Shifts in soil microbiomes caused by biocides and facade eluates as an innovative ecotoxicological risk assessment

DISSERTATION

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To my parents

In gratitude for the support and opportunities you have given me

„Sage nie: *Ich kann nicht,* sage stets: *Ich will.*
Stets das Ziel vor Augen, dann erreichst Du viel!“
– Frau Margit Unger, Grundschullehrerin, aus meinem Poesiealbum.

Dieser Satz begleitet mich seit meiner Kindheit.
Was einst als Ermutigung begann, wurde zu einer Haltung,
die meinen Lebensweg prägt und auch diese Arbeit getragen hat.

Structural Note

This dissertation investigates the ecotoxicological effects of biocides and facade eluates from building materials on soil microbial communities, with a particular focus on metabolically active fractions. It begins with an introduction to the topic, outlining the environmental relevance of biocides in urban soils and the limitations of current assessment methods. The synopsis presents the central aims and hypotheses, which are explored through three experimental studies (Biocide-I, Biocide-II, and Biocide-III). The articles from myself (first authorship) that form the basis of this research are reprinted in chapter 3, including the supplementary material. The general discussion recapitulates the findings, highlighting the differential responses of active versus total microbial communities, the functional consequences of biocide exposure, and the implications for urban soil health and regulatory frameworks.

For clarity, key terms used throughout this dissertation are defined as follows. Ecotoxicological effect/impact is used within this dissertation to describe disturbances or harmful impacts on soil microorganisms and their functions caused by pollutants (e.g., biocides, facade eluates), potentially impairing the stability and resilience of the soil ecosystem. Soil health is used as generic term, describing an intact soil environment in which soil organisms function without disturbances or negative impacts, ensuring the stability and resilience of the entire soil ecosystem.

Core articles that form basis of the main text:

Biocide-I:
Reiß, F., Kiefer, N., Purahong, W., Borken, W., Kalkhof, S., & Noll, M. (2024). Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials. Science of the Total Environment, 912, 168689.
doi: 10.1016/j.scitotenv.2023.168689. (IF 2022 = 9.8)

Biocide-II:
Reiß, F., Kiefer, N., Reiß, P., Kalkhof, S., & Noll, M. (2025). Facade eluates affect active and total soil microbiome. *Environmental Pollution*, 364, 125242.
doi: 10.1016/j.envpol.2024.125242. (IF 2023 = 7.3)

Biocide-III:
Reiß, F., Kiefer, N., Tanunchai B., Reiß, P., Kalkhof, S., & Noll, M. (2026). Continuous intake of facade eluates affects active and total soil microbiome. *Biology and Fertility of Soils*, 62, 75–95.
doi: 10.1007/s00374-025-01955-9 (IF 2024 = 5.6)

Chapter 4 provides a list of all peer reviewed articles generated during my thesis.

Abbreviations

Abbreviation	**Full name**
µg	Microgram
A- (Prefix)	Active
ARGs	Antibiotic Resistance Genes
ASTM	Advancing Standards Transforming Markets
ASV	Amplicon Sequence Variant
ATP	Adenosine triphosphate
B-29d	Soil treated with facade eluates without biocides for 29 days
B-62	Soil treated with facade eluates without biocides for 62 days
BIT	benzisothiazolinone
bp	Base pair
BPR	European Biocide Regulation
BrdU	Bromodeoxyuridine
C	Carbon
CEC	Cation Exchange Capacity
CLPP	Community-level Physiological Profiling
CMIT	Chloromethylisothiazolinone
COMLEAM	Construction Material Leaching Model
C_{org}	microbial organic matter
d	Day
D-B	Facade eluates from DIN testing without biocides
DIN	*Deutsche Institut für Normung e. V.,* German Institute for Standardization
DNA	Deoxyribonucleic acid
dsDNA	double-stranded DNA
D-UOF	Facade eluates from DIN testing containing biocides
F-B	Facade eluates from free weathering experiment without biocides
F-UOF	Facade eluates from free weathering experiment containing biocides
g	Gramm
h	Hour
I	Isoproturon
IF	Impact Factor
IO	Isoproturon + Octhilinone
ISO	International Organization for Standardization

Abbreviations

Abbreviation	**Full name**
ITS	Internal Transcribed Spacer
MIT	Methylisothiazolinone
N	Nitrogen
NGS	Next-Generation Sequencing
NMDS	Non-metric Multidimensional Scaling
NO_3^-	Nitrate
O	Octhilinone
OECD	Organisation for Economic Co-operation and Development
OTU	Operational Taxonomic Units
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PELMO	Pesticide Leaching Model
qPCR	quantitative PCR
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RT-qPCR	reverse transcription qPCR
RW-29d	Soil treated with rainwater for 29 days
RW-62d	Soil treated with rainwater for 62 days
SIP	Stable Isotope Probing
SIR	Substrate-induced Respiration
SIS	Stormwater Infiltration Systems
ssDNA	Single-stranded DNA
SO_4^{2-}	Sulfate
T	Terbutryn
T- (Prefix)	Total
T0	Untreated soil
TI	Terbutryn + Isoproturon
TIO	Terbutryn + Isoproturon + Octhilinone
TO	Terbutryn + Octhilinone
UBA	*Umweltbundesamt*; German Environment Agency
UOF-29	Soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days
UOF-62d	Soil treated with facade eluates containing in-can preservatives and film preservatives for 62 days
UV	Ultraviolet
W	Water control

Abstract

Biocides are incorporated into building materials to reduce microbial growth and biodeterioration. However, these substances gradually leach into the surrounding soil near the buildings. Therefore, this dissertation presents a comprehensive ecotoxicological assessment of the impact of biocides and facade eluates from building materials on soil microbial communities, with a particular focus on metabolically active microbial members (BrdU-immunocapture technique). Through three experimental studies—Biocide-I, Biocide-II, and Biocide-III—conducted in controlled laboratory and outdoor microcosm settings, the research investigates how biocidal compounds and facade eluates affect soil microbial abundance, diversity, community composition, and functional traits.

Biocide-I examined the effects of three commonly used biocides—terbutryn, isoproturon, and octhilinone—applied individually and in mixtures. Results showed that metabolically active soil microbial communities responded more strongly than total DNA-based communities. Combined biocide treatments significantly reduced bacterial and fungal gene copy numbers and altered community composition on genus level. Despite these shifts, soil respiration and physicochemical parameters remained stable, suggesting short-term buffering through functional redundancy. Biocide-II focused on the ecotoxicological effects of facade eluates generated through standardized immersion testing and natural weathering. Eluates contained both active biocides and formulation components such as binders and heavy metals. The study revealed that even eluates from biocide-free facade s caused measurable changes in microbial communities. Active fractions were particularly sensitive, with notable shifts in bacterial and fungal diversity and function. Indicator taxa such as *Pseudarthrobacter* and *Trichoderma* emerged as potential biomarkers for stress response. Biocide-III simulated real-world exposure by applying facade eluates repeatedly over 62 days in outdoor microcosms. This long-term study demonstrated progressive alterations in microbial composition and function. Bacterial abundance declined, while fungal richness initially increased before dropping due to delayed leaching of film preservatives. Dominant taxa such as *Pseudoalteromonas* and stress-tolerant fungi were enriched, indicating adaptation to chronic exposure. Functional analyses showed increased fermentation and ureolysis, suggesting microbial stress responses and potential bioremediation traits.

Across all studies, metabolically active microbes proved to be reliable indicators of sub-lethal stress. Total microbial communities often masked these effects, underscoring the importance of targeting active fractions in ecotoxicological assessments. Functional annotation revealed that short-term ecosystem processes were buffered, while prolonged exposure could exhaust microbial redundancy and impair soil health. The findings highlight critical gaps in current EU biocide regulations, which focus on individual compounds and overlook the complex mixtures and chronic soil effects observed in this research. Moreover, no indicator organisms are assessed for evaluating the ecotoxicological effects on more complex communities such as the soil microbiome. The dissertation advocates for integrated risk assessment frameworks that incorporate active microbial metrics, long-term and mixture exposure scenarios, and functional validation. Urban soils, which play essential roles in nutrient cycling, water retention, carbon storage, and biodiversity support, should be recognized as a critical and protect-worthy environment.

Zusammenfassung

Biozide werden in Baustoffen eingesetzt, um mikrobielle Besiedelung und Biodeterioration zu verlangsamen. Durch Witterungseinflüsse gelangen diese Substanzen jedoch zunehmend in die umliegenden Böden in Gebäudenähe. Ziel dieser Dissertation ist eine umfassende ökotoxikologische Bewertung der Auswirkungen von Bioziden und Fassaden-Eluaten auf Bodenmikroorganismen, mit besonderem Fokus auf metabolisch aktive Gemeinschaften, analysiert mittels BrdU-Immunocapture-Methode. Dazu wurden drei experimentelle Studien - Biocide-I, Biocide-II und Biocide-III - unter kontrollierten Laborbedingungen sowie in möglichst realitätsnahen Freiland-Mikrokosmen durchgeführt. Es wurden die Effekte einzelner Biozide, ihrer Kombinationen und von Fassaden-Eluaten auf mikrobielle Abundanz, Diversität, Zusammensetzung und Funktion untersucht.

In der Studie *Biocide-I* wurde die Wirkung der Biozide Terbutryn, Isoproturon und Octhylisothiazolinon, einzeln und kombiniert auf das Bodenmikrobiom untersucht. Die Analysen belegten, dass metabolisch aktive mikrobielle Gemeinschaften deutlich empfindlicher auf Biozid-Exposition reagiert haben als die totalen mikrobiellen Gemeinschaften. Mischungen der Biozide führten zu einem besonders starken Rückgang der Genkopienzahlen von Bakterien und Pilzen sowie zu deutlichen Veränderungen in der Gemeinschaftszusammensetzung. Trotz dieser Veränderungen blieben die Bodenatmung und physikochemischen Parameter stabil. Dies weist auf eine kurzfristige Pufferung durch funktionelle Redundanz hin.

Biocide-II untersuchte die ökotoxikologischen Effekte von Fassadenabläufen, die durch standardisierte Immersionstests (DIN EN 16105) und natürliche Verwitterung erzeugt wurden. Die Eluate enthielten sowohl aktive Biozide als auch Formulierungskomponenten wie Bindemittel und Schwermetalle. Die Studie belegte, dass selbst Abläufe von Fassaden frei von Bioziden messbare Veränderungen in mikrobiellen Gemeinschaften verursachten. Selbst Abläufe von Fassaden welche frei von Bioziden waren verursachten messbare Veränderungen in mikrobiellen Gemeinschaften. Besonders die aktiven Fraktionen reagierten empfindlich, mit deutlichen Verschiebungen in bakterieller und pilzlicher Diversität und Funktion. Indikatortaxa wie *Pseudarthrobacter* und *Trichoderma* wurden als potenzielle Biomarker für Stress identifiziert.

Innerhalb der *Biocide-III* Studie wurde eine realistische Langzeitexposition durch wiederholte Anwendung von Fassaden-Eluaten in Freiland-Mikrokosmen über 62 Tage simuliert. Es zeigte sich eine kontinuierliche Veränderung der mikrobiellen Zusammensetzung und Funktion. Die bakterielle Abundanz nahm ab, während die pilzliche Diversität zunächst anstieg, später jedoch durch verzögerte Auswaschung von Filmkonservierungsmitteln wieder sank. Dominante Taxa wie *Pseudoalteromonas* und stressresistente Pilze wurden angereichert – ein Hinweis auf Anpassung an den Stress durch das Bodenmikrobiom. Funktionelle Analysen zeigten eine Zunahme fermentativer Prozesse und Ureolyse, was auf mikrobielle Stressantworten und potenzielle Bioremediations-Eigenschaften hindeutet.

In allen Studien erwiesen sich metabolisch aktive Mikroorganismen als zuverlässige Indikatoren für subletalen Stress. Die Analyse der totalen DNA verdeckten häufig diese Effekte, was die Bedeutung der aktiven Fraktionen in ökotoxikologischen Bewertungen unterstreicht. Funktionelle Annotationen zeigten, dass kurzfristige Prozesse zwar gepuffert werden, aber eine langfristige Belastung, die mikrobielle Redundanz erschöpfen und die Bodenfruchtbarkeit beeinträchtigen kann. Die Ergebnisse deuten auf Lücken in der aktuellen EU-Biozid Verordnung (Verordnung Nr. 528/2012) hin, die sich auf Einzelstoffe konzentriert und komplexe Mischungen sowie langfristige Effekte vernachlässigt. Zudem werden keine Indikatororganismen für die Bewertung der ökotoxikologischen Effekte auf komplexere Gemeinschaften wie das Bodenmikrobiom untersucht. Die Dissertation zeigt, dass Risikobewertungsansätze, das aktive Mikrobiom, Langzeitexpositionen, Mischtoxizitäten und funktionelle Bewertung mit einbezogen werden sollten. Städtische Böden, die eine wesentliche Rolle in den Nährstoffkreisläufen, bei der Wasserspeicherung, der Kohlenstoffspeicherung und der Förderung der Biodiversität spielen, müssen als wichtige und schützenswerte Umwelt anerkannt werden.

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1 Introduction

1.1 The use of biocides in building materials

Biocides are used in building materials to protect them against the occurrence of microorganisms. The use of biocides is regulated by law under the European Biocide Regulation (BPR) no. 528/2012 (European Parliament, Council of the European Union, 2012). Biocides serve two main purposes in coating materials. First, in-can preservatives (product type 6; BPR) are added to prevent microbial growth in the liquid state, both before application and during storage. Second, film preservatives (product type 7; BPR) are used to provide long-term protection for the facade once the coating is dry after application (Reiß et al., 2021). Approximately 25% of the annually produced biocides are used in building materials (Paulus, 2005).

Algae, fungi, bacteria, and lichens can grow as phototrophic or chemo-lithotrophic organisms on facades (Breuer et al., 2012; Krueger et al., 2013). The presence of microorganisms on buildings can lead to color changes due to colored biofilms. Additionally, some dwelling microorganisms can cause biodeterioration in building materials, especially in painted surfaces (Negi and Sarethy, 2019). Mansour and Al-Dawery (2018) have demonstrated the effectiveness of antimicrobial strategies using nano-structured photocatalysts for self-cleaning surfaces in preventing biodeterioration and biofilm formation, biocides are still commonly incorporated into paint and building materials in conventional practices.

The lack of knowledge regarding the microbial growth on building materials and associated biodeterioration results in the application of a broad range of biocides in the products instead of a specific microorganism target approach (Reiß et al., 2021). Buildings are constructed using a variety of materials e.g. paints, renders, and wood. Each product contains different mixtures of herbicides, bactericides, algaecides, fungicides, and metal ions. To ensure biological efficiency, biocides must be water-soluble and can thus enter the environment by leaching from the facade (Burkhardt et al., 2012; Hensen et al., 2018; Linke et al., 2021) (**Figure 1**).

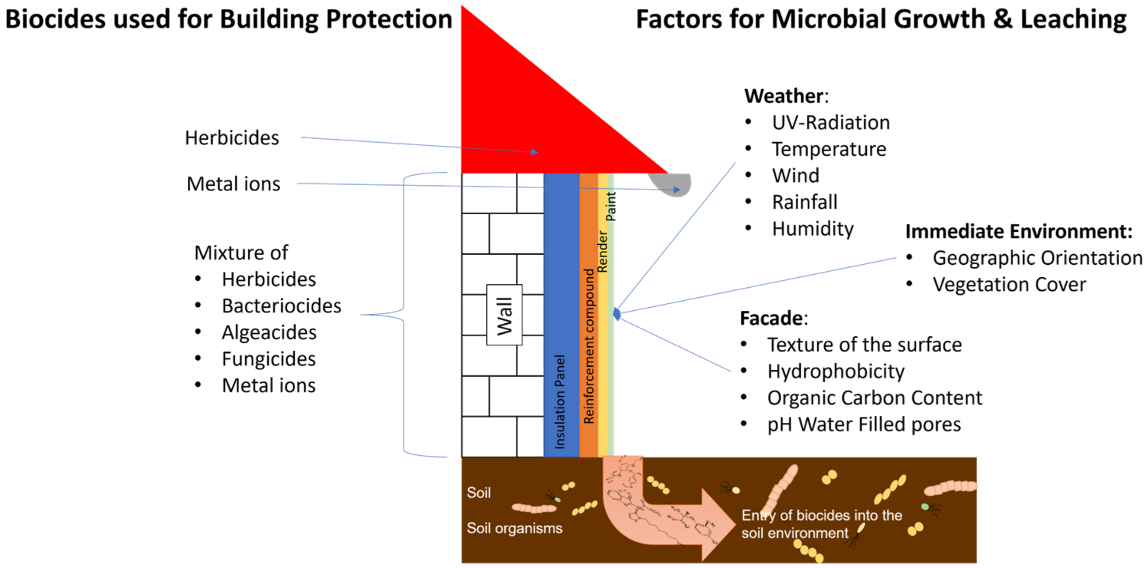


Figure 1: Schematic overview of facade eluate entry and microbial growth factors. The left side illustrates how facade eluates and their components enter the soil environment. The right side highlights factors affecting microbial growth and leaching on facades. Biocides must be water-soluble to remain biologically effective; however, this solubility also allows them to leach from facades during wetting events, such as rain or dew. The runoff water from facades — referred to as facade eluates in this study — can reach soils in the immediate vicinity of buildings. Scheme adapted from Reiß et al.(2021).

Factors like weather, ultraviolet (UV) radiation, and material degradation influence the rate and type of biocide release (Paijens et al., 2020). The combination released into the environment from these products is complex and difficult to control, as the variety of material combinations is vast and the degradation of biocides already occurs on the facades caused by weather influences (Bollmann et al., 2016, 2017). As a result, the biocide mixture that ends up in the environment is unpredictable, making it challenging to assess and manage its impact effectively. These individual biocides, biocidal degradation products and mixtures might not only affect the target organisms but also prokaryotic and eukaryotic non target organisms (Reiß et al., 2021).

1.2 Ecological importance of soil microbial communities and the impact of biocides

Soil microbial communities are fundamental to ecosystem functioning, as they regulate processes such as organic matter decomposition, nutrient cycling, carbon sequestration, water regulation, and biomass production (**Figure 2**), thereby supporting biodiversity and sustaining human activities (Gayan et al., 2023; Sheeba et al., 2011; Van Der Heijden et al., 2008). The abundance, diversity, and activity of these communities directly influence soil resilience and long-term productivity across agricultural, urban, and peri-urban environments.

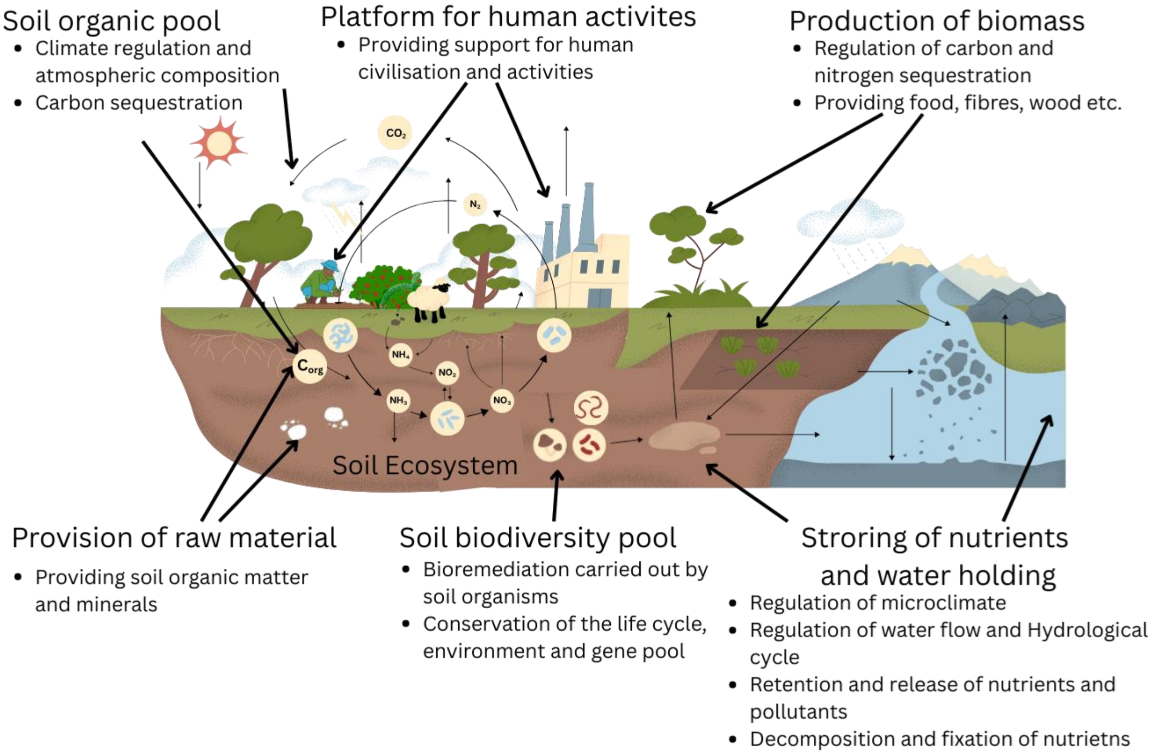


Figure 2: Key roles of soil ecosystems. The figure emphasizes the essential functions of soils, including carbon sequestration, nutrient cycling, support of biodiversity, biomass production, and regulation of water. These functions form the foundation for many human activities, highlighting the critical importance of healthy soil ecosystems. Own representation based on data from Gayan et al. (2023) and visualized using Canva (2025).

At the same time, soils are increasingly exposed to anthropogenic stressors, including heavy metals, synthetic fertilizers, pesticides, and biocides leached from construction materials (Reiß et al., 2021). Compared to agricultural pesticides, which are applied in regulated doses and times, biocides enter soils irregularly, in diluted forms, and locally concentrated around buildings (Heidorn, 2002). These differences are particularly relevant for urban soils, which tend to be compacted, structurally altered, and mixed with waste material, in contrast to well-aerated agricultural soils. As a result, findings from agricultural pesticide research cannot always be directly applied to biocides in urban contexts. The environmental fate of biocides is governed by soil properties (e.g., texture, pH, organic matter, microbial biomass), hydro-climatic conditions, and the physicochemical characteristics of the compounds (e.g., concentration, solubility, chemical structure) (Arias-Estévez et al., 2008; Reiß et al., 2021; Walker et al., 1999). Sorption, desorption, degradation, and leaching processes determine bioavailability and persistence, with degradation products sometimes exhibiting higher ecotoxicity or persistence than parent compounds (Bollmann et al., 2017).Recent advances, such as coupling the *Construction Material Leaching Model* (COMLEAM) with the *Pesticide Leaching Model* PELMO, have shown that persistent biocides, including film preservatives, can accumulate in the upper soil layers, posing long-term contamination risks (Kiefer et al., 2025).

Assessing the ecological significance of these impacts requires methodological innovation. Conventional ecotoxicological tests often rely on general enzyme activity or a few model organisms, providing only an incomplete view of microbial responses. In contrast, advanced approaches such as next-generation sequencing, metaproteomics, and fingerprinting techniques offer high-resolution insights into community composition and functional shifts. Integrating these tools with enzyme activity profiles and respiration metrics would allow a more accurate assessment of microbiome functionality under biocide stress. Importantly, site-specific risk assessments that consider local soil conditions and degradation kinetics are essential to avoid underestimation of long-term ecological risks.

1.3 Methods for evaluating terrestrial ecotoxicity

1.3.1 Conventional ecotoxicological methods

Ecotoxicity studies investigate the anthropogenic effects and environmental impacts on aquatic and terrestrial ecosystems (Kobetičová and Černý, 2017). A variety of conventional assays for testing the ecotoxicological impact on a broad range of model organisms are available (Reiß et al., 2021). Most methods are standardized by international organizations like Organization for Economic Co-operation and Development (OECD), International Organization for Standardization (ISO), Advancing Standards Transforming Markets (ASTM), and German Institute for Standardization (DIN, Deutsche Institut für Normung e. V.) (Erhirhie et al., 2018). The chosen model organisms reflect diverse trophic levels in the ecosystem and a variety of factors like reproduction, mortality, health, behavior, growth, and metabolism are analyzed (Erhirhie et al., 2018; Kobetičová and Černý, 2017). These methods were examined in detail as part of the review article Reiß et al. (2021). The literature survey revealed that the broad diversity of ecotoxicological assays and corresponding guidelines is currently available only for aquatic environments, and is lacking for soil environments (Reiß et al., 2021). Looking at the guidelines available for the soil environment, mainly overall soil microbial activity is the measure. For example, nitrogen (N) transformation activity (OECD 216) (OECD, 2000a) and the carbon (C) transformation test (OECD 217) (OECD, 2000b) address the whole soil microbial activity. These methods lack information regarding the impact of distinct microbial taxa on soil ecosystems functioning. Due to functional redundancy of the soil microbiome, many microorganisms show the capacity to turn over N and/or C (Wertz et al., 2006). This could lead to an underestimation of compound toxicity since functional redundancy can preserve the overall turnover rate, and most of the functional taxa must be affected by the compound to cause an effect. Model organisms commonly used to assess terrestrial ecotoxicity include the dehydrogenase activity of *Arthrobacter globiformis* (EN ISO 18187) (DIN German Institute for Standardization e. V, 2024) and the reproduction test of the earthworm species *Eisenia fetida* (OECD 222) (OECD, 2016a). Current guidelines for terrestrial ecotoxicological assays offer several advantages, such as cost-effectiveness, rapid execution, and high reproducibility. However, a significant limitation of these methods is their inability to capture the effects on the soil microbiome's composition and functions. These assays primarily focus on a limited number of model microorganisms —*A. globiformis* and *E. fetida*— which represent only a small fraction of the overall soil biodiversity and its associated ecosystem functions (Reiß et al., 2021).

To address these limitations, multi-species assays have been developed that incorporate a broader range of soil organisms and ecological interactions. For example, the MS-3 multispecies soil system integrates macro-decomposers such as earthworms (*Eisenia andrei*) and woodlice (*Armadillidium vulgare*), along with plant species like lettuce (*Lactuca sativa*) and mustard (*Sinapis alba*), to assess the ecotoxicological effects of benzene, toluene, ethylbenzene and xylene (BTEX) compounds in soil (da Silva Júnior et al., 2019). This approach allows for the simultaneous evaluation of multiple endpoints, including organism survival, biomass, seed germination, and enzymatic activity, offering a more holistic view of soil health. Other standardized multi-species assays have been developed to complement single-species tests and provide a more ecologically relevant assessment of soil toxicity. The Enchytraeid Reproduction Test (OECD 220) (OECD, 2016b), for instance, uses *Enchytraeus albidus* to evaluate reproductive toxicity in soil environments. Similarly, the Collembola Reproduction Test (ISO 11267) involves springtails such as *Folsomia candida*, which are important decomposers and serve as sensitive indicators of soil contamination (DIN German Institute for Standardization e. V, 2023). Plant-based assays, such as those described in OECD 208 (OECD, 2006), assess seedling emergence and growth across various plant species, offering insights into phytotoxicity and soil–plant interactions.

1.3.2 Evaluating microbial activity as an indicator of ecotoxicological assessment in terrestrial environments

Toxic effects of substances like pesticides on soils are typically evaluated by measuring the functional responses of the soil, with microbial activity often serving as a key indicator (Imfeld and Vuilleumier, 2012). These measurements are usually compared to reference values from unpolluted soils in the same environment. However, this approach does not directly reflect the toxicity of the substance on members of the soil microbial community, as it relies on indirect comparisons rather than measuring the actual impact on microbial status quo. Additionally, current ecotoxicological assays focus on single biocides, overlooking the accumulation of these substances in soil or the combined effects of multiple biocides and formulation components commonly used in building materials. This limitation complicates the extrapolation of results to real-world scenarios, such as facades. Assessing multiple test substances is crucial, as terrestrial environments like soil exhibit significant variability in absorption capacity, which is likely to result in higher toxicity rates than those indicated by existing ecotoxicological assays. These assays can be differentiated in cultivation-dependent and -independent methods. Pros and cons of these methods were extensively reviewed by Imfeld and Vuilleumier (2012) for cultivation-dependent methods and Reiß et al. (2021) for cultivation-independent methods.

1.3.2.1 Global soil microbial indicator as indicator for terrestrial ecotoxicity - Cultivation-dependent methods

The microbial contribution to nutrient cycles in soil can be quantitatively assessed using global physiological parameters such as microbial biomass C, microcalorimetry, [^{3}H]-leucine or [^{3}H]-thymidine incorporation, substrate-induced respiration (SIR), and the soil enzymes (Yang et al., 2024). These parameters are compared to substance-free control experiments. These methods bear the advantage of being inexpensive and easy to measure. The loss of enzymatic activity was also often used as a measure to evaluate the ecotoxicity of biocides within soils (Imfeld and Vuilleumier, 2012). Enzymatic assays use e.g., dehydrogenase, phosphatase, and urease to assess the activity of the soil microbiome (Andréa et al., 2000; Dungan et al., 2003; Rehman et al., 2024). As previously described, individual strains are commonly used to assess terrestrial ecotoxicology via the motility, growth, viability, adenosine triphosphate (ATP) content, bioluminescence, oxygen uptake, nitrification, or heat production of the respective model strain(s) (Bitton and Koopman, 1992). Cultivation-dependent methods share similar drawbacks with conventional approaches. These are laboratory-based methods involving single-parameter analyses. Consequently, the ecotoxicological effect of the test substance is not sufficiently depicted. Solely, the minor cultivable fraction of the soil microbial community is reflected due to the incubation step during the test procedure. Thereby, overgrowth can disrupt experimental investigations, and over- or underestimation of the ecotoxicological assessment must be taken into account (Fernández-Marcos, 2024; Reiß et al., 2021; Zengler, 2008). Community-level physiological profiling (CLPP) (Biolog® Plates) involves inoculating a soil-derived microbial suspension into wells, incubating under standardized conditions, and monitoring C source utilization to generate a “metabolic fingerprint” for each sample (Sofo and Ricciuti, 2019). CLPP provides functional insights, is a fast-forward and cost-efficient method that owes high reproducibility and comparability (Thiele-Bruhn et al., 2020). Nevertheless, Biolog® plates on conventional media set-up mainly detect fast-growing and aerobic microbes, overlooking slow-growing or anaerobic species, which limits ecological output (Sofo and Ricciuti, 2019; Thiele-Bruhn et al., 2020).

1.3.2.2 Analysis of soil microbial community composition as indicator for terrestrial ecotoxicity - cultivation independent methods

To overcome the above-described limitations of cultivation-dependent methods, cultivation-independent molecular methods have gained prominence. Among these, Polymerase Chain Reaction (PCR)-based techniques combined with amplicon sequencing (e.g., 16S Ribosomal ribonucleic acid (rRNA) gene and Internal Transcribed Spacer (ITS) region profiling, and quantitative PCR (qPCR) as well as reverse transcription qPCR (RT-qPCR), have found widespread application in assessing microbial responses to environmental pollutants, including biocides (Imfeld and Vuilleumier, 2012; Lasota et al., 2019; Silva et al., 2020). These methods enable species-specific insights and can detect shifts in microbial diversity, abundance, and community structure. However, they also have limitations. PCR amplification bias, primer mismatches, and the presence of relic deoxyribonucleic acid (DNA) can lead to over- or underestimation of taxa (Amend et al., 2010; von Wintzingerode et al., 1997). Furthermore, discrepancies between relative sequence abundances and actual microbial activity challenge the interpretation of results (Amend et al., 2010; Louca et al., 2018). For instance, DNA isolated from soil represents a complex mixture comprising (i) fragments from non-viable or lysed cells, (ii) extracellular or relic DNA that can remain stable in soils for extended periods (Nielsen et al., 2007), (iii) DNA from dormant microbial populations, (iv) genetic material from viable cells (Emerson et al., 2017), and (v) DNA from microbes that can rapidly transition to an active state in response to minimal nutrient inputs, often within minutes or hours (Blagodatskaya and Kuzyakov, 2013; de Nobili et al., 2001).

Analysis of phylotype abundance or functional genes enables characterization of the soil microbiome (Imfeld and Vuilleumier, 2012; Widenfalk et al., 2008). Due to limitations of single-method approaches, recent research increasingly advocates for integrative, multi-omics methodologies. Metagenomics and metatranscriptomics provide insights into the genetic potential and active gene expression within microbial communities, while metaproteomics and metabolomics elucidate protein expression and metabolic pathways (Martinez-Alonso et al., 2019; Qian and Hettich, 2017). These methods allow researchers to move beyond taxonomy and explore functional responses, ecological processes, and metabolic activities *in situ*.

In particular, stable isotope probing (SIP), especially protein-SIP, has proven valuable in identifying metabolically active microbial taxa involved in the degradation or transformation of environmental contaminants, including biocides (Jakobs-Schönwandt et al., 2010; Li et al., 2019; Seifert et al., 2012). Another promising approach to specifically identify active microbes in soil is bromodeoxyuridine (BrdU) immunocapture method combined with Illumina amplicon sequencing. BrdU labeling allows to distinguish between total genomic DNA from DNA retrieved from metabolic active organisms (Purahong et al., 2022; Wahdan et al., 2021). Previous studies have demonstrated that the method can be used for meso- and microcosm studies as well as for natural habitats (Allison and Treseder, 2008; Bravo et al., 2013; Goldfarb et al., 2011; Grubisic et al., 2017; Hjort et al., 2007; Kelly et al., 2016; Taniguchi et al., 2015; Walters and Field, 2006). The BrdU-immunocapture method offers a distinct advantage by enabling researchers to differentiate DNA from metabolically active microorganisms from the total DNA pool, thus providing clearer insights into the active members of microbial communities and their real-time responses to environmental stressors such as biocides (Purahong et al., 2022; Reiß et al., 2025, 2024; Wahdan et al., 2021). This approach allows for comprehensive profiling of both total and metabolically active microbial communities within a given ecosystem, providing valuable insights into microbial dynamics, functionality, and ecological roles. These insights might otherwise be overlooked due to interference from relic or extracellular DNA in traditional DNA-based methods.

However, the method also has drawbacks. It can be labor-intensive, requires careful optimization of incubation and immunocapture protocols. Additionally, BrdU incorporation primarily targets actively dividing cells, possibly underestimating slow growing but functionally significant microorganisms (Artursson and Jansson, 2003; Borneman, 1999; van Elsas and Boersma, 2011). Among the available activity-based labeling techniques, BrdU immunocapture proved to be the most suitable method for gaining initial insights into the active soil microbiome due to its relatively low cost, straightforward setup, and broad applicability. In comparison, DNA-SIP and RNA-SIP provide higher functional specificity by linking microbial identity to substrate assimilation or transcriptional activity, respectively. DNA-SIP is ideal for identifying microbes that incorporate a specific labeled substrate into their DNA during growth, while RNA-SIP captures early metabolic responses at the transcript level (Verastegui et al., 2014), offering greater temporal resolution (Manefield et al., 2002). Despite their strengths, both SIP approaches require costly isotopes, specialized equipment, and complex protocols, making BrdU a practical first step in uncovering the active fraction of microbial communities under environmental stress.

1.4 Impact of biocides on the soil microbiome

Over the past decades, the application of biocides in building materials has steadily increased, driven by demands for durable and low-maintenance surfaces**.** Biocides are increasingly added to paints, plasters, and coatings to protect surfaces from microbial growth, as modern architecture often lacks facade protection measures such as roof overhangs. Today, biocides are widely used in various construction products, raising growing concerns about their environmental fate and impact. While leaching behavior and aquatic ecotoxicological risks are well documented (Burkhardt et al., 2012; Paijens et al., 2020), their impact on terrestrial environments, especially on the soil microbiome, remains poorly understood. Soils, as terminal sinks for many urban runoff contaminants, are particularly vulnerable to the accumulation and effects of leached biocides. This section addresses the current state of research on the effects of biocides on the soil ecosystem and builds on the findings of the review by Reiß et al. (2021).

1.4.1 Impact of pesticides

Soil microbial communities are vital for ecosystem functioning, driving essential processes such as nutrient cycling, organic matter decomposition, and pollutant degradation. Despite this, terrestrial ecotoxicological assessments of biocides from construction materials have received limited scientific attention, with existing soil tests providing only fragmented insights into microbial diversity and activity (Bandow et al., 2020). Comparatively, the impact of agricultural pesticides — including herbicides, fungicides, and insecticides — on soil microbiomes has been extensively studied.

These chemicals are known to cause significant shifts in microbial community composition, diversity, and function (Meena et al., 2020; Singh et al., 2020). Sensitive microbes are often inhibited, allowing non-sensitive species to dominate, potentially leading to altered ecosystem functions (Supreeth et al., 2016). Some microbes even contribute to bioremediation by degrading pesticides (Oro et al., 2024; Yasir et al., 2025), though such resilience is context-dependent and often disrupted by high or prolonged pesticide exposure. Notably, many pesticides used in agriculture are also applied as biocides in building materials, such as terbutryn and isoproturon. Both have demonstrated negative effects on key soil microbial functions like nitrification and N fixation (Maharana et al., 2025; Singh and Wright, 2002). Comparable data and research regarding biocides in building materials are currently lacking. Since the application of biocides used in agriculture differs significantly from those in construction contexts, existing testing protocols are not readily applicable. This is due to differences in exposure pathways and the agricultural research's often misleading focus and experimental design (Reiß et al., 2021). In agricultural settings, biocides are typically applied at low concentrations over large areas and only for the duration of the growing season (Heidorn, 2002; Vormeier et al., 2023). Therefore, the extensive body of agricultural biocide research offers only limited transferability to the environmental and toxicological impacts of biocides used in building materials.

1.4.2 Impact of biocides on building materials

This subsection addresses the effects of biocides used in building materials on the soil microbiome. While most previous studies have focused on individual biocides and a narrow range of target organisms, little attention has been given to the combined toxicity of biocide mixtures on the broader and more diverse microbial communities. These further limit the applicability of current agricultural research to the context of building materials, where microbial communities and exposure conditions differ markedly. Emerging research on biocides from building materials suggests compounds such as octhilinone and terbutryn can persist in soil and disrupt microbial functions, particularly under long-term exposure (Bollmann et al., 2017; Fernández-Calviño et al., 2021).

A developing concern in recent years is the accumulation of biocides in urban stormwater infiltration systems (SIS), where contaminated runoff from impermeable surfaces is directed into engineered soil beds. While these systems are designed to manage hydrological flow and improve water quality, they may inadvertently function as repositories for biocidal compounds. A 2023 investigation found that SIS soils exhibit elevated concentrations of terbutryn, diuron, and isothiazolinones, depending on infiltration rates, system age, and soil porosity (Linke et al., 2023). Although the detected concentrations are often below acutely toxic thresholds, the chronic exposure of soil biota to low levels of multiple biocides raises new ecotoxicological questions. Over time, accumulation may lead to compound effects on microbial communities, particularly in the upper soil horizons, which are critical for organic matter processing and plant–microbe interactions.

A particularly urgent development in biocide research is the growing evidence that environmental exposure to certain biocides may promote the selection of antibiotic resistance genes (ARGs) in soil microorganisms. This co-selection arises because some biocides and antibiotics target similar cellular mechanisms, leading to cross-resistance (Sousa et al., 2025). Recent studies have demonstrated that sub-inhibitory concentrations of common biocides such as benzalkonium chloride and isothiazolinones can enrich for resistant microbial strains in soils (Langsrud et al., 2003; Tandukar et al., 2013). This process poses a dual risk: ecological disturbance and the potential transfer of resistance genes to human–associated pathogens through horizontal gene transfer. The linkage between environmental biocide contamination and public health concerns, especially in densely populated urban area, represents a critical research frontier that was only peripherally addressed in earlier studies (Sousa et al., 2025).

Many terrestrial ecotoxicity studies still rely on traditional methods such as standard plate counts, indirect assessments like SIR, or low-resolution molecular techniques to evaluate the ecotoxicological impacts of biocides on soil microbial communities (Reiß et al., 2021). To accurately distinguish the effects of biocides within the complex composition and functions of soil microbial communities, high-resolution techniques, such as DNA-based methods, are essential. Short term tests may underestimate risks, emphasizing the need for prolonged and more holistic evaluations. Additionally, mixed toxicity of biocides as found in facade eluates were barely investigated. In summary, there is a critical knowledge gap in understanding how leached biocides from building materials affect the soil microbiome, with significant implications for soil health, ecological balance, and environmental policy.

This underscores the urgent need for more advanced, long-term, and molecular-based ecotoxicological assessments to inform safe biocide application and regulation.

1.5 Applied methods to study the influence of biocides of building materials on the soil microbiome

To investigate the ecotoxicological effects of biocides from building materials on soil microbial communities, a multi-methodological approach was employed in this dissertation. This chapter outlines the methods and analytical techniques used to assess the soil microbiome after biocide exposure.

1.5.1 Soil microcosm experiment

Soil microcosms are simplified, controllable, small-scale models of natural soil environments used to simulate and study ecological processes, such as nutrient cycling, microbial interactions, and contaminant behavior. These systems are designed to replicate key components of soil ecosystems while allowing for precise manipulation of variables and maintaining most other conditions constant. Microcosm studies are widely applied in environmental science, soil ecology, and ecotoxicology, especially to investigate the effects of pollutants, climate change, or agricultural practices on soil organisms and functions. They enable high experimental control, replicability, standardization, cost effectiveness, and temporal efficiency (Römbke et al., 2006). In contrast, soil microcosms lack ecological realism as no multi-trophic interactions are feasible, and soil heterogeneity might be disrupted. Additionally, boundaries potentially alter moisture retention, temperature gradients, and the behavior of soil organisms (Kuan et al., 2006). However, soil microcosms are increasingly used for different kinds of research questions. As for example, the effect of soil moisture on gas fluxes (Subramaniam et al., 2024), the increase of cation availability (Nicolitch et al., 2019), different pollutants such as microplastics (Sun et al., 2024) on the soil microbial diversity, as well as bioremediation of the pesticide atrazine (Sagarkar et al., 2014), or degradation of other pollutants (Barra Caracciolo et al., 2013). Due to the above-described advantages and the high statistical power through replication, soil microcosm studies were chosen to gain first insight into the effects of biocides in Biocide-I and facade eluates Biocide-II on soil microbial community.

1.5.2 DNA metabarcoding as a tool for soil microbial diversity and functional assessment

As outlined in chapter 1.3.2.2, cultivation-independent approaches provide important advantages for assessing ecotoxicological risks in terrestrial ecosystems. Among them, DNA metabarcoding has emerged as a high-throughput molecular technique capable of identifying diverse taxa within complex environmental matrices such as soil (Drummond et al., 2015; Kirse et al., 2021; Köninger et al., 2023; Taberlet et al., 2012). This approach offers powerful insights into soil biodiversity and has become an essential tool in microbial ecology, land-use monitoring, and soil health assessments.

The workflow typically begins with the extraction of environmental DNA from soil using commercially available kits optimized for the simultaneous recovery of bacterial and fungal template DNA. Ribosomal marker regions are then amplified through PCR with universal primers (e.g., V3–V4 of 16S for bacteria or ITS2 for fungi). The resulting amplicons are sequenced using next-generation platforms such as Illumina MiSeq, NovaSeq, or, more recently, long-read technologies like Oxford Nanopore (Bolyen et al., 2019). Following sequencing, a structured bioinformatic workflow is used to convert raw reads into ecologically interpretable taxonomic units. The first step involves quality filtering, adapter trimming, and primer removal using tools like Cutadapt or QIIME2's built-in plugins (Bolyen et al., 2019). Subsequently, two main strategies can be used to group sequences: Either Operational Taxonomic Units (OTUs) clustering, a method that clusters sequences to OTUs based on a similarity threshold (commonly 97%) using tools such as VSEARCH or USEARCH (Edgar, 2010; Rognes et al., 2016). On the other hand, Amplicon Sequence Variants (ASVs) were calculated using denoising algorithms such as DADA2 or Deblur infer ASVs, which provide single-nucleotide resolution and improved reproducibility across studies (Callahan et al., 2016). After dereplication and chimera removal, the representative sequences are taxonomically classified using reference databases—SILVA for bacteria (Quast et al., 2013) and UNITE for fungi (Nilsson et al., 2019). Classification can be carried out via naïve Bayes classifiers, BLAST alignment, or other k-mer-based methods available in tools like QIIME2 and mothur.

To enable comparisons across samples, sequencing depth is normalized using rarefaction or other scaling methods. Downstream analyses include alpha and beta diversity metrics (Chapter 1.5.5) . For visualization and statistical modeling, various R packages like

QIIME2, Phyloseq, or the vegan are available (Bolyen et al., 2019). This comprehensive workflow allows for high-resolution, scalable profiling of soil microbial communities, with applications ranging from ecological monitoring to sustainable agriculture and pollution assessment. Thereby, this approach has been extensively applied in biodiversity research involving soil microorganisms like bacteria and fungi (Donhauser et al., 2023; Hernández-Lara et al., 2022; Labouyrie et al., 2023).

Beyond taxonomic profiling, functional annotation provides ecological context by predicting the metabolic capabilities and ecological roles of microbial communities. Based on sequencing data, computational tools compare these sequences to reference databases that contain known gene functions, metabolic pathways, or ecological traits. By mapping taxa to these references, researchers can infer the functions likely present in the community, such as nutrient cycling, organic matter decomposition, or symbiotic interactions. For fungi, functional guilds or trait databases provide ecological classifications (e.g., saprotroph, pathogen, symbiont), enabling assessment of shifts in community function under different environmental conditions. This approach allows functional insights without directly measuring gene expression or enzymatic activity.

For bacteria, functional profiles can be inferred from 16s rRNA gene data using tools like PICRUSt2 (Douglas et al., 2020) and Tax4Fun2 (Aßhauer et al., 2015), which map microbial taxa to functional pathways in databases like KEGG (Kanehisa and Goto, 2000). Another commonly used tool for bacterial communities is FAPROTAX (Functional Annotation of Prokaryotic Taxa), which assigns functional traits (e.g. nitrification, methanogenesis, sulfur respiration) to prokaryotic taxa based on curated literature linking taxonomy to metabolism (Louca et al., 2016). FAPROTAX is particularly effective for soil and aquatic microbial ecology due to its focus on biogeochemical processes.

For fungi, functional annotation is typically taxon-based. Tools like FUNGuild classify fungal OTUs or ASVs into ecological guilds such as saprotrophs, symbiotrophs (e.g., mycorrhizal fungi), and pathotrophs (Nguyen et al., 2016). This approach enables researchers to evaluate ecological strategies of fungal communities and their shifts across gradients of soil management, disturbance, or vegetation type. In addition to guild classification, emerging approaches now emphasize fungal functional traits, which provide a more nuanced understanding of ecological strategies. Trait-based fungal ecology is supported by curated databases such as FunFun and FungalTraits (Flores-Moreno et al., 2019; Krivonos et al., 2023), which compile data from literature and culture collections to assign trait values to fungal taxa (Põlme et al., 2021). These databases enable researchers to examine how life history strategies and functional roles vary across communities and environments, especially under pressures such as land-use change or climate stress.

One key benefit of this technique is its ability to recover the full spectrum of biodiversity within a soil system, including cryptic or previously undescribed species that are often overlooked by traditional methods (Arrigoni et al., 2016; Hebert et al., 2003; Klarica et al., 2012). It enables the analysis of the entire community, including its diversity, complexity of interspecies networks, and the dissimilarity between different communities (Semenov, 2021). Additionally, functional annotation provides a crucial bridge between community composition and ecosystem functioning, offering insights into the roles of soil microbes in nutrient cycling, plant health, C turnover, and ecological resilience.

1.5.3 BrdU immunocapture technique to analyze active microbial community members

Despite the many advantages of DNA metabarcoding shown above, the method cannot identify the active pool of organisms within the high biodiversity of soils and the presence of extracellular DNA (Semenov, 2021). The BrdU immunocapture technique is an alternative to ribonucleic acid (RNA) sequencing and SIP to determine metabolically active microorganisms within environmental samples. The BrdU immunocapture technique operates on the principle of supplying BrdU — a synthetic thymidine analog — in excess to the extracellular environment, where it is transported into metabolically active cells and incorporated into newly synthesized DNA during replication, effectively replacing thymidine (**Figure 3**).

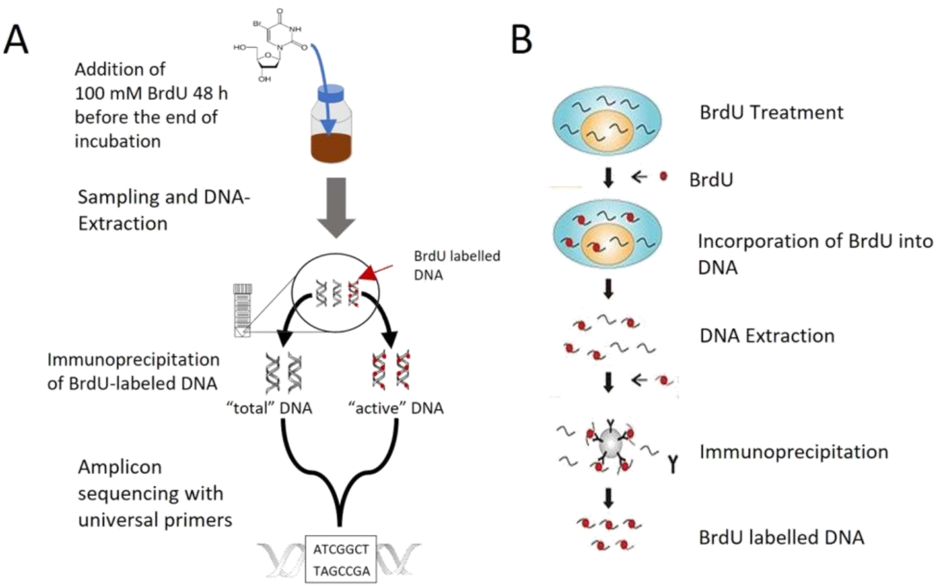


Figure 3: Schematic overview of the soil microcosm experiment and BrdU-based microbiome analysis. (A) Experimental setup for analyzing the active soil microbiome. The red arrow indicates the step of immunoprecipitation. (B) Detailed procedure for the incorporation of BrdU into replicating genomic DNA and the subsequent enrichment of BrdU-labeled DNA by immunoprecipitation. This approach allows assessment of ecotoxicological effects by comparing the total versus active soil microbiomes. Scheme translated from Reiss and Noll (2025).

This incorporation highlights the presence of proliferating microbial populations *in situ* (Borneman, 1999; Urbach et al., 1999). DNA from these active organisms, now labeled with BrdU, can be selectively isolated from the total community DNA using anti-BrdU antibodies in an immunocapture process. Although BrdU incorporation is not universal across all microbial taxa (Wahdan et al., 2021), most tested bacterial strains are capable of incorporating BrdU, and representatives from all major bacterial phyla have been identified using this method. BrdU uptake by fungi in environmental samples has rarely been addressed systematically and thus remains poorly documented beyond a handful of cultured species. This gap is noteworthy given their ecological importance, as fungi often show limited thymidine incorporation in vitro (Sivakumar et al., 2004), yet evidence from field and microcosm studies indicates that BrdU labeling can indeed capture fungal activity under natural conditions, including soil and litter decomposition (Treseder et al., 2014), microcosms experiments (Reiß et al., 2025, 2024; Wahdan et al., 2021), soil with mycorrhizae (Artursson and Jansson, 2003), and deadwood in forests (Purahong et al., 2022).

1.5.4 Quantitative real-time PCR assay

Quantitative PCR (qPCR) is a widely used molecular method for assessing microbial biomass in environmental samples (Kralik and Ricchi, 2017). It provides a sensitive and specific means of quantifying bacterial and fungal DNA in complex matrices such as soil, without the biases of cultivation-based techniques. qPCR quantifies gene copy numbers in DNA extracts by amplifying target genes in real-time and detecting fluorescence signals generated by intercalating dyes (e.g., SYBR Green) or probe-based systems (e.g., TaqMan) (Tajadini et al., 2014). In both SYBR Green and TaqMan qPCR methods, the intensity of fluorescence released correlates directly with the amplification of microbial DNA, allowing for the quantification of specific microbial genes. In SYBR Green assays, the fluorescent dye binds to newly formed double-stranded DNA (dsDNA), emitting a signal that increases with each qPCR cycle (Tajadini et al., 2014). In contrast, the TaqMan method uses sequence-specific dual-labeled probes that anneal to single-stranded DNA (ssDNA). During the extension phase, Taq polymerase cleaves these probes through its 5′ to 3′ exonuclease activity, separating the fluorophore from the quencher. This results in fluorescence emission and the continued synthesis of dsDNA (Tajadini et al., 2014). For microbial biomass estimation, bacteria are typically quantified using primers targeting the 16S rRNA gene, and Fungi are quantified using primers targeting the ITS region, which offers higher taxonomic resolution for fungal communities. For quantification the primer pair 341f-785R was used for bacteria and the primer set ITS4 and fITS7 for fungi. The same primer pairs were used for quantification and sequencing for better comparability.

