0.6	0.5	0.6	0.3	0.4	0.5	0.6	0.6	0.7	0.6	0.7	0.4	0.6	0.3	0.8	0.5	0.1	0.1	0.1	0.6	0.5	0.4	
<i>Methylobacteriaceae</i> (4)	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.1	0.3	0.3	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.0	0.3	0.2	0.1	0.0	0.1	0.2	0.2	0.2	
<i>Phyllobacteriaceae</i> (3)	0.2	0.3	0.3	0.3	0.5	0.5	0.9	0.5	0.4	0.2	0.5	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.5	0.3	0.5	0.2	0.7	0.4	0.1	0.1	0.1	0.2	0.2	0.3	
<i>Rhizobiaceae</i> (2)	0.1	0.0	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	
<i>Rhodobacteraceae</i> (2)	0.2	0.3	0.3	0.3	0.3	0.2	0.7	0.4	0.4	0.3	0.5	0.3	0.2	0.4	0.3	0.5	0.6	0.4	0.3	0.4	0.4	0.3	0.4	0.2	0.1	0.0	0.1	0.2	0.2	0.3	
<i>Roseiarcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Xanthobacteraceae</i> (5)	1.3	1.2	1.0	1.9	1.3	1.2	2.8	1.2	1.3	1.3	2.0	1.6	1.1	0.8	1.3	1.2	2.2	1.4	1.3	1.0	1.2	0.8	1.4	1.2	0.2	0.2	0.2	0.9	1.2	0.9	
Unassigned <i>Rhizobiales</i> (15)	0.1	0.2	0.2	0.2	0.2	0.2	0.6	0.2	0.2	0.2	0.4	0.3	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.1	0.1	
<i>Rhodobacteraceae</i> (4)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	
<i>Acetobacteraceae</i> (8)	0.1	0.1	0.1	0.1	0.1	0.2	0.5	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.1	0.1	

Phyla, Class, Family <sup>b</sup>	Sampling Time:										30h																			
	0h										30h																			
	Treatment:	C 1	C 2	C 3	Ce	Ch	Pe	Xy	Md	Da	C 1	C 2	C 3	Cel 1	Cel 2	Cel 3	Ch 1	Ch 2	Ch 3	Pe 1	Pe 2	Pe 3	Xy 1	Xy 2	Xy 3	Md 1	Md 2	Md 3	Da 1	Da 2
	Relative Abundance (%)																													
<i>Rhodospirillaceae</i> (13)	0.4	0.3	0.4	0.4	0.6	0.3	0.6	0.3	0.4	0.3	0.6	0.6	0.4	0.4	0.6	0.6	0.6	0.4	0.4	0.3	0.5	0.1	0.6	0.3	0.1	0.1	0.1	0.4	0.4	0.3
Unassigned <i>Rhodospirillales</i> (33)	0.6	0.6	0.9	0.6	1.1	0.6	1.2	0.6	0.7	0.6	1.2	1.2	0.5	0.7	1.0	0.6	0.6	0.7	0.6	0.5	1.0	0.2	0.5	0.3	0.2	0.1	0.1	0.7	0.6	0.4
<i>Holospiraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned																														
<i>Rickettsiales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Betaproteobacteria</i> ,																														
<i>Alcaligenaceae</i> (3)	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Burkholderiaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (10)	0.2	0.2	0.4	0.3	0.5	0.1	1.0	0.3	0.3	0.0	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0
<i>Oxalobacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Burkholderiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Methylophilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Neisseriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (29)	0.2	0.1	0.2	0.2	0.3	0.2	1.4	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0
<i>Rhodocyclaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (20)	0.1	0.1	0.2	0.1	0.3	0.1	0.7	0.1	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<i>Deltaproteobacteria</i> ,																														
<i>Bacteriovoraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bedellovibrionaceae</i> (15)	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1
<i>Desulfurellaceae</i> (32)	0.6	0.5	0.9	0.4	0.9	0.4	1.1	0.5	0.5	0.3	1.0	0.9	0.3	0.4	0.4	0.3	0.5	0.4	0.3	0.3	0.6	0.2	0.5	0.3	0.1	0.1	0.1	0.6	0.5	0.3
<i>Desulfuro-</i>																														
<i>monadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (14)	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1
<i>Archangiaceae</i> (7)	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0
<i>Haliangiaceae</i> (42)	0.2	0.3	0.3	0.3	0.3	0.2	0.7	0.1	0.1	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.4	0.2	0.3	0.1	0.2	0.1	0.3	0.2	0.1	0.0	0.0	0.2	0.2	0.2
<i>Myxococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicytidaceae</i> (5)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1
<i>Polyangiaceae</i> (22)	0.2	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.1	0.2	0.1
<i>Sandaracinaceae</i> (21)	0.1	0.1	0.3	0.1	0.2	0.1	0.6	0.0	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (58)	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.2	0.1	0.3	0.4	0.4	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.1	0.3	0.2	0.0	0.0	0.1	0.2	0.4	0.2
<i>Oligoflexaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned																														
<i>Oligoflexales</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Syntrophaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Deltaproteobacteria</i> (13)	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gammaproteobacteria</i> ,																														
<i>Aeromonadaceae</i> (2) [GPT-1]	38	32	28	25	28	51	11	29	37	1.3	1.8	2.3	3.2	3.1	3.6	2.2	2.2	3.0	5.4	3.0	3.4	34	2.0	1.5	35	34	31	13	13	23
<i>Shewanellaceae</i> (1)	0.3	0.2	0.2	0.2	0.2	0.7	0.1	0.1	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1
<i>Cellvibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haliaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spongiibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (4) [GPT-4, GPT-5]	2.7	2.8	2.9	2.4	4.1	4.3	1.7	5.0	6.5	1.0	1.0	1.6	0.8	0.8	1.1	0.7	0.6	1.0	2.7	3.6	3.1	16	0.3	0.2	33	37	37	12	11	12
<i>Coxiellaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.																	

Phyla, Class, Family <sup>b</sup>	Sampling Time:			0h						30h																					
	Treatment:	C 1	C 2	C 3	Ce	Ch	Pe	Xy	Md	Da	C 1	C 2	C 3	Cel 1	Cel 2	Cel 3	Ch 1	Ch 2	Ch 3	Pe 1	Pe 2	Pe 3	Xy 1	Xy 2	Xy 3	Md 1	Md 2	Md 3	Da 1	Da 2	Da 3
	Relative Abundance (%)																														
<i>Xanthomonadaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Xanthomonadales</i> (18)	0.2	0.2	0.3	0.1	0.2	0.1	1.7	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.1	0.2	0.1	
Unassigned <i>Gamma-proteobacteria</i> (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Saccharibacteria</i></b> , Unassigned <i>Saccharibacteria</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Spirochaetae</i></b> , <i>Spirochaetes</i> , <i>Spirochaetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>SR1_Absconditabacteria</i></b> , Unassigned <i>SR1_Absconditabacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Tectomicrobia</i></b> , Unassigned <i>Tectomicrobia</i> (10)	1.0	0.8	1.0	0.9	1.0	0.7	1.0	0.6	0.6	0.6	0.8	0.9	0.6	0.4	0.9	0.5	0.7	0.7	0.6	0.2	0.7	0.1	0.6	0.3	0.1	0.1	0.1	0.6	0.6	0.6	
<b><i>Tenericutes</i></b> , <i>Mollicutes</i> , <i>Anaeroplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Entomoplasmatales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mycoplasmataceae</i> (4) [ <b>GPT-3</b> ]	20	26	14	29	17	20	13	30	23	22	12	7.4	27	25	20	28	24	27	21	18	18	16	27	21	5.5	4.8	7.8	21	14	13	
<b><i>TM6_Dependentiae</i></b> , Unassigned <i>TM6-Dependentiae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Verrucomicrobia</i></b> , <i>OPB35 soil group</i> (45)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	
<i>Opiritae</i> , <i>Opiritaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Opiritae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Spartobacteria</i> , <i>Chthonio-</i> <i>bacteraceae</i> (28)	0.1	0.1	0.2	0.2	0.3	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	
<i>Xiphinemato-</i> <i>bacteraceae</i> (4)	0.7	1.4	1.0	1.0	2.1	1.3	0.7	2.3	3.1	0.9	3.0	0.8	1.5	3.5	2.6	2.1	1.6	1.9	2.6	2.0	2.7	0.6	1.9	1.6	0.6	0.6	1.3	0.8	1.6		
Unassigned <i>Chthoniobacterales</i> (24)	0.3	0.3	0.4	0.4	0.7	0.2	0.4	0.6	0.5	0.3	0.5	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.4	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	
<i>Verrucomicrobiae</i> , <i>Verrucomicrobiaceae</i> (14)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Verrucomicrobiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Verrucomicrobia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Archaea</b> <b><i>Euryarchaeota</i></b> , <i>Methanobacteria</i> , <i>Methanobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Thermoplasmatales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Thaumarchaeota</i></b> , Unassigned <i>Thaumarchaeota</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

<sup>a</sup>Samples of the three replicates of the 16S rRNA gene (A) control treatment at 0 h and 30 h, 16S rRNA (B) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Treatments: C, unsupplemented control; Cel, cellulose; Ch, chitin; Pe, pectin; Xy, xylan; Md, maltodextrin; Da, dextran. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes from Figure 33 are bold and in brackets.



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h			30h					
	Treatment:	C1	C2	C3	GI	St	C1	C2	C3	GI	St
Relative Abundance (%)											
<i>Rubrobacteriaceae</i> (3)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Unassigned <i>Rubrobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermoleophila</i> ,											
<i>Conexibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gaiellaceae</i> (5)	1.9	1.9	2.3	1.6	1.5	1.3	1.5	1.4	0.8	0.2	
Unassigned <i>Gaiellales</i> (30)	3.5	4.2	4.3	3.4	2.6	2.0	2.6	2.6	1.2	0.5	
<i>Parviterribacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Patulibacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Solirubrobacteraceae</i> (4)	0.5	0.6	0.7	0.5	0.5	0.4	0.6	0.6	0.1	0.1	
Unassigned <i>Solirubrobacteriales</i> (38)	2.4	2.4	3.0	2.1	1.8	1.6	1.8	2.2	0.4	0.4	
<b>Armatimonadetes,</b>											
<i>Armatimonadia</i> ,											
Unassigned <i>Armatimonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Bacteroidetes,</b>											
<i>Bacteroidia</i> ,											
<i>Bacteroidaceae</i> (6)	1.4	0.0	0.0	0.2	0.0	0.0	0.2	0.6	0.4	0.2	1.1
<i>Porphyromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rikenellaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia</i> ,											
<i>Cytophagaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteria</i> ,											
<i>Flavobacteriaceae</i> (9)	0.2	0.3	0.2	0.2	0.2	0.2	0.5	0.8	0.4	0.0	0.0
<i>Sphingobacteria</i> ,											
<i>Chitinophagaceae</i> (5)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1,</b>											
Unassigned <i>BRC1</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae,</b>											
<i>Chlamydia</i> ,											
<i>Parachlamydiaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi,</b>											
<i>Anaerolineae</i> ,											
<i>Anaerolineaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ardenticatenia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Caldilineae</i> ,											
<i>Caldilineaceae</i> (10)	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0
<i>Chloroflexia</i>											
<i>AKIW781</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Roseiflexaceae</i> (4)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0
<i>Gitt-GS-136</i> (4)	0.3	0.3	0.4	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1
<i>JG30-KF-CM66</i> (8)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>KD4-96</i> (10)	1.7	2.0	2.4	1.6	1.4	1.3	1.4	1.1	0.4	0.2	
<i>Ktedonobacteria</i> ,											
Unassigned <i>Ktedonobacteria</i> (7)	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>S085</i> (11)	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>SBR2076</i> (5)	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>SJA-15</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermomicrobia</i> ,											
Unassigned <i>Thermomicrobia</i> (26)	0.4	0.5	0.4	0.4	0.3	0.2	0.2	0.3	0.1	0.1	
<i>TK10</i> (12)	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	
<b>Cyanobacteria,</b>											
<i>Chloroplast</i> ,											
Unassigned <i>Chloroplast</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyanobacteria</i> ,											
Unassigned <i>Cyanobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Elusimicrobia,</b>											
<i>Elusimicrobia</i> ,											
Unassigned <i>Elusimicrobia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Firmicutes,</b>											



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h			30h					
	Treatment:	C1	C2	C3	GI	St	C1	C2	C3	GI	St
	Relative Abundance (%)										
<i>Rhodobiaceae</i> (2)	1.0	1.0	0.9	0.9	0.7	0.7	0.7	0.7	0.7	0.1	0.0
<i>Rhodospirillaceae</i> (13)	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.0
Unassigned Rhodospirillales (13)	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0
Unassigned Rhizobiales (8)	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0
<i>Sphingomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (3)	2.5	2.6	3.1	2.4	2.1	1.7	1.9	2.3	0.7	0.3	0.0
Unassigned <i>Alphaproteobacteria</i> (13)	0.4	0.5	0.5	0.4	0.4	0.3	0.4	0.4	0.1	0.1	0.0
<i>Betaproteobacteria</i> ,											
<i>Alcaligenaceae</i> (1)	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Burkholderiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (7)	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
<i>Gallionellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (17)	0.3	0.2	0.3	0.2	0.1	0.1	0.2	0.1	0.0	0.0	0.0
<i>Oxalobacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (11)	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Deltaproteobacteria</i> ,											
<i>Archangiaceae</i> (5)	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
<i>Bdellovibrionaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfobulbaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfurellaceae</i> (19)	0.8	0.7	0.9	0.6	0.6	0.4	0.4	0.7	0.2	0.1	0.0
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (11)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Haliangiaceae</i> (24)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.0
<i>Myxococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (20)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Nannocystaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselocystidaceae</i> (1)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Polyangiaceae</i> (17)	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Sandaracinaceae</i> (14)	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Oligoflexales</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Deltaproteobacteria</i> (11)	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
<i>Gammaproteobacteria</i> ,											
<i>Aeromonadaceae</i> (3) [GPT-1]	22	22	23	24	27	24	20	19	60	66	0.0
<i>Coxiellaceae</i> (1)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
<i>Enterobacteriaceae</i> (5) [GPT-4, GPT-5]	1.4	2.4	1.5	2.0	1.9	1.7	2.0	2.2	4.0	5.2	0.0
<i>Legionellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (2)	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0
<i>Shewanellaceae</i> (4)	8.4	8.6	8.8	5.7	6.4	15	15	16	1.1	1.0	0.0
<i>Xanthomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Gammaproteobacteria</i> (14)	0.2	0.2	0.3	0.1	0.2	0.2	0.1	0.1	0.0	0.0	0.0
<i>Saccharibacteria</i> ,											
Unassigned <i>Saccharibacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spirochaetae</i> ,											
<i>Spirochaetales</i> ,											
<i>Spirochaetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tectomicrobia</i> ,											
Unassigned <i>Tectomicrobia</i> (11)	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.2	0.1	0.0	0.0
<i>Tenericutes</i> ,											
<i>Mollicutes</i> ,											
<i>Mycoplasmataceae</i> (5) [GPT-3]	10	8.8	6.7	12	12	8.4	7.2	8.7	5.4	3.3	0.0
Unassigned <i>Entomoplasmatales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobia</i> ,											
<i>OPB35 soil group</i> (24)	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Spartobacteria</i> ,											
<i>Chthoniobacteraceae</i> (10)	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xiphinematobacteraceae</i> (3)	0.9	1.0	0.8	0.7	1.0	1.0	1.0	0.7	0.4	0.1	0.0

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h			30h					
	Treatment:	C1	C2	C3	GI	St	C1	C2	C3	GI	St
Unassigned Chthoniobacterales (17)		1.0	1.4	1.3	1.2	1.1	0.8	0.9	0.9	0.4	0.2
<i>Verrucomicrobiae</i> , <i>Verrucomicrobiaceae</i> (4)		0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0

**(B) 16S rRNA**

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h			30h									
	Treatment:	C1	C2	C3	GI	St	C1	C2	C3	GI1	GI2	GI3	St1	St2	St3
<b>Acidobacteria,</b>															
<i>Acidobacteria</i> ,															
<i>Acidobacteriaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatellia</i> ,															
<i>Blastocatellaceae</i> (4)		0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holophagae</i> ,															
Unassigned <i>Holophagae</i> (4)		0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solibacteres</i> ,															
<i>Solibacteraceae</i> (12)		0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (5)		0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (45)		0.5	0.3	0.7	0.6	0.7	0.2	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Subgroup_11 (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_18 (1)		0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>															
<i>Acidimicrobia</i> ,															
<i>Acidimicrobiaceae</i> (12)		0.6	0.6	1.3	0.7	0.6	0.5	0.6	0.7	0.2	0.1	0.1	0.1	0.1	0.1
<i>Iamiaceae</i> (5)		0.2	0.1	0.1	0.2	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Acidimicrobia</i> (40)		0.9	1.2	1.9	1.0	1.0	0.6	0.7	1.3	0.2	0.3	0.2	0.2	0.2	0.3
<i>Actinobacteria</i> ,															
<i>Acidotherrmaceae</i> (5)		0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Catenulisporaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellulomonadaceae</i> (1)		0.0	0.1	0.2	0.1	0.2	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.0
<i>Demequinaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Dermacoccaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (2)		0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geodermatophilaceae</i> (1)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glycomycetaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (3)		0.1	0.1	0.3	0.1	0.1	0.1	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Kineosporiaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Microbacteriaceae</i> (6)		0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micrococcaceae</i> (2)		0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micromonosporaceae</i> (18)		0.9	0.9	1.8	0.8	0.7	0.6	0.9	0.8	0.2	0.2	0.1	0.2	0.1	0.2
<i>Mycobacteriaceae</i> (5)		0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nakamurellaceae</i> (1)		0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nocardiaceae</i> (5)		0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nocardioideae</i> (19)		0.4	0.6	1.4	0.6	0.6	0.4	0.8	0.5	0.1	0.1	0.1	0.1	0.2	0.2
<i>Promicromonosporaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Propionibacteriaceae</i> (6)		0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.0	0.1	0.0	0.1	0.0	0.0
<i>Pseudonocardiaceae</i> (10)		0.2	0.3	0.9	0.3	0.3	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1
<i>Sporichthyaceae</i> (3)		0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	Treatment:	0h					30h								
		C1	C2	C3	GI	St	C1	C2	C3	GI1	GI2	GI3	St1	St2	St3
Relative Abundance (%)															
Streptomycetaceae (4)		0.2	0.2	0.8	0.5	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.0	0.1	0.1
Streptosporangiaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Thermomonosporaceae (3)		0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Actinobacteria (2)		0.0	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Coriobacteria,															
Coriobacteriaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Coriobacteria (21)		0.3	0.6	0.9	0.4	0.5	0.2	0.6	0.4	0.1	0.1	0.1	0.0	0.0	
Rubrobacteria,															
Rubrobacteriaceae (3)		0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	
Unassigned Rubrobacteria (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Thermoleophila,															
Conexibacteraceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Gaiellaceae (5)		0.4	0.5	0.6	0.5	0.6	0.3	0.5	0.5	0.1	0.1	0.2	0.1	0.1	
Unassigned Gaiellales (30)		0.9	0.8	1.4	1.3	1.1	0.4	1.1	0.8	0.2	0.3	0.2	0.2	0.2	
Parviterribacteraceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Patulibacteraceae (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Solirubrobacteraceae (4)		0.7	0.7	1.3	0.9	0.9	0.4	0.7	0.7	0.1	0.2	0.1	0.2	0.1	
Unassigned Solirubrobacterales (38)		0.5	0.7	0.9	0.9	0.4	0.3	0.5	0.4	0.2	0.1	0.1	0.1	0.2	
Armatimonadetes,															
Armatimonadia,															
Unassigned Armatimonadales (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Bacteroidetes,															
Bacteroidia,															
Bacteroidaceae (6)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
Porphyromonadaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
Rikenellaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Cytophagia,															
Cytophagaceae (6)		0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Flavobacteria,															
Flavobacteriaceae (9)		0.1	0.0	0.0	0.1	0.0	0.0	0.4	0.1	0.1	0.0	0.0	0.1	0.0	
Sphingobacteria,															
Chitinophagaceae (5)		0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Sphingobacteriaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned Sphingobacteriales (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
BRC1,															
Unassigned BRC1 (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Chlamydiae,															
Chlamydiae,															
Parachlamydiaceae (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Chloroflexi,															
Anaerolineae,															
Anaerolineaceae (6)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Ardenticatenia (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Caldilineae,															
Caldilineaceae (10)		0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Chloroflexia															
AKIW781 (1)		0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Roseiflexaceae (4)		0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	
Gitt-GS-136 (4)		0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
JG30-KF-CM66 (8)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
KD4-96 (10)		0.5	0.5	0.9	0.6	0.4	0.2	0.5	0.4	0.2	0.1	0.1	0.1	0.1	
Ktedonobacteria,															
Unassigned Ktedonobacteria (7)		0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
S085 (11)		0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
SBR2076 (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
SJA-15 (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Thermomicrobia,															
Unassigned Thermomicrobia (26)		0.1	0.2	0.2	0.4	0.4	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	
TK10 (12)		0.2	0.1	0.4	0.2	0.2	0.1	0.2	0.3	0.1	0.0	0.0	0.0	0.1	
Cyanobacteria,															



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h					30h								
	Treatment:		C1	C2	C3	GI	St	C1	C2	C3	GI1	GI2	GI3	St1	St2	St3
			Relative Abundance (%)													
<i>Beijerinckiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (3)			0.5	0.6	0.8	0.6	0.7	0.4	0.7	0.6	0.1	0.1	0.1	0.2	0.1	0.1
<i>Caulobacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Hyphomicrobiaceae</i> (4)			0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Methylobacteriaceae</i> (3)			0.1	0.2	0.2	0.1	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0
<i>Phyllobacteriaceae</i> (3)			0.2	0.0	0.4	0.2	0.1	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhizobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (3)			0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobiaceae</i> (2)			0.2	0.1	0.4	0.2	0.2	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.1
<i>Rhodospirillaceae</i> (13)			0.2	0.3	1.0	0.3	0.3	0.5	0.5	0.4	0.2	0.0	0.1	0.1	0.1	0.1
Unassigned Rhodospirillales (13)			0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1
Unassigned Rhizobiales (8)			0.0	0.0	0.2	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (3)			0.7	1.3	1.7	0.9	1.0	0.5	0.8	1.1	0.2	0.2	0.1	0.1	0.3	0.2
Unassigned <i>Alphaproteobacteria</i> (13)			0.2	0.2	0.6	0.2	0.4	0.1	0.3	0.3	0.1	0.0	0.0	0.0	0.1	0.1
<i>Betaproteobacteria</i> ,																
<i>Alcaligenaceae</i> (1)			0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Burkholderiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (7)			0.0	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gallionellaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (17)			0.1	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oxalobacteraceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (11)			0.1	0.1	0.2	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Deltaproteobacteria</i> ,																
<i>Archangiaceae</i> (5)			0.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bdellovibrionaceae</i> (5)			0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfobulbaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfurellaceae</i> (19)			0.2	0.1	0.6	0.2	0.4	0.1	0.1	0.2	0.0	0.1	0.0	0.1	0.0	0.1
<i>Desulfuromonadaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (11)			0.0	0.1	0.2	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haliangiaceae</i> (24)			0.1	0.2	0.4	0.2	0.3	0.2	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.1
<i>Myxococcaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (20)			0.1	0.1	0.2	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (2)			0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicystidaceae</i> (1)			0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Polyangiaceae</i> (17)			0.3	0.0	0.4	0.2	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.1
<i>Sandaracinaceae</i> (14)			0.1	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
<i>Vulgatibacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Oligoflexales</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Deltaproteobacteria</i> (11)			0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gammaproteobacteria</i> ,																
<i>Aeromonadaceae</i> (3) [GPT-1]			27	21	5.0	27	33	21	25	21	57	61	76	66	67	60
<i>Coxiellaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (5) [GPT-4, GPT-5]			2.5	3.0	0.4	3.8	4.8	1.9	2.0	1.9	5.1	6.1	5.2	7.5	6.4	6.5
<i>Legionellaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (2)			0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<i>Shewanellaceae</i> (4)			3.1	2.3	0.7	1.3	1.7	2.4	5.1	4.3	0.2	0.4	0.5	0.5	0.4	0.4
<i>Xanthomonadaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Gammaproteobacteria</i> (14)			0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saccharibacteria</i> ,																
Unassigned <i>Saccharibacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spirochaetae</i> ,																
<i>Spirochaetaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tectomicrobia</i> ,																
Unassigned <i>Tectomicrobia</i> (11)			0.4	0.6	0.9	0.4	0.7	0.5	0.7	0.5	0.2	0.1	0.1	0.2	0.2	0.1
<i>Tenericutes</i> ,																

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h					30h							
	Treatment:	C1	C2	C3	Gl	St	C1	C2	C3	Gl1	Gl2	Gl3	St1	St2	St3
		Relative Abundance (%)													
<i>Mollicutes</i> ,															
<i>Mycoplasmataceae</i> (5) [GPT-3]	21	28	41	26	21	25	17	18	8.2	7.2	2.9	5.3	4.8	6.4	
Unassigned <i>Entomoplasmatales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Verrucomicrobia</i></b> ,															
<i>OPB35 soil group</i> (24)	0.2	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Spartobacteria</i> ,															
<i>Chthoniobacteraceae</i> (10)	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Xiphinematobacteraceae</i> (3)	0.7	1.2	1.8	1.0	1.0	0.8	0.7	1.0	0.5	0.2	0.2	0.2	0.2	0.2	
Unassigned <i>Chthoniobacterales</i> (17)	0.3	0.1	0.4	0.3	0.2	0.1	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.1	
<i>Verrucomicrobiae</i> ,															
<i>Verrucomicrobiaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

<sup>a</sup>Samples of the three replicates of the 16S rRNA gene (A) control treatment at 0 h and 30 h, 16S rRNA (B) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Treatments: C, unsupplemented control; Gl, glycogen; St, starch. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes from Figure 33 are bold and in brackets.