1.5.5 Statistical approaches for microbial community analysis

Across Biocide-I, Biocide-II, and Biocide-III, statistical analyses were structured to assess the impact of biocide treatments on both total and metabolically active microbial communities. All datasets were first summarized at the genus level, and the normality of alpha diversity indices (OTU richness, Shannon index, Simpson index, Pielou’s Evenness) and richness estimators (Bias-Corrected Chao1, abundance-based coverage estimator ACE) was evaluated using the Shapiro-Wilk test. Depending on normality, parametric tests (one-way ANOVA with Tukey post hoc) or non-parametric tests (Kruskal-Wallis ANOVA with Dunn post hoc) were applied to assess treatment effects on alpha diversity and total bacterial and fungal gene copy numbers (Hothorn et al., 2008). Rarefaction analyses were performed to evaluate sampling depth and richness coverage, ensuring reliable diversity estimates (Noll et al., 2005).

Community composition was explored using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities (Clarke et al., 2014; Clarke, 1993). NMDS provides an ordination that visualizes complex, high-dimensional microbial datasets in a low-dimensional space, facilitating interpretation of overall community patterns. To statistically test the significance of compositional differences between treatment groups, permutational multivariate analysis of variance (PERMANOVA or NPMANOVA) was applied (Hammer et al., 2001; Legendre and Anderson, 1999), accounting for the multivariate nature of microbial communities and functional profiles. Environmental variables were fitted to the NMDS ordinations using the envfit function to assess their explanatory power, with significance determined through permutation tests (typically 999 permutations) (Oksanen et al., 2025).

Indicator species analysis was used to identify bacterial and fungal genera significantly associated with specific biocide treatments (de Cáceres and Legendre, 2009), revealing taxa driving the observed differences in community composition. In Biocide-III, analyses were extended using linear models incorporating Group, Time, and Group × Time interactions, along with estimated marginal means (EMMs) and pairwise comparisons to detect temporal dynamics in community responses (Lenth, 2025). Multiple testing corrections, such as the Benjamini-Hochberg false discovery rate (FDR), were applied to control type I error across comparisons.

The multivariate and univariate approaches applied across the three experiments provide a comprehensive framework for evaluating microbial responses at multiple ecological scales. Advantages include the ability to capture subtle shifts in microbial community structure and function, integrate environmental covariates, and detect treatment-specific indicator taxa. Limitations include sensitivity of PERMANOVA to differences in group dispersion, potential bias from choice of dissimilarity metrics, and challenges in interpreting ordination axes ecologically(Clarke et al., 2014; Clarke, 1993; Legendre and Anderson, 1999). Despite these constraints, the combination of alpha diversity metrics, NMDS, PERMANOVA, and indicator species analysis allowed robust and reproducible assessment of biocide effects on soil microbial communities, including both total and metabolically active fractions, and supported the identification of key taxa and functional responses relevant for ecotoxicological risk assessment.

2 Synopsis

Within this dissertation three biocide experiments — Biocide-I, Biocide-II, and Biocide-III — were conducted to investigate the effects of biocides and facade eluates on soil microbial communities. Biocide-I and Biocide-II were carried out under controlled laboratory conditions, whereas Biocide-III was designed to mimic the facade eluate uptake of soils in the proximity of the building. Together, these experiments provide a comprehensive assessment of biocide-related impacts under both controlled and environmentally relevant conditions.

2.1 Aims and hypotheses

Facade eluates contain a complex mixture of biocides and formulation components derived from building materials, including paints and renders. These substances may be mobilized through various leaching processes and subsequently enter surrounding soils. While previous research has characterized the chemical composition of facade eluates, their impact on soil microbial communities — particularly active microbial fractions — remains poorly understood. This dissertation investigates how facade eluates and their constituent biocides influence soil microbial composition and function, with a focus on both bacterial and fungal communities and their active subsets, analyzed using the BrdU immunocapture technique.

2.1.1 Biocide-I: Individual biocides and biocide combinations

The first study (Biocide-I, 3.2) evaluated the effects of various biocides. This study aimed to determine how specific biocides, and their combinations affect the composition and functional dynamics of total and active soil bacterial and fungal communities The research addressed critical gaps in knowledge regarding the specific impacts of biocides, the differential responses of active versus total microbial communities, and the potential additive, synergistic, or antagonistic effects of combined biocide exposure.

2.1.2 Biocide-II: Facade eluates

The second study (Biocide-II, 3.3) assessed the effects of complex facade eluates, comprising biocides, heavy metals, binders, and fillers, on soil microbial communities. Runoff from biocide-treated and untreated facades was generated via two leaching protocols: the standardized DIN EN 16105 immersion test and a natural weathering trial. Both total and active bacterial and fungal communities were analyzed to identify taxa that are positively or negatively affected by facade eluates. This study addressed important knowledge gaps concerning the ecotoxicological effects of real-world facade eluates, the potential impact of biocide-free facade components, and the influence of leaching protocol on eluates toxicity and thus microbial responses.

2.1.3 Biocide-III: Multiple entries of facade eluates

The third study (Biocide-III, 3.4) investigated the effects of repeated facade runoff applications under outdoor conditions, simulating real-world, recurrent exposure. This study evaluated changes in total and active bacterial and fungal communities, soil physicochemical properties, and microbial functions over time. By doing so, it addressed the lack of long-term studies, the limited understanding of cumulative impacts on active versus total microbial communities, and the need for data on temporal dynamics of microbial responses to recurring biocidal stress.

2.1.4 Overarching hypotheses

Based on the three studies, the dissertation tested the following overarching hypotheses:

H1 **Selective impact on active communities:** Facade eluates and biocides primarily alter the composition and function of **metabolically active microbial communities** – as they directly interfere with ongoing biogeochemical processes such as carbon degradation, nitrogen transformations, and redox reactions. Consequently, total communities remain comparatively stable due to functional redundancy.

H2 **Biocide-specific effects:** Individual biocides and biocide combinations differentially affect microbial taxa, with fungi hypothesized to be more sensitive to fungicidal compounds, whereas some bacteria may benefit from reduced competition or utilization of biocide compounds as a substrate.

H3 **Cumulative and protocol-dependent effects:** Repeated or combined exposures, as well as the type of eluate and leaching procedure, modulate the magnitude and direction of microbial community shifts. Repeated or combined exposures can cause cumulative alterations in key ecosystem functions including nutrient cycling (carbon, nitrogen, phosphorus), biofilm structural integrity, resource turnover, and microbial interactions (competition, inhibition, facilitation). These factors may generate nonlinear responses in metabolic rates, such as enhanced nutrient mobilization, shifts in aerobic–anaerobic redox processes, or altered organic matter degradation.

H4 **Functional stability through redundancy:** Despite compositional changes, overall microbial biogeochemical functions remain stable because biocide-sensitive taxa are replaced by functionally equivalent, biocide-insensitive taxa.

Collectively, these hypotheses address critical gaps regarding the ecotoxicological effects of both defined biocides and complex facade eluates on soil microbial communities, particularly regarding active microbial fractions, long-term exposure, and real-world leaching scenarios.

2.2 Study design and study sites

To answer hypotheses of Biocide-I and Biocide-II soil microcosm experiments were conducted under laboratory conditions for 29 days, and single application of biocides, biocide mixtures, or facade eluates (**Figure 4**). To investigate the effects of facade eluates under the most realistic conditions possible, the Biocide-III incubation was conducted outdoors.

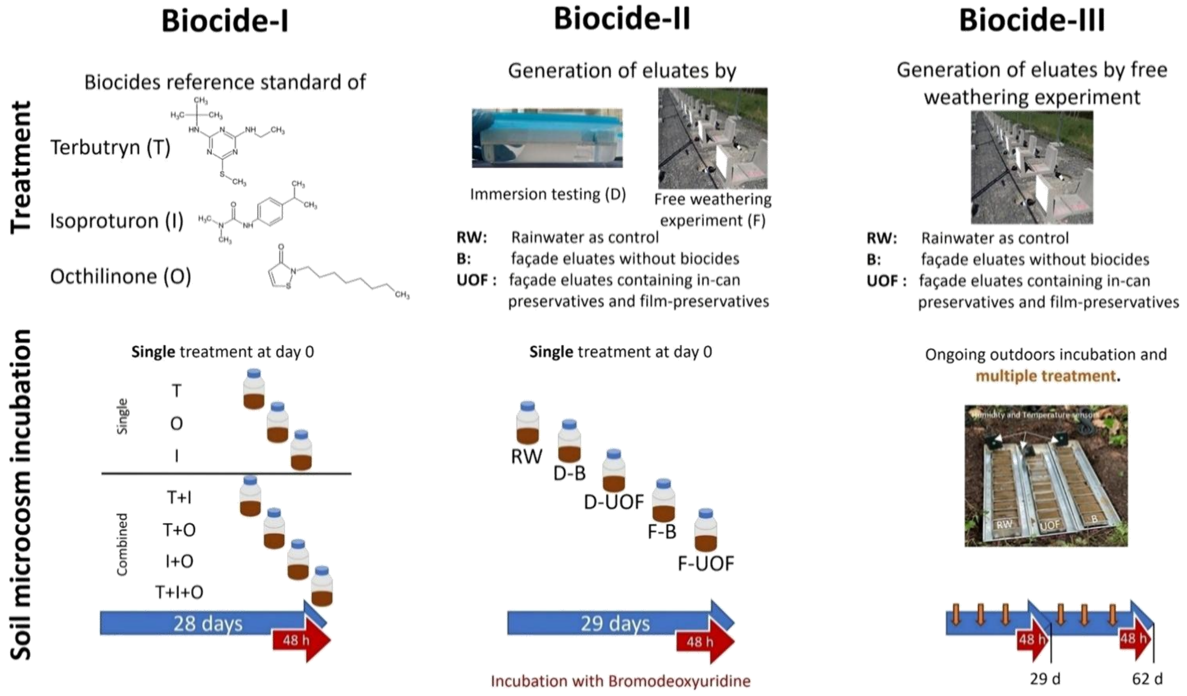


Figure 4: Overview of the three incubation experiments Biocide-I to -III with the corresponding characteristics and differences.

All studies were conducted using RefeSol-02A, sourced from the Fraunhofer Institute for Molecular Biology and Applied Ecology, chosen for its lack of pesticide use for at least the past five years and its suitability for biological tests according to OECD terrestrial ecotoxicological guidelines (Schlich and Hund-Rinke, 2015).

2.2.1 Biocide-I microcosm study

To evaluate the ecotoxicological effects of commonly used biocides and their mixtures on soil microbial communities, the study employed soil microcosms treated with terbutryn (T), isoproturon (I), and octhilinone (O) — both individually and in combinations — at environmentally relevant concentrations not exceeding 10 µg g^{-1} (Kiefer et al., 2024). Microbial activity was assessed using BrdU labeling to identify metabolically active bacteria and fungi. After 28 days of incubation, DNA extraction and immunocapture techniques enabled separation of total and “active” DNA, which were analyzed through amplicon sequencing (16S rRNA gene and ITS) to assess community structure and function. Gene copy numbers, soil respiration, and physicochemical parameters were also measured to evaluate microbial biomass and activity.

2.2.2 Biocide-II microcosm study

To assess the impact of facade eluates on soil microbial communities, this study treated soil microcosms with runoff generated from building facade s using two leaching procedures: standardized immersion testing (DIN EN 16105) and natural weathering. Eluates were derived from facades with and without biocides. The biocidal facade samples contained the in-can preservative Acticide SR 2081 (Thor GmbH, Speyer, Germany), which consists of benzisothiazolinone (BIT), chloromethylisothiazolinone (CMIT), and methylisothiazolinone (MIT). Additionally, the film preservative Acticide MKB3 (Thor GmbH, Speyer, Germany) was used, comprising terbutryn, octhilinone, zinc pyrithione, and zinc oxide. Facade eluates were applied over a 29-day incubation period. To ensure realistic concentrations facade eluates were diluted depending on the soil/facade ratio Additionally, high comparability between the Biocide-I and Biocide-II studies was ensured by using the identical laboratory experimental setup and standardized processes.

2.2.3 Biocide-III soil incubation study

Biocide-I and Biocide-II provided first insights into the ecotoxicological effects of biocides and facade eluates on the soil microbiome. However, laboratory soil microcosm experiments and the single application of biocides do not reflect the reality in the proximity of the buildings. Therefore, study Biocide-III was designed to mimic the realistic entry of facade eluates to soils. As only a small percentage of the rain reaches the facade and thereby leaches biocides and formulation components, it is necessary to consider the amounts of rain and the resulting dilution of facade eluate next to the building. Furthermore, soil microcosms in stainless steel boxes were placed outdoors in Coburg, Bavaria, Germany, from June to August 2021. These were inserted into the ground to maintain ambient soil temperature. Microcosms were protected against rain by a lid that allowed ventilation and enabled them to dry completely during rain-free periods. In line with the previous studies, 48 h before each sampling point BrdU labelling was conducted. The sampling points were after 29 days together with Biocide-II, experiments were conducted in parallel, and after 62 days. Downstream analytical methods were identical to Biocide-II.

2.3 General discussion

2.3.1 Recapitulation of the findings

The studies conducted in this dissertation provide initial insights into the effects of biocides and facade eluates on the composition and function of soil microbial communities. As reviewed by Reiß et al. (2021), data providing detailed species-specific information on the effects of biocides of building materials on the soil microbiome are rather scarce. Therefore, these studies were designed as follow-up investigations aimed at gaining more detailed information about bacterial and fungal stress responses to the respective treatments with increasing complexity and realism to the scenarios at the proximity of the buildings.

Biocide-I (Chapter 3.2) found that biocides commonly used in building materials significantly impact the active soil microbial community, affecting its diversity, richness, composition, and functional patterns. While the overall soil physicochemical parameters and soil respiration were not notably altered by the biocide treatments, the active microbial community showed considerable changes. Combined biocide treatments had a greater impact than single treatments, leading to a significant reduction in bacterial and fungal gene copy numbers. Specifically, the combination of terbutryn, isoproturon, and octhilinone (TIO) showed the largest decrease in both bacterial and fungal gene copy numbers compared to the respective water control. In terms of richness, active bacterial and fungal richness was not significantly affected by biocide treatment when compared to water control. However, total (T-) bacterial richness was highest in the T-TIO treatment and significantly different from the total terbutryn treatment (T-T). Total fungal richness showed no significant alteration due to biocide treatments compared to the respective water control. The study also observed shifts in the community composition and functional patterns, particularly in the active microbial communities. For instance, certain bacterial and fungal taxa benefited from the presence of biocides, while others were negatively impacted, as indicated by indicator species analysis. These findings highlight the sensitivity of the active soil microbiome to biocides and suggest its potential use as an ecotoxicological measure (Reiß et al., 2024).

Biocide-II (Chapter 3.3) found that facade eluates, whether produced by laboratory immersion testing or natural weathering, significantly affected both the total and active soil microbiome. Application of these eluates led to a reduction in total bacterial and fungal gene copy numbers, with the highest microbial abundance observed in untreated soils. Notably, active bacterial and fungal richness was lower than total richness and was significantly changed by eluate treatments. Community composition was clearly altered, especially in active communities, with dominance of *Pseudarthrobacter* in bacteria and reduced fungal diversity. Functional shifts were more pronounced in the fungal microbiome than in bacteria, with key functions and indicator taxa changing depending on the type of eluate applied. Effects occurred even with facade eluates lacking added biocides, suggesting that other components in facade materials (e.g., heavy metals, binders) also impact soil microbiota. Certain taxa benefited from eluate exposure, while others were omitted, highlighting both resistance and sensitivity within the soil microbiome. Overall, the results indicate that facade runoff, regardless of biocide content or leaching method, poses an environmental risk to soil microbial community structure and function, underscoring the need for further research and ecotoxicological assessment (Reiß et al., 2025).

The main findings of Biocide-III (Chapter 3.4) are that repeated exposure of soils to facade eluates, especially those containing biocides, led to major shifts in soil microbial communities over a two-month period. Bacterial gene abundances significantly declined with facade eluate exposure, particularly with biocidal treatments, while fungal richness increased in those same treatments. These changes were more pronounced within metabolically active microbial fractions than in the total community, as shown by BrdU-labeling and high-throughput sequencing. Indicator species analysis revealed distinct bacterial and fungal taxa linked to facade eluate exposure, including the loss of some rare and functionally important groups found only in untreated or rainwater-treated soils. Prolonged eluate exposure also shifted microbial functional potentials: bacterial communities showed increased ureolytic activity, a stress-response trait, while fungal communities experienced a decline in plant pathogens and a relative increase in photoautotrophic and saprotrophic taxa. Overall, these facade eluate-induced changes altered both the structure and predicted functions of soil microbiomes, reducing bacterial abundance, increasing fungal diversity, selectively omitting sensitive taxa, and promoting traits linked to environmental stress tolerance. The results highlight ecological risks posed by runoff from building materials, suggesting that biocides in facade products should be considered environmental hazards due to their disruption of soil microbial community composition and key ecosystem functions (Reiß et al., 2026).

Investigating the effects of biocides on the soil microbiome is essential for a comprehensive understanding of their ecological impact. Soil microbial communities are fundamental to ecosystem processes such as nutrient cycling, organic matter decomposition, and the maintenance of soil fertility and plant health (Fierer, 2017). Biocides leaching from building materials have the potential to disrupt these microbial assemblages, leading to reductions in diversity and alterations in functional capacity that could compromise soil health and ecosystem stability. Given that the active fraction of the microbiome is particularly sensitive to biocide exposure (3.2 Biocide-I Figure 3, Table 2; 3.3 Biocide-II Figure 3, Table 2; 3.4 Biocide-III, Figure 4), such disturbances may have immediate and significant effects on key soil processes, including biological (e.g. soil respiration, N and C cycling), chemical (e.g., nutrient availability, pH buffering) or physical processes (e.g., water retention, soil structure) (Chapter 1.2). Additionally, the persistence and accumulation of biocides in the environment underscore the importance of assessing both short term and long-term consequences, not only for targeted organisms but also for the broader soil ecosystem. Understanding these dynamics is vital for informed risk assessments and for guiding sustainable management practices regarding the use of biocides in urban and agricultural settings.

In all three studies I aimed to evaluate the impact of facade-derived biocides and their mixtures on soil microbial communities, with a particular focus on comparing responses of total versus metabolically active communities (3.2 Biocide-I, 3.3 Biocide-II, and 3.4 Biocide-III). In this chapter, I will provide an overview, comparison and discussion of the studies conducted. I will evaluate the relevance of the key findings for the ecotoxicological assessment of biocides originating from building materials.

2.3.2 Impact of biocides and facade eluates on soil microbial gene copy numbers

To monitor shifts in soil microbial communities following exposure to biocides and facade eluates, gene copy numbers for bacteria and fungi were quantified. Across all studies, bacterial abundance consistently declined. This decline was particularly notable following biocide treatments, especially in mixtures, despite the total biocide concentration being identical to that of single biocide applications (3.2 Biocide-I, Figure 1 A). As discussed in Biocide-I (3.2), these effects may arise from indirect interactions within the soil food web or from direct action on unidentified microbial targets (Reiß et al., 2024). Several studies reported a decline of bacterial gene copy numbers pesticide application of diverse chemical groups, e.g., triazines and carbamates (Feld et al., 2015; Milosevic and Govedarica, 2002). Consistent with these findings, facade eluate treatments also led to reduced bacterial gene copy numbers. Surprisingly, this effect was observed even in soils that had been treated with facade eluates without biocides, after a single facade eluate application compared to the respective water controls (3.3 Biocide-II, Figure 1 A). The most substantial bacterial reduction occurred in treatment T-F-UOF after both a single exposure (3.3 Biocide-II, Figure 1 A) and repeated applications in Biocide-III (3.4 Biocide-III, Figure 2 A). In Biocide-III, no bacterial recovery was observed during the 62-day incubation period, despite conditions that favored growth—evidenced by a doubling of bacterial gene copy numbers in the untreated rainwater control (3.4 Biocide-III, Figure 2A). At first glance, these findings seem unexpected, given that film-preservative biocides are not designed to target bacteria. However, it is important to note that at the beginning of a facade 's lifespan, both film preservatives and in-can preservatives leach out. Therefore, the observed decline in bacterial gene copy numbers is likely due to a combination of indirect ecological effects — such as trophic cascades, apparent competition, and trait-mediated indirect effects (as proposed in 3.2 Biocide-I) — as well as direct toxic effects from in-can preservatives such as BIT, CMIT, and MIT. Kiefer et al. (2024) confirmed that high concentrations of these substances were present in the facade eluates used in this study, especially in early-stage eluates, which were dominated by BIT.

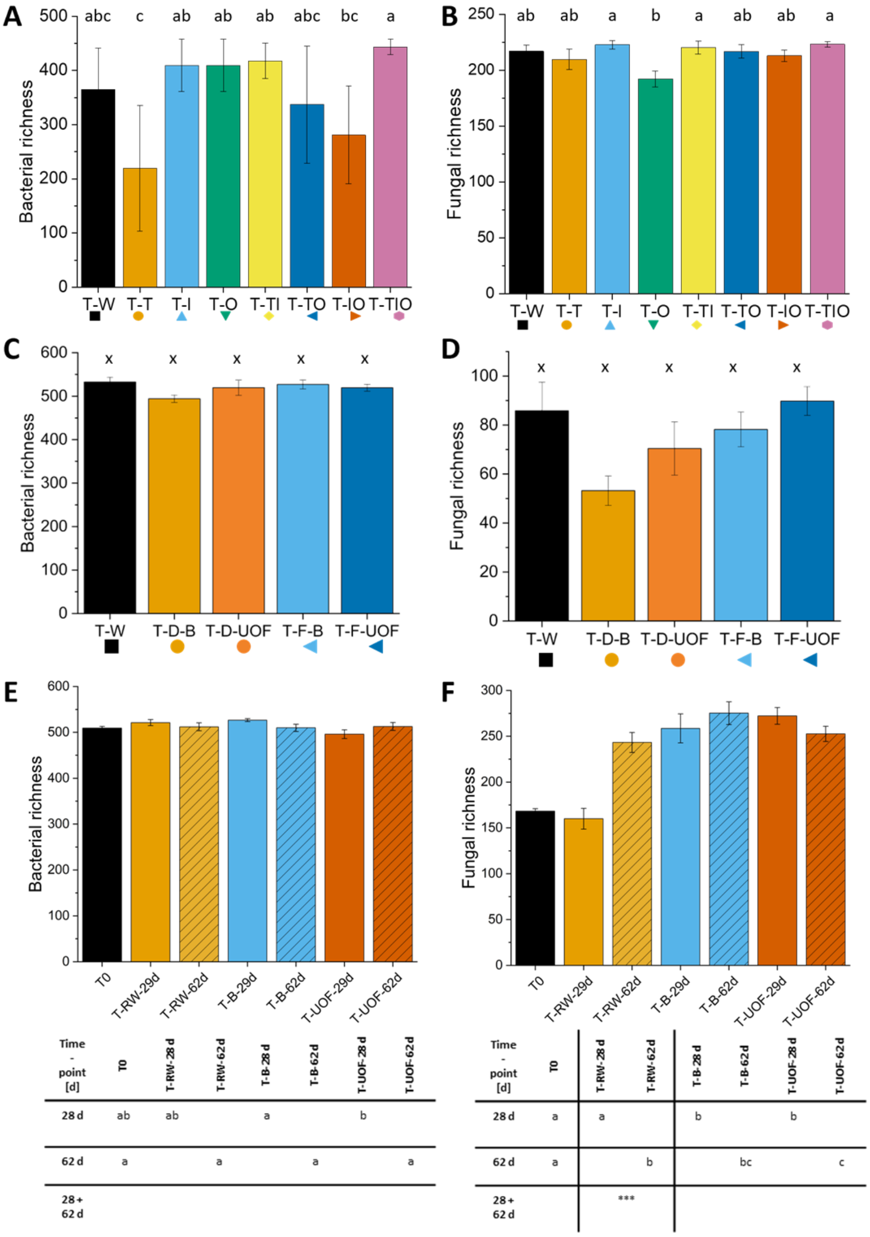
Fungal gene copy numbers followed similar trends across the studies but were generally less affected than bacteria. While fungi are typically considered more sensitive to biocides due to being primary targets, their reductions were less pronounced. In Biocide-I (3.2), fungal gene copy numbers were reduced only in the combined biocide treatments (3.2 Biocide-I, Figure 1B), suggesting lower sensitivity to terbutryn, isoproturon, and octhilinone. In Biocide-II (3.3), fungal gene copy numbers remained stable after a single facade eluate application under laboratory conditions (Figure 1B). Interestingly, in Biocide-III (3.4), fungal gene copy numbers increased during the first 29 days of incubation (3.4 Biocide-III, Figure 2B). This rise was greater in facade eluate-treated soils compared to water controls, suggesting fungi may have benefited from released ecological niches and increased nutrient availability due to competitor suppression and substrate input via the eluates. However, by day 62, a reversal was observed: fungal abundance dropped compared to the corresponding water-treated soil (T-RW-62d). This decrease may be attributed to changes in eluate composition over time, with film preservatives, known to target fungi, beginning to leach (Kiefer et al., 2024). Alternatively, the cumulative effect of repeated biocide applications may have raised soil concentrations to toxic levels (Linke et al., 2023), or biocidal degradation products formed through UV or microbial processes may have become increasingly harmful to fungi (Bollmann et al., 2017).

2.3.3 Contrasting effects of facade eluates and biocide treatments on soil physicochemical properties and microbial alpha diversity

Despite the differences in exposure sources, ranging from defined mixtures in Biocide-I to facade eluates in single (Biocide-II) and multiple application (Biocide-III), the results revealed consistent microbial responses. Soil microbial community structure correlates with soil physicochemical properties (Fierer, 2017). To prevent misinterpretation of the results, soil physicochemical properties were analyzed for significant changes within each experiment. Across all studies, no significant changes due to the treatments were observed. Only time-dependent changes were detected in Biocide-III, specifically for pH, NO_3^-, and SO_4^{2-} (3.4 Biocide-III). This observation suggests that this treatment had minimal impact on the broader soil ecosystem. The detected changes likely reflect natural soil processes rather than direct or indirect effects of the biocide, indicating a high degree of resilience within the soil microbial and chemical environment. Overall, Biocide-III did not cause significant ecological disruption under the tested conditions, as most soil parameters remained stable over time. These changes occurred consistently at each subsequent sampling point, but not between different treatments. Therefore, changes observed in the microbial community in study Biocide-III can be attributed to the presence of facade eluates (Reiß et al., 2026).

In line with the expectation that not all bacteria and fungi detected in soil total DNA are metabolically active, overall richness in active communities was consistently lower than total richness across all experiments, irrespective of treatment. For both Biocide-I and Biocide-II, bacterial and fungal richness, evenness, and diversity indices (Shannon and Simpson) did not differ significantly from their respective water controls in the total DNA samples, indicating no measurable effect of biocide or facade eluate treatments on total community

structure (**Figure 5**). Evenness values further demonstrated an equal distribution of species within total communities across treatments in microcosm experiments. Similarly, no significant differences in bacterial richness were observed after repeated facade eluate applications compared with water controls. However, after 29 days of incubation, bacterial richness was significantly reduced in eluates containing biocides (T-UOF-29d) compared with eluates without biocides (T-B-29d). By the final sampling point (T-UOF-62d), bacterial richness had recovered across all treatments, resulting in comparable richness levels after 62 days of incubation (**Figure 5** E).



Time - point [d]	T0	T-RW-28 d	T-RW-62d	T-B-28 d	T-B-62d	T-UOF-28 d	T-UOF-62d
28 d	ab	ab		a		b	
62 d	a		a		a		a
28 + 62 d							

Time - point [d]	T0	T-RW-28 d	T-RW-62d	T-B-28 d	T-B-62d	T-UOF-28 d	T-UOF-62d
28 d	a	a		b		b	
62 d	a		b		bc		c
28 + 62 d		***					

Figure 5: Overview of total bacterial (A, C, E) and fungal (B, D, F) richness in soil microcosms across the three biocide experiments: Biocide-I (A, B), Biocide-II (C, D), and Biocide-III (E, F). In Biocide-I and Biocide-II, statistically significant differences between treatments were determined using one-way non-parametric multivariate analysis ($p < 0.05$), indicated by different letters above the bar charts. For Biocide-III, differences were assessed using linear models with interaction terms (Group × Time), followed by estimated marginal means and pairwise comparisons ($p < 0.05$; $n = 5$). Significant differences within the same time point are denoted by different letters, while differences between time points are marked with asterisks (*). Treatment abbreviations: W – water control; T – Terbutryn; I – Isoproturon; O – Octhilinone; TI – Terbutryn + Isoproturon; TO – Terbutryn + Octhilinone; IO – Isoproturon + Octhilinone; TIO – Terbutryn + Isoproturon + Octhilinone; D-B – facade eluates from DIN testing without biocides; D-UOF – facade eluates from DIN testing containing biocides; F-B – facade eluates from free weathering without biocides; F-UOF – facade eluates from free weathering containing biocides; T0 – untreated soil; RW-29d/62d –rainwater for 29 or 62 days; B-29d/62d – facade eluates without biocides for 29 or 62 days; UOF-29d/62d – facade eluates containing in-can and film preservatives for 29 or 62 days.

In contrast to bacteria, fungal total richness increased after 29 days of incubation in laboratory experiments compared to water controls (**Figure 5** D, F). Evenness remained stable and showed no treatment-specific shifts. Consistently, fungal total richness also increased after repeated facade eluate applications, although evenness was reduced compared with both the rainwater control and untreated soil (T0). Similar results had been recently shown for fungal richness within brownfield samples (Mejia et al., 2023). During the 62-day incubation, fungal richness continued to increase in the rainwater control and T-B-62d, whereas a marked decline occurred in T-UOF-62d (3.4 Biocide-III, Figure 3B). This decline corresponded with the observed reduction in fungal abundance and is likely attributable to the onset of film-preservative leaching (section 2.3.2). At this point, T-UOF-62d also exhibited lower evenness relative to other treatments, supporting the assumption that facade eluates shape fungal communities through the disappearance or emergence of specific taxa. Reduced evenness reflects the dominance of only a few species, an effect also evident in active bacterial and fungal communities following facade eluate treatment. This suggests that analyses of active communities may provide earlier indications of community alterations compared with total DNA analyses.

Although active bacterial and fungal communities exhibited treatment-specific compositional changes, no significant differences in richness or evenness were observed in Biocide-I (3.2 Biocide-I, Figure 2B, D; Supplementary Information Table A.2, A.3) or after multiple facade eluate applications in Biocide-III (3.4 Biocide-III, Supplementary Information, Figure S2) compared to the respective water controls. In Biocide-II, however, bacterial and fungal richness differed significantly between the treatments A-D-UOF and A-F-B, although neither differed significantly from their respective water controls (3.3 Biocide-II, Figure 2 B, D). Notably, the A-D-UOF bacterial community displayed the lowest evenness. This reduced richness and evenness is likely attributable to elevated pH values and high biocide concentrations generated by the DIN EN 16105 laboratory leaching procedure, which employs harsh elution conditions (Kiefer et al., 2024; Reiß et al., 2025).

Across experiments, soils treated with facade eluates consistently exhibited reduced active bacterial evenness compared with water controls, suggesting that only a few dominant taxa were able to proliferate, while many others persisted at comparatively low abundances. This shift in community structure is consistent with previous reports of microbial responses to pesticide applications (Farthing et al., 2020; Mallet et al., 2019; Wang et al., 2020; Zhang et al., 2019). The same pattern was evident after both single and multiple facade eluate applications. Accordingly, facade eluate treatments shaped fungal communities toward lower diversity, with dominance of taxa harboring resistance traits (Maharana et al., 2025; Vischetti et al., 2020).

2.3.4 Facade eluates and biocides reshape soil microbiomes: insights into beta-diversity, active communities, and regulatory gaps

In line with observations for bacterial and fungal alpha diversity, beta diversity was significantly altered following exposure to facade eluates and biocides, with distinct responses observed between total and active microbial communities. At the genus level, total bacterial community composition generally remained unaffected after incubation with biocides, biocide combinations, or facade eluates, regardless of single or multiple applications. Consistent with expectations, total soil microbial communities in the Biocide-I study were largely unaffected by biocide treatments, except for the harshest combined biocide treatment (TIO), which induced significant changes. Although significant changes were anticipated for D-UOF and F-UOF treatments in Biocide-II due to this observation, only the T-D-B treatment showed notable alterations compared to water control, indicating that components of render and paint formulations can impact soil bacterial communities. Although the overall community pattern appeared unchanged in Biocide-III, significant differences emerged between sampling points at 29 and 62 days for RW and B treatments, as well as compared to untreated soil prior to incubation. Comparisons between treatments revealed that after 29 days, T-B-29d and T-UOF-29d communities differed significantly from their respective water controls. I therefore conclude that soil bacterial beta diversity is influenced not only by biocide concentration but also by the mode of entry, as the overall concentration was identical in Biocide-II and Biocide-III. After 62 days, significant deviations from rainwater controls were observed only in T-B-62d, but not in T-UOF-62d. Consistent with the Biocide-II findings, B eluates exerted a stronger impact on soil bacterial communities than UOF-eluates. Notably, even facade eluates free of biocides altered both soil bacterial and fungal communities, likely due to the presence of various non-biocidal substances that can act as stressors. During immersion testing and natural weathering, paints and renders release not only the intended biocides but also additional compounds such as heavy metals, binders, fillers, pigments, and degradation products (e.g., BIT, metal oxides), which may exhibit toxicity or alter soil chemistry (e.g., pH, ionic strength) (Reiß et al., 2026, 2025). In line with the present findings for the soil microbiome, biocide free facade eluates also induced significant effects in OECD test systems 219 (OECD, 2023) and 225 (OECD, 2007), affecting the higher organisms *Lumbriculus variegatus* and *Chironomus riparius*, respectively (Kiefer et al., 2024). For the toxicity studies conducted by Kiefer et al. (2024), the same dilution of facade eluates was applied.

While total bacterial communities retained taxonomic diversity across treatments, active bacterial communities were dominated by distinct genera as already suspected by the alpha diversity results. *Paenibacillus* and *Arthrobacter* in Biocide-I (Fig.3 A), *Pseudarthrobacter* in Biocide-II (3.3 Biocide-II, Figure 3 B), and *Pseudoalteromonas* in Biocide-III (3.4 Biocide-III; Figure 4 B). These genera are known for their resilience in polluted environments and their capacity to degrade chemical pollutants such as biocides (Bafana et al., 2010; Navarro-Torre et al., 2017; Scheublin et al., 2014; Villa et al., 2020; Wang et al., 2019; Zan et al., 2021). The variation in dominant taxa among the studies is surprising but may be partly explained by methodological limitations. In many amplicon-based microbiome studies, *Arthrobacter* and *Pseudarthrobacter* — despite being closely related — often cannot be reliably distinguished due to limited sequence divergence in the V3–V4 region targeted by the commonly used 341F/785R primer pair (~444 bp). Consequently, these genera may be misclassified under the same genus in short amplicon studies, especially when reference databases still group *Pseudarthrobacter* within *Arthrobacter* (Busse, 2016). In contrast to these closely related genera, *Pseudoalteromas* dominant in Biocide-III is a gram-negative rod-shaped bacterium unrelated to the dominant genera of Biocide-I and -II. Its prevalence in Biocide-III likely reflects the incubation conditions, which differed from the more stable laboratory soil microcosms of Biocide-I and II. In Biocide-III, environmental parameters such as temperature and soil moisture varied with rainfall, which may be the most critical additional factor influencing the soil microbiome. *Pseudoalteromonas* is not typically found in soil but is associated with wet and high-salinity soils (Handayani et al., 2022; Iijima et al., 2009; Navarro-Torre et al., 2020). Its dominance in Biocide-III is partly due to elevated soil moisture caused by frequent rainfall, as well as its resilience to heavy metals. Additionally, the higher abundance in soils treated with facade eluates compared to rainwater controls suggests it may utilize eluate components as substrates, likely due to its ability to degrade aromatic compounds (Wang et al., 2018). Future studies should validate this hypothesis using soil metaproteomics or stable isotope probing.

Biocide and facade eluate exposure markedly reshaped fungal beta diversity, as demonstrated by multivariate analyses of community composition. In the Biocide-I study, only the combined treatment T-TIO significantly affected total bacterial communities, whereas total fungal communities were already altered under single treatments (T-I) and in combinations (T-TO, T-IO, T-TIO) compared to their respective water controls (Fig.3 C). Consistent with these findings, facade eluates containing biocides (UOF) also caused significant shifts. Interestingly, even biocide-free B eluates induced pronounced changes in total fungal communities after single and repeated applications (3.3 Biocide-II, Figure 3 C; 3.4 Biocide-III; Figure 4 C,D). This suggests that either co-formulants and degradation products contribute to the observed toxicity, or that certain fungal species are capable of metabolizing compounds present in the facade eluates, thereby gaining growth and selection advantage. This challenges the assumption that biocide-free construction materials are ecologically benign. UOF eluates, by contrast, only affected total fungal beta diversity after repeated applications.

In line with observed evenness results, fungal communities exposed to biocide stress shifted toward dominance by taxa such as *Gibellulopsis* and unclassified *Sordariales* (3.2 Biocide-I, Fig.3 C,D; 3.3 Biocide-II, Figure 3 C; 3.4 Biocide-III; Figure 4 C,D). Eluates of B strongly shaped total fungal composition across studies, while effects on active fungal beta diversity were limited under single laboratory applications (29 days). However, A-F-UOF showed significant alterations in Biocide-II, and after 62 days of repeated exposures, active fungal communities (A-B-62d and A-UOF-62d) diverged from both A-RW-62d and untreated soil (T0). This highlights the importance of long-term, repeated low-dose exposures that mimic real environmental conditions. Active fungal communities showed the strongest compositional shifts, often enriched in uncharacterized taxa or stress-tolerant groups such as *Chlorosarcinopsis*, *Mortariella*, *Trichoderma*, and unclassified *Lasiosphaeriaceae*. Notably, although active fungal alpha diversity remained similar in Biocide-I, beta diversity revealed distinct community compositions under treatments A-IO and A-TIO (Fig.3 D). This demonstrates that communities can maintain within-sample diversity while undergoing marked structural turnover.

Biocide-I further revealed strong effects of combined biocide applications, underlining that current ecotoxicological assessments — which evaluate only single compounds — are insufficient (DIN German Institute for Standardization e. V, 2018; Reiß et al., 2024). Extrapolating from single compounds to mixture scenarios risks underestimating impacts. Moreover, the results with B eluates emphasize that not only active ingredients but also formulation additives influence ecotoxicological outcomes. Thus, existing regulations fail to adequately account for the risks associated with complex building materials (see also section 1.1). Both, total and active bacterial and fungal communities were altered by facade eluates and biocides, with the most pronounced effects observed in metabolically active fractions. This suggests that active communities are reliable indicators of sublethal environmental stress, irrespective of the exposure source.

Across diverse environmental contexts — from urban brownfields to facade-derived eluates and industrial soil pollution — soil microbial communities reveal remarkable sensitivity and adaptability to anthropogenic chemical stressors. Mejia et al. (2023) have shown long-term contamination by heavy metals, petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and volatile organics in a former rail yard significantly shaped microbial diversity across depths. Similarly, the results of this dissertation show that facade eluates — even those without biocides — prompt strong changes in the active soil microbiome, to a greater extent, than the total microbial community, underscoring the vulnerability of metabolically active soil bacteria and fungi. Meanwhile, Sazykina et al. (2022) demonstrated that PAHs and toxic elements reduce microbial diversity but favor pollution-tolerant phyla while yielding predictive bioindicator taxa. Together, these studies suggest that despite the different types of stressors — whether from brownfield legacy pollution, building materials, or industrial contaminants — active and depth-resolved microbial profiling consistently revealed shifts in richness, composition, and functional potential. This convergence emphasizes the need for integrative, multi-marker DNA-based methods to both diagnose soil health and identify tolerant or indicative microbial taxa for monitoring and remediation.

Nonetheless, certain methodological considerations apply: while beta diversity analyses are effective in detecting compositional shifts, they remain descriptive in nature and are influenced by factors such as sequencing depth and the choice of distance metrics (Alberdi et al., 2018). While they highlight turnover in microbial composition, they do not directly reveal functional mechanisms underlying the observed shifts. Functional annotation was applied here to partly overcome this limitation, but predictions still rely on reference databases and remain indirect; direct functional validation is needed (Sansupa et al., 2021). BrdU immunocapture further enriches for actively replicating taxa but misses slow-growing or metabolically active yet non-dividing organisms. This limitation is especially relevant for fungi, where DNA replication is often decoupled from metabolic activity (Singer et al., 2017).

Since BrdU uptake is not well reported (see 1.5.3), it is worth taking a closer look at the most abundant fungi found within the active fungal community. The study indicates that the ability to take up and incorporate thymidine analogs such as BrdU is highly variable across lineages. As expected, plants (e.g. *Brassica*) and green algae (e.g. *Chlorophyta*, *Chlorosarcinopsis*, *Coccomyxa*, *Parietochloris*) are capable of BrdU incorporation, consistent with prior reports that BrdU is routinely used as a marker of S-phase DNA synthesis in higher plants and algal cultures (Galbraith, 1989). It should be noted that the detection of *Brassica* and green algae (e.g., *Chlorophyta*, *Coccomyxa*, *Parietochloris*) likely reflects co-extracted DNA and primer cross-amplification, which are common in environmental sequencing of fungal communities. Such taxa are not fungi themselves but represent ecologically associated organisms whose DNA was captured alongside fungal sequences in our samples predominantly within outdoors Biocide-III (3.4) experiment. In contrast, most filamentous fungi and yeasts (e.g. *Fusarium*, *Mortierella*, *Malassezia*, *Cyberlindnera*) are generally considered inefficient at thymidine uptake due to the lack of nucleoside transporters and thymidine kinase activity (Boeke et al., 1987; Gossen and Bujard, 1992). Interestingly, the compilation suggests that some fungal lineages not previously examined may also be amenable to BrdU labeling under certain conditions, particularly those associated with aquatic or algal symbioses. To our knowledge, this is the first study to highlight the potential for BrdU labeling in these underexplored taxa, which opens new avenues for tracking DNA synthesis in diverse microbial eukaryotes. Nonetheless, efficiency is expected to be species- and condition-dependent, and systematic testing will be required to establish robust protocols across genera**.** Consequently, the “active community” assessed here represents only a subset of the true metabolically active microbiome.

Despite these caveats, the pronounced treatment-specific alterations in BrdU-labeled and total fractions strongly suggest that facade eluates impose stress on the soil microbiome. Future studies should complement BrdU labeling with approaches such as stable isotope probing (e.g., ^{13}C, ^{15}N), metatranscriptomics, or single-cell activity assays to capture additional dimensions of microbial activity. Extending monitoring beyond 62 days will also be essential to determine whether active communities recover or whether facade eluates leave persistent ecological legacies. Together, such efforts would verify the utility of active microbes as bioindicators of sublethal stress and clarify the long-term functional consequences of facade runoff on soil microbiomes.

2.3.5 Functional consequences for the soil microbiome

Functional annotation was used to provide insights into the mechanisms underlying compositional changes. Functional shifts, such as alterations in bacterial metabolic traits and fungal trophic modes, were noted across studies. All investigations distinguished between total and active microbial communities, revealing that the active community was far more responsive to biocide or facade eluate exposure.

Bacterial functions were consistently dominated by chemoheterotrophy/aerobic chemoheterotrophy across all studies. In Biocide-I, combined biocide treatments produced the strongest functional alterations, particularly in active communities, whereas single biocide applications induced milder changes (3.2 Biocide-I, Figure 4). The strongest effects were observed after TIO treatment in the active bacterial functional composition, even though no significant changes compared to the water control were detected in the overall community composition. This highlights that compositional analyses, as described in Section 2.3.4, lack functional resolution and may therefore overlook important effects on the soil microbiome. Despite these functional shifts, overall soil respiration and physicochemical properties often remained unchanged, suggesting functional redundancy that buffers ecosystem processes in the short term (Reiß et al., 2024). Facade eluates generated in the laboratory leaching experiment led to more pronounced alterations in bacterial functional community composition (3.3 Biocide-II, Figure 4). While the D-UOF eluate significantly altered total bacterial functional composition, no significant changes were observed in the active bacterial functions after this treatment. In contrast, active bacterial functions were strongly affected by eluates from biocide-free facade samples, regardless of the leaching experiment. Interestingly, fermentation increased in abundance in Biocide-II (3.3) and Biocide-III (3.4). This trend was already visible in Biocide-I for combined biocide treatments, suggesting that enzymes activated by fermentation may contribute positively to the bioremediation of polluted soils, either through direct degradation of biocides or indirectly as biostimulants. Previous studies have shown that fermentation-derived biostimulants promoted degradation of the herbicide oxyfluorfen (Tejada et al., 2022), facilitated the bioremediation of soils contaminated with benzo[a]pyrene (Xie and Cui, 2025), and supported the removal of heavy metals from polluted soils (Zhang et al., 2017). Fermentation can thus lower environmental and health risks by reducing the bioavailability of pollutants.

In line with the single-application Biocide-II study, no significant effects of facade eluates were observed compared to the respective water controls, except that A-B-29d and A-UOF-29d differed significantly. Interestingly, ureolysis increased across all active soil communities, including the controls, in Biocide-III. I therefore assume this observation was not caused by facade eluates. Ureolysis is strongly linked to soil properties and environmental factors, and the increase could result from incubation conditions (3.4 Biocide-III), as it was not observed in the laboratory microcosm studies Biocide-I and Biocide-II. However, significant alterations were detected in total bacterial functions between facade eluate-treated soils and the respective rainwater controls after 62 days of incubation. Surprisingly, in Biocide-III no significant alterations were detected in the BrdU-retrieved active bacterial community, whereas the total community displayed clear functional shifts compared to untreated soil and the rainwater control after 62 days. This discrepancy from the other studies can be explained by the methodological principle of BrdU labeling, which selectively targets bacteria actively undergoing DNA replication during the labeling period (Artursson and Jansson, 2003; Borneman, 1999). This subset is typically dominated by fast-growing and opportunistic taxa that are relatively resilient to environmental stressors (Artursson and Jansson, 2003; van Elsas and Boersma, 2011). Consequently, the functional composition of the BrdU-positive fraction is buffered by high redundancy and generalist traits, masking subtle effects of facade eluates. In contrast, the total community encompasses both dividing and non-dividing taxa, including slow-growing or stress-sensitive populations, which are more likely to be suppressed or altered under long-term exposure (Blagodatskaya and Kuzyakov, 2013; Lennon and Jones, 2011). Therefore, significant functional differences in the total community after prolonged incubation likely reflect cumulative impacts on non-dividing or dormant taxa, which remain undetected in the BrdU-active fraction. This result underlines the necessity of investigating both active and total microbial members to gain the most comprehensive insight into treatment effects.

Fungal functional profiles differed markedly. Total fungi were characterized by soil and litter saprotrophs and plant pathogens, which remained relatively stable. Active fungi, however, shifted toward soil saprotrophs and photoautotrophs, while plant pathogenic taxa declined. In Biocide-I, total soil fungal functions remained stable across treatments, with the sole exception of terbutryn-treated soil (T-T), which differed significantly from the water control (3.2 Biocide-I, Figure 4). While active bacterial functional compositions were highly similar across samples, active fungal functional community patterns showed greater variability — though these differences were not statistically significant compared to their respective controls. In contrast, active fungal communities were significantly affected by UOF eluates. Biocide-II showed partly contradictory results: T-D-B differed significantly from the water control within the total fungal community, whereas F-UOF eluates significantly affected the active fungal community (A-F-UOF). Interestingly, photoautotrophic algae increased in Biocide-II (3.3, Figure 4) and Biocide-III (3.4, Figure 5) compared to Biocide-I. I presume this increase was caused by inoculation from rainwater introduced via facade eluates. This effect was absent in the Biocide-II water control, which did not contain rainwater, but present in Biocide-III, where the control treatment consisted of natural rainwater from the weathering site. Rainwater often harbors diverse viable microorganisms, including algae, fungi and bacteria, which are washed out from the atmosphere or collected from roof surfaces during precipitation (Peter et al., 2014; Zhang et al., 2025). These organisms can inoculate soils upon deposition, potentially altering community composition and function.

As terbutryn — used for film preservation and present in UOF eluates — targets Photosystem II and inhibits photosynthesis, it is not surprising that photoautotrophic functions decreased in UOF-treated samples across studies (Guardiola et al., 2012). In line with the assumption that film-preservative biocides increase in later eluate sampling points, photoautotrophic fungi were lowest in A-UOF-62d. Similarly, the observed decrease in soil saprotrophs in Biocide-I was reproduced: UOF eluates reduced soil saprotrophs in Biocide-II compared to the respective B-eluate treatment, and this effect was also observed in A-UOF-29d in Biocide-III. Our results demonstrate that significant functional alterations became detectable only after 62 days of incubation when comparing facade eluate-treated soils with the respective water controls. This suggests that short-term functional redundancy initially buffered ecosystem processes, but cumulative stress effects emerged over longer exposure periods. Such delayed responses are consistent with evidence that microbial communities may appear functionally stable in the short term, while prolonged disturbance can gradually disrupt slower-growing or stress-sensitive populations, leading to measurable shifts in community function (Blagodatskaya and Kuzyakov, 2013; Lennon and Jones, 2011). Regarding plant pathogenic fungi, their dominance in Biocide-I was not reproduced in later studies. Plant pathogenic fungi remained at low abundance (<5%) in active fungal functional communities after single facade eluate treatment. The same was observed in Biocide-III, except for A-UOF-29d, where plant pathogens exceeded 10%. This increase could either result from the disruption of microbial community members that normally suppress pathogens or from indirect stimulation of pathogen virulence (Jeyaseelan et al., 2024).

While functional annotation provided important insights into microbial responses, several limitations of this approach should be considered. First, functional predictions are based on marker-gene data and reference databases, which remain incomplete and may misrepresent the metabolic potential of environmental taxa (Langille et al., 2013; Louca et al., 2018). Second, BrdU labeling selectively captures actively replicating organisms, thereby excluding metabolically active but non-dividing populations and biasing results toward fast-growing, opportunistic taxa (Artursson and Jansson, 2003; Borneman, 1999; van Elsas and Boersma, 2011). Furthermore, microcosm incubation conditions cannot fully reproduce field dynamics, and treatment effects may be confounded by incubation artifacts or microbial inoculation from rainwater-derived eluates. Importantly, observed functional shifts did not consistently translate into changes in soil respiration or physicochemical properties, highlighting that functional redundancy can buffer ecosystem processes in the short term (Lennon and Jones, 2011; Reiß et al., 2024). Despite these constraints, the presented results are useful and necessary as they provide first insights into how facade-derived biocides and eluates affect microbial functional composition. Future studies should verify and extend these findings by combining functional annotation with shotgun metagenomics, metatranscriptomics, or enzyme activity assays to directly measure functional genes and processes (Anantharaman et al., 2016; Prosser, 2015). Long-term field experiments and isotope tracing approaches would further help disentangle short term redundancy from persistent ecosystem impacts, and distinguish chemical from inoculation effects of facade eluates (Jansson and Hofmockel, 2020; Papp et al., 2018).

Across all studies, the key insight is that active microbial fractions provide a sensitive window into ecotoxicological impacts, revealing stress-adaptation (ureolysis, fermentation) and selective enrichment of tolerant taxa, while total communities may mask many of these early-stage effects. As described above BrdU labelling can also lead to misinterpretation when only analyzing the active microbes. This underscores the importance of targeting metabolically active as well as the total microbes when assessing the ecological risk of facade-derived pollutants.

2.3.6 Indicator-taxa reveal stress-resistant and sensitive microbial signatures

Across all three studies, indicator species analyses showed that facade eluates and biocides not only enriched certain stress-tolerant microbes but also consistently led to the omission of sensitive taxa from active soil communities. In the controlled biocide exposure experiment (3.2 Biocide-I), active bacterial indicators included *Aminobacter* (linked to ureolysis) and *Bacillus*, as well as fungal taxa such as *Metarhizium* and *Protosiphon*, reflecting their ability to withstand or metabolize biocides. However, other groups that are typically abundant in soils, including some *Acidobacteria* and saprotrophic fungi, were absent in biocide treatments, pointing to their vulnerability (Reiß et al., 2024). In the facade eluate single application study (3.3 Biocide-II), eluate treatments enriched *Proteobacteria*, while sensitive bacterial groups such as *Paenarthrobacter*, despite known resistance traits, and members of *Actinobacteria*, *Planctomycetotoa*, and *Verrucomicrobiota* largely disappeared from eluate-treated soils. Fungal communities showed a similar pattern: eluates promoted stress-tolerant taxa like *Trichoderma*, algal indicators such as *Chlorosarcinopsis*, and degradation-associated *Lasiosphaeriaceae*, but omitted sensitive groups including *Aureobasidium*, *Cercozoa*, and *Gamsia* (Reiß et al., 2025). The outdoor microcosm multiple facade eluate treatment study (3.4 Biocide-III) likewise identified bacterial indicators such as *Comamonas*, *Paracoccus*, and *Glutamicibacter*, all associated with bioremediation potential, but at the same time fungal taxa like *Saccharomyces* and *Aureobasidium*—well known for their sensitivity to pesticides and pH shifts—were excluded from eluate soils. Other sensitive omissions included host-dependent chytrids (*Rhizophydiales*) and soil fungi such as *Gamsia* (Reiß et al., 2026).

While the indicator analysis offers valuable initial insights into how facade eluates and biocides influence soil microbiomes, several limitations must be considered. The associations between indicator taxa and treatments are correlative, not than causal —certain bacteria and fungi may co-occur with facade eluates, but direct selection cannot be confirmed (Legendre, 2012). Taxonomic resolution is often low, with some indicators unclassified at family or order level, making ecological interpretations speculative. This is further complicated by database biases (Louca et al., 2018; Nilsson et al., 2019), as taxonomic and functional assignments rely on reference datasets skewed toward well-studied groups.

Rainwater inoculation may also confound results, potentially explaining the presence of genera like *Pseudoalteromonas*, which are unlikely soil residents (Dang and Lovell, 2016).The short experimental duration (29-62 days) limits conclusions about longer-term microbial resilience and recovery (Allison and Martiny, 2008).

Interpreting omitted taxa poses additional challenges. Absence in sequencing data does not confirm elimination — taxa may persist below detection thresholds or within the rare biosphere, which is difficult to capture with amplicon sequencing (Jousset et al., 2017). Functional losses are inferred from taxonomy rather than validated through metagenomics or soil function assays (Fierer, 2017), and microbial responses are context-dependent, with some taxa potentially re-emerging under different conditions (García et al., 2018). Despite these constraints, this study is among the first to apply high-resolution indicator analysis to microbiomes affected by biocide or facade eluate treatments, offering indicative rather than definitive results. Future research should integrate metagenomics, metatranscriptomics, and soil enzyme assays to link taxonomic shifts with functional changes (Delgado-Baquerizo et al., 2016; Prosser, 2015). Long-term field studies and controlled inoculation experiments could help disentangle treatment effects from rainwater microbes, while stable isotope probing or cultivation of candidate indicators would clarify their ecological roles (McMahon et al., 2011; Radajewski et al., 2000). Overall, facade eluates and biocides act as strong selective filters — some microbes thrive under stress or by degrading pollutants, while others are consistently lost, reducing community diversity. Both enriched and omitted taxa serve as complementary markers for assessing the ecological risks of facade-derived pollutants.

2.3.7 Experimental development: From controlled conditions to realistic outdoor exposure

The experimental design followed a stepwise approach. Biocide-I established a controlled soil microcosm framework to investigate the effects of biocides and their combinations, building on principles of standard terrestrial ecotoxicological assessments. Such assessments are typically conducted in standardized microcosms with reference soils and model organisms, focusing on well-defined endpoints to ensure comparability, reproducibility, and regulatory acceptance. In the experiments presented herein, however, model organisms were replaced by DNA metabarcoding coupled with BrdU immunocapture technique, which enabled the analysis of total and active soil microbial communities and thus provided a higher-resolution insight into microbiome responses.

All experiments were conducted with RefeSol-02A, a well characterized sandy loam soil from the standardized RefeSol soil system (Bussian et al., 2005). The use of this reference soil ensures reproducibility across laboratories and is recognized in several OECD terrestrial ecotoxicological guidelines (Schlich and Hund-Rinke, 2015) as well as by the German Environment Agency (Römbke et al., 2021). Its physicochemical and biological properties, including microbial activity, are well documented, and the soil has been free from pesticide application and major contamination for at least five years (Jänsch et al., 2005). This low background ensures that observed microbial responses can be attributed specifically to the test substances, such as biocides and facade eluates. Since sandy loam is representative of many Central European soils, RefeSol-02A provides a robust foundation for ecotoxicological testing. In accordance with standard soil ecotoxicological assessment, single biocides and their combinations were spiked into the soil and compared with untreated controls. Biocide-I therefore served as the initial experiment to establish baseline knowledge of biocide effects in soil microcosms without additional substances, providing the foundation for the subsequent Biocide-II and Biocide-III studies.

Building on this, Biocide-II and Biocide-III introduced increasingly realistic exposure regimes. In Biocide-II effects of eluates generated from different leaching methods were compared. Laboratory immersion tests (DIN EN 16105) provided reproducible eluates but lacked environmental drivers, whereas natural weathering generated eluates that, despite lower biocide concentrations, exerted higher ecotoxicity on aquatic and sediment organisms (Kiefer et al., 2024). This comparison highlighted how leaching methods shape pollutant composition, bioavailability, and ultimately microbial community responses (Kiefer et al., 2024; Reiß et al., 2026; Sazykina et al., 2022). Biocide-II investigated a single application of facade eluates under controlled moisture, whereas Biocide-III simulated repeated rainfall-driven applications outdoors, exposing soils to fluctuating temperatures, natural drying–rewetting cycles, and dynamic eluate composition. While stainless steel microcosms introduced artificial boundaries (Kuan et al., 2006), they allowed treatment-related differences to be reliably detected under semi-natural conditions. Importantly, Biocide-III was the first study to investigate prolonged, repeated biocide applications in soil microcosms, marking a methodological novelty in this field.

Taken together, these experiments reflect the established practice of soil microcosm studies in ecotoxicology while extending them into the underexplored domain of biocide-contaminated soils. The use of a standardized soil such as RefeSol-02A ensures comparability with existing test systems, whereas the incorporation of facade eluates provides an environmentally relevant contaminant source. Although future work should include a broader range of soil types and undisturbed field systems to capture ecological variability, the present approach demonstrates that combining DNA metabarcoding with the BrdU immunocapture technique offers a promising and innovative tool for terrestrial ecotoxicological risk assessment. This methodology enables earlier and more detailed detection of ecotoxicological effects on the soil microbiome than conventional approaches.

2.3.8 Implication for urban soil health

Recent research has uncovered critical insights into how microbial communities—key drivers of urban soil health — respond to environmental stressors such as biocides and facade runoff. Metabolically active microbes, including bacteria and fungi, act as early warning indicators, reacting more sensitively than the total DNA-based community. This sensitivity makes them valuable for detecting sub-lethal stress that may precede broader ecological degradation (Schloter et al., 2018). Exposure to biocides and facade eluates leads to a marked reduction in microbial abundance and diversity (see 2.3.3, 2.3.4), disrupting community composition and threatening essential soil functions such as nutrient cycling, organic matter decomposition, and C sequestration (Bollmann et al., 2017). These disruptions undermine the ecological integrity of urban soils, which are already under pressure from compaction, pollution, and land sealing. Alarmingly, even runoff from facades without intentional biocides can carry heavy metals, binders, and degradation products, which reshape microbial communities and pose hidden ecological risks (Vermeirssen et al., 2018). This highlights the need for more sustainable urban design and material choices that consider soil health impacts. Especially because urban soils are exposed to a variety of anthropogenic and natural environmental risks (Fiorentino et al., 2025).

Although functional redundancy among microbes can temporarily buffer ecosystem processes like soil respiration, this resilience is not infinite. Prolonged exposure to pollutants may exhaust this redundancy, leading to long-term declines in soil fertility, reduced C storage capacity, and impaired water regulation—functions that are vital for climate adaptation and urban sustainability (Chen et al., 2022). Certain microbial taxa respond to biocide exposure and could serve as biomarkers for monitoring soil health. Their use in environmental assessments could improve early detection of stress and guide targeted soil management strategies (Schloter et al., 2018). However, current EU biocide regulations are insufficient, as they focus on individual compounds and overlook the complex mixtures and chronic effects on soil ecosystems (European Parliament, Council of the European Union, 2012; Reiß et al., 2024). Regulation (EU) No 528/2012 emphasizes product safety but lacks provisions for long-term soil health monitoring and microbial indicators. To safeguard urban soil health, there is an urgent need for integrated risk assessment frameworks that incorporate active microbial metrics, long-term and mixture exposure scenarios, and ecosystem function indicators.

Soils are vital to ecosystems (Chapter 1.2), their rich biodiversity underpins multiple ecosystem functions, making them key to climate resilience and sustainable urban development. However, these soils are increasingly threatened by stressors like pollution, biocide runoff, and land sealing, which disrupt microbial communities and degrade soil functions. In order to safeguard their long-term health and functionality, soils must be recognized as critical ecosystems worth protecting. Therefore, terrestrial ecotoxicological risk assessment needs to be improved to identify indicators of soil microbiome stress.

2.4 Conclusion

This dissertation provides a comprehensive assessment of the impact of biocides and facade eluates from building materials on soil microbial communities, with a particular focus on metabolically active microbial fractions. Across three experimental studies — Biocide-I, Biocide-II, and Biocide-III — conducted under controlled laboratory conditions and realistic outdoor scenarios, the overarching Hypothesis H1 that metabolically active microbial fractions are more sensitive indicators of environmental stress than total DNA-based communities could be consistently confirmed. Active microbial communities responded more strongly to both pure biocide treatments and complex facade eluates, revealing significant shifts in microbial abundance, diversity, and community composition.

Targeted analyses in Biocide-I revealed that combined biocide treatments (terbutryn + isoproturon + octhilinone) significantly reduced bacterial and fungal gene copy numbers and altered microbial community structures, confirming Hypothesis H2. Despite these disruptions, core ecosystem functions like soil respiration and physicochemical properties remained stable short-term (Hypothesis H4), suggesting functional redundancy buffers ecosystem
processes — though this resilience may not hold under prolonged exposure.

Biocide-II showed that even runoff from biocide-free materials affected microbial communities, supporting hypothesis that co-released additives (e.g., heavy metals, binders) pose ecological risks (see 2.1.2). Laboratory eluates had stronger ecotoxicological effects than naturally weathered ones, with active microbial fractions more impacted. Indicator taxa such as *Trichoderma*, *Chlorosarcinopsis*, and *Lasiosphaeriaceae* emerged as biomarkers of stress.

Biocide-III simulated repeated runoff events, confirming Hypothesis H3: exposure mode and frequency shape microbial responses. Repeated exposure led to progressive shifts — bacterial decline, transient fungal richness, and delayed effects from film preservatives. Consistent biomarkers across studies included *Paenarthrobacter* decline and enrichment of stress-tolerant genera like *Tetracladium*, *Pseudoalteromonas*, and *Trichoderma*, suggesting potential for soil health monitoring.

Functional shifts (e.g., increased fermentation, ureolysis) indicated microbial stress and possible bioremediation traits, especially in active communities. Importantly, stochastic processes (dispersal, colonization, extinction) played a larger role than expected, challenging the assumption that microbial responses are solely driven by environmental gradients and highlighting the need for long-term, replicated studies. The findings of this dissertation expose critical gaps in current regulatory frameworks, particularly within the EU Biocide Regulation (Regulation No. 528/2012), which focuses on individual compounds and overlooks the effects of mixtures and chronic exposure. To improve ecological relevance, future risk assessments should incorporate active microbial metrics and long-term and mixture exposure scenarios. Building on these insights, future research is now poised to extend investigations to a broader range of soil types, particularly urban soils, to account for variability in sorption behavior, pH, and organic matter content. Long-term field trials exceeding the 62-day incubation period are needed to assess the persistence of microbial alterations and potential for functional recovery. Moreover, complementary "omics" approaches — including metatranscriptomics, shotgun metagenomics, stable isotope probing, and enzyme activity assays — should be integrated to validate the functional relevance of observed taxonomic shifts.

Altogether, this dissertation significantly advances our understanding of how biocides and facade runoff influence soil microbial ecology. It emphasizes the need to recognize urban soils as critical ecological infrastructure and to develop construction materials and regulatory strategies that minimize adverse impacts on soil health and ecosystem services.

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3 Declaration of contribution and publications

3.1 Author contribution

3.1.1 Contribution to joint publication Biocide-I

Title: Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials

Author: Fabienne Reiß, Nadine Kiefer, Witoon Purahong, Werner Borken, Stefan Kalkhof, Matthias Noll

Status: Published

Publication: Science of the Total Environment

Publisher: Elsevier

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Own contribution: samples and metadata collection (90% and 10% were carried out by NK), DNA analysis and DNA extraction (100%), PCR library preparation for sequencing (100 % by LGC Genomics GmbH (Berlin, Germany)), microbial taxonomy and data analyses, visualization (100%), manuscript writing (80% and 20% were carried out by NK, and MN).

Author contribution: FR and MN conceived and designed the study. FR and NK collected samples and metadata. WP and SK contributed reagents and laboratory equipment. FR led DNA analysis, bioinformatics, and the microbial taxonomy and data analyses. FR also led the data visualization. FR, NK, and MN wrote the manuscript. MN and SK supervised FR and NK. SK and MN acquired funding and administered the project. WP, WB, SK, and MN reviewed and gave comments and suggestions for the manuscript. All of the authors gave final approval for manuscript submission

3.1.2 Contribution to joint publication Biocide-II

Title: Facade eluates affect active and total soil microbiome

Author: Fabienne Reiß, Nadine Kiefer, Pascal Reiß, Stefan Kalkhof, Matthias Noll

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Author contribution: FR and MN conceived and designed the study. FR and NK conducted the investigation and contributed to methodology development. FR, NK, and PR carried out data analysis, with PR supporting the software and formal analysis. FR curated the data and led the visualization. FR, NK, and MN wrote the manuscript. MN and SK supervised the project. SK and MN acquired funding and administered the project. SK and MN reviewed and provided comments and suggestions on the manuscript. All authors approved the final version of the manuscript for submission.

3.1.3 Contribution to joint publication Biocide-III

Title: Continuous intake of facade eluates affects active and total soil microbiome

Author: Fabienne Reiß, Nadine Kiefer, Benjawan Tanunchai, Pascal Reiß, Stefan Kalkhof, Matthias Noll

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Author contribution: FR and MN conceived and designed the study. FR and NK conducted 4the investigation and contributed to methodology development. FR, NK, and PR carried out data analysis, with PR supporting the software and formal analysis. FR curated the data and led the visualization. FR and MN wrote the manuscript. MN and SK supervised the project. SK and MN acquired funding and administered the project. SK and MN reviewed and provided comments and suggestions on the manuscript. All authors approved the final version of the manuscript for submission.

3.2 Biocide-I: Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials

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Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials

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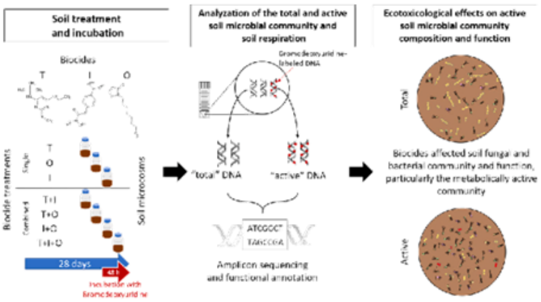
HIGHLIGHTS

- Soil physico-chemical parameters were not altered due to the biocide treatment.
- Effects on total soil microbial community (active and inactive DNA) due to the biocide treatment were moderate.
- Biocides directly affected active soil microbial community.
- Combined biocide treatment had greater impact than single biocide treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Combinations of biocides are commonly added to building materials to prevent microbial growth and thereby cause degradation of the façades. These biocides reach the environment by leaching from façades posing an environmental risk. Although ecotoxicity to the aquatic habitat is well established, there is hardly any data on the ecotoxicological effects of biocides on the soil habitat. This study aimed to characterize the effect of the biocides terbutryn, isoproturon, octhilinone, and combinations thereof on the total and metabolically active soil microbial community composition and functions. Total soil microbial community was retrieved directly from the nucleic acid extracts, while the DNA of the active soil microbial community was separated after bromodeoxyuridine labeling. Bacterial 16S rRNA gene and fungal internal transcribed spacer region gene-based amplicon sequencing was carried out for both active and total, while gene copy numbers were quantified only for the total soil microbial community. Additionally, soil respiration and physico-chemical parameters were analyzed to investigate overall soil microbial activity. The bacterial and fungal gene copy numbers were significantly affected by single biocides and combined biocide soil treatment but not soil respiration and physico-chemical parameters. While the total soil microbiome experienced only minor effects from single and combined biocide treatment, the active soil microbiome was significantly impacted in its diversity, richness, composition, and functional patterns. The

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active bacterial richness was more sensitive than fungal richness. However, the adverse effects of the biocide combination treatments on soil bacterial richness were highly dependent on the identities of the biocide combination. Our results demonstrate that the presence of biocides frequently used in building materials affects the active soil microbiome. Thereby, the approach described herein can be used as an ecotoxicological measure for the effect on complex soil environments in future studies.

1. Introduction

Biocides are commonly added to renders and paints to protect them against microbial growth during manufacture, storage, and after application (Bollmann et al., 2014; Burkhardt et al., 2011; European Parliament, Council of the European Union, 2012). Typically, three to five active ingredients are combined to protect the façade against a broad spectrum of microbial activity (Burkhardt et al., 2007). Biocides must be water-soluble for biological efficiency and can thus enter the environment by leaching from the façade (Burkhardt et al., 2012; Hensen et al., 2018; Linke et al., 2021; Paijens et al., 2020). Façades are coated by combining different products (e.g., paint, renders). This leads to a complex and rarely controllable combination of active substances from many possible material combinations continuously released into the environment (Reiß et al., 2021). Moreover, the biocides are designed to decrease microbial colonization along the lifetime of a façade, and industry guarantees up to fifteen years of service time.

Some biocides applied to building materials are also used as agricultural pesticides (Wittmer et al., 2011). The impact of agriculturally used biocides on the soil microbiome was mainly investigated for crop applications (Bromilow et al., 1996). A recent study highlights plant-soil microbiome feedback in an agricultural setup after pesticide treatment that induces resistance to above-ground herbivorous pests in horticultural crops (Pineda et al., 2020). Additionally, similar findings and data are missing for biocides in building materials. Since knowledge of the ecotoxicity of agricultural sites is not transferable to the toxicity of biocides used in building materials, testing guidelines are not directly transferable due to the difference in route of intake as well as the misleading focus and experimental setup of the previous agricultural-focused research (Reiß et al., 2021). However, in agriculture, the use of biocides is fundamentally different, as the area-wide but low-concentration application is intended only for the respective growing season (Heidorn, 2002). Thus, the findings on biocide effects acquired from the far more vast experience within the field of agriculture are only transferable to a limited extent to the scenario addressed herein.

A number of distinct factors govern accumulation of biocides in soil. Some of these factors are described as the climatic conditions (e.g., temperature, precipitation), the chemical properties of the soil (e.g., texture, organic matter content, pH, porosity, hydraulic conductivity), the chemical nature of the biocide (e.g., water-solubility, volatility, charge, molecular size, functional groups), and their interactions (e.g., sorption of biocides on soil particles, bio-availability of biocides), which has been reviewed in detail previously (Arias-Estévez et al., 2008). The toxicity of biocides against the soil microbiome depends on the nature of the chemical, the dose, the length of exposure, and the route of entry or absorption by the soil body (Kumar et al., 2012). The presence of biocides can lead to different effects on members of the soil microbiome like dormancy (Tidwell et al., 2015), cell lysis (Amjad, 2010), and changes in microbial activity (Bünemann et al., 2006). Some microorganisms survive biocide presence via efflux pump activity (Tegene and Tenkegna, 2020), modification of target structures (Sinning et al., 1989), and biocide degradation or inactivation (Kaufman and Kearney, 1970). Bollmann et al. (2017) showed by measuring the bioluminescence inhibition of *Aliivibrio fischeri* that the biocide octhilinone frequently used in building materials had the highest toxicity, followed by its degradation products as compared to terbutryn and its degradation products (Bollmann et al., 2017). Field data from soils obtained around houses suspected to be renovated with biocide-containing materials within the last five years supports the above results (Bollmann et al., 2017).

Biocide emission is expected to be very high after recent renovation with biocide-containing paints and/or renders. Among the characterized biocides, terbutryn, diuron, and triazoles showed low degradation rates in laboratory settings. Biocides of the triazine class can reduce the soil enzymatic activity and culturable microbial fraction over long time exposure due to their residual effects in soil, which is interpreted as high toxicity (Milosevic and Govedarica, 2002). Bacterial and fungal growth and substrate-induced respiration (SIR) assay showed that terbutryn caused microbial growth inhibition after 40 days of soil exposure (Fernández-Calviño et al., 2021). Most of these terrestrial ecotoxicity studies are still based on standard plate count, indirect measures such as SIR, or low-resolution molecular approaches to assess the ecotoxicological effect of biocides on the soil microbial community (Reiß et al., 2021).

Moreover, research has focused on the effects of single biocides on a small range of solely target organisms and not the mixed toxicity of combinations on a diverse soil microbial community composition. Often biocide usage does not affect the target organisms but also non-target organisms, especially in complex microbial community compositions, which were, for instance, frequent in soil environments. In order to effectively discriminate between the biocide effects within a complex microbial composition and function of a soil system, a high-resolution methodology, such as a DNA-based approach, is necessary. The DNA pool in soil consists of a mixture of (i) DNA in non-intact cells, (ii) relic or “extracellular” DNA able to persist for many years in soils (Nielsen et al., 2007), (iii) DNA of dormant microbes, (iv) DNA of living cells (Emerson et al., 2017), and (v) DNA of potentially active microbes that change activity state within minutes to few hours depending on trace quantities of specific nutrients (Blagodatskaya and Kuzyakov, 2013; Nobili et al., 2001). Bromodeoxyuridine (BrdU) immunocapture method combined with Illumina amplicon sequencing is a straightforward approach to differentiate the total genomic DNA from DNA derived from active metabolic organisms, as explained earlier (Purahong et al., 2022; Wahdan et al., 2021). BrdU incorporation has been shown to detect active bacteria in microcosms (Bravo et al., 2013; Kelly et al., 2016) and diverse natural habitats (Allison and Treseder, 2008; Goldfarb et al., 2011; Grubisic et al., 2017; Hjort et al., 2007; Taniguchi et al., 2015; Walters and Field, 2006). Wahdan et al. (2021) outlined that many studies had been conducted analyzing total and active (BrdU immunocaptured) microbial communities, which showed significant differences between these two communities. Further, the soil microbiome's next-generation sequencing data was affiliated with functional databases to assign its functional patterns (Sansupa et al., 2021; Tanunchai et al., 2022). BrdU-based phylogenetic analysis with functional assignment enables the quantification of the biocidal effect on the soil microbiome and identification of biocide-sensitive and in-sensitive microbiome members since the soil biodiversity allows assessment of the biocidal effects on the ecosystem services and functions (Reiß et al., 2021).

The study herein aimed to analyze the effect of three single biocides, terbutryn (herbicide), isoproturon (herbicide), and octhilinone (fungicide) commonly applied in building materials and combinations thereof on the total and active soil bacterial and fungal community composition; to investigate whether specific bacterial or fungal taxa benefit or lose due to the biocide presence, to determine if single biocide soil treatment cause a less intensive shift in the bacterial and fungal community composition and quantity as compared to biocide combinations with the same total biocide concentration, and whether soil microbial functions

are altered by respective biocide treatment. We expect biocide treatment to change the active soil bacterial and fungal community composition and function but not alter the corresponding total soil bacterial and fungal community composition and function. We hypothesize that fungal taxa, in particular, and to a lesser extent bacterial taxa, will be sensitive to biocide treatment as some biocides (octhilinone, terbutryn) are known to be fungicidal (i). On the other hand, we expect that other microbes will gain an advantage due to the inactivation of competitors and the addition of biocides as substrate (ii). We assume that combinations of biocides have a greater reduction of biocide-sensitive but an increase of biocide-insensitive members of the active microbial community composition than its single biocide application, which would be reflected in the phylogenetic and functional levels (iii). We expect that the overall soil physico-chemical parameters and microbial functions will not be altered by biocide treatment as biocide-sensitive microbes will be replaced on an equal footing by biocide-insensitive microbes (iv).

2. Methods

2.1. Soil incubation

The soil RefeSol-02A was obtained from the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Schmallenberg, Germany). RefeSol-02A was selected based on available documentation showing that pesticide use has not occurred for at least 40 years. The German Federal Environment Agency (UBA) recommended this soil as a reference for soil biological tests since it matches the OECD terrestrial ecotoxicological guidelines (Schlich and Hund-Rinke, 2015). RefeSol-02A soil was taken from a light humic stagnic luvisol and had a silty loamy texture and a sub-acidic pH (further soil properties are given in Table A.1).

For the experiment, 100 g field-moist and sieved (2 mm) soil were filled in 250 mL glass jars and adjusted to 50 % of the maximum water holding capacity. Soil microcosms ($n = 5$ per treatment) were either treated with double distilled water (water control; W) or with 10 μg g^{-1} of single biocides terbutryn (T) (2-*N*-*tert*-butyl-4-*N*-ethyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine, DRE-C17320000, LGC Standards GmbH, Wesel, Germany), isoproturon (I) ([*N*-(4-isopropylphenyl)-*N*′, *N*′-dimethylurea], DRE-C14470000, LGC Standards GmbH), or octhilinone (O) (2-octyl-1,2-thiazol-3-one, DRE-C15711500, LGC Standards GmbH) or biocides mixtures thereof 5 μg g^{-1} terbutryn + 5 μg g^{-1} isoproturon (TI), 5 μg g^{-1} terbutryn + 5 μg g^{-1} octhilinone (TO), 5 μg g^{-1} isoproturon + 5 μg g^{-1} octhilinone (IO), and 3.3 μg g^{-1} terbutryn + 3.3 μg g^{-1} isoproturon + 3.3 μg g^{-1} octhilinone (TIO) for 28 days at room temperature. Biocide treatments were named with a T- or A- prefix if results were retrieved from the total or active microbiome fraction. These concentrations were based on biocide emission rates described by Bollmann et al. (2017). Water loss through evaporation was controlled weekly by weighing and compensated by addition of double deionized water.

2.2. Soil respiration

CO_2 production and CH_4 consumption were measured after incubation at 1, 7, and 28 days. Glass jars were closed using lids with septa 24 h before measurement. The microcosms' gas phase was mixed using a gas-tight syringe during sampling. Afterward, an aliquot of 6 mL from 150 mL headspace was transferred to an exetainer (818 W; 5.9 mL, Labco Limited, Lampeter, Great Britain) for the gas measurement. The CO_2 and CH_4 concentrations were analyzed using a gas chromatograph equipped with a flame ionization detector and reduction unit (SRI 8610C, SRI Instruments, Torrance, CA, USA) at the department of Soil Ecology at the University Bayreuth, as described earlier (Peršoh and Borken, 2017). Certified gas standards (1000, 3000, and 10,000 ppm CO_2, 1.3, 2, 10, 30, and 100 ppm CH_4, Riessner Gase, Lichtenfels, Germany) were used to calibrate the gas chromatograph.

2.3. Soil chemical parameters

Soil pH was determined according to DIN EN 15933:2012-11 (DIN German Institute for Standardization e. V, n.d.-c). To estimate the organic soil content, the ignition loss was analyzed as described in DIN18128:2002-12 (DIN German Institute for Standardization e. V, n.d.-a). Soil anions were analyzed by ion-chromatography. Soil (5 g) and ultrapure water (25 mL) were mixed in tubes for 45 min and centrifuged at room temperature (25 min; 4695.6*g). Afterward, 12.5 mL supernatant was transferred to a fresh tube and filtered first with a 15 μm filter and second with a 0.22 μm filter. Before each measurement, all samples were degassed by ultrasound.

2.4. Quantification of biocides

Biocide concentrations were determined in the soil in triplicates of each treatment. For this purpose, soil (3 g) was weighed into weakly binding vials (Sarstedt AG, Nümbrecht, Germany). Methanol (15 mL) (MeOH, HPLC gradient grade, Carl Roth GmbH&Co.KG, Karlsruhe, Germany) was added, and the samples were shaken for 1 min and sonicated for 15 min at 25 °C in an ultrasonic bath for biocide extraction. Afterward, the samples were centrifuged at 1472*g at 5 °C for 10 min. The supernatants were transferred to a round-neck flask. This procedure was repeated three times. The supernatants from the extraction cycles were combined and concentrated to <0.5 mL using a vacuum rotary evaporator. The concentrate was diluted to 5 mL with a solution of 50/50 MeOH/H_2O (LC-MS grade, Carl Roth GmbH&Co.KG) and filtered with a 0.2 μm polyamide filter, and measured using high-performance liquid chromatography (HPLC) equipped with a UV detector (Waters Corporation Milford, Massachusetts, US). Separation was performed by a gradient method (flow rate of 0.2 mL*min^{-1}, using 70 % water with 0.1 % formic acid and 30 % MeOH for 3 min followed by a linear increased MeOH gradient up to 70 % after 5 min being maintained for additional 4 min). The analytes were quantified at a wavelength of 280 nm (O), 240 nm (T), and 227 nm (I). As stated above, T, I, or O was used for the quantification. Quantification was done using an external calibration curve (concentration range 1–10 ppm, linear fit, $R \geq 0.99$).

2.5. In-situ bromodeoxyuridine labeling, soil DNA extraction, and immunocapture

Soil samples were incubated with 100 mM BrdU (B5002, Merck, Darmstadt, Germany) solution at room temperature from day 26 for 48 h to the end of incubation. According to the manufacturers' instructions, DNA was extracted from all microcosms using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Europe GmbH, Freiburg im Breisgau, Germany). We refer to this DNA as “total DNA”, analogous to Wahdan et al. (2021). BrdU-labeled DNA was isolated from the “total DNA” by an immunocapture approach described earlier (McMahon et al., 2011). Briefly, for each sample, 2 μL monoclonal BrdU antibodies (1 mg μL^{-1} mouse anti-BrdU, clone BU-33, Sigma-Aldrich) were mixed with 18 mL denatured herring sperm DNA (1.25 mg mL^{-1} in phosphate buffer saline (PBS), Promega) and incubated for 45 min at 30 °C to form antibody-herring sperm DNA complex. Denatured DNA sample (20 μL) was then added to antibody-herring sperm DNA complex and incubated for 30 min at 30 °C to capture BrdU-labeled DNA. After incubation, the mixture was added to 6.26 μL aliquots of washed Dynabeads™ goat anti-mouse IgG (Invitrogen) in PBS–bovine serum albumin solution (PBS–BSA) and incubated under slow rotation for 35 min. The Dynabead complex (Dynabead-BrdU antibodies-BrdU-labeled DNA) was washed with 100 μL PBS–BSA eight times by adding the wash solution and trapping the complex with a magnetic particle concentrator (DynaMag™, Invitrogen™). BrdU-labeled DNA was released from the washed Dynabeads by adding 25 μL BrdU solution (1.7 mM in PBS–BSA) and then incubated under slow rotation for 35 min. The obtained immunocaptured DNA is now referred to as “active” DNA isolated from metabolically microbial active cells, as explained earlier (Wahdan et al., 2021).

2.6. *Quantitative PCR of soil DNA extracts*

The quantitative PCR (qPCR) for "total DNA" was performed to quantify the gene copy numbers of fungal internal transcribed spacer (ITS) regions with the primer set fITS7 (5′-GTGARTCATCGAATCTTTG-3′) (Ihrmark et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1994) and of the bacterial 16S rRNA gene with the primer set BAC341f (5′-CCTACGGGNGGCWGCAG-3′) and BAC758R (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). Quantifying each independent replicate in technical triplicates in 96-well plates using the CFX96™ Real-Time System C1000™ Thermal Cycler (Bio-Rad Laboratories GmbH, Feldkirchen, Germany), and nuclease-free master mix blanks were run as negative controls. Gene copy numbers were calculated by comparing PCR-cycle threshold values (Ct) to a standard curve of triplicate 10-fold dilutions of genomic DNA (gDNA) extracted from a known concentration of *Escherichia coli* K12 (DSM 423) and *Fusarium solani* (DSM 1164) by employing the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit according to manufacturer's instructions (Zymo Research Europe GmbH). The genomic DNA concentration per PCR reaction of *E. coli* and *F. solani* standard ranged from 1×10^9 to 5×10^3 and 6.51×10^6 to 65.1 gene copies, respectively.

2.7. *Amplicon sequencing of the soil microbiome*

"Total DNA" and "active DNA" samples were further analyzed by amplicon sequencing. The 16S rRNA gene and ITS region were amplified with the same primer sets used for the qPCR analysis to create amplicon sequencing libraries for the 85 "total" and "active" DNA samples, respectively. Inline barcodes and Illumina sequencing adapters were added to the amplicon sequence libraries using the Nextera CT Index Kit (Illumina, San Diego, CA, USA) and MiSeq Reagent Kit v3 600 cycles (Illumina) according to the manufacturer's instructions. PCR products for library preparation were purified by AMPure XP beads (Beckman Coulter, Brea, CA, USA). The sequencing of libraries was performed by 300 bp paired-end sequencing on an Illumina MiSeq platform (Illumina MiSequ V3; Illumina) based on a standard protocol from the manufacturer. Amplicon sequencing library preparation, sequencing, and sequence quality check were carried out by LGC Genomics GmbH (Berlin, Germany). The active and total microbiome's bacterial 16S rRNA and fungal ITS gene sequences were deposited in the NCBI nucleotide sequence databases under accession PRJNA870307.

2.8. *Bioinformatics*

Raw data pre-processing with demultiplexing, sorting, adapter trimming, and merging reads were congregated using Illumina bcl2fastq2 conversion software v2.20 and BBMerge v34.48 (Bushnell et al., 2017). The sequence quality of the reads was controlled with the FastQC software, version 0.11.8 (Andrews, 2010). Sequence pre-processing and Operational Taxonomic Units (OTUs) picking from amplicons was conducted using mothur 1.35.1 (Schloss et al., 2009). Sequences were aligned against the 16S mothur-Silva SEED r138 reference alignment depending on their Phred quality score over 33. (Glöckner et al., 2017; Quast et al., 2013; Yilmaz et al., 2014). Filtering of short alignments and reduction of sequencing errors was carried out by pre-clustering, where a maximum of one base mismatch per 100 bases within a cluster was allowed. Chimeras were eliminated with the UCHIME algorithm (Edgar et al., 2011). Afterward, taxonomical classification of the sequences against the Silva reference classification was conducted, and sequences of other domains of life were removed for OTU picking. OTUs were selected and assigned to a taxonomic level by clustering at the 99 % identity level using the average neighbor method with cluster.split (Edgar, 2018; Nilsson et al., 2019). Thereby OTU tables for DNA samples were generated. Sequence counts for fungi ranged from 1927 to 313,770 per sample and 1775 to 8,873,105 per sample for bacteria. The datasets were then rarefied to 9068 sequence counts for bacteria and 29,555 sequence reads for fungi, resulting in Good's Coverage estimates above 98 % for all samples.

Ecological and metabolic functions of detected bacterial OTUs were predicted using the Functional Annotation Tool of Prokaryotic Taxa v.1.1 (FAPROTAX) database (Louca et al., 2016; Sansupa et al., 2021). The functions of each phylogenetic taxa were annotated using literature on cultivable strains. FungalTraits database (Põlme et al., 2021), a specific functional prediction tool, was used to taxonomically parse fungal genera by ecological guild independent of the sequencing method. Bacterial and fungal function count tables for each DNA sample were generated.

2.9. *Statistics*

Statistical analyses were performed after OTUs were taxonomically summarized at genus level. Normal distribution of each dataset was tested via OriginPro (OriginLab Corporation, Northampton, MA, USA) by Shapiro-Wilk Test ($p < 0.05$). Rarefaction analysis, as well as the estimation of alpha diversity (OTU richness, Shannon index, Simpson index, Pielou's Evenness), and OTU richness estimators (Bias-Corrected Chao1 and an abundance-based coverage estimator (ACE)), were performed for "total" DNA and "active" DNA samples in RStudio (Version 2022.02.1, RStudio, Inc., Boston, MA, USA) and the package vegan 2.5-7 (Oksanen et al., 2022; R Core Team, 2022).

Alpha diversity indices were tested for normal distribution by Shapiro-Wilk Test ($p < 0.05$). Significant effects ($p < 0.05$) on total and active alpha diversity indices due to the biocide treatment were calculated either by one-way ANOVA with a post hoc adjusted Tukey test if the dataset was normally distributed, or Kruskal-Wallis ANOVA with a post hoc Dunn Test, if the dataset was not normally distributed, using the OriginPro (OriginLab Corporation). The same statistical procedure was used to analyze the effects of biocide treatments on total bacterial and fungal gene copy numbers. Non-metric multidimensional scaling (NMDS) was conducted using the "metaMDS" function in the vegan 2.5-7 package.

Permutation multivariate analysis of variance (NPMANOVA) based on Bray-Curtis similarity was performed using the software PAST 2.17c (Hammer et al., 2001) to analyze the differences between the different biocide treatments for active and total microbial communities and functions. Results were visualized by OriginPro (OriginLab Corporation). Afterward, Goodness-of-fit statistics (R^2) of environmental variables fitted to the NMDS ordination of active and total bacterial and fungal communities were calculated using the "envfit" function in the vegan 2.5-7 package, with p values based on 999 permutations (Oksanen et al., 2022; R Core Team, 2022). As explained by Noll et al. (2005), relative abundances were calculated for each sample and visualized with OriginPro (OriginLab Corporation).

The biocide treatment's significantly distinctive bacterial and fungal genera were identified using indicator species analysis conducted using the "multipatt" function in the indicspecies package (Cáceres and Legendre, 2009), which calculates indicator values (Stat value) with the "r.g." function.

3. Results

3.1. *Combined biocide treatment significantly decreased bacterial and fungal gene copy numbers*

The highest gene copy numbers were found in the total bacterial ($4.5 *10^7*g^{-1}$) and fungal ($2.2*10^5 *g^{-1}$) community of the water control treatment. Single biocide treatment had no significant effect on total bacterial (Fig. 1a) and fungal gene copy numbers compared to water control treatment (Fig. 1b). Total bacterial and fungal gene copy

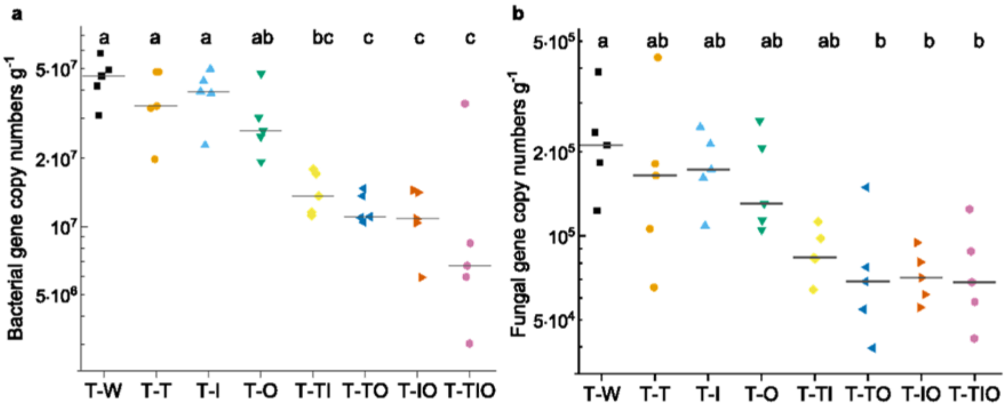

Fig. 1. Gene copy numbers of total (prefix T-) bacteria (a) and fungi (b) after different biocide(s) treatments (n = 5). Median (—) with individual data points are shown per treatment. According to Tukey's test, different letters indicate statistically significant differences ($p < 0.05$, n = 5). Outliers were identified by Grubbs test ($p = 0.05$) and not considered for the statistics. Water control (W, ■); Terbutryn (T, ●); Isoproturon (I, ▲); Octhilinone (O, ▼); Terbutryn + Isoproturon (TI, ◆); Terbutryn + Octhilinone (TO, ◀); Isoproturon + Octhilinone (IO, ▶); Terbutryn + Isoproturon + Octhilinone (TIO, ●).

numbers were significantly reduced by combined biocide treatments T-TI ($p_{Bacteria} = 1.782*10^{-5}$; $p_{Fungi} = 0.072$), T-TO ($p_{Bacteria} = 5.495*10^{-6}$; $p_{Fungi} = 0.043$), T-IO ($p_{Bacteria} = 3.132*10^{-6}$; $p_{Fungi} = 0.032$), and T-TIO ($p_{Bacteria} = 7.621*10^{-7}$; $p_{Fungi} = 0.039$) in comparison to water control treatment (Fig. 1a and b). Total bacterial gene copy numbers of the treatments T-T, T-I, and T-O were significantly higher than T-TO ($p_T = 6.68*10^{-4}$, $p_I = 1.98*10^{-4}$, $p_O = 0.03$), T-IO ($p_T = 3.79*10^{-4}$, $p_I = 1.12*10^{-4}$, $p_O = 0.02$), and T-TIO ($p_T = 5.82*10^{-5}$, $p_I = 1.84*10^{-5}$, $p_O = 0.003$). Total fungal gene copy numbers for T-TI did not differ significantly ($p = 0.07198$) from the water control treatment results. Upon comparison of combined biocide treatments, both total bacterial and fungal gene copy numbers were not altered between the treatment T-TI and T-TO, T-IO, or T-TIO. The largest decrease in both bacterial and fungal gene copy numbers were observed in treatment T-TIO. Total soil fungal and bacterial gene copy numbers were reduced by about 66.5 % and 74 % respectively upon the T-TIO treatment compared to the water control treatment (fungi: $p = 0.039$; bacteria: $p = 7.621*10^{-7}$).

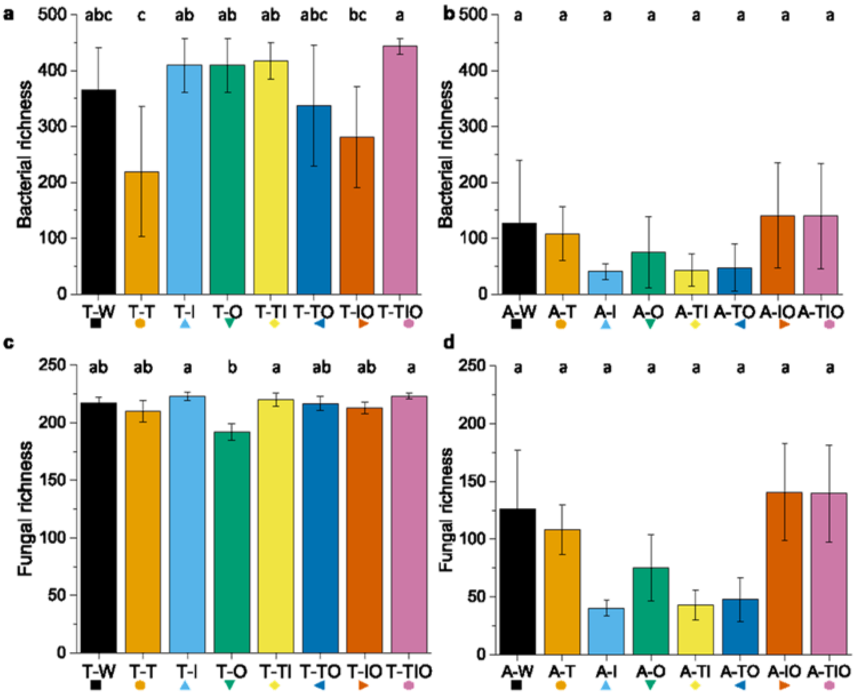

Fig. 2. Richness of total (prefix T-) and active (prefix A-) bacterial (a; b) and fungal community composition (c; d) after respective biocide(s) treatments (n = 5). Statistically significant differences were tested by ANOVA and Kruskal-Wallis-ANOVA ($p < 0.05$) and indicated by different letters. Error bars represent the standard error. Total (T-); Active (A-); Water control (W, ■); Terbutryn (T, ●); Isoproturon (I, ▲); Octhilinone (O, ▼); Terbutryn + Isoproturon (TI, ◆); Terbutryn + Octhilinone (TO, ◀); Isoproturon + Octhilinone (IO, ▶); Terbutryn + Isoproturon + Octhilinone (TIO, ●).

3.2. Biocide treatment significantly altered bacterial and fungal alpha diversity

The sampled soils were found to contain 3,843,009 sequences corresponding to 659 bacterial genera for active DNA and 895 for total DNA. For fungi, 6,712,328 sequence counts corresponding to 162 genera for active and 322 for total DNA were determined. Active bacterial and fungal richness was not significantly affected by the biocide treatment. Overall, active richness was detected to be lower than the total (compare Fig. 2a with Fig. 2b and Fig. 2c with Fig. 2d). Total bacterial richness was the highest for T-TIO (443 genera), significantly different from the T-T treatment (219 genera, $p = 0.001$). T-T was significantly diverged from T-I ($p = 0.009$), T-O ($p = 0.008$), and T-TI ($p = 0.010$). No significant effects in the total bacterial richness could be observed when the various biocides treatments were compared to the respective water control. For the total fungal richness, no biocide treatment resulted in significant alteration compared to the respective water control. The lowest richness was obtained for T-O (192 genera), whereas the highest value was found for T-TIO (223 genera). Due to its low richness, T-O was significantly different from T-I ($p = 0.018$), T-TI ($p = 0.039$), and T-TIO ($p = 0.016$).

Total and active bacterial and fungal alpha diversity indices were affected by the treatments (Table A.2) but were not significantly changed compared to the respective water controls (Table A.3). However, bacterial Shannon and ACE index of T-T was significantly different from that of T-TI as well as T-TIO, which was supported by significant effects in the Simpson index from T-T. The Shannon and Simpson index of T-IO was significantly changed in comparison to T-TIO for bacteria (Table 1a). The ACE index from A-TI an A-TO was significantly different from A-IO, looking at the active bacterial community composition (Table 1a). The active fungal community was also significantly affected by A-IO as opposed to A-TI (Table 1b). Chao1 of the fungal community composition upon T-O was significantly altered compared to T-I, T-TI, and T-TIO. The latter was also significantly changed in the ACE index compared to T-O (Table 1b).

3.3. Combined biocide treatment shifted the microbial community composition and function

Combined and single biocide treatments affected total and active bacterial and fungal community composition. T-TIO affected the total bacterial community composition, which differed significantly from the respective water control ($p = 0.0463$) and all biocide treatments except T-I (Fig. 3a, Table A.5a). Relative sequence read abundance of dominant bacterial families within the total water control, such as *Pseudomonadaceae*, decreased within the communities of biocide-treated soils (Fig. 3a, Table A. 4). Similarly, the total fungal community composition was significantly affected by single biocide treatment T-I as well as the combined treatments T-TO, T-IO, and T-TIO in comparison to the respective water control (Fig. 3c, Table A.5b). The fungal community composition of T-IO differed significantly from the single biocide treatments T-T, T-I, and the combined T-TI (Fig. 3c, Table A.5b). The relative sequence read abundance of dominant members of the active fungal community, for example members of the family *Nectariaceae* and unclassified members of the order Sodariales, were reduced in the biocide treatments compared to the respective water control (Fig. 3c, Table A. 4).

Table 1

Excerpt of p-Values of the active (prefix A-) and total (prefix T-) bacterial (a) and fungal (b) alpha diversity statistical analysis. Full overview of the determined p-Values is provided in Supplementary Table A3 ($p < 0.05$ highlighted in red; $n = 5$). W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO).

		Treatment comparison	Shannon	Simpson	Pielou's Eveness	Chao1	ACE
a	Bacteria	*T-T | T-TI*	0.3767	1	1	0.0906	0.0312
		T-T | T-TIO	0.0070	0.0010	0.0298	0.0787	0.0365
		T-IO | T-TIO	0.0338	0.0477	0.1064	0.1357	0.1241
		A-TI | A-IO	1	1	1	0.0768	0.0305
		A-TO | A-IO	1	0.9984	1	0.1839	0.0246
b	Fungi	*T-O | T-I*	0.6007	1	1	0.0304	0.0236
		T-O | T-TI	1	1	1	0.0235	0.0261
		T-O | T-TIO	1	1	1	0.0578	0.0306
		A-TI | A-IO	0.0222	0.0479	0.0840	0.2573	1

Active bacterial community compositions were not significantly affected due to the different biocide treatments compared to the respective water control. Significant differences were detected for the single biocide treatment A-O compared to A-T and A-I (Table 2a). Additionally, A-T sample was significantly altered compared with A-TI. The active fungal community composition of A-IO was significantly changed compared to A-T, A-I, A-TI, and A-TO (Table 2b). The relative sequence read abundance of members of the order Saccharimonadales was reduced in all treatments containing terbutryn (Table A. 4). Active fungal community composition was significantly altered due to A-IO and A-TIO in comparison the respective water control (Fig. 3d, Table 2b). The A-IO treatment was significantly different from all biocide treatments except for the A-TO and A-TIO (Table 2b). The active fungal community composition of A-TIO significantly diverged from the A-T, A-I, and A-TO (Table 2b). Dominant relative sequence read abundance of members of the family *Mortariellaceae* was reduced in the biocide-treated active fungal communities compared to the respective water control (Table A. 4).

Most bacterial indicator taxa were found in the A-TIO treatment (44 taxa, details see Table A.6) reflecting mainly chemoheterotrophic functions (Table 3). Members of the genera *Bacillus*, *Cutibacterium*, *Gaiella*, and the family *Vicinamibacteraceae* were the most abundant indicator taxa (Table 3). Whereas TRA3-20, *Romboutsia*, *Pseudonocardia*, and unclassified members of *Myoxococcaceae* were the indicator taxa with the highest Stat value of the A-TIO treatment (Table A.6). *Aminobacter* was the indicator taxon for the A-I treatment (Table 3). Three indicator taxa were identified for the A-IO treatment. The most abundant indicator taxa were KD4-96, followed by unclassified members of *Adhaeribacter* and Rhiziobiales (Table 3). For the water control, two indicator taxa *Pseudomonas*, and Phaselicystis, and 65 bacterial taxa were determined as only present within A-W treatment (Table A 4). Most abundant by the biocide's presence displaced taxa are uncultured *Acidimicrobiaceae*, Bacteroidetes VC2.1 Bac22 and KD3-10 (Table A. 4).

Most fungal indicator taxa of the active fungal community were identified in the A-IO treatment with manifold functions (Table 3). A-IO's indicator taxa with the highest abundance were *Metarhizium*, followed by unclassified members of *Clavulinaceae* and Helotiales, *Ilyonectria*, and *Micractinium* (Table 3). *Protosiphon* was the indicator taxon for the A-O treatment (Table 3). Unclassified members of the family *Pyronemataceae* were identified as indicator taxa for the A-TO treatment. Similar to the bacterial community, 19 fungal taxa were determined as only present in the respective water control A-W (Table A. 4). Most abundant omitted taxa were unclassified members of *Hydnodontaceae*, *Chlorella*, and unclassified members of *Peniophoraceae* and *Barnettozyma* (Table A. 4).