**Table A3.** Summary of all detected families in the saccharide experiment based on 16S rRNA gene (A) and 16S rRNA (B) analysis (Section 3.1.4).<sup>a</sup>

**(A) 16S rRNA genes**

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h							30h							
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C1	C2	C3	A	Ce	G	Ga	X
		Relative Abundance (%)															
<b><i>Acidobacteria</i></b> ,																	
<i>Acidobacteria</i> ,																	
<i>Acidobacteriaceae</i> (12)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0
<i>Blastocatellia</i> ,																	
<i>Blastocatellaceae</i> (14)	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1
Unassigned <i>Acidobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holophagae</i> ,																	
Unassigned <i>Holophagae</i> (7)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1
<i>Solibacteres</i> ,																	
<i>Solibacteraceae</i> (26)	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.2	0.3	0.3	0.1	0.1	0.2	0.2	0.2
Subgroup_2 (5)	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
Subgroup_5 (6)	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
Subgroup_6 (83)	2.8	3.1	3.4	2.9	3.0	2.7	2.9	3.1	3.1	1.2	1.6	1.9	0.9	0.8	1.0	0.9	1.5
Subgroup_11 (6)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_15 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (17)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2
Subgroup_18 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (13)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (6)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Actinobacteria</i></b> ,																	
<i>Acidimicrobia</i> ,																	
<i>Acidimicrobiaceae</i> (19)	1.4	1.7	1.8	1.6	1.8	1.7	1.8	1.6	1.6	1.0	1.3	1.4	0.8	0.9	0.8	0.9	1.4
<i>Iamiaceae</i> (9)	0.2	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h						30h									
	Treatment:		C1	C2	C3	A	Ce	G	Ga	X	C1	C2	C3	A	Ce	G	Ga	X
Relative Abundance (%)																		
Unassigned <i>Acidimicrobiales</i> (51)			2.3	2.3	2.5	2.5	2.5	2.5	2.2	2.7	1.2	1.7	1.7	0.9	1.0	1.2	1.1	1.4
<i>Actinobacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Catenulisporaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycobacteriaceae</i> (9)			1.5	1.3	1.3	1.3	1.1	1.3	1.3	1.3	0.6	0.7	0.9	0.4	0.4	0.5	0.4	0.7
<i>Nocardiaceae</i> (8)			2.0	2.0	2.3	2.2	2.1	2.3	2.0	2.0	0.9	1.3	1.4	0.8	0.6	0.7	0.8	1.1
<i>Acidothermaceae</i> (5)			0.2	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1
<i>Cryptosporangiaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (4)			0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
<i>Geodermatophilaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nakamurellaceae</i> (2)			0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sporichthyaceae</i> (3)			0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1
Unassigned <i>Frankiales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glycomycetaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Kineosporiaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellulomonadaceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.2
<i>Demequinaceae</i> (1)			0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (2)			0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0.2
<i>Microbacteriaceae</i> (5)			0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Micrococcaceae</i> (2)			0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1
<i>Promicromonosporaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micromonosporaceae</i> (25)			1.4	1.3	1.5	1.3	1.2	1.4	1.2	1.1	0.7	0.9	1.0	0.5	0.6	0.5	0.5	0.9
<i>Nocardioideae</i> (27)			2.0	2.0	2.3	2.2	2.1	2.3	2.0	2.0	0.9	1.3	1.4	0.8	0.6	0.7	0.8	1.1
<i>Propionibacteriaceae</i> (9)			0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.2
<i>Pseudonocardaceae</i> (10)			0.5	0.4	0.6	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.3
<i>Streptomycetaceae</i> (3)			0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.5	0.3	0.3	0.4	0.2	0.1	0.3	0.2	0.4
<i>Streptosporangiaceae</i> (5)			0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1
<i>Thermomonosporaceae</i> (8)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Unassigned <i>Actinobacteria</i> (3)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1
<i>Coriobacteria</i> ,																		
<i>Coriobacteriaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>MB-A2-108</i> (22)			2.2	2.2	2.5	2.4	2.3	2.6	2.3	2.3	1.2	1.4	1.6	0.8	0.8	0.9	0.9	1.2
<i>Rubrobacteria</i> ,																		
<i>Rubrobacteriaceae</i> (3)			0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Thermoleophila</i> ,																		
<i>Gaiellaceae</i> (7)			2.3	2.6	2.8	2.4	2.5	2.5	2.4	2.6	1.3	1.6	1.9	1.0	1.0	1.0	1.2	1.6
Unassigned <i>Gaiellaceae</i> (37)			3.8	4.0	4.2	3.9	4.0	3.8	3.9	4.4	1.9	2.8	2.7	1.5	1.3	1.6	1.6	2.2
<i>Conexibacteraceae</i> (2)			0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solirubrobacteraceae</i> (3)			0.5	0.5	0.7	0.5	0.5	0.6	0.5	0.5	0.2	0.4	0.5	0.2	0.2	0.2	0.2	0.3
Unassigned <i>Solirubrobacterales</i> (58)			2.7	2.8	3.4	2.9	2.8	2.9	2.5	2.9	1.3	1.9	1.9	1.1	1.1	1.2	0.9	1.3
Unassigned <i>Actinobacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Armatimonadetes,</b>																		
<i>Armatimonadia</i> ,																		
Unassigned <i>Armatimonadales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chthonomonadetes</i> ,																		
<i>Chthonomonadaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthonomonadales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Armatimonadetes</i> (9)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes,</b>																		
<i>Bacteroidia</i> ,																		
<i>Prolixibacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagaceae</i> (24)			0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1
<i>Flavobacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteriaceae</i> (9)			0.3	0.2	0.1	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1
Unassigned <i>Flavobacteriales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteria</i> (40)			0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.2
<i>Lentimicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saprospiraceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h						30h								
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C1	C2	C3	A	Ce	G	Ga	X
Relative Abundance (%)																	
<i>Fibrobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Fibrobacterales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Firmicutes,</b>																	
<i>Bacilli,</i>																	
<i>Alicyclobacillaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bacillaceae</i> (22)	1.1	1.0	1.0	1.1	1.1	1.2	1.1	1.0	5.1	3.5	3.8	5.3	2.0	5.1	3.9	4.4	
<i>Paenibacillaceae</i> (39)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.6	0.8	1.0	0.5	0.2	0.6	0.4	0.9	
<i>Pasteuriaceae</i> (1)	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	
<i>Planococcaceae</i> (9)	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	
<i>Sporolactobacillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Staphylococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Thermoactinomycetaceae</i> (11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Bacilli</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Clostridia,</i>																	
<i>Caldicoprobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Clostridiaceae</i> (25) [GPT-6, GPT-8]	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.2	12	8.5	8.1	6.2	4.0	5.2	7.5	6.2	
<i>Defluviitaleaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Eubacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Gracilibacteraceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Heliobacteriaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Lachnospiraceae</i> (14)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.8	1.1	1.0	1.0	0.3	1.0	0.7	1.0	
<i>Peptococcaceae</i> (7)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.4	0.2	0.3	0.0	0.2	
<i>Peptostreptococcaceae</i> (7) [GPT-2]	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	23	13	11	9.3	4.3	8.1	17	9.4	
<i>Ruminococcaceae</i> (31)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	
Unassigned <i>Clostridiales</i> (19)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	
<i>Halanaerobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Thermoanaerobacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Clostridia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Erysipelotrichia,</i>																	
<i>Erysipelotrichaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Limnochordia,</i>																	
<i>Limnochordaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Limnochordales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Negativicutes,</i>																	
<i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Veillonellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Fusobacteria,</b>																	
<i>Fusobacteriales,</i>																	
<i>Fusobacteriaceae</i> (1) [GPT-7]	0.1	0.2	0.1	0.3	0.2	0.2	0.4	0.4	6.2	3.4	4.4	21	23	11	7.1	5.1	
<b>Gemmatimonadetes,</b>																	
<i>Gemmatimonadetes,</i>																	
<i>Gemmatimonadaceae</i> (32)	0.9	0.9	0.9	1.0	0.8	0.9	1.0	0.9	0.4	0.6	0.6	0.3	0.3	0.4	0.3	0.5	
Unassigned <i>Gemmatimonadetes</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Latescibacteria,</b>																	
Unassigned <i>Latescibacteria</i> (20)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Nitrospirae,</b>																	
<i>Nitrospira,</i>																	
<i>Nitrospiraceae</i> (8)	0.2	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	
Unassigned <i>Nitrospirales</i> (12)	1.2	1.4	1.4	1.2	1.3	1.2	1.3	1.4	0.6	0.7	0.8	0.4	0.4	0.5	0.5	0.6	
<b>Planctomycetes,</b>																	
<i>OM190</i> (33)	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	
<i>Phycisphaerae,</i>																	
<i>Phycisphaeraceae</i> (26)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tepidisphaeraceae</i> (57)	0.9	1.0	1.0	0.9	1.0	1.1	1.2	1.0	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.6	
Unassigned <i>Phycisphaerae</i> (15)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	
<i>Planctomycetacia,</i>																	
<i>Planctomycetaceae</i> (539)	5.9	6.1	6.4	6.4	6.7	6.8	6.6	6.4	3.4	4.3	4.3	2.8	3.9	3.6	3.2	4.9	
Unassigned <i>Planctomycetes</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Proteobacteria,</b>																	



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h						30h									
	Treatment:		C1	C2	C3	A	Ce	G	Ga	X	C1	C2	C3	A	Ce	G	Ga	X
Relative Abundance (%)																		
<b>Gammaproteobacteria,</b>																		
<i>Acidiferrobacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Aeromonadaceae</i> (4) [GPT-1]			5.0	5.2	4.2	6.8	5.2	6.1	7.2	8.6	3.3	2.5	3.7	15	20	20	3.3	6.4
<i>Shewanellaceae</i> (1)			0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.5	0.6	0.7	0.1	0.0	0.2	0.3	0.9
<i>Cellvibrionaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Halieaceae</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (5) [GPT-4, GPT-5]			0.5	0.5	0.5	0.8	0.6	0.8	0.9	0.9	1.2	0.7	1.0	5.3	7.1	4.5	22	4.1
<i>Coxiellaceae</i> (11)			0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Legionellaceae</i> (11)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Methylococcaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Thiotrichales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthomonadaceae</i> (9)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1
Unassigned <i>Xanthomonadales</i> (24)			0.7	0.7	0.8	0.7	0.5	0.7	0.5	0.6	0.2	0.3	0.4	0.2	0.2	0.2	0.2	0.3
Unassigned <i>Gammaproteobacteria</i> (16)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1
Unassigned <i>Proteobacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Saccharibacteria,</b>																		
Unassigned <i>Saccharibacteria</i> (18)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia,</b>																		
<i>Spirochaetes,</i>																		
<i>Brevinemataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spirochaetaceae</i> (1)			0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Tectomicrobia</i> (12)			0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.5	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2
<b>Tenericutes,</b>																		
<i>Mollicutes,</i>																		
<i>Haloplasmataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (7) [GPT-3]			23	21	17	18	20	17	18	16	13	19	16	9.2	12	13	8.9	18
Unassigned <i>Entomoplasmatales</i> (2)			0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haloplasmataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>TM6_Dependentiae,</b>																		
Unassigned <i>TM6_Dependentiae</i> (8)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia,</b>																		
<i>OPB35 soil group</i> (64)			0.6	0.5	0.5	0.6	0.5	0.6	0.6	0.6	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2
<i>Spartobacteria,</i>																		
<i>Spartobacteria</i> (34)			0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
<i>Chthoniobacteraceae</i> (5)			1.2	1.4	1.3	1.4	1.3	1.4	1.7	1.6	1.0	1.4	1.0	0.8	1.0	1.0	0.8	1.1
<i>Xiphinematobacteraceae</i> (31)			2.8	3.5	3.4	3.6	3.6	3.8	3.5	3.4	2.1	2.4	2.4	1.7	2.0	1.7	2.0	2.4
<i>Opitutae,</i>																		
<i>Opitutaceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobiae,</i>																		
<i>Verrucomicrobiaceae</i> (24)			0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Verrucomicrobiales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Verrucomicrobia</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archaeae</b>																		
<b>Euryarchaeota,</b>																		
<i>Methanomicrobia,</i>																		
<i>Methanosarcinaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Thaumarchaeota,</b>																		
Unassigned <i>Thaumarchaeota</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

## (B) 16S rRNA

Phyla, Class, Family <sup>b</sup>	Sampling Time: 0h								30h																			
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C1	C2	C3	A1	A2	A3	Ce1	Ce2	Ce3	G1	G2	G3	Ga1	Ga2	Ga3	X1	X2	X3	
Relative Abundance (%)																												
<b>Acidobacteria,</b>																												
<i>Acidobacteria,</i>																												
<i>Acidobacteriaceae</i> (12)	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
<i>Blastocatellia,</i>																												
<i>Blastocatellaceae</i> (14)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Acidobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holophagae,</i>																												
Unassigned <i>Holophagae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solibacteres,</i>																												
<i>Solibacteraceae</i> (26)	0.7	0.6	0.7	0.6	0.7	0.6	0.5	0.6	0.2	0.4	0.4	0.5	0.2	0.2	0.3	0.2	0.3	0.4	0.4	0.4	0.2	0.5	0.3	0.3	0.4	0.4	0.4	0.4
<i>Subgroup_2</i> (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
<i>Subgroup_5</i> (6)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
<i>Subgroup_6</i> (83)	0.9	0.9	1.3	1.0	1.0	1.0	0.9	0.8	0.3	0.4	0.6	0.7	0.5	0.2	0.3	0.3	0.3	0.7	0.4	0.7	0.2	0.4	0.4	0.5	0.5	0.5	0.4	0.4
<i>Subgroup_11</i> (6)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_15</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_17</i> (17)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Subgroup_18</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_22</i> (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_25</i> (6)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>																												
<i>Acidimicrobia,</i>																												
<i>Acidimicrobiaceae</i> (19)	1.2	0.9	1.1	1.1	1.1	1.3	1.0	1.0	0.6	1.0	1.1	1.1	0.6	0.5	0.9	0.7	0.7	0.7	0.8	0.7	0.5	0.8	0.8	0.8	0.9	0.9	0.9	0.9
<i>Iamiaeae</i> (9)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Acidimicrobiales</i> (51)	1.2	0.9	1.2	0.8	0.9	0.9	0.9	1.0	0.6	0.8	1.0	1.3	0.9	0.4	0.7	0.7	0.7	0.9	0.8	1.2	0.5	1.0	1.1	1.1	1.1	1.3	0.9	0.9
<i>Actinobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Catenulisporaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycobacteriaceae</i> (9)	0.3	0.2	0.5	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.3	0.3	0.3	0.2	0.2	0.2
<i>Nocardiaceae</i> (8)	0.8	0.7	1.2	0.7	0.6	0.6	0.6	0.6	0.5	0.6	0.7	0.9	0.6	0.3	0.4	0.3	0.5	0.8	0.5	0.7	0.4	0.5	0.6	0.6	0.6	0.8	0.7	0.7
<i>Acidothermaceae</i> (5)	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Cryptosporangiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (4)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0
<i>Geodermatophilaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nakamurellaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sporichthyaceae</i> (3)	0.1	0.0	0.2	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Frankiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glycomycetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Kineosporiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Cellulomonadaceae</i> (1)	0.2	0.1	0.3	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.3	0.3	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.3	0.1	0.2	0.2	0.3	0.3	0.3	0.4	0.4
<i>Demequinaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (2)	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2
<i>Microbacteriaceae</i> (5)	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Micrococcaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Promicromonosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micromonosporaceae</i> (25)	0.7	0.8	1.3	0.8	0.7	0.8	0.8	0.7	0.5	0.9	1.0	0.9	0.6	0.4	0.7	0.5	0.6	1.1	0.7	0.9	0.4	0.8	0.9	0.9	1.1	0.9	0.9	0.9
<i>Nocardioidaceae</i> (27)	0.8	0.7	1.2	0.7	0.6	0.6	0.6	0.6	0.5	0.6	0.7	0.9	0.6	0.3	0.4	0.3	0.5	0.8	0.5	0.7	0.4	0.5	0.6	0.6	0.6	0.8	0.7	0.7
<i>Propionibacteriaceae</i> (9)	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.3	0.1	0.0	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1
<i>Pseudonocardaceae</i> (10)	0.2	0.2	0.4	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.5	0.3	0.1	0.2	0.2	0.2	0.4	0.2	0.3	0.1	0.2	0.3	0.3	0.2	0.2	0.2	0.2
<i>Streptomycetaceae</i> (3)	0.3	0.3	0.5	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.2	0.1	0.3	0.2	0.4	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3
<i>Streptosporangiaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermomonosporaceae</i> (8)	0.0	0.0	0.1	0.0	0.0	0.0	0.0																					

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h							30h																	
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C 1	C 2	C 3	A 1	A 2	A 3	Ce 1	Ce 2	Ce 3	G 1	G 2	G 3	Ga 1	Ga 2	Ga 3	X 1	X 2	X 3
		Relative Abundance (%)																									
<i>Coriobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>MB-A2-108</i> (22)	0.2	0.2	0.4	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.3	0.3	0.3	0.3	0.3
<i>Rubrobacteria</i>																											
<i>Rubrobacteriaceae</i> (3)	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	
<i>Thermoleophilla</i>																											
<i>Gaiellaceae</i> (7)	0.3	0.4	0.5	0.4	0.3	0.2	0.4	0.4	0.2	0.2	0.3	0.3	0.4	0.4	0.1	0.2	0.2	0.2	0.4	0.3	0.5	0.1	0.4	0.3	0.3	0.4	0.3
Unassigned <i>Gaiellaceae</i> (37)	0.5	0.5	0.9	0.5	0.5	0.5	0.5	0.7	0.4	0.3	0.6	0.7	0.5	0.2	0.4	0.3	0.4	0.7	0.5	0.7	0.3	0.5	0.6	0.7	0.7	0.5	
<i>Conexibacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Patulibacteraceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Solirubrobacteraceae</i> (3)	0.3	0.4	0.8	0.4	0.3	0.3	0.4	0.5	0.2	0.2	0.2	0.4	0.5	0.5	0.2	0.2	0.2	0.2	0.5	0.4	0.6	0.2	0.4	0.3	0.4	0.6	0.4
Unassigned <i>Solirubrobacterales</i> (58)	0.4	0.3	0.7	0.4	0.4	0.4	0.3	0.4	0.2	0.2	0.4	0.4	0.4	0.3	0.2	0.2	0.2	0.2	0.4	0.3	0.5	0.2	0.4	0.4	0.4	0.5	0.5
Unassigned <i>Actinobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Armatimonadetes</i>																											
<i>Armatimonadia</i>																											
Unassigned <i>Armatimonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Chthonomonadetes</i>																											
<i>Chthonomonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Chthonomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Armatimonadetes</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bacteroidetes</i>																											
<i>Bacteroidia</i>																											
<i>Prolixibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Cytophagia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Cytophagaceae</i> (24)	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<i>Flavobacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Flavobacteriaceae</i> (9)	0.7	0.5	0.2	0.6	0.5	0.7	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	
Unassigned <i>Flavobacteriales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Sphingobacteria</i> (40)	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2
<i>Lentimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Saprospiraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Sphingobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Sphingobacteriales</i> (18)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Bacteroidetes</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>BJ-169</i>																											
Unassigned <i>BJ-169</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>BRC1</i>																											
Unassigned <i>BRC1</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Chlamydiae</i>																											
<i>Chlamydiae</i>																											
<i>Chlamydiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Parachlamydiaceae</i> (70)	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1
<i>Simkaniaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Chlamydiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Chlorobi</i>																											
<i>Chlorobia</i>																											
Unassigned <i>Chlorobiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Chloroflexi</i>																											
<i>Anaerolineae</i>																											
<i>Anaerolineaceae</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Caldilineae</i>																											
<i>Caldilineaceae</i> (7)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	
<i>Chloroflexia</i>																											
<i>Roseiflexaceae</i> (5)	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2
Unassigned <i>Kallotenuales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Gltt-GS-136</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>JG30-KF-CM66</i> (11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>JG37-AG-4</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>KD4-96</i> (12)	0.5	0.3	0.5	0.5	0.4	0.4	0.3	0.3	0.2	0.3	0.4	0.5	0.4	0.4	0.1	0.3	0.3	0.2	0.3	0.3	0.5	0.2	0.3	0.5	0.4	0.5	0.4
<i>Ktedonobacteria</i>																											



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h							30h																	
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C 1	C 2	C 3	A 1	A 2	A 3	Ce 1	Ce 2	Ce 3	G 1	G 2	G 3	Ga 1	Ga 2	Ga 3	X 1	X 2	X 3
Relative Abundance (%)																											
<i>Erysipelotrichia</i> , <i>Erysipelotrichaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Limnochordia</i> , <i>Limnochordaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Limnochordales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Negativicutes</i> , <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Veillonellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria</b> , <i>Fusobacteriales</i> , <i>Fusobacteriaceae</i> (1) [GPT-7]	0.4	0.4	0.2	0.6	0.5	0.4	0.8	1.0	2.7	0.7	0.5	1.6	2.4	13	5.4	3.4	5.1	1.1	1.2	0.8	2.5	0.8	1.0	0.6	0.5	0.3	
<b>Gemmatimonadetes</b> , <i>Gemmatimonadetes</i> , <i>Gemmatimonadaceae</i> (32)	0.2	0.1	0.5	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.3	0.3	0.2	0.1	0.1	0.2	0.1	0.3	0.3	0.3	0.1	0.2	0.3	0.3	0.3	0.2	
Unassigned <i>Gemmatimonadetes</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Latescibacteria</b> , Unassigned <i>Latescibacteria</i> (20)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Nitrospirae</b> , <i>Nitrospira</i> , <i>Nitrospiraceae</i> (8)	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	
Unassigned <i>Nitrospirales</i> (12)	0.5	0.3	0.7	0.5	0.3	0.3	0.4	0.5	0.4	0.4	0.4	0.9	0.9	0.2	0.4	0.4	0.4	0.6	0.6	0.9	0.3	0.4	0.8	0.7	0.9	0.6	
<b>Planctomycetes</b> , OM190 (33)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	
<i>Phycisphaerae</i> , <i>Phycisphaeraceae</i> (26)	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tepidisphaeraceae</i> (57)	0.7	0.7	0.7	0.6	0.7	0.8	0.7	0.7	0.2	0.4	0.3	0.6	0.4	0.2	0.4	0.3	0.3	0.4	0.5	0.5	0.3	0.3	0.5	0.7	0.9	0.4	
Unassigned <i>Phycisphaerae</i> (15)	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	
<i>Planctomycetacia</i> , <i>Planctomycetaceae</i> (539)	11	11	11	9.3	12	12	11	9.7	8.0	13	10	9.6	6.6	6.1	10	8.7	8.7	11	11	9.2	6.9	13	12	13	12	13	
Unassigned <i>Planctomycetes</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Proteobacteria</b> , <i>Alphaproteobacteria</i> , <i>Caulobacteraceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
<i>Hyphomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bradyrhizobiaceae</i> (5)	1.2	1.5	1.7	1.7	1.4	1.3	1.3	1.7	0.7	1.0	1.5	1.6	0.9	0.6	1.0	0.6	0.9	1.6	1.0	1.1	0.4	1.3	1.0	1.0	1.0	1.1	
<i>Hyphomicrobiaceae</i> (6)	0.3	0.3	0.4	0.3	0.4	0.3	0.2	0.2	0.3	0.3	0.5	0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.4	0.3	0.3	0.3	0.3	
<i>Methylobacteriaceae</i> (4)	0.2	0.2	0.3	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	
<i>Phyllobacteriaceae</i> (3)	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.1	0.4	0.3	0.3	0.2	0.3	
<i>Rhizobiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Rhodobiaceae</i> (2)	0.5	0.5	0.5	0.5	0.5	0.6	0.3	0.2	0.5	0.8	0.7	0.4	0.2	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.6	0.5	0.5	0.4	0.6	
<i>Roseiarcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Xanthobacteraceae</i> (6)	1.7	2.1	3.0	2.1	1.9	2.3	1.9	1.2	1.3	2.2	2.5	2.0	1.4	0.8	1.4	1.2	1.3	2.1	1.9	1.8	0.8	2.0	1.9	1.8	2.0	2.2	
Unassigned <i>Rhizobiales</i> (14)	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.1	0.3	0.2	0.3	0.1	0.3	0.3	0.2	0.2	0.3	
<i>Rhodobacteraceae</i> (6)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<i>Acetobacteraceae</i> (10)	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.1	0.2	0.2	0.3	0.2	0.2	
<i>Rhodospirillaceae</i> (14)	0.4	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.6	0.6	0.7	0.7	0.5	0.3	0.4	0.3	0.4	0.7	0.5	0.5	0.2	0.7	0.6	0.5	0.5	0.6	
Unassigned <i>Rhodospirillales</i> (32)	0.8	0.7	0.9	0.8	0.7	0.6	0.7	0.7	0.6	0.8	1.0	1.1	0.9	0.4	0.6	0.5	0.6	1.1	0.8	0.9	0.4	0.9	1.0	0.8	0.8	0.9	
<i>Anaplasmataceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Holosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mitochondria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Rickettsiales</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Sphingomonadaceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Sphingomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Betaproteobacteria</i> , <i>Alcaligenaceae</i> (2)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	
<i>Burkholderiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0h							30h																	
	Treatment:	C1	C2	C3	A	Ce	G	Ga	X	C 1	C 2	C 3	A 1	A 2	A 3	Ce 1	Ce 2	Ce 3	G 1	G 2	G 3	Ga 1	Ga 2	Ga 3	X 1	X 2	X 3
Relative Abundance (%)																											
<i>Mollicutes</i> ,																											
<i>Haloplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (7) [GPT-3]	46	48	35	43	48	47	41	39	30	47	41	24	27	22	35	41	36	28	32	29	19	37	32	34	36	43	
Unassigned <i>Entomoplasmatales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Haloplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>TM6_Dependentiae</i></b>																											
Unassigned <i>TM6_Dependentiae</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Verrucomicrobia</i></b> ,																											
OPB35 soil group (64)	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.5	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.3	0.2	
<i>Spartobacteria</i> ,																											
<i>Spartobacteria</i> (34)	0.3	0.2	0.1	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.2	
<i>Chthoniobacteraceae</i> (5)	1.6	1.4	0.7	1.8	1.8	1.6	1.5	1.2	2.2	2.6	2.6	2.0	1.0	1.4	2.1	1.4	1.3	1.1	1.6	1.1	1.0	2.7	1.7	1.8	1.4	1.6	
<i>Xiphinematobacteraceae</i> (31)	0.5	0.5	0.5	0.5	0.6	0.4	0.4	0.4	0.2	0.2	0.4	0.5	0.3	0.2	0.4	0.4	0.3	0.3	0.4	0.2	0.4	0.5	0.6	0.4	0.4		
<i>Opitutae</i> ,																											
<i>Opitutaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Opitutae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Verrucomicrobiae</i> ,																											
<i>Verrucomicrobiaceae</i> (24)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	
Unassigned <i>Verrucomicrobiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Verrucomicrobia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Archaea</b>																											
<b><i>Euryarchaeota</i></b> ,																											
<i>Methanomicrobia</i> ,																											
<i>Methanosarcinaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b><i>Thaumarchaeota</i></b> ,																											
Unassigned <i>Thaumarchaeota</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

<sup>a</sup>Samples of the three replicates of the 16S rRNA gene (A) control treatment at 0 h and 30 h, 16S rRNA (B) control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Treatments: C, unsupplemented control; A, *N*-acetylglucosamine; Ce, cellobiose; G, glucose; Ga, galacturonic acid; X, xylose. Table modified and used with permission from Zeibich *et al.*, 2019a.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes from Figure 33 are bold and in brackets.

**Table A4.** Summary of all detected families in the yeast cell lysate experiment based on 16S rRNA gene and 16S rRNA analysis (Section 3.2.2).<sup>a</sup>

Phylum, Class, Family <sup>c</sup>	Sampling Time:		0 h		6 h		12 h		20 h	
	Treatment <sup>b</sup> :	D	R	C.R	L.R	C.R	L.R	C.R	L.R	
Relative Abundance (%)										
<b><i>Actinobacteria</i></b> ,										
<i>Acidimicrobia</i> ,										
<i>Acidimicrobiaceae</i> (8)		2.1	1.2	1.7	0.6	1.5	0.6	1.3	0.7	
TM214 group (4)		1.0	0.3	0.4	0.2	0.6	0.2	0.3	0.2	
Unassigned <i>Acidimicrobiales</i> (6)		1.0	0.4	0.6	0.3	0.8	0.2	0.5	0.2	
<i>Actinobacteria</i> ,										
<i>Mycobacteriaceae</i> (2)		0.5	0.2	0.2	0.1	0.2	0.0	0.1	0.0	
<i>Nakamurellaceae</i> (1)		0.3	0.3	0.3	0.1	0.4	0.1	0.2	0.1	
<i>Cellulomonadaceae</i> (2)		0.1	0.5	0.6	0.3	0.7	0.2	0.5	0.2	
<i>Intrasporangiaceae</i> (1)		0.4	0.3	0.5	0.1	0.6	0.1	0.4	0.1	
<i>Microbacteriaceae</i> (5)		1.7	0.7	1.0	0.4	1.6	0.3	0.9	0.4	
<i>Micrococccaceae</i> (1)		0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.1	
<i>Micromonosporaceae</i> (1)		0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	

Phylum, Class, Family <sup>c</sup>	Sampling Time:		6 h		12 h		20 h		
	Treatment <sup>b</sup> :	0 h		C.R	L.R	C.R	L.R	C.R	L.R
		D	R						
					Relative Abundance (%)				
<i>Nocardioideaceae</i> (7)		1.7	0.6	0.7	0.3	0.9	0.3	0.7	0.3
<i>Propionibacteriaceae</i> (3)		0.5	0.3	0.4	0.1	0.4	0.1	0.3	0.1
<i>Streptomyceaceae</i> (2)		0.6	0.4	0.3	0.1	0.4	0.1	0.2	0.1
Unassigned <i>Actinobacteria</i> (4)		2.2	1.0	1.1	0.4	1.1	0.4	0.8	0.4
<i>Thermoleophilia</i> ,									
<i>Gaiellaceae</i> (2)		0.8	0.2	0.2	0.1	0.3	0.1	0.1	0.1
Unassigned <i>Gaiellaceae</i> (5)		1.9	0.3	0.3	0.1	0.5	0.1	0.3	0.1
<i>Patulibacteraceae</i> (1)		0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.1
<i>Solirubrobacteraceae</i> (2)		0.2	0.4	0.4	0.1	0.5	0.1	0.3	0.1
Unassigned <i>Solirubrobacteraceae</i> (3)		1.1	0.2	0.2	0.1	0.3	0.1	0.2	0.1
<b>Chloroflexi</b> ,									
Unassigned <i>Chloroflexi</i> (3)		1.5	0.5	0.5	0.2	0.7	0.2	0.4	0.3
<b>Firmicutes</b> ,									
<i>Bacilli</i> ,									
<i>Bacillaceae</i> (4)		1.5	2.0	1.3	1.9	1.6	0.4	1.0	0.5
<i>Paenibacillaceae</i> (1)		0.0	0.0	0.0	0.1	0.2	0.1	0.1	0.2
<i>Clostridia</i> ,									
<i>Clostridiaceae</i> (10) [CL8, CL10, CL18, CL15]		0.0	0.0	0.1	1.0	0.6	4.3	0.7	5.9
<i>Lachnospiraceae</i> (5) [CL6]		0.0	0.0	0.0	0.0	0.0	0.5	0.0	3.4
<i>Peptostreptococcaceae</i> (14) [CL2, CL5]		0.2	0.4	4.6	37	12.1	38.6	17	28
<b>Proteobacteria</b> ,									
<i>Alphaproteobacteria</i> ,									
<i>Bradyrhizobiaceae</i> (1)		1.2	1.8	1.7	0.6	1.2	0.4	1.0	0.4
<i>Hyphomicrobiaceae</i> (2)		0.2	0.2	0.3	0.1	0.3	0.1	0.2	0.1
<i>Methylobacteriaceae</i> (2)		0.2	0.3	0.2	0.1	0.3	0.1	0.2	0.1
<i>Phyllobacteriaceae</i> (1)		0.3	0.4	0.5	0.2	0.5	0.1	0.3	0.1
<i>Rhodobiaceae</i> (1)		1.4	0.6	0.7	0.3	0.7	0.2	0.6	0.2
<i>Xanthobacteraceae</i> (4)		2.5	1.1	1.3	0.4	1.6	0.4	1.2	0.4
<i>Acetobacteraceae</i> (1)		0.0	0.2	0.1	0.1	0.1	0.0	0.1	0.1
<i>Rhodobacteraceae</i> (3)		0.2	0.8	0.7	0.3	0.7	0.3	0.4	0.3
<i>Rhodospirillaceae</i> (1)		0.0	0.1	0.1	0.0	0.1	0.0	0.1	0.0
Unassigned <i>Rhodospirillales</i> (6)		0.0	0.8	1.2	1.2	0.5	1.6	0.4	1.1
<i>Deltaproteobacteria</i> ,									
<i>Nitrospinaceae</i> (3)		0.2	0.8	0.8	0.3	0.8	0.3	0.7	0.3
<i>Sorangineae</i> (2)		0.1	0.3	0.3	0.1	0.4	0.1	0.2	0.1
Unassigned <i>Deltaproteobacteria</i> (2)		0.6	0.5	0.3	0.2	0.4	0.1	0.3	0.1
<i>Gammaproteobacteria</i> ,									
<i>Aeromonadaceae</i> (3) [CL7]		5.8	3.0	0.4	10	0.4	8.9	0.6	7.2
<i>Shewanellaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Enterobacteriaceae</i> (7) [CL4]		0.6	0.4	0.2	3.5	0.3	8.6	0.6	15
<b>Planctomycetes</b> ,									
<i>Planctomycetacia</i> ,									
<i>Planctomycetaceae</i> (13)		0.8	1.1	1.6	0.8	1.3	0.8	2.0	0.8
<b>Tenericutes</b> ,									
<i>Mollicutes</i> ,									
<i>Mycoplasmataceae</i> (4)		6.0	37	27	14	24	10	27	13
<b>Verrucomicrobia</b> ,									
<i>Spartobacteria</i> ,									
<i>Xiphinematobacteraceae</i> (2)		3.3	4.2	6.5	2.5	3.2	2.5	5.1	2.5
Unassigned <i>Chthoniobacteriales</i> (1)		1.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1