The functional patterns of the total bacterial community composition were not significantly shifted after any biocide treatment compared to the respective water control treatment (Fig. 4a). In turn, the biocide treatment significantly affected the functional pattern of the active bacterial community composition (Fig. 4b, Table 4a). The combined biocide treatments A-TI, A-IO, and A-TIO significantly differed from the respective water control. The functional pattern of A-TI was significantly

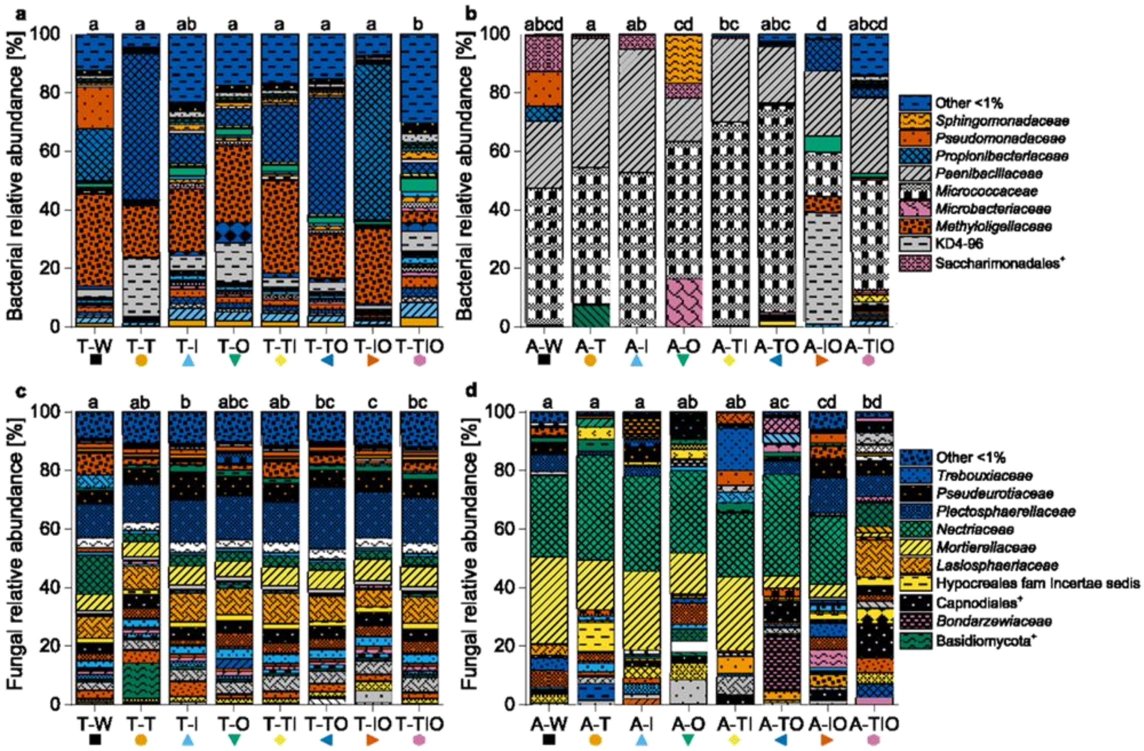

Fig. 3. Bacterial (a; b) and fungal community composition on family level (c; d) after biocide treatments (n = 5). The respective total (prefix T-; a; c) and active community composition (prefix A-; b; d) are indicated. The short legend highlights the fungal and bacterial families with a > 1 % (bacterial) and a > 9 % (fungi) relative abundance. Different letters indicate statistically significant differences according to one-way non-parametric multivariate analysis ($p < 0.05$) based on Bray-Curtis similarities (Tables 2, A.5). Table A 4 contains the bacterial and fungal relative abundances on family level per treatment. Total (T-); Active (A-); Water control (W, ■); Terbutryn (T, ●); Isoproturon (I, ▲); Octhilinone (O, ▼); Terbutryn + Isoproturon (TI, ◆); Terbutryn + Octhilinone (TO, ◀); Isoproturon + Octhilinone (IO, ▶); Terbutryn + Isoproturon + Octhilinone (TIO, ●). Unclassified members of the taxon are marked with ⁺.

different from all single biocide treatments and A-IO (Fig. 4b, Table 4a). Bacterial functional pattern of A-T differed significantly from A-TI, A-IO and A-TIO.

In contrast to the total bacteria, the functional patterns of total fungal community were significantly affected by T-T compared to the respective water control (Fig. 4c). The biocide treatment significantly affected the functional pattern of the active fungal community but not compared to the respective water control (Fig. 4d, Table 4b). The fungal functional pattern of A-T differed significantly from A-IO and A-TIO. Fungal functional pattern of A-I was significantly altered compared to the combined treatments A-TO, A-IO, and A-TIO. For the combined biocide treatments, fungal functional pattern was significantly different between A-TO and A-TIO (Fig. 4d, Table 4b).

The environmental parameters only had slight effects on fungal and bacterial community compositions (Table A.7). The soil respiration solely affected the total bacterial and fungal community composition. In contrast, total bacterial functional pattern was significantly affected by soil organic content and the amount of fluoride in the soil (Table A.8). The total fungal functional pattern was solely significantly affected by the amount of nitrate. Additionally, bacterial and fungal active richness significantly affected the respective functional patterns of the community compositions after biocide treatment (Table A.8).

4. Discussion

Biocides did not alter the main soil physico-chemical parameters (hypothesis iv; Table A.9). However, fungal and bacterial gene copy numbers (Fig. 1), community compositions (Figs. 2, 3), and functional patterns (Fig. 4) were affected after 28 days of treatment, particularly with combinations of biocides. Biocide combinations caused tremendous shifts in the active fungal and bacterial community compositions (Figs. 2, 3) and functions (Fig. 4) as compared to single biocide treatments. In contrast, the total (comprising active and inactive DNA) community composition and functions were affected to a much lesser extent by single and combined biocide treatments.

4.1. *Biocide treatment had a higher impact on the number of bacteria than on fungi*

The biocide treatment caused a reduction in total bacterial and fungal gene copy numbers, which is in line with the results previously observed (Milosevic and Govedarica, 2002). Moreover, single biocide treatments had a minor effect on bacterial and fungal gene copy numbers in comparison to the combined biocide treatment effect, even if the sum of biocide concentration within each treatment was the same. The biocide treatments reduced the bacterial gene copy numbers (Fig. 1a) to a similar extent as fungal gene copy numbers (Fig. 1b). Since terbutryn and octhilinone are known to be fungicidal, we presume that the effects on bacteria are not solely caused by the presence of biocide but also by the reduction or inactivation of associated fungi. This indicates that treatment with biocide combinations might be more effective than treatment with single biocides. The single biocide isoproturon had the lowest effect on bacterial and fungal gene copy numbers, followed by terbutryn and octhilinone (Fig. 1). As expected in hypothesis

Table 2

One-way non-parametric multivariate analysis based on the Bray-Curtis similarities of the active (prefix A-) bacterial (a) and fungal community compositions (b) ($p < 0.05$ highlighted in red; n = 5). W: Water control (■); T: Terbutryn (●); I: Isoproturon (▲); O: Octhilinone (▼); Terbutryn + Isoproturon (TI (◆)); Terbutryn + Octhilinone (TO (◀)); Isoproturon + Octhilinone (IO (▶)); Terbutryn + Isoproturon + Octhilinone (TIO (⬢)).

a Active bacterial community composition

		■	●	▲	▼	◆	◀	▶	⬢
		A-W	**A-T**	**A-I**	**A-O**	**A-TI**	**A-TO**	**A-IO**	**A-TIO**
■	**A-W**	1	0.0788	0.223	0.2202	0.0932	0.2355	0.0683	0.1908
●	**A-T**		1	0.9779	0.016	0.0144	0.1963	0.0248	0.0848
▲	**A-I**			1	0.0391	0.0612	0.2429	0.0145	0.1286
▼	**A-O**				1	0.5357	0.2668	0.0551	0.3341
◆	**A-TI**					1	0.4708	0.0327	0.3663
◀	**A-TO**						1	0.0386	0.3721
▶	**A-IO**							1	0.113
⬢	**A-TIO**								1

b Active fungal community composition

		■	●	▲	▼	◆	◀	▶	⬢
		A-W	**A-T**	**A-I**	**A-O**	**A-TI**	**A-TO**	**A-IO**	**A-TIO**
■	**A-W**	1	0.5923	0.9618	0.4297	0.1686	0.0859	0.0152	0.0076
●	**A-T**		1	0.807	0.6263	0.2563	0.2888	0.015	0.0082
▲	**A-I**			1	0.3129	0.3241	0.1971	0.0152	0.0171
▼	**A-O**				1	0.6502	0.7655	0.0244	0.0592
◆	**A-TI**					1	0.2399	0.0237	0.0737
◀	**A-TO**						1	0.0565	0.0294
▶	**A-IO**							1	0.0901
⬢	**A-TIO**								1

iii, the combination of all three biocides showed the highest decrease in bacterial gene copy numbers.

4.2. Biocides had only a minor impact on the total soil microbial community

Similar to the result above, single biocide treatments altered the richness of total bacterial community composition (comprising active and inactive DNA) less than the combined biocide treatments (Fig. 2, Table A.2) (hypothesis iii). The highest richness values were detected for the T-TIO treatment for both bacteria and fungi. No significant differences were observed in comparison with the respective water control for bacterial and fungal communities. Total bacterial alpha diversity of T-T sample was significantly changed compared to single biocide treatments and T-TIO. (Fig. 2a, Table 1). T-IO treatment led to significant changes within the alpha diversity compared to the T-TIO treatment (Table 1). This occurrence might be a result of higher observed diversity within T-TIO. In contrast, no significant changes in active bacterial richness between treatments and the respective water control were obtained. The ACE index was significantly lower within the A-TI and A-TO compared to the A-IO sample (Fig. 2b, Tables A.2, A.3), which aligns with the richness results since a higher ACE index indicates higher diversity. Similar results were found for fungal alpha diversity. Total fungal community was mainly affected by T-O, resulting in significant differences within ACE and Chao1 indices compared to T-I, T-TI, and T-TIO (Fig. 2c, Tables 1, A.2, A.3), which is caused by the significantly lower richness within T-I. In contrast, solely A-IO differed significantly from the A-TI treatment in terms of active fungal alpha diversities. This result disproves the initial expectation that biocides are ineffective in changing total soil microbial community composition (comprising active and inactive DNA).

4.3. Biocide combination showed the highest impact on soil microbial community composition

The effect of the biocide combinations on the active and total bacterial and fungal community composition significantly differed from the majority of single biocide treatments (Fig. 3, Tables 2, A.5). The total bacterial community composition was significantly affected by T-TIO in comparison to the water control, and all other biocide treatments except T-I (Fig. 3a, Table A.5). Total fungal community composition was strongly affected by the presence of biocides. T-I and the combined biocide treatments (except T-TI) shifted the total fungal community composition compared to the respective water control. Similar shifts were recently observed in sediments with glutaraldehyde or aldehyde-releasing single biocide treatments (Shi et al., 2021). However, both the environment and the biocides involved differed from the work herein. This supports our expectation (hypothesis iii) that combinations of biocides have a greater impact on the soil microbial community composition than single biocide treatments.

The methodology of BrdU application to retrieve active members of the microbial communities has been frequently shown in recent environmental community studies (McMahon et al., 2011; Purahong et al., 2022; Wahdan et al., 2021), demonstrating its universal applicability in many environmental habitats (e.g., rhizosphere, arctic soil, deadwood) and leading to reliable results. Each single biocide treatment reduced the richness of the active fungal and bacterial community composition (Fig. 2b, d). In contrast, the total bacterial and fungal richness increased due to the combined biocide treatment compared to the respective water control (Fig. 2a, c, Tables A.2, A.3).

Moreover, the active soil bacterial and fungal community composition was altered by the combined biocide treatments (Table 2), which to the authors' knowledge has not been studied before. No significant effect could be observed for active bacterial community composition compared to the respective water control. Interestingly, TIO treatment had no significant effects on active bacterial community composition, even though effects could be observed for the total community of T-TIO sample (Fig. 3a, b, Tables 2a, A.5). However, further significant differences between the different biocide treatments were recognized (Fig. 3b, Table 2a). The biocide octhilinone (A-O) had significant effects on the active bacterial community composition compared to A-T and A-I treatment (Fig. 3b, Table 2a). Since the BrdU method enables a more detailed picture of the effects on active bacterial community composition, the effect of the stand-alone octhilinone treatment could be shown even though it was not detected at the total community level (Fig. 3a, Table A.5). These results additionally indicate that the total community compositions were either (i) not affected, (ii) dormant, (iii) contained nucleic acids from necromass, or (iv) were in a sporulated form, which masks biocide interacting community members. This obfuscation could also lead to the differences between the bacterial community composition of A-TIO and T-TIO described above.

The highest effects on fungal community composition were observed for the combined biocide treatment A-IO. Interestingly, A-IO and A-TIO treatments showed significant effects, and the A-TO treatment did not, leading us to the assumption that the presence of isoproturon in combination with octhilinone might drive this effect. As the fungicide octhilinone can directly interact with fungi by inhibiting enzymatic activity as well as bacteria (Silva et al., 2020), an effect was expected (hypothesis i). Even though isoproturon interacts with different target sites on the photosystem (Guardiola et al., 2012), significant effects on bacteria and fungi could be observed in combination with octhilinone. Only phototrophic microorganisms can be considered as target organisms of isoproturon. But these comprise only a tiny proportion of the soil

Table 3

Indicator species analysis of active bacteria and fungi after biocide treatment showing the Stat value and significance of each species and its function, ordered after the relative abundance. W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO). Only genera with a relative abundance >0.5 % are shown for A-TIO, and all other indicator species of the A-TIO treatment can be found in the Supplementary Table A8. Unclassified members of the taxon are marked with $^{+}$.

	Biocide treatment	Stat value	p-Value	Significance	Taxa	Relative abundance [%]	Function
Active bacteria	I	0.577	0.0174	*	*Aminobacter*	0.024	Ureolysis
	IO	0.752	0.0009	***	KD4-96	45.035	NA
		0.610	0.0244	*	Rhizobiales^{+}	0.015	Plant pathogen
		0.527	0.0466	*	*Adhaeribacter*	0.018	Aerobic chemoheterotrophy
	TIO	0.591	0.0099	**	*Bacillus*	1.644	Chemoheterotrophy; aerobic chemoheterotrophy
		0.575	0.0058	**	*Vicinamibacteraceae*	1.156	Chemoheterotrophy; aerobic chemoheterotrophy
		0.632	0.0097	**	*Cutibacterium*	1.141	Pathogen
		0.611	0.0058	**	*Gaiella*	1.046	Chemoheterotrophy; aerobic chemoheterotrophy
	TIO	0.611	0.0121	*	Gaiellales uncultured	1.017	Chemoheterotrophy; aerobic chemoheterotrophy
Active bacteria		0.554	0.0155	*	Nocardioides	0.932	Aerobic chemoheterotrophy; aromatic compound degradation; chemoheterotrophy
		0.538	0.0095	**	*Candidatus* Udaeobacter	0.817	Chemoheterotroph
		0.576	0.0244	*	67-14	0.737	NA
		0.526	0.0050	**	RB41	0.725	Chemoheterotroph
		0.546	0.0290	*	IMCC26256	0.645	NA
		0.676	0.0020	**	*Romboutsia*	0.597	Fermentation; chemoheterotrophy
		0.610	0.0211	*	MB-A2-108	0.582	NA
	W	0.425	0.0272	*	*Pseudomonas*	0.024	Chemoheterotrophy; aerobic chemoheterotrophy
		0.541	0.0384	*	*Phaselicystis*	13.659	Chemoheterotrophy; aerobic chemoheterotrophy
Active fungi	IO	0.595	0.0075	**	*Metarhizium*	0.744	Animal parasite
		0.603	0.0088	**	*Clavulinaceae*$^{+}$	0.605	Ectomycorrhizal
		0.583	0.0013	**	Helotiales^{+}	6.164	Litter saprotroph
	IO	0.538	0.0082	**	*Ilyonectria*	3.245	Plant pathogen
Active fungi		0.580	0.0088	**	*Micractinium*	1.049	Photoautotrophic
	O	0.528	0.0432	*	*Protosiphon*	2.186	Photoautotrophic
	TO	0.483	0.0381	*	*Pyronemataceae*$^{+}$	1.349	Soil saprotroph
	IO + W	0.591	0.0183	*	Sordariales^{+}	6.760	Soil saprotroph

Significant levels: 0 < ‘***’ < 0.001 < ‘**’ < 0.01 < ‘*’ < 0.05 < ‘.’ < 0.1 < ‘ ’ < 1.

bacterial and fungal community composition (Fig. 4) and thereby do not cause such drastic overall effects. Nevertheless, most fungi and bacteria can be referred to as non-target groups of both biocides. Therefore, these observations can be explained by indirect effects due to the food web or direct effects from currently unidentified target sites.

From the regulatory perspective, ecotoxicological effects on the soil, as well as aquatic environments, were assessed only for single biocides treatments (DIN German Institute for Standardization e. V, n.d.-b). The effect of biocide combinations is currently extrapolated from the single biocide's recorded toxicity. Findings herein show that such extrapolations from single biocide treatments to combined biocide treatments were misleading and likely to lead to an underestimation, significantly impacting the accuracy of the ecotoxicological assessment. Such findings have already been found in aquatic ecotoxicological surveys (Wang et al., 2011). However, this study is one of the first to contribute to a better understanding of terrestrial ecotoxicological findings.

4.4. Distinct bacterial or fungal taxa benefit or lose due to the biocide treatment

Indicator species analyses for each treatment was conducted to assess the treatment effect on the active bacterial and fungal community compositions on the genus level. The indicator species analysis is a statistical approach to determine which taxa are significantly associated with biocide treatments by indicator values. This index is used to assess the predictive value of taxa as indicators of the respective biocide treatments (Cáceres and Legendre, 2009). Multiple chemoheterotrophic bacteria were identified as indicators in A-I, A-IO, and A-TIO treatment (Table 3). *Aminobacter* has been determined as indicator for the A-I treatment (Table 3). This genus is capable of ureolysis (Maynaud et al., 2012) and has been described to degrade pesticide residues in contaminated soils (Sørensen et al., 2007). Within the A-IO treatment, three indicator taxa have been shown (Table 3). The highest sequence read abundance taxa is KD4-96, frequently detected in contaminated soils (Gołębiewski et al., 2014; Wegner and Liesack, 2017). Nevertheless, their functions in those soils remain unclear (Kujala et al., 2018). Unclassified members of the order Rhizobiales, some slow-growing members of which were reported to be less affected by the presence of herbicides, were identified as indicator of A-IO treatment containing the herbicide isoproturon (van Rensburg and Strijdom, 1984). As a third indicator of A-IO, *Adhaeribacter* was determined (Table 3). *Adhaeribacter* has been described to degrade nonylphenol by nitration of aromatic compounds (Luque-Almagro et al., 2006). Such nitration could be beneficial in coping with biocide stress by mineralization and/or non-specific transformation (Kulkarni and Chaudhari, 2007). Moreover, this transformation is also beneficial to target organisms as it reduces the concentration of bio-available biocides.

Most soil indicator microorganisms were found in the active bacterial community composition in the A-TIO treatment, which was accompanied by a higher richness compared to the other treatments of the active bacterial community compositions (Fig. 2; Tables 3, A.2, A.8). *Bacillus* was the indicator taxon with the highest sequence read abundance within A-TIO (Table 3). Members of the genus *Bacillus* can degrade diuron, a phenylurea herbicide such as isoproturon (Ngigi et al., 2011; Tegene and Tenkegna, 2020). The degradation of diuron and isoproturon is initiated by the decarboxylation of the ureiod group of the phenylurea, which can be catalyzed by enzymes of the members of the genus *Bacillus* (Wallnöfer, 1969). Family members of *Vicinamibacteraceae*, a further indicator taxon of A-TIO, were described to be prevalent within contaminated sediments and to be able to degrade complex organic compounds (Zhang et al., 2022). *Cutibacterium* was identified as another indicator taxon for A-TIO (Table 3). This taxon has shown resistance against antibiotics in soil by tetracycline-resistance genes (Pérez-Valera et al., 2019). Since genes for biocides resistance by efflux pump activity have been reported in other closely related taxa (Wand et al., 2019), it was herein presumed that *Cutibacterium* also copes with

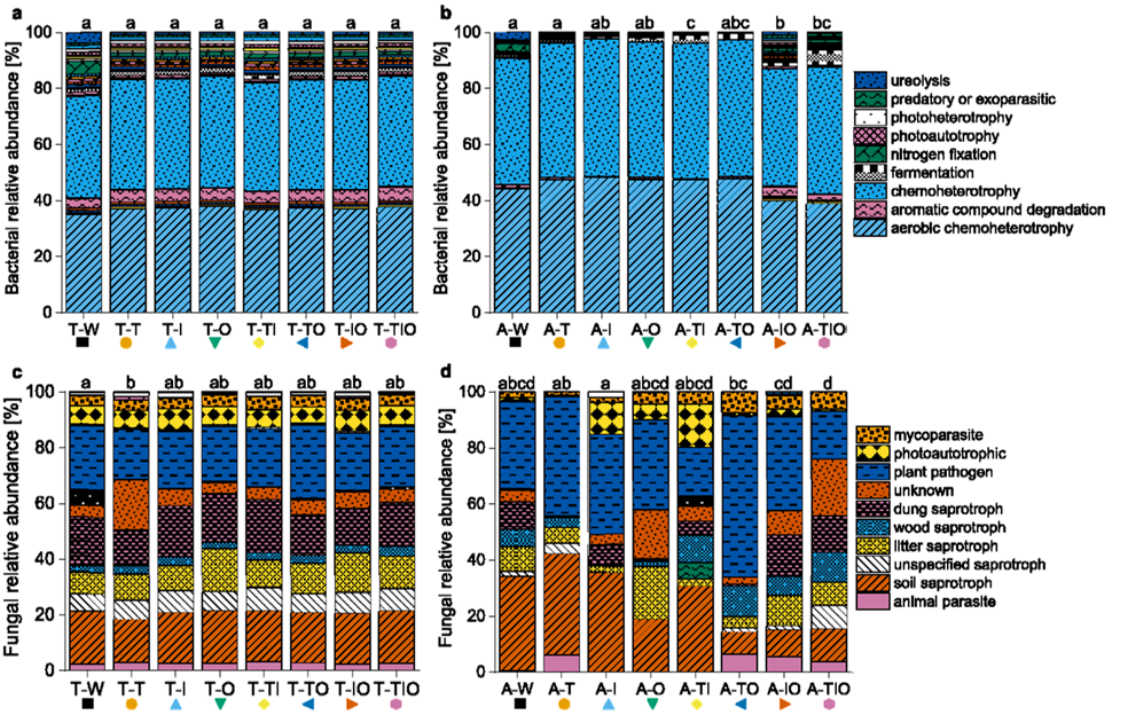

Fig. 4. Bacterial function composition on OTU Level (a; b) and fungal function composition on genus level (c; d) after biocide treatments (n = 5). The respective total (prefix T; a; c) and active composition (prefix A; b; d) are indicated. Short legend highlights bacterial functions with a > 5 % relative abundance. Complete legend, including an indication of all functions, can be found in the Fig. A. 1. Different letters indicate statistically significant differences according to one-way non-parametric multivariate analysis ($p < 0.05$) (Table 4). Total (T-); Active (A-); Water control (W, ■); Terbutryn (T, ●); Isoproturon (I, ▲); Octhilinone (O, ▼); Terbutryn + Isoproturon (TI, ◆); Terbutryn + Octhilinone (TO, ◀); Isoproturon + Octhilinone (IO, ▶); Terbutryn + Isoproturon + Octhilinone (TIO, ●).

the biocide stress by efflux pump activity. *Gaiella* was identified as another indicator taxon (Table 3), and Du et al. (2022) also identified this genus as indicator taxa for contaminated soil sites. The highest Stat value and therefore, the best association with A-TIO was found for TRA3-20 (Table A.8), which responded positively to the presence of pesticides and insecticides in soils resulting in a higher abundance (Balázs et al., 2020; Grenier et al., 2023). Based on these findings, TRA3-20 is expected to cope with the different biocides used in this study.

Members of *Pseudomonas* and *Phaselicystis* were identified as bacterial indicator taxa for the water control treatment (Table 3). Even though some members of the genus *Pseudomonas* are capable of degrading isoproturon (Dwivedi et al., 2011) and enzymatic cleavage of triazine rings (Karns, 1999), Members of the family *Pseudomonadaceae* were very sensitive towards the used biocides within this study as their relative sequence read abundances of the active and total bacterial community composition were reduced in the biocide treatments (Fig. 3a, b). Thereby, it is presumed that the detected *Pseudomonas* are sensitive towards the used biocides and combinations within this study. Li et al. (2020) showed that *Pseudomonas* was sensitive towards the biocide octhilinone and did prove that the toxicity is higher within biocide mixtures. *Phaselicystis* was found in the total bacterial community of the water control and in all biocide treatments of the total community (Table A. 4), which has been described to form myxospores (Garcia et al., 2009). In contrast, this genus was absent in most of the active bacterial community composition biocide treatments but present respective water control. Therefore, it is possible that the majority of members of this genus built myxospores to cope with the biocide stress.

Indicator taxa of the active fungal community composition were found in the treatments A-O, A-TO, and to the highest extent within A-IO sample (5 taxa, Table 3). *Protosiphon* could be identified as indicator for the A-O treatment. Since this otherwise well described genus was not known as an isothiazolinone degrader, it can be hypothesized it to benefit from the bio-available release of substrates by octhilinone-sensitive species or by inactivation of competitors or their predators such as protists. This affiliation to the described functional traits are supportive for hypothesis ii, but still needs to be verified in the future by substrate flux or establish predator-prey dynamics measurement in a similar habitat. Unclassified members of the family *Pyronemataceae* were determined as indicator species for the A-TO treatment. A recent study reported members of this family as indicator taxon in soil fungal communities after fungicide treatment (Schmidt, 2023). Further research is necessary regarding this family to elucidate the reasons for this biocide feedback. *Metarhizium* was identified as indicator taxon with the highest sequence read abundance for treatment A-IO (Table 3), which is able degrade Atrazine by involving cytochrome 450 (Szewczyk et al., 2020). The fungal degradation of methylisothiazolinone by cytochrome 450 was already shown recently (Nowak et al., 2020), which is based on the same basic chemical structure as octhilinone. Thereby, it stands to reason that members of the genus *Metarhizium* were involved in octhilinone degradation and therefore profited by presence of this biocide. Unclassified members of *Clavulinaceae*, Helotiales and *Ilynectria* were found as indicator taxa in the A-IO treatment (Table 3), which were so far not described in biocide-polluted soil environments. Moreover, *Micractinium* (Table 3), known to tolerate organic pollution (Palmer, 1969), was reported as indicator taxon for treatment A-IO. These findings verify the expectation herein that some bacterial or fungal taxa benefited from the presence of single biocides and biocide combinations (hypothesis ii). Due to this, these taxa should be further analyzed in future studies.

Moreover, some genera were only present in the respective water

Table 4
One-way non-parametric multivariate analysis based on the Bray-Curtis similarities of the active (prefix A-) bacterial (a) and fungal functional patterns (b). ($p < 0.05$ highlighted in red; n = 5). W: Water control (■); T: Terbutryn (●); I: Isoproturon (▲); O: Octhilinone (▼); Terbutryn + Isoproturon (TI (◆)); Terbutryn + Octhilinone (TO (◀)); Isoproturon + Octhilinone (IO (▶)); Terbutryn + Isoproturon + Octhilinone (TIO (●)).

a			■	●	▲	▼	◆	◀	▶	●
			A-W	**A-T**	**A-I**	**A-O**	**A-TI**	**A-TO**	**A-IO**	**A-TIO**
Active bacterial functional patterns	■	**A-W**	1	0.7389	0.6744	0.0570	0.0079	0.1516	0.0135	0.0461
	●	**A-T**		1	0.5184	0.0570	0.0074	0.1245	0.0149	0.026
	▲	**A-I**			1	0.1135	0.0086	0.1420	0.0518	0.1422
	▼	**A-O**				1	0.0386	0.1891	0.0641	0.4938
	◆	**A-TI**					1	0.6453	0.0070	0.4389
	◀	**A-TO**						1	0.0504	0.5029
	▶	**A-IO**							1	0.2968
	●	**A-TIO**								1

b			■	●	▲	▼	◆	◀	▶	●
			A-W	**A-T**	**A-I**	**A-O**	**A-TI**	**A-TO**	**A-IO**	**A-TIO**
Active fungal functional patterns	■	**A-W**	1	0.6582	0.745	0.4611	0.7676	0.0788	0.5259	0.2667
	●	**A-T**		1	0.2202	0.1594	0.1234	0.0918	0.0077	0.0081
	▲	**A-I**			1	0.2100	0.5644	0.0420	0.0486	0.0153
	▼	**A-O**				1	0.4547	0.2177	0.4134	0.2723
	◆	**A-TI**					1	0.1296	0.3150	0.4480
	◀	**A-TO**						1	0.2041	0.0324
	▶	**A-IO**							1	0.7650
	●	**A-TIO**								1

control but absent in any biocide treatment (Table A 4). For the active bacterial community composition, omission of 65 community members was identified. *Acidimicrobiaceae* showed the highest sequence read abundance within the omitted taxa followed by unclassified members of the order Bacterioidetes VC2.1 Bac22, and KD3-10. *Acidimicrobiaceae* are known to build dormant prophages within soils (Kieft and Anantharaman, 2021), and KD3-10 is member of the family *Myxococcaceae* which can form spores (Garcia and Müller, 2014). As described above for *Phaselicystis*, *Acidimicrobiaceae* and KD3-10 were absent in most of the active bacterial community composition biocide treatments and had the highest sequence read abundance in the respective water control. Therefore, it can be hypothesized that many of these were either dormant or sporulated in response to the presence of biocides. Not only in the bacterial but also in the fungal community compositions, 19 omitted microbes were found after biocide treatment (Table A 4). Most abundant taxa were *Hydnodontaceae*, *Chlorella*, and unclassified members of *Peniophoraceae* and *Barnettozyma*. Given that all biocides had *Chlorella* as target organism either by the inhibition of the photosystem (terbutryn, isoproturon) or the enzymatic activity (octhilinone), the omission is not surprising (Reiß et al., 2021). The other listed examples of omitted fungal and bacterial community members after biocide treatment were so far not described in literature. Therefore, their response to soil pollution or biocides should be further explored. More research is needed to understand the mechanisms of fungal and bacterial absence. These findings confirmed the second hypothesis that distinct bacterial or fungal taxa benefit or lose due to the biocide treatment.

4.5. Biocides had a higher impact on fungal functional patterns than bacterial

Not only was the microbial community composition altered by the biocide treatment, but the functional bacterial and fungal patterns were also affected (Fig. 4). No differences were detected between the different biocide treatments on the total bacterial functional patterns. Overall main functional assignments of the active and total bacterial community composition were nitrification and aerobic chemoheterotrophy. In contrast, the functional patterns of the active bacterial community was significantly altered by the combined treatments A-TI and A-IO in comparison to the respective water control and other biocide treatments (Figs. 3, 4b).

Whereas the effects of the biocides on bacterial functional patterns were relatively scarce, alterations of the total fungal functional patterns were detected upon treatment T-T (Figs. 3, 4c). Moreover, considerable shifts in the functional patterns of the active fungal community were observed (Fig. 4d). While the single biocide treatments did not differ, the combined biocide treatments A-TO, A-IO, and A-TIO showed tremendous shifts in their functional patterns compared to the respective water control and A-I. The highest decrease in the functional patterns was found in soil saprotrophs due to the combined biocide treatments (Fig. 4d).

Although differences were found in the functional patterns of the soil microbiome due to biocide treatments, it did not significantly alter the soil physico-chemical parameters and the overall respiration. This aligns with our expectation (hypothesis iv) that the biocide treatments do not affect soil's microbial functions and thereby maintain environmental functioning by functional redundancy. Functional redundancy of the soil microbial community compositions will buffer the loss or inactivation of important functional community members in this short-term observation. The long-term effects of biocide mixtures on microbial soil functions should be further addressed in future studies.

Biocides are used to protect façades, renders, and paints against microbial growth and biodeterioration. Although the targets of these biocides, which are leached out from building materials, are not soil, they affect the soil microbial community composition and its functional patterns. Biocide amount and combinations in soils caused by continuous release from buildings is an important source of soil pollution that should be considered in future environmental research.

5. Conclusions

Our results showed that the combined entry of biocides frequently used in building materials into the soil does not significantly alter soil respiration, or physico-chemical properties. However, biocides tremendously affect active soil microbial communities and their associated functions. The BrdU immunocapture method enabled the investigation of the members of the soil microbiome that benefited or lost due to the biocide treatments. The results of this study underline the importance of further in-depth research in this field, particularly in light of consequentially large amounts of biocide presently used in building materials. For instance, a metaproteomics survey can facilitate our understanding of microbial metabolic feedback on biocide presence in the soil environment.

Moreover, a more extended experiment would be beneficial to address long-term effects on the soil microbiome. It must be noted that the soils were treated with biocide directly and mixed after the biocide's addition to guarantee even distribution. This artificial laboratory experiment neglects the pathway of biocide entry and concentration from building materials and distribution in soil. Therefore, the effect of leachates from façades on the soil microbiome should be addressed in future studies to reframe a more realistic risk scenario of soil contamination. The results herein demonstrate that the active soil microbiome is

more sensitive than the total soil microbiome to the effects of biocides frequently used in building materials. Thus, it can be used as an ecotoxicological measure for studying the impacts of biocide treatments on soil biodiversity and functions.

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CRediT authorship contribution statement

Fabienne Reiß: Writing – original draft, Conceptualization, Investigation, Methodology, Validation, Formal analysis, Data curation, Visualization. **Nadine Kiefer:** Writing – original draft, Investigation, Methodology, Validation, Resources. **Witoon Purahong:** Writing – review & editing, Resources. **Werner Borken:** Writing – review & editing, Investigation, Resources. **Stefan Kalkhof:** Funding acquisition, Project administration, Writing – review & editing. **Matthias Noll:** Funding acquisition, Supervision, Conceptualization, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Table A4 (Excel format) from the supplementary material is accessible via the website mentioned above

Table A.1 RefeSol 02A soil characteristics as analysed by the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) according to DIN (Fraunhofer IME 2020).

	Sand [wt%]	**Silt** [wt%]	**Clay** [wt%]	**pH$_{CaCl2}$**	**C$_{org}$** [%]	**N$_{total}$** [g*kg^{-1}]	**CEC$_{effective}$** [mmolc* kg^{-1}]	**Watercapacity** [g*kg^{-1}]
RefeSol 02A	2.30	82.00	15.70	6.54	1.04	1.20	81.20	471.00

Table A.2 Diversity indices of total (prefix T-; a) and active (prefix A-; b) bacterial, total (c) and active (d) fungal community composition after biocide treatment (n=5). W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO).

		Treatment	**Shannon Index**	**Simpson Index**	**Pielou's Evenness**	**Chao1***	**ACE****
a	**Total bacteria**	*T-W*	3.02±0.57	0.73±0.10	0.51±0.09	475.22±38.26	475.48±29.73
		T-T	1.37±0.56	0.37±0.12	0.24±0.09	344.43±67.78	341.79±55.38
		T-I	4.02±0.46	0.89±0.05	0.67±0.07	522.88±23.43	517.00±20.89
		T-O	3.45±0.66	0.83±0.07	0.59±0.9	503.33±9.10	489.67±6.72
		T-TI	3.21±0.71	0.73±0.13	0.54±0.11	554.33±10.26	456.01±15.84
		T-TO	2.90±0.81	0.69±0.15	0.49±0.13	463.14±56.87	115.01±51.44
		T-IO	1.89±0.60	0.47±0.14	0.33±0.10	389.73±46.40	390.58±40.22
		T-TIO	4.74±0.02	0.98±5.4*10^{-4}	0.78±0.003	551.69±15.40	544.21±6.44
b	**Active bacteria**	*A-W*	1.18±0.35	0.50±0.11	0.24±0.06	215.40±58.0	218.00±56.15
		A-T	0.65±0.16	0.26±0.09	0.14±0.03	193.04±21.42	225.22±16.82
		A-I	0.62±0.24	0.28±0.12	0.14±0.05	85.65±11.63	155.09±36.19
		A-O	0.85±0.13	0.37±0.03	0.22±0.03	113.89±32.69	143.07±36.40
		A-TI	0.89±0.13	0.47±0.08	0.27±0.06	61.81±18.31	73.41±21.72
		A-TO	0.63±0.13	0.41±0.11	0.19±0.03	72.45±27.24	36.11±22.88
		A-IO	1.24±0.34	0.50±0.08	0.25±0.05	228.76±34.61	242.31±28.37
		A-TIO	2.63±0.78	0.66±0.18	0.54±0.16	164.00±43.19	160.24±41.64

		Treatment	Shannon Index	Simpson Index	Pielou's Evenness	Chao1*	ACE**
c	**Total fungi**	*T-W*	3.72±0.18	0.94±0.01	0.69±0.03	220.22±4.79	220.44±4.80
		T-T	4.02±0.02	0.96±0.002	0.75±0.006	216.19±9.75	214.45±9.69
		T-I	4.03±0.025	0.95±0.009	0.73±0.02	227.93±4.72	226.88±4.23
		T-O	3.83±0.09	0.95±0.006	0.73±0.02	198.55±6.37	195.91±7.55
		T-TI	3.99±0.06	0.96±0.002	0.74±0.009	228.83±5.10	226.52±5.52
		T-TO	3.95±0.032	0.96±0.004	0.73±0.009	224.71±6.26	223.09±6.15
		T-IO	3.89±0.055	0.96±0.004	0.73±0.008	216.46±6.25	216.31±6.26
		T-TIO	3.99±0.02	0.96±0.002	0.74±0.004	225.61±2.23	225.95±2.16
d	**Active fungi**	*A-W*	1.83±0.59	0.76±0.09	0.67±0.07	13.25±5.56	4.03±8.05
		A-T	1.62±0.65	0.73±0.16	0.79±0.03	11.4±7.96	12.19±10.22
		A-I	1.45±0.36	0.69±0.12	0.62±0.16	10.25±2.99	4.3. ±6.45
		A-O	1.60±0.39	0.73±0.09	0.65±0.13	13.3±4.97	7.73±11.59
		A-TI	1.12±0.59	0.55±0.23	0.52±0.21	9.6±2.70	4.42±6.05
		A-TO	1.75±0.15	0.58±0.29	0.55±0.28	21.1±9.96	24.51±17.37
		A-IO	2.57±0.46	0.89±0.03	0.81±0.01	27.7±17.84	17.58±27.43
		A-TIO	2.58±1.27	0.85±0.14	0.81±0.06	17.38±10.11	18.47±12.81

** Bias-Corrected Chao1, ** Abundance-Based Coverage estimator.*

Table A 3: p-Values from the statistical diversity analysis indices of active (prefix A-) and total (prefix T-)bacterial (a) and fungal (b) community composition after biocide treatment ($p < 0.05$ highlighted in red; n = 5). W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO).

		Treatment comparison	Shannon	Simpson	Pielou's Eveness	Chao1*	ACE**
a	**Total bacteria**	*T-I | T-IO*	0.2129	0.9763	0.7965	0.3059	0.9864
		T-I | T-O	0.9969	1	1	1	1
		T-I | T-TI	0.9761	1	1	0.9994	1
		T-I | T-TIO	0.9878	1	1	0.9995	1

	Total bacteria	*T-I* | *T-TO*	0.8736	1	1	0.9622	1
		T-IO| *T-TIO*	0.0338	0.0477	0.1064	0.1357	0.1241
		T-O| *T-IO*	0.5836	1	1	0.5724	1
		T-O| *T-TI*	1	1	1	0.9917	1
		T-O| *T-TIO*	0.7801	1	1	0.9917	1
		T-O| *T-TO*	0.9974	1	1	0.9973	1
		T-T | *T-I*	0.0588	0.3080	0.2850	0.0651	0.3724
		T-T | *T-IO*	0.9983	1	1	0.9921	1
		T-T | *T-O*	0.2373	1	0.9763	0.1835	1
		T-T | *T-TI*	0.0338	1	1	0.0906	0.0312
		T-T | *T-TIO*	0.0070	0.0100	0.0298	0.0787	0.0365
		T-T | *T-TO*	0.6107	1	1	0.4454	1
		T-TI | *T-IO*	0.7578	1	1	0.1532	0.1009
		T-TI | *T-TIO*	0.6091	0.7433	1	1	1
		T-TI | *T-TO*	0.9999	1	1	0.7955	1
		T-TO | *T-IO*	0.9261	1	1	0.8953	1
		T-TO | *T-TIO*	0.3754	1	1	0.7711	1
		T-W | *T-I*	0.9297	1	1	0.9893	1
		T-W | *T-IO*	0.8684	1	1	0.7999	1
		T-W | *T-O*	0.9996	1	1	0.9997	1
		T-W | *T-T*	0.5092	1	1	0.3267	1
		T-W | *T-TI*	1	1	1	0.8875	1
		T-W | *T-TIO*	0.4692	0.8530	1	0.8743	1
		T-W | *T-TO*	1	1	1	1	1
b	**Active bacteria**	*A-I* | *A-IO*	1	0.8288	1	0.6461	0.6373
		A-I | *A-O*	1	0.9988	1	1	1
		A-I | *A-TI*	1	0.9222	1	1	0.7073

	Active bacteria	*A-I* | *A-TIO*	0.6908	0.2441	0.6426	1	1
		A-I | *A-TO*	1	0.9900	1	1	0.6533
		A-IO| *A-TIO*	1	0.9672	1	0.2248	0.7026
		A-O| *A-IO*	1	0.9883	1	0.4175	0.4817
		A-O| *A-TI*	1	0.9984	1	1	0.8406
		A-O| *A-TIO*	1	0.5761	1	1	1
		A-O| *A-TO*	1	1	1	1	0.7969
		A-T | *A-I*	1	1	1	1	0.8361
		A-T | *A-IO*	1	0.7396	1	1	1
		A-T | *A-O*	1	0.9940	1	1	0.7015
		A-T | *A-TI*	1	0.8601	1	0.2357	0.0690
		A-T | *A-TIO*	1	0.18022	0.4779	1	0.8822
		A-T | *A-TO*	1	0.9719	1	0.5152	0.0566
		A-TI | *A-IO*	1	1	1	0.0768	0.0305
		A-TI | *A-TIO*	1	0.9074	1	1	0.6421
		A-TI | *A-TO*	1	0.9999	1	1	1
		A-TO | *A-IO*	1	0.9984	1	0.1839	0.0246
		A-TO | *A-TIO*	0.9112	0.7225	1	1	0.5864
		A-W | *A-I*	1	0.8276	1	1	0.8983
		A-W | *A-IO*	1	1	1	1	0.9996
		A-W | *A-O*	1	0.9881	1	1	0.7864
		A-W | *A-T*	1	0.7382	1	1	1
		A-W | *A-TI*	1	1	1	0.3002	0.0956
		A-W | *A-TIO*	1	0.9676	1	1	0.9323
		A-W | *A-TO*	1	0.9983	1	0.6426	0.0788
c	**Total fungi**	*T-I* | *T-IO*	1	1	1	0.8740	0.9224
		T-I | *T-O*	0.6007	1	1	0.0304	0.0236
		T-I | *T-TI*	1	1	1	1	1

		T-I | *T-TIO*	1	1	1	1	1
		T-I | *T-TO*	1	1	1	0.9999	0.9998
		T-IO| *T-TIO*	1	1	1	0.9577	0.9507
		T-O| *T-IO*	1	1	1	0.4345	0.3042
		T-O| *T-TI*	1	1	1	0.0235	0.0261
		T-O| *T-TIO*	1	1	1	0.0578	0.0306
		T-O| *T-TO*	1	1	1	0.0732	0.0660
		T-T | *T-I*	1	1	1	0.8601	0.8386
		T-T | *T-IO*	1	1	1	1	1
		T-T | *T-O*	0.8569	1	1	0.4542	0.4208
		T-T | *T-TI*	1	1	1	0.8100	0.8574
		T-T | *T-TIO*	1	1	1	0.9507	0.8844
		T-T | *T-TO*	1	1	1	0.9709	0.9724
		T-TI | *T-IO*	1	1	1	0.8263	0.9345
		T-TI | *T-TIO*	1	1	1	0.9999	1
		T-TI | *T-TO*	1	1	1	0.9997	0.9999
		T-TO | *T-IO*	1	1	1	0.9757	0.9932
		T-TO | *T-TIO*	1	1	1	1	1
		T-W | *T-I*	1	1	1	0.9834	0.9950
		T-W | *T-IO*	1	1	1	0.9998	0.9997
		T-W | *T-O*	1	1	1	0.2143	0.1271
		T-W | *T-T*	1	1	1	0.9997	0.9968
		T-W | *T-TI*	1	1	1	0.9693	0.9965
		T-W | *T-TIO*	1	1	1	0.9981	0.9981
		T-W | *T-TO*	1	1	1	0.9994	1
d	**Active fungi**	*A-I* | *A-IO*	0.2357	0.5523	0.5196	0.6988	1
		A-I | *A-O*	1	1	1	1	1

A-I | A-TI	1	0.8745	0.9625	1	1
A-I | A-TIO	1	0.8005	0.5361	1	1
A-I | A-TO	1	0.9567	0.9912	1	1
A-IO| A-TIO	1	0.9999	1	1	1
A-O| A-IO	0.4779	0.7537	0.7094	1	1
A-O| A-TI	1	0.7054	0.8717	1	1
A-O| A-TIO	1	0.9327	0.7250	1	1
A-O| A-TO	1	0.8487	0.9489	1	1
A-T | A-I	1	1	0.7389	1	1
A-T | A-IO	0.7420	0.7778	1	0.3599	1
A-T | A-O	1	1	0.8808	1	1
A-T | A-TI	1	0.6791	0.1932	1	1
A-T | A-TIO	1	0.9441	1	1	1
A-T | A-TO	1	0.8284	0.2866	1	1
A-TI | A-IO	0.0222	0.0479	0.0840	0.2573	1
A-TI | A-TIO	0.1999	0.1186	0.0889	1	1
A-TI | A-TO	1	1	1	1	1
A-TO | A-IO	1	0.0872	0.1380	1	1
A-TO | A-TIO	1	0.1999	0.1454	1	1
A-W | A-I	1	0.9987	0.9996	1	1
A-W | A-IO	1	0.8861	0.8196	1	1
A-W | A-O	1	1	1	1	1
A-W | A-T	1	1	0.9421	1	1
A-W | A-TI	1	0.5339	0.7750	1	1
A-W | A-TIO	1	0.9834	0.8324	1	1
A-W | A-TO	1	0.7017	0.8874	1	1

** Bias-Corrected Chao1, ** Abundance-Based Coverage estimator.*

Table A 4: Bacterial and fungal relative abundance on genus level per treatment group. Genera that are only present in A-W are marked with ~. Unclassified members of the taxon are marked with^{+}. EXCEL File Reiß_Supplementarymaterial_TableA.4

Table A.5 One-way non-parametric multivariate analysis based on the Bray-Curtis similarities of the total (prefix T-); bacterial (a) and fungal communities (b) ($p < 0.05$ highlighted in red; n = 5). W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO).

a			■	●	▲	▼	◆	◀	▶	⬢
			T-W	**T-T**	**T-I**	**T-O**	**T-TI**	**T-TO**	**T-IO**	**T-TIO**
Total bacterial community composition	■	**T-W**	1	0.1521	0.4710	0.5498	0.9928	0.3793	0.4023	0.0463
	●	**T-T**		1	0.0895	0.1204	0.2134	0.6464	0.9122	0.0092
	▲	**T-I**			1	0.8925	0.7794	0.2603	0.0929	0.0708
	▼	**T-O**				1	0.7352	0.2717	0.0734	0.0173
	◆	**T-TI**					1	0.5567	0.4420	0.0236
	◀	**T-TO**						1	0.7659	0.0468
	▶	**T-IO**							1	0.0079
	⬢	**T-TIO**								1

b			■	●	▲	▼	◆	◀	▶	⬢
			T-W	**T-T**	**T-I**	**T-O**	**T-TI**	**T-TO**	**T-IO**	**T-TIO**
Total fungal community composition	■	**T-W**	1	0.3749	0.0148	0.0678	0.0697	0.0386	0.0073	0.0243
	●	**T-T**		1	0.4899	0.6621	0.5970	0.7830	0.0203	0.3764
	▲	**T-I**			1	0.3465	0.1690	0.1850	0.0074	0.0622
	▼	**T-O**				1	0.6819	0.8030	0.1546	0.5581
	◆	**T-TI**					1	0.5757	0.0070	0.6580
	◀	**T-TO**						1	0.0602	0.9037
	▶	**T-IO**							1	0.2198
	⬢	**T-TIO**								1

Table A.6 Indicator species analysis of active bacterial and fungal community compositions after biocide treatment showing the Stat value and significance of each species and its function ordered after the Statvalue. Unclassified members of the taxon are marked with $^{+}$.

	Biocide treatment	**Stat value**	**p-value**	**Significance**	**Relative abundance [%]**	**Taxa**
Active bacteria	I	0.577	0.0174	*	0.024	*Aminobacter*
	IO	0.752	0.0009	***	45.035	KD4-96
		0.610	0.0244	*	0.015	Rhizobiales $^{+}$
		0.527	0.0466	*	0.018	*Adhaeribacter*
	TIO	0.683	0.0088	**	0.055	TRA3-20
		0.676	0.0020	**	0.597	*Romboutsia*
		0.665	0.0025	**	0.347	*Pseudonocardia*
		0.654	0.0096	**	0.108	*Myxococcaceae* $^{+}$
		0.648	0.0014	**	0.226	*Curvibacter*
		0.645	0.0165	*	0.185	Subgroup 17
		0.641	0.0083	**	0.172	*Solirubrobacter*
		0.636	0.0173	*	0.264	*Clostridia* $^{+}$
		0.632	0.0097	**	1.141	*Cutibacterium*
		0.621	0.0052	**	0.292	*Bradyrhizobium*
		0.618	0.0232	*	0.269	*Ellin 6055*
		0.617	0.0262	*	0.161	*Candidatus Xiphinematobacter*
		0.611	0.0058	**	1.046	*Gaiella*
		0.611	0.0121	*	1.017	Gaiellales uncultured
		0.610	0.0211	*	0.582	MB-A2-108
		0.605	0.0192	*	0.112	*Tumebacillus*
		0.601	0.0250	*	0.106	*Catellatospora*
		0.591	0.0165	*	0.148	*Planifilum*
		0.591	0.0267	*	0.276	*Xanthobacteraceae* $^{+}$
		0.591	0.0099	**	1.644	*Bacillus*

		0.591	0.0088	**	0.086	DS-100
		0.589	0.0324	*	0.262	*Micromonosporaceae* +
		0.581	0.0087	**	0.071	*Luteolibacter*
		0.577	0.0146	*	0.137	Rokubacteriales
		0.576	0.0209	*	0.258	Gaiellales +
		0.576	0.0244	*	0.737	67-14
		0.575	0.0058	**	1.156	*Vicinamibacteraceae*
		0.569	0.0380	*	0.073	RBG-13-54-9
		0.565	0.0265	*	0.068	*Ellin6067*
		0.556	0.0277	*	0.131	*Pelomonas*
		0.554	0.0155	*	0.932	*Nocardioides*
		0.546	0.0290	*	0.645	IMCC26256
		0.544	0.0124	*	0.256	*Luedemannella*
		0.538	0.0095	**	0.817	*Candidatus* Udaeobacter
		0.537	0.0467	*	0.237	*Gemmataceae* uncultured
		0.536	0.0245	*	0.055	*Nitrospira*
		0.535	0.0213	*	0.288	*Sporosarcina*
		0.526	0.0050	**	0.725	RB41
		0.522	0.0314	*	0.442	*Mycobacterium*
		0.513	0.0170	*	0.002	Vicinamibacterales uncultured
		0.496	0.0127	*	0.018	*Chitinophagaceae* uncultured
		0.492	0.0395	*	0.090	*Gemmatimonadaceae* +
		0.488	0.0346	*	0.139	*Pajaroellobacter*
		0.478	0.0241	*	0.137	*Solibacillus*
	W	0.541	0.0384	*	0.024	*Phaselicystis*
		0.425	0.0272	*	13.659	*Pseudomonas*
Active fungi	IO	0.603	0.0088	**	0.605	*Clavulinaceae*+
		0.595	0.0075	**	0.744	*Metarhizium*
		0.583	0.0013	**	6.164	Helotiales +
		0.580	0.0088	**	1.049	*Micractinium*

	0.538	0.0082	**	3.245	*Ilyonectria*
O	0.528	0.0432	*	2.186	*Protosiphon*
TO	0.483	0.0381	*	1.349	*Pyronemataceae* +
IO + W	0.591	0.0183	*	6.760	Sordariales +

*Significant levels: 0 < '***' < 0.001 < '**' < 0.01 < '*' < 0.05 < '.' < 0.1 < ' ' < 1*

Table A.7 Effect of the environmental parameters on the composition of the active and total soil bacterial and fungal community composition after biocide treatment.

		Total bacteria		Active bacteria		Total fungi		Active fungi	
	Variable	R^2	p-Value	R^2	p-Value	R^2	p-Value	R^2	p-Value
Community composition	Gene copy numbers	0.351		-		0.157		-	
	Number of biocides	0.036		0.673		0.060		0.673	
	Biocide concentration	0.018		0.015		0.039		0.015	
	pH	0.047		0.017		0.090		0.017	
	C_{org}	0.040		0.003		0.021		0.003	
	Fl	0.011		0.063		0.037		0.063	
	Cl_2	0.040		0.071		0.036		0.071	.
	NO_3	0.067		0.015		0.128	.	0.015	
	PO_4	0.009		0.008		0.039		0.008	
	SO_4	0.045		0.079		0.027	.	0.079	
	CO_2	0.013	*	0.042		0.315	**	0.042	
	CH_4	0.241		0.047		0.019		0.047	

*Significant levels: 0 < '***' < 0.001 < '**' < 0.01 < '*' < 0.05 < '.' < 0.1 < ' ' < 1*

Table A.8 Effect of the environmental parameters on the active and total the functional community composition of soil bacterial and fungal communities after biocide treatment.

		Total bacteria		Active bacteria		Total fungi		Active fungi	
	Variable	R^2	p-Value	R^2	p-Value	R^2	p-Value	R^2	p-Value
Community function	OTU Richness	0.071		0.365	***	0.022		0.181	*
	Gene copy numbers	0.047		-		0.122		-	
	Number of biocides	0.072		0.038		0.102		0.090	.
	Biocide concentration	0.086		0.045		0.051		0.034	
	pH	0.010		0.110		0.021		0.042	
	C_{org}	0.105	*	0.070		0.022		0.001	
	Fl	0.180	*	0.055		0.010		0.003	
	Cl_2	0.077		0.015		0.113		0.035	
	NO_3	0.206	.	0.000		0.171	*	0.012	
	PO_4	0.068		0.012		0.080		0.087	
	SO_4	0.152	.	0.004		0.032		0.051	
	CO_2	0.007		0.021		0.110		0.056	
	CH_4	0.071		0.009		0.017		0.147	.

*Significant levels: 0 < '***' < 0.001 < '**' < 0.01 < '*' < 0.05 < '.' < 0.1 < ' ' < 1*

Table A.9 Physico-chemical properties of the soil after 28 days of biocide incubation. W: Water control; T: Terbutryn; I: Isoproturon; O: Octhilinone; Terbutryn + Isoproturon (TI); Terbutryn + Octhilinone (TO); Isoproturon + Octhilinone (IO); Terbutryn + Isoproturon + Octhilinone (TIO).

Biocide treatment	***W***	***T***	***I***	***O***	***TI***	***TO***	***IO***	***TIO***
Biocide concentration	0±0	1.9±0.1	2.7±0.1	0±0	6.1±0.4	2.6±0.3	3.6±0.1	5.9±0.4
pH	7.1±0.1	7.1±0.0	7.3±0.0	7.2±0.0	7.3±0.0	7.3±0.0	7.3±0.0	7.3±0.0
C_{org} [%]	3.68±0.03	3.66±0.09	3.67±0.12	3.74±0.25	3.73±0.04	3.65±0.07	3.81±0.03	3.71±0.12
Fl^- [µg(biocide)*g(soil)$^{-1}$]	4.23±0.44	4.32±0.45	3.90±0.36	3.53±0.37	4.08±0.72	3.55±1.68	4.88±0.86	3.36±1.50
Cl^- [µg(biocide)*g(soil) $^{-1}$]	6.67±1.73	9.08±2.08	6.26±0.81	5.52±0.67	9.96±4.19	7.09±0.97	8.79±1.14	6.89±0.49
$NO3^-$ [µg(biocide*g(soil) $^{-1}$]	58.84±41.18	113.93±3.99	74.47±20.24	101.96±10.70	105.66±3.14	91.43±32.29	86.15±26.12	103.49±20.50
PO_4^{3-} [µg(biocide)*g(soil) $^{-1}$]	0.69±0.84	0.79±0.97	0.54±0.66	0.00±0.00	0.66±0.81	0.64±0.93	1.34±1.24	1.35±0.70

Biocide treatment	***W***	***T***	***I***	***O***	***TI***	***TO***	***IO***	***TIO***
SO_4^{2-} [µg(biocide)*g(soil) $^{-1}$]	13.82±10.47	16.37±1.60	13.55±1.00	14.01±1.02	15.32±2.09	16.27±1.07	14.63±5.51	15.36±0.37
CO_2 [ppm]	2026.42±359.50	1987.89±185.00	1937.81±126.22	2085.22±296.17	2016.28±68.69	1936.52±1919.71	2158.52±96.82	1955.10±169.14
CH_4 [ppm]	2.19±0.96	2.04±0.92	2.17±0.97	1.86±0.96	2.09±0.91	2.11±0.93	2.18±0.96	2.12±0.92

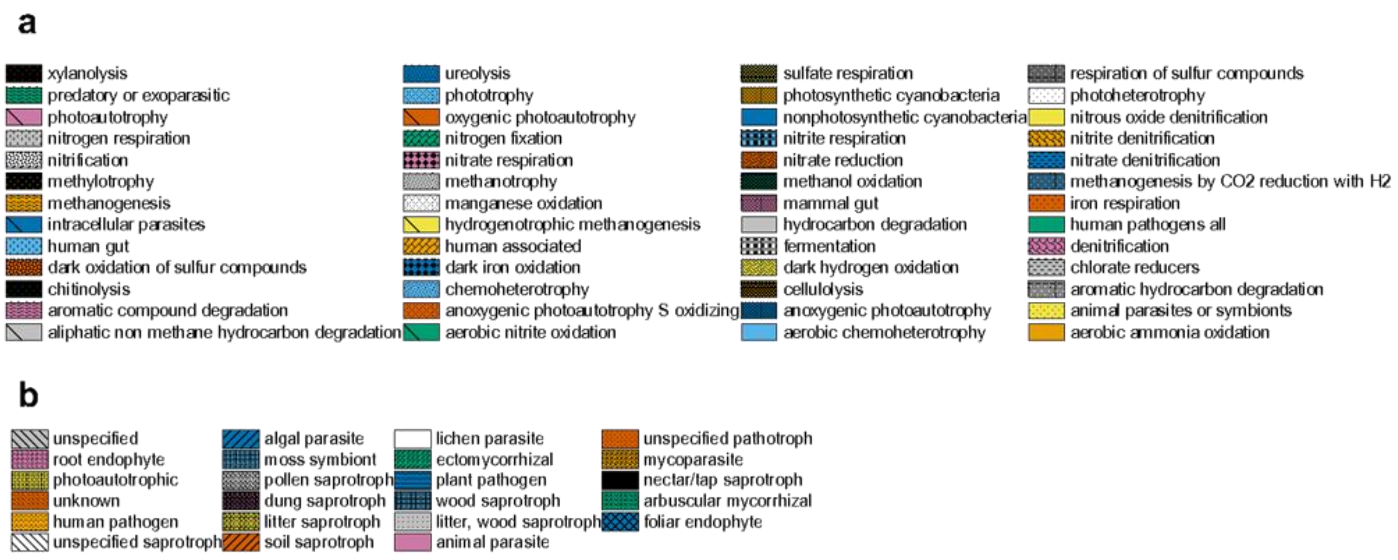

Fig. A. 1: Full legend of all bacterial (a) and fungal (b) functions found in the active community.

3.3 Biocide-II: Facade eluates affect active and total soil microbiome

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Facade eluates affect active and total soil microbiome☆

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ABSTRACT

The application of biocides in building materials has become a prevalent practice to mitigate the growth of microorganisms such as algae, fungi, and bacteria on the façades. These can leach out from the material and reach the nearby soil environment. This study aimed to characterize the effect of façade eluates generated within different leaching experiments on total and metabolic active soil microbial community composition and functions. Façade eluates were produced by immersion testing DIN EN 16105 and a natural weathering experiment. Afterward, soil microcosms were treated with the respective façade eluate and incubated for 29 days. Subsequently, the active and total soil microbial community compositions were investigated.

Fungal internal transcribed spacer region gene and bacterial 16S rRNA gene were sequenced for active (bromodeoxyuridine labeled DNA) microbial community and total community. Façade eluates reduced total bacterial and fungal gene copy numbers. Overall, active bacterial and fungal richness was reduced and altered in community composition in comparison to the total richness and composition, respectively. Façade eluates retrieved of façade samples without biocides did alter the soil microbial communities to the same extent as façade eluates with biocides. Additionally, members of the active microbiome that benefit from the presence of façade eluates and omitted ones could be identified. Our result demonstrated that façade eluates affect active and total soil microbial community composition and function regardless of the leaching procedure and biocides addition.

1. Introduction

Biocides are used in paints and renders to protect them against microbial growth during storage, manufacture, and after application (Bollmann et al., 2014; Burkhardt et al., 2011; European Parliament, 2012). Façade constructions combine different products (e.g., paint, renders) with various chemical compositions, which may contain different biocides and biocide concentrations. This leads to a complex and rarely controllable combination of biocides that were released into the environment (Reiβ et al., 2021). Biocides are water-soluble for biological efficiency and thus enter the environment by leaching from the façade (Burkhardt et al., 2012; Hensen et al., 2018; Linke et al., 2021; Paijens et al., 2020). Biocides are used to reduce microbial colonization throughout the life of a façade, and the manufacturer guarantees that no microbial discoloration will occur for up to fifteen years. Some biocides are also used as pesticides in agriculture and are detected in surface waters at the same rate as commonly used pesticides (Wittmer et al., 2011). Even though some studies were conducted to study the effects of pesticides on the soil microbiome (Bromilow et al., 1996; Pineda et al., 2020), similar findings and data are relatively scarce for biocides in building materials. Agricultural application occurs at distinct time points, about a large area, and is highly regulated by European law (2021). Biocide combinations leach sporadically and uncontrolled from various building materials throughout the year, diluted to high concentrations, and selectively spread around the building.

Biocide emission is expected to be very high after recent renovation with biocide-containing paints or renders. Leaching of biocides is a process that is affected by multiple parameters previously described by Paijens et al. (2020). The leaching behavior of biocides has been previously studied by artificial or natural weathering experiments (Kiefer

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et al., 2024). DIN testing or tests with a weathering chamber have been used for leaching experiments in the laboratory (Vermeirssen et al., 2018; Schoknecht et al., 2020; Wangler et al., 2012; Schoknecht et al., 2013). These methods, especially DIN EN 16105 (European Committee for Standardization, 2011), benefit from a fast and highly reproducible test procedure (Schoknecht et al., 2013). However, these tests poorly reflect *in situ* conditions as they are conducted with relatively high water-contact times and do not account for weather parameters such as UV irradiation (Bandow et al., 2020). Consequently, different leaching procedures result in varying eluate compositions from render and paint components, including biocides and heavy metals, binders, fillers, and pigments. Most leaching and toxicity studies have primarily focused on biocides, often neglecting the presence of these additional façade components in the eluates. Moreover, artificial testing typically uses deionized water, while natural weathering involves rainwater, affecting leachability and leaching rate. This difference is significant because leachates from mineral-based renders and paints are very alkaline, leading to higher alkalinity in the eluates, a factor not adequately considered in current toxicity testing. The release and effect of biocides on the environment is well studied especially for the aquatic environment (Thomas and Brooks, 2010; Konstantinou and Albanis, 2004).

Once biocides are released, the accumulation of biocides in soils below the façades is governed by a number of distinct factors (Arias-Estévez et al., 2008). Main factors are described as the chemical properties of the soil (e.g., texture, organic matter content, pH, porosity, hydraulic conductivity), the chemical nature of the biocide (e.g., water-solubility, volatility, charge, molecular size, functional groups), the climatic conditions (e.g., temperature, precipitation), and their interactions (e.g., sorption of biocides on soil particles, bioavailability of biocides). The factors for pesticide accumulation in soils have been extensively reviewed by Arias-Estévez et al. (2008), and their long-term fate have been recently shown by Riedo et al. (2023). The toxic effects of biocides on the soil microbiome depend on the nature of the chemical, the dose, the duration of exposure, and the route of entry or absorption by the soil organism (Kumar et al., 2012). The presence of biocides can lead to different effects on members of the soil microbiome like alterations in microbial activity (Bünemann et al., 2006), dormancy (Tidwell et al., 2015), and cell lysis (Amjad, 2010; Paulus, 2005). Some microorganisms survive biocide presence through modification of target structures (Sinning et al., 1989), efflux pump activity (Tegene and Tenkegna, 2020), and biocide degradation or inactivation (Kaufman and Kearney, 1970). Previous studies showed that biocides frequently used in building materials have toxic effects on *Aliivibrio fischeri* (Bollmann et al., 2017) and the soil microbiome (Reiβ et al., 2024).

Terrestrial ecotoxicity studies are still based on standard plate count, indirect measures such as substrate-induced respiration (SIR), or low-resolution molecular approaches to assess the ecotoxicological effect of biocides on the soil microbial community (Reiβ et al., 2021). Biocides of the triazine class show residual effects in soil and can thereby reduce the soil enzymatic activity and culturable microbial fraction over long-term exposure (Milosevic and Govedarica, 2002). SIR, bacterial, and fungal growth assay showed that terbutryn caused microbial growth inhibition after 40 days of soil exposure (Fernández-Calviño et al., 2021). Moreover, research has focused on the effects of single biocides on a small range of target organisms and not the mixed toxicity of biocide combinations on a diverse soil microbial community composition. Often, biocide usage does not only affect the target organisms but also non-target organisms, especially in complex microbial community compositions, which are present in soil environments. High-resolution methodology, such as a DNA-based approach, is necessary to effectively discriminate between the biocide effects within a complex microbial composition and function of a soil system. Since the DNA pool in soil consists of a mixture of DNA, which hampers the DNA-based approaches by the presence of relic DNA as previously described by (Purahong et al., 2022; Wahdan et al., 2021; Reiβ et al., 2024). To overcome this drawback, Bromodeoxyuridine (BrdU) immunocapture method combined with Illumina amplicon sequencing is a straightforward approach to differentiate the total genomic DNA from DNA derived from metabolically active organisms, as explained earlier (Purahong et al., 2022; Wahdan et al., 2021; Reiβ et al., 2024). BrdU incorporation has been shown to detect active bacteria in microcosms (Bravo et al., 2013; Kelly et al., 2016) and diverse natural habitats (Hjort et al., 2007; Grubisic et al., 2017; Goldfarb et al., 2011; Taniguchi et al., 2015; Walters and Field, 2006; Allison and Treseder, 2008). Many studies have analyzed total and active (BrdU immunocaptured) microbial communities, which showed significant differences between these two fractions of the same community (Wahdan et al., 2021).

Further, next generation sequencing data of the soil microbiome was affiliated with functional databases to receive its functional composition (Tanunchai et al., 2022; Sansupa et al., 2021). Reiβ et al. (2024) showed that biocides frequently used in building materials affect soil microbial community composition and function. Even though the effects on the total soil microbiome were rather scarce, the active soil microbiome was significantly altered in function and composition due to the single but more pronounced in the combined treatment with biocide standard solutions. However, under realistic conditions not only the biocides are carried in, but also degradation products thereof and further components of façade matrices. These complex eluate compositions could also harm or promote the soil microbiome. For example, heavy metals like zinc harm the soil microbiome and decrease bacterial richness and diversity (Fortunato et al., 2021), whereas silica nanoparticles promote soil microbial growth (Rangaraj et al., 2014).

Therefore, this study aimed (i) to analyze the effect of façades leachates generated within a DIN EN 16105 immersion testing or a natural weathering trial on the total and active soil bacterial and fungal community composition and function; (ii) to test if leachates of façades with and without biocides affect the total and active soil bacterial and fungal community composition; and (iii) to investigate whether certain bacterial or fungal taxa benefit or decline in response to the presence of leachates. This research will provide insights into the environmental risks associated with façade runoff, especially with the increasing use of biocides in urban environments. We anticipate that the respective leachate treatment will modify the composition and function of the active soil bacterial and fungal communities without affecting the total soil bacterial and fungal communities. In addition, we expect that the overall soil microbial functions will remain unchanged as biocide-insensitive microbes will replace biocide-sensitive microbes. We predict differences in the composition and function of active soil bacterial and fungal communities when treated with water control, biocide-free, or biocide-containing façade leachates, depending on the leaching procedure used. We also expect leachates without biocides to still induce changes within the soil microbial community.

2. Material and methods

2.1. Façade eluates production by immersion testing and natural weathering experiment

The samples consisted of a render and paint layer prepared based on the formulations previously described by Schoknecht et al. (2013). They were applied to expanded polystyrene panels (EPS, provided by Saint-Gobain Weber) for the immersion testing and on L-stones for the natural weathering experiment (F) (Fig. S1). The immersion testing (D) was conducted according to DIN EN 16105 (European Committee for Standardization, 2011). The on EPS prepared façade eluate samples were exposed to deionized water in nine immersion cycles of 1 h immersion and 4 h of drying. The elautes generated within the immersion cycles were combined for the following soil incubations. Details regarding the natural weathering experiment were previously described by Kiefer et al. (2024). Briefly, façade without biocides, containing no in-can and no film preservatives (B), and façade containing both in-can and film preservatives (UOF) in the render and paint layer were tested.

Each façade combination was placed in triplicates per direction and two weathering sides for the natural weathering experiment. As in-can preservative ACTICIDE SR 2081 (Thor GmbH, Speyer, Germany) and ACTICIDE MKB3 (Thor GmbH) as film perseverative were added to the UOF façade formulation as follows: 350 ppm within the render formulation and 500 ppm within the paint formulation as explained earlier Kiefer et al., (2024). For one month after the end of each rain period (at least half a day without a predicted rain event), the eluate samples of each façade type were taken, and the runoff volume was determined. After collection, the eluates were filtered, the pH of the eluates was measured, and the samples were stored at −20 °C until soil treatment.

2.2. *Soil incubation*

The soil RefeSol-02A was obtained from the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Schmallenberg, Germany). RefeSol-02A was selected based on available documentation showing that pesticide use has not occurred for at least 40 years. RefeSol soils are suitable for biological tests since they match OECD terrestrial ecotoxicological guidelines (Schlich and Hund-Rinke, 2015). Field-fresh sieved RefeSol-02A soil was used for the experiments, which is a silt loam, sub-acidic, and light humic stagnic luvisol. Detailed data on the soil is supplied in Supplementary Table S1. Soil incubation was performed analog to Reiß et al. (2024). Shortly described, 100 g field-moist and sieved (2 mm) soil was filled in 250 mL glass jars and adjusted to 50% of the maximum water holding capacity using the façade eluates and rainwater as diluent. Soil microcosms (n = 5 per treatment) were either treated with 150 mL rainwater (water control; W) or 35 mL façade eluates (B or UOF) diluted with 115 mL rainwater for 28 days at room temperature. Since only a few percent of the rainfall is reaching the façade and thereby biocide-containing façade runoff, a dilution factor of 5.19 was calculated for both B and UOF, as explained earlier Kiefer et al. (2024). Therefore, the experimental runoff factor of the rain events was determined, and the soil/façade area ratio (0.1/0.4) was considered. The façade eluates differed in the leached façade sample (B or UOF) and leaching method (immersion testing, D or natural weathering, F). This results in five different treatment groups: (i) water control (W), (ii) biocide-free sample generated by immersion testing (D-B), (iii) biocide-containing samples generated by immersion testing (D-UOF), (iv) biocide-free sample generated within the natural weathering experiment (F-B), (v) biocide-containing samples generated within the natural weathering experiment (F-UOF). Water loss through evaporation was controlled weekly by weighing and compensated by the addition of double deionized water.

2.3. *Soil chemical parameters*

Soil pH per microcosm (n = 5 independent replicates) was determined according to DIN EN 15933:2012–11 (DIN German Institute for Standardization e.V). Soil anions were analyzed by ion chromatography, as Reiß et al. (2024) explained earlier. Briefly, 5 g of soil was mixed with 25 mL ultrapure water in tubes for 45 min and centrifuged at room temperature (25 min; 4695.6*g). Afterward, 12.5 mL supernatant was transferred to a fresh tube and filtered first with a 15 μm filter and second with a 0.22 μm filter. The external calibration curve was generated using the multielement ion chromatography anion standard solution (Honeywell Fluka™, Seelze, Germany). Before each measurement, all samples, standards, and eluent (1.7 mM $NaHCO_3$; 1.8 mM Na_2CO_3; 2% Acetone) were degassed by ultrasound. Afterward, the ion chromatography was performed using a Metrohm 883 basic IC Plus device equipped with a Metrosep A Supp 4 column (length 250 mm, inner diameter 4 mm). Separation was performed by method with a flow rate of 1 mL*min^{-1} and the measuring time was 20 min.

2.4. *Quantification of biocides in facade eluates*

Biocide concentrations were determined in each façade eluates sample (n = 3 independent replicates) analogous to Kiefer et al. (2024). Samples were filtered with a 0.2 μm polyamide filter and measured using high-performance liquid chromatography (HPLC) equipped with a UV detector (Waters Corporation Milford, Massachusetts, US). Separation was performed by a gradient method with a flow rate of 0.2 mL*min^{-1}, using 70% water with 0.1% formic acid and 30% MeOH for 3 min followed by a linear increased MeOH content up to 70% after 6 min being maintained for additional 4 min. Afterward, 70% water content was reached again until min 14 and held until the end of each run (20 min). The samples were quantified at a wavelength of 280 nm for methylisothiazolinone (MIT), octylisothiazolinone (OIT), methylchloroisothiazolinone (CMIT), terbutryn (T) at 240 nm, and benzisothiazolinone (BIT) at 317 nm using an external calibration curve.

2.5. *In-situ bromodeoxyuridine labeling, soil DNA extraction, and immunocapture*

Soil samples were treated with 100 mM BrdU (B5002gtz, Merck, Darmstadt, Germany) solution for 48 h as previously described by Reiß et al. (2024). Afterward, DNA was extracted from all microcosms using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit according to the manufacturer's protocol (Zymo Research Europe GmbH, Freiburg im Breisgau, Germany). Similar to previous studies, we refer to this DNA as "total DNA" (Wahdan et al., 2021; Reiß et al., 2024). As described earlier, BrdU-labeled DNA was isolated from the "total DNA" by an immunocapture approach (Hanson C. and McMahon S., 2006; McMahon et al., 2011). First, antibody-herring sperm DNA complex was allowed to form by adding 2 μL monoclonal BrdU antibodies (1 mg μL^{-1} mouse anti-BrdU, clone BU-33, Sigma-Aldrich, St. Louis, USA) to 18 μL denatured herring sperm DNA (1.25 mg mL^{-1} in phosphate buffer saline (PBS), Promega, Madison, USA) at room temperature for 45 min. An antibody-herring sperm DNA complex was added to each denatured DNA sample (20 μL) and incubated for 30 min to capture BrdU-labeled DNA. After incubation, 6.26 μL of prepped Dynabeads™ goat anti-mouse IgG (Invitrogen, Waltham, USA) in PBS – bovine serum albumin solution (PBS – BSA) was added to the mixture and incubated under slow rotation for 35 min. The Dynabead complex (Dynabead-BrdU antibodies-BrdU-labeled DNA) was washed with 100 μL PBS – BSA eight times by adding the wash solution and trapping the complex with a magnetic particle concentrator (Dynal). BrdU-labeled DNA was released from the washed Dynabeads by adding 25 μL BrdU solution (1.7 mM in PBS – BSA) and then incubated under slow rotation for 35 min. The obtained immunocapture DNA is now referred to as "active" DNA isolated from metabolically microbial active cells, as explained earlier (Wahdan et al., 2021; Reiß et al., 2024).

2.6. *Quantitative PCR and amplicon sequencing of the soil microbiome*

DNA samples were analyzed by amplicon sequencing and quantitative PCR using the fungal internal transcribed spacer (ITS) DNA region with the primer set fITS7 (5′-GTGARTCATCGAATCTTTG-3′) (Ihrmark et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1994) and the bacterial 16S rRNA gene with the primer set BAC341f (5′-CCTACGGGNGGCWGCAG-3′) and BAC758R (5′-GACTACHVG GGTATCTAAKCC-3′) (Klindworth et al., 2013).

The quantitative PCR (qPCR) for "total DNA" was performed by quantifying each independent replicate in three technical triplicates in 96-well plates using the CFX96™ Real-Time System C1000™ Thermal Cycler (Bio-Rad Laboratories GmbH, Feldkirchen, Germany), and nuclease-free master mix blanks were run as negative controls. Gene copy numbers were calculated by comparing PCR-cycle threshold values (C_T) to a standard curve of triplicate 10-fold dilutions of genomic DNA (gDNA) extracted from a known concentration of *Escherichia coli* K12

(DSM 423) or *Fusarium solani* (DSM 1164) by employing the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit according to manufacturer's instructions (Zymo Research Europe GmbH). The genomic DNA concentration per PCR reaction of *E. coli* and *F. solani* standard ranged from 1×10^9 to 1×10^4 and 1×10^7 to 100 gene copies, respectively.

Amplicon sequencing libraries were created for the 50 ″total" and "active" DNA samples. Inline barcodes and Illumina sequencing adapters were added to the amplicon sequence libraries by the Nextera CT Index Kit (Illumina, San Diego, CA, USA) and MiSeq Reagent Kit v3 600 cycles (Illumina) according to the manufacturer's instructions. For library preparation, PCR products were purified by AMPure XP beads (Beckman Coulter, Brea, CA, USA). The sequencing of libraries was performed by 300 bp paired-end sequencing on an Illumina MiSeq platform (Illumina MiSequ V3; Illumina) based on a standard protocol from the manufacturer. Amplicon sequencing library preparation, sequencing, and sequence quality checks were carried out by LGC Genomics GmbH (Berlin, Germany). The active and total microbiome's bacterial 16S rRNA and fungal ITS gene sequences were deposited in the NCBI nucleotide sequence databases under accession PRJNA1074951.

2.7. Bioinformatics

Raw data pre-processing including demultiplexing, sorting, adapter trimming, and merging reads were assembled using Illumina bcl2fastq2 conversion software v2.20 and BBMerge v34.48 (Bushnell et al., 2017). The sequence quality of the reads was controlled with the FastQC software, version 0.11.8 (Andrews, 2010). Afterward, sequence pre-processing and Operational Taxonomic Units (OTUs) picking from amplicons was performed using mothur 1.35.1 (Schloss et al., 2009). Sequences were aligned against the 16S mothur-Silva SEED r138 reference alignment depending on their Phred quality score over 33 (Quast et al., 2013; Yilmaz et al., 2014; Glöckner et al., 2017). Short alignments were filtered, and reduction of sequencing errors was conducted by pre-clustering, allowing a maximum of one base mismatch per 100 bases within a cluster. Chimeras were eliminated by UCHIME algorithm (Edgar et al., 2011). Taxonomical classification of the sequences against the Silva reference classification was conducted, and sequences of other domains of life were removed for OTU picking. OTUs were chosen and assigned to a taxonomic level by clustering at the 99% identity level using the average neighbor method with cluster split (Edgar, 2018; Nilsson et al., 2019). Thereby OTU tables for DNA samples were generated. Sequence counts for bacteria ranged before rarefaction analysis from 5106 – 113,795 per sample and 1615 - 8,105,383 per sample for fungi. The datasets were then rarefied to 11,765 sequence counts for bacteria and 10,000 sequence reads for fungi, resulting in Good's Coverage estimates above 99% for bacteria and over 93% for fungi.

Ecological and metabolic functions of identified bacterial OTUs were predicted using the Functional Annotation Tool of Prokaryotic Taxa v.1.1 (FAPROTAX) database (Louca et al., 2016; Sansupa et al., 2021). The functions of each phylogenetic taxa were annotated using literature on cultivable strains. FungalTraits database (Põlme et al., 2020), a specific functional prediction tool, was used to taxonomically analyze fungal genera by ecological guild independent of the sequencing method. Function count tables were generated for each sample's sequencing dataset for bacteria and fungi.

2.8. Statistics

Statistical analyses were performed after OTUs were taxonomically summarized at the genus level. The normal distribution of each dataset was tested via OriginPro 2022 (OriginLab Corporation, Northampton, MA, USA) by Shapiro-Wilk Test ($p < 0.05$). Rarefaction analysis, as well as the estimation of alpha diversity (OTU richness, Shannon index, Simpson index, 'Pielou's Evenness), OTU richness estimators (bias-corrected Chao1), and an abundance-based coverage estimator (ACE), were performed for total and active DNA samples in RStudio (Version 2022.02.1, RStudio, Inc, Boston, MA, USA) and the package vegan 2.5–7 (R Core Team, 2022; Oksanen et al., 2022).

Alpha diversity indices were tested for normal distribution by Shapiro-Wilk Test ($p < 0.05$). Significant effects ($p < 0.05$) on total and active alpha diversity indices due to the biocide treatment were calculated either by one-way ANOVA with a post hoc adjusted Tukey test if the dataset was normally distributed, or Kruskal-Wallis ANOVA with a post hoc Dunns Test, if the dataset was not normally distributed, using the OriginPro (Version 2022; OriginLab Corporation). Non-metric multidimensional scaling (NMDS) was conducted using the "metaMDS" function in the vegan 2.5–7 package.

Permutation multivariate analysis of variance (NPMANOVA) based on Bray-Curtis similarity was performed using the software PAST 2.17c (Hammer et al., 2001) to analyze the differences between the different biocide treatments for active and total microbial communities and functions. Results were visualized by OriginPro (Version 2022. OriginLab Corporation). Afterward, Goodness-of-fit statistics (R^2) of environmental variables fitted to the NMDS ordination of active and total bacterial and fungal communities were calculated using the "envfit" function in the vegan 2.5–7 package, with p values based on 999 permutations (R Core Team, 2022; Oksanen et al., 2022). As explained by Noll et al. (2005), relative abundances were calculated for each sample and visualized with OriginPro (Version 2022; OriginLab Corporation).

The biocide treatment's significantly distinctive bacterial and fungal genera were identified using indicator species analysis conducted using the "multipatt" function in the indicspecies package (Cáceres and Legendre, 2009), which calculates indicator values (Stat value) with the "r.g." function.

3. Results

3.1. Façade eluates contain mainly in-can preservatives after 29 days of weathering

The façade eluates mean pH values after the natural weathering experiment before dilution for the soil incubation were 8.2 ± 0.1 for F-B and 8.2 ± 0.1 for F-UOF and within the immersion testing pH values were 10.0 ± 0.1 for D-B and 10.2 ± 0.1 for D-UOF. However, the soil pH remained at pH approx. 7.2 after incubation with the façade eluates for 28 days (see Table S6). The concentration of biocides leached out within the different leaching experiments were previously published and described in detail by Kiefer et al. (2024). The relevant concentrations are shown in Table 1.

The leached amount of biocides varied between the different façade samples and the leaching experiments. Mainly, BIT was found within the façade eluates. Highest biocides amount was found within F-UOF facade eluates with 1.08 $mg{*}L^{-1}$. Followed by D-UOF containing 0.268 $mg{*}L^{-1}$. Even within the facades without biocides small amounts of BIT was found.

3.2. Fungal and bacterial gene copy numbers decreased due to the presence of façade eluates

Façade eluates reduced the bacterial and fungal gene copy numbers of the total DNA in comparison to the respective water control (Fig. 1). Highest gene copy numbers were observed in the water control for bacteria ($4.2{*}10^5{*}g^{-1}$) and fungi ($1.6{*}10^4{*}g^{-1}$). The bacterial gene copy number of the T-F-UOF treatment was significantly reduced compared to T-D-UOF ($p = 0.01531$), T-F-B treatment ($p = 0.01531$), and the respective water control ($p = 0.01178$). Façade eluates, irrespective of the leaching procedure, caused a reduction in bacterial gene copy numbers (Fig. 1 A). In contrast, no significant differences were found between the treatments and the respective water control in the fungal gene copies.

Table 1
Concentration of biocides in façade eluates after immersion testing (D) and natural weathering experiment (F). D-B: Façade eluates from DIN testing from facades without biocides; D-UOF: Façade eluates from DIN testing from facades containing biocides; F-B: Façade eluates from natural weathering experiment from facades without biocides; F-UOF: Façade eluates from natural weathering experiment from facades containing biocides; MIT: Methylisothiazolinone; CMIT: Methylchloroisothiazolinone; T: Terbutryn; OIT: Octylisothiazolinone; BIT: Benzisothiazolinone.

	Concentration biocide in façade eluates [mg*L^{-1}]			
	D-B	D-UOF	F-B	F-UOF
MIT	0.00	0.01	0.00	0.53
CMIT	0.00	0.00	0.00	0.00
BIT	0.25	1.29	0.14	4.92
T	0.00	0.03	0.00	0.00
OIT	0.00	0.07	0.00	0.17
	Concentration after dilution for soil incubation [mg*L^{-1}]			
	D-B	D-UOF	F-B	F-UOF
MIT	0.00	0.00	0.00	0.10
CMIT	0.00	0.00	0.00	0.00
BIT	0.05	0.25	0.03	0.95
T	0.00	0.00	0.00	0.00
OIT	0.00	0.01	0.00	0.03
Sum	0.05	0.268	0.03	1.08

3.3. *Façade eluates showed higher effects on fungal alpha diversity indices*

Façade eluates showed no significant effects on total bacterial and fungal richness (Fig. 2A; C). Solely, the total bacterial ACE index of the treatment T-D-B was significantly different from the respective water control (**Supplementary Table S2**; p = 0.048). Chao1 ($p = 0.048$), Shannon ($p = 0.031$), and Simpson ($p = 0.031$) index of the total fungal communities were significantly different between the T-F-UOF and T-D-B treatment (**Supplementary Table S2**). In comparison, the active samples showed a lower richness overall than the total bacterial and fungal communities (Fig. 2B; D). Only A-D-UOF and A-F-B treatments differed significantly in the active fungal richness (Fig. 2D), while other treatments were not significantly different (Supplementary Table S1). The A-D-UOF and A-F-B treatments were only significantly different in the bacterial richness but not in other alpha diversity indices (**Supplementary Table S2**). Overall, a higher richness in the active community could be obtained in the A-D-B and A-F-B treatments.

3.4. *Façade eluates altered bacterial and fungal community compositions*

The presence of façade eluates significantly affected both fungal and bacterial community composition (Fig. 3). Moreover, Bray-Curtis similarities showed significant differences between the treatments on the active and total community composition (Table 2). T-D-B's bacterial community was significantly altered compared to T-F-UOF and the respective water control (T-W) (Fig. 3A–Table 2A). T-F-UOF was significantly different from the T-D-UOF treatment. Members of the genus *Curvibacter* were solely present in the total bacterial community composition of T-W (Fig. 3A–Supplementary Table S3). Active bacterial community compositions were significantly altered between A-D-B and A-F-B in comparison to the respective water control (A-W) (Fig. 3B–Table 2A). The most dominant taxon in the active bacterial community was *Pseudarthrobacter* (>55% relative sequence read abundances within all treatment groups). At the same time, the genera *Microbacterium* and *Sphingomonas* (both family Micrococcaceae) were enriched within all treatments except the respective water control (Fig. 3B).

In contrast to the total bacterial community composition, the total fungal community composition was only significantly altered in the T-D-B treatment compared to the respective water control (T-W), T-F-B, and T-F-UOF treatments (Fig. 3C – Table 2B). The main different taxa in T-D-B treatment were the dominant presence of unclassified members of the class Sordariales (Fig. 3C). Members of the genus *Aureobasidium* were solely present in the water control treatment but absent in any other treatment of the total fungal community composition. The A-F-OUF treatment showed a significant shift in the fungal community composition in comparison to the respective water control (A-W) as well as to the corresponding A-F-B treatment without biocides (Fig. 3D – Table 2B). Unknown genera were the most dominant taxa in the active fungal community after all facade eluate treatments except A-D-UOF (Fig. 3D – Supplementary Table S3), and *Chlorosarcinopsis* and *Sclerococcum* were solely present in the A-D-UOF and A-F-UOF treatments, respectively (Fig. 3D – Supplementary Table S3). Members of the genus *Tetracladium* were enriched in the active fungal community composition of all treatments compared to the respective water control (A-W). Relative sequence read abundances of unclassified members of the phylum Chlorophyta and of the kingdom Viridiplantae were enriched in the treatments A-D-B and A-F-B in comparison to the other treatments. Even though soil physico-chemical parameters remained unchanged (Supplementary Table S3) during the eluate-based soil incubation, soil microbial community composition was significantly affected. However, significant environmental parameters for the above presented effects were not identified by EnvFit analysis (Supplementary Table S4).

3.5. *Functional alteration of soil microbial community by the presence of façade eluates in soil*

Like the community composition, the composition of the microbial

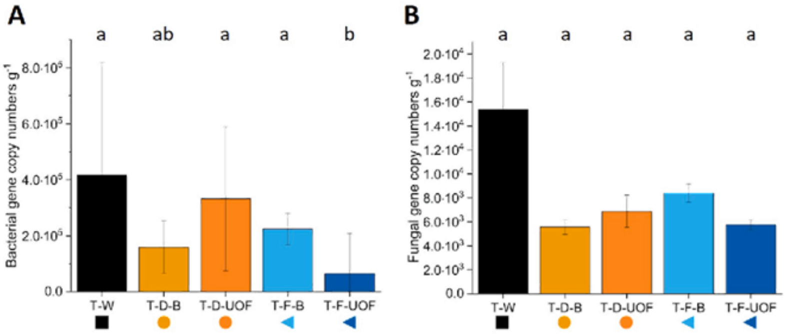

Fig. 1. Gene copy numbers of total bacterial (a) and fungal gene copy numbers (b) after different leachate treatments (n = 5). According to Dunns test, different letters indicate statistically significant differences ($p. < 0.05$, n = 5). T- Total; W: Water control (■); D-B: Façade eluates from DIN testing without biocides (●); D-UOF: Façade eluates from DIN testing containing biocides (●); F-B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀).

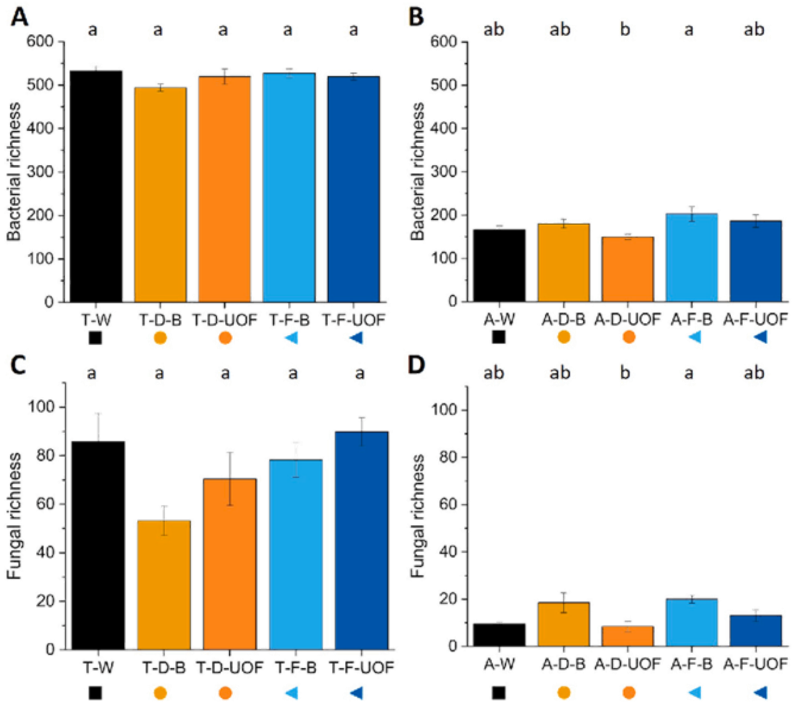

Fig. 2. Richness on genus level of total and active bacterial (**A; B**) and fungal community composition (**C; D**) after respective façade treatments (n = 5). Statistically significant differences are indicated by different letters above the bar charts (Tukey Test; $p < 0.05$). T- Total; A- Active; W: Water control (■); D-B: Façade eluates from DIN testing without biocides (●); D-UOF: Façade eluates from DIN testing containing biocides (●); F-B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀).

functions was significantly altered due to the eluate treatments (Fig. 4). The total and active bacterial functional community composition was dominated by chemoheterotrophs, and fermenters were more present in the active bacterial community (Fig. 4A; B). Bacterial functional community composition in T-D-UOF was significantly different from T-F-B and T-F-UOF treatment and the respective control (T-W) (Fig. 4A - Supplementary Table S5). Likewise, A-F-B but also A-D-B treatments showed significant differences in the active functional community compared to the respective control (Fig. 4B – Supplementary Table S5).

Compared to the bacterial functional community effects on fungal functional community were more prominent (Fig. 4). The active fungal functional community composition differed significantly from that of the total. Dominant functions of total fungal community were litter and soil saprotrophs and plant pathogens (Fig. 4C). Most fungal functions could not be annotated for the active community, although prominent changes were revealed. Sooty mold fungi and mycoparasites were solely present in the active water control (A-W) (Fig. 4D). In contrast, photoautotrophic Viridiplantae were present in higher sequence read abundances in treatments except for A-F-UOF treatment. Interestingly, lichen parasites showed a higher sequence read abundance in the treatments A-D-UOF and A-F-UOF compared to other treatments. Solely, the A-F-UOF treatment was significantly different from the A-F-B treatment and the respective water control (Fig. 4D – Supplementary Table S5). Significant environmental parameters for the alteration of functional community compositions were not identified by EnvFit analysis (Supplementary Table S6).

3.6. Distinct fungal and bacterial taxa benefit from the presence of façade eluates

Indicator analysis showed that defined bacterial and fungal taxa were significantly correlated with the façade eluate treatments or the respective water controls (Fig. 5 - Supplementary Table S7). As a result, 18 taxa for total bacteria, 30 taxa for active bacteria, ten taxa for total fungi, and seven taxa for active fungi were extracted as indicator taxa for the respective treatment (Fig. 5). In addition, the indicator analysis revealed that these taxa were associated with the soils treated with B and UOF eluates regardless of the leaching procedure (Fig. 5 A - Supplementary Table S7, Figs. S2 and S3). The bacterial indicators were mainly assigned to chemotrophs, while fungal indicators were assigned to saprotrophs.

Most indicator taxa (18 taxa) of the active bacterial community composition of the treatment A-F-UOF showed a broad phylogenetic distribution, including the orders Bacilli, Bacteroidia, and S0134 terrestrial group (Fig. 5A - Supplementary Table S7). Most bacterial indicators of the treatments A-D-UOF and A-F-UOF were members of the Phylum Proteobacteria. Indicator taxa of the treatments A-D-B and A-F-B were mainly associated with members of the order Actinobacteria, whereas the respective water control was associated with the orders Actinobacteria, Planctomycetes, Verrumicrobiae, and Clostridia (Fig. 5 A). Sequencing analysis showed that bacterial 118 taxa were solely present in the water control (A-W and T-W). From these taxa, 59 taxa were found in the active water control (A-W) and 61 taxa in the total water control (T-W), with 305 taxa present in both active and total water control. The latter included members of the bacterial genera *Leuconostoc* and *Plesiomonas* (Supplementary Table S3).

Only a few fungal indicator taxa were identified (Fig. 5B - Supplementary Table S7), mainly found in the A-D-B treatment, and were only members of the order Sordariomycetes. Indicator taxa in the A-F-B treatment were unclassified members of the green algal genus *Chlorosarcinopsis*, whereas unclassified members of the genus *Capnodiales* were observed in the respective water control (A-W). In contrast to the

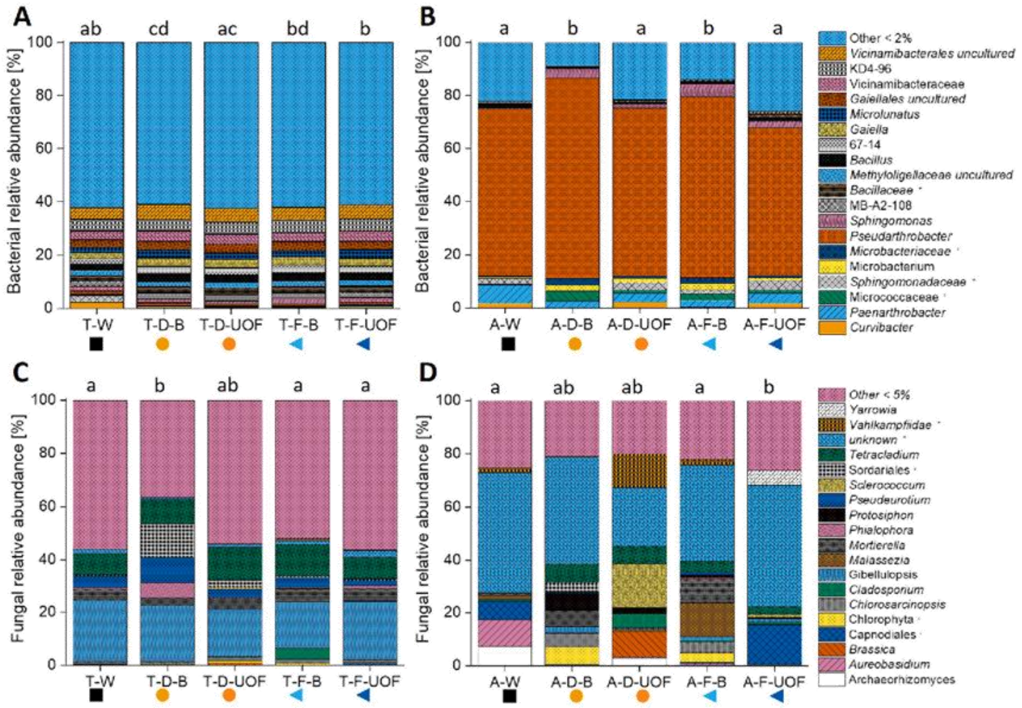

Fig. 3. Bacterial (A; B) and fungal community composition on genus level (C; D) after biocide treatments (n = 5). The respective total (prefix T-; A; C) and active community composition (prefix A-; B; D) are indicated. The short legend highlights fungal and bacterial genera with a >2% (bacterial) and a >5% (fungi) relative abundance. Different letters indicate statistically significant differences according to one-way non-parametric multivariate analysis ($p < 0.05$, Table 2). Complete relative abundances can be found in Supplementary Table S3. W: Water control (■); D–B: Façade eluates from DIN testing without biocides (●); D–UOF: Façade eluates from DIN testing containing biocides (●); F–B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀); $^+$: unclassified members.

bacterial community compositions only 30 fungal taxa were omitted after soil treatment with any façade eluate. Twenty-four indicator taxa were solely present in total water control (T-W), and six taxa in active water control (A-W) (Supplementary Table S3).

4. Discussion

Façade eluates generated within DIN EN 16105 immersion testing and generated within a natural weathering experiment did not affect soil physico-chemical parameters after 28 days of incubation (**Supplementary Table S3**). However, total fungal and bacterial gene copy numbers were decreased (Fig. 1) due to the presence of façade eluates. Active fungal and bacterial richness (Fig. 2), community (Fig. 3), and functional (Fig. 4) compositions were altered. At the same time, the total community composition and function were affected to a much lesser extent by the façade eluates treatment compared. In addition, taxa were identified that were present/increased in relative sequence read abundances or absent/decreased due to the eluate's presence (Fig. 5).

4.1. Eluate treatments decreased bacterial gene copy numbers and active soil microbial richness

All façade eluate treatments, even the blank formulations of both leaching procedures, caused a reduction in total fungal and bacterial gene copy numbers (Fig. 1). Similar results were obtained in previous studies due to the presence of herbicides in an agricultural environment (Milosevic and Govedarica, 2002) as well as for single biocides and biocide combinations in the same soil environment (Reiß et al., 2024). As previously obtained by Reiß et al. (2024), fungal gene copy numbers were less affected by the presence of façade eluates than the bacterial gene copy numbers. The bacterial gene copy numbers of treatment T-F-UOF were significantly reduced in comparison to all other treatments except T-D-B (Fig. 1), which is in line with our expectations due to the high amount of biocides within F-UOF façade eluate (Table 1). However, our gene copy numbers were relatively low compared to other studies, and future experiments should explore a wider variety of soil types with contrasting microbial biomass levels to determine if similar results can be observed. Bacterial and fungal total richness were not altered due to the façade eluate treatments and reduced within the active communities in comparison to the total richness (Fig. 2), which is in line to previous results observed for RefeSol – 02A (Reiß et al., 2024). With respect to the active microbial richness, fungal and bacterial richness were slightly increased compared to the respective water control. Notably, a significant difference was discovered between active fungal and bacterial richness of the D-UOF and F-B treatments (Fig. 2B, D), which can be explained in two ways. On the one hand, DIN leaching procedure was harsh and led to higher pH values in the eluates, potentially having more drastic effects on the soil microbiome. However, after the addition of façade eluates (pH 8.2/8.2, 10.0, 10.2) no differences between treatments (approximately pH 7.2 in each treatment) (Table S3) were observed after 28 days of incubation, most likely due to the soil's buffering capacity. On the other hand, eluates did not only consist of biocides and their degradation products, as heavy metals, binders, fillers, and pigments can be leached out of the façades and affect the soil microbiome even without the addition of biocides.

Table 2

One-way non-parametric multivariate analysis based on the Bray-Curtis similarities of the total (prefix T-) and active (prefix A-) bacterial (**A**) and fungal community compositions (**B**) ($p < 0.05$ highlighted in **bold**; n = 5). W: Water Control (■); D-B: Façade eluates from DIN testing without biocides (●); D-UOF: Façade eluates from DIN testing containing biocides (●); F-B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀).