Phylum, Class, Family <sup>c</sup>	Sampling Time:		30 h							
	Treatment <sup>b</sup> :		D.C	D.L	C1.R	C2.R	C3.R	L1.R	L2.R	L3.R
Relative Abundance (%)										
<b>Actinobacteria,</b>										
<i>Acidimicrobia,</i>										
<i>Acidimicrobiaceae</i> (8)			1.4	1.3	2.0	1.9	1.5	0.7	1.2	0.9
TM214 group (4)			0.6	0.6	0.6	0.5	0.4	0.3	0.3	0.3
Unassigned <i>Acidimicrobiales</i> (6)			0.6	0.5	0.7	0.6	0.6	0.3	0.4	0.3
<i>Actinobacteria,</i>										
<i>Mycobacteriaceae</i> (2)			0.2	0.3	0.2	0.1	0.2	0.1	0.1	0.1
<i>Nakamurellaceae</i> (1)			0.1	0.2	0.4	0.4	0.3	0.3	0.2	0.1
<i>Cellulomonadaceae</i> (2)			0.2	0.1	0.6	0.7	0.6	0.3	0.3	0.3
<i>Intrasporangiaceae</i> (1)			0.3	0.3	0.5	0.4	0.4	0.2	0.2	0.2
<i>Microbacteriaceae</i> (5)			0.9	1.2	1.5	1.4	1.6	1.1	0.6	0.5
<i>Micrococcaceae</i> (1)			0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
<i>Micromonosporaceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Nocardioideae</i> (7)			1.0	1.0	0.9	0.9	0.7	0.3	0.4	0.3
<i>Propionibacteriaceae</i> (3)			0.2	0.2	0.4	0.4	0.4	0.2	0.2	0.2
<i>Streptomycetaceae</i> (2)			0.3	0.4	0.3	0.3	0.4	0.3	0.2	0.2
Unassigned <i>Actinobacteria</i> (4)			1.0	1.0	1.0	0.9	0.7	0.4	0.5	0.4
<i>Thermoleophilla,</i>										
<i>Gaiellaceae</i> (2)			0.4	0.4	0.2	0.2	0.2	0.1	0.1	0.1
Unassigned <i>Gaiellaceae</i> (5)			0.8	0.9	0.4	0.4	0.3	0.2	0.2	0.2
<i>Patulibacteraceae</i> (1)			0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1
<i>Solirubrobacteraceae</i> (2)			0.2	0.2	0.4	0.4	0.3	0.2	0.2	0.1
Unassigned <i>Solirubrobacteraceae</i> (3)			0.4	0.5	0.3	0.2	0.3	0.1	0.1	0.1
<b>Chloroflexi,</b>										
Unassigned <i>Chloroflexi</i> (3)			0.9	0.8	0.6	0.6	0.5	0.3	0.4	0.3
<b>Firmicutes,</b>										
<i>Bacilli,</i>										
<i>Bacillaceae</i> (4)			3.3	1.3	1.5	1.4	1.8	1.1	0.8	0.7
<i>Paenibacillaceae</i> (1)			0.5	0.1	0.1	0.2	0.1	0.1	0.1	0.1
<i>Clostridia,</i>										
<i>Clostridiaceae</i> (10) [CL8, CL10, CL18, CL15]			8.5	11	0.9	1.1	1.1	11	8.6	11
<i>Lachnospiraceae</i> (5) [CL6]			0.5	1.3	0.1	0.1	0.1	11	8.7	6.4
<i>Peptostreptococcaceae</i> (14) [CL2, CL5]			17	29	7.0	9.9	11	20	19	23
<b>Proteobacteria,</b>										
<i>Alphaproteobacteria,</i>										
<i>Bradyrhizobiaceae</i> (1)			0.5	0.6	0.9	0.6	0.8	0.3	0.4	0.5
<i>Hyphomicrobiaceae</i> (2)			0.1	0.2	0.3	0.3	0.2	0.1	0.2	0.1
<i>Methylobacteriaceae</i> (2)			0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1
<i>Phyllobacteriaceae</i> (1)			0.1	0.1	0.2	0.2	0.3	0.1	0.1	0.1
<i>Rhodobiaceae</i> (1)			0.7	0.8	0.7	0.6	0.7	0.3	0.5	0.3
<i>Xanthobacteraceae</i> (4)			1.0	1.1	1.4	1.6	1.1	0.8	0.6	0.5
<i>Acetobacteraceae</i> (1)			0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0
<i>Rhodobacteraceae</i> (3)			0.1	0.1	0.4	0.4	0.4	0.3	0.3	0.2
<i>Rhodospirillaceae</i> (1)			0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0
Unassigned <i>Rhodospirillales</i> (6)			0.5	0.4	0.2	1.5	1.4	0.7	0.6	0.7
<i>Deltaproteobacteria,</i>										
<i>Nitrospinaceae</i> (3)			0.1	0.1	0.5	0.5	0.2	0.1	0.2	0.2
<i>Sorangiiineae</i> (2)			0.1	0.1	0.4	0.5	0.3	0.2	0.2	0.1
Unassigned <i>Deltaproteobacteria</i> (2)			0.2	0.1	0.2	0.2	0.1	0.0	0.0	0.0
<i>Gammaproteobacteria,</i>										
<i>Aeromonadaceae</i> (3) [CL7]			4.8	0.2	0.1	0.2	0.1	1.3	0.9	1.2
<i>Shewanellaceae</i> (1)			2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (7) [CL4]			1.4	12	0.3	0.3	0.2	10	9.4	13
<b>Planctomycetes,</b>										
<i>Planctomycetacia,</i>										
<i>Planctomycetaceae</i> (13)			0.7	0.4	2.9	2.6	2.0	0.8	1.4	1.2
<b>Tenericutes,</b>										
<i>Mollicutes,</i>										
<i>Mycoplasmataceae</i> (4)			8.7	2.1	24	28	29	13	13	11
<b>Verrucomicrobia,</b>										

Phylum, Class, Family <sup>c</sup>	Sampling Time:	30 h							
	Treatment <sup>b</sup> :	D.C	D.L	C1.R	C2.R	C3.R	L1.R	L2.R	L3.R
Relative Abundance (%)									
<i>Spartobacteria</i> ,									
<i>Xiphinematobacteraceae</i> (2)		2.7	1.8	5.9	5.2	4.9	2.4	4.7	4.6
Unassigned <i>Chthoniobacteriales</i> (1)		0.9	0.2	0.1	0.1	0.1	0.0	0.0	0.0

<sup>a</sup>Listed are families that had at least one phylotype with  $\geq 1,000$  reads. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Abbreviations and treatments: D, 16S rRNA genes; R, 16S rRNA; C, unsupplemented control; L, *S.cerevisiae* lysate treatment. RNA or DNA samples of the three replicates were always pooled except for RNA samples at 30 h. Identification numbers (e.g., C1) indicate the respective replicates.

<sup>c</sup>The number of phylotypes with  $\geq 1,000$  reads are shown in parenthesis. Abundant responsive phylotypes from Figure 43 are bold and in brackets.

**Table A5.** Summary of all detected families in the protein and RNA experiment based on 16S rRNA gene and 16S rRNA analysis (Section 3.2.4).<sup>a</sup>

Phylum, Class, Family <sup>c</sup>	Sampling Time:	0 h						10 h			20 h		
	Treatment <sup>b</sup> :	p.D	p.R	C.D	C.R	r.D	r.R	p.R	C.R	r.R	p.R	C.R	r.R
Relative Abundance (%)													
<b>Actinobacteria</b> ,													
<i>Acidimicrobia</i> ,													
<i>Acidimicrobiaceae</i> (7)		1.2	0.8	1.2	1.0	1.2	1.0	0.7	1.1	1.0	0.5	1.1	0.7
Unassigned <i>Acidimicrobiales</i> (7)		1.3	0.8	1.3	0.7	1.3	0.8	0.7	1.0	0.8	0.5	0.9	0.7
<i>Actinobacteria</i> ,													
<i>Mycobacteriaceae</i> (2)		0.4	0.1	0.4	0.1	0.4	0.1	0.0	0.0	0.0	0.0	0.1	0.0
Unassigned <i>Corynebacteriales</i> (1)		0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0
<i>Cellulomonadaceae</i> (1)		0.1	0.3	0.1	0.3	0.1	0.3	0.3	0.4	0.3	0.2	0.4	0.3
<i>Intrasporangiaceae</i> (1)		0.4	0.2	0.4	0.3	0.4	0.2	0.3	0.3	0.3	0.2	0.3	0.3
<i>Microbacteriaceae</i> (5)		1.0	1.3	1.0	1.4	0.9	1.3	0.9	1.3	0.8	0.6	0.9	0.6
<i>Micrococcaceae</i> (1)		0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Micromonosporaceae</i> (2)		0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.1	0.2	0.2
<i>Nakamurellaceae</i> (1)		0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2
<i>Nocardiodaceae</i> (4)		0.8	0.4	0.8	0.4	0.8	0.4	0.3	0.5	0.4	0.2	0.4	0.3
<i>Propionibacteriaceae</i> (3)		1.0	0.4	0.9	0.4	0.9	0.5	0.3	0.5	0.4	0.3	0.5	0.3
<i>Pseudonocardiaceae</i> (3)		0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.7	0.6	0.3	0.6	0.5
<i>Streptomycetaceae</i> (2)		0.5	0.4	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3
DA023 group (13)		2.3	1.8	2.2	1.5	2.2	1.5	1.1	1.4	1.2	0.5	0.9	0.9
Unassigned <i>Holophagae</i> (1)		0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0
<i>Thermoleophila</i> ,													
<i>Gaiellaceae</i> (1)		0.5	0.1	0.5	0.1	0.5	0.2	0.2	0.2	0.2	0.1	0.2	0.2
Unassigned <i>Gaiellales</i> (8)		2.3	0.5	2.2	0.5	2.2	0.5	0.5	0.7	0.6	0.3	0.6	0.5
<i>Patulibacteraceae</i> (1)		0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Solirubrobacteraceae</i> (4)		0.8	1.0	0.9	1.0	0.9	1.0	0.8	1.2	0.9	0.5	1.1	0.8
Unassigned <i>Solirubrobacteriales</i> (6)		1.2	0.3	1.3	0.3	1.3	0.3	0.2	0.3	0.3	0.2	0.3	0.2
Unassigned <i>Actinobacteria</i> (5)		1.6	0.6	1.7	0.6	1.6	0.7	0.6	0.9	0.7	0.4	0.7	0.6
<b>Chloroflexi</b> ,													
<i>Caldilineae</i> ,													
<i>Caldilineaceae</i> (1)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chloroflexia</i> ,													
<i>Chloroflexaceae</i> (1)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
KD4-96 group (4)		2.6	0.9	2.7	0.9	2.8	0.9	0.7	1.0	0.7	0.5	0.7	0.6
Unassigned <i>Thermomicrobia</i> (2)		0.3	0.1	0.4	0.1	0.4	0.1	0.1	0.1	0.1	0.0	0.1	0.1
Unassigned <i>Chloroflexi</i> (3)		0.5	0.2	0.6	0.2	0.5	0.2	0.1	0.2	0.2	0.1	0.2	0.1

Phylum, Class, Family <sup>c</sup>	Sampling Time:		0 h				10 h			20 h				
	Treatment <sup>b</sup> :		p.D	p.R	C.D	C.R	r.D	r.R	p.R	C.R	r.R	p.R	C.R	r.R
Relative Abundance (%)														
<b>Firmicutes</b>														
<i>Bacilli</i>														
<i>Bacillaceae</i> (6)			0.6	0.6	0.6	0.6	0.6	0.8	0.4	0.6	1.1	0.3	0.5	0.8
<i>Clostridia</i>														
<i>Clostridiaceae</i> (9) [PR7, PR12]			0.2	0.3	0.2	0.2	0.2	0.2	3.4	0.5	0.5	7.8	0.8	0.6
<i>Lachnospiraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Peptostreptococcaceae</i> (4) [PR2, PR8]			0.2	0.2	0.2	0.1	0.1	0.1	17	1.4	0.8	26	4.1	0.9
Unassigned <i>Clostridiales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
<b>Fusobacteria</b>														
<i>Fusobacteria</i>														
<i>Fusobacteriaceae</i> (1) [PR6]			0.0	0.0	0.1	0.0	0.0	0.0	0.5	0.1	0.2	4.0	0.8	0.1
<b>Nitrospirae</b>														
<i>Nitrospira</i>														
<i>Nitrospiraceae</i> (1)			0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0
Unassigned <i>Nitrospirales</i> (3)			0.5	0.3	0.5	0.3	0.5	0.3	0.2	0.3	0.3	0.2	0.3	0.4
<b>Planctomycetes</b>														
<i>Planctomycetacia</i>														
<i>Planctomycetaceae</i> (64)			5.9	8.8	6.1	9.0	6.8	9.1	7.0	9.3	9.5	5.3	10	10
Unassigned <i>Phycisphaerae</i> (7)			1.2	1.0	1.0	0.8	1.3	0.9	0.6	0.7	1.0	0.3	0.7	0.8
Unassigned <i>Planctomycetales</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0
<b>Proteobacteria</b>														
<i>Alphaproteobacteria</i>														
<i>Bradyrhizobiaceae</i> (1)			0.7	1.2	0.6	1.1	0.6	1.2	1.0	1.3	1.0	0.7	1.2	0.7
<i>Hyphomicrobiaceae</i> (2)			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1
<i>Methylobacteriaceae</i> (2)			0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.3	0.2
<i>Phyllobacteriaceae</i> (1)			0.2	0.3	0.2	0.3	0.2	0.3	0.3	0.4	0.3	0.2	0.3	0.2
<i>Rhodobiaceae</i> (1)			1.1	0.4	1.0	0.4	1.0	0.4	0.3	0.4	0.3	0.2	0.4	0.2
<i>Xanthobacteraceae</i> (7)			2.4	1.4	2.4	1.2	2.2	1.4	1.1	1.5	1.2	0.8	1.5	0.9
Unassigned <i>Rhizobiales</i> (1)			0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0
<i>Rhodobacteraceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Rhodospirillaceae</i> (7)			0.6	1.3	0.6	1.2	0.6	1.3	1.3	1.7	1.4	0.8	1.5	1.2
Unassigned <i>Rhodospirillales</i> (4)			0.4	0.6	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.3	0.6	0.5
<i>Betaproteobacteria</i>														
<i>Alcaligenaceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Comamonadaceae</i> (1)			0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Deltaproteobacteria</i>														
<i>Nitrospinaceae</i> (2)			0.1	0.7	0.1	0.6	0.1	0.6	0.6	0.6	0.6	0.3	0.5	0.5
<i>Cystobacteraceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.1
<i>Nannocystineae</i> (1)			0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1
<i>Sorangineae</i> (1)			0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1
Unassigned <i>Deltaproteobacteria</i> (2)			0.5	0.4	0.5	0.4	0.5	0.4	0.3	0.5	0.4	0.3	0.4	0.3
<i>Gammaproteobacteria</i>														
<i>Aeromonadaceae</i> (4) [PR3]			0.4	0.1	0.3	0.1	0.3	0.8	1.3	0.3	12	0.7	0.3	25
<i>Shewanellaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (3) [PR33]			0.1	0.3	0.1	0.1	0.1	0.2	0.6	0.1	0.4	0.7	0.2	0.7
<i>Sinobacteraceae</i> (1)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0
<b>Tenericutes</b>														
<i>Mollicutes</i>														
<i>Mycoplasmataceae</i> (3)			13	29	13	32	11	28	20	26	21	20	28	15
Unassigned <i>Mollicutes</i> (2)			0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1
<b>Verrucomicrobia</b>														
<i>Spartobacteria</i>														
<i>Chthoniobacteraceae</i> (1)			0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.0
<i>Xiphinematobacteraceae</i> (2)			4.5	5.0	4.9	4.4	5.5	5.3	5.0	5.6	4.2	3.7	4.6	2.8
Unassigned <i>Chthoniobacteriales</i> (8)			3.7	0.7	4.3	0.8	4.6	0.8	0.5	0.6	0.5	0.3	0.5	0.4
<i>Verrucomicrobiae</i>														
<i>Verrucomicrobiaceae</i> (1)			0.2	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0

Phylum, Class, Family <sup>c</sup>	Sampling Time:				30 h								
	Treatment <sup>b</sup> :	p.D	p1.R	p2.R	p3.R	C.D	C1.R	C2.R	C3.R	r.D	r1.R	r2.R	r3.R
Relative Abundance (%) <sup>b</sup>													
<b>Actinobacteria,</b>													
<i>Acidimicrobiia,</i>													
<i>Acidimicrobiaceae</i> (7)	0.6	0.6	0.6	0.6	1.2	1.0	1.0	0.8	0.6	0.8	0.7	1.1	
Unassigned <i>Acidimicrobiales</i> (7)	0.5	0.4	0.4	0.5	1.3	0.9	0.8	0.7	0.5	0.7	0.6	0.8	
<i>Actinobacteria,</i>													
<i>Mycobacteriaceae</i> (2)	0.3	0.0	0.0	0.0	0.3	0.1	0.1	0.1	0.2	0.1	0.0	0.0	
Unassigned <i>Corynebacteriales</i> (1)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
<i>Cellulomonadaceae</i> (1)	0.1	0.2	0.2	0.2	0.3	0.4	0.4	0.3	0.1	0.3	0.3	0.5	
<i>Intrasporangiaceae</i> (1)	0.2	0.1	0.2	0.2	0.4	0.3	0.3	0.2	0.2	0.2	0.2	0.2	
<i>Microbacteriaceae</i> (5)	0.4	0.5	0.6	0.6	0.9	1.1	0.8	0.8	0.4	1.0	0.7	1.2	
<i>Micrococcaceae</i> (1)	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<i>Micromonosporaceae</i> (2)	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.2	0.1	0.2	0.2	0.2	
<i>Nakamurellaceae</i> (1)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.0	0.2	0.1	0.2	
<i>Nocardioideaceae</i> (4)	0.3	0.2	0.2	0.2	0.7	0.3	0.5	0.3	0.3	0.3	0.3	0.4	
<i>Propionibacteriaceae</i> (3)	0.4	0.3	0.3	0.3	1.0	0.4	0.5	0.4	0.4	0.4	0.3	0.5	
<i>Pseudonocardiaceae</i> (3)	0.2	0.3	0.4	0.3	0.5	0.5	0.6	0.5	0.3	0.5	0.5	0.7	
<i>Streptomycetaceae</i> (2)	0.2	0.1	0.2	0.2	0.5	0.3	0.4	0.3	0.2	0.3	0.3	0.4	
DA023 group (13)	0.8	0.2	0.4	0.4	1.8	0.7	0.8	0.4	0.6	0.6	0.7	0.6	
Unassigned <i>Holophagae</i> (1)	0.1	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.1	
<i>Thermoleophilia,</i>													
<i>Gaiellaceae</i> (1)	0.2	0.1	0.1	0.1	0.5	0.2	0.2	0.2	0.2	0.2	0.1	0.2	
Unassigned <i>Gaiellales</i> (8)	1.0	0.3	0.3	0.4	2.4	0.7	0.6	0.6	1.0	0.6	0.5	0.7	
<i>Patulibacteraceae</i> (1)	0.1	0.0	0.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<i>Solirubrobacteraceae</i> (4)	0.3	0.4	0.5	0.5	0.8	1.0	1.0	0.8	0.3	0.8	0.7	1.1	
Unassigned <i>Solirubrobacterales</i> (6)	0.5	0.2	0.2	0.2	1.1	0.3	0.3	0.3	0.5	0.2	0.2	0.3	
Unassigned <i>Actinobacteria</i> (5)	0.7	0.4	0.4	0.4	1.6	0.7	0.8	0.7	0.7	0.6	0.5	0.8	
<b>Chloroflexi,</b>													
<i>Caldilineae,</i>													
<i>Caldilineaceae</i> (1)	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	
<i>Chloroflexia,</i>													
<i>Chloroflexaceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	
KD4-96 group (4)	1.1	0.4	0.4	0.5	2.9	0.9	0.7	0.6	1.1	0.7	0.7	0.9	
Unassigned <i>Thermomicrobia</i> (2)	0.2	0.0	0.1	0.1	0.4	0.1	0.1	0.1	0.2	0.1	0.1	0.1	
Unassigned <i>Chloroflexi</i> (3)	0.2	0.1	0.1	0.1	0.6	0.2	0.2	0.1	0.2	0.1	0.2	0.2	
<b>Firmicutes</b>													
<i>Bacilli</i>													
<i>Bacillaceae</i> (6)	1.7	0.4	0.3	0.4	1.0	0.6	0.7	0.7	1.1	1.2	1.2	1.8	
<i>Clostridia</i>													
<i>Clostridiaceae</i> (9) [PR7, PR12]	14	11	13	12	1.5	1.4	1.1	1.3	0.8	1.8	1.7	3.0	
<i>Lachnospiraceae</i> (1)	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.7	1.1	1.1	2.2	
<i>Peptostreptococcaceae</i> (4) [PR2, PR8]	18	18	21	23	3.4	4.1	3.6	3.5	1.3	2.2	2.4	4.5	
Unassigned <i>Clostridiales</i> (1)	0.2	0.5	0.4	0.6	0.1	0.3	0.4	0.4	0.0	0.0	0.0	0.0	
<b>Fusobacteria,</b>													
<i>Fusobacteriaceae</i> (1) [PR6]	12	11	7.9	6.3	1.4	0.6	1.6	0.5	0.6	0.2	0.2	0.2	
<b>Nitrospirae,</b>													
<i>Nitrospira,</i>													
<i>Nitrospiraceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	
Unassigned <i>Nitrospirales</i> (3)	0.2	0.1	0.1	0.2	0.6	0.2	0.3	0.2	0.2	0.3	0.2	0.3	
<b>Planctomycetes</b>													
<i>Planctomycetacia,</i>													
<i>Planctomycetaceae</i> (64)	3.2	5.7	5.6	6.1	6.6	9.2	9.5	6.4	3.4	8.1	8.3	10.6	
Unassigned <i>Phycisphaerae</i> (7)	0.6	0.3	0.3	0.3	1.0	0.5	0.5	0.3	0.6	0.5	0.5	0.5	
Unassigned <i>Planctomycetales</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	
<b>Proteobacteria,</b>													
<i>Alphaproteobacteria,</i>													
<i>Bradyrhizobiaceae</i> (1)	0.3	0.5	0.6	0.6	0.6	1.0	1.2	0.9	0.2	0.6	0.8	1.0	

Phylum, Class, Family <sup>c</sup>	Sampling Time:		30 h										
	Treatment <sup>b</sup> :		p.D	p1.R	p2.R	p3.R	C.D	C1.R	C2.R	C3.R	r.D	r1.R	r2.R
Relative Abundance (%) <sup>b</sup>													
<i>Hyphomicrobiaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2
<i>Methylobacteriaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.2
<i>Phyllobacteriaceae</i> (1)	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.1	0.2	0.2	0.3
<i>Rhodobiaceae</i> (1)	0.4	0.3	0.2	0.2	0.9	0.5	0.5	0.3	0.3	0.3	0.2	0.2	0.4
<i>Xanthobacteraceae</i> (7)	0.9	0.7	0.7	0.8	2.1	1.5	1.6	1.2	0.8	0.9	0.9	0.9	1.2
Unassigned <i>Rhizobiales</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Rhodobacteraceae</i> (1)	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Rhodospirillaceae</i> (7)	0.3	0.8	0.8	0.8	0.6	1.2	1.4	1.1	0.3	1.1	1.0	1.6	1.6
Unassigned <i>Rhodospirillales</i> (4)	0.2	0.3	0.4	0.3	0.5	0.5	0.6	0.4	0.2	0.5	0.4	0.5	0.5
<i>Betaproteobacteria</i> ,													
<i>Alcaligenaceae</i> (1)	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1
<i>Comamonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1
<i>Deltaproteobacteria</i> ,													
<i>Nitrospinaceae</i> (2)	0.1	0.2	0.3	0.2	0.1	0.3	0.5	0.3	0.1	0.4	0.5	0.5	0.5
<i>Cystobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1
<i>Nannocystineae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1
<i>Sorangineae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1
Unassigned <i>Deltaproteobacteria</i> (2)	0.2	0.2	0.2	0.2	0.4	0.3	0.3	0.3	0.2	0.3	0.3	0.4	0.4
<i>Gammaproteobacteria</i> ,													
<i>Aeromonadaceae</i> (4) [PR3]	4.4	0.3	0.4	0.3	2.2	0.1	0.3	0.1	45	18	21	22	22
<i>Shewanellaceae</i> (1)	0.7	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.5	0.2	0.2	0.2	0.2
<i>Enterobacteriaceae</i> (3) [PR33]	0.8	0.4	0.4	0.3	0.2	0.1	0.1	0.1	4.0	2.3	2.6	3.4	3.4
<i>Sinobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tenericutes</i> ,													
<i>Mollicutes</i> ,													
<i>Mycoplasmataceae</i> (3)	6.8	18.0	16	16	7.8	32	28	19	6.5	22	18	30	30
Unassigned <i>Mollicutes</i> (2)	0.1	0.2	0.2	0.2	0.1	0.3	0.3	0.2	0.1	0.2	0.2	0.3	0.3
<i>Verrucomicrobia</i> ,													
<i>Spartobacteria</i> ,													
<i>Chthoniobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1
<i>Xiphinematobacteraceae</i> (2)	2.8	4.6	4.5	3.7	4.3	4.4	5.2	3.9	3.0	3.0	5.1	6.4	6.4
Unassigned <i>Chthoniobacterales</i> (8)	2.1	0.3	0.3	0.2	3.4	0.6	0.6	0.3	1.8	0.4	0.3	0.5	0.5
<i>Verrucomicrobiae</i> ,													
<i>Verrucomicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>Listed are families that had at least one phylotype with  $\geq 1,000$  reads. Table modified and used with permission from Zeibich *et al.*, 2018.

<sup>b</sup>Abbreviations and treatments: D, 16S rRNA genes; R, 16S rRNA; C, unsupplemented control; r, RNA treatment; p, protein treatment. RNA or DNA samples of the three replicates were always pooled except for RNA samples at 30 h. Identification numbers (e.g., C1) indicate the respective replicates.

<sup>c</sup>The number of phylotypes with  $\geq 1,000$  reads are shown in parenthesis. Abundant responsive phylotypes from Figure 43 are bold and in brackets.

**Table A6.** Summary of all detected families in the amino acid experiment based on 16S rRNA gene (A) and 16S rRNA (B) analysis (Sectio 3.2.7).<sup>a</sup>

**(A) 16S rRNA genes**

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						10 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Relative Abundance (%)																
<i>Acidobacteria</i> ,																
<i>Acidobacteria</i> ,																
<i>Acidobacteriaceae</i> (16)	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1
<i>Blastocatellia</i> ,																



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						10 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
	Relative Abundance (%)															
<i>Gaiellaceae</i> (11)			2.3	2.3	2.3	2.2	2.3	2.4	2.3	2.1	1.2	1.5	1.8	1.9	2.4	2.4
Unassigned <i>Gaiellales</i> (72)			4.7	4.7	4.8	4.4	4.5	4.6	4.5	3.1	2.5	2.9	3.8	3.8	4.5	4.4
<i>Conexibacteraceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Parviterribacteraceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (15)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
<i>Solirubrobacteraceae</i> (15)			0.6	0.6	0.7	0.6	0.6	0.6	0.7	0.7	0.4	0.5	0.6	0.7	0.8	0.8
Unassigned <i>Solirubrobacterales</i> (26)			0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.4	0.6	0.7	0.7	0.8	0.8
Unassigned <i>Thermoleophilia</i> (67)			2.4	2.5	2.3	2.4	2.3	2.2	2.6	2.0	1.5	1.8	2.1	2.1	2.4	2.2
Unassigned <i>Actinobacteria</i> (34)			2.1	2.0	2.1	1.9	1.9	2.1	2.0	1.1	1.0	1.2	1.5	1.5	1.7	1.7
<b>Armatimonadetes,</b>																
<i>Armatimonadia,</i>																
Unassigned <i>Armatimonadales</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chthonomonadaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthonomonadales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Armatimonadetes</i> (21)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes,</b>																
<i>Bacteroidia,</i>																
<i>Prolixibacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagaceae</i> (33)			0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flammeovirgaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteria,</i>																
<i>Flavobacteriaceae</i> (21)			0.6	0.6	0.6	0.6	0.5	0.4	0.5	0.7	0.3	0.4	0.5	0.5	0.2	0.3
<i>Sphingobacteriia,</i>																
<i>Chitinophagaceae</i> (48)			0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Lentimicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saprospiraceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriia</i> (10)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacteroidetes</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BJ-169,</b>																
Unassigned <i>BJ-169</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1,</b>																
Unassigned <i>BRC1</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae,</b>																
<i>Chlamydiae,</i>																
<i>Chlamydiaceae</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Parachlamydiaceae</i> (154)			0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
<i>Simkaniaceae</i> (11)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Waddliaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chlamydiales</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlorobi,</b>																
<i>Chlorobia,</i>																
Unassigned <i>Chlorobia</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi,</b>																
<i>Anaerolineae,</i>																
<i>Anaerolineaceae</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ardenticatenia</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Caldilineae,</i>																
<i>Caldilineaceae</i> (15)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chloroflexia,</i>																
<i>Roseiflexaceae</i> (8)			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.1	0.1	0.2	0.2	0.2	0.2
Unassigned <i>Chloroflexia</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ktedonobacteria,</i>																
<i>Ktedonobacterales,</i>																
<i>Ktedonobacteraceae</i> (9)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermosporotrichaceae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacterales</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteria</i> (18)			0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						10 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
	Relative Abundance (%)															
Unassigned Clostridiales (17)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Thermoanaerobacterales (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Clostridia (34)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erysipelotrichia</i> ,																
<i>Erysipelotrichaceae</i> (18)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Limnochordia</i> ,																
<i>Limnochordaceae</i> (23)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Limnochordales</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Negativicutes</i> ,																
<i>Veillonellaceae</i> (20)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Selenomonadales</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Firmicutes</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria</b> ,																
<i>Fusobacteria</i> ,																
<i>Fusobacteriaceae</i> (10) [GPT-5]			10	11	11	12	13	14	14	21	38	36	22	19	14	17
<i>Leptotrichiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Gemmatimonadetes</b> ,																
<i>Gemmatimonadetes</i> ,																
<i>Gemmatimonadaceae</i> (37)			0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.4	0.4	0.4	0.5	0.5
<i>Longimicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Gemmatimonadetes</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Hydrogenedentes</b> ,																
Unassigned <i>Hydrogenedentes</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Latescibacteria</b> ,																
Unassigned <i>Latescibacteria</i> (30)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Nitrospira</b> ,																
<i>Nitrospira</i> ,																
<i>Nitrospiraceae</i> (8)			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2
Unassigned <i>Nitrospira</i> (18)			0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.5	0.3	0.4	0.4	0.5	0.6	0.5
<b>Parcubacteria</b> ,																
Unassigned <i>Parcubacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Planctomycetes</b> ,																
<i>Phycisphaerae</i> ,																
<i>Phycisphaeraceae</i> (38)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidisphaeraceae</i> (82)			0.8	0.6	0.7	0.6	0.6	0.6	0.6	0.2	0.3	0.4	0.5	0.5	0.6	0.5
Unassigned <i>Phycisphaerales</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Phycisphaerae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planctomycetacia</i> ,																
<i>Planctomycetaceae</i> (733)			4.5	4.0	4.1	3.8	4.0	4.0	3.7	2.2	2.3	2.5	3.0	3.3	3.9	3.7
Unassigned <i>Planctomycetes</i> (82)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1
<b>Proteobacteria</b> ,																
<i>Alphaproteobacteria</i> ,																
<i>Caulobacteraceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Beijerinckiaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (5)			1.2	1.2	1.1	1.1	1.0	1.2	1.0	0.7	0.6	0.7	0.9	0.9	1.1	1.2
<i>Hyphomicrobiaceae</i> (8)			0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.1	0.2	0.2	0.3	0.2	0.3	0.3
<i>Methylobacteriaceae</i> (4)			0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
<i>Methylocystaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phyllobacteriaceae</i> (3)			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2
<i>Rhizobiaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobiaceae</i> (2)			1.3	1.2	1.2	1.3	1.3	1.2	1.1	0.9	0.6	0.8	1.0	0.9	1.2	1.1
<i>Roseiarcaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (9)			3.7	3.4	3.4	3.4	3.3	3.2	3.1	2.1	1.7	2.0	2.5	2.6	3.2	3.1
Unassigned <i>Rhizobiales</i> (19)			0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.1	0.2	0.2	0.2	0.3	0.2
<i>Rhodobacteraceae</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Acetobacteraceae</i> (15)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Rhodospirillaceae</i> (19)			0.5	0.4	0.4	0.3	0.3	0.4	0.4	0.2	0.2	0.2	0.3	0.3	0.3	0.3
Unassigned <i>Rhodospirillales</i> (25)			0.6	0.6	0.6	0.5	0.5	0.6	0.5	0.5	0.3	0.4	0.5	0.5	0.6	0.6
<i>Anaplasmataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holospiraceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						10 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
	Relative Abundance (%)															
<b>RsaHf231</b>																
Unassigned <i>RsaHf231</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Saccharibacteria</b>																
Unassigned <i>Saccharibacteria</i> (78)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Spirochaetae</b>																
<i>Spirochaetes</i>																
<i>Spirochaetaeaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Brevinemataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Synergistetes</b>																
<i>Synergistia</i>																
<i>Synergistaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia</b>																
Unassigned <i>Tectomicrobia</i> (21)			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2
<b>Tenericutes</b>																
<i>Mollicutes</i>																
Unassigned <i>Entomoplasmatales</i> (3)			0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1
<i>Haloplasmataceae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (19)			13	13	12	14	13	9.7	12	10	7.5	7.7	8.9	10	11	10
<b>TM6_Dependentiae</b>																
Unassigned <i>TM6_Dependentiae</i> (40)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia</b>																
Unassigned OPB35 soil group (87)			0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2
<i>Opitutae</i>																
<i>Opitutaceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria</i>																
<i>Chthoniobacteraceae</i> (46)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Chthoniobacterales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DA101 soil group (28)			3.4	3.5	3.4	3.6	3.3	3.3	3.3	2.1	1.7	2.3	2.6	2.7	3.1	3.0
<i>Xiphinematobacteraceae</i> (9)			1.2	1.1	1.1	1.1	1.0	1.2	1.1	0.5	0.7	0.8	0.9	1.0	1.1	1.1
Unassigned <i>Spartobacteria</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobiae</i>																
<i>Verrucomicrobiaceae</i> (22)			0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Unassigned <i>Verrucomicrobia</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archeae</b>																
<b>Thaumarchaeota</b>																
Unassigned <i>Thaumarchaeota</i> (9)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	Sampling Time:		22 h						30 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
	Relative Abundance (%)															
<b>Acidobacteria</b>																
<i>Acidobacteria</i>																
<i>Acidobacteriaceae</i> (16)			0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatellia</i>																
<i>Blastocatellaceae</i> (23)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Holophagae</i>																
Unassigned <i>Holophagae</i> (12)			0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Solibacteres</i>																
<i>Solibacteraceae</i> (34)			0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Subgroup_11 (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (16)			0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1
Subgroup_22 (16)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (9)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (10)			0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1



Phyla, Class, Family <sup>b</sup>	Sampling Time:		22 h						30 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
Relative Abundance (%)																
<i>Armatimonadia</i> ,																
Unassigned <i>Armatimonadales</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chthonomonadaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthonomonadales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Armatimonadetes</i> (21)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes</b> ,																
<i>Bacteroidia</i> ,																
<i>Prolixibacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagaceae</i> (33)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flammeovirgaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteria</i> ,																
<i>Flavobacteriaceae</i> (21)			0.7	0.3	0.2	0.6	0.6	0.1	0.2	0.8	0.3	0.2	0.4	0.5	0.1	0.1
<i>Sphingobacteria</i> ,																
<i>Chitinophagaceae</i> (48)			0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Lentimicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saprosiraceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriia</i> (10)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacteroidetes</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacteroidetes</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BJ-169</b> ,																
Unassigned <i>BJ-169</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1</b> ,																
Unassigned <i>BRC1</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae</b> ,																
<i>Chlamydiae</i> ,																
<i>Chlamydiaceae</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Parachlamydiaceae</i> (154)			0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Simkaniaceae</i> (11)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Waddliaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chlamydiales</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlorobi</b> ,																
<i>Chlorobia</i> ,																
Unassigned <i>Chlorobia</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi</b> ,																
<i>Anaerolineae</i> ,																
<i>Anaerolineaceae</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ardenticatenia</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Caldilineae</i> ,																
<i>Caldilineaceae</i> (15)			0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chloroflexia</i> ,																
<i>Roseiflexaceae</i> (8)			0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1
Unassigned <i>Chloroflexia</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ktedonobacteria</i> ,																
<i>Ktedonobacteriales</i> ,																
<i>Ktedonobacteraceae</i> (9)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermosporotrichaceae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteriales</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteria</i> (18)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Thermomicrobia</i> ,																
<i>Thermomicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Thermomicrobia</i> (56)			0.3	0.2	0.2	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.2	0.3	0.3	0.3
Unassigned <i>Chloroflexi</i> (111)			1.9	1.0	0.9	1.1	1.4	2.0	1.9	1.7	1.1	1.0	1.1	1.3	1.8	1.5
<b>Cyanobacteria</b> ,																
<i>Chloroplast</i> ,																
Unassigned <i>Chloroplast</i> (34)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyanobacteria</i> ,																
Unassigned <i>Cyanobacteria</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gastranaerophilales</i> ,																



Phyla, Class, Family <sup>b</sup>	Sampling Time:		22 h						30 h							
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
			Relative Abundance (%)													
Unassigned <i>Selenomonadales</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Firmicutes</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria,</b>																
<i>Fusobacteriia,</i>																
<i>Fusobacteriaceae</i> (10) [GPT-5]			21	39	44	30	25	17	22	18	33	39	23	20	14	23
<i>Leptotrichiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Gemmatimonadetes,</b>																
<i>Gemmatimonadetes,</i>																
<i>Gemmatimonadaceae</i> (37)			0.5	0.2	0.2	0.3	0.3	0.5	0.4	0.4	0.2	0.3	0.3	0.4	0.5	0.3
<i>Longimicrobiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Gemmatimonadetes</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Hydrogenedentes,</b>																
Unassigned <i>Hydrogenedentes</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Latescibacteria,</b>																
Unassigned <i>Latescibacteria</i> (30)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Nitrospirae,</b>																
<i>Nitrospira,</i>																
<i>Nitrospiraceae</i> (8)			0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Nitrospira</i> (18)			0.5	0.3	0.3	0.4	0.4	0.5	0.6	0.4	0.3	0.3	0.3	0.4	0.4	0.4
<b>Parcubacteria,</b>																
Unassigned <i>Parcubacteria</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Planctomycetes,</b>																
<i>Phycisphaerae,</i>																
<i>Phycisphaeraceae</i> (38)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidisphaeraceae</i> (82)			0.5	0.3	0.3	0.3	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.4	0.5	0.4
Unassigned <i>Phycisphaerales</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Phycisphaerae</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planctomycetacia,</i>																
<i>Planctomycetaceae</i> (733)			3.1	1.7	1.9	2.1	2.5	2.7	3.3	3.1	2.0	1.8	2.1	2.7	3.4	2.7
Unassigned <i>Planctomycetes</i> (82)			0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Proteobacteria,</b>																
<i>Alphaproteobacteria,</i>																
<i>Caulobacteraceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Beijerinckiaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (5)			0.8	0.5	0.5	0.6	0.7	0.9	0.9	0.8	0.5	0.5	0.6	0.7	0.8	0.8
<i>Hyphomicrobiaceae</i> (8)			0.3	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.1	0.2	0.2	0.3	0.2
<i>Methylobacteriaceae</i> (4)			0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Methylocystaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phyllobacteriaceae</i> (3)			0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2
<i>Rhizobiaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobiaceae</i> (2)			1.1	0.5	0.5	0.5	0.6	1.0	1.0	0.9	0.6	0.6	0.7	0.8	1.1	0.9
<i>Roseiarcaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (9)			2.7	1.4	1.4	1.4	1.9	2.3	2.6	2.4	1.6	1.4	1.7	2.0	2.7	2.2
Unassigned <i>Rhizobiales</i> (19)			0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
<i>Rhodobacteraceae</i> (6)			0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Acetobacteraceae</i> (15)			0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Rhodospirillaceae</i> (19)			0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
Unassigned <i>Rhodospirillales</i> (25)			0.5	0.2	0.2	0.3	0.4	0.5	0.5	0.5	0.3	0.2	0.2	0.4	0.5	0.4
<i>Anaplasmataceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holosporaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mitochondria</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rickettsiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Rickettsiales</i> (7)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Alphaproteobacteria</i> (19)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Betaproteobacteria,</i>																
<i>Alcaligenaceae</i> (2)			0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
<i>Burkholderiaceae</i> (10)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Phyla, Class, Family <sup>b</sup>	Sampling Time:		22 h						30 h						
	Treatment:		C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly
	Relative Abundance (%)														
<i>Synergistaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia</b> , Unassigned <i>Tectomicrobia</i> (21)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<b>Tenericutes</b> , <i>Mollicutes</i> , Unassigned <i>Entomoplasmatales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haloplasmataceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (19)	9.7	7.3	5.4	5.9	6.5	8.4	8.0	8.6	7.6	5.9	7.2	7.2	10	9.5	
<b>TM6_Dependentiae</b> , Unassigned <i>TM6_Dependentiae</i> (40)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia</b> , Unassigned OPB35 soil group (87)	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Opitutae</i> , <i>Opitutaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria</i> , <i>Chthoniobacteraceae</i> (46)	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0
Unassigned <i>Chthoniobacterales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DA101 soil group (28)	2.8	1.5	1.5	1.7	2.0	2.6	2.7	2.6	1.7	1.7	1.6	2.1	2.5	2.1	
<i>Xiphinematobacteraceae</i> (9)	0.9	0.6	0.6	0.7	0.9	0.9	1.0	1.0	0.7	0.6	0.7	0.9	1.0	0.9	
Unassigned <i>Spartobacteria</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobiae</b> , <i>Verrucomicrobiaceae</i> (22)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Verrucomicrobia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archeae</b> <b>Thaumarchaeota</b> , Unassigned <i>Thaumarchaeota</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

## (B) 16S rRNA

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h							10 h						22 h									
	Treatment:		C1	C2	C3	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly	C	CAA	Glu	Asp	Thr	Ala/Gly	Val/Gly
	Relative Abundance (%)																								
<b>Acidobacteria</b> , <i>Acidobacteria</i> , <i>Acidobacteriaceae</i> (16)	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0
<i>Blastocatellia</i> , <i>Blastocatellaceae</i> (23)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Holophagae</i> , Unassigned <i>Holophagae</i> (12)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Solibacteres</i> , <i>Solibacteraceae</i> (34)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
Subgroup_11 (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (16)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (16)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (10)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Subgroup_6 (113)	1.0	0.8	0.9	0.8	0.8	0.9	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.7	0.8	1.0	0.8	0.8	0.6	0.5	0.5	0.6	0.6	0.8	0.8
Unassigned <i>Acidobacteria</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria</b> , <i>Acidimicrobia</i> , <i>Acidimicrobiaceae</i> (30)	1.3	1.2	1.1	1.2	1.2	1.3	1.0	1.2	1.2	1.2	1.2	0.8	1.0	1.1	1.2	1.3	1.4	1.1	0.6	0.7	0.9	1.0	1.2	1.3	
<i>Iamiaceae</i> (12)	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	
Unassigned <i>Acidimicrobiales</i> (70)	1.2	1.1	1.1	1.3	1.3	1.3	1.4	1.5	1.5	1.4	0.9	1.2	1.3	1.2	1.3	1.5	1.2	0.8	0.8	1.1	1.1	1.5	1.5		

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h							10 h						22 h										
	Treatment:	C1	C2	C3	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly	C	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly	C	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly		
																									Relative Abundance (%)	
<i>Actinobacteria</i> ,																										
<i>Actinospicaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Catenulisporaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mycobacteriaceae</i> (12)		0.5	0.4	0.5	0.5	0.5	0.7	0.7	0.8	0.7	0.7	0.4	0.5	0.6	0.5	0.5	0.6	0.4	0.3	0.3	0.3	0.4	0.6	0.6	0.6	0.6
<i>Nocardiaceae</i> (12)		0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
<i>Acidothermaceae</i> (10)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
<i>Cryptosporangiaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (5)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
<i>Geodermatophilaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nakamurellaceae</i> (5)		0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
<i>Sporichthyaceae</i> (6)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Glycomycetaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Kineosporiaceae</i> (5)		0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Beutenbergiaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Brevibacteriaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellulomonadaceae</i> (1)		0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
<i>Demequinaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Dermabacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Dermacoccaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (4)		0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.3	0.3
<i>Microbacteriaceae</i> (15)		0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Micrococcaceae</i> (4)		0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
<i>Promicromonosporaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sanguibacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micromonosporaceae</i> (44)		1.1	0.9	0.9	1.0	1.0	1.3	0.9	1.1	1.1	1.3	0.8	0.9	1.0	1.0	1.1	1.3	1.0	0.7	0.6	0.7	0.8	1.4	1.3	1.3	1.3
<i>Nocardiodiaceae</i> (41)		1.3	1.1	1.1	1.3	1.2	1.4	1.1	1.3	1.3	1.2	0.8	1.1	1.2	1.1	1.3	1.4	1.0	0.7	0.6	0.9	0.9	1.2	1.3	1.3	1.3
<i>Propionibacteriaceae</i> (11)		0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
<i>Pseudonocardiaceae</i> (20)		0.8	0.7	0.7	0.6	0.6	0.7	0.5	0.7	0.6	0.7	0.4	0.5	0.6	0.6	0.7	0.7	0.5	0.4	0.4	0.5	0.6	0.6	0.7	0.7	0.7
<i>Streptomyces</i> (5)		0.5	0.4	0.4	0.5	0.4	0.5	0.4	0.4	0.5	0.5	0.3	0.4	0.4	0.4	0.5	0.5	0.5	0.4	0.3	0.3	0.3	0.5	0.5	0.5	0.5
<i>Nocardiopepsaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Streptosporangiaceae</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<i>Thermomonosporaceae</i> (15)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Frankiales</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Actinobacteria</i> (2)		0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
<i>Coriobacteria</i> ,																										
<i>Coriobacteriaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rubrobacteria</i> ,																										
<i>Rubrobacteriaceae</i> (6)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Thermoleophilia</i> ,																										
<i>Gaiellaceae</i> (11)		0.8	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.5	0.7	0.8	0.7	0.8	0.9	0.8	0.5	0.5	0.6	0.6	0.7	0.9	0.9	0.9
Unassigned <i>Gaiellales</i> (72)		1.4	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4	1.5	0.9	1.2	1.4	1.2	1.5	1.6	1.3	0.9	0.9	1.1	1.1	1.4	1.5	1.5	1.5
<i>Conexibacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Parviterribacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (15)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solirubrobacteraceae</i> (15)		1.0	1.0	0.9	1.0	0.9	1.0	1.0	1.1	1.0	1.0	0.7	0.9	1.0	1.1	1.2	1.3	1.0	0.7	0.7	0.8	0.8	1.0	1.1	1.1	1.1
Unassigned <i>Solirubrobacterales</i> (26)		0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.5	0.4	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.4
Unassigned <i>Thermoleophilia</i> (67)		0.5	0.4	0.4	0.4	0.5	0.6	0.3	0.4	0.5	0.5	0.3	0.5	0.5	0.5	0.6	0.6	0.6	0.3	0.3	0.3	0.4	0.6	0.6	0.5	0.5
Unassigned <i>Actinobacteria</i> (34)		0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.3	0.4	0.5	0.4	0.5	0.5	0.4	0.3	0.2	0.3	0.4	0.5	0.5	0.5	0.5
<i>Armatimonadetes</i> ,																										
<i>Armatimonadia</i> ,																										
Unassigned <i>Armatimonadales</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chthonomonadaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthonomonadales</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Armatimonadetes</i> (21)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0





Phyla, Class, Family <sup>b</sup>	Sampling Time:										10 h						22 h								
	Treatment:										Relative Abundance (%)						Relative Abundance (%)								
	C1	C2	C3	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly		C	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly		C	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly
<i>Fusobacteriaceae</i> (10) [GPT-5]	8.6	13	13	9.6	13	10	12	10	10	20	33	29	20	19	13	13	20	33	34	25	22	12	15		
<i>Leptotrichiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Gemmatimonadetes,</b>																									
<i>Gemmatimonadetes,</i>																									
<i>Gemmatimonadaceae</i> (37)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.2	0.2	0.3	0.3	0.3		
<i>Longimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Gemmatimonadetes</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Hydrogenedentes,</b>																									
Unassigned <i>Hydrogenedentes</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Latescibacteria,</b>																									
Unassigned <i>Latescibacteria</i> (30)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Nitrospirae,</b>																									
<i>Nitrospira,</i>																									
<i>Nitrospiraceae</i> (8)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2		
Unassigned <i>Nitrospira</i> (18)	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.5	0.3	0.4	0.4	0.4	0.5	0.6	0.5	0.3	0.4	0.3	0.4	0.6	0.5		
<b>Parcubacteria,</b>																									
Unassigned <i>Parcubacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Planctomycetes,</b>																									
<i>Phycisphaerae,</i>																									
<i>Phycisphaeraceae</i> (38)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Tepidisphaeraceae</i> (82)	0.6	0.6	0.5	0.5	0.6	0.6	0.7	0.6	0.5	0.5	0.3	0.4	0.5	0.5	0.7	0.6	0.5	0.2	0.3	0.4	0.4	0.6	0.5		
Unassigned <i>Phycisphaerales</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Phycisphaerae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Planctomycetacia,</i>																									
<i>Planctomycetaceae</i> (733)	8.1	8.1	7.3	7.5	8.0	7.8	6.5	7.2	8.0	8.5	4.9	6.4	6.6	7.7	10	9.1	8.1	4.6	4.6	5.5	7.3	10	9.3		
Unassigned <i>Planctomycetes</i> (82)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1		
<b>Proteobacteria,</b>																									
<i>Alphaproteobacteria,</i>																									
<i>Caulobacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Beijerinckiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Bradyrhizobiaceae</i> (5)	2.0	1.7	1.7	1.6	1.6	1.7	0.9	1.3	1.4	1.0	1.1	1.2	1.1	1.4	1.6	1.6	1.5	1.0	0.8	0.9	1.4	1.5	1.5		
<i>Hyphomicrobiaceae</i> (8)	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2		
<i>Methylobacteriaceae</i> (4)	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2		
<i>Methylocystaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Phyllobacteriaceae</i> (3)	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.2	0.2	0.2	0.2		
<i>Rhizobiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Rhodobiaceae</i> (2)	0.3	0.3	0.2	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.2	0.2	0.4	0.3		
<i>Roseiarcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Xanthobacteraceae</i> (9)	1.3	1.1	1.1	1.2	1.1	1.3	1.2	1.1	1.3	1.0	0.8	1.0	1.1	1.0	1.4	1.3	1.1	0.8	0.7	0.9	0.9	1.4	1.4		
Unassigned <i>Rhizobiales</i> (19)	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.3		
<i>Rhodobacteraceae</i> (6)	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1		
<i>Acetobacteraceae</i> (15)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2		
<i>Rhodospirillaceae</i> (19)	0.6	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.6	0.4	0.3	0.4	0.4	0.5	0.6	0.6	0.4	0.3	0.3	0.4	0.6	0.5	0.6		
Unassigned <i>Rhodospirillales</i> (25)	0.6	0.6	0.5	0.5	0.5	0.5	0.4	0.6	0.6	0.5	0.4	0.4	0.4	0.5	0.6	0.6	0.5	0.4	0.4	0.4	0.6	0.6	0.7		
<i>Anaplastaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Holosporaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Mitochondria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Rickettsiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Rickettsiales</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Sphingomonadaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Sphingomonadales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Alphaproteobacteria</i> (19)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2		
<i>Betaproteobacteria,</i>																									
<i>Alcaligenaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1		
<i>Burkholderiaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Comamonadaceae</i> (17)	0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.1	0.2	0.2	0.2	0.3		
<i>Oxalobacteraceae</i> (15)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Neisseriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h							10 h						22 h										
	Treatment:	C1	C2	C3	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly	C		Glu	Asp	Thr	Ala/ Gly	Val/ Gly	C		Glu	Asp	Thr	Ala/ Gly	Val/ Gly		
											CAA	Glu						CAA	Glu						Asp	Thr
		Relative Abundance (%)																								
<i>Gallionellaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (30)		0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2
<i>Rhodocyclaceae</i> (9)		0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Betaproteobacteria</i> (41)		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	
<i>Deltaproteobacteria</i> ,																										
<i>Bacteriovoraceae</i> (12)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bdellovibrionaceae</i> (54)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	
<i>Desulfarculaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Desulfobulbaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Desulfurellaceae</i> (41)		0.6	0.5	0.5	0.6	0.5	0.6	0.4	0.6	0.6	0.5	0.4	0.4	0.5	0.5	0.6	0.6	0.5	0.4	0.3	0.4	0.5	0.6	0.7	0.7	
<i>Desulfuromonadaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Geobacteraceae</i> (26)		0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	
<i>Archangiaceae</i> (20)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	
<i>Haliangiaceae</i> (74)		0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.2	0.2	0.3	0.4	0.3	0.3	
<i>Myxococcaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Nannocystaceae</i> (6)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Phaselicyclidaceae</i> (14)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	
<i>Polyangiaceae</i> (48)		0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.4	0.3	0.3	
<i>Sandaracinaceae</i> (38)		0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	
<i>Vulgatibacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Myxococcales</i> (177)		0.4	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.3	0.3	0.2	0.1	0.2	0.3	0.4	0.4	0.4	
<i>Oligoflexaceae</i> (29)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Oligoflexales</i> (86)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Syntrophaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Deltaproteobacteria</i> (19)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Gammaproteobacteria</i> ,																										
<i>Acidiferrobacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Aeromonadaceae</i> (8) [GPT-1]		15	19	20	16	17	13	19	17	16	18	16	14	17	15	11	12	12	15	10	18	14	12	8.9	8.9	
<i>Shewanellaceae</i> (3)		2.0	2.4	2.4	2.8	2.9	2.6	3.1	3.1	2.9	2.6	2.3	3.3	2.4	2.9	2.4	2.6	2.1	3.2	3.2	3.0	4.2	2.4	2.5	2.5	
<i>Cellvibrionaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Haliaceae</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Enterobacteriaceae</i> (12) [GPT-2], [GPT-3]		5.6	4.9	5.3	6.0	5.1	5.1	5.0	5.9	5.8	3.4	6.2	5.2	5.3	4.5	4.5	5.3	3.9	6.5	13	9.1	4.5	5.4	5.3	5.3	
<i>Coxiellaceae</i> (35)		0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Legionellaceae</i> (30)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Methylococcaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Oleiphilaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Moraxellaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Pseudomonadaceae</i> (4)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	
Unassigned <i>Thiotrichales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Xanthomonadaceae</i> (12)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Xanthomonadales</i> (24)		0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	
Unassigned <i>Gammaproteobacteria</i> (30)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Proteobacteria</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>RsaHf231</i> ,																										
Unassigned <i>RsaHf231</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Saccharibacteria</i> ,																										
Unassigned <i>Saccharibacteria</i> (78)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Spirochaetae</i> ,																										
<i>Spirochaetes</i> ,																										
<i>Spirochaetaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Brevinemataceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Synergistetes</i> ,																										
<i>Synergistia</i> ,																										
<i>Synergistaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tectomicrobia</i> ,																										

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h								10 h						22 h							
	Treatment:	C1	C2	C3	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly	Relative Abundance (%)						C	CAA	Glu	Asp	Thr	Ala/ Gly	Val/ Gly	
											C	CAA	Glu	Asp	Thr	Ala/ Gly								Val/ Gly
Unassigned <i>Tectomicrobia</i> (21)		0.9	0.8	0.8	0.8	0.7	0.7	0.5	0.8	0.8	0.7	0.5	0.5	0.6	0.7	0.9	0.8	0.7	0.6	0.5	0.7	0.9	0.7	1.0
<b>Tenericutes,</b>																								
<i>Mollicutes,</i>																								
Unassigned <i>Entomoplasmatales</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haloplasmataceae</i> (7)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (19)		21	18	18	22	21	23	24	21	21	13	13	14	17	17	21	19	17	12	9.4	13	14	14	17
<b>TM6_Dependentiae,</b>																								
Unassigned <i>TM6_Dependentiae</i> (40)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia,</b>																								
Unassigned OPB35 soil group (87)		0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
<i>Opitutae,</i>																								
<i>Opitutaceae</i> (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria,</i>																								
<i>Chthoniobacteraceae</i> (46)		0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Chthoniobacterales</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DA101 soil group (28)		1.0	0.9	0.8	1.0	0.8	1.0	0.9	1.0	1.0	1.0	0.6	0.8	0.9	0.8	0.8	0.9	1.0	0.4	0.6	0.6	0.5	1.0	0.7
<i>Xiphinematobacteraceae</i> (9)		2.3	2.0	1.9	2.0	1.8	2.0	0.8	1.6	1.8	1.5	1.1	1.3	1.2	1.8	1.4	1.8	1.7	1.0	0.9	1.0	1.9	1.9	1.9
Unassigned <i>Spartobacteria</i> (7)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobiae,</i>																								
<i>Verrucomicrobiaceae</i> (22)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Verrucomicrobia</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archaea</b>																								
<b>Thaumarchaeota,</b>																								
Unassigned <i>Thaumarchaeota</i> (9)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	Sampling Time:		30 h																			
	Treatment:	C1	C2	C3	CAA 1	CAA 2	CAA 3	Glu 1	Glu 2	Glu 3	Asp 1	Asp 2	Asp 3	Thr 1	Thr 2	Thr 3	Ala/ Gly1	Ala/ Gly2	Ala/ Gly3	Val/ Gly1	Val/ Gly2	Val/ Gly3
<b>Acidobacteria,</b>																						
<i>Acidobacteria,</i>																						
<i>Acidobacteriaceae</i> (16)		0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatellia,</i>																						
<i>Blastocatellaceae</i> (23)		0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Holophagae,</i>																						
Unassigned <i>Holophagae</i> (12)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solibacteres,</i>																						
<i>Soilbacteraceae</i> (34)		0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2
Subgroup_11 (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (16)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (16)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (9)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (10)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Subgroup_6 (113)		0.7	0.6	0.6	0.5	0.5	0.4	0.4	0.5	0.4	0.6	0.4	0.6	0.5	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.5
Unassigned <i>Acidobacteria</i> (8)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>																						
<i>Acidimicrobiia,</i>																						
<i>Acidimicrobiaceae</i> (30)		1.2	1.0	1.2	0.8	0.8	0.8	0.7	0.8	0.7	0.9	0.8	0.8	0.9	1.0	1.0	1.2	1.2	1.0	1.1	1.0	1.0
<i>Iamiaeae</i> (12)		0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1
Unassigned <i>Acidimicrobiales</i> (70)		1.2	1.1	1.2	0.9	0.8	0.8	0.7	0.8	0.7	0.9	0.9	0.8	1.0	1.0	1.0	1.1	1.1	1.0	1.3	0.9	1.0
<i>Actinobacteria,</i>																						
<i>Actinospicaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Catenulisporaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Phyla, Class, Family <sup>b</sup>	Sampling Time:			30 h																		
	Treatment:	C1	C2	C3	CAA 1	CAA 2	CAA 3	Glu 1	Glu 2	Glu 3	Asp 1	Asp 2	Asp 3	Thr 1	Thr 2	Thr 3	Ala/ Gly1	Ala/ Gly2	Ala/ Gly3	Val/ Gly1	Val/ Gly2	Val/ Gly3
		Relative Abundance %																				
<i>Cytophagaceae</i> (33)	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
<i>Flammeovirgaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteria</i> , <i>Flavobacteriaceae</i> (21)	1.5	1.3	1.3	0.7	0.7	0.5	0.6	0.4	0.3	0.5	0.6	0.6	0.6	0.7	0.8	0.1	0.1	0.1	0.2	0.1	0.1	0.1
<i>Sphingobacteria</i> , <i>Chitinophagaceae</i> (48)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Lentimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saprospiraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (14)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteria</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacteroidetes</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BJ-169</b> , Unassigned <i>BJ-169</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1</b> , Unassigned <i>BCR1</i> (14)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae</b> , <i>Chlamydiae</i> , <i>Chlamydiaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Parachlamydiaceae</i> (154)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Simkaniaceae</i> (11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Waddliaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chlamydiales</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlorobi</b> , <i>Chlorobia</i> , Unassigned <i>Chlorobia</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi</b> , <i>Anaerolineae</i> , <i>Anaerolineaceae</i> (14)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ardenticatenia</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Caldilineae</i> , <i>Caldilineaceae</i> (15)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Chloroflexia</i> , <i>Roseiflexaceae</i> (8)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Chloroflexia</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ktedonobacteria</i> , <i>Ktedonobacterales</i> , <i>Ktedonobacteraceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermosporotrichaceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacterales</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteria</i> (18)	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
<i>Thermomicrobia</i> , <i>Thermomicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Thermomicrobia</i> (56)	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Unassigned <i>Chloroflexi</i> (111)	1.1	1.0	1.1	0.8	0.8	0.7	0.6	0.8	0.7	0.9	0.7	0.8	0.8	0.9	0.9	1.0	1.0	0.9	1.1	0.9	0.9	0.9
<b>Cyanobacteria</b> , <i>Chloroplast</i> , Unassigned <i>Chloroplast</i> (34)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyanobacteria</i> , Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gastranaerophilales</i> , Unassigned <i>Gastranaerophilales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Obscuribacterales</i> , Unassigned <i>Obscuribacterales</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Cyanobacteria</i> (11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Deferribacteres</b> , Unassigned <i>Deferribacteres</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Deinococcus-Thermus</b> , <i>Deinococci</i> ,																						