A			■	●	●	◀	◀
			T-W	**T-D-B**	**T-D-UOF**	**T-F-B**	**T-F-UOF**
Total Bacteria	■	**T-W**	1	**0.0383**	0.1752	0.8293	0.8133
	●	**T-D-B**		1	0.5463	0.0686	**0.0478**
	●	**T-D-UOF**			1	**0.0468**	**0.0307**
	◀	**T-F-B**				1	0.7493
	◀	**T-F-UOF**					1
			A-W	**A-D-B**	**A-D-UOF**	**A-F-B**	**A-F-UOF**
Active Bacteria	■	**A-W**	1	**0.0057**	0.332	**0.0098**	0.1181
	●	**A-D-B**		1	**0.0065**	0.1911	**0.0083**
	●	**A-D-UOF**			1	**0.0075**	0.2432
	◀	**A-F-B**				1	**0.0071**
	◀	**A-F-UOF**					1

B			**T-W**	**T-D-B**	**T-D-UOF**	**T-F-B**	**T-F-UOF**
Total Fungi	■	**T-W**	1	**0.0456**	0.4105	0.6743	0.9441
	●	**T-D-B**		1	0.25	**0.0182**	**0.0306**
	●	**T-D-UOF**			1	0.416	0.6522
	◀	**T-F-B**				1	0.5424
	◀	**T-F-UOF**					1
			A-W	**A-D-B**	**A-D-UOF**	**A-F-B**	**A-F-UOF**
Active Fungi	■	**A-W**	1	0.061	**0.0463**	0.1409	**0.0164**
	●	**A-D-B**		1	0.2201	0.4291	0.0776
	●	**A-D-UOF**			1	0.0962	0.0809
	◀	**A-F-B**				1	**0.0179**
	◀	**A-F-UOF**					1

4.2. Active and total community composition are altered due to the eluate's presence

Façade eluates altered bacterial and fungal community composition (Fig. 3). The total bacterial community of T-D-B and T-F-B altered significantly from the respective water control but were not significantly different from their respective UOF treatment. T-D-UOF and T-F-UOF were significantly different from each other even though T-D-B and T-F-B were not (Fig. 3A). The total bacterial community composition was taxonomically highly diverse in line with the richness results. Over 60% comprises genera that make up less than 2% of the total community composition. In contrast to the total bacterial community, active bacterial community was dominated by *Pseudoathrobacter* (Fig. 3B) and thereby less diverse. *Pseudoathrobacter* is described as gaining abundance in polluted soils (Villa et al., 2020), being capable of immobilizing heavy metals, and removing Cd and Pb (Wang et al., 2019). Façade formulation and the biocide mixtures such as ACTICIDE SR 2081 used in this study commonly contain zinc oxide and zinc pyrithion, which is water-soluble and can be leached out and form part of the treatment eluate. We presume that *Pseudoathrobacter* benefits from the presence of metals within the soils and the thereby gains abundance within the communities. The active bacterial community compositions of the treatments A-D-B and A-F-B differed significantly from the respective water control and their respective UOF treatment (Fig. 3B), which can be partly based on the presence of heavy metals in the case of A-D-B, whereas the effect of A-F-OUF might be increased due to the high amount of biocides within F-UOF façade eluates (Table 1).

Eluate treatments affected the total fungal community composition to a lesser extent than the total bacterial community composition. Similarly, genera with low sequence read abundances made up more than half of the total relative abundances, and the other half was dominated by a few taxa such as *Gibellulopsis* (approx. 20% in all

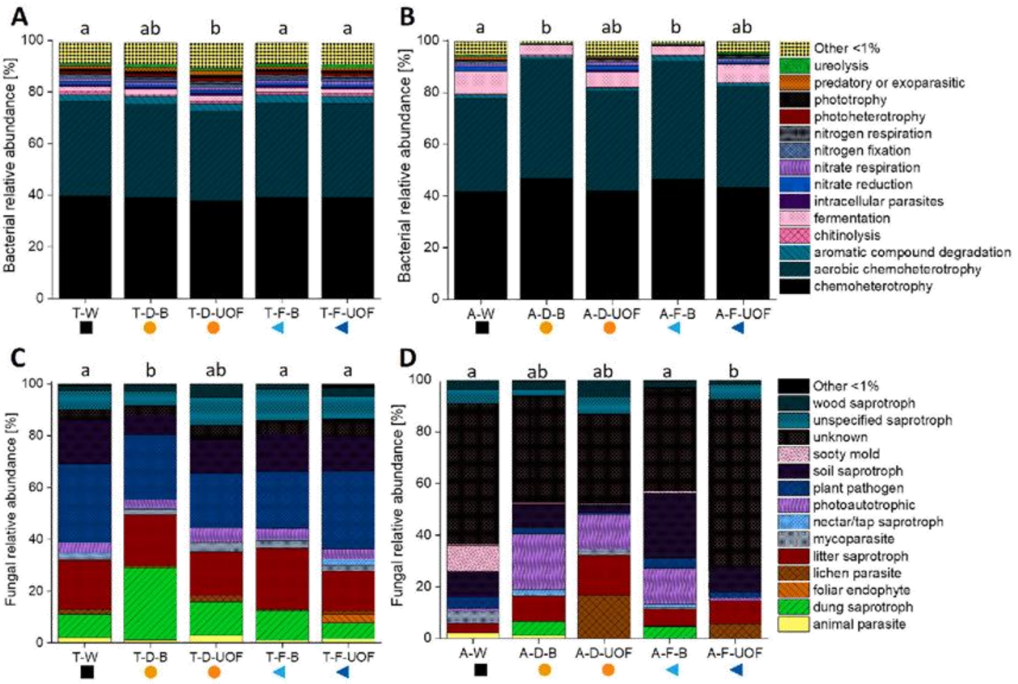

Fig. 4. Bacterial function composition on OTU Level (**A**; **B**) and fungal function composition on genus level (**C**; **D**) after biocide treatments (n = 5). The respective total (prefix T-; **A**; **C**) and active composition (prefix A-; **B**; **D**) are indicated. Short legend highlights bacterial functions with a >1% relative abundance. Different letters indicate statistically significant differences according to one-way non-parametric multivariate analysis ($p < 0.05$). W: Water control (■); D-B: Façade eluates from DIN testing without biocides (●); D-UOF: Façade eluates from DIN testing containing biocides (●); F-B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀).

treatments expect water control) and *Tetracladium* (approx. 10%) (Fig. 3C). *Tetracladium* is known to cope with pesticide presence within a lab study (Nagai, 2020) and was shown to demethylate biocides such as isoproturon (Rønhede et al., 2005). However, such information about members of the genus *Gibellulopsis* is lacking but is needed to interpret their enrichment. In line with the previous results, T-D-B treatment differed significantly from other treatments and respective water control except for T-D-UOF. In contrast, the low abundant community members of the active fungal community were reduced up to 20% relative sequence read abundances of the active community composition and replaced by mainly unknown genera (Fig. 3D). In addition, the treatment A-F-OUF differed significantly from A-F-B but not from the other treatments. Unclassified members of the algal phylum Chlorophyta showed increased relative sequence read abundances in the treatment A-D-B and A-F-B. This result is in line with the findings of Kiefer et al. (2024) that algae showed slight toxic effects towards the identical façade eluates D-UOF and F-UOF after dilution (Table 1) within DIN 38412 L33:1991–03 (Scenedesmus e. V).

4.3. *Fungal functions are more affected by the presence of façade eluates than bacterial functions*

Likewise, changes in the fungal and bacterial community composition also changes in the functional community composition were observed due to the presence of façade eluates. The primary functional assignments of the total and active bacterial community compositions were chemoheterotrophs and aerobic chemoheterotrophs (Fig. 4A, B), which is in line with the same soil after single/multiple biocide additions (Reiß et al., 2024). The active bacterial functional composition was significantly affected in the A-D-B and A-F-B treatment as already observed for the active bacterial community composition (compare Fig. 3 with Fig. 4). The proportion of the functional members smaller than 1% relative sequence read abundances was almost entirely displaced by the dominant chemotrophic members (Fig. 4B). Overall active bacterial functional community composition was very similar across all treatments. Fermentation increased in the relative sequence read abundances compared to the total functional community composition. This finding was also observed for treatments with biocide combinations within RefeSol-02A (Reiß et al., 2024). In contrast, the active fungal functional community compositions were very different from the total functional community (Fig. 4C and D) and were significantly altered by the same treatments as the fungal community composition (compare Fig. 3 with Fig. 4). As already observed for the active fungal community composition, functional composition was dominated by unknown genera (Fig. 3D). Interestingly, lichen parasites were enriched in the relative sequence read abundances in A-D-UOF and A-F-UOF treatment compared to the respective water control and the total fungal community (Fig. 4D). This effect can be explained by the high amount of *Sclerococcum* within A-D-UOF and A-F-UOF compared to the other treatments (Fig. 3D). Since *Sclerococcum* is not known to bear resistance against biocides and further antimicrobial agents we presume that the gain in abundance might be due to the reduction of competitors or the release of bioavailable substrates from sensitive species within the environment (Reiß et al., 2024). In contrast, photoautotrophic taxa were nearly eliminated in the A-F-UOF treatment. Plant pathogenic fungi were very dominant in the total functional community composition (>20% relative sequence read abundances) compared to the active functional community composition (<5% in all treatments) (Fig. 4C, D). All treatments' total fungal functional community composition (especially in the T-D-B treatment) showed a slight decline in soil saprotrophs.

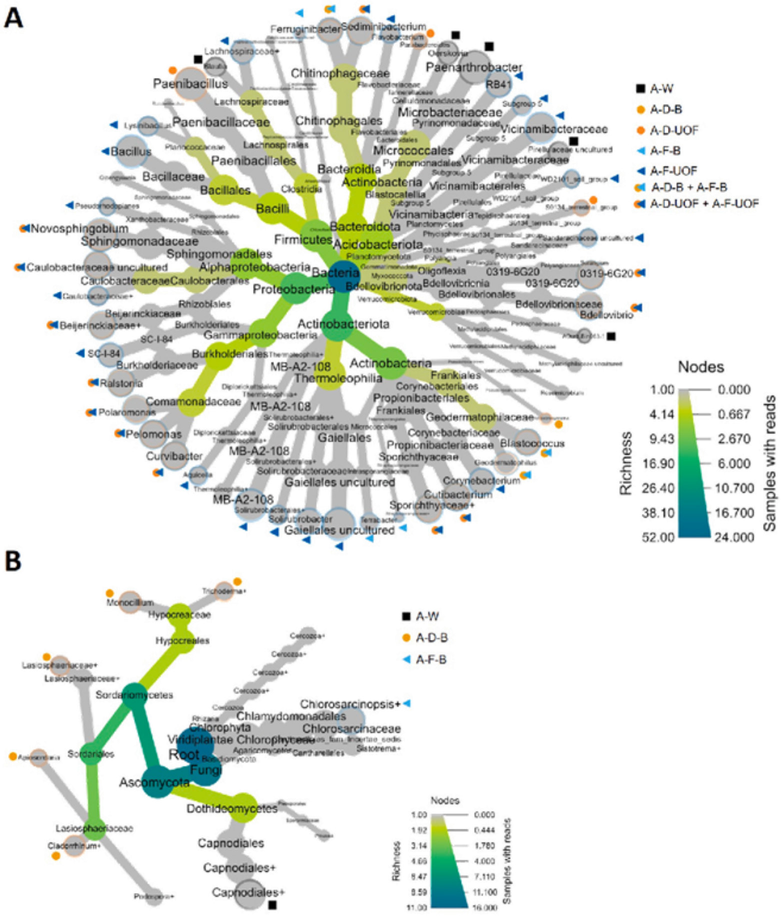

Fig. 5. Heat tree representation of the active bacterial (**A**) and fungal (**B**) indicator taxa of the respective treatment indicated by symbols and colored frame of the indicator nodes. The color code resembles the richness and the node size of the respective sequence read abundances. Unclassified members of the taxon are marked with $^+$. W: Water control (■); D-B: Façade eluates from DIN testing without biocides (●); D-UOF: Façade eluates from DIN testing containing biocides (●); F-B: Façade eluates from natural weathering experiment without biocides (◀); F-UOF: Façade eluates from natural weathering experiment containing biocides (◀). Information to the total community indicators can be found in the Supplementary Figs. S2 and S3. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

This aligns with our recent study of biocide combinations but not after single biocide treatments (Reiβ et al. (2024). In contrast to the total soil fungal functional composition, the amount of soil saprotrophs was not altered within the active fungal functional community after façade eluates treatment, and overall functions of the active community were not affected (Fig. 4D). We thereby presume that the biocides and their quantity (Table 1) used within the façade formulations are not as harmful to soil saprotrophs as those used within the study of Reiβ et al. (2024), as the significance of the shifts was lower in this study even though the methodology was identical.

4.4. Façade eluates of B and UOF lead to joint indicator taxa independent of the leaching procedure

The bacterial and fungal community members were assessed using indicator species analysis, a statistical approach to determine which taxa were significantly assigned to the treatment based on the indicator value (Cáceres and Legendre, 2009). Taxa were identified as indicators for the B- and UOF-treated soils independent of the leaching procedure. The A-D-B and A-F-B treatments were characteristic of the bacterial indicators *Ferruginibacter, Blastocoocus*, and *Geodermatophilus*, belonging to the *Geodermatophilaceae* (Fig. 5A). Members of the bacterial family *Geodermatophilaceae* are known to be extremophilic and stress-resistant (Tekaya et al., 2012). This shows that even eluates from façade B altered soil microbial community composition without biocides due to the formulation components such as heavy metals, binders, fillers, and pigments. The A-D-UOF and A-F-OUF treatment revealed indicator taxa mainly in Alphaproteobacteria and Gammaproteobacteria (Fig. 5A). One of the discovered Alphaproteobacteria was *Novosphigobium*, which was previously described as being able to cope with man-made environments and is resistant to antibiotics (Vaz-Moreira et al., 2011). In addition, *Cutibacterium* (Actinomycetota) was identified as indicator for the A-D-UOF and A-F-UOF treatments, which was previously also found as indicator taxa for soil treatment with biocide mixtures Reiβ et al. (2024).

In agreement with the functional annotation and our previous study, most bacterial indicators were chemotrophs, and the fungal indicators were saprotrophs (Reiβ et al., 2024). The bacterial indicator for the A-D-UOF treatment with high sequence read abundances was *Paenibacillus* (Fig. 5A), which is known to cope with a high variety of stress factors like high temperatures, biocides, pressure, and UV exposure (Pandey et al., 2023). Thereby, *Paenibacillus* survived extreme conditions as part of the D-UOF leachate in soil, characterized by a higher biocide concentration (Table 1) and alkaline pH value. The eluate composition and biocide concentrations differed between the leaching procedures (compare A-F-UOF and A-D-UOF), which caused different indicator taxa and possibly resulted in a different successional development in the composition of the bacterial community (Fig. 3). For example, A-F-UOF eluates contained UV degradation products, which showed higher toxic effects on bacteria in an earlier study (Bollmann et al., 2017). A detailed description of the composition of the façade eluates was published in our parallel study by Kiefer et al. (2024). *Bacillus* and members of the order Gaiellales were identified as indicator taxa in our previous study as well as for the A-F-UOF treatment (Reiß et al., 2024), indicating similar response activities in RefeSol 02-A. In addition, *Flavobacterium* was determined as an indicator taxa for the A-F-UOF treatment (Fig. 5A), and they are known to be multidrug resistant by a putative set of resistance genes and mechanisms (Králová et al., 2021; Otur et al., 2023; McCann et al., 2019). In contrast to the high amount of indicator taxa found for the eluate-treated soils, only three indicator taxa were found for the respective water control. Those genera *Oerskova*, ADurb-Bin063-1, and *Paenarthrobacter* were sensitive to the eluates treatment and significantly correlated to the water control. Even though some species of the genus *Paenarthrobacter* were previously described to degrade the herbicide atrazine in soils (Jia et al., 2021) or to show a wide range of antimicrobial-resistant genes (Rosas-Díaz et al., 2021), we are the first to describe this genus as sensitive towards the presence of biocides. We presume that the combination of different eluted biocides and façade matrix components affect *Paenarthrobacter* within this study.

In comparison to the active bacterial indicator taxa, only a few active fungal indicator taxa were identified (Fig. 5B). Unclassified members of the order Capnodiales were determined as fungal indicator taxon for the water control. Members of Capnodiales showed high sensitivity toward environmental stress in soils within a field study (Hernandez et al., 2021). It can be presumed that Capnodiales are very sensitive to stressors like biocides or habitat changes. Interestingly, only the A-D-B and A-F-B treatment bared indicators in the active fungal community composition (Fig. 5B). Fungal indicator taxa assigned to the A-D-B treatment were all from the class Sodariomycetes. As an example of this class, unclassified members of the family *Lasiosphaeriaceae* are known to degrade polycyclic aromatic hydrocarbon and are resistant to organic compounds within rhizosphere and fertile soils (Li et al., 2020). It can be hypothesized that this genus benefits from the presence of the A-D-B eluate. Moreover, members of the genus *Trichoderma* were previously described to promote the soil environment during saline-alkaline stress (Fu et al., 2021). Façade eluates are due to the high pH of the paint and render formulations very alkaline independent of the presence of biocides. Unclassified members of soil microalgae *Chlorosarcinopsis* were identified as indicator taxon of A-F-B treated soils. Members of the genus *Chlorosarcinopsis* can cope with stress conditions by producing antioxidative substances to protect their cells (Khani-Juyabad et al., 2019).

Indicator analysis showed taxa that are significantly correlated to the treatment and benefit thereof, but many taxa were omitted due to the presence of façade eluates. Looking at the total fungal community, *Aureobasidium* was omitted within all treatment groups and was solely found in the total water control (Supplementary Table 3). A recent mini-review stated that stress resistance of most of *Aureobasidium* species is still unknown (Rensink et al., 2024). Our study is the first description of stress sensitivity against façade eluates by members of the genus *Aureobasidium*. Unclassified members of *Cercozoa* were also displaced by the façade eluate treatment in all groups. Previous studies suggested *Cercozoa* species as possible bioindicators due to their characteristics like great sensitivity, world-wide distribution, and short life cycle (Freitas et al., 2022). Interestingly, *Gamsia* was also omitted in all treatment groups even though species of this genus were described to be persistent and gaining abundance on cave walls after being cleaned with biocides (Martin-Pozas et al., 2024). It can be hypothesized that *Gamsia* might be omitted due to further components like zinc, for example, of the façade eluates than the presence of biocides and their degradation products. Similar results were obtained for the genus *Schizispora*, which was solely present in the water control of active fungal communities. Opposing studies show that some strains obtain a high tolerance against heavy metals, polycyclic aromatic hydrocarbons, and dye decolorizing activity (Min et al., 2015). Contrary, *Leuconostoc* and *Plesiomonas* were displaced from total and active bacterial community compositions due to the façade eluate treatment (UOF and B, Fig. 3). *Leuconostoc* was previously reported to be highly sensitive towards biocides in laboratory conditions during sucrose extraction (Šereš et al., 2017). Finally, members of the genus *Plesiomonas* were also identified as indicator species, and the species *Plesiomonas shigelloides* has been described to evolve co-resistance to different antibiotics (Ekundayo and Okoh, 2020). Such co-resistance of *P. shigelloides* was achieved by horizontal gene transfer in polluted environmental habitats (Yin et al., 2020). Therefore, more research is needed if and how façade leachates stimulate horizontal gene transfer events.

5. Conclusion

Our results demonstrated for the first time that façade eluates lead to alterations of the soil microbiome regardless of the leaching procedure and the concentration of biocides. Render formulations without biocide additions caused significant alteration of the soil microbial community composition. Overall, these results provide a first insight into the effects of façade eluates on the soil microbiome and its functions. Compared to the previous studies the experimental design was more realistic since façade eluates were produced by leaching experiments considering degradation products and drying times. Our results urge further studies on the effects of façade eluates on the soil microbiome. The methods used allowed for the identification of active microbiome members that benefit from the presence of façade eluates and those that are negatively affected. These results could help establish ecotoxicological measures for assessing soil contamination and health in the future and serve as a basis for investigating the effects on plants and other organisms surrounding buildings.

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CRediT authorship contribution statement

Fabienne Reiß: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nadine Kiefer:** Writing – original draft, Validation, Resources, Methodology, Investigation. **Pascal Reiß:** Software, Formal analysis. **Stefan Kalkhof:** Writing – review & editing, Project administration, Funding acquisition. **Matthias Noll:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125242.

Data availability

Data is availaible via PRJNA1074951 on NCBI

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Biocide-II: Supplementary Information

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Table S3 (Excel format) from the supplementary material is accessible via the website mentioned above.

Table S1: RefeSol 02A soil characteristics as analysed by the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) according to DIN ISO 11277:2002-08 for the soil texture (1), DIN EN 15933:2012-11 for the pH (2), DIN ISO 11260:2018-11 for the Cation Exchange Capacity (CECeffective) (3), and DIN EN ISO 14240-1:2011-09 for analyzation of the microbial organic matter (Corg) (4, 5).

	Sand [wt%]	**Silt** [wt%]	**Clay** [wt%]	**pH_{CaCl2}**	**C_{org}** [%]	**N_{total}** [$g*kg^{-1}$]	**$CEC_{effective}$** [$mmolc* kg^{-1}$]	**Watercapacity** [$g*kg^{-1}$]
RefeSol 02A	2.30	82.00	15.70	6.54	1.04	1.20	81.20	471.00

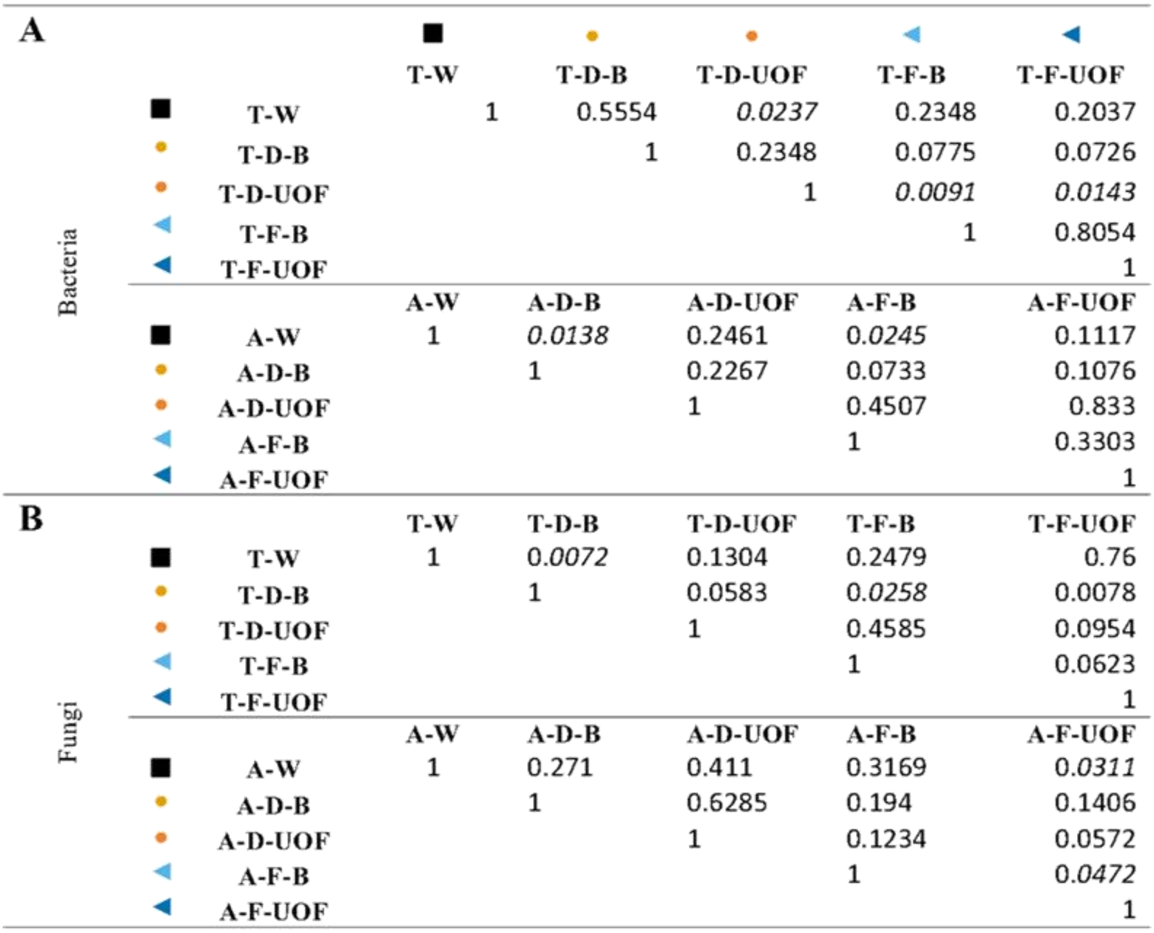
Table S2: Alpha diversities of total and active bacterial and fungal community after facade eluates treatment. Statistically significant differences are indicated by different letters in the significance (*Sig.*) column (Tukey Test; $p < 0.05$). The respective total (prefix T-) and active composition (prefix A-) are indicated. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides; SE: Standard Error.

		Richness			**Chao1**			**ACE**			**Shannon**			**Simpson**			**Eveness**		
	Sample	*Mean*	*SE*	*Sig.*	*Mean*	*SE*	*Sig.*	*Mean*	*SE*	*Sig.*	*Mean*	*SE*	*Sig.*	*Mean*	*SE*	*Sig.*	*Mean*	*SE*	*Sig.*
Bacteria	**T-W**	533	11	A	615.352	16.877	A	618.868	15.955	A	4.940	0.030	A	4.94	0.03	A	0.787	0.003	A
	T-D-B	494	9	A	536.909	14.815	A	536.602	14.182	B	4.905	0.017	A	4.91	0.02	A	0.791	0.003	A
	T-D-UOF	520	18	A	589.559	33.184	A	587.122	30.903	AB	4.957	0.017	A	4.96	0.02	A	0.793	0.004	A
	T-F-B	527	10	A	610.598	15.599	A	607.041	17.928	AB	4.945	0.023	A	4.94	0.02	A	0.789	0.002	A
	T-F-UOF	519	8	A	601.562	17.483	A	597.641	11.631	AB	4.909	0.024	A	4.91	0.02	A	0.785	0.003	A
	A-W	166	9	AB	170.028	8.886	B	168.428	9.091	B	2.150	0.122	AB	2.15	0.12	AB	0.421	0.022	AB
	A-D-B	180	10	AB	272.676	19.974	A	276.015	15.953	A	1.369	0.072	B	1.37	0.07	B	0.263	0.011	B
	A-D-UOF	150	6	B	169.320	19.529	B	169.545	19.116	B	2.086	0.431	AB	2.09	0.43	AB	0.418	0.087	AB
	A-F-B	203	17	A	296.815	22.710	A	301.516	28.406	A	1.604	0.146	B	1.60	0.15	B	0.302	0.025	B

	A-F-UOF	186	15	AB	192.670	15.688	B	4.599	1.779	C	6.295	0.410	A	3.03	0.46	A	0.583	0.094	A
Fungi	**T-W**	86	12	A	93.240	12.142	AB	6.514	3.119	A	3.253	0.102	AB	3.25	0.10	AB	0.741	0.041	A
	T-D-B	53	6	A	56.500	6.093	B	3.679	1.809	A	2.664	0.277	B	2.66	0.28	B	0.672	0.057	A
	T-D-UOF	70	11	A	78.600	12.197	AB	7.455	3.049	A	3.235	0.109	AB	3.24	0.11	AB	0.776	0.047	A
	T-F-B	78	7	A	79.800	8.220	AB	1.987	1.301	A	3.266	0.101	AB	3.27	0.10	AB	0.753	0.023	A
	T-F-UOF	90	6	A	97.500	7.834	A	6.812	3.838	A	3.360	0.088	A	3.36	0.09	A	0.749	0.022	A
	A-W	9	1	AB	10.267	1.253	A	8.500	1.500	A	1.420	0.195	A	1.42	0.20	A	0.636	0.073	A
	A-D-B	19	4	AB	19.000	4.198	A	23.712	4.741	A	1.888	0.375	A	1.89	0.37	A	0.648	0.093	A
	A-D-UOF	8	2	B	11.400	3.415	A	16.006	6.671	A	1.511	0.248	A	1.51	0.25	A	0.737	0.098	A
	A-F-B	20	2	A	22.700	3.270	A	24.400	3.601	A	1.846	0.159	A	1.85	0.16	A	0.618	0.048	A
	A-F-UOF	13	2	AB	18.350	5.095	A	21.098	6.008	A	1.133	0.140	A	1.13	0.14	A	0.464	0.057	A

Table S3: Bacterial and fungal relative abundance on genus level per treatment group. The respective total (prefix T-) and active composition (prefix A-) are indicated. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides Ommitted species are marked in **Bold** within the total communities and *italics* within the active communities. EXCEL File Reiß_ TableS3.xlsx

Table S4: One-way non-parametric multivariate analysis based on the Bray-Curtis similarities of the total (prefix T-) and active (prefix A-) bacterial (**A**) and fungal functional community compositions (**B**) ($p < 0.05$ highlighted in italics; n = 5). W: Water Control (■); D-B: Facade eluates from DIN testing without biocides (●); D-B: Facade eluates from DIN testing containing biocides (●); F-B: Facade eluates from free weathering experiment without biocides (◀); and F-UOF: Facade eluates from free weathering experiment containing biocides (◀).



A			■	●	●	◀	◀
			T-W	**T-D-B**	**T-D-UOF**	**T-F-B**	**T-F-UOF**
Bacteria	■	**T-W**	1	0.5554	*0.0237*	0.2348	0.2037
	●	**T-D-B**		1	0.2348	0.0775	0.0726
	●	**T-D-UOF**			1	*0.0091*	*0.0143*
	◀	**T-F-B**				1	0.8054
	◀	**T-F-UOF**					1
			A-W	**A-D-B**	**A-D-UOF**	**A-F-B**	**A-F-UOF**
	■	**A-W**	1	*0.0138*	0.2461	*0.0245*	0.1117
	●	**A-D-B**		1	0.2267	0.0733	0.1076
	●	**A-D-UOF**			1	0.4507	0.833
	◀	**A-F-B**				1	0.3303
	◀	**A-F-UOF**					1
B			**T-W**	**T-D-B**	**T-D-UOF**	**T-F-B**	**T-F-UOF**
Fungi	■	**T-W**	1	*0.0072*	0.1304	0.2479	0.76
	●	**T-D-B**		1	0.0583	*0.0258*	0.0078
	●	**T-D-UOF**			1	0.4585	0.0954
	◀	**T-F-B**				1	0.0623
	◀	**T-F-UOF**					1
			A-W	**A-D-B**	**A-D-UOF**	**A-F-B**	**A-F-UOF**
	■	**A-W**	1	0.271	0.411	0.3169	*0.0311*
	●	**A-D-B**		1	0.6285	0.194	0.1406
	●	**A-D-UOF**			1	0.1234	0.0572
	◀	**A-F-B**				1	*0.0472*
	◀	**A-F-UOF**					1

Table S5: Indicator species analysis of Total (Prefx T-) and active (Prefix A-) bacterial and fungal community compositions after eluate treatment showing the Stat value and significance of each species and its function ordered after the Stat value. Unclassified members of the taxon are marked with $^{+}$. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

	Treatment	Stat value	p-value	Significance	Taxa	Relative abundance [%]	Function
Bacteria	T-W	0.850	0.0006	***	*Corynebacterium*	0.356	NA
		0.712	0.0006	***	*Flavobacterium*	0.211	Aerobic chemoheterotrophy, chemoheterotrophy
		0.650	0.0220	*	*Qipengyuania*	0.009	Aerobic chemoheterotrophy, chemoheterotrophy
		0.635	0.0062	**	*Roseimicrobium*	0.075	Aerobic chemoheterotrophy, chemoheterotrophy
	T-D-B	0.798	0.0029	**	Methylacidiphilaceae uncultured	0.012	Methanotroph

	0.608	0.0217	*	B1-7BS	0.080	NA
	0.583	0.0330	*	Thermoleophilia $^{+}$	0.088	NA
T-D-UOF	0.6	0.0412	*	BIyi10	0.015	Aerobic chemoheterotrophy, chemoheterotrophy
T-F-B	0.739	0.0198	*	Sar202 Clade	0.005	Heterotrophy
	0.661	0.0069	**	vadinHA49	0.037	Chemoorganotroph
	0.649	0.0330	*	Peptostreptococcales - Tisserales $^{+}$	0.012	Fermentation, chemoheterotrophy
	0.632	0.0213	*	*Pseudogracilibacillus*	0.007	Aerobic chemoheterotrophy, chemoheterotrophy
	0.576	0.0470	*	*Acetivibrio*	0.015	Fermentation, chemoheterotrophy
T-F-UOF	0.691	0.0212	*	*Herminiimonas*	0.006	Heterotrophy
	0.691	0.0207	*	*Sulfuriferula*	0,006	Chemolithoautotrophy

	0.667	0.0128	*	PAUC26F	0.021	Acidophilic chemoheterotrophy
	0.619	0.0251	*	*Herbiconiux*	0.013	Aerobic chemoheterotrophy, chemoheterotrophy
	0.612	0.0415	*	*Epulopiscium*	0.010	Heterotrophy
A-W	0.864	0.0020	**	*Blautia*	0.039	Fermentation, chemoheterotrophy
	0.635	0.0222	*	ADurb.Bin063-1	0.022	Anaerobic chemoorganotrophs
	0.611	0.0113	*	*Paenarthrobacter*	6.960	Aromatic compound degradation
	0.569	0.0309	*	Pirellulaceae uncultured	0.015	Heterotrophy
	0.550	0.0472	*	*Oerskovia*	0.257	Fermentation, chemoheterotrophy
A-D-B	0.691	0.0196	*	*Alloactinosynnema*	0.004	NA

A-D-UOF	0.691	0.0019	**	*Paenibacillus*	1.610	Aerobic chemoheterotrophy, chemoheterotrophy
	0.612	0.0413	*	S0134 terrestrial group	0.039	NA
	0.593	0.0430	*	*Parabacteroides*	0.049	Fermentation, chemoheterotrophy
A-F-B	0.786	0.0018	**	*Terrabacter*	0.032	Aerobic chemoheterotrophy, chemoheterotrophy
	0.691	0.0225	*	Caldilineaceae uncultured	0.005	Aerobic, facultatively anaerobic chemoorganotrophy
	0.590	0.0225	*	Streptosporangiaceae $^{+}$	0.009	Aerobic chemoheterotrophy, chemoheterotrophy
A-F-UOF	0.725	0.0037	**	Solirubrobacterales $^{+}$	0.063	Aerobic chemoheterotrophy, chemoheterotrophy
	0.715	0.0225	*	*Aquicella*	0.054	Chemoorganotrophy

0.712	0.0214	*	*Flavobacterium*	0.063	Aerobic chemoheterotrophy, chemoheterotrophy
0.686	0.0055	**	*Corynebacterium*	0.280	NA
0.682	0.0049	**	RB41	0.139	Chemoheterotroph
0.675	0.0031	**	*Pseudorhodoplanes*	0.058	Aerobic chemoheterotrophy, chemoheterotrophy
0.663	0.0087	**	MB-A2-108	0.347	NA
0.648	0.0093	**	*Lysinibacillus*	0.073	Insect pathogen
0.636	0.0160	*	Gaiellales uncultured	0.461	Aerobic chemoheterotrophy, chemoheterotrophy
0.635	0.0091	**	Vicinamibacteraceae	0.241	Chemoheterotrophy
0.613	0.0354	*	WD2101 soil group	0.090	Chemoorganotrophy
0.607	0.0243	*	Lachnospiraceae $^{+}$	0.182	Fermentation

		0..595	0.0486	*	Subgroup 5	0.024	Heterotrophy
		0.577	0.0027	**	*Bacillus*	0.490	Aerobic chemoheterotrophy, chemoheterotrophy
		0.573	0.0445	*	Caulobacteraceae $^+$	0.185	Aerobic chemoheterotrophy, chemoheterotrophy
		0.562	0.0296	*	SC-I-84	0.112	Aerobic, facultatively anaerobic chemoorganotroph
		0.556	0.0486	*	*Solirubrobacter*	0.126	Aerobic chemoheterotrophy, chemoheterotrophy
		0.534	0.0449	*	Sandaracinaceae uncultured	0.019	Aerobic chemoheterotrophy, chemoheterotrophy
Bacteria	T-D-B + T-F-B	0.556	0.0496	*	*Rummeliibacillus*	0.026 +0.039	Heterotrophy
	T-D-UOF + T-F-UOF	0.569	0.0361	*	*Sorangium*	0.061 +0.078	Cellulolysis, chemoheterotrophy

A-D-B + A-F-B	0.610	0.0197	*	*Ferruginibacter*	0.134 + 0.150	Aerobic chemoheterotrophy, chemoheterotrophy
	0.564	0.0443	*	*Blastococcus*	0.128 + 0.170	Aerobic chemoheterotrophy, chemoheterotrophy
	0.558	0.0482	*	*Geodermatophilus*	0.028+ 0.031	Manganese oxidation
A-D-UOF + A-F-UOF	0.739	0.0017	**	Caulobacteraceae uncultured	0.881 +0.913	Chemoorganotrophy
	0.674	0.0049	**	X0319-6G20	0.153 + 0.153	Obligate predators
	0.674	0.0064	**	*Ralstonia*	0.258 + 0.643	Plant pathogen
	0.668	0.0059	**	Beijerinckiaceae $^{+}$	0.146 + 0.179	Chemoorganotrophy
	0.667	0.0075	**	*Curvibacter*	2.139 + 1.741	Heterotrophy
	0.662	0.0070	**	*Novosphingobium*	0.207 + 0.502	Aromatic compound degradation, chemoheterotrophy
	0.643	0.0100	**	*Bdellovibrio*	0.100 + 0.085	Predatory, exoparasitic

		0.600	0.02334	*	*Pelomonas*	1.615 + 0.819	Chemoorganotrophy
		0.578	0.0373	*	*Polaromonas*	0.116 + 0.087	Chemoorganotrophy
		0.560	0.0448	*	*Sediminibacterium*	0.172 + 0.111	Chemoorganotrophy
		0.548	0.0390	*	*Cutibacterium*	0.457 + 1.180	Pathogen
		0.545	0.0484	*	Sporichthyaceae $^{+}$	0.216 + 0.216	Heterotrophy
Fungi	T-W	0.556	0.0424	*	*Cercozoa* $^{+}$	0.134	Dung saprotroph
		0.537	0.0429	*	*Sistotrema*	3.77	Litter saprotroph
		0.419	0.0211	*	Basidiobolales $^{+}$	0.158	Unspecified saprotroph
	T-D-UOF	0.697	0.0234	*	*Eocronartium*	0.264	Plant pathogen
		0.649	0.0132	*	*Oidiodendron*	0.106	Soil saprotroph
		0.509	0.0403	*	*Preussia*	1.346	Dung saprotroph
		0.501	0.0396	*	*Tetragoniomyces*	0.432	Mycoparasite

	T-F-B	0.587	0.0386	*	Scenedesmaceae $^{+}$	0.178	Photoautotrophic
		0.572	0.0006	***	*Podospora*	0.966	Soil saprotroph
	T-F-OUF	0.639	0.0183	*	Trebuxiophyceae $^{+}$	0.476	Photoautotrophic
	A-W	0.762	0.0027	**	Capnoidales $^{+}$	6.714	Unknown
	A-D-B	0.559	0.0206	*	*Monocillium*	1.66	Litter saprotroph
		0.538	0.0219	*	*Cladorrhinum*	0.933	Unspecified saprotroph
		0.495	0.0204	*	*Trichoderma*	0.204	Mycoparasite
		0.485	0.0219	*	Lasiosphaeriaceae $^{+}$	0.736	Unspecified saprotroph
		0.463	0.0219	*	*Apiosordaria*	0.311	Dung saprotroph
	A-F-B	0.553	0.0491	*	*Chlorosarcinopsis*	5.079	Photoautotrophic

Table S6: Physico-chemical properties of the soil after 28 days of eluate incubations. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

Eluate treatment	**W**	**D-B**	**D-UOF**	**F-B**	**F-UOF**
pH	7.2±0.0	7.1±0.0	7.1±0.0	7.2±0.0	7.2±0.0
Fl^{-} [µg(biocide)*g(soil)$^{-1}$]	7.0±0.04	6.3±0.03	6.3±0.18	6.4±0.15	6.7±0.13
Cl^{-} [µg(biocide)*g(soil)$^{-1}$]	2.7±0.04	3.04±0.17	2.94±0.23	3.8±0.06	4.0±0.07
NO3^{-} [µg(biocide*g(soil)$^{-1}$]	77.52±0.62	83.0±0.43	77.53±4.1	84.49±3.2	78.08±1.37
PO$_4^{3-}$ [µg(biocide)*g(soil)$^{-1}$]	2.89±0.21	2.16±0.06	2.57±0.21	1.88±0.13	2.7±0.16
SO$_4^{2-}$ [µg(biocide)*g(soil)$^{-1}$]	7.9±0.2	9.1±0.6	7.64±0.18	12.78±0.33	14.71±1.32

Table S7: Effect of the environmental parameters on the composition of the active and total soil bacterial and fungal community composition after *eluate* treatment.

		Total bacteria		**Active bacteria**		**Total fungi**		**Active fungi**	
		R^2	p -value	R^2	p -value	R^2	p -value	R^2	p -value
Community composition	Gene copy numbers	0.066	0.427	-	-	0.038	0.588	-	-
	Eluate	0.031	0.729	0.020	0.801	0.087	0.356	0.101	0.334
	pH	0.152	0.170	0.359	*0.006*	0.107	0.297	0.019	0.795
	Fl^-	0.168	0.126	0.231	0.067	0.065	0.497	0.217	0.068
	NO_3	0.017	0.805	0.292	*0.022*	0.020	0.803	0.166	0.153
	SO_4	0.366	*0.011*	0.196	0.109	0.219	0.060	0.072	0.453

Table S 8: Effect of the environmental parameters on the functional composition of the active and total soil bacteria and fungi after eluate treatment.

		Total bacteria		**Active bacteria**		**Total fungi**		**Active fungi**	
		R^2	p -value	R^2	p -value	R^2	p -value	R^2	p -value
Functional composition	Gene copy numbers	0.180	0.098			0.266	0.055	-	-
	Eluate	0.002	0.981			0.005	0.948	0.076	0.452
	pH	0.077	0.396	0.177	0.120	0.086	0.375	0.081	0.404
	Fl^-	0.010	0.898	0.146	0.193	0.097	0.324	0.299	0.029
	NO_3	0.194	0.089	0.179	0.106	0.131	0.205	0.366	0.015
	SO_4	0.078	0.399	0.026	0.744	0.109	0.282	0.073	0.461

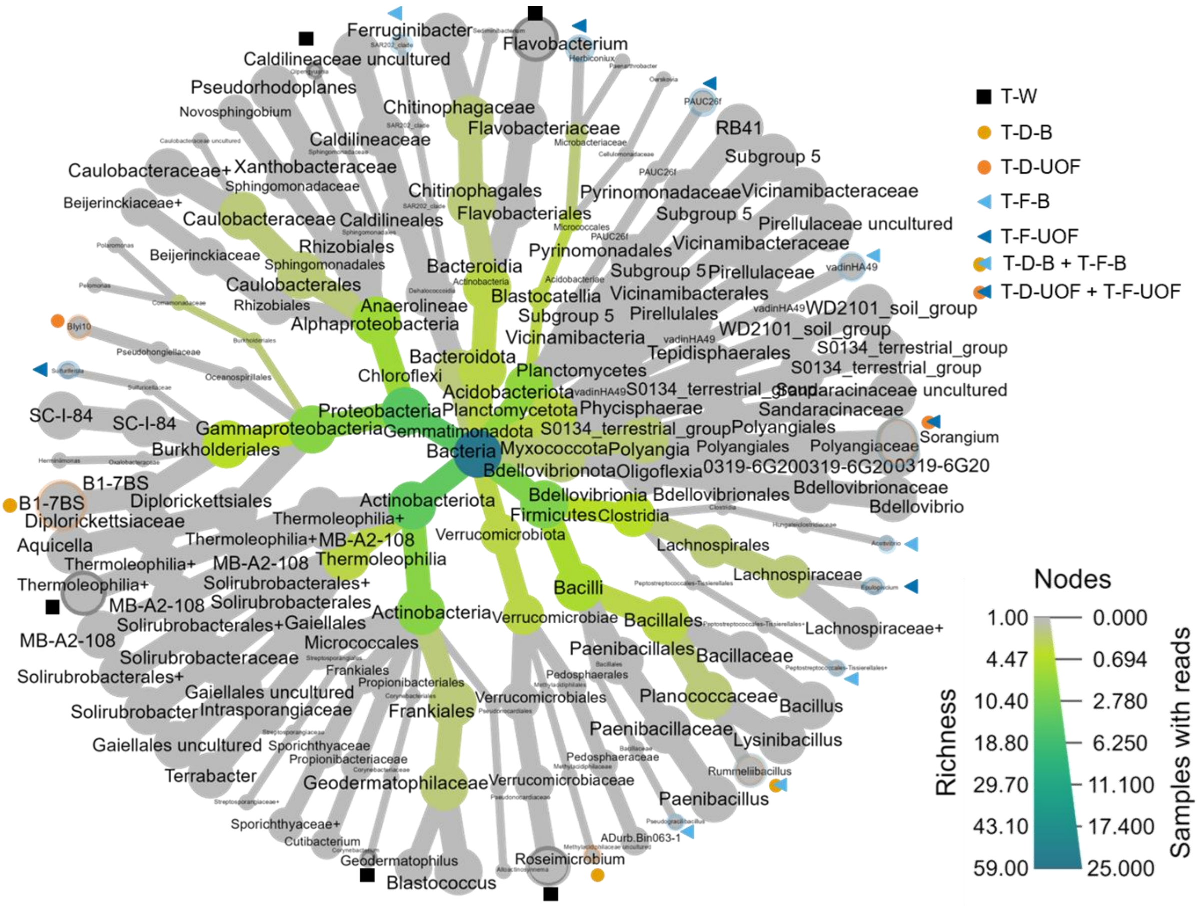


Figure S1: Heat tree representation of the total bacterial indicator taxa. The color code resembles the richness and the node size the respective sample reads. Unclassified members of the taxon are marked with $^{+}$. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

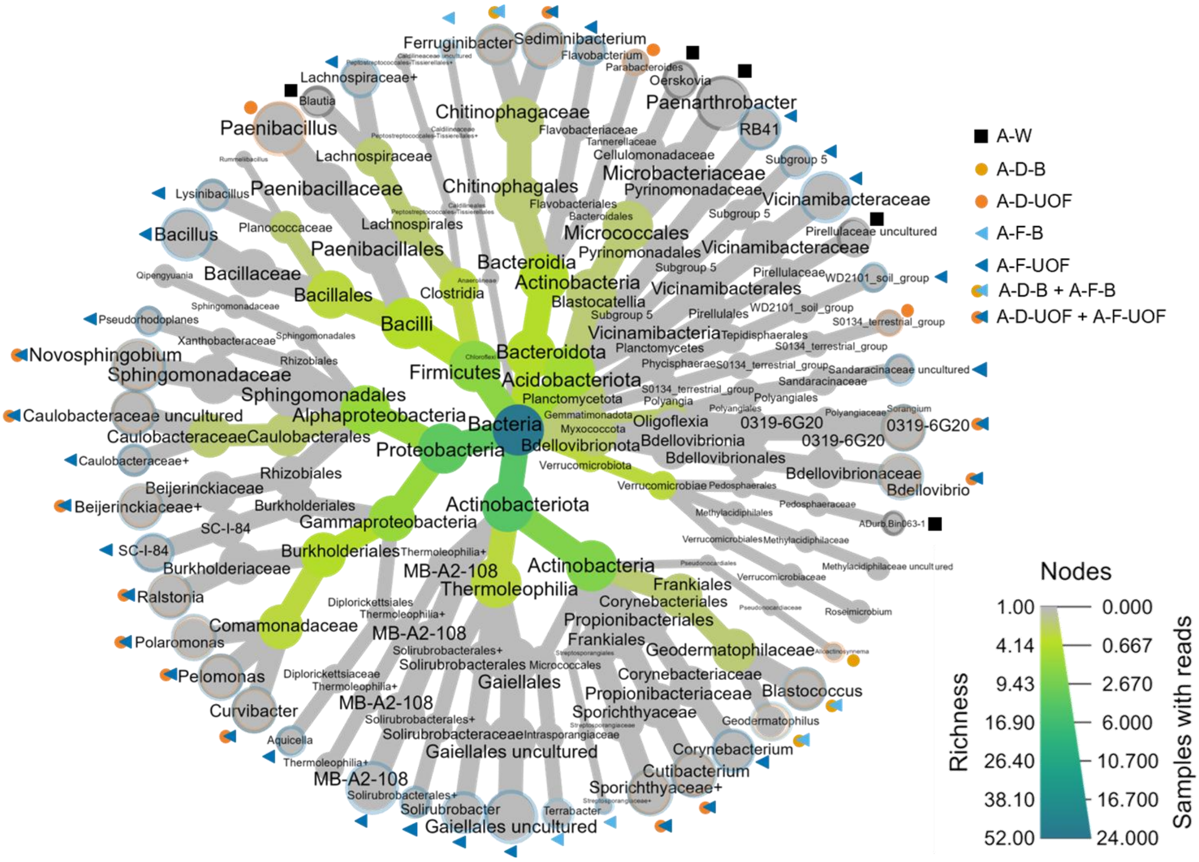


Figure S2: Heat tree representation of the active bacterial indicator taxa. The color code resembles the richness and the node size the respective sample reads. Unclassified members of the taxon are marked with $^+$. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

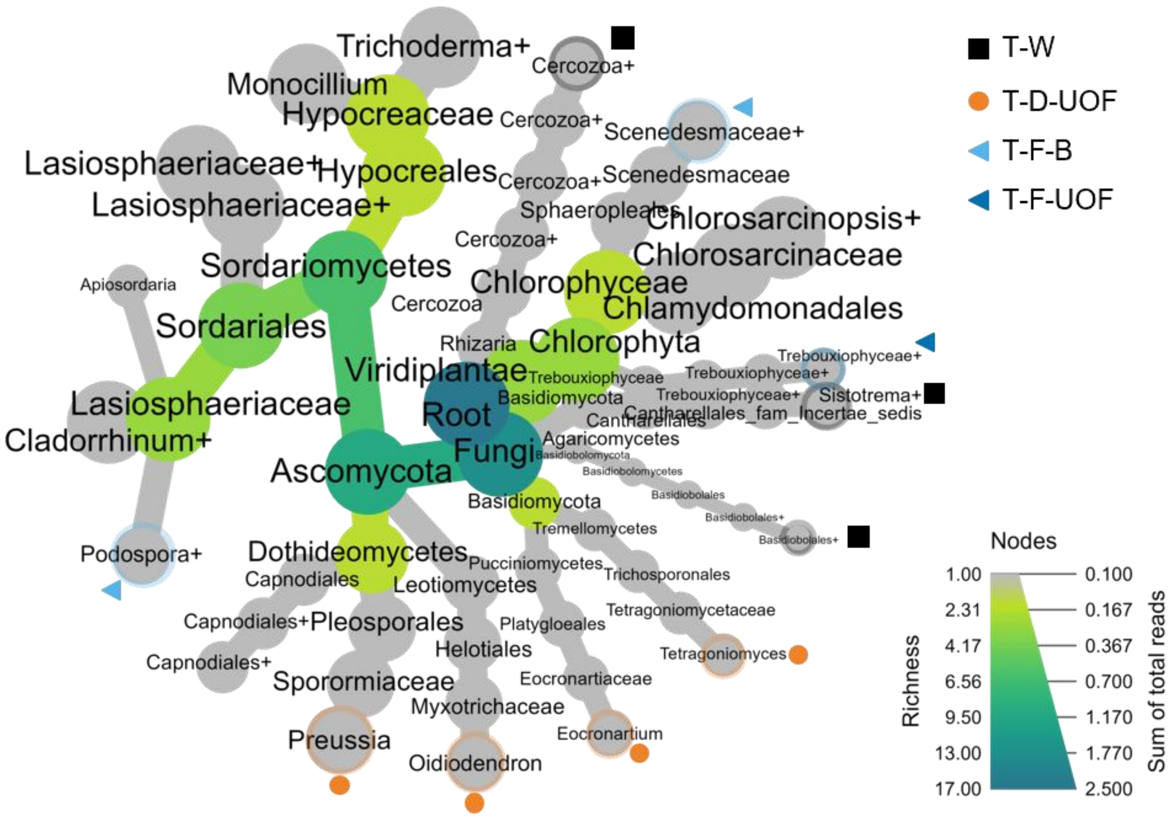


Figure S3: Heat tree representation of the total fungal indicator taxa. The color code resembles the richness and the node size the respective sample reads. Unclassified members of the taxon are marked with $^+$. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

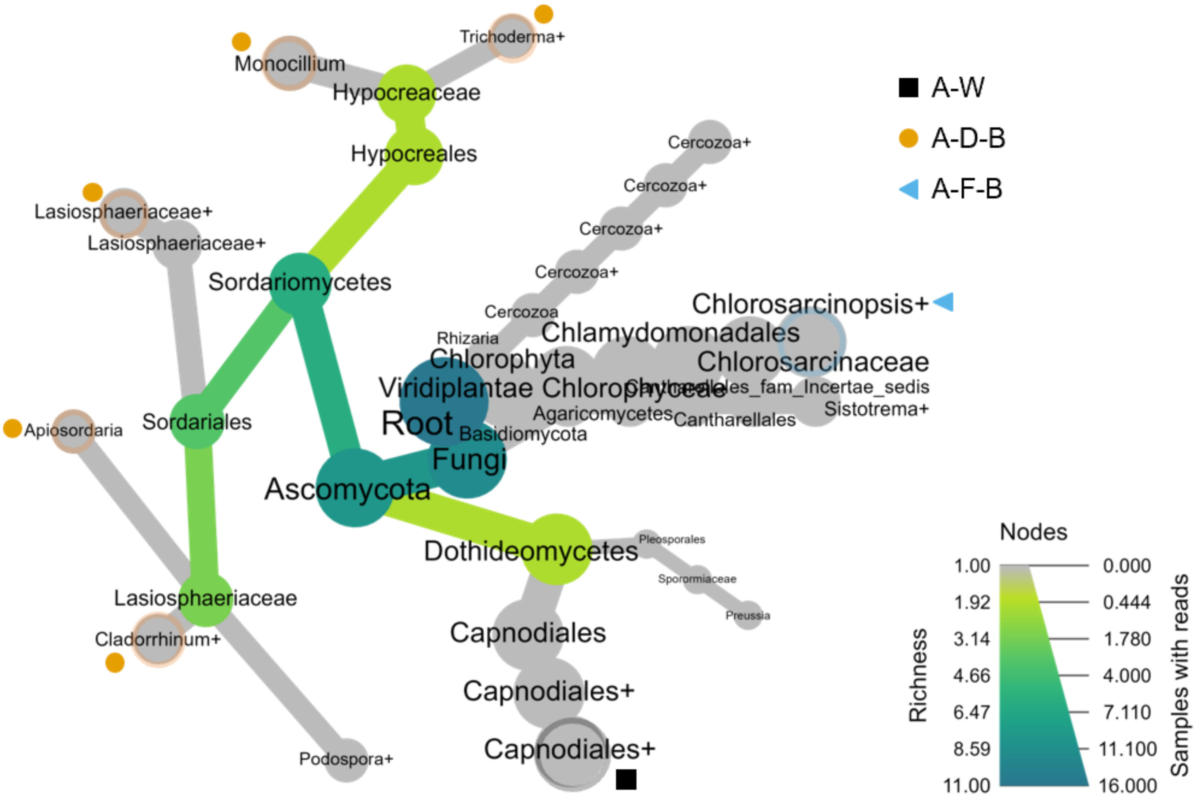


Figure S4: Heat tree representation of the active fungal indicator taxa. The color code resembles the richness and the node size the respective sample reads. Unclassified members of the taxon are marked with $^{+}$. W: Water control; D-B: Facade eluates from DIN testing without biocides; D-B: Facade eluates from DIN testing containing biocides; F-B: Facade eluates from free weathering experiment without biocides; F-UOF: Facade eluates from free weathering experiment containing biocides

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3.4 Biocide-III: Continuous intake of facade eluates affects active and total soil microbiome

Biology and Fertility of Soils
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ORIGINAL PAPER

Continuous intake of facade eluates affects active and total soil Microbiome

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Abstract
Urban surfaces such as building facades release chemical compounds into the environment through rain-induced runoff. Facade eluates typically consist of a mixture of biocides and heavy metals that can pose an environmental risk. Even though, leaching of these substances and their aquatic ecotoxicity is well determined, data regarding their terrestrial ecotoxicological effects on the soil microbiome are still scarce. In this study, we investigated the temporal effects of repeated facade eluate applications on the composition and function of soil bacterial and fungal communities. Facade runoff was collected under natural weathering conditions from facades without and with biocides and applied to outdoor soil microcosms over a two-month period. High-throughput amplicon sequencing and qPCR analyses revealed that facade eluate exposure significantly altered microbial community composition and reduced bacterial gene copy numbers, while increased fungal richness over time. Active (Bromodeoxyuridine labelled DNA) communities showed stronger and more distinct treatment responses than total communities. Functional profiling indicated increased prevalence of stress-associated traits such as ureolysis in bacteria and shifts in fungal trophic modes, including reduced plant pathogens and increased photoautotrophs, which were supported by indicator species analysis. Our findings highlight that repeated facade runoff applications and prolonged incubation can alter the soil microbial community. These results emphasize the ecological risks posed by facade eluate on the soil microbiome and the consideration of biocides from building materials as environmental risk.