Phyla, Class, Family <sup>b</sup>	Sampling Time:			30 h																		
	Treatment:	C1	C2	C3	CAA 1	CAA 2	CAA 3	Glu 1	Glu 2	Glu 3	Asp 1	Asp 2	Asp 3	Thr 1	Thr 2	Thr 3	Ala/ Gly1	Ala/ Gly2	Ala/ Gly3	Val/ Gly1	Val/ Gly2	Val/ Gly3
	Relative Abundance %																					
<i>Gemmatimonadetes</i>																						
<i>Gemmatimonadaceae</i> (37)	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1
<i>Longimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Gemmatimonadetes</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Hydrogenedentes</i>																						
Unassigned <i>Hydrogenedentes</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Latescibacteria</i>																						
Unassigned <i>Latescibacteria</i> (30)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrospirae</i>																						
<i>Nitrospira</i>																						
<i>Nitrospiraceae</i> (8)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1
Unassigned <i>Nitrospira</i> (18)	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4
<i>Parcubacteria</i>																						
Unassigned <i>Parcubacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planctomycetes</i>																						
<i>Phycisphaerae</i>																						
<i>Phycisphaeraceae</i> (38)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidisphaeraceae</i> (82)	0.6	0.5	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.4
Unassigned <i>Phycisphaerales</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Phycisphaerae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planctomycetacia</i>																						
<i>Planctomycetaceae</i> (733)	8.9	7.7	7.8	6.1	6.9	5.7	5.6	6.7	6.2	7.2	6.2	6.3	7.7	8.1	8.6	8.9	8.3	8.2	8.1	6.9	8.6	8.6
Unassigned <i>Planctomycetes</i> (82)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
<i>Proteobacteria</i>																						
<i>Alphaproteobacteria</i>																						
<i>Caulobacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Beijerinckiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (5)	1.6	1.3	1.1	1.2	0.9	1.0	1.0	0.8	0.8	1.0	0.9	0.9	1.2	1.1	1.1	1.3	1.3	1.1	1.2	1.3	1.4	1.4
<i>Hyphomicrobiaceae</i> (8)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
<i>Methylobacteriaceae</i> (4)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
<i>Methylocystaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phyllobacteriaceae</i> (3)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
<i>Rhizobiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobacteriaceae</i> (2)	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2
<i>Roseiarcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (9)	1.1	1.0	1.0	0.8	0.9	0.8	0.7	0.8	0.8	1.0	0.8	0.8	0.9	0.8	0.9	1.0	1.1	1.0	1.1	1.0	0.9	0.9
Unassigned <i>Rhizobiales</i> (19)	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
<i>Rhodobacteraceae</i> (6)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0
<i>Acetobacteraceae</i> (15)	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
<i>Rhodospirillaceae</i> (19)	0.5	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.5	0.4	0.5	0.5
Unassigned <i>Rhodospirillales</i> (25)	0.5	0.5	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.4	0.5	0.4	0.5	0.5
<i>Anaplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holosporeaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mitochondria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rickettsiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Rickettsiales</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Alphaproteobacteria</i> (19)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
<i>Betaproteobacteria</i>																						
<i>Alcaligenaceae</i> (2)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Burkholderiaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (17)	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
<i>Oxalobacteraceae</i> (15)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Neisseriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gallionellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (30)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
<i>Rhodocyclaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Phyla, Class, Family <sup>b</sup>	Sampling Time:			30 h																				
	Treatment:			C1	C2	C3	CAA 1	CAA 2	CAA 3	Glu 1	Glu 2	Glu 3	Asp 1	Asp 2	Asp 3	Thr 1	Thr 2	Thr 3	Ala/ Gly1	Ala/ Gly2	Ala/ Gly3	Val/ Gly1	Val/ Gly2	Val/ Gly3
	Relative Abundance %																							
Unassigned <i>Entomoplasmatales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haloplasmataceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (19)	16	16	17	14	17	15	11	13	13	16	14	16	13	18	16	19	19	17	17	16	17			
<b><i>TM6_Dependentiae</i></b> , Unassigned <i>TM6_Dependentiae</i> (40)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Verrucomicrobia</i></b> , Unassigned OPB35 soil group (87)	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Opitutae</i> , <i>Opitutaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria</i> , <i>Chthoniobacteraceae</i> (46)	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned <i>Chthoniobacterales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DA101 soil group (28)	0.9	0.7	0.8	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.8	0.7	0.6	0.5	0.6	0.6	0.7	0.6	0.6	0.6	0.5	0.5	0.5	0.5
<i>Xiphinematobacteraceae</i> (9)	2.0	1.6	1.5	1.7	1.1	1.5	1.4	0.9	1.1	1.2	1.3	1.0	1.7	1.5	1.6	1.9	1.8	1.5	1.9	1.7	1.9			
Unassigned <i>Spartobacteria</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobiae</i> , <i>Verrucomicrobiaceae</i> (22)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Verrucomicrobia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archaea</b> <i>Thaumarchaeota</i> , Unassigned <i>Thaumarchaeota</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22h, or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Abbreviations: C, unsupplemented control; CAA, casamino acids; Glu, glutamate; Asp, aspartate; Thr, threonine; Ala, alanine, alanine; Gly, glycine; Val, valine. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes and phylotypes from Figure 59 are bold and in brackets.

**Table A7.** Summary of all detected families in the ribose experiment based on 16S rRNA gene and 16S rRNA analysis (Section 3.2.9).<sup>a</sup>

Phyla, Class, Family <sup>b</sup>	16S rRNA Genes				16S rRNA																	
	Sampling Time:		0 h				30 h				0 h			30 h								
	Treatment:		C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R1	R2	R3		
	Relative Abundance (%)																					
<b><i>Acidobacteria</i></b> , <i>Acidobacteria</i> , <i>Acidobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatella</i> , <i>Blastocatellaceae</i> (4)	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holophagae</i> , Unassigned <i>Holophagae</i> (4)	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solibacteres</i> , <i>Solibacteraceae</i> (12)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1
Subgroup_5 (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (46)	1.4	1.2	1.2	1.1	0.7	0.7	0.9	0.4	0.5	0.3	0.7	0.8	0.2	0.3	0.3	0.2	0.1	0.1	0.1	0.0	0.1	0.3
Subgroup_11 (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (4)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_18 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	Sampling Time: Treatment:	16S rRNA Genes								16S rRNA									
		0 h				30 h				0 h				30 h					
		C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R1	R2	R3
		Relative Abundance (%)																	
Subgroup_25 (3)		0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>																			
Acidimicrobia,																			
Acidimicrobiaceae (13)		0.8	1.1	1.4	0.9	0.7	1.0	0.8	0.3	0.6	0.6	1.3	0.5	0.5	0.6	0.7	0.5	0.3	0.5
Unassigned Acidimicrobiales (40)		1.8	1.8	2.3	1.7	1.1	1.4	1.4	0.7	0.9	1.2	1.9	0.9	0.6	0.7	1.3	0.8	0.8	1.0
Iamiaceae (5)		0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.0	0.2	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.1
Actinobacteria,																			
Acidothermaceae (4)		0.3	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.0	0.1	0.1	0.0	0.1	0.1
Catenulisporaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cellulomonadaceae (1)		0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.1	0.2	0.1	0.1	0.0
Demequinaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dermacoccaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Frankiaceae (2)		0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0
Geodermatophilaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glycomycetaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intrasporangiaceae (3)		0.2	0.2	0.3	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.3	0.2	0.1	0.3	0.1	0.1	0.1	0.1
Kineosporiaceae (2)		0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microbacteriaceae (7)		0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.0
Micrococcaceae (2)		0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0
Micromonosporaceae (18)		1.1	1.1	1.3	1.0	0.6	0.9	0.8	0.4	0.9	0.9	1.8	1.0	0.6	0.9	0.8	0.6	0.6	0.6
Mycobacteriaceae (5)		0.4	0.5	0.5	0.4	0.3	0.3	0.3	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
Nakamurellaceae (1)		0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Nocardiaceae (5)		0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nocardioideaceae (18)		1.3	1.4	1.6	1.2	0.9	1.1	1.0	0.4	0.4	0.6	1.4	0.8	0.4	0.8	0.5	0.3	0.5	0.4
Promicromonosporaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Propionibacteriaceae (6)		0.5	0.4	0.4	0.4	0.2	0.3	0.3	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.1
Pseudonocardaceae (10)		0.5	0.5	0.5	0.4	0.3	0.5	0.4	0.1	0.2	0.3	0.9	0.6	0.2	0.3	0.2	0.3	0.3	0.3
Sporichthyaceae (3)		0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.0
Streptomycetaceae (4)		0.8	0.7	0.7	0.6	0.5	0.5	0.6	0.3	0.2	0.2	0.8	0.5	0.3	0.3	0.3	0.2	0.1	0.2
Streptosporangiaceae (2)		0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thermomonosporaceae (3)		0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Actinobacteria (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Coriobacteria,																			
Coriobacteriaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rubrobacteria,																			
Rubrobacteriaceae (3)		0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.0
Thermoleophilia,																			
Conexibacteraceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Thermoleophila (28)		1.5	1.6	1.9	1.5	1.1	1.2	1.4	0.5	0.3	0.4	0.5	0.5	0.2	0.2	0.3	0.3	0.2	0.3
Gaiellaceae (5)		1.9	1.9	2.3	1.7	1.3	1.5	1.4	0.7	0.4	0.5	0.6	0.7	0.3	0.5	0.5	0.3	0.2	0.5
Unassigned Gaiellales (28)		3.5	4.2	4.3	3.0	2.0	2.6	2.6	1.1	0.8	0.8	1.4	1.0	0.4	1.0	0.8	0.5	0.5	1.0
Parviterribacteraceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Patulibacteraceae (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solirubrobacteraceae (4)		0.5	0.6	0.7	0.4	0.4	0.6	0.6	0.2	0.7	0.7	1.3	0.9	0.4	0.7	0.7	0.5	0.5	0.8
Unassigned Solirubrobacterales (13)		0.9	0.8	1.1	0.7	0.4	0.7	0.8	0.3	0.3	0.3	0.4	0.4	0.1	0.3	0.1	0.2	0.2	0.2
Unassigned Actinobacteria (23)		2.2	2.8	3.2	2.0	1.6	1.6	1.8	0.7	0.3	0.6	0.9	0.5	0.2	0.6	0.4	0.3	0.2	0.3
<b>Armatimonadetes,</b>																			
Armatimonadia,																			
Unassigned Armatimonadia (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes,</b>																			
Bacteroidia,																			
Bacteroidaceae (5)		1.4	0.0	0.0	0.0	0.2	0.6	0.4	0.3	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
Porphyromonadaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rikenellaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cytophagia,																			

Phyla, Class, Family <sup>b</sup>	Sampling Time: Treatment:	16S rRNA Genes								16S rRNA									
		0 h				30 h				0 h				30 h					
		C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R1	R2	R3
		Relative Abundance (%)																	
Cytophagaceae (6)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flavobacteriia, Flavobacteriaceae (9)		0.2	0.3	0.2	0.2	0.5	0.8	0.4	0.2	0.1	0.0	0.0	0.1	0.0	0.4	0.1	0.1	0.1	0.2
Sphingobacteriia, Chitinophagaceae (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphingobacteriaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Sphingobacteriales (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1</b> , Unassigned BRC1 (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae</b> , Chlamydiae, Parachlamydiaceae (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi</b> , Anaerolineae, Anaerolineaceae (6)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ardenticatenia (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caldilineae, Caldilineaceae (10)		0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Chloroflexia, Roseiflexaceae (4)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.1
Unassigned Kallotenuales (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ktedonobacteria, Unassigned Ktedonobacteriales (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Ktedonobacteria (6)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Thermomicrobia, Unassigned Thermomicrobia (27)		0.4	0.5	0.4	0.3	0.2	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Unassigned Chloroflexi (51)		2.4	2.7	3.2	2.3	1.7	1.9	1.4	0.8	0.9	0.9	1.5	0.9	0.4	0.8	0.8	0.4	0.5	0.6
<b>Cyanobacteria</b> , Chloroplast, Unassigned Chloroplast (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyanobacteria, Unassigned Cyanobacteria (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Euryarchaeota</b> , Methanomicrobia, Methanocellaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Firmicutes</b> , Bacilli, Alicyclobacillaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacillaceae (6)		0.6	0.5	0.4	0.5	0.5	0.5	0.6	0.3	0.4	0.2	0.6	0.7	0.1	0.4	0.3	0.4	0.3	0.4
Lactobacillaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Lactobacillales (1)		0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paenibacillaceae (18)		0.1	0.1	0.2	0.1	0.1	0.2	0.2	1.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	1.4	1.7	1.4
Pasteuriaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Planococcaceae (4)		0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptococcaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thermoactinomycetaceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Bacilli (2)		0.6	0.7	0.5	0.8	0.3	0.3	0.3	0.1	0.4	0.5	0.7	0.6	0.3	0.2	0.3	0.2	0.2	0.2
Clostridia, Christensenellaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Clostridiaceae (16) [A14]		0.1	0.1	0.0	0.1	0.8	1.0	1.0	0.6	0.0	0.1	0.2	0.2	2.0	1.2	1.5	0.8	1.3	1.1
Unassigned Clostridiales (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0
Eubacteriaceae (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gracilibacteraceae (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heliobacteriaceae (3)		0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lachnospiraceae (13)		0.0	0.0	0.0	0.0	0.5	0.7	0.8	0.8	0.0	0.0	0.0	0.1	1.5	0.8	0.9	0.6	0.7	0.6
Peptococcaceae (4)		1.4	1.5	1.3	1.8	1.0	1.1	0.9	1.0	1.2	1.3	2.3	0.8	1.2	0.9	0.9	1.3	1.2	0.9
Peptostreptococcaceae (4) [GPT-4], [A8]		0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.0	0.1	0.2	0.1	0.1	0.0	0.1
Ruminococcaceae (11)		0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1

Phyla, Class, Family <sup>b</sup>	Sampling Time: Treatment:	16S rRNA Genes								16S rRNA									
		0 h				30 h				0 h				30 h					
		C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R1	R2	R3
		Relative Abundance (%)																	
Unassigned Clostridia (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erysipelotrichia</i> , <i>Erysipelotrichaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Negativicutes</i> , <i>Veillonellaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria</b> , <i>Fusobacteria</i> , <i>Fusobacteriaceae</i> (1) [GPT-5]		14	13	9.8	19	20	19	18	20	22	19	1.8	12	27	22	25	20	16	14
<b>Gemmatimonadetes</b> , <i>Gemmatimonadetes</i> , <i>Gemmatimonadaceae</i> (14)		0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.1	0.1	0.1	0.2	0.1	0.0	0.2	0.2	0.1	0.0	0.1
<i>Latescibacteria</i> , Unassigned <i>Latescibacteria</i> (4)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Nitrospirae</b> , <i>Nitrospira</i> , <i>Nitrospiraceae</i> (5)		0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Nitrospirales</i> (10)		0.6	0.7	0.7	0.4	0.4	0.5	0.4	0.2	0.1	0.2	0.5	0.4	0.2	0.5	0.2	0.3	0.3	0.3
<b>Planctomycetes</b> , <i>Phycisphaerae</i> , <i>Phycisphaeraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Phycisphaerae</i> (7)		0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Unassigned <i>Phycisphaerales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidicisphaeraceae</i> (34)		0.4	0.6	0.6	0.5	0.4	0.4	0.4	0.2	0.4	0.5	0.8	0.4	0.2	0.2	0.4	0.2	0.2	0.3
<i>Planctomycetacia</i> , <i>Planctomycetaceae</i> (331)		2.6	2.9	3.6	2.5	2.0	2.5	1.8	1.0	7.1	7.1	15	6.1	5.9	8.1	8.5	5.4	5.4	5.8
Unassigned <i>Planctomycetes</i> (17)		0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
<b>Proteobacteria</b> , <i>Alphaproteobacteria</i> , <i>Acetobacteraceae</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1
<i>Beijerinckiaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (3)		0.7	0.8	0.8	0.7	0.5	0.5	0.5	0.2	0.5	0.6	0.8	0.6	0.4	0.7	0.6	0.2	0.3	0.5
<i>Caulobacteraceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Hyphomicrobiaceae</i> (4)		0.2	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.1
<i>Methylobacteriaceae</i> (3)		0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.0	0.1	0.2	0.2	0.2	0.0	0.1	0.1	0.1	0.2	0.1
<i>Phyllobacteriaceae</i> (3)		0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.0	0.2	0.0	0.4	0.2	0.1	0.0	0.3	0.0	0.0	0.1
<i>Rhizobiaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (2)		1.0	1.0	0.9	0.6	0.7	0.7	0.7	0.2	0.2	0.1	0.4	0.2	0.0	0.2	0.1	0.1	0.1	0.1
<i>Rhodospirillaceae</i> (13)		0.4	0.5	0.5	0.5	0.4	0.4	0.4	0.1	0.2	0.3	1.0	0.5	0.5	0.5	0.4	0.3	0.2	0.4
Unassigned <i>Rhodospirillales</i> (23)		0.5	0.5	0.6	0.4	0.4	0.4	0.4	0.1	0.3	0.3	0.7	0.6	0.2	0.5	0.4	0.3	0.5	0.4
<i>Sphingomonadaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (3)		2.5	2.6	3.1	2.1	1.7	1.9	2.3	0.7	0.7	1.3	1.7	1.0	0.5	0.8	1.1	0.6	0.5	0.7
Unassigned <i>Rhizobiales</i> (12)		0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.3	0.1	0.0	0.1	0.0	0.0	0.1	0.1
<i>Betaproteobacteria</i> , <i>Alcaligenaceae</i> (1)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Burkholderiaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (8)		0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gallionellaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (16)		0.3	0.2	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.0	0.1
<i>Oxalobacteraceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (11)		0.1	0.2	0.2	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.0	0.1	0.0
<i>Deltaproteobacteria</i> , Unassigned <i>Oligoflexales</i> (5)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	16S rRNA Genes								16S rRNA										
	Sampling Time:	0 h				30 h				0 h				30 h					
		Treatment:	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R	C1	C2	C3	R1	R2
		Relative Abundance (%)																	
<i>Archangiaceae</i> (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.0	0.1
<i>Bdellovibrionaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfobulbaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfurellaceae</i> (19)	0.8	0.7	0.9	0.6	0.4	0.4	0.7	0.2	0.2	0.2	0.1	0.6	0.5	0.1	0.1	0.2	0.1	0.1	0.2
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (9)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.2	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0
<i>Haliangiaceae</i> (26)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.2	0.4	0.3	0.2	0.1	0.2	0.1	0.1	0.1
<i>Myxococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicytidaceae</i> (1)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
<i>Polyangiaceae</i> (17)	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.0	0.4	0.2	0.1	0.2	0.1	0.0	0.1	0.1
<i>Sandaracinaceae</i> (13)	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (31)	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.3	0.2	0.0	0.0	0.2	0.2	0.1	0.1	0.2
Unassigned <i>Deltaproteobacteria</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Gammaproteobacteria</i>,</b>																			
<b><i>Aeromonadaceae</i> (3) [GPT-1]</b>	22	22	23	22	24	20	19	42	27	21	5.0	34	21	25	21	39	41	44	
<i>Coxiellaceae</i> (1)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Enterobacteriaceae</i> (5) [GPT-2], [GPT-3]</b>	1.4	2.4	1.5	1.6	1.7	2.0	2.2	5.0	2.5	3.0	0.4	3.5	1.9	2.0	1.9	4.1	5.0	4.1	
<i>Legionellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (2)	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0
<i>Shewanellaceae</i> (4)	8.4	8.6	8.8	7.9	15	15	16	10	3.1	2.3	0.7	3.9	2.4	5.1	4.3	4.5	3.6	4.9	
<i>Xanthomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Xanthomonadales</i> (12)	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Unassigned <i>Gammaproteobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Saccharibacteria</i>,</b>																			
Unassigned <i>Saccharibacteria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Spirochaetae</i>,</b>																			
<i>Spirochaetes</i> ,																			
<i>Spirochaetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Tectomicrobia</i>,</b>																			
Unassigned <i>Tectomicrobia</i> (10)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.4	0.6	0.9	0.9	0.5	0.7	0.5	0.4	0.3	0.6	
<b><i>Tenericutes</i>,</b>																			
<i>Mollicutes</i> ,																			
Unassigned <i>Entomoplasmatales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (5)	10	8.8	6.7	10	8.4	7.2	8.7	5.4	21	28	41	17	25	17	18	12	12	8.8	
<b><i>Verrucomicrobia</i>,</b>																			
<i>OPB35 soil group</i> (29)	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1
<i>Opitutae</i> ,																			
<i>Opitutaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria</i> ,																			
<i>Chthoniobacteraceae</i> (10)	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthoniobacterales</i> (17)	1.0	1.4	1.3	1.3	0.8	0.9	0.9	0.3	0.3	0.1	0.4	0.2	0.1	0.1	0.2	0.1	0.0	0.1	
<i>Xiphinematobacteraceae</i> (3)	0.9	1.0	0.8	1.1	1.0	1.0	0.7	0.4	0.7	1.2	1.8	0.8	0.8	0.7	1.0	0.4	0.7	0.5	
<i>Verrucomicrobiae</i> ,																			
<i>Verrucomicrobiaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archeae</b>																			
<b><i>Thaumarchaeota</i>,</b>																			
Unassigned <i>Thaumarchaeota</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>Samples of the three replicates of the 16S rRNA gene control treatment at 0 h and 30 h, 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Abbreviations: C, unsupplemented control; R, ribose. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes from Figure 59 are bold and in brackets.



Phyla, Class, Family <sup>b</sup>	Sampling Time:				10 h				22 h		30 h					
	Treatment:				C		G		C		G		F		S	
					C	G	F	S	C	G	C	G	C	G	F	S
				Relative Abundance (%)												
Unassigned Actinobacteria (4)	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Coriobacteria,																
Coriobacteriaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rubroacteria,																
Rubrobacteriaceae (4)	0.2	0.3	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Thermoleophilia,																
Gaiellaceae (7)	2.6	2.1	1.9	1.9	1.2	0.5	1.1	0.6	1.2	0.8	1.4	0.8				
Parviterribacteraceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Patulibacteraceae (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solirubrobacteraceae (4)	0.4	0.5	0.5	0.4	0.4	0.1	0.3	0.2	0.3	0.3	0.5	0.3	0.3	0.3	0.5	0.3
Unassigned Solirubrobacterales (16)	0.9	0.8	0.8	0.7	0.6	0.2	0.3	0.3	0.5	0.3	0.7	0.4				
Unassigned Gaiellales (27)	4.6	3.9	3.7	3.3	2.5	0.8	1.7	1.2	1.9	1.3	2.6	1.6				
Unassigned Thermoleophilla (36)	2.2	1.8	1.6	1.3	1.2	0.5	0.9	0.5	1.0	0.7	1.2	0.7				
Unassigned Actinobacteria (23)	2.5	2.7	2.6	1.9	1.4	0.4	1.3	0.5	1.1	0.8	1.2	0.9				
Armatimonadetes,																
Unassigned Armatimonadetes (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacteroidetes,																
Bacteroidia,																
Bacteroidaceae (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.6
Porphyromonadaceae (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Prevotellaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rikenellaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphingobacteria																
Chitinophagaceae (9)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cytophagia,																
Cytophagaceae (11)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Flavobacteriia,																
Flavobacteriaceae (7)	0.7	0.9	0.7	0.6	0.4	0.1	0.3	0.1	0.2	0.0	0.1	0.1				
Sphingobacteria,																
Saprospiraceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphingobacteriaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Sphingobacteriales (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BRC1,																
Unassigned BRC1 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chlamydiae,																
Chlamydiae,																
Chlamydiaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Parachlamydiaceae (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chlorobi,																
Chlorobiales,																
Unassigned Chlorobiales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroflexi,																
Anaerolineae																
Anaerolineaceae (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Ardenticatenia (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caldilineae,																
Caldilineaceae (11)	0.3	0.2	0.2	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1				
Chloroflexia,																
Unassigned Kallotenuales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Roseiflexaceae (6)	0.2	0.3	0.2	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ktedonobacteria,																
Ktedonobacteraceae (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thermosporotrichaceae (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Ktedonobacterales (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Ktedonobacteria (5)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned Thermomicrobia (28)	0.5	0.6	0.5	0.3	0.2	0.1	0.3	0.1	0.3	0.2	0.3	0.2				
Unassigned Chloroflexi (41)	2.9	2.3	2.8	2.1	1.4	0.5	1.4	0.5	1.4	0.8	1.6	1.1				
Cyanobacteria,																
Chloroplast,																
Unassigned Chloroplast (5)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>	Sampling Time:				0 h				10 h		22 h		30 h			
	Treatment:	C	G	F	S	C	G	C	G	C	G	F	S			
		Relative Abundance (%)														
<i>Cyanobacteria</i> ,																
Unassigned <i>Cyanobacteria</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Cyanobacteria</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Elusimicrobia</b> ,																
Unassigned <i>Elusimicrobia</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Fibrobacteres</b> ,																
<i>Fibrobacteria</i> ,																
<i>Fibrobacteraceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Firmicutes</b> ,																
<i>Bacilli</i> ,																
<i>Aerococcaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Alicyclobacillaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Bacillaceae</i> (9)		1.3	1.3	0.8	0.9	0.8	0.4	1.1	0.8	1.8	1.5	2.0	1.1			
<i>Enterococcaceae</i> (1)		0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Lactobacillaceae</i> (2)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0		
<i>Paenibacillaceae</i> (22)		0.2	0.1	0.1	0.1	0.0	0.0	0.2	0.1	0.3	0.2	0.2	0.2			
<i>Planococcaceae</i> (7)		0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1		
<i>Staphylococcaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Thermoactinomycetaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Streptococcaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0		
Unassigned <i>Lactobacillales</i> (1)		0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1		
Unassigned <i>Bacilli</i> (2)		0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Clostridia</i> ,																
<i>Clostridiaceae</i> (22) [A14]		0.4	0.4	0.4	0.2	0.5	0.2	1.7	1.0	2.5	2.5	2.5	2.1			
Unassigned <i>Clostridiales</i> (7)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1			
<i>Eubacteriaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Gracilibacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Hellobacteriaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Lachnospiraceae</i> (24)		0.1	0.0	0.1	0.0	0.0	0.0	0.9	0.3	4.9	2.7	5.7	6.0			
<i>Peptococcaceae</i> (5)		1.0	1.2	0.8	0.9	1.1	0.5	0.7	0.5	0.9	0.8	0.9	0.6			
<i>Peptostreptococcaceae</i> (6) [GPT-4], [A8]		0.2	0.1	0.2	0.2	0.8	0.1	2.5	1.0	2.9	2.6	5.1	1.9			
<i>Ruminococcaceae</i> (28)		0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	1.6			
<i>Erysipelotrichia</i> ,																
<i>Erysipelotrichaceae</i> (6)		0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.1			
<i>Limnochordia</i> ,																
Unassigned <i>Limnochordales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Negativicutes</i> ,																
<i>Acidaminococcaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Veillonellaceae</i> (9)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Fusobacteria</b> ,																
<i>Fusobacteriia</i> ,																
<i>Fusobacteriaceae</i> (1) [GPT-5]		1.8	2.8	3.0	4.2	20	12	29	23	22	21	17	35			
<b>Gemmatimonadetes</b> ,																
<i>Gemmatimonadaceae</i> (15)		0.3	0.3	0.4	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1			
Unassigned <i>Gemmatimonadetes</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Latescibacteria</b> ,																
Unassigned <i>Latescibacteria</i> (8)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Nitrospirae</b> ,																
<i>Nitrospira</i> ,																
<i>Nitrospiraceae</i> (5)		0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0			
Unassigned <i>Nitrospirales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Nitrospira</i> (12)		0.7	0.9	0.8	0.7	0.5	0.1	0.4	0.1	0.4	0.3	0.4	0.3			
<b>Planctomycetes</b> ,																
<i>Phycisphaerae</i> ,																
<i>Phycisphaeraeaceae</i> (9)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Tepidisphaeraeaceae</i> (40)		0.9	0.9	1.1	0.6	0.4	0.1	0.4	0.2	0.3	0.3	0.3	0.2			