Keywords Facade eluates · Multiple treatment · Outdoors incubation experiment · Soil microbiome · Bacteria · Bromodeoxyuridine · Fungi · Terrestrial ecotoxicological effects

Introduction

Biocides are commonly incorporated into paints and renders to prevent microbial growth during storage, manufacture, and after application (Burkhardt et al. 2011; European Parliament, Council of the European Union 2012; Bollmann et al. 2014). These materials are used in facade constructions, which combine different products with varying biocide concentrations and chemical compositions. This results in a highly variable and uncontrollable release of biocides into the surrounding environment (Paijens et al. 2020; Linke et al. 2021; Kiefer et al. 2024). Typical biocidal compounds include isothiazolinones (e.g., OIT, DCOIT), carbamates (IPBC), phenylureas (Diuron, Terbutryn), and metal-based agents (zinc or copper pyrithione) (Reiß et al. 2021). Formulations usually combine algicidal and fungicidal agents to ensure broad-spectrum and long-lasting protection. The

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main purpose of biocides in building materials is to minimize microbial growth on façades over their lifespan, with manufacturers frequently offering protection guarantees of up to fifteen years. Similar to agricultural pesticides, biocides are found in surface waters at comparable frequencies (Wittmer et al. 2011). Although the effects of pesticides on soil microbiomes have been widely studied (Bromilow et al. 1996; Pineda et al. 2020), there is a limited information on the environmental impact of biocides released from building materials. Unlike agricultural applications, which are highly regulated and occur at specific times (Heidorn 2002), biocides from building materials leach sporadically and uncontrollably throughout the year, resulting in localized and varied concentrations (Reiß et al. 2021).

Biocide emissions are especially elevated after recent renovations that use biocide-containing paints or renders. According to Paijens et al. (2020), the leaching behavior of these biocides is affected by a variety of factors. Laboratory leaching methods do not accurately replicate in situ conditions, such as varying water contact times and UV irradiation, which affect the leachate composition (Bandow et al. 2020). Facade eluates can contain biocides, heavy metals, binders, fillers, and pigments. These substances reach the soil environment with every rain event, can accumulate, and their impact on soil depends on various factors, including soil properties, biocide chemistry, and climatic conditions (Arias-Estévez et al. 2008). Previous studies have shown that biocides embedded in building materials can present ecotoxicological hazards. For instance, the model organism *Aliivibrio fischeri* was impacted by terbutryn and its breakdown products (Bollmann et al. 2017). Kiefer et al. (2024) showed that facade eluates caused toxic effects on several sediment and aquatic organisms within standardized ecotoxicity tests.

Terrestrial ecotoxicity studies and the corresponding advantages and disadvantages have been discussed in previous publications (Reiß et al. 2021, 2024, 2025). In brief, most rely on standard plate counts, indirect methods like substrate-induced respiration (SIR), or low-resolution molecular techniques, lacking detailed resolution. To simulate natural conditions and understand the effects of facade eluates on soil microbiomes, high-resolution DNA-based methodologies, such as Bromodeoxyuridine (BrdU) immunocapture coupled with Illumina amplicon sequencing, should be used. This approach differentiates between total genomic DNA and DNA from metabolically active organisms (McMahon et al. 2011; Purahong et al. 2022), providing a clearer picture of biocide impacts without affecting the soil microcosm experiment negatively by changing substrate availability for example (Reiß et al. 2024). Studies using BrdU incorporation have successfully detected active bacteria and partly also active fungi in various environments, highlighting significant differences between total and active microbial communities (McMahon et al. 2011; Wahdan et al. 2021; Purahong et al. 2022; Reiß et al. 2024).

The ecotoxicological effects on soil microbiomes depend on the biocide's chemical nature, dose, exposure duration, and entry route (Kumar et al. 2012). Biocides and biocide combinations frequently used in building materials caused significant changes in the composition of the active soil microbial community, where distinct bacterial and fungal taxa were reduced due to the presence of biocides, and others gained in abundance and benefited (Reiß et al. 2024). Similar results were obtained for single treatment of facade eluates in soil microcosm studies (Reiß et al. 2025), and even facade eluates of surfaces without biocides impacted the composition of the active soil microbial community. However, the repeated entry of facade eluates, with and without biocides, has not yet been addressed in the literature and will be investigated in this study. The preservatives used on the sampled facades in the natural weathering experiment were preselected to be as realistic as possible while encompassing a broad spectrum of the active ingredients described above (Kiefer et al. 2024). To systematically and realistically pursue this approach, facade eluates were collected after each rain event and applied to soil microcosm set up, which was sheltered by a roof and soil barrier to prevent external water sources.

This study aims (i) to investigate the effect of facade eluates (with and without biocides) on the composition of the total and active soil microbial community in garden soil; (ii) to determine whether soil physico-chemical properties and soil microbial functions are altered by repeated facade eluate treatments. We expect that facade eluate treatments will change the composition and function of the active bacterial and fungal communities without significantly affecting the total soil microbial community. Additionally, the introduction of facade eluates containing biocides is expected to cause a greater shift in the active microbial community compared to eluates without biocides.

Methods

Soil incubation

The reference soil RefeSol-02 A was used for the incubation experiment and purchased from the Fraunhofer Institute for Molecular Biology and Applied Ecology IME in Schmallenberg, Germany. This soil was chosen due to existing records confirming that no pesticides had been applied to it for at least the last decade. RefeSol soils are well-suited for biological testing, as they comply with

OECD terrestrial ecotoxicological standards (Schlich and Hund-Rinke 2015). For the experiments, freshly collected and sieved RefeSol-02 A soil was used. The silt loam, sub-acidic soil has the following properties: 2.3 wt% sand, 82.0 wt% silt, 15.7 wt% clay, pH 6.54, 1.04% organic carbon, 1.2 g kg^{-1} soil nitrogen, 81.20 mmolc kg^{-1} cation exchange capacity, and 471.00 g kg^{-1} water-holding capacity. Soil incubation was carried out outdoors in Coburg, Bavaria, Germany, using stainless steel boxes filled with 100 g of soil from June to August 2021. These boxes were placed in the ground to guarantee the same soil temperature as the surroundings and had drainage holes to allow excess eluate to drain away (Fig. 1). Additionally, boxes were sheltered by a roof and soil barrier to prevent external water and pollution sources. For each treatment, ten soil boxes were used and received the same amount of diluted eluate (B; UOF) or rainwater as a control (RW) after every rain event. Soil was allowed to completely dry if there was a duration without rain events during the testing period. Soil samples were taken after 29 and 62 days of outdoor incubation. Soil samples were incubated with 100 mM BrdU (B5002, Merck, Darmstadt, Germany) solution at trial 48 h before the sampling time point.

Soil chemical properties

Soil pH was measured in accordance with DIN EN 15933:2012-11. The standard defines a 1:5 soil-to-suspension volume ratio (i.e., 1 part soil, 5 parts liquid) for preparing samples to measure pH (DIN German Institute for Standardization e. V.). Anion analysis in the soil was carried out using ion chromatography on a Metrohm 883 Basic IC Plus system equipped with a Metrosep A Supp 4–250/4.0 column. For sample preparation, 5 g of soil was combined with 25 mL of ultrapure water in a tube and agitated for 45 min. The suspension was then centrifuged at room temperature for 25 min at 4695.6 × g. From the resulting supernatant, 12.5 mL was carefully transferred to a new tube and filtered in two steps—initially through a 15 µm filter, followed by a 0.22 µm filter. An external calibration curve was established using a multielement anion standard solution for ion chromatography (Honeywell Fluka™, Seelze, Germany). Prior to analysis, all samples, standards, and eluents were degassed using an ultrasonic bath. The eluent solution consisted of 1.7 mM sodium bicarbonate ($NaHCO_3$), 1.8 mM sodium carbonate (Na_2CO_3), and 2% acetone. Chromatographic separation was conducted at a flow rate of 1 mL/min with a total runtime of 20 min.

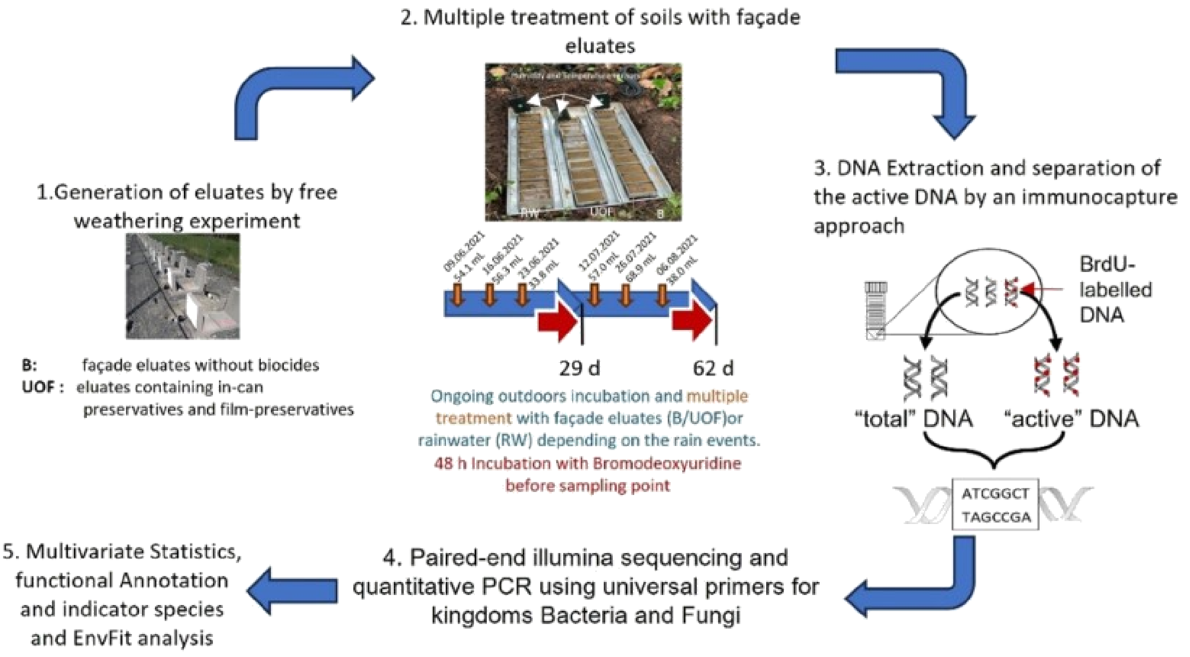


Fig. 1 Overview of the experimental workflow. Starting with the eluate generation (**1**), the soil incubation (**2**), the DNA extraction (**3**), (**4**) the amplicon sequencing analysis, and (**5**) statistical analyses. During (**2**) soil was treated with rainwater (**RW**), facade eluates without biocides (**B**), and facade eluates containing in-can preservatives and film preservatives (**UOF**) as indicated by the orange arrows showing the date and amount of treatment. For identification of active soil microbial members, soil microcosms were treated with Bromodeoxyuridine 48 h before destructive soil sampling. Samples were taken after 29 and 62 days. Soil moisture and temperature were monitored via humidity and temperature sensors

Quantification of biocides in facade eluates

As outlined in Kiefer et al. (2024), biocide concentrations in each facade eluate ($n=3$) were quantified. In brief, samples were first filtered through a 0.2 µm polyamide membrane and subsequently analyzed via high-performance liquid chromatography (HPLC) equipped with a UV detector (Waters Corporation, Milford, Massachusetts, USA). Compound separation was achieved using a gradient elution protocol at a flow rate of 0.2 mL·min^{-1}. The mobile phase initially consisted of 70% water with 0.1% formic acid and 30% methanol (MeOH) for the first 3 min. This was followed by a linear gradient increasing the MeOH concentration to 70% over the next 6 min, which was then held constant for 4 min. The mobile phase was then returned to its initial composition and maintained for the remainder of the 20-minute run. Quantification of methylisothiazolinone (MIT), octylisothiazolinone (OIT), methyl-chloroisothiazolinone (CMIT), and terbutryn (T) was conducted at a wavelength of 240 nm, whereas benzisothiazolinone (BIT) was detected at 317 nm. External calibration curves were used for all analytes.

Isolation of soil DNA and Immunoprecipitation of BrdU-incorporated DNA

DNA was extracted from all microcosms using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Europe GmbH, Freiburg im Breisgau, Germany), following the manufacturer's protocol using 250 mg of soil. This DNA is referred to as "total DNA," in line with terminology used in prior studies (Wahdan et al. 2021; Purahong et al. 2022; Reiß et al. 2024, 2025). BrdU-labeled DNA was subsequently isolated from the total DNA using an established immunocapture technique (Hanson and McMahon 2006; McMahon et al. 2011). Briefly, for each sample, 2 µL of monoclonal anti-BrdU antibody (1 mg·µL^{-1}, clone BU-33, mouse-derived, Sigma-Aldrich, St. Louis, USA) was combined with 18 mL of denatured herring sperm DNA (1.25 mg·µL^{-1} in phosphate-buffered saline [PBS]; Promega, Madison, USA) and incubated at 30 °C for 45 min to facilitate antibody–DNA complex formation. Next, 20 µL of denatured sample DNA was added to the complex and incubated for an additional 30 min at 30 °C to enable binding of BrdU-incorporated DNA.

The mixture was then combined with 6.26 µL of pre-washed Dynabeads™ coated with goat anti-mouse IgG (Invitrogen, Waltham, USA) in PBS with bovine serum albumin (PBS–BSA) and gently rotated for 35 min. The resulting Dynabead–antibody–DNA complex was subjected to eight washing steps with 100 µL PBS–BSA, using a magnetic particle concentrator (Dynal) to retain the complex after each wash. Finally, BrdU-labeled DNA was eluted by adding 25 µL of a 1.7 mM BrdU solution in PBS–BSA and incubating under slow rotation for 35 min. This purified BrdU-labeled DNA is referred to as "active DNA," representing the DNA of metabolically active microorganisms, as previously defined in the literature (Wahdan et al. 2021; Purahong et al. 2022; Reiß et al. 2024, 2025).

Quantitative PCR of soil DNA extracts

Quantitative PCR (qPCR) targeting "total DNA" was conducted to quantify fungal and bacterial gene copy numbers. For fungi, the internal transcribed spacer (ITS) region was amplified using the primer pair fITS7 (5'- GTGARTCATCGAATCTTTG-3') (Ihrmark et al. 2012) and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') (White et al. 1994). For bacteria, the 16S rRNA gene was targeted using primers BAC341f (5'-CCTACGGGNGGCWGCAG-3') and BAC758R (5'-GACTACHVGGGTATCTAAKCC-3') (Klindworth et al. 2013). Each qPCR reaction (20 µL total volume) contained iTaq Universal SYBR® Green Supermix (1×; Bio-Rad), forward and reverse primers (300 nM each), PCR Enhancer (1×; biotechrabbit, Berlin, Germany), and 1 µL template DNA. Each biological replicate was analyzed in technical triplicates using a 96-well plate format on the C1000 Touch thermocycler combined with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The cycling program consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, annealing at 52 °C (16S rRNA gene) or 52.7 °C (ITS) for 30 s, and extension at 60 °C for 30 s. Reactions were completed with a melt curve analysis from 65 °C to 95 °C, increasing by 0.5 °C every 5 s. Nuclease-free master mix blanks were included as negative controls in each run.

Gene copy numbers were determined by comparing sample cycle threshold (CT) values to standard curves generated from 10-fold serial dilutions of genomic DNA from *Escherichia coli* K12 (DSM 423) and *Fusarium solani* (DSM 1164). These standards were prepared using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Europe GmbH) following the manufacturer's protocol. For *E. coli*, DNA standards ranged from 1×10^9 to 1×10^4 gene copies per reaction, while for *F. solani*, the range was from 1×10^7 to 1×10^2 gene copies per reaction.

Amplicon sequencing of the soil Microbiome

Microbial communities from both "total DNA" and "active DNA" fractions were characterized through 16S rRNA gene and ITS region amplicon sequencing using the Illumina MiSeq platform, following the protocol described by Weißbecker et al. (2020). For bacterial community profiling, the

V3–V4 region of the 16S rRNA gene was amplified using the universal primer pair BAC341f and BAC758R (Klindworth et al. 2013), both modified with Illumina adapter sequences. Fungal communities were assessed by targeting the ITS2 region using the universal primer pair fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1994), also including Illumina adapter overhangs.

PCR reactions were performed in 20 µL volumes using 5× HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia) on ABI Veriti thermo cyclers (Applied Biosystems, Carlsbad, CA, USA). For bacterial amplification, the cycling conditions included an initial denaturation at 95 °C for 15 min, followed by 30 cycles of 95 °C for 20 s, 55 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 7 min and a hold at 4 °C. Fungal PCR was performed with the same initial denaturation (95 °C for 15 min), followed by 30 cycles of 95 °C for 20 s, 54 °C for 40 s, and 72 °C for 1 min, with a final extension of 72 °C for 10 min.

Amplification products were checked via gel electrophoresis. For each sample, three PCR replicates were pooled in equimolar amounts and purified using the Agencourt AMPure XP kit (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT indices were added to the fungal amplicons. The final libraries were subjected to paired-end sequencing (2 × 300 bp) using the MiSeq Reagent Kit v3 on an Illumina MiSeq system (Illumina Inc., San Diego, CA, USA) at the Department of Soil Ecology, Helmholtz Centre for Environmental Research, Germany.

All bacterial 16S rRNA and fungal ITS gene sequences from both total and active microbial communities were deposited in the NCBI nucleotide sequence database under BioProject accession number PRJNA1199236.

Bioinformatics

Raw data preprocessing, including demultiplexing, sorting, adapter trimming, and merging of reads, was performed using Illumina's bcl2fastq conversion software v2.20 alongside BBMerge v34.48 (Bushnell et al. 2017). The quality of the sequences was assessed with FastQC version 0.11.8 (Babraham Bioinformatics 2020). Subsequent sequence preprocessing and Operational Taxonomic Units (OTUs) identification from amplicons were carried out using Mothur 1.35.1 (Schloss et al. 2009). Sequences were aligned to the 16S Mothur-Silva SEED r119 reference alignment, selecting those with Phred quality scores exceeding 33 (Whelan et al. 2019). To reduce sequencing errors and exclude short alignments, pre-clustering was applied, allowing a maximum of one nucleotide mismatch per 100 bases within each cluster. Chimeric sequences were identified and removed using the UCHIME algorithm (Edgar et al. 2011). Taxonomic classification was then conducted against the Silva reference database, excluding sequences from non-target domains prior to OTU picking. OTUs were clustered at 99% sequence identity and assigned taxonomic levels accordingly (Edgar 2018; Nilsson et al. 2019; Kõljalg et al. 2020). This procedure generated OTU tables for DNA samples, with fungal sequence counts ranging from 13,279 to 127,649 and bacterial counts from 13,652 to 70,945. Rarefaction was applied to ensure optimal Good's Coverage across samples, resulting in a rarefaction depth of 20,000 reads for bacteria (95% Good's Coverage) and 45,000 reads for fungi (99% Good's Coverage).

We acknowledge that amplicon sequence variants (ASVs), generated via denoising algorithms in platforms such as QIIME 2 or USEARCH 12, offer higher resolution and improved reproducibility compared to traditional OTUs. In the present study, however, we deliberately employed an OTU-based approach to maintain methodological consistency and ensure comparability with our previous work (Reiß et al. 2024, 2025). This practice remains common in current microbial ecology research (Shigyo et al. 2024; Yoon et al. 2024; Maslovska et al. 2025). To enhance taxonomic resolution and reduce potential biases associated with the conventional 97% clustering threshold, we applied a 99% identity threshold for OTU clustering. Previous studies have demonstrated that OTU-based analyses remain suitable for addressing large-scale ecological diversity questions (Joos et al. 2020; Jeske and Gallert 2022). For internal validation, we re-analyzed our prior dataset at the ASV-based methods. The results revealed only marginal differences in community composition and diversity indices. A Mantel test comparing ASV- and OTU-based abundance distance matrices yielded a high correlation ($r=0.9385$, $p=0.001$), indicating that overall community patterns were nearly identical. Based on these findings, we proceeded with 99% OTU clustering in the current study to maintain consistency across datasets generated under the same funding framework.

The ecological and metabolic functions of bacterial OTUs were inferred using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database v1.1 (Louca et al. 2016; Sansupa et al. 2021), which annotates functions based on literature from cultivable strains. For fungi, the FungalTraits database (Põlme et al. 2021), a specialized tool for functional prediction, was employed to classify fungal genera into ecological guilds irrespective of the sequencing methodology. Functional count tables for both bacterial and fungal communities were then generated for each DNA sample.

Statistics

Following taxonomically summarization at genus level, statistical analysis were performed. The Shapiro-Wilk test ($p < 0.05$), implemented in OriginPro 2024 (OriginLab Corporation, Northampton, MA, USA), was used to assess normality in each dataset. Rarefaction analysis and alpha diversity (OTU richness) estimation were carried out for all samples using RStudio (version 2024.04.2, RStudio, Inc., Boston, MA, USA) with the *vegan* package version 2.6–8.6 (Oksanen et al. 2025).

Normal distribution of alpha diversity indices was evaluated again using the Shapiro-Wilk test ($p < 0.05$). All subsequent statistical analyses were conducted in R (version 4.4.1, R Core Team). Linear models including interaction terms were used to examine the effects of Group, Time, and their interaction (Group × Time) on each response variable. Estimated marginal means were computed via the *emmeans* package version 1.10.6 (Lenth 2025), and pairwise comparisons were performed to detect statistically significant differences. The same modelling approach was applied to analyze the impact of biocide treatments on bacterial and fungal gene copy numbers.

Relative abundance data for each sample were calculated as described by Noll et al. (2005) and visualized using OriginPro (Version 2024, OriginLab Corporation). Differences in microbial community composition across facade eluate treatments were assessed using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities. This was conducted using the *metaMDS* function from the *vegan* package, with genus-level relative abundance data square-root transformed to minimize the dominance effect of abundant taxa. Permutational multivariate analysis of variance (PERMANOVA) was run with 999 permutations using the *adonis2* function to test for significant differences between treatments. Pairwise group comparisons were further explored through a custom pairwise PERMANOVA workflow, applying *adonis2* independently to each group combination.

To correct for multiple testing, resulting p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR). Group-level distinctions were visualized using the *multcompLetters* function from the *multcompView* package version 0.1–10 (Hothorn et al. 2008), assigning significance letters to indicate statistically similar or different groups at $\alpha = 0.05$.

Visual representations of statistical results were generated in OriginPro (OriginPro v.2024, OriginLab Corporation, Northampton, MA, USA). The *envfit* function from the *vegan* package was employed to evaluate the goodness-of-fit (R^2 values) of environmental variables fitted to the NMDS ordinations for both total and active bacterial and fungal communities, with significance based on 999 permutations (Oksanen et al. 2025).

Indicator species analysis was conducted to determine which bacterial and fungal genera were significantly linked to the UOF, RW or B treatments (see Fig. 1). This was performed using the *multipatt* function from the *indicspecies* package version 1.7.15 (Cáceres and Legendre 2009), with indicator values computed via the "r.g." method. To pinpoint genera uniquely present in specific sample groups, a presence/absence filter was applied. Genus-level count data were converted to long format and merged with sample metadata, linking each observation to its respective group. A genus was marked as "present" in a group if it appeared in at least one sample from that group. Genera exclusively found in the T0 or RW groups and absent in all other groups were classified as group specific. This analysis was carried out in R (version 4.4.1) using the *dplyr* (Wickham et al. 2023), *tidyr* (Wickham et al. 2024b), and *readr* (Wickham et al. 2024a) packages.

Natural weathering experiment

The natural weathering experiment was previously described by Kiefer et al. (2024). Briefly, the facade eluates were sampled from concrete L-stones on which render and paint samples were applied and placed in the vicinity of Coburg, Germany. In this study, facade eluates were retrieved from surfaces without biocides, containing neither in-can nor film preservatives (B), and samples containing both in-can and film preservatives (UOF) in the render and paint layer as introduced earlier (Kiefer et al. 2024). The in-can preservative ACTICIDE SR 2081 (Thor GmbH, Speyer, Germany) consists of Benzisothiazolinone, Chloromethylisothiazolinone, and Methylisothiazolinone. The film perseverative ACTICIDE MKB3 (Thor GmbH, Speyer, Germany) contains Terbutryn, Octhilinone, Zinc pyrithione, and Zinc oxide. Both preservatives were added to the formulations of UOF facade samples as follows: 350 ppm within the render formulation and 500 ppm within the paint formulation. Each UOF and B facade eluates were sampled from three independent L-stone surfaces with south-east (weathering site) and north-west orientation (non-weathering site), respectively. Following each rainfall event, defined as a period of at least 12 h without any forecasted precipitation, the corresponding facade runoff was collected, and the volume of the runoff was measured. The collected eluates were then filtered (MN 615, 4–12 µm, cellulose, Marchery-Nagel, Düren, Germany), their pH levels recorded, and the samples were stored at −20 °C until further use in soil treatments. As only a small proportion of total rainfall contributes to biocide-containing facade runoff, a dilution factor of 5.19 was applied. The dilution factor was calculated based on the first three rainfall events from the natural weathering experiment, using the soil-to-facade ratio of 0.1 to 0.4 as described for a standard building by ECHA for product type 10 (ECHA website 2022). The runoff factor for each rain event was calculated accordingly. Further details regarding the calculation of the dilution factor can be found in Kiefer et al. (2024).

Results

Incubation of soils with multiple applications of eluates and rainwater alters soil physicochemical properties compared to the untreated soil

Analysis of soil physicochemical properties revealed that both the method of incubation and type of treatment significantly influenced soil properties, regardless of the specific treatment applied (Table S1). Notably, significant differences were observed between T0 and all incubated soil samples (Table S2), as well as across different sampling dates. For soils treated with UOF eluates, significant changes in pH, NO_3^-, and SO_4^{2-} were observed between sampling dates (p_{pH} = 0.001; p_{NO3-}= 9.21·10^{-6}; p_{SO4} = 0.0001) and compared to T0 (p_{pH} = 6.6 × 10^{-6}; p_{NO3-} = 1.0 × 10^{-13}; p_{SO4} = 1.0 × 10^{-13}). Similarly, soils treated with B eluates showed significant temporal changes in F^- (p=0.013), Cl^- (p=0.01), NO_3^- (p=1.0 × 10^{-15}), and PO_4^{3-} concentration (p=0.009), as well as significant differences from T0 (p_{F-} = 1.0 × 10^{-7}; p_{Cl-} = 1.0 × 10^{-13}; p_{NO3^-} = 1.0 × 10^{-13}). Control soils treated with rainwater also exhibited significant changes over time in Cl^- (p=0.002), NO_3^- (p=8.2 × 10^{-11}), and PO_4^{3-} concentration (p=0.006), as well as significant deviations from T0 (p_{pH} = 1.0 × 10^{-9}; p_{F-} = 4.92 × 10^{-4}; p_{Cl-} = 1.0 × 10^{-13}; p_{NO3^-} = 1.0 × 10^{-13}; p_{SO4} = 1.0 × 10^{-13}) (Table S2).

Multiple treatment of facade eluates leads to a reduction of fungal and bacterial gene copy numbers over time

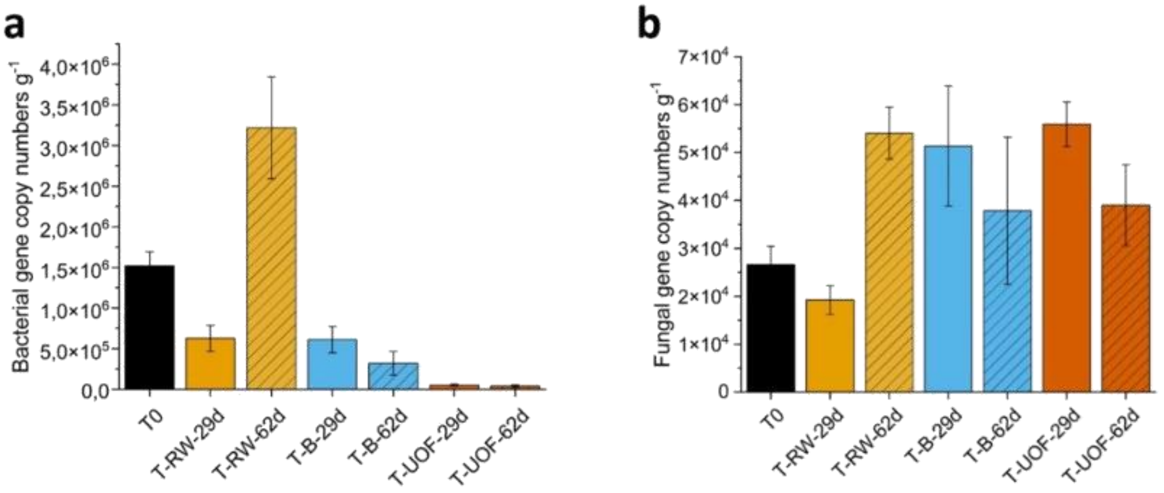
A multi-way ANOVA revealed significant effects on treatment, time, and their interaction on total bacterial (Fig. 2a). Highest bacterial gene copy numbers were observed in T-RW-62d (3.22 × 10^6 g^{-1}) and lowest at T-UOF-62d (3.8 × 10^5 g^{-1}). At the 29 d sampling point T-UOF-29d had significantly lower bacterial gene copy numbers than T0 treatment (1.52 × 10^6 g^{-1}) (p=0.002). Bacterial gene copy numbers of T-RW increased significantly from 29 days (6.26 × 10^5 g^{-1}) to 62 days (p=0.01), whereas no significant temporal change was detected in other facade eluate treatments (p>0.05). At 62 day sampling point, all treatments differed significantly from T0. T-RW-62d was significantly higher than T0 (p=0.0003), T-B-62d (p=8.56 × 10^{-7}) and T-UOF-62d (p=7.37 × 10^{-9}).

For fungal gene copy numbers, the multi-way ANOVA also indicated significant effects of treatment, time, and their interaction (Fig. 2b). Highest fungal gene copy numbers were recorded in T-UOF-29d (5.59 × 10^4 g^{-1}) and the lowest at T-RW-29d (1,92 × 10^4 g^{-1}). At 29 days, T-RW-29d was significantly lower than T-B-29d (5.14 × 10^4 g^{-1}) (p=0.048) and T-UOF-29d (p=0.019). A significant temporal increase was observed in T-RW from 29 to 62 days (5.4 × 10^4·g^{-1}, p=0.005).

Alteration of bacterial and fungal alpha diversity over time and with facade eluate treatment

Total bacterial richness (Fig. 3a) was significantly influenced by treatment, time, and their interaction (Table S3). Facade eluate treatment T-B-29d (496.0 ± 9.5) and T-UOF-29d (509.8 ± 8.0) showed reduced richness relative to controls and differed significantly from each other (p=0.018). No significant temporal increase except for a slight rise in T-B (to 512.0 ± 8.7) and T-UOF (to 512.8 ± 8.5) at 62 d was observed. Active bacterial richness values were markedly lower across all treatments and showed no significant differences (Fig. S1, Table S3). A-RW-29d increased from 247.6 ± 8.8 to 265.6 ± 9.8 (A-RW-62d) over the incubation time. Facade eluate treated soils remained lowest active bacterial richness overall (A-B-29d: 238.0 ± 12.9; A-UOF-29d: 256.8 ± 14.9), with only minimal recovery at 62 d (A-B-62d: 256.8 ± 7.8; A-UOF-62d: 246.6 ± 12.1). Patterns in ACE index mirrored richness trends, with T0 and RW maintaining the highest values (ACE = 583.6–624.1), while ACE index of façade eluate treated soils — especially active bacterial alpha diversity — remained significantly reduced (ACE = 272.6–308.9) (Table S3). Shannon and Simpson diversity indices followed the same structure: T0 and RW exhibited higher diversity (Shannon ≈ 4.69–4.78; Simpson ≈ 0.75–0.77) than soils treated with facade eluates, whose active indices often fell below Shannon = 2.5 and Simpson = 0.46, particularly in A-B-29d and A-UOF-29d (Table S3). Evenness was consistently reduced in active bacteria (≈0.30–0.46) compared to total communities (≈0.75–0.77), with A-UOF-29d showing the lowest bacterial evenness (0.37 ± 0.02) and differed significantly from the respective water control (p=0.03) (Table S3).

Fungal richness (Fig. 3b) displayed similar treatment effects (Table S4). In the total community, untreated soils (T0) retained high richness (168.2 ± 2.8 at 29 d) and increased significantly with rainwater treatment over time (T-RW-62d: 243.4 ± 11.0; p_{T0} = 5.14 × 10^{-5}; $p_{T\text{-}RW\text{-}29d}$ = 1.93 × 10^{-6}). Additionally, T-B-29d and T-UOF-29d were significantly different from T0 ($p_{T\text{-}B\text{-}29d}$ = 2.62 × 10^{-6};



Time-point [d]	T0	T-RW-28 d	T-RW-62 d	T-B-28 d	T-B-62 d	T-UOF-28 d	T-UOF-62 d
28 d	a	ab		ab		b	
62 d	a		b		c		c
28 + 62 d		***					

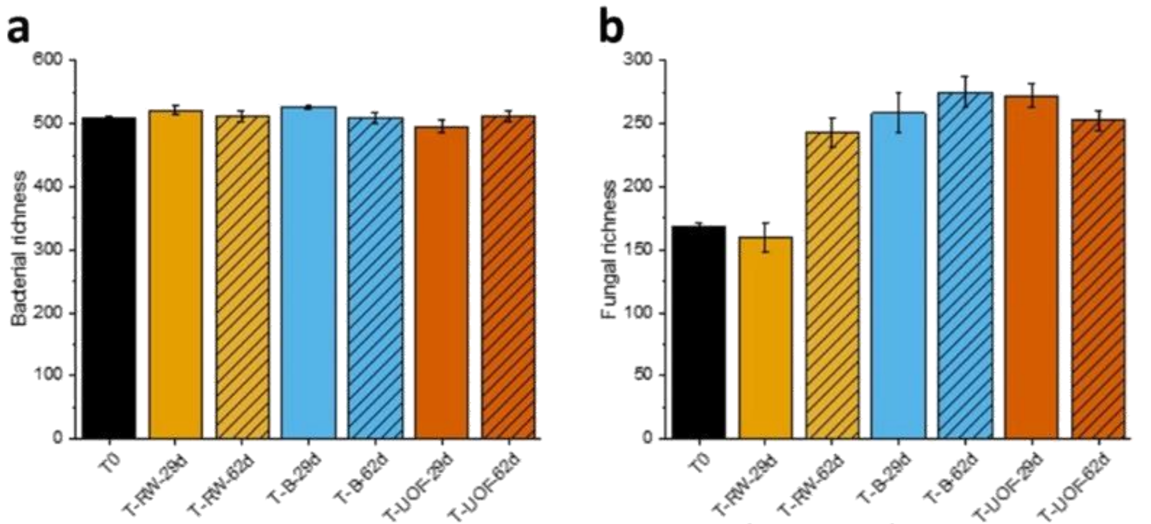
Time-point [d]	T0	T-RW-28 d	T-RW-62 d	T-B-28 d	T-B-62 d	T-UOF-28 d	T-UOF-62 d
28 d	a	b		a		a	
62 d	a		a		a		a
28 + 62 d		***					

Fig. 2 Gene copy numbers of total (prefix T-) bacteria (**a**) and fungi (**b**) after facade eluate treatment ($n=5$). Statistically significant differences were tested using linear models with interaction terms (Group × Time), followed by estimated marginal means with pairwise comparisons. ($p<0.05$; $n=5$). Significant differences are indicated by different letters for within the same time points and by asterisks (*) between time points. Untreated soil (T0); soil treated with rainwater for 29 days (T-RW-29d) or 62 days (T-RW-62d); soil treated with facade eluates without biocides for 29 days (T-B-29d) or 62 days (T-B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (T-UOF-29d) or 62 days (T-UOF-62d)

$p_{T\text{-}UOF\text{-}29d}$ = 1.8×10^{-7}) and the respective water control ($p_{T\text{-}B\text{-}29d}$ = 5.16×10^{-7}; $p_{T\text{-}UOF\text{-}29d}$ = 3.72×10^{-8}). This was also observed for the latter sampling point at 62 days. Fungal richness of T-UOF-62d (252.6 ± 8.4) and T-B-62d (275.2 ± 12.4), were significantly higher than T0 ($p_{T\text{-}B\text{-}62d}$ = 1.01×10^{-7}; $p_{T\text{-}UOF\text{-}62d}$ = 8.72×10^{-6}). The differences observed between T-RW-62d and T-UOF-62d remained significant ($p=5.79\times10^{-5}$). Active fungal richness was reduced (48.6–56.0 at 29 d), with only slight increases in rainwater control treatments by 62 days (e.g., A-RW-62d: 51.4 ± 5.6) (Table S3). ACE values confirmed this reduction, especially for facade eluate treatments (e.g., A-UOF-62d: 82.2 ± 4.3) (Table S4). Shannon and Simpson values in active fractions were much lower (Shannon ≈ 1.2–2.1; Simpson ≈ 0.30–0.52) than in total communities (Shannon ≈ 3.9–4.07; Simpson ≈ 0.71–0.78). Evenness remained highest in T0 and RW total communities (≈0.72–0.78) and lowest in facade eluate active communities, with A-B-62d (0.30 ± 0.02) and A-UOF-62d (0.31 ± 0.03) showing the most pronounced declines (Table S4). This decline was also observed for the Shannon and Simpson indices and was significant compared to A-RW-62d ($p<0.002$). Facade eluate treatments reduced bacterial and fungal alpha diversity, with active fractions showing the strongest declines in richness, evenness, and Shannon and Simpson indices. Total communities were less affected, while rainwater controls maintained high diversity and evenness over time, highlighting the sensitivity of active microbial communities to treatment-induced shifts.

Incubation time and facade eluate treatment altered active bacterial and fungal community composition

Multiple treatments with facade eluates over time altered both bacterial and fungal community compositions (Fig. 4). Bray–Curtis dissimilarity analysis confirmed that



Time - point [d]	T0	T-RW-28 d	T-RW-62 d	T-B-28 d	T-B-62 d	T-UOF-28 d	T-UOF-62 d
28 d	ab	ab		a		b	
62 d	a		a		a		a
28+ 62 d							

Time - point [d]	T0	T-RW-28 d	T-RW-62 d	T-B-28 d	T-B-62 d	T-UOF-28 d	T-UOF-62 d
28 d	a	a		b		b	
62 d	a		b		bc		c
28+ 62 d		***					

Fig. 3 OTU richness of total (prefix T-) bacterial (**a**) and fungal (**b**) community after respective façcade eluate treatment ($n = 5$). Statistically significant differences were tested using linear models with interaction terms (Group × Time), followed by estimated marginal means with pairwise comparisons ($p < 0.05$; $n = 5$). Significant differences are indicated by different letters within the same time points and by asterisks (*) between time points. Untreated soil (T0); soil treated with rainwater for 29 days (T-RW-29d) or 62 days (T-RW-62d); soil treated with facade eluates without biocides for 29 days (T-B-29d) or 62 days (T-B-62d), soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (T-UOF-29d) or 62 days (T-UOF-62d). OTU richness of the active bacterial and fungal community can be found in the supplementary Fig. S1

communities from treated soils diverged significantly from untreated controls (T0) (Fig. 4a, c). NMDS ordination further revealed that total communities were relatively stable and tightly clustered (Fig. 4b, d).

For the bacterial community composition, significant treatment and time effects were detected (Table S5). T-RW-29d differed significantly from T-B-29d ($p = 0.007$) and T-UOF-29d ($p = 0.027$). After 62 days, T-B-62d separated from both T-RW-62 d ($p = 0.019$) and T-UOF-62d ($p = 0.048$). Temporal changes were also evident, with most treatments differing significantly from their earlier sampling points (Fig. 4a, Table S5). Active bacterial communities showed even stronger divergence. A-B-29d and A-B-62d differed from nearly all other active treatments significantly, including A-RW-29d ($p = 0.008$), A-RW-62d ($p = 0.009$), A-UOF-29d ($p = 0.045$), A-UOF-62d ($p = 0.035$), as well as from each other ($p = 0.004$). Compositinally, active bacterial communities were dominated by *Pseudoalteromonas* (>50% relative sequence read abundances), followed by members of the order Micrococcales. NMDS ordination reflected these patterns: while total bacterial communities clustered tightly, active communities spread more widely, with A-B-29d and A-B-62d positioned apart along the NMDS2 axis (Fig. 4b).

For the fungal community composition, facade eluate treatments produced even more pronounced compositional changes (Fig. 4c**)**. At 29 days, T-RW-29d fungal community composition differed significantly from T-B-29d ($p = 0.009$) and T-UOF-29d ($p = 0.014$). By 62 day, significant temporal shifts were observed within T-RW ($p = 0.011$) and T-UOF- ($p = 0.026$), while T-B-62d differed significantly from T-RW-62d ($p = 0.028$) and T-UOF-62d ($p = 0.030$). Fungal communities were dominated by *Mortierella*, *Gibellulopsis*, and *Chlorosacinopsis* in both total and active, though many

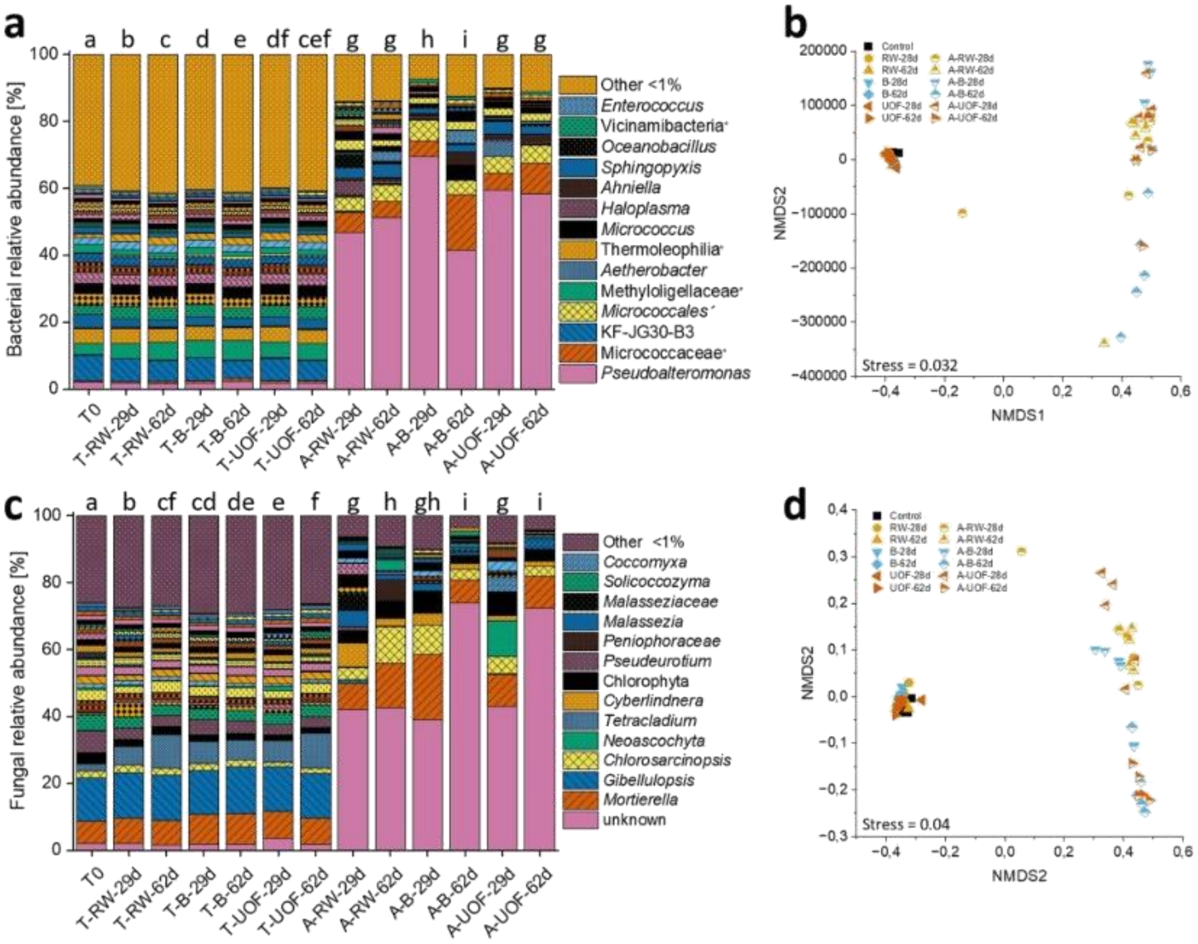


Fig. 4 Bacterial (**a**) and fungal community composition on genus level (**c**) after facade eluate treatments ($n=5$). The legend highlights fungal and bacterial genera with a > 1% relative abundance. Different letters indicate statistically significant differences between groups based on pairwise PERMANOVA (Adonis) with a significance threshold of $p<0.05$. Detailed information regarding the data and the p-values can be found in Tables S1 and S2. The corresponding NMDS plots show the Bray-Curtis dissimilarities for bacterial (stress = 0.03) (**b**) and fungal community compositions (stress = 0.04) (**d**) across replicates. Prefix T- Total samples; Prefix A- Active samples; untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with facade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d)

taxa in the active fungal communities remained unidentified (Fig. 4c).

Comparisons between total and active communities showed consistent significant differences across all treatments (Fig. 4c, d; Table S6). Active fungal communities displayed particularly strong response to facade eluate. After 29 days, no significant differences in active fungal communities were observed across treatments. However, by 62 days, A-RW-62d differed significantly from both A-B-62d ($p=0.013$) and A-UOF-62d ($p=0.007$). Temporal changes were also observed for A-RW ($p=0.040$) and A-UOF ($p=0.015$). NMDS ordination revealed clustering of all active fungal communities at 29 d, whereas by 62 d, intra-group variation increased with A-B-62d and A-UOF-62d forming a distinct cluster in the lower right quadrant of the ordination plot (Fig. 4**d)**. Overall, facade eluate treatments induced stronger compositional shifts in fungal than bacterial communities, with active fractions responding more dynamically than total communities.

Multiple facade eluate treatments alter soil microbial functions

Facade eluate treatments significantly altered both bacterial and fungal functional composition compared to the untreated soil (T0) (Fig. 5). Across all communities, the

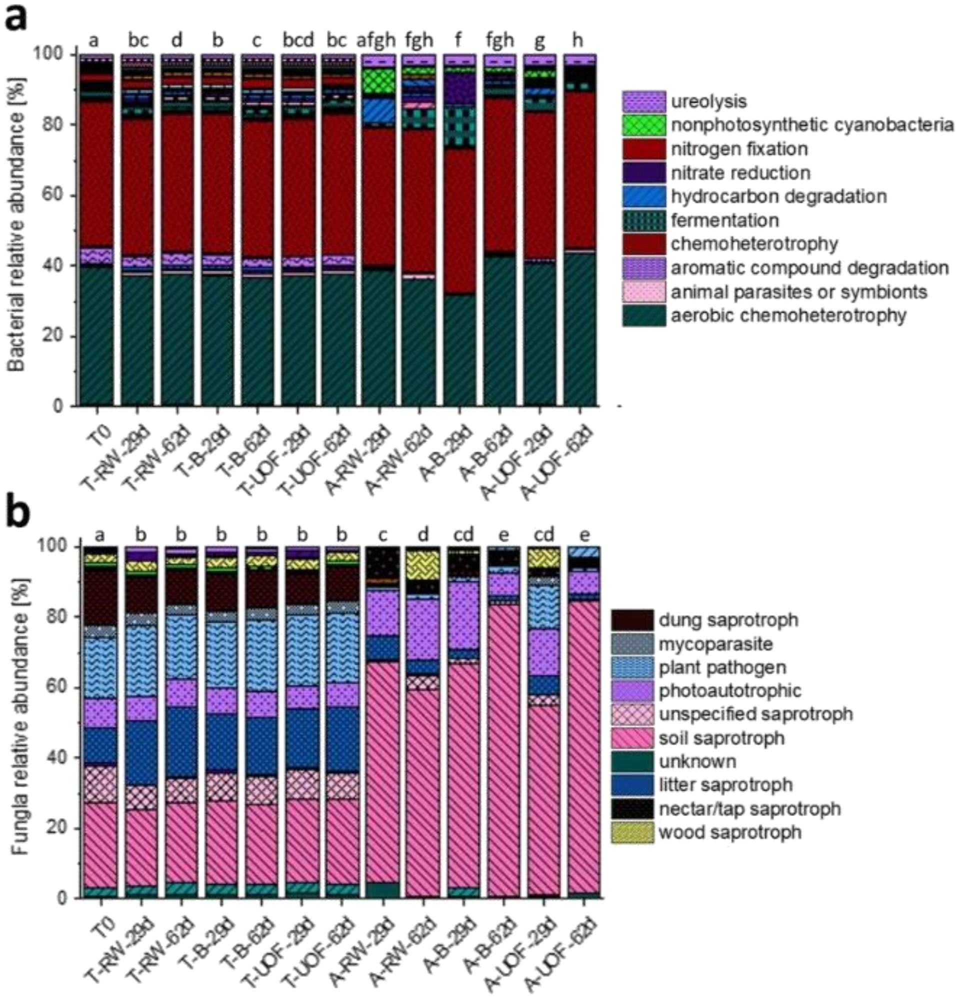


Fig. 5 Bacterial function composition on OTU Level (**a**) and fungal function composition on genus level (**b**) after biocide treatments ($n = 5$). Short legend highlights the top 10 of the bacterial and fungal most abundant functions. A complete legend, including an indication of all functions, can be found in Fig. S2. Different letters indicate statistically significant differences between groups based on pairwise PERMANOVA (Adonis) with a significance threshold of $p < 0.05$. Total samples (Prefix T-); active samples (Prefix A-); untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with facade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d)

dominant predicted bacterial functions were aerobic chemoheterotrophs and chemoheterotrophs, while fungal functions were primarily saprotrophy and plant pathogenicity.