Phyla, Class, Family <sup>b</sup>	Sampling Time:				10 h				22 h				30 h				
	Treatment:	0 h				C		G		C		G		C		G	
		C	G	F	S	C	G	C	G	C	G	F	S				
		Relative Abundance (%)															
Unassigned <i>Phycisphaerae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				
<i>Planctomycetacia</i> ,																	
<i>Planctomycetaceae</i> (400)	3.8	3.9	3.7	2.7	2.4	0.7	2.0	1.0	2.1	1.6	1.9	1.3					
Unassigned <i>Planctomycetes</i> (23)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0					
<b>Proteobacteria,</b>																	
<i>Alphaproteobacteria</i>																	
<i>Acetobacteraceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Bradyrhizobiaceae</i> (2)	0.7	0.9	0.8	0.6	0.4	0.1	0.4	0.1	0.4	0.2	0.3	0.2					
<i>Caulobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Holosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Hyphomicrobiaceae</i> (6)	0.9	0.8	0.8	0.7	0.4	0.2	0.4	0.2	0.4	0.2	0.4	0.4					
<i>Methylobacteriaceae</i> (3)	0.1	0.1	0.2	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1					
<i>Phyllobacteriaceae</i> (3)	0.4	0.3	0.4	0.3	0.2	0.0	0.1	0.1	0.2	0.1	0.1	0.2					
<i>Rhizobiaceae</i> (2)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Rhizobiales</i> (9)	0.3	0.3	0.3	0.2	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1					
<i>Rhodobacteraceae</i> (5)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1					
<i>Rhodobiaceae</i> (2)	1.9	1.6	1.7	1.1	0.8	0.3	0.7	0.2	0.7	0.4	0.9	0.6					
<i>Rhodospirillaceae</i> (12)	0.6	1.0	1.0	0.5	0.4	0.1	0.4	0.2	0.3	0.2	0.4	0.3					
Unassigned <i>Rhodospirillales</i> (13)	0.6	0.5	0.7	0.4	0.3	0.1	0.3	0.1	0.3	0.2	0.3	0.2					
<i>Sphingomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Sphingomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Xanthobacteraceae</i> (4)	2.8	2.7	2.5	2.0	1.3	0.4	1.2	0.6	1.0	0.6	1.1	1.0					
Unassigned <i>Rickettsiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Alphaproteobacteria</i> (12)	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0					
<i>Betaproteobacteria</i> ,																	
<i>Alcaligenaceae</i> (2)	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0					
<i>Burkholderiaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Comamonadaceae</i> (9)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Oxalobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Neisseriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Nitrosomonadaceae</i> (17)	0.3	0.4	0.4	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.2					
<i>Rhodocyclaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Betaproteobacteria</i> (20)	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1					
<i>Deltaproteobacteria</i> ,																	
<i>Bdellovibrionaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Desulfovibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1					
<i>Desulfurellaceae</i> (21)	1.3	1.1	1.3	1.0	0.6	0.2	0.6	0.3	0.5	0.4	0.6	0.3					
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Geobacteraceae</i> (8)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Archangiaceae</i> (5)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0					
<i>Haliangiaceae</i> (32)	0.2	0.2	0.2	0.1	0.2	0.0	0.1	0.0	0.1	0.1	0.1	0.1					
<i>Myxococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Myxococcales</i> (45)	0.2	0.2	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.1					
<i>Nannocystaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Phaselicytidaceae</i> (3)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Polyangiaceae</i> (18)	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0					
<i>Sandaracinaceae</i> (19)	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0					
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Oligoflexaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Oligoflexales</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Unassigned <i>Deltaproteobacteria</i> (8)	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Epsilonproteobacteria</i> ,																	
<i>Campylobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
<i>Gammaproteobacteria</i>																	
<i>Aeromonadaceae</i> (2) [GPT-1]	17	15	20	29	26	67	17	48	16	35	15	12					
<i>Coxiellaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0					
<i>Enterobacteriaceae</i> (6) [GPT-2], [GPT-3]	1.6	1.8	2.5	3.0	3.2	2.4	3.4	2.6	3.1	2.2	3.3	3.0					
<i>Halleaeae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					



Phyla, Class, Family <sup>b</sup>	Sampling Time:																						
	0 h						10 h		22 h		30 h												
	Treatment:	C1	C2	C3	G	F	S	C	G	C	G	C1	C2	C3	G1	G2	G3	F1	F2	F3	S1	S2	S3
	Relative Abundance (%)																						
Subgroup_17 (9)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_18 (1)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (6)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (49)	0.8	0.5	0.6	0.7	0.8	0.6	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
Subgroup_9 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>																							
<i>Acidimicrobia,</i>																							
<i>Acidimicrobiaceae</i> (17)	1.3	1.5	1.2	1.2	1.2	1.1	0.7	0.3	0.7	0.3	0.8	0.7	0.6	0.3	0.4	0.4	0.9	0.8	0.7	0.6	0.6	0.7	0.7
<i>lamiaceae</i> (8)	0.1	0.1	0.3	0.2	0.1	0.2	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1
Unassigned <i>Acidimicrobiales</i> (39)	1.8	1.6	1.9	1.7	1.6	1.5	1.0	0.5	0.9	0.4	1.0	0.9	0.8	0.3	0.6	0.4	0.7	0.9	0.9	1.1	0.8	0.6	0.8
<i>Actinobacteria,</i>																							
<i>Acidotherrmaceae</i> (4)	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
<i>Actinomycetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bifidobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bogoriellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellulomonadaceae</i> (1)	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Corynebacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cryptosporangiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Demequinaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (3)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
<i>Geodermatophilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (3)	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1
<i>Kineosporiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Microbacteriaceae</i> (6)	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0
<i>Micrococcaceae</i> (1)	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1
<i>Micromonosporaceae</i> (18)	0.7	0.9	0.9	1.2	0.8	0.8	0.6	0.2	0.4	0.2	0.5	0.4	0.5	0.2	0.3	0.3	0.8	0.4	0.5	0.5	0.4	0.5	0.5
<i>Mycobacteriaceae</i> (5)	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Nakamurellaceae</i> (2)	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Nocardiaceae</i> (6)	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nocardoidaceae</i> (25)	1.1	0.8	1.0	0.9	0.8	0.7	0.4	0.2	0.4	0.2	0.4	0.5	0.4	0.2	0.2	0.2	0.7	0.4	0.5	0.5	0.4	0.5	0.5
<i>Promicromonosporaceae</i> (2)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.2	0.1	0.1
<i>Propionibacteriaceae</i> (6)	0.2	0.2	0.1	0.3	0.2	0.3	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.1	0.1
<i>Pseudonocardiaceae</i> (10)	0.5	0.5	0.6	0.7	0.7	0.6	0.4	0.1	0.4	0.2	0.4	0.3	0.2	0.2	0.2	0.1	0.5	0.4	0.3	0.4	0.2	0.4	0.4
<i>Sporichthyaceae</i> (3)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
<i>Streptomyces</i> (4)	0.4	0.3	0.3	0.3	0.5	0.3	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.3	0.3	0.2	0.3	0.2	0.2	0.2
<i>Streptosporangiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermomonosporaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Frankiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Actinobacteria</i> (4)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0
<i>Coriobacteria,</i>																							
<i>Coriobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rubrobacteria,</i>																							
<i>Rubrobacteriaceae</i> (4)	0.1	0.2	0.3	0.2	0.3	0.2	0.1	0.1	0.2	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Thermoleophilia,</i>																							
<i>Gaiellaceae</i> (7)	0.7	0.7	0.7	0.8	0.7	0.7	0.5	0.2	0.5	0.2	0.4	0.3	0.4	0.1	0.2	0.2	0.3	0.2	0.3	0.5	0.3	0.3	0.3
<i>Parviterribacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solirubrobacteraceae</i> (4)	0.9	0.8	1.0	1.0	0.8	0.8	0.5	0.2	0.5	0.2	0.5	0.4	0.4	0.2	0.3	0.3	0.4	0.3	0.4	0.7	0.5	0.4	0.4
Unassigned <i>Solirubrobacterales</i> (16)	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.2	0.4	0.2	0.3	0.3
Unassigned <i>Gaiellales</i> (27)	1.1	1.2	1.1	1.0	1.1	0.9	0.6	0.3	0.4	0.2	0.4	0.5	0.6	0.2	0.3	0.2	0.5	0.4	0.6	0.9	0.6	0.5	0.5
Unassigned <i>Thermoleophilia</i> (36)	0.6	0.3	0.4	0.3	0.3	0.3	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.3	0.4	0.1	0.2	0.2
Unassigned <i>Actinobacteria</i> (23)	0.5	0.4	0.4	0.5	0.5	0.4	0.2	0.1	0.2	0.1	0.4	0.3	0.2	0.1	0.1	0.1	0.3	0.2	0.4	0.3	0.2	0.2	0.2
<b>Armatimonadetes,</b>																							
Unassigned <i>Armatimonadetes</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes,</b>																							
<i>Bacteroidia,</i>																							
<i>Bacteroidaceae</i> (5)	0.0	0.1	0.0	0.0	0.0	0.3	0.0	0.0	9.3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Porphyromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Phyla, Class, Family <sup>b</sup>		Sampling Time:																						
		0 h						10 h		22 h		30 h												
Treatment:		C1	C2	C3	G	F	S	C	G	C	G	C1	C2	C3	G1	G2	G3	F1	F2	F3	S1	S2	S3	
		Relative Abundance (%)																						
	<i>Prevotellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Rikenellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Sphingobacteria</i>																							
	<i>Chitinophagaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Cytophagia</i>																							
	<i>Cytophagaceae</i> (11)	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Flavobacteria</i>																							
	<i>Flavobacteriaceae</i> (7)	0.2	0.4	0.2	0.3	0.5	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.2	0.2	0.0	0.0	0.1	
	<i>Sphingobacteria</i>																							
	<i>Saprospiraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Sphingobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Sphingobacteriales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>BRC1</b>																							
	Unassigned <i>BRC1</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Chlamydiae</b>																							
	<i>Chlamydiae</i>																							
	<i>Chlamydiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Parachlamydiaceae</i> (13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Chlorobi</b>																							
	<i>Chlorobiales</i>																							
	Unassigned <i>Chlorobiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Chloroflexi</b>																							
	<i>Anaerolineae</i>																							
	<i>Anaerolineaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Ardenticatenia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Caldilineae</i>																							
	<i>Caldilineaceae</i> (11)	0.1	0.0	0.1	0.2	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0
	<i>Chloroflexia</i>																							
	Unassigned <i>Kallotenuales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Roseiflexaceae</i> (6)	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
	<i>Ktedonobacteria</i>																							
	<i>Ktedonobacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Thermosporotrichaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Ktedonobacterales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Ktedonobacteria</i> (5)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Thermomicrobia</i> (28)	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1
	Unassigned <i>Chloroflexi</i> (41)	0.6	0.8	0.8	1.0	0.9	0.6	0.5	0.2	0.5	0.2	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.4	0.4
	<b>Cyanobacteria</b>																							
	<i>Chloroplast</i>																							
	Unassigned <i>Chloroplast</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Cyanobacteria</i>																							
	Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Elusimicrobia</b>																							
	Unassigned <i>Elusimicrobia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Fibrobacteres</b>																							
	<i>Fibrobacteria</i>																							
	<i>Fibrobacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Firmicutes</b>																							
	<i>Bacilli</i>																							
	<i>Aerococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Alicyclobacillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Bacillaceae</i> (9)	1.3	1.2	1.5	1.1	1.3	1.2	0.8	0.5	0.7	0.6	1.1	1.1	1.2	1.0	1.0	0.7	1.6	1.3	1.4	1.2	0.6	0.9	
	<i>Enterococcaceae</i> (1)	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.8	1.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.7	0.0	0.0	0.0	
	<i>Lactobacillaceae</i> (2)	0.1	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	<i>Paenibacillaceae</i> (22)	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1

		Sampling Time:																							
		0 h						10 h		22 h		30 h													
Treatment:		C1	C2	C3	G	F	S	C	G	C	G	C1	C2	C3	G1	G2	G3	F1	F2	F3	S1	S2	S3		
Phyla, Class, Family <sup>b</sup>		Relative Abundance (%)																							
	<i>Planococcaceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	<i>Staphylococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Thermoactinomyces</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Streptococcaceae</i> (3)	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.3
	Unassigned <i>Lactobacillales</i> (1)	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	Unassigned <i>Bacilli</i> (2)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Clostridia</i> ,	<i>Clostridiaceae</i> (22) [A14]	0.3	0.4	0.2	0.3	0.4	0.3	0.7	0.4	1.6	1.6	2.4	2.0	2.1	3.4	4.1	3.6	3.1	3.2	3.5	2.2	2.0	1.9		
	Unassigned <i>Clostridiales</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2		
	<i>Eubacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Gracilibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Heliobacteriaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Lachnospiraceae</i> (24)	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.0	1.1	0.5	3.1	4.1	4.3	3.0	3.2	4.0	2.5	3.5	5.6	5.2	4.7	4.8		
	<i>Peptococcaceae</i> (5)	0.7	0.7	0.9	0.6	0.8	0.8	0.7	0.3	0.5	0.5	0.7	0.4	0.7	0.4	0.6	0.5	0.7	0.8	1.0	0.7	0.8	0.8		
	<i>Peptostreptococcaceae</i> (6) [GPT-4], [A8]	0.1	0.1	0.2	0.0	0.2	0.2	1.8	0.5	3.1	2.4	3.2	3.1	3.5	3.6	4.3	4.2	7.9	7.6	9.4	3.1	2.6	2.2		
	<i>Ruminococcaceae</i> (28)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1		
<i>Erysipelotrichia</i> ,	<i>Erysipelotrichaceae</i> (6)	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0		
<i>Limnochordia</i> ,	Unassigned <i>Limnochordales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Negativicutes</i> ,	<i>Acidaminococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Veillonellaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Fusobacteria</b> ,	<i>Fusobacteriaceae</i> (1) [GPT-5]	1.3	1.5	0.6	1.3	1.2	1.4	13	10	20	22	23	18	17	16	15	15	15	16	11	22	29	30		
<b>Gemmatimonadetes</b> ,	<i>Gemmatimonadaceae</i> (15)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1		
	Unassigned <i>Gemmatimonadetes</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Latescibacteria</b> ,	Unassigned <i>Latescibacteria</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Nitrospirae</b> ,	<i>Nitrospira</i> ,																								
	<i>Nitrospiraceae</i> (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0		
	Unassigned <i>Nitrospirales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	Unassigned <i>Nitrospira</i> (12)	0.4	0.5	0.5	0.5	0.5	0.4	0.3	0.1	0.3	0.1	0.3	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.3	0.3		
<b>Planctomycetes</b> ,	<i>Phycisphaerae</i> ,																								
	<i>Phycisphaeraeaceae</i> (9)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Tepidisphaeraeaceae</i> (40)	0.7	1.2	1.1	1.2	0.8	0.4	0.3	0.1	0.3	0.1	0.6	0.2	0.3	0.1	0.1	0.1	0.3	0.4	0.2	0.1	0.2	0.1		
	Unassigned <i>Phycisphaerae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Planctomycetacia</i> ,	<i>Planctomycetaceae</i> (400)	8.2	9.8	8.6	8.4	7.5	5.6	4.1	1.5	4.3	1.8	5.8	5.1	5.6	2.1	2.8	2.9	6.8	6.6	5.1	3.7	5.2	4.7		
	Unassigned <i>Planctomycetes</i> (23)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Proteobacteria</b> ,	<i>Alphaproteobacteria</i>																								
	<i>Acetobacteraceae</i> (6)	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0		
	<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Bradyrhizobiaceae</i> (2)	0.7	0.8	0.9	1.0	1.1	0.9	0.5	0.2	0.5	0.3	0.5	0.4	0.4	0.2	0.2	0.2	0.4	0.4	0.3	0.4	0.2	0.5		
	<i>Caulobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Holosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	<i>Hyphomicrobiaceae</i> (6)	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.1	0.2	0.1	0.1	0.3	0.2	0.0	0.1	0.0	0.2	0.1	0.2	0.3	0.2	0.1		
	<i>Methylbacteriaceae</i> (3)	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.2		
	<i>Phyllobacteriaceae</i> (3)	0.2	0.2	0.3	0.5	0.2	0.3	0.2	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2		
	<i>Rhizobiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	Unassigned <i>Rhizobiales</i> (9)	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0		
	<i>Rhodobacteraceae</i> (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0		
	<i>Rhodobiaceae</i> (2)	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.0	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2		
	<i>Rhodospirillaceae</i> (12)	0.7	0.7	0.9	1.2	1.1	1.0	0.5	0.2	0.4	0.2	0.5	0.6	0.4	0.3	0.3	0.2	0.6	0.7	0.5	0.7	0.4	0.6		

Phyla, Class, Family <sup>b</sup>	Sampling Time:																					
	0 h						10 h		22 h		30 h											
	Treatment:	C1	C2	C3	G	F	S	C	G	C	G	C1	C2	C3	G1	G2	G3	F1	F2	F3	S1	S2
Relative Abundance (%)																						
Unassigned <i>Rhodospirillales</i> (13)	0.4	0.4	0.4	0.7	0.6	0.6	0.3	0.1	0.3	0.1	0.2	0.4	0.3	0.1	0.1	0.1	0.3	0.3	0.2	0.4	0.2	0.3
<i>Sphingomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (4)	0.5	0.8	0.7	1.0	0.6	0.6	0.3	0.1	0.4	0.1	0.4	0.5	0.4	0.1	0.2	0.1	0.4	0.4	0.3	0.6	0.3	0.3
Unassigned <i>Rickettsiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Alphaproteobacteria</i> (12)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
<i>Betaproteobacteria</i> ,																						
<i>Alcaligenaceae</i> (2)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<i>Burkholderiaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (9)	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oxalobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Neisseriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (17)	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0
<i>Rhodocyclaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (20)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Deltaproteobacteria</i> ,																						
<i>Bdellovibrionaceae</i> (9)	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0
<i>Desulfovibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfurellaceae</i> (21)	0.4	0.4	0.5	0.6	0.7	0.6	0.4	0.1	0.3	0.1	0.3	0.2	0.3	0.1	0.1	0.1	0.2	0.1	0.3	0.4	0.2	0.4
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (8)	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Archangiaceae</i> (5)	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1
<i>Haliangiaceae</i> (32)	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.0	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.3	0.2	0.2
<i>Myxococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (45)	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.0	0.1	0.1	0.3	0.2	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.3	0.1	0.2
<i>Nannocystaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicystidaceae</i> (3)	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1
<i>Polyangiaceae</i> (18)	0.3	0.2	0.4	0.3	0.3	0.2	0.1	0.0	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.0	0.2	0.1	0.2	0.3	0.2	0.2
<i>Sandaracinaceae</i> (19)	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.0	0.1	0.0	0.0	0.2	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.1
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oligoflexaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Oligoflexales</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Deltaproteobacteria</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Epsilonproteobacteria</i> ,																						
<i>Campylobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gammaproteobacteria</i>																						
<i>Aeromonadaceae</i> (2) [GPT-1]	23	21	15	16	17	22	31	62	23	46	20	23	20	46	41	39	24	18	15	16	13	13
<i>Coxiellaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (6) [GPT-2], [GPT-3]	3.3	3.1	7.0	3.1	5.8	7.2	6.0	4.0	4.1	4.1	3.0	3.5	3.5	4.6	4.0	4.6	4.0	4.2	4.7	4.6	3.2	4.2
<i>Haliaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Legionellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Shewanellaceae</i> (2)	1.8	1.5	0.9	1.2	1.2	0.8	1.3	0.7	1.7	0.7	2.1	3.3	3.3	1.9	1.5	1.6	1.5	2.3	1.3	1.4	1.5	1.5
<i>Xanthomonadaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Xanthomonadales</i> (14)	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Unassigned <i>Gammaproteobacteria</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saccharibacteria</i> ,																						
Unassigned <i>Saccharibacteria</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spirochaetae</i> ,																						
<i>Spirochaetales</i> ,																						
<i>Spirochaetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tectomicrobia</i>																						
Unassigned <i>Tectomicrobia</i> (16)	0.8	0.8	1.2	1.2	1.4	1.4	0.8	0.3	0.8	0.3	0.6	0.8	0.8	0.5	0.5	0.4	1.0	0.8	0.6	1.0	0.7	0.8
<i>Tenericutes</i> ,																						
<i>Mollicutes</i> ,																						
<i>Mycoplasmataceae</i> (6)	36	33	32	37	35	35	25	12	15	13	21	22	25	12	15	17	19	23	27	21	26	22

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h					10 h		22 h		30 h												
	Treatment:		C1	C2	C3	G	F	S	C	G	C	G	C1	C2	C3	G1	G2	G3	F1	F2	F3	S1	S2	S3
Relative Abundance (%)																								
Unassigned <i>Entomoplasmatales</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>TM6_Dependentiae</i></b> , Unassigned <i>TM6_Dependentiae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b><i>Verrucomicrobia</i></b> , Unassigned <i>OPB35 soil group</i> (29)		0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria</i> , <i>Chthoniobacteraceae</i> (16)		0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xiphinematobacteraceae</i> (3)		1.4	2.2	2.3	2.8	2.4	1.8	1.3	0.5	1.0	0.8	1.7	1.2	1.0	0.6	0.7	0.5	1.0	1.3	1.5	0.9	1.0	1.2	
Unassigned <i>Chthoniobacterales</i> (19)		0.3	0.4	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	
<i>Opiritae</i> , <i>Opiritaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opiritae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Verrucomicrobiae</i> , <i>Verrucomicrobiaceae</i> (7)		0.0	0.0	3.8	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Archeae																								
<i>Thaumarchaeota</i> , Unassigned <i>Thaumarchaeota</i> (5)																								
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>Samples of the three replicates of the 16S rRNA control treatment at 0 h, and all 16S rRNA treatments at 30 h were analyzed separately. Samples of the three replicates were pooled for each of the other treatments at 0 h, 10 h, 22h, or 30 h. Identification numbers (e.g., C1) indicate the respective replicates. Abbreviations: C, unsupplemented control; S, succinate; F, formate; G, glucose. Table modified and used with permission from Zeibich *et al.*, 2019b.

<sup>b</sup>The number of phylotypes are shown in parenthesis. Abundant responsive group phylotypes from Figure 59 are bold and in brackets.

**Table A9.** Summary of all detected families in the yeast extract experiment with gut content (A) and soil (B) treatments based on 16S rRNA gene and 16S rRNA analysis (Section 3.3.2).<sup>a</sup>

**(A) Gut Content**

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h					40 h							
	Treatment <sup>a</sup> :		C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R
Relative Abundance (%)															
<b><i>Acidobacteria</i></b> ,															
<i>Acidobacteria</i> ,															
<i>Acidobacteriaceae</i> (7)		0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatellia</i> ,															
<i>Blastocatellaceae</i> (25)		0.3	0.3	0.4	0.5	0.2	0.2	0.3	0.1	0.1	0.1	0.2	0.0	0.0	0.0
<i>Holophagae</i> ,															
Subgroup_10 (10)		0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_7 (9)		0.2	0.2	0.2	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Unassigned <i>Acidobacteriales</i> (6)		0.1	0.1	0.2	0.1	0.2	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Solibacteres</i> ,															
<i>Solibacteraceae</i> (25)		0.4	0.3	0.3	0.2	0.6	0.7	0.2	0.0	0.3	0.2	0.3	0.0	0.0	0.1
Subgroup_11 (6)		0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (22)		0.5	0.7	0.6	0.8	0.3	0.2	0.3	0.1	0.0	0.1	0.1	0.0	0.0	0.0
Subgroup_22 (26)		0.2	0.3	0.3	0.4	0.2	0.3	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Subgroup_25 (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (106)		5.3	5.5	6.0	4.4	4.0	2.6	3.4	0.5	0.8	0.9	1.1	0.2	0.2	0.1
Unassigned <i>Acidobacteria</i> (25)		0.3	0.2	0.2	0.2	0.2	0.4	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<b><i>Actinobacteria</i></b>															
<i>Actinobacteria</i> ,															



Phyla, Class, Family <sup>b</sup>		Sampling Time:		0 h				40 h								
		Treatment <sup>a</sup> :	C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R	E3.R
		Relative Abundance (%)														
<b>BRC1,</b>	<i>Sphingobacteriaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Unassigned <i>Sphingobacteriales</i> (27)	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae,</b>	Unassigned BCR1 (12)	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
	<i>Chlamydiae,</i>															
	<i>Parachlamydiaceae</i> (45)	0.1	0.2	0.2	0.1	0.1	0.0	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.0	
	<i>Simkaniaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Chlamydiales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Chlorobi,</b>	<i>Chlorobia,</i>															
	Unassigned <i>Chlorobiales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Chloroflexi,</b>	<i>Anaerolineae,</i>															
	<i>Anaerolineaceae</i> (20)	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	
	Unassigned <i>Ardenticatenia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Caldilineae,</i>															
	<i>Caldilineaceae</i> (12)	0.4	0.4	0.4	0.3	0.2	0.1	0.2	0.0	0.2	0.1	0.3	0.0	0.0	0.0	
	<i>Chloroflexia,</i>															
	Unassigned <i>Kallotenuales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Roseiflexaceae</i> (12)	0.4	0.4	0.4	0.4	0.5	0.2	0.2	0.0	0.2	0.1	0.2	0.0	0.0	0.0	
<b>Cyanobacteria,</b>	<i>Chloroplast,</i>															
	<i>Trebouxiophyceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Chloroplast</i> (6)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Cyanobacteria</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	
	<i>Cyanobacteria,</i>															
	Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Chloroflexi,</b>	JG30-KF-CM66 group (13)	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	KD4-96 group (13)	2.9	3.2	2.9	4.8	1.0	1.1	1.8	0.3	0.5	0.4	0.6	0.1	0.1	0.1	
	<i>Ktedonobacteria,</i>															
	<i>Ktedonobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Thermosporotrichaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Ktedonobacterales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Ktedonobacteria</i> (12)	0.3	0.3	0.3	0.3	0.3	0.1	0.2	0.0	0.2	0.1	0.2	0.0	0.0	0.0	
	<i>Melainabacteria,</i>															
	Unassigned <i>Obscuribacterales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	S085 group (11)	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	
	SBR2076 group (10)	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Thermomicrobia,</i>															
	Unassigned <i>Thermomicrobia</i> (33)	0.6	0.8	0.8	0.6	0.5	0.4	0.6	0.1	0.2	0.3	0.3	0.0	0.0	0.1	
	TK10 group (15)	0.3	0.3	0.3	0.4	0.5	0.8	0.2	0.0	0.2	0.1	0.2	0.1	0.0	0.1	
	Unassigned <i>Chloroflexi</i> (7)	0.4	0.3	0.4	0.6	0.1	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	
<b>Deinococcus-Thermus,</b>	<i>Deinococci,</i>															
	Unassigned <i>Deinococci</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Elusimicrobia,</b>	<i>Elusimicrobia,</i>															
	Unassigned <i>Elusimicrobia</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Fibrobacteres,</b>	<i>Fibrobacteria,</i>															
	<i>Fibrobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Firmicutes,</b>	<i>Bacilli,</i>															
	<i>Alicyclobacillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Bacillaceae</i> (16) [E19]	2.0	1.6	1.7	0.9	2.2	1.0	11	3.0	5.5	6.2	5.7	1.9	1.7	1.7	
	<i>Paenibacillaceae</i> (24)	0.2	0.2	0.2	0.1	0.2	0.1	1.5	0.5	0.5	0.4	0.8	0.3	0.4	0.2	
	<i>Pasteuriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Planococcaceae</i> (7)	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h					40 h							
	Treatment <sup>a</sup> :	C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R	E3.R
<i>Planctomycetacia</i> ,															
<i>Planctomycetaceae</i> (505)		8.2	8.2	8.6	8.6	20	12	6.9	1.8	17	19	15	4.3	4.2	3.7
Unassigned <i>Planctomyces</i> (15)		0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<b>Proteobacteria</b> ,															
<i>Alphaproteobacteria</i> ,															
<i>Caulobacteraceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Hyphomonadaceae</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (2)		1.4	1.5	1.5	1.2	2.4	2.3	0.9	0.2	1.8	1.6	2.3	0.2	0.3	0.2
<i>Hyphomicrobiaceae</i> (7)		1.2	1.2	1.2	0.9	1.1	0.9	0.8	0.1	0.9	0.9	1.3	0.1	0.1	0.1
<i>Methylobacteriaceae</i> (4)		0.3	0.3	0.3	0.2	0.3	0.4	0.2	0.0	0.2	0.4	0.4	0.0	0.0	0.0
<i>Phyllobacteriaceae</i> (3)		0.5	0.4	0.4	0.3	0.5	0.6	0.2	0.0	0.3	0.3	0.4	0.1	0.0	0.1
<i>Rhizobiaceae</i> (3)		0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Rhodobiaceae</i> (2)		1.6	1.5	1.5	1.0	0.6	0.2	1.5	0.3	0.7	0.6	0.7	0.1	0.0	0.1
<i>Xanthobacteraceae</i> (4)		4.6	4.8	4.8	4.2	2.8	1.5	3.3	0.7	2.3	2.4	2.3	0.3	0.3	0.2
Unassigned <i>Rhizobiales</i> (14)		0.5	0.5	0.5	0.5	0.5	0.4	0.2	0.1	0.4	0.2	0.3	0.1	0.0	0.0
<i>Rhodobacteraceae</i> (4)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0
<i>Acetobacteraceae</i> (6)		0.1	0.1	0.1	0.1	0.2	0.3	0.1	0.0	0.2	0.1	0.1	0.0	0.0	0.0
<i>Rhodospirillaceae</i> (15)		0.8	0.7	0.7	0.6	0.8	1.0	0.3	0.1	0.6	0.7	0.6	0.1	0.1	0.1
Unassigned <i>Rhodospirillales</i> (32)		1.3	1.3	1.3	1.5	1.5	1.7	0.7	0.2	0.9	0.8	1.2	0.1	0.2	0.1
<i>Holospiraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Rickettsiales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (6)		0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Unassigned <i>Sphingomonadales</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Betaproteobacteria</i> ,															
<i>Alcaligenaceae</i> (2)		0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.0	0.1	0.2	0.2	0.0	0.0	0.0
<i>Burkholderiaceae</i> (4)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (12)		0.3	0.3	0.3	0.2	1.0	0.9	0.2	0.0	0.2	0.1	0.4	0.0	0.0	0.0
<i>Oxalobacteraceae</i> (7)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Neisseriaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (35)		0.6	0.7	0.6	0.8	0.3	0.4	0.3	0.0	0.1	0.1	0.2	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (10)		0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SC-1-84 group (22)		0.6	0.7	0.7	0.3	0.5	0.1	0.8	0.1	0.1	0.1	0.1	0.0	0.0	0.0
TRA3-20 group (5)		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (3)		0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Deltaproteobacteria</i> ,															
<i>Bacteriovoraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bdellovibrionaceae</i> (16)		0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.0	0.0
<i>Desulfarculaceae</i> (37)		2.0	2.1	2.1	1.8	1.4	0.8	1.2	0.2	0.5	0.6	1.1	0.2	0.1	0.2
<i>Desulfuromonadaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (14)		0.3	0.3	0.3	0.4	0.4	0.4	0.2	0.1	0.1	0.2	0.4	0.0	0.0	0.0
<i>Archangiaceae</i> (9)		0.1	0.2	0.1	0.1	0.3	0.2	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1
<i>Haliangiaceae</i> (57)		0.5	0.5	0.4	0.2	0.8	0.3	0.4	0.0	0.5	0.7	0.7	0.1	0.1	0.1
<i>Myxococcaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (3)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicytidaceae</i> (5)		0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.0	0.2	0.2	0.2	0.0	0.0	0.0
<i>Polyangiaceae</i> (28)		0.2	0.2	0.1	0.0	0.5	0.2	0.1	0.1	0.3	0.4	0.5	0.1	0.0	0.1
<i>Sandaracinaceae</i> (23)		0.1	0.1	0.1	0.1	0.4	0.1	0.1	0.0	0.3	0.2	0.3	0.1	0.0	0.0
<i>Vulgatibacteraceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (71)		0.4	0.4	0.5	0.3	1.0	0.6	0.2	0.0	0.5	0.6	0.5	0.1	0.1	0.1
NB1-j group (13)		0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oligoflexaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Oligoflexales</i> (24)		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Gammaproteobacteria</i> ,															
<i>Acidiferrobacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Aeromonadaceae</i> (2) [E3]		0.3	0.2	0.2	1.0	1.1	1.2	3.3	5.9	0.2	0.8	0.5	2.3	2.9	2.7
<i>Shewanellaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellvibrionaceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haliaceae</i> (2)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spongibacteraceae</i> (1)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



**(B) Soil**

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h				40 h									
	Treatment <sup>a</sup> :		C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R	E3.R
Relative Abundance (%)																
<b>Acidobacteria,</b>																
<i>Acidobacteria,</i>																
<i>Acidobacteriaceae</i> (7)			0.2	0.1	0.2	0.2	0.1	0.0	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.0
<i>Blastocatellia,</i>																
<i>Blastocatellaceae</i> (25)			0.4	0.4	0.5	1.3	0.3	0.8	0.8	0.1	0.3	0.1	0.2	0.0	0.0	0.0
<i>Holophagae,</i>																
Subgroup_10 (10)			0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_7 (9)			0.4	0.3	0.3	0.1	0.0	0.1	0.4	0.0	0.0	0.1	0.1	0.0	0.0	0.0
Unassigned <i>Acidobacteriales</i> (6)			0.3	0.3	0.3	0.3	0.2	0.1	0.3	0.1	0.1	0.2	0.1	0.0	0.0	0.0
<i>Solibacteres,</i>																
<i>Solibacteraceae</i> (25)			0.6	0.6	0.6	0.4	0.6	0.9	0.7	0.1	0.9	0.9	0.9	0.1	0.1	0.1
Subgroup_11 (6)			0.1	0.1	0.3	0.2	0.1	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Subgroup_17 (22)			0.9	0.8	0.7	0.8	0.4	0.4	0.9	0.1	0.3	0.3	0.3	0.0	0.0	0.0
Subgroup_22 (26)			0.4	0.3	0.3	1.0	0.3	0.6	0.5	0.1	0.4	0.3	0.2	0.0	0.0	0.0
Subgroup_25 (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (106)			10	9.2	9.3	9.9	3.8	3.5	11	1.3	2.9	2.5	3.1	0.4	0.2	0.3
Unassigned <i>Acidobacteria</i> (25)			0.4	0.5	0.5	0.5	0.4	0.4	0.6	0.1	0.2	0.2	0.3	0.0	0.0	0.0
<b>Actinobacteria</b>																
<i>Actinobacteria,</i>																
<i>Acidimicrobiaceae</i> (18)			1.7	1.7	1.4	1.5	1.8	1.6	1.2	0.2	1.8	1.9	1.6	0.1	0.2	0.2
<i>lamiaceae</i> (8)			0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.0	0.2	0.1	0.1	0.0	0.0	0.0
Unassigned <i>Acidimicrobiales</i> (54)			3.7	3.4	3.2	3.0	2.8	2.2	2.7	0.4	2.5	2.7	2.2	0.1	0.1	0.3
<i>Actinobacteria,</i>																
<i>Actinomycetaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Catenulisporaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycobacteriaceae</i> (8)			1.0	0.9	1.0	0.6	0.7	0.3	0.9	0.1	0.5	0.4	0.7	0.0	0.0	0.0
<i>Nocardiaceae</i> (7)			0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Acidotherrmaceae</i> (5)			0.5	0.4	0.5	0.6	0.4	0.4	0.3	0.0	0.4	0.3	0.4	0.0	0.0	0.0
<i>Frankiaceae</i> (3)			0.3	0.2	0.2	0.1	0.4	0.2	0.1	0.0	0.2	0.3	0.4	0.0	0.0	0.0
<i>Geodermatophilaceae</i> (1)			0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
<i>Nakamurellaceae</i> (3)			0.1	0.1	0.1	0.1	0.3	0.3	0.1	0.0	0.2	0.2	0.2	0.0	0.0	0.0
<i>Sporichthyaceae</i> (4)			0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.2	0.1	0.0	0.0	0.0
Unassigned <i>Frankiales</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Kineosporiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellulomonadaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Brevibacteriaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Demequinaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (3)			0.2	0.3	0.2	0.1	0.4	0.3	0.2	0.0	0.3	0.4	0.4	0.0	0.0	0.0
<i>Microbacteriaceae</i> (5)			0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micrococcaceae</i> (3)			0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.0	0.1	0.2	0.3	0.0	0.0	0.0
<i>Promicromonosporaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Micromonosporaceae</i> (30)			2.7	2.7	2.4	1.3	3.7	1.6	2.6	0.4	3.8	4.1	3.3	0.1	0.1	0.2
<i>Nocardiodiaceae</i> (21)			1.9	2.1	1.9	1.6	2.4	2.0	2.0	0.2	2.0	2.0	2.1	0.1	0.2	0.1
<i>Propionibacteriaceae</i> (5)			0.3	0.3	0.2	0.1	0.2	0.1	0.2	0.0	0.3	0.1	0.2	0.0	0.0	0.0
<i>Pseudonocardiaceae</i> (11)			0.5	0.5	0.4	0.2	0.9	0.6	0.3	0.1	0.8	1.0	0.7	0.1	0.1	0.1
<i>Streptomyetaceae</i> (2)			1.1	1.1	1.0	0.6	1.3	0.9	0.9	0.1	1.3	1.2	1.5	0.0	0.0	0.0
<i>Streptosporangiaceae</i> (4)			0.2	0.2	0.2	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Thermomonosporaceae</i> (6)			0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Unassigned <i>Actinobacteria</i> (3)			0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
KIST-JY010 group (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MB-A2-108 group (21)			2.8	3.1	2.7	2.8	1.0	0.7	2.6	0.4	0.9	0.9	0.8	0.1	0.1	0.1
<i>Rubrobacteria,</i>																
<i>Rubrobacteriaceae</i> (6)			0.5	0.5	0.3	0.3	0.6	0.4	0.2	0.0	0.5	0.6	0.6	0.0	0.0	0.0
<i>Thermoleophilia,</i>																
<i>Gaiellaceae</i> (6)			3.5	3.8	2.9	3.0	1.6	1.3	2.9	0.4	1.1	0.9	1.2	0.1	0.1	0.1
Unassigned <i>Gaiellales</i> (32)			6.6	6.9	5.3	4.6	2.0	1.7	4.7	0.8	1.5	1.2	1.6	0.1	0.1	0.1



Phyla, Class, Family <sup>b</sup>		Sampling Time:		0 h				40 h								
		Treatment <sup>a</sup> :		C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R
		Relative Abundance (%)														
	Unassigned <i>Obscuribacterales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S085 group (11)	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.0	0.2	0.1	0.2	0.0	0.0	0.0	0.0
	SBR2076 group (10)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Thermomicrobia</i> ,															
	Unassigned <i>Thermomicrobia</i> (33)	0.8	0.9	0.7	0.4	0.6	0.3	1.0	0.1	0.4	0.4	0.5	0.0	0.0	0.0	
	TK10 group (15)	0.3	0.4	0.3	0.2	0.5	0.6	0.2	0.0	0.5	0.6	0.6	0.0	0.0	0.0	
	Unassigned <i>Chloroflexi</i> (7)	0.5	0.6	0.4	0.8	0.2	0.1	0.5	0.0	0.1	0.1	0.1	0.0	0.0	0.0	
	<b>Deinococcus-Thermus,</b>															
	<i>Deinococci</i> ,															
	Unassigned <i>Deinococci</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Elusimicrobia,</b>															
	<i>Elusimicrobia</i> ,															
	Unassigned <i>Elusimicrobia</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Fibrobacteres,</b>															
	<i>Fibrobacteria</i> ,															
	<i>Fibrobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<b>Firmicutes,</b>															
	<i>Bacilli</i> ,															
	<i>Alicyclobacillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Bacillaceae</i> (16) [E19]	1.2	1.3	2.1	0.6	1.1	0.7	1.4	7.9	1.6	1.4	1.8	6.2	7.4	6.3	
	<i>Paenibacillaceae</i> (24)	0.2	0.1	0.3	0.1	0.1	0.0	0.2	0.3	0.1	0.1	0.1	0.9	0.8	0.8	
	<i>Pasteuriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Planococcaceae</i> (7)	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	
	<i>Staphylococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Thermoactinomycetaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Aerococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Streptococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Lactobacillales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Bacillales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Bacilli</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Clostridia</i> ,															
	<i>Caldicoprobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Christensenellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Clostridiaceae</i> (20) [E6, E13, E16, E17]	0.1	0.1	0.5	0.2	0.0	0.1	0.3	8.7	0.2	0.1	0.1	23	21	21	
	<i>Eubacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Gracilibacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Hellobacteriaceae</i> (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Lachnospiraceae</i> (15)	0.0	0.0	0.2	0.1	0.0	0.0	0.0	1.9	0.0	0.0	0.0	1.7	1.6	1.8	
	<i>Peptococcaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Peptostreptococcaceae</i> (5) [E5, E4]	0.0	0.0	0.3	0.3	0.1	0.1	0.1	21	0.0	0.1	0.1	9.4	13	12	
	<i>Ruminococcaceae</i> (16)	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Unassigned <i>Clostridiales</i> (12)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.2	0.2	
	<i>Thermoanaerobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Erysipelotrichia</i> ,															
	<i>Erysipelotrichaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Limnochordia</i> ,															
	<i>Limnochordaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Negativicutes</i> ,															
	<i>Veillonellaceae</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.3	0.4	
	Unassigned <i>Selenomonadales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<b>Fusobacteria,</b>															
	<i>Fusobacteria</i> ,															
	Unassigned <i>Fusobacteriales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	<b>Gemmatimonadetes,</b>															
	<i>Gemmatimonadetes</i> ,															
	<i>Gemmatimonadaceae</i> (51)	2.0	1.8	1.8	4.3	1.9	1.1	1.6	0.2	1.4	1.5	1.7	0.1	0.0	0.1	
	Unassigned <i>Gemmatimonadetes</i> (6)	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
	<b>Latescibacteria,</b>															
	Unassigned <i>Latescibacteria</i> (37)	0.5	0.5	0.5	1.3	0.6	1.8	0.6	0.1	0.5	0.5	0.6	0.0	0.0	0.1	
	<b>Nitrospirae,</b>															



Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h					40 h							
	Treatment <sup>a</sup> :	C1.D	C2.D	C3.D	E.D	C.R	E.R	C.D	E.D	C1.R	C2.R	C3.R	E1.R	E2.R	E3.R
<i>Bdellovibrionaceae</i> (16)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Desulfarculaceae</i> (37)	1.8	2.0	2.1	1.6	1.4	1.0	1.0	2.0	0.2	0.9	1.0	1.1	0.1	0.1	0.1
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (14)	0.3	0.2	0.4	0.2	0.5	0.6	0.6	0.4	0.1	0.4	0.5	0.4	0.0	0.0	0.1
<i>Archangiaceae</i> (9)	0.2	0.2	0.2	0.0	0.2	0.1	0.2	0.2	0.0	0.9	0.7	1.1	0.0	0.1	0.0
<i>Haliangiaceae</i> (57)	0.8	0.7	0.6	0.7	1.2	2.1	2.1	0.7	0.1	0.7	0.7	0.8	0.1	0.1	0.1
<i>Myxococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicytidaceae</i> (5)	0.2	0.2	0.2	0.1	0.2	0.3	0.3	0.2	0.0	0.1	0.2	0.2	0.0	0.0	0.0
<i>Polyangiaceae</i> (28)	0.2	0.2	0.2	0.1	0.3	0.7	0.7	0.2	0.0	0.2	0.3	0.2	0.0	0.0	0.1
<i>Sandaracinaceae</i> (23)	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Vulgatibacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (71)	0.5	0.6	0.5	0.5	0.8	1.5	1.5	0.4	0.0	0.6	0.4	0.5	0.0	0.1	0.0
NB1-j group (13)	0.3	0.4	0.3	0.7	0.2	0.2	0.2	0.4	0.0	0.2	0.1	0.1	0.0	0.0	0.0
<i>Oligoflexaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Oligoflexales</i> (24)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Gammaproteobacteria,</b>															
<i>Acidiferrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Aeromonadaceae</i> (2) [E3]	0.0	0.0	0.2	0.1	0.0	0.2	0.2	0.2	21	0.0	0.0	0.1	37	28	22
<i>Shewanellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cellvibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haliaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spongilbacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (4) [E2, E314]	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	24	0.0	0.1	0.1	15	20	28
<i>Coxiellaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Legionellaceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Halomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oceanospirillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pasteurellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Moraxellaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.1	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (2)	0.0	0.1	0.1	0.1	0.1	0.3	0.2	0.2	0.0	0.1	0.2	0.1	0.0	0.0	0.0
Unassigned <i>Thiotrichales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solimonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthomonadaceae</i> (14)	0.1	0.2	0.1	0.3	0.1	0.2	0.2	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Unassigned <i>Xanthomonadales</i> (26)	0.9	1.1	1.2	1.5	1.3	1.5	1.5	1.0	0.1	0.6	0.7	0.7	0.0	0.0	0.1
Unassigned <i>Gammaproteobacteria</i> (14)	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Saccharibacteria,</b>															
Unassigned <i>Saccharibacteria</i> (1)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Spirochaetae,</b>															
<i>Spirochaetes,</i>															
<i>Spirochaetaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia</b>															
Unassigned <i>Tectomicrobia</i> (12)	0.4	0.5	0.7	0.3	3.0	2.8	2.8	0.4	0.1	3.1	3.2	3.5	0.2	0.2	0.2
<b>Tenericutes</b>															
<i>Mollicutes,</i>															
<i>Mycoplasmataceae</i> (5)	0.0	0.0	0.6	0.3	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Haloplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Entomoplasmatales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>TM6-Dependentiae</b>															
Unassigned <i>TM6-Dependentiae</i> (5)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia</b>															
OPB35 group,															
Unassigned OPB35 group (74)	0.9	0.8	1.2	2.1	0.8	2.3	2.3	1.4	0.2	0.9	0.9	1.0	0.1	0.2	0.2
<i>Opitutae,</i>															
<i>Opitutaceae</i> (4)	0.0	0.0	0.0	0.1	0.1	0.4	0.4	0.0	0.0	0.1	0.2	0.1	0.0	0.0	0.0
Unassigned <i>Opitutae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria,</i>															
<i>Chthoniobacteraceae</i> (32)	0.3	0.2	0.4	0.8	0.2	0.5	0.5	0.3	0.1	0.2	0.3	0.2	0.1	0.1	0.0
Unassigned <i>Chthoniobacteriales</i> (23)	2.5	2.7	3.3	3.5	0.7	1.0	1.0	3.7	0.7	0.5	0.6	0.4	0.1	0.1	0.1



Phyla, Class, Family <sup>a</sup>	Sampling Time:		0 h						5 h		10 h		20 h		30 h					
	Treatment:		U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
	Relative Abundance (%)																			
<i>Dermabacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Dermacoccaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (4)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.0	0.0	0.1
<i>Geodermatophilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Glycomycetaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (3)	0.6	0.6	0.6	0.7	0.4	0.6	0.6	0.6	0.6	0.7	0.6	1.0	0.5	1.0	0.9	1.1	0.4	0.4	0.5	0.5
<i>Kineosporiaceae</i> (1)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Microbacteriaceae</i> (11)	0.6	0.5	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.8	0.4	0.7	0.8	0.8	0.2	0.3	0.3	0.3
<i>Micrococcaceae</i> (3)	0.4	0.4	0.4	0.2	0.3	0.3	0.3	0.2	0.4	0.3	0.4	0.2	0.5	0.7	0.5	0.2	0.2	0.3	0.3	0.3
<i>Micromonosporaceae</i> (28)	1.3	1.3	1.4	0.9	1.0	0.9	0.9	1.1	1.6	1.1	2.1	1.1	2.2	2.2	2.4	0.9	0.7	0.8	0.8	0.8
<i>Mycobacteriaceae</i> (8)	1.4	1.4	1.4	0.9	1.0	0.9	0.9	1.0	1.3	0.9	1.3	1.0	1.3	1.6	1.6	0.9	0.6	0.8	0.8	0.8
<i>Nakamurellaceae</i> (2)	0.3	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2
<i>Nocardiaceae</i> (9)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Nocardioidaceae</i> (28)	2.5	2.6	2.6	2.5	2.1	2.4	2.6	2.5	3.0	2.1	3.6	2.0	3.4	3.7	4.1	1.8	1.5	1.5	1.5	1.5
<i>Promicromonosporaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Propionibacteriaceae</i> (10)	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.4	0.3	0.1	0.1	0.2	0.1	0.2
<i>Pseudonocardiaceae</i> (12)	0.9	1.0	0.9	0.5	0.7	0.6	1.0	0.8	1.2	0.7	1.2	0.8	1.1	1.3	1.4	0.5	0.6	0.6	0.6	0.6
<i>Sporichthyaceae</i> (3)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1
<i>Streptomyces</i> (6)	1.1	1.2	1.2	0.9	0.9	0.9	1.0	1.0	1.3	0.8	1.7	0.8	1.9	1.8	1.9	0.7	0.5	0.7	0.5	0.7
<i>Streptosporangiaceae</i> (3)	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.0	0.1
<i>Thermomonosporaceae</i> (8)	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Actinobacteria</i> (3)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Coriobacteria</i> ,																				
<i>Coriobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Coriobacteria</i> (23)	2.4	2.7	2.5	2.1	2.0	2.1	1.6	1.9	2.5	1.9	2.8	1.6	3.0	3.3	3.7	1.6	1.4	1.4	1.4	1.4
<i>Rubrobacteria</i> ,																				
<i>Rubrobacteriaceae</i> (3)	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
<i>Thermoleophilia</i> ,																				
<i>Coxiobacteraceae</i> (3)	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Gaiellaceae</i> (5)	1.9	2.2	2.1	1.5	1.4	1.5	1.7	1.7	2.0	1.4	2.2	1.4	2.1	2.1	2.4	1.2	1.0	1.5	1.5	1.5
<i>Parviterribacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Solirubrobacteraceae</i> (4)	0.5	0.7	0.6	0.7	0.5	0.6	0.6	0.9	0.8	0.7	0.8	0.7	0.7	0.7	0.9	0.6	0.4	0.5	0.5	0.5
Unassigned <i>Solirubrobacterales</i> (54)	2.6	3.0	2.6	2.1	1.8	1.9	2.1	2.5	3.3	2.4	3.8	2.4	3.4	3.7	4.3	1.7	1.4	1.6	1.6	1.6
Unassigned <i>Thermoleophilia</i> (44)	2.6	2.8	2.5	2.3	1.9	2.0	2.0	2.4	2.5	2.0	3.1	2.1	3.0	3.3	3.7	1.7	1.6	1.6	1.6	1.6
<b>Armatimonadetes,</b>																				
Unassigned <i>Armatimonadia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chthonomonadetes,</i>																				
<i>Chthonomonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Chthonomonadetes</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Armatimonadetes</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes,</b>																				
<i>Bacteroidia,</i>																				
<i>Bacteroidaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Porphyromonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Prevotellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rikenellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia,</i>																				
<i>Cytophagaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Flavobacteriia,</i>																				
<i>Flavobacteriaceae</i> (9)	1.5	1.5	1.6	1.8	2.0	2.2	0.0	1.2	0.0	1.2	0.0	0.8	0.0	0.0	0.0	0.6	0.4	0.5	0.5	0.5
<i>Sphingobacteria,</i>																				
<i>Chitinophagaceae</i> (23)	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1
<i>Lentimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Saprosiraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingobacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (4)	0.0	0.0	0.0	0.2	0.2	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
<b>BJ-169,</b>																				
Unassigned <i>BJ-169</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1,</b>																				



Phyla, Class, Family <sup>p</sup>	Sampling Time:		0 h						5 h		10 h		20 h		30 h					
	Treatment:		U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
	Relative Abundance (%)																			
<i>Clostridiaceae</i> (27)	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.5	0.2	1.8	0.3	0.4	0.3	3.5	3.5	2.6	
<i>Defluviitaleaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Eubacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Clostridiales</i> (17)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.3	0.3	0.2		
<i>Gracilibacteraceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0		
<i>Halanaerobiaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Hellobacteriaceae</i> (21)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0		
<i>Lachnospiraceae</i> (20)	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	1.9	0.3	0.2	0.3	3.7	4.9	4.0	
<i>Peptococcaceae</i> (5)	1.0	0.9	0.9	0.8	0.8	0.8	0.8	1.5	0.6	1.9	0.4	2.2	0.4	2.9	3.0	3.0	0.6	0.5	0.4	
<i>Peptostreptococcaceae</i> (5) [D5]	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.5	1.2	0.8	1.1	1.0	2.9	0.8	0.6	1.4	1.3	1.2	
<i>Ruminococcaceae</i> (39)	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	
<i>Syntrophomonadaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Thermoanaerobacteraceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Clostridia</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Erysipelotrichia</i> , <i>Erysipelotrichaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Limnochordia</i> , <i>Limnochordaceae</i> (17)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	
Unassigned <i>Limnochordaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Negativicutes</i> , <i>Veillonellaceae</i> (11)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Unassigned <i>Negativicutes</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Fusobacteria</b> , <i>Fusobacteria</i> , <i>Fusobacteriaceae</i> (1) [D1]	5.0	4.9	5.0	14	16	13	13	15	13	18	17	19	12	11	12	9.2	16	18	15	
<b>Gemmatimonadetes</b> , <i>Gemmatimonadetes</i> , <i>Gemmatimonadaceae</i> (22)	0.6	0.6	0.5	0.4	0.5	0.6	0.6	0.2	0.4	0.1	0.4	0.2	0.4	0.2	0.2	0.2	0.3	0.3	0.4	
Unassigned <i>Gemmatimonadetes</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Latescibacteria</b> , Unassigned <i>Latescibacteria</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<b>Nitrospirae</b> , <i>Nitrospira</i> , <i>Nitrospiraceae</i> (6)	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	
Unassigned <i>Nitrospirales</i> (10)	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.1	0.6	0.1	0.5	0.0	0.5	0.1	0.1	0.1	0.5	0.5	0.6	
<b>Planctomycetes</b> , <i>Phycisphaerae</i> , <i>Phycisphaeraceae</i> (16)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Tepididysphaeraceae</i> (46)	1.0	0.9	0.9	0.8	0.9	0.9	0.9	0.2	0.8	0.1	0.6	0.0	0.6	0.1	0.0	0.0	0.7	0.6	0.8	
Unassigned <i>Phycisphaerae</i> (7)	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	
<i>Planctomycetacia</i> , <i>Planctomycetaceae</i> (456)	4.9	4.7	4.4	3.8	4.0	4.2	4.2	2.8	3.7	3.3	3.4	2.4	3.5	3.2	3.8	3.9	4.1	3.5	3.7	
Unassigned <i>Planctomycetes</i> (28)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	
<b>Proteobacteria</b> , <i>Alphaproteobacteria</i> , <i>Acetobacteraceae</i> (8)	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<i>Anaplasmataceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bejerinckiacae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Bradyrhizobiaceae</i> (3)	1.4	1.4	1.4	0.9	1.1	1.2	1.2	0.7	1.1	1.0	1.0	1.3	0.9	1.1	1.6	1.7	0.9	0.8	0.9	
<i>Caulobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Holospiraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Hyphomicrobiaceae</i> (5)	0.3	0.4	0.4	0.3	0.2	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.3	0.5	0.6	0.7	0.2	0.2	0.2	
<i>Methylobacteriaceae</i> (5)	0.4	0.4	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.2	0.4	0.2	0.4	0.4	0.5	0.2	0.2	0.2	
<i>Methylocystaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Mitochondria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Phyllobacteriaceae</i> (3)	0.5	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.4	0.3	0.5	0.2	0.6	0.7	0.9	0.2	0.2	0.3	
<i>Rhizobiaceae</i> (3)	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1	
<i>Rhodobacteraceae</i> (5)	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.0	0.0	0.0	
<i>Rhodobiaceae</i> (2)	1.5	1.6	1.5	1.2	1.1	1.1	1.1	1.1	1.2	1.4	1.0	1.8	0.9	2.4	2.5	2.9	0.9	0.9	0.8	



Phyla, Class, Family <sup>p</sup>	Sampling Time:		0 h			5 h		10 h		20 h		30 h							
	Treatment:	U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
<b>Relative Abundance (%)</b>																			
<b>Synergistetes,</b>																			
<i>Synergistia,</i>																			
<i>Synergistaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia,</b>																			
Unassigned <i>Tectomicrobia</i> (8)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.0	0.2	0.0	0.0	0.0	0.2	0.1	0.1	
<b>Tenericutes,</b>																			
<i>Mollicutes,</i>																			
<i>Haloplasmataceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mycoplasmataceae</i> (5) [D3]	12	10	12	9.9	8.5	8.9	7.9	5.8	3.1	4.9	0.3	3.6	0.4	0.2	0.2	6.0	5.5	4.2	
Unassigned <i>Mollicutes</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>TM6_Dependentiae,</b>																			
Unassigned <i>TM6_Dependentiae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia,</b>																			
<i>OPB35 soil group</i> (42)	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	
<i>Opiritatae,</i>																			
<i>Opiritaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opiritatae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria,</i>																			
<i>Chthoniobacteraceae</i> (20)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Xiphinematobacteraceae</i> (4)	1.2	1.1	1.0	0.9	0.9	1.1	1.3	0.8	1.4	0.8	1.7	0.9	2.2	2.4	2.3	0.9	0.8	1.0	
Unassigned <i>Spartobacteria</i> (25)	2.9	2.9	2.6	2.3	2.2	2.4	1.2	2.1	1.1	1.8	1.3	1.8	1.4	2.0	2.3	2.3	2.0	2.0	
<i>Verrucomicrobiae,</i>																			
<i>Verrucomicrobiaceae</i> (10)	0.4	0.4	0.4	0.4	0.4	0.4	0.7	0.3	0.4	0.2	0.3	0.2	1.1	0.8	0.7	0.2	0.3	0.1	
Unassigned <i>Verrucomicrobiae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archeae</b>																			
<b><i>Thaumarchaeota,</i></b>																			
Unassigned <i>Thaumarchaeota</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

### (B) 16S rRNA

Phyla, Class, Family <sup>p</sup>	Sampling Time:		0 h			5 h		10 h		20 h		30 h							
	Treatment:	U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
<b>Relative Abundance (%)</b>																			
<b>Acidobacteria,</b>																			
<i>Acidobacteria,</i>																			
<i>Acidobacteriaceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Blastocatellia,</i>																			
<i>Blastocatellaceae</i> (9)	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
<i>Holophagae,</i>																			
Unassigned <i>Holophagae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solibacteres,</i>																			
<i>Solibacteraceae</i> (17)	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.3	0.2	0.2	
Subgroup_2 (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_5 (7)	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_6 (78)	0.4	0.5	0.6	0.4	0.9	0.7	0.1	0.8	0.1	0.7	0.0	0.4	0.1	0.1	0.1	0.6	0.3	0.5	
Subgroup_11 (4)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_13 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_17 (14)	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_18 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_22 (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subgroup_25 (5)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria,</b>																			
<i>Acidimicrobia,</i>																			
<i>Acidimicrobiaceae</i> (21)	0.8	1.0	0.7	0.6	0.5	0.7	0.7	0.9	0.7	0.6	0.5	0.7	0.8	0.8	0.7	0.9	0.7	0.7	
<i>lamiaceae</i> (11)	0.1	0.2	0.2	0.0	0.1	0.0	0.1	0.3	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	



Phyla, Class, Family <sup>a</sup>	Sampling Time:		0 h						5 h		10 h		20 h		30 h						
	Treatment:		U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3	
	Relative Abundance (%)																				
<i>Flavobacteria</i> ,																					
<i>Flavobacteriaceae</i> (9)	1.0	0.9	1.1	1.8	1.7	2.4	0.1	0.7	0.0	0.9	0.0	1.6	0.0	0.0	0.0	0.4	0.7	0.7			
<i>Sphingobacteria</i> ,																					
<i>Chitinophagaceae</i> (23)	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0		
<i>Lentimicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Saprospiraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<i>Sphingobacteriaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Unassigned <i>Sphingobacteriales</i> (4)	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>BJ-169</b> ,																					
Unassigned BJ-169 (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>BRC1</b> ,																					
Unassigned BRC1 (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
<b>Chlamydiae</b> ,																					
<i>Chlamydiae</i> ,																					
<i>Parachlamydiaceae</i> (40)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0			
<i>Simkaniaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Unassigned <i>Chlamydiae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Chloroflexi</b> ,																					
<i>Anaerolineae</i> ,																					
<i>Anaerolineaceae</i> (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Caldilineae</i> ,																					
<i>Caldilineaceae</i> (10)	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1			
<i>Chloroflexia</i> ,																					
<i>Roseiflexaceae</i> (5)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1			
Unassigned <i>Chloroflexia</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Ktedonobacteria</i> ,																					
<i>Ktedonobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Thermosporotrichaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Unassigned <i>Ktedonobacteria</i> (11)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Thermomicrobia</i> ,																					
<i>Thermomicrobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Unassigned <i>Thermomicrobia</i> (33)	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.1	0.2	0.1			
Unassigned <i>Chloroflexi</i> (67)	0.7	0.8	1.0	0.5	0.7	0.5	0.6	0.7	0.9	0.8	0.7	0.5	1.0	0.7	1.2	0.6	0.5	0.5			
<b>Cyanobacteria</b> ,																					
<i>Chloroplast</i> ,																					
Unassigned <i>Chloroplast</i> (12)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Cyanobacteria</i> ,																					
Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Unassigned <i>Cyanobacteria</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Deferribacteres</b> ,																					
<i>Deferribacteres</i> ,																					
<i>Deferribacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Deinococcus-Thermus</b> ,																					
Unassigned <i>Deinococci</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Elusimicrobia</b> ,																					
Unassigned <i>Elusimicrobia</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Euryarchaeota</b> ,																					
<i>Methanomicrobia</i> ,																					
<i>Methanosarcinaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Fibrobacteria</b> ,																					
<i>Fibrobacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Unassigned <i>Fibrobacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<b>Firmicutes</b> ,																					
<i>Bacilli</i> ,																					
<i>Alicyclobacillaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Bacillaceae</i> (31)	0.7	0.9	1.0	0.6	0.6	0.7	1.3	0.8	1.6	0.7	2.4	0.7	2.9	3.8	3.4	0.9	1.0	0.9			
<i>Lactobacillaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Leuconostocaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
<i>Paenibacillaceae</i> (55)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.2			
<i>Pasteuriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						5 h		10 h		20 h		30 h					
	Treatment:		U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
<i>Planococcaceae</i> (7)			0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.0
<i>Sporolactobacillaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermoactinomycetaceae</i> (14)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacilli</i> (8)			0.7	0.5	0.7	0.4	0.6	0.4	0.6	0.5	1.4	0.3	0.4	0.2	0.2	0.1	0.0	0.4	0.2	0.3
<i>Clostridia</i> ,																				
<i>Caldicoprobacteraceae</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Christensenellaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Clostridiaceae</i> (27)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.1	2.1	0.4	0.1	0.1	4.5	4.4	4.3
<i>Defluviitaleaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Eubacteriaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Clostridiales</i> (17)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3	0.2	0.2	
<i>Gracilibacteraceae</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Halanaerobiaceae</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Helio bacteriaceae</i> (21)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lachnospiraceae</i> (20)			0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.2	1.4	0.2	0.2	0.2	3.0	3.3	2.8
<i>Peptococcaceae</i> (5)			0.3	0.3	0.2	0.4	0.4	0.5	0.6	0.4	0.7	0.2	0.9	0.3	1.7	2.1	1.8	0.3	0.4	0.3
<i>Peptostreptococcaceae</i> (5) [D5]			0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.7	1.9	1.0	4.2	1.2	10	8.1	7.9	1.8	1.6	1.6
<i>Ruminococcaceae</i> (39)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.2	0.2	
<i>Syntrophomonadaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermoanaerobacteraceae</i> (5)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Clostridia</i> (6)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erysipelotrichia</i> ,																				
<i>Erysipelotrichaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Limnochordia</i> ,																				
<i>Limnochordaceae</i> (17)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Limnochordaceae</i> (3)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Negativicutes</i> ,																				
<i>Veillonellaceae</i> (11)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Negativicutes</i> (2)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria</b> ,																				
<i>Fusobacteria</i> ,																				
<i>Fusobacteriaceae</i> (1) [D1]			14	12	11	25	19	24	24	19	26	27	42	24	27	31	27	22	25	22
<b>Gemmatimonadetes</b> ,																				
<i>Gemmatimonadetes</i> ,																				
<i>Gemmatimonadaceae</i> (22)			0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.2	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1
Unassigned <i>Gemmatimonadetes</i> (4)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Latescibacteria</b> ,																				
Unassigned <i>Latescibacteria</i> (10)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Nitrospirae</b> ,																				
<i>Nitrospira</i> ,																				
<i>Nitrospiraceae</i> (6)			0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1
Unassigned <i>Nitrospirales</i> (10)			0.2	0.3	0.4	0.1	0.2	0.3	0.0	0.3	0.1	0.3	0.0	0.2	0.1	0.0	0.0	0.1	0.2	0.2
<b>Planctomycetes</b> ,																				
<i>Phycisphaerae</i> ,																				
<i>Phycisphaeraceae</i> (16)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidisphaeraceae</i> (46)			0.6	0.7	0.6	0.5	0.6	0.5	0.2	0.9	0.1	0.6	0.0	0.5	0.0	0.0	0.0	0.3	0.3	0.3
Unassigned <i>Phycisphaerae</i> (7)			0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planctomycetacia</i> ,																				
<i>Planctomycetaceae</i> (456)			9.9	10	9.5	6.5	7.4	6.9	9.7	12	10	8.7	6.7	8.6	7.4	10	11	9.3	8.6	8.6
Unassigned <i>Planctomycetes</i> (28)			0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.1
<b>Proteobacteria</b> ,																				
<i>Alphaproteobacteria</i> ,																				
<i>Acetobacteraceae</i> (8)			0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Anaplasmatocaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Beijerinckiaceae</i> (1)			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (3)			1.5	1.4	1.3	0.9	1.1	1.0	0.8	1.1	1.3	0.9	1.1	1.0	1.2	1.8	1.9	0.9	0.8	1.1

Phyla, Class, Family <sup>b</sup>	Sampling Time:		0 h						5 h		10 h		20 h		30 h					
	Treatment:		U1	U2	U3	D1	D2	D3	U	D	U	D	U	D	U1	U2	U3	D1	D2	D3
	Relative Abundance (%)																			
<i>Caulobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Holosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Hyphomicrobiaceae</i> (5)	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.4	0.4	0.3	0.1	0.1	0.1
<i>Methylobacteriaceae</i> (5)	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.4	0.4	0.4	0.4	0.1	0.2	0.3
<i>Methylocystaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mitochondria</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phyllobacteriaceae</i> (3)	0.5	0.6	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.4	0.2	0.4	0.2	0.6	0.7	0.6	0.3	0.3	0.3	0.3
<i>Rhizobiaceae</i> (3)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (5)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (2)	0.3	0.2	0.5	0.2	0.2	0.2	0.2	0.2	0.3	0.7	0.2	0.4	0.2	0.9	0.6	0.9	0.2	0.3	0.3	0.3
<i>Rhodospirillaceae</i> (14)	0.8	0.9	1.1	0.6	0.6	0.8	0.8	0.8	0.8	1.1	0.6	0.9	0.8	0.8	0.6	0.9	0.8	0.6	0.7	0.7
<i>Rickettsiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (5)	1.5	1.8	2.1	0.9	1.0	1.0	1.0	1.1	1.7	2.3	1.2	1.4	1.1	1.6	1.9	2.1	1.1	1.0	1.1	1.1
Unassigned <i>Rhodospirillales</i> (22)	0.5	0.7	0.9	0.4	0.4	0.4	0.4	0.6	0.6	0.7	0.5	0.3	0.4	0.4	0.3	0.3	0.6	0.5	0.4	0.4
Unassigned <i>Rhizobiales</i> (17)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1
<i>Betaproteobacteria</i> ,																				
<i>Alcaligenaceae</i> (4)	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Burkholderiaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (8)	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<i>Nitrosomonadaceae</i> (20)	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
<i>Oxalobacteraceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (26)	0.1	0.0	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
<i>Deltaproteobacteria</i> ,																				
<i>Archangiaceae</i> (10)	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1
<i>Bacteriovoraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bdellovibrionaceae</i> (17)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.2	0.2	0.0	0.0	0.1	0.1
<i>Desulfarculaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfobivibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfurellaceae</i> (25)	0.3	0.2	0.4	0.2	0.3	0.2	0.2	0.1	0.2	0.1	0.2	0.0	0.2	0.1	0.0	0.1	0.2	0.2	0.2	0.2
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Haliangiaceae</i> (33)	0.2	0.1	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.3	0.1	0.2	0.2
<i>Myxococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
<i>Oligoflexaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phaselicytidaceae</i> (4)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0
<i>Polyangiaceae</i> (23)	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1
<i>Sandaracinaceae</i> (16)	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0
<i>Syntrophaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Vulgatibacteraceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Myxococcales</i> (57)	0.1	0.1	0.2	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0
Unassigned <i>Deltaproteobacteria</i> (37)	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
<i>Epsilonproteobacteria</i> ,																				
<i>Helicobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gammaproteobacteria</i> ,																				
<i>Aeromonadaceae</i> (2) [D4, D179]	21	23	22	25	22	25	25	22	24	17	22	5.9	18	3.7	3.8	4.0	15	16	19	19
<i>Coxiellaceae</i> (6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Enterobacteriaceae</i> (7)	4.4	5.0	4.9	5.0	5.4	5.4	5.4	4.2	3.7	4.4	4.1	2.4	3.5	3.7	2.3	2.7	4.5	3.3	3.6	3.6
<i>Haliaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Legionellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Methylococcaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (4)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.4	0.4	1.1	1.1
<i>Shewanellaceae</i> (2) [D2]	3.4	3.1	4.4	2.1	2.0	2.7	2.7	4.2	2.6	6.6	4.1	14	8.6	15	10	11	6.2	5.2	6.5	6.5
<i>Spongiibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Succinivibrionaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthomonadaceae</i> (7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



Treatment:	WB A	WB B	WB C	Turf A	Turf B	Turf C	Soil A	Soil B	Soil C	Turf 1	Turf 2	Turf 3	Soil 1	Soil 2	Soil 3
<b>Phyla, Class, Family<sup>b</sup></b>	<b>Relative Abundance (%)</b>														
<i>Blastocatellaceae</i> (4)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0
<i>Holophagae</i> , Unassigned <i>Holophagae</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Solibacteres</i> , <i>Solibacteraceae</i> (12)	0.0	0.0	0.0	0.3	0.5	0.0	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2
<i>Subgroup_5</i> (5)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.1	0.1	0.2	0.2	0.1
<i>Subgroup_6</i> (43)	0.0	0.0	0.5	1.0	1.5	0.3	1.0	1.5	0.8	0.7	0.9	0.5	1.5	1.6	0.9
<i>Subgroup_11</i> (3)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
<i>Subgroup_17</i> (4)	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_18</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_22</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Subgroup_25</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Actinobacteria</b> , <i>Acidimicrobia</i> , <i>Acidimicrobiaceae</i> (13)	0.0	0.1	0.2	1.7	1.5	0.5	3.1	1.2	0.6	1.0	0.9	0.9	1.6	1.3	1.4
Unassigned <i>Acidimicrobiales</i> (39)	0.3	0.3	0.5	1.9	2.3	0.8	4.3	2.5	1.7	1.4	1.5	1.4	3.2	3.9	3.4
<i>Iamiaeae</i> (5)	0.0	0.0	0.0	0.1	0.1	0.1	0.7	0.1	0.0	0.1	0.3	0.2	0.3	0.1	0.2
<i>Actinobacteria</i> , <i>Acidothermaceae</i> (8)	2.3	1.4	1.7	0.1	0.1	0.2	0.5	0.5	0.1	0.1	0.2	0.1	0.4	0.5	0.3
<i>Cellulomonadaceae</i> (1)	0.0	0.1	0.1	0.1	0.0	0.1	1.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
<i>Demequinaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Dermacoccaceae</i> (1)	0.3	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Frankiaceae</i> (3)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.0	0.1	0.2	0.1	0.2	0.2	0.1
<i>Geodermatophilaceae</i> (1)	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
<i>Glycomycetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Intrasporangiaceae</i> (3)	0.0	0.1	0.6	0.2	0.2	0.1	0.8	0.2	0.1	0.2	0.3	0.2	0.3	0.3	0.3
<i>Kineosporiaceae</i> (3)	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Microbacteriaceae</i> (7)	1.0	1.2	1.2	0.2	0.2	0.2	0.5	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1
<i>Micrococcaceae</i> (2)	0.5	1.1	1.3	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.1	0.2	0.2	0.1
<i>Micromonosporaceae</i> (18)	0.0	0.0	0.1	1.2	1.1	1.4	2.7	1.8	1.0	0.7	1.0	0.8	2.4	1.4	1.8
<i>Mycobacteriaceae</i> (7)	0.1	0.1	0.3	0.5	0.1	0.4	0.1	0.2	0.1	0.2	0.5	0.2	0.2	0.1	0.2
<i>Nakamurellaceae</i> (1)	0.0	0.0	0.0	0.1	0.1	0.0	0.5	0.0	0.0	0.2	0.1	0.1	0.1	0.0	0.1
<i>Nocardiaceae</i> (4)	0.0	0.1	0.0	0.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
<i>Nocardioidaceae</i> (19)	1.1	0.8	1.4	1.2	1.1	0.5	1.5	0.9	0.7	0.7	1.1	0.9	1.8	1.3	1.2
<i>Promicromonosporaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Propionibacteriaceae</i> (5)	0.0	0.0	0.0	0.3	0.2	0.2	0.8	0.5	0.1	0.3	0.4	0.3	0.5	0.6	0.3
<i>Pseudonocardiaceae</i> (11)	0.0	0.1	0.1	1.3	1.0	0.7	0.6	0.7	0.4	1.0	1.2	0.8	1.3	1.0	0.9
<i>Sporichthyaceae</i> (3)	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.2	0.1	0.1	0.1	0.1
Unassigned <i>Frankiales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Streptomycetaceae</i> (4)	0.4	0.3	0.3	0.6	0.7	0.4	1.0	0.4	0.5	0.5	0.8	0.5	0.8	1.0	1.0
<i>Streptosporangiaceae</i> (2)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Thermomonosporaceae</i> (3)	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0
Unassigned <i>Actinobacteria</i> (1)	0.0	0.0	0.0	0.2	0.0	0.1	1.1	0.0	0.0	0.1	0.1	0.2	0.1	0.1	0.0
<i>Coriobacteria</i> , <i>Coriobacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rubrobacteria</i> , <i>Rubrobacteriaceae</i> (3)	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.4	0.2	0.1	0.1	0.2	0.3	0.3	0.2
<i>Thermoleophila</i> , <i>Conexibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gaiellaceae</i> (5)	0.0	0.1	0.2	0.8	0.9	0.8	1.2	1.8	0.7	1.0	1.3	0.9	1.4	1.7	0.9
Unassigned <i>Gaiellales</i> (29)	0.3	0.3	0.5	1.3	1.6	1.1	1.7	3.3	1.1	1.3	1.7	1.3	2.3	2.1	1.7
<i>Parviterribacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Patulibacteraceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Solirubrobacteraceae</i> (4)	0.0	0.1	0.2	1.1	1.5	1.1	1.9	1.4	0.7	0.7	1.4	1.0	1.5	1.4	0.9
Unassigned <i>Solirubrobacterales</i> (13)	0.8	0.6	0.7	0.5	0.7	0.6	0.8	0.7	0.3	0.5	0.8	0.5	0.8	0.7	0.7
Unassigned <i>Thermoleophila</i> (27)	0.2	0.2	0.3	0.6	0.5	0.4	1.1	0.7	0.4	0.4	0.8	0.6	0.6	0.7	0.5
Unassigned <i>Actinobacteria</i> (18)	0.0	0.0	0.1	0.4	0.8	0.3	1.1	1.8	0.7	0.5	0.8	0.5	1.2	1.3	1.2
<b>Armatimonadetes</b> , Unassigned <i>Armatimonadetes</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Bacteroidetes</b> , <i>Bacteroidia</i> ,															

Treatment:	WB A	WB B	WB C	Turf A	Turf B	Turf C	Soil A	Soil B	Soil C	Turf 1	Turf 2	Turf 3	Soil 1	Soil 2	Soil 3
Phyla, Class, Family <sup>b</sup>	Relative Abundance (%)														
<i>Bacteroidaceae</i> (5)	0.1	1.4	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>Porphyromonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Prevotellaceae</i> (2)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rikenellaceae</i> (2)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cytophagia</i> ,															
<i>Cytophagaceae</i> (7)	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
<i>Flavobacteria</i> (13)	4.5	16	2.7	1.8	0.7	0.4	0.3	2.5	0.6	0.5	0.2	0.4	0.0	0.2	0.2
<i>Sphingobacteria</i> ,															
<i>Chittinophagaceae</i> (9)	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
<i>Sphingobacteriaceae</i> (6)	0.3	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Sphingobacteriales</i> (2)	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>BRC1</b> ,															
Unassigned <i>BRC1</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chlamydiae</b> ,															
<i>Chlamydiae</i> ,															
<i>Parachlamydiaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Chloroflexi</b> ,															
<i>Anaerolineae</i> ,															
<i>Anaerolineaceae</i> (5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
<i>Caldilineae</i> ,															
<i>Caldilineaceae</i> (8)	0.0	0.0	0.1	0.1	0.3	0.0	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.3	0.1
<i>Chloroflexia</i> ,															
<i>Roseiflexaceae</i> (6)	0.0	0.0	0.0	0.1	0.3	0.0	0.2	0.3	0.2	0.1	0.1	0.2	0.3	0.3	0.2
Unassigned <i>Kallotenuales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ktedonobacteria</i> ,															
Unassigned <i>Ktedonobacterales</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Ktedonobacteria</i> (6)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
<i>Thermomicrobia</i> ,															
Unassigned <i>Thermomicrobia</i> (22)	0.0	0.1	0.1	0.2	0.6	0.2	0.8	0.2	0.2	0.2	0.4	0.3	0.2	0.5	0.2
Unassigned <i>Chloroflexi</i> (44)	0.1	0.0	0.4	1.1	1.7	0.6	2.7	2.3	1.0	1.1	1.9	1.4	2.4	2.8	2.3
<b>Cyanobacteria</b> ,															
<i>Chloroplast</i> ,															
Unassigned <i>Chloroplast</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cyanobacteria</i> ,															
Unassigned <i>Cyanobacteria</i> (2)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
<b>Deinococcus-Thermus</b> ,															
<i>Deinococci</i> ,															
Unassigned <i>Deinococci</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Elusimicrobia</b> ,															
<i>Elusimicrobia</i> ,															
Unassigned <i>Elusimicrobia</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Firmicutes</b> ,															
<i>Bacilli</i> ,															
<i>Bacillaceae</i> (4)	1.0	1.1	1.4	0.8	0.5	1.2	0.5	0.7	0.5	0.7	1.9	1.0	0.8	0.8	0.6
<i>Lactobacillaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Paenibacillaceae</i> (13)	0.1	0.1	0.3	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.1
<i>Pasteuriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Planococcaceae</i> (4)	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
<i>Streptococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Thermoactinomycetaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Bacilli</i> (2)	0.0	0.0	0.0	0.0	1.4	1.1	0.0	0.0	0.0	0.4	0.9	0.3	0.4	0.3	0.2
<i>Clostridia</i> ,															
<i>Clostridiaceae</i> (9)	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2
Unassigned <i>Clostridiales</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Eubacteriaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gracilibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lachnospiraceae</i> (13)	0.1	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Treatment:	WB A	WB B	WB C	Turf A	Turf B	Turf C	Soil A	Soil B	Soil C	Turf 1	Turf 2	Turf 3	Soil 1	Soil 2	Soil 3
<b>Phyla, Class, Family<sup>b</sup></b>	<b>Relative Abundance (%)</b>														
<i>Peptococcaceae</i> (2)	0.3	2.6	0.1	0.4	0.5	0.8	0.3	1.6	0.3	0.8	1.7	1.7	1.8	1.5	1.2
<i>Peptostreptococcaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ruminococcaceae</i> (10)	0.4	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
<i>Erysipelotrichia</i> , <i>Erysipelotrichaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Fusobacteria</b> , <i>Fusobacteriia</i> , Unassigned <i>Fusobacteriales</i> (1)	0.2	4.6	0.1	0.6	3.0	3.3	0.1	4.1	17.1	3.0	1.6	2.4	0.1	0.9	1.8
<b>Gemmatimonadetes</b> , <i>Gemmatimonadetes</i> , <i>Gemmatimonadaceae</i> (13)	0.0	0.0	0.1	0.1	0.2	0.1	0.2	0.6	0.1	0.1	0.1	0.1	0.3	0.3	0.1
<b>Latescibacteria</b> , Unassigned <i>Latescibacteria</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Nitrospirae</b> , <i>Nitrospira</i> , <i>Nitrospiraceae</i> (4)	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0
Unassigned <i>Nitrospirales</i> (10)	0.0	0.0	0.1	0.4	0.9	0.2	1.1	1.4	0.5	0.3	0.5	0.3	1.1	1.1	0.4
<b>Planctomycetes</b> , <i>OM190</i> (10)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Phycisphaerae</i> , Unassigned <i>Phycisphaerae</i> (5)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Tepidisphaeraceae</i> (35)	0.0	0.0	0.1	0.6	1.1	0.1	1.6	0.5	0.2	0.3	0.2	0.5	0.6	1.1	0.4
<i>Planctomycetacia</i> , <i>Planctomycetaceae</i> (343)	4.3	3.2	8.3	11	16	8.6	20	14	5.6	9.1	6.1	9.7	7.9	10.5	7.0
Unassigned <i>Planctomycetes</i> (4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Proteobacteria</b> , <i>Alphaproteobacteria</i> , <i>Acetobacteraceae</i> (7)	0.2	0.1	0.2	0.3	0.3	0.1	0.3	0.1	0.1	0.2	0.1	0.3	0.3	0.2	0.2
<i>Beijerinckiaceae</i> (1)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Bradyrhizobiaceae</i> (3)	0.1	0.1	0.2	2.5	1.7	0.7	2.0	1.1	0.8	1.3	1.5	1.0	2.0	1.9	1.7
<i>Caulobacteraceae</i> (4)	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Erythrobacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Hyphomicrobiaceae</i> (4)	0.1	0.1	0.1	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.5	0.3	0.3
<i>Methylobacteriaceae</i> (3)	0.0	0.0	0.1	0.7	0.3	0.1	0.4	0.1	0.0	0.3	0.6	0.3	0.3	0.5	0.4
<i>Phyllobacteriaceae</i> (3)	0.0	0.1	0.1	0.6	0.5	0.1	0.7	0.2	0.2	0.4	0.4	0.4	0.5	0.5	0.4
<i>Rhizobiaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodobacteraceae</i> (4)	0.1	0.1	0.1	0.1	0.1	0.0	0.4	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.1
<i>Rhodobiaceae</i> (2)	0.1	0.1	0.1	0.5	0.5	0.2	0.5	0.1	0.2	0.2	0.4	0.3	0.7	0.4	0.6
<i>Rhodospirillaceae</i> (12)	0.0	0.0	0.3	2.2	1.9	1.0	2.3	1.6	0.8	1.5	1.9	1.1	2.9	2.4	2.6
Unassigned <i>Rhodospirillales</i> (25)	0.1	0.1	0.3	2.3	1.5	0.9	2.5	0.6	0.4	0.9	1.6	1.1	1.2	1.8	1.1
Unassigned <i>Rickettsiales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Roseiarcaceae</i> (1)	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sphingomonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Xanthobacteraceae</i> (3)	0.1	0.1	0.4	2.3	2.6	1.3	4.0	2.4	0.9	1.6	2.5	1.8	2.8	2.5	2.3
Unassigned <i>Rhizobiales</i> (12)	0.0	0.0	0.1	0.5	0.5	0.1	0.4	0.4	0.1	0.2	0.3	0.3	0.4	0.6	0.5
<b>Betaproteobacteria</b> , <i>Alcaligenaceae</i> (2)	0.0	0.0	0.1	0.3	0.3	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.6	0.4	0.1
<i>Burkholderiaceae</i> (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Comamonadaceae</i> (10)	0.1	0.3	0.1	0.3	0.3	0.0	0.6	0.2	0.1	0.1	0.3	0.1	0.4	0.3	0.1
<i>Gallionellaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Methylophilaceae</i> (1)	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitrosomonadaceae</i> (16)	0.0	0.0	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.3	0.4	0.1
<i>Oxalobacteraceae</i> (3)	0.2	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Rhodocyclaceae</i> (4)	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Betaproteobacteria</i> (11)	0.0	0.0	0.1	0.2	0.2	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.4	0.1	0.1
<b>Deltaproteobacteria</b> , Unassigned <i>Oligoflexales</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Archangiaceae</i> (4)	0.0	0.0	0.0	0.1	0.2	0.2	0.3	0.3	0.1	0.1	0.2	0.1	0.2	0.3	0.1
<i>Bdellovibrionaceae</i> (6)	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1
<i>Desulfobulbaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Desulfovibrionaceae</i> (1)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Treatment:	WB A	WB B	WB C	Turf A	Turf B	Turf C	Soil A	Soil B	Soil C	Turf 1	Turf 2	Turf 3	Soil 1	Soil 2	Soil 3
<b>Phyla, Class, Family<sup>b</sup></b>	<b>Relative Abundance (%)</b>														
<i>Desulfurellaceae</i> (21)	0.0	0.0	0.1	0.5	0.9	0.4	0.7	1.1	0.7	0.5	0.8	0.3	1.6	1.7	1.0
<i>Desulfuromonadaceae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Geobacteraceae</i> (10)	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.2	0.0	0.2	0.3	0.2	0.3	0.4	0.1
<i>Haliangiaceae</i> (24)	0.0	0.0	0.1	0.3	0.3	0.2	0.3	0.1	0.1	0.2	0.4	0.2	0.4	0.2	0.3
<i>Myxococcaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nannocystaceae</i> (2)	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
<i>Phaseolycystidaceae</i> (1)	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0
<i>Polyangiaceae</i> (18)	0.0	0.1	0.0	0.2	0.3	0.3	1.6	0.1	0.1	0.1	0.4	0.3	0.5	0.3	0.2
<i>Sandaracinaceae</i> (14)	0.0	0.1	0.0	0.2	0.2	0.1	0.7	0.1	0.0	0.1	0.1	0.2	0.2	0.2	0.0
<i>Vulgatibacteraceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Unassigned <i>Myxococcales</i> (30)	0.0	0.0	0.1	0.2	0.3	0.3	2.3	0.3	0.2	0.2	0.4	0.3	0.7	0.4	0.4
Unassigned <i>Deltaproteobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Gammaproteobacteria,</b>															
<i>Aeromonadaceae</i> (3)	10	8.5	7.9	8.7	9.8	31	5.3	16	24	19	11	14	1.0	6.1	13
<i>Coxiellaceae</i> (1)	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.0	0.0	0.1
<i>Enterobacteriaceae</i> (6)	15	8.4	6.4	8.1	2.7	2.8	0.7	0.2	0.6	5.6	4.8	4.9	0.2	0.5	0.8
<i>Moraxellaceae</i> (2)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Oleiphilaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonadaceae</i> (3)	14	21	9.8	1.3	0.4	0.4	0.0	0.0	0.0	1.2	0.5	0.5	0.0	0.0	0.0
<i>Shewanellaceae</i> (3)	0.1	1.0	0.2	0.9	0.9	1.3	1.2	4.4	8.2	4.8	3.2	3.7	0.2	1.2	3.3
<i>Xanthomonadaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Xanthomonadales</i> (11)	0.0	0.0	0.1	0.1	0.2	0.0	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Unassigned <i>Gammaproteobacteria</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Spirochaetes,</b>															
<i>Spirochaetes,</i>															
<i>Spirochaetaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Tectomicrobia,</b>															
Unassigned <i>Tectomicrobia</i> (11)	0.0	0.1	0.1	1.2	1.8	0.6	1.8	1.6	0.8	1.4	1.8	1.3	2.3	2.7	1.9
<b>Tenericutes,</b>															
<i>Mollicutes,</i>															
<i>Mycoplasmataceae</i> (5)	40	19	46	26	19	15	6.6	17	21	27	29	32	34	25	29
<b>TM6 Dependientiae,</b>															
Unassigned <i>TM6 Dependientiae</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Verrucomicrobia,</b>															
<i>OPB35 soil group</i> (24)	0.0	0.0	0.0	0.2	0.4	0.0	0.5	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2
<i>Opiritae,</i>															
<i>Opiritaceae</i> (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unassigned <i>Opiritaceae</i> (1)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spartobacteria,</i>															
<i>Chthoniobacteraceae</i> (10)	0.0	0.0	0.0	0.1	0.2	0.0	0.4	0.0	0.0	0.2	0.1	0.0	0.1	0.2	0.1
Unassigned <i>Chthoniobacterales</i> (17)	0.0	0.0	0.0	0.4	0.7	0.1	0.5	0.1	0.2	0.2	0.4	0.5	0.5	0.5	0.3
<i>Xiphinematobacteraceae</i> (3)	0.0	0.0	0.0	3.3	3.4	0.5	2.3	0.5	1.1	2.2	1.3	2.0	2.4	3.2	3.1
<i>Verrucomicrobiae,</i>															
<i>Verrucomicrobiaceae</i> (5)	0.0	0.1	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Archaea</b>															
<b>Thaumarchaeota,</b>															
Unassigned <i>Thaumarchaeota</i> (2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>Capital letter assigned to a substrate [e.g., Turf A] indicate the respective individual. Number assigned to a substrate [e.g., Turf 1] indicate the respective replicate of the three replicate analyses of pooled gut content from approximately 20 individuals per substrate. Abbreviations: WB, worm bedding.

<sup>b</sup>The number of phylotypes are shown in parenthesis.

## **10. (EIDESSTATTLICHE) VERSICHERUNGEN UND ERKLÄRUNGEN**

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Hiermit versichere ich eidesstattlich, dass ich die Arbeit selbstständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe (vgl. Art. 64 Abs. 1 Satz 6 BayHSchG).

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Bayreuth, 21 Oktober 2019

Ort, Datum