For the bacterial functional community composition, treatment and time effects were detected (Table S5). In total communities no significant differences were detected among treatments at 29 days (Fig. 5a; Table S5). However, by day 62, T-RW-62d differed significantly from T-B-62d ($p = 0.010$) and T-UOF-62d ($p = 0.021$). Temporal shifts were also evident for T-RW ($p = 0.014$) and between T-B ($p = 0.018$). In the active bacterial communities, functional profiles diverged more strongly. Aromatic compound degradation function was absent, whereas ureolysis was enriched. In A-RW-29d, non-photosynthetic cyanobacteria and members capable of hydrocarbon degradation gained prominence, whereas nitrate reduction and fermentation were dominant in A-B-29d. Significant differences were observed between A-B-29d and A-UOF-29d ($p = 0.028$). Notably, A-UOF was the only group showing significant temporal change between 29 and 62 days ($p = 0.005$) (Fig. 5a).

The total fungal functional community composition was primarily composed of soil and litter saprotrophs and plant pathogens (Fig. 5b). Although all treatments differed significantly from the untreated soil (T0), no significant temporal changes were detected between 29 and 62 days (Fig. 5b, Table S6). In contrast, the active fungal functional communities, soil saprotrophs were dominant, followed by photoautotrophs, whereas plant pathogens were nearly absent. At 29 days, no treatment effects were detected. However, by 62 days, A-RW-62d differed significantly from A-B-62d ($p=0.012$) and A-UOF-62d ($p=0.006$). Additionally, all active fungal functional communities exhibited significant temporal changes relative to their earlier timepoints (Fig. 5b, Table S6).

In summary, facade eluate exposure altered functional composition in both bacteria and fungi, but active fractions were more dynamic than total communities. While total bacterial and fungal functions remained relatively stable, active fractions displayed pronounced shifts over time, with bacterial functions primarily affected by UOF eluates and fungal functions by both B and UOF eluates.

Microbial indicator taxa and selective omission under multiple facade eluate treatments

Indicator analysis identified distinct bacterial and fungal taxa associated with facade eluate treatments (Fig. 6, Fig. S3). In total, 64 out of 1,092 bacterial and 42 out of 425 fungal taxa were significantly correlated with a single treatment. Bacterial indicators were predominantly classified as chemotrophs, whereas fungal indicators were mainly saprotrophs (Table S7). For bacterial community, indicator taxa were more prevalent in the active than in the total communities. The highest number of indicator taxa was observed in A-RW-29d (17 taxa), followed by A-B-62d (7 taxa) (Fig. 6a). In both A-RW-29d (17) and A-RW-62d (5), the indicators spanned a broad phylogenetic range. *Pseudoalteromonas* dominated A-RW-29d in sequence abundance (46.66%, Stat$=$0.505, $p=0.0005$), while *Rhizorhapis* showed the strongest correlation (Stat$=$0.684, $p=0.0016$). In A-RW-62d, unclassified Silvanigrellaceae and Cytophagales emerged as dominant indicators (Stat$=$0.590 and 0.645, respectively). In contrast, facade eluate treatments yielded fewer indicators: A-B-29d (2 taxa), A-B-62d (7), A-UOF-29d (2), and A-UOF-62d (4), with the absence of Alteromonadales, Firmicutes, Elusimicrobiota, and Cyanobacteria (Fig. 6a). For A-B-29d, *Comamonas* was the most abundant indicator (0.031%, Stat$=$0.566, $p=0.0077$), while unclassified *Kineosporiaceae* showed the strongest correlation (Stat$=$0.736, $p=0.0002$). By 62 days, uncultured *Devosiaceae* (Stat$=$0.907, $p=0.0001$) and unclassified *Micrococcales* (4.21%; Stat$=$0.893, $p=0.0001$) characterized A-B-62d. For UOF treatments, *Demequina* (Stat$=$0.717, $p=0.0003$) and *Janthinobacterium* (Stat$=$0.614, $p=0.0002$) were significant indicators at 29 days, while *Glutamicibacter* (Stat$=$0.746, $p=0.0002$), *Paracoccus* (Stat$=$0.635; $p=0.0001$), and *Taibaiella* (Stat$=$0.628 $p=0.0005$) were characteristic for A-UOF-62d (Fig. 6a; Table S7).

For the fungal community, indicator taxa were more numerous in the total than in the active fraction (Fig. S3b). The strongest correlations occurred at T0 (8 taxa), followed by T-UOF-62d (7), and T-UOF-29d (6). In the active fungal community, most indicators were identified in soils treated with rainwater: *Saccharomyces* (Stat$=$0.669, $p=0.0035$) and unclassified *GS02* (Stat$=$0.557, $p=0.0001$) in A-RW-29d, and *Taxus* (Stat$=$0.684, $p=0.0006$) in A-RW-62d. For facade eluate treatments, *Chlorococcum* (Stat$=$0.488, $p=0.0436$) was characteristic of A-B-29d, while *Rhizophydium* (Stat$=$0.748, $p=0.0001$) and unclassified *Rhizophydiales* (Stat$=$0.778, $p=0.0002$) were strong indicators in A-B-62d. In A-UOF-29d, *Operculomyces* (Stat$=$0.503, $p=0.0401$) and *Trebuxia* (Stat$=$0.516, $p=0.0263$) were significant indicators, whereas A-UOF-62d was dominated by Eukaryota kgd *incertae sedis*, the strongest fungal indicator overall (Stat$=$0.933, $p=0.0001$) (Fig. S3b; Table S7).

Overall, active fractions revealed treatment- and time-specific indicator taxa more clearly than total communities. Rainwater treatments promoted broad phylogenetic responses among bacteria and fungi, whereas facade eluates were associated with a narrower set of bacterial taxa and distinct fungal saprotrophs and chytrids.

Omission of bacterial and fungal genera after repeated treatment with facade eluates

Several bacterial and fungal taxa were absent in facade eluate-treated soils and occurred exclusively in untreated (T0) or rainwater-treated (RW) samples (Table S8**).** In total, 29 bacterial and nine fungal taxa were omitted after incubation with facade eluates but persisted under rainwater treatment.

In the total bacterial community, T0 soil contained unique taxa such as *Anabaena PCC-7122*, *Nodularia PCC-9350*, and *mle1-8*, along with unclassified Bacteroidota, Geobacteraceae, and Thermomicrobiales. RW-treated soils (29 d and 62 d) harbored distinct genera including *Azospira*, *Cellvibrio*, *Dojkabacteria*, and *Listeria*, as well as unclassified Acidobacteriota and Desulfotomaculales. In the fungal community, T0 soils contained only unclassified members of RW-treated soils, by contrast, included diverse genera such as *Arrhenia*, *Dioszegia*, *Liberomyces*, *Pochonia*, and *Rhizophlyctis*, in addition to unclassified Botryosphaeriaceae. Within the active bacterial community of RW-treated soils, the highest number of exclusive bacterial taxa was observed, including *Alicyclobacillus*, *Asticcacaulis*, *Dietzia*, *Gordonia*, *Idiomarina*, and *Syntrophobacter*. Additional unclassified members, such as Neisseriaceae,

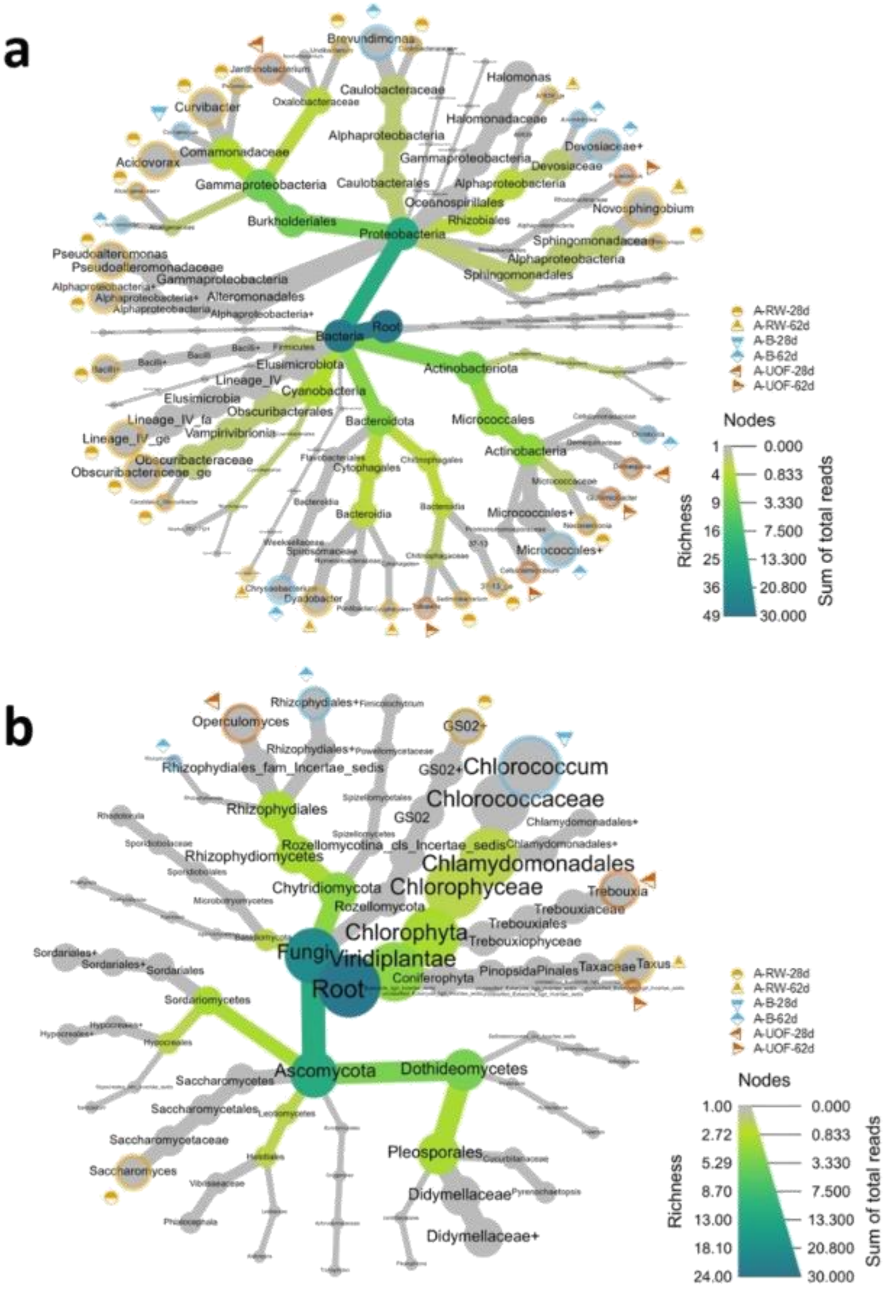


Fig. 6 Heat tree representation of the active bacterial (**a**) and fungal (**b**) indicator taxa of the respective treatment indicated by symbols and colored frame of the indicator nodes. The color code resembles the richness, and the node size of the respective sequence read abundances. Unclassified members of the taxon are marked with +. Untreated soil (T0); soil treated with rainwater for 29 days (A-RW-29d) or 62 days (A-RW-62d); soil treated with facade eluates without biocides for 29 days (A-B-29d) or 62 days (A-B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (A-UOF-29d) or 62 days (A-UOF-62d)

Saccharimonadaceae, Silvanigrellaceae, and uncultured Rhodospirillaceae, were also restricted to rainwater treatements. For the active fungal community, *Thelephora* and unclassified members of the family Ulmaceae were unique to RW-treated soils (Table S8).

Overall, facade eluates suppressed several bacterial and fungal taxa that persisted under rainwater incubation, with the strongest omission effects observed in the active fractions.

Discussion

This study demonstrates that facade eluates exert strong and selective effects on soil microbial communities. Despite significant temporal shifts in soil physico-chemical properties, no significant differences were observed between the treatments at individual sampling points. Therefore, the differences that occur at the respective sampling times can be attributed to the presence of facade eluates. Bacterial abundances declined consistently in facade-treated soils, while fungi were less affected or even increased, suggesting differential sensitivity of soil microbial groups to facade-derived compounds. Repeated applications revealed that bacterial recovery was impaired, particularly in biocide-containing eluates, whereas fungi remained relatively resilient.

Repeated treatment of soils with facade eluates increased fungal gene copy numbers

Repeated treatment with facade eluates led to a reduction in total bacterial gene copy numbers over time compared to the untreated soil T0 (Fig. 2). Consistent with previous findings, this decrease was observed in soils treated with both types of facade eluates, regardless of whether the formulation contained biocides (Reiß et al. 2025). Prolonged incubation with repeated facade eluate applications showed that the bacterial community did not recover in terms of gene copy numbers; instead, it continued to decline. In contrast, bacterial gene copy numbers in the rainwater control after 62 days (T-RW-62d) showed signs of recovery compared to earlier sampling point after 29 days and façade-treated soils. This suggests that the observed bacterial decline was primarily caused by the façade eluates, particularly those containing biocides (UOF), rather than by incubation conditions. Although the preservative mixtures ACTICIDE SR 2081 and ACTICIDE MKB3 (Thor GmbH) used in this study are targeted toward, fungi and algae rather than bacteria façade eluates still had a stronger effect on bacteria than fungi. The stronger bacterial response may be attributed to non-target effects of façade ingredients. Zinc pyrithione, a component of ACTICIDE MKB3, has been shown to reduce bacterial DNA content in sediments (Groth Petersen et al. 2004). Moreover, the loss of bacterial gene copy numbers may not be solely attributed to biocides, but also to other components in render and paint formulations, such as heavy metals, fillers, binders, and pigments. Zinc oxide, frequently used as an additive in facades, is known to exert toxic effects on soil bacteria as it can inhibit soil microbial biomass and enzyme activities (Abdelmeged et al. 2023; Strekalovskaya et al. 2024). In contrast to bacteria, fungal gene copy numbers increased over time with repeated facade eluate exposure, compared to T0 and T-RW-29d (Fig. 2b). After 62 days, fungal gene copy numbers were not significantly affected by facade eluate treatments, indicating that repeated exposure did not negatively impact soil fungal gene copy numbers (Reiß et al. 2025). In contrast, fungal communities appear more tolerant, possibly due to physiological adaptations, functional redundancy, or greater resistance to stress compounds. A limitation of this study is that the non-sterile rainwater may have introduced microbial inocula, particularly fungal spores that disperse more readily than bacteria (Tipton et al. 2019); however, as all treatments received the same rainwater, relative comparisons among treatments remain valid. While qPCR detects DNA rather than exclusively viable cells, potentially including extracellular DNA or remnants of dead organisms, this limitation is unlikely to alter the observed treatment-driven trends. In line with previous studies using RefeSol-02 A, gene copy numbers were relatively low compared. Therefore, future studies should comprise soil types with contrasting microbial biomass levels for comparison. These contrasting trajectories suggest that facade eluates act as ecological filters selectively altering the microbial balance in soils.

Treatment-induced changes in soil microbial alpha diversity

Consistent with the single-application experiment, active bacterial and fungal richness was lower than total richness, and differences between active communities and the rainwater control were not significant (Reiß et al. 2025). In contrast, repeated treatment with UOF eluates reduced total fungal richness compared to the rainwater control, indicating that prolonged exposure exerts selective pressure on fungal communities. Bacterial richness, however, recovered by 62 days, likely due to the rapid leaching of in-can preservatives and the thereby lower biocide concentration in later facade eluate treatments (Kiefer et al. 2024). Eluates and rainwater were not sterile filtered, so observed richness increases may partly stem from external microbial inputs. Surviving taxa may also have benefited from resources released by the death of sensitive microbes. Interestingly, facade eluates tended to increase microbial richness rather than reduce it, a pattern consistent with previous biocide-related Refesoil-02 A studies (Reiß et al. 2024, 2025) and even observed in specialized fungal assemblages such as those on honeybees (Reiß et al. 2023). This suggests that the complex mixture of stressors in facade eluates promotes community diversification, preventing the typical richness decline expected under toxic stress.

Diversity indices further revealed lower evenness in eluate-treated soils, showing that community structure shifted toward dominance by a few tolerant species rather than outright species loss. Similar effects of biocides and heavy metals on microbial evenness and alpha diversity have been reported in aquatic and soil systems (Alhajjar et al. 2021; Signorini et al. 2023). These findings highlight that facade eluates reshape microbial community balance through altered dominance patterns, with potential consequences for soil ecosystem functioning.

Temporal shifts in soil microbial communities induced by facade eluate treatments

Prolonged incubation with facade eluates significantly altered both the active and total bacterial and fungal community compositions. While total communities displayed low intra-group variation, the active communities revealed more pronounced treatment effects emphasizing that metabolically active taxa are more responsive indicators of stress. This is consistent with our earlier findings that eluate composition and exposure regime jointly influence soil microbial community (Reiß et al. 2025).

Repeated applications of biocide-containing eluates in particular led to shifts in both bacterial and fungal community composition over time (Fig. 4). The enrichment of *Pseudoalteromonas* in active bacterial communities underscores their capacity to dominate under persistent stress, supported by their generic potential for hydrocarbon degradation and resistance to heavy metals (Navarro-Torre et al. 2017; Zan et al. 2021). Similarly, changes in the relative abundance of fungal taxa highlighting contrasting resilience: *Mortierella* persisted under exposure due to its pollutant-degrading capabilities (Ellegaard-Jensen et al. 2013; Wang et al. 2022), whereas sensitive genera such as *Gibellulopsis* declined (Lin et al. 2025). The rise of *Chlorosacinopsis*, a microalgae with known stress tolerance, suggests that eluates create selective conditions favoring *Chlorosacinopsis* due to its capability to tolerate harsh conditions and lower susceptibility to elevated zinc levels (Vasistha et al. 2021).

Functional profiles reflected these compositional changes but with a delayed response (Fig. 5). Consistent with previous findings, bacterial functional composition remained largely unaffected by facade eluates after 29 days of repeated treatment (Reiß et al. 2024, 2025). Functional profiling revealed that bacterial communities largely retained core functions aerobic heterotrophy and chemotrophy, though significant shifts emerged after prolonged exposure likely due to cumulative biocide input, toxic degradation products, or microbial adaption (Arias-Estévez et al. 2008; Kumar et al. 2012; Bollmann et al. 2017; Elekhnawy et al. 2020). These findings suggest that prolonged exposure and repeated application of facade runoff can alter total bacterial functional capacities over time. Elevated ureolysis in active bacteria indicates an adaptive stress response, enabling pH buffering and nitrogen salvage under chemically altered soil conditions (Mobley and Hausinger 1989).

Fungal functional responses were more dynamic than bacterial ones. Although total fungal functions remained stable, active communities diverged after extended treatment, showing increased photoautotrophy and reduced pathogenic taxa. The dominant fungal functions across all treatments remained associated with soil and litter saprotrophs and plant pathogens (Reiß et al. 2024, 2025). This aligns with literature showing that microbial communities, particularly fungi, exhibit functional redundancy, where different taxa can fulfil similar roles, resulting in stable functional profiles despite compositional shifts (Qin et al. 2023). In line with findings for active bacterial communities prolonged treatment with biocides led to significant alterations within active fungal community after 62 days (A-UOF-62d). Active communities are known to be more responsive to environmental perturbations due to the sensitivity of metabolically active organisms to stressors (Zhang et al. 2023).

Together, these findings suggest that facade eluates do not simply suppress soil microbiota but restructure communities by favoring stress-tolerant taxa and functional pathways. The result is a shift in community dominance and functional balance, with potential consequences for soil ecosystem services, including nutrient cycling and pathogen suppression. While this study provides new insights into the effects of façade eluates on soil microbiomes after prolonged treatment and incubation, some limitations must be acknowledged. First, functional predictions were derived from amplicon sequencing (FAPROTAX, FungalTraits), which provides only an indirect approximation of community function and cannot fully resolve metabolic processes. Metagenomic or metatranscriptomic approaches as well as enzyme activity assays would be necessary to validate the functional shifts inferred here. Second, the BrdU-labeling technique used to capture active fractions primarily targets bacterial DNA synthesis, while fungal activity is only partially represented. As a result, the response of active fungal communities and their ecological role may be underestimated. Finally, the microcosm design does not fully reflect field conditions, where environmental heterogeneity, microbial dispersal, and dilution processes may buffer eluate effects. These factors should be considered when extrapolating our findings. Despite these constraints, the controlled setup allowed us to isolate façade eluate impacts and identify clear microbial responses.

Bacterial and fungal indicators of environmental stress from facade eluates

The bacterial and fungal community members were analyzed using indicator species analysis, a statistical method that identifies taxa significantly associated with specific treatments based on their indicator values (Cáceres and Legendre 2009). Indicator species analysis revealed distinct microbial responses to facade eluate treatments (Fig. 6, Fig. S3, Table S6). For bacterial communities, most indicators were associated with the rainwater controls (A-RW), suggesting that passive microbial inoculation from rainfall contributed strongly to early community composition. The high abundance of *Pseudoalteromonas*, a marine- and aerosol-associated genus (Zheng et al. 2023), highlights rainwater as a significant microbial input to soil, rather than an adaptation of this taxon to terrestrial conditions. Similarly, *Rhizorhapis* and members of *Silvanigrellaceae* and *Cytophagales*, which were negatively affected by facade eluates, point to a sensitivity of these lineages to chemical stress (Rezaei Somee et al. 2021; Yavari-Bafghi et al. 2023). In case of *Rhizorhapis* this result is surprising as they are known for harboring metal resistance genes (Siles et al. 2022). *Silvanigrellaceae* is a relatively newly described bacterial family (Pitt et al. 2020), and this study is the first to document their sensitivity to facade eluates.

In contrast, eluate-treated soils selected for stress tolerant or pollutant degrading taxa. *Comamonas* and members of Kineosporiaceae, indicator taxa A-B-29d treatment, are known for their roles in bioremediation and environmental resilience (Bagwell et al. 2008; Hussein et al. 2024; Frederico et al. 2025). By 62 days, more specialized indicator taxa emerged in A-B-62d, including *Devosiaceae*, *Micrococcales*, and *Arsenicitalea*, all of which are associated with heavy-metal resistance and persistence in contaminated soils (Mu et al. 2016; Talwar et al. 2020; Vargas-Suárez et al. 2021; Zeng et al. 2022). Same was observed for the indicator taxa of UOF treated soils: After 29 days, *Demequina* and *Janthinobacterium* and after 62 days taxa like *Glutamicibacter*, *Paracoccus*, and *Taibaiella* were identified as indicators. These taxa are known to possess a diverse array of enzymes capable of degrading complex carbon sources (Du et al. 2007; Weiland-Bräuer et al. 2017; Gao et al. 2021, 2023; Zhang et al. 2024), as well as resistance to antibiotics and heavy metals (Belikov et al. 2021 Li et al. 2022), and have been associated with soil remediation after pollution events (Diallo et al. 2021). This shift suggests progressive community adaptation under prolonged chemical exposure, with tolerant taxa increasingly dominating the active fraction.

Fungal indicator taxa provided complementary insights. In rainwater controls, taxa such as *Saccharomyces* and unclassified GS02 lineages appeared, indicating sensitivity to eluates and potential use as sentinel organisms for chemical disturbance (Fai and Grant 2009; Estève et al. 2009). In treated soils, taxa with known pollution tolerance or remediation potential (e.g., *Chlorococcum*, *Trebouxia*) were enriched (Lv et al. 2017; Barreno et al. 2022; Morsi et al. 2023), while parasitic groups like *Rhizophydiales* were detected later, likely reflecting indirect effects mediated by their algal hosts (Powell 1993; Ibelings et al. 2004). Collectively, these patterns indicate that fungal communities not only respond directly to eluate chemistry but also indirectly via altered host–parasite or symbiotic interactions.

While the indicator species analysis provided valuable insights into treatment-specific microbial responses, some limitations should be acknowledged. The associations identified are correlative rather than strictly causal, meaning that facade eluates may not directly select for all observed indicator taxa. Furthermore, several indicators remained unclassified at higher taxonomic levels, and functional interpretations are therefore speculative and shaped by database biases that favor well-studied groups. Finally, the 62-day incubation period may not fully capture long-term community reassembly or resilience. Despite these caveats, the consistent detection of functionally relevant taxa across treatments highlights the utility of indicator analysis in revealing ecological shifts driven by facade eluates. These findings underscore two key ecological dynamics. First, rainwater itself introduces microbial taxa that can confound interpretations of soil community change if not considered explicitly. Second, repeated eluate exposure selects for resilient, pollutant-tolerant taxa in both bacterial and fungal communities, while sensitive groups decline. Over time, this process may reduce taxonomic diversity but enrich functional guilds specialized in stress tolerance and pollutant degradation, potentially reshaping soil ecosystem functioning.

Multiple inputs of facade eluates displace bacterial and fungal taxa

The omission of 29 bacterial and 9 fungal taxa in facade eluate-treated samples highlights the selective pressure exerted by façade runoff (Table S8). In contrast, rainwater (RW)-treated soils retained higher microbial diversity, suggesting that natural inputs support community stability while façade eluates act as a stressor. These findings align with studies showing that contaminants leaching from urban surfaces, such as heavy metals, biocides, and organic residues that negatively affect microbial communities by altering physicochemical soil properties and introducing toxicants (Wang et al. 2018; Knappenberger et al. 2022).

Of particular concern is the disappearance of functionally important groups. Taxa observed at baseline (T0), including *Anabaena*, *Nodularia*, and members of Geobacteraceae and Thermomicrobiales, are linked to nitrogen fixation and metal reduction (Bhardwaj et al. 2024; Xu et al. 2024). Their loss indicates a potential decline in ecosystem services that underpin nutrient cycling and soil health. Conversely, rainwater controls retained unique taxa with beneficial functions, such as *Azospira* and *Cellvibrio* (nutrient cycling, organic matter decomposition) and *Pochonia* and *Rhizophlyctis* (pathogen suppression) (Berg et al. 2020), underscoring that unpolluted inputs can help maintain ecological resilience (Jousset et al. 2017). The consistent disappearance of uncultured or poorly characterized lineages, including *Silvanigrellaceae*, *Saccharimonadaceae*, and *Rhodospirillaceae*, highlights the vulnerability of the "rare biosphere". Although these taxa are typically low in abundance, they play disproportionate roles in buffering communities against environmental disturbances and enabling recovery after stress (Jousset et al. 2017). Their loss may therefore reduce long-term adaptability of soil microbiomes.

Functionally specialized groups such as *Syntrophobacter* and Desulfotomaculales, involved in sulfur cycling and syntrophic degradation, were also excluded by facade eluate exposure. This supports the hypothesis that façade runoff not only alters taxonomic composition but also diminishes functional redundancy. The erosion of this redundancy could compromise essential biogeochemical processes, such as carbon and sulfur turnover, increasing the vulnerability of soils exposed to repeated façade runoff events (Banerjee et al. 2019). It should be noted that the interpretation of these results comes with several caveats. First, observed absences are correlative, and it cannot be proven that facade eluates directly caused the disappearance of individual taxa. Second, many of the omitted groups remain unclassified at family or order level, and their ecological roles remain speculative. Third, database biases toward well-studied taxa limit the accuracy of assignments and functional predictions, meaning the contribution of rare or novel lineages may be underestimated. Finally, the experimental timeframe of 62 days may be too short to fully capture longer-term recovery or resilience dynamics. Nevertheless, as the first study to investigate facade eluate impacts on soil microbiomes, these findings provide an important starting point and highlight directions for future work on rare biosphere stability and functional redundancy in contaminated soils.

Conclusion

This study demonstrates for the first time that repeated application of facade eluates, generated under natural weathering conditions, can significantly and persistently alter soil microbial communities. Biocide-containing eluates in particular suppressed bacterial abundances, shifted richness patterns, and reshaped active microbial communities, while simultaneously fostering fungal proliferation. Indicator and functional analyses further revealed that facade eluates selectively excluded sensitive and functionally important taxa, reducing redundancy in key biogeochemical processes such as nitrogen and sulfur cycling. While interpretations are limited by the correlative nature of community analyses, incomplete taxonomic resolution, and the relatively short experimental timeframe, the consistent patterns observed across total and active fractions highlight a clear ecological risk. These findings underline the importance of considering facade design and biocide usage in the context of soil health and ecosystem services. As the first study to address this issue, it provides a foundation for future research into the long-term resilience of soil microbiomes under repeated pollutant exposure and calls for integrating microbial endpoints into environmental assessments of building materials.

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Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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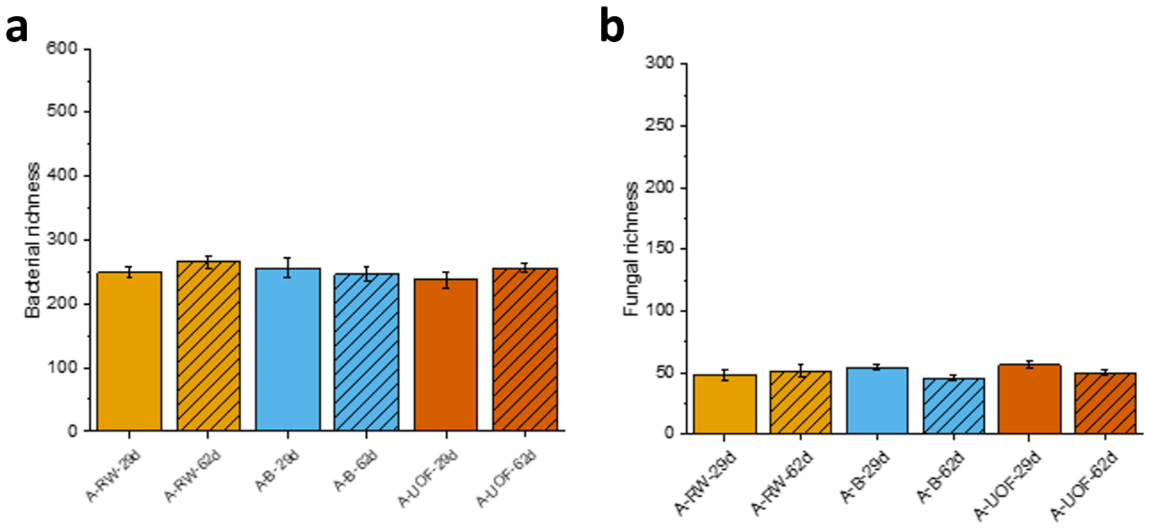
Figures



Time - point [d]	A-RW-28 d	A-RW-62 d	A-B-28 d	A-B-62 d	A-UOF-28 d	A-UOF-62 d
28 d	a		a		a	
62 d		a		a		a
28 + 62 d						

Time - point [d]	A-RW-28 d	A-RW-62 d	A-B-28 d	A-B-62 d	A-UOF-28 d	A-UOF-62 d
28 d	a		a		a	
62 d		a		a		a
28 + 62 d						

Figure S1 OTU richness of active (prefix A-) bacterial (A) and fungal (b) community after respective facade eluate treatment (n = 5). Statistically significant differences were tested using linear models with interaction terms (Group × Time), followed by estimated marginal means with pairwise comparisons. ($p < 0.05$; n = 5). Untreated soil (T0); soil treated with rainwater for 29 days (A-RW-29d) or 62 days (A-RW-62d); soil treated with facade eluates without biocides for 29 days (A-B-29d) or 62 days (A-B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (A-UOF-29d) or 62 days (A-UOF-62d).

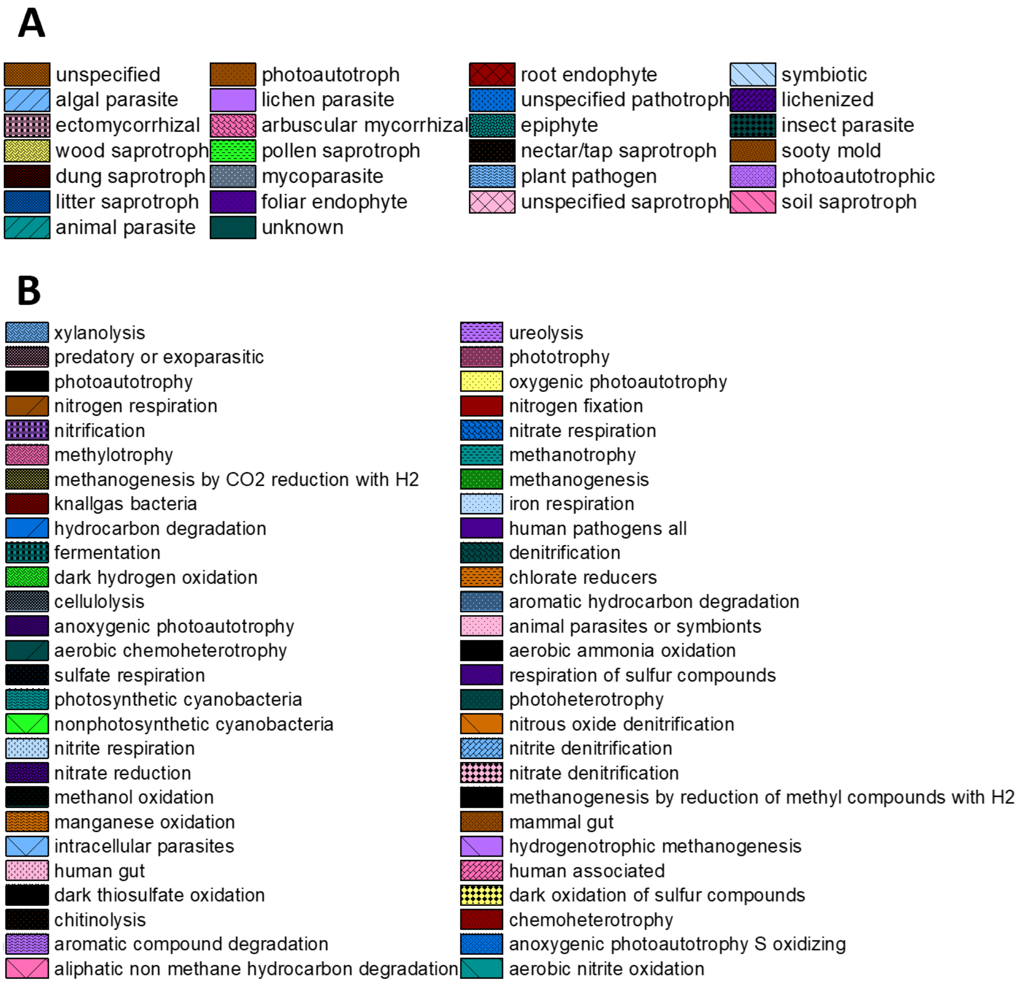


Figure S2 Full legend of bacterial (A) and fungal (B) functions of Figure 6.

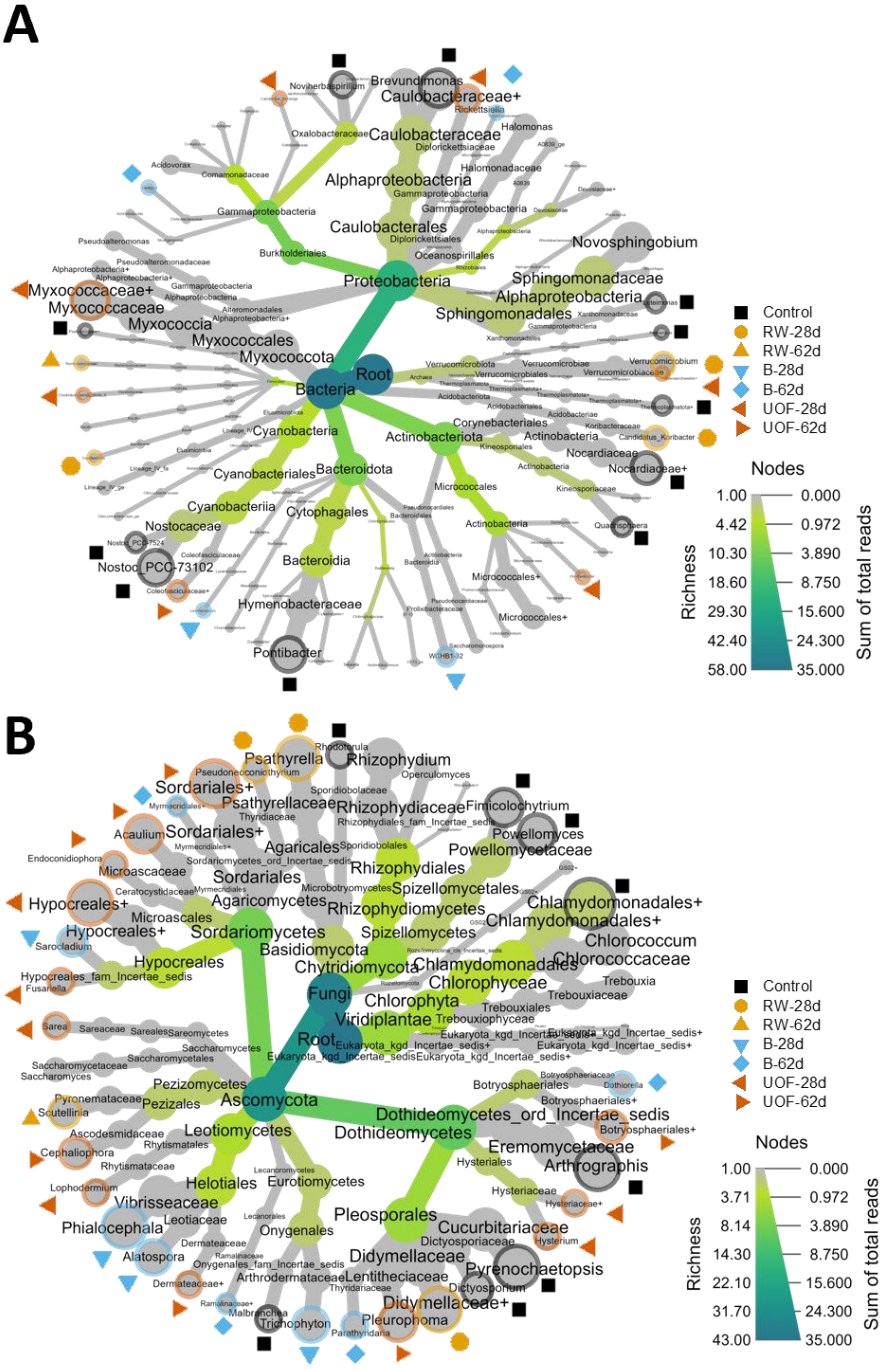


Figure S3 Heat tree representation of the active bacterial (A) and fungal (B) indicator taxa of the respective treatment indicated by symbols and colored frame of the indicator nodes. The color code resembles the richness and the node size of the respective sequence read abundances. Unclassified members of the taxon are marked with +. Untreated soil (T0); soil treated with rainwater for 29 days (A-RW-29d) or 62 days (A-RW-62d); soil treated with facade eluates without biocides for 29 days (A-B-29d) or 62 days (A-B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (A-UOF-29d) or 62 days (A-UOF-62d).

Tables

Table S1: Soil physico-chemical parameters before (T0) and after incubation with multiple entries of rain water or façade eluates for 29 or 62d days. Untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with façade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with façade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d).

Group	pH	Fl$^-$ [µg(biocide)×g(soil)-1]	Cl$^-$ [µg(biocide)×g(soil) -1]	NO$_3^-$ [µg(biocide×g(soil) -1]	PO$_4^{3-}$ [µg(biocide)×g(soil)$^{-1}$]	SO$_4^{2-}$ [µg(biocide)×g(soil)$^{-1}$]	Soil moisture (gravimetric %)	C$_{org}$ [%]
T0	6.68	4.79	10.04	115.98	2.43	24.71	24.15	1.04
RW-29d	6.97	6.16	0.38	4.49	2.75	2.27	26.29	2
B-29d	7.03	7.18	0.73	6.17	2.82	2.95	24.73	1.9
UOF-29d	7.11	7.53	0.80	8.08	3.16	5.21	27.20	1.9
RW-62d	6.93	5.52	NA	16.85	3.27	1.42	22.29	2.1
B-62d	7.00	6.26	2.00	23.83	4.16	1.75	21.83	2
UOF-62d	6.98	7.34	0.17	14.45	2.99	1.60	23.56	1.9

Table S2: Statistically significant differences (p-values) of soil physico-chemical parameters between the timepoints tested using linear models with interaction terms (Group x Time), followed by estimated marginal means with pairwise comparisons ($p < 0.05$; n = 5)

Group 1	Group 2	Timepoint	pH	F$^-$	Cl$^-$	NO$_3^-$	PO$_4^-$	SO$_4^-$	Soil moisture
B	Control	28	1.59E-07	1.30E-07	1.00E-13	1.00E-13	0.93612796	1.00E-13	1.00E-13
B	RW	28	0.32040053	0.03328386	0.87520801	0.5157566	0.99882661	0.84106038	1.00E-13
B	UOF	28	0.00197198	0.76687621	0.99903736	0.40591374	0.89735182	0.04391831	1.00E-13
Control	RW	28	1.50E-09	4.92E-04	1.00E-13	1.00E-13	0.97170413	1.00E-13	1.00E-13
Control	UOF	28	6.60E-12	9.29E-09	1.00E-13	1.00E-13	0.58134214	1.00E-13	1.00E-13
RW	UOF	28	0.13282167	0.00279866	0.81002166	0.02765511	0.83261901	0.0056569	1.00E-13
B	Control	62	3.80E-06	2.33E-04	1.00E-13	1.00E-13	0.01066573	1.00E-13	1.00E-13
B	RW	62	0.22245085	1	1	1	1	1	1.00E-13
B	UOF	62	0.44031572	0.02111835	0.00264862	4.46E-08	0.12389763	0.9977219	1.00E-13

Control	RW	62	1.59E-08	2.33E-04	1.00E-13	1.00E-13	0.01066573	1.00E-13	1.00E-13
Control	UOF	62	5.37E-08	3.74E-08	1.00E-13	1.00E-13	0.81366749	1.00E-13	1.00E-13
RW	UOF	62	0.96933195	0.02111835	0.00264862	4.46E-08	0.12389763	0.9977219	1.00E-13

Table S3: Bacterial alpha diversity indices and their statistical differences. Statistically significant differences were tested using linear models with interaction terms (Group × Time), followed by estimated marginal means with pairwise comparisons. ($p < 0.05$; n = 5). Significant differences are indicated by different letters for within the same time points and by asterisks (*) between time points. Prefix T- Total samples; Prefix A- Active samples; untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with façade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with façade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d).

Bacteria	Richness					ACE					Shannon					Simpson					Evenness				
			Significance					*Significance*					*Significance*					*Significance*					*Significance*		
Sample	*Mean*	*SE*	*28 d*	*62 d*	*28d + 62d*	*Mean*	*SE*	*28 d*	*62 d*	*28d + 62d*	*Mean*	*SE*	*28 d*	*62 d*	*28d + 62d*	*Mean*	*SE*	*28 d*	*62 d*	*28d + 62d*	*Mean*	*SE*	*28 d*	*62 d*	*28d + 62d*
T0	509.20	3.62	ab	a		583.58	3.94	a	a		4.69	0.01	b	a		4.69	0.01	b	a		0.75	0.00	a	a	
RW-29d	521.20	6.55	ab			594.54	7.70	a		*	4.75	0.01	a			4.75	0.01	a			0.76	0.00	a		***
RW-62d	526.60	3.26		a		624.09	7.65		b		4.75	0.01		b		4.75	0.01		b		0.76	0.00		b	
B-29d	496.00	9.49	a			604.06	16.93	a			4.72	0.01	a			4.72	0.01	a			0.76	0.00	a		
B-62d	512.00	8.67		a		628.57	15.81		ab		4.78	0.02		ac		4.78	0.02		ac		0.77	0.00		a	
UOF-29d	509.80	7.96	b			617.41	10.69	a			4.72	0.01	ab			4.72	0.01	ab			0.76	0.00	a		
UOF-62d	512.80	8.49		a		610.53	10.76		ab		4.74	0.01		bc		4.74	0.01		bc		0.76	0.00		a	
A-RW-29d	247.60	8.80	x			272.59	9.02	x			2.45	0.36	x			2.45	0.36	x			0.44	0.07	x		
A-RW-62d	265.60	9.84		x		304.14	6.64		x		2.41	0.17		x		2.41	0.17		x		0.43	0.03		x	
A-B-29d	238.00	12.86	x			280.82	19.44	x			1.67	0.15	xy			1.67	0.15	xy			0.30	0.02	xy		
A-B-62d	256.80	7.76		x		308.97	14.60		x		2.53	0.08		x		2.53	0.08		x		0.46	0.01		x	
A-UOF-29d	256.80	14.91	x			308.91	17.46	x			2.05	0.13	y		***	2.05	0.13	y		***	0.37	0.02	y		***
A-UOF-62d	246,60	12,11		x		281,11	11,57		x		2,11	0,17		x		2,11	0,17		x		0,38	0,03		x	

Table S5: Results of pairwise PERMANOVA (Adonis) with a significance threshold of $p < 0.05$ of bacterial Bray-Curtis dissimilarities for composition and function. Prefix T- Total samples; Prefix A- Active samples; Untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with facade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d).

		Composition		**Function**	
Group1	**Group2**	**R^2**	**p-Value**	**R^2**	**p-Value**
A-B-29d	*A-B-62d*	0.65465993	0.004	0.15904701	0.223
A-B-29d	*A-RW-29d*	0.1666459	0.008	0.14571912	0.281
A-B-29d	*A-RW-62d*	0.23219946	0.009	0.08339797	0.517
A-B-29d	*A-UOF-29d*	0.24847055	0.045	0.32207759	0.028
A-B-29d	*A-UOF-62d*	0.27791128	0.035	0.32913162	0.019
A-B-29d	*B-29d*	0.96196421	0.007	0.46120024	0.004
A-B-29d	*B-62d*	0.958948	0.007	0.45256666	0.005
A-B-29d	*T0*	0.965185	0.011	0.36363109	0.007
A-B-29d	*RW-29d*	0.96573145	0.011	0.43235784	0.008
A-B-29d	*RW-62d*	0.96678885	0.008	0.51720697	0.008
A-B-29d	*UOF-29d*	0.9602876	0.005	0.45938451	0.011
A-B-29d	*UOF-62d*	0.96235096	0.007	0.42709016	0.006
A-B-62d	*A-RW-29d*	0.23919085	0.007	0.04059292	0.664
A-B-62d	*A-RW-62d*	0.30925718	0.024	0.03324091	0.873
A-B-62d	*A-UOF-29d*	0.53985567	0.009	0.29169111	0.060
A-B-62d	*A-UOF-62d*	0.39383634	0.039	0.24626575	0.123
A-B-62d	*B-29d*	0.93518557	0.012	0.41405622	0.006
A-B-62d	*B-62d*	0.93115551	0.005	0.41617218	0.007
A-B-62d	*T0*	0.9376974	0.008	0.33919440	0.013
A-B-62d	*RW-29d*	0.9389189	0.008	0.39307151	0.008
A-B-62d	*RW-62d*	0.94005114	0.01	0.47484977	0.013
A-B-62d	*UOF-29d*	0.9330474	0.007	0.41202184	0.011
A-B-62d	*UOF-62d*	0.93501991	0.005	0.37633480	0.010
A-RW-29d	*A-RW-62d*	0.09872863	0.623	0.07012187	0.643
A-RW-29d	*A-UOF-29d*	0.1279785	0.256	0.25675044	0.075
A-RW-29d	*A-UOF-62d*	0.12880732	0.265	0.17133875	0.203
A-RW-29d	*B-29d*	0.67026732	0.011	0.30111942	0.006
A-RW-29d	*B-62d*	0.6622332	0.009	0.30552010	0.008
A-RW-29d	*T0*	0.66928552	0.011	0.23801546	0.104
A-RW-29d	*RW-29d*	0.67445803	0.015	0.28547870	0.005
A-RW-29d	*RW-62d*	0.67821429	0.009	0.36227334	0.009
A-RW-29d	*UOF-29d*	0.66730597	0.006	0.31751119	0.009
A-RW-29d	*UOF-62d*	0.66905121	0.008	0.27681717	0.032
A-RW-62d	*A-UOF-29d*	0.14168231	0.165	0.26780759	0.073
A-RW-62d	*A-UOF-62d*	0.11982474	0.377	0.26998491	0.067
A-RW-62d	*B-29d*	0.85009546	0.006	0.36591746	0.006
A-RW-62d	*B-62d*	0.84360244	0.011	0.35226749	0.008

A-RW-62d	*T0*	0.8483365	0.006	0.29631220	0.035
A-RW-62d	*RW-29d*	0.85312348	0.014	0.32539961	0.008
A-RW-62d	*RW-62d*	0.85518386	0.012	0.45359631	0.008
A-RW-62d	*UOF-29d*	0.84706721	0.008	0.37528524	0.012
A-RW-62d	*UOF-62d*	0.84821546	0.015	0.32101705	0.014
A-UOF-29d	*A-UOF-62d*	0.15023039	0.228	0.57256343	0.005
A-UOF-29d	*B-29d*	0.9440863	0.007	0.44703603	0.006
A-UOF-29d	*B-62d*	0.94059151	0.008	0.48708894	0.008
A-UOF-29d	*T0*	0.94597875	0.008	0.57340328	0.012
A-UOF-29d	*RW-29d*	0.94792138	0.008	0.48448580	0.007
A-UOF-29d	*RW-62d*	0.94915565	0.007	0.38615530	0.023
A-UOF-29d	*UOF-29d*	0.9420887	0.012	0.39807091	0.036
A-UOF-29d	*UOF-62d*	0.94389585	0.008	0.46271917	0.007
A-UOF-62d	*B-29d*	0.94218564	0.004	0.73736032	0.010
A-UOF-62d	*B-62d*	0.93841063	0.011	0.71649759	0.008
A-UOF-62d	*T0*	0.94464803	0.009	0.55734905	0.006
A-UOF-62d	*RW-29d*	0.94599325	0.011	0.68874215	0.014
A-UOF-62d	*RW-62d*	0.9470735	0.004	0.77648998	0.011
A-UOF-62d	*UOF-29d*	0.94013757	0.008	0.71404033	0.009
A-UOF-62d	*UOF-62d*	0.94196027	0.009	0.69757936	0.008
B-29d	*B-62d*	0.25942474	0.021	0.27605811	0.018
B-29d	*T0*	0.64545822	0.012	0.86629706	0.009
B-29d	*RW-29d*	0.50376002	0.007	0.31054301	0.050
B-29d	*RW-62d*	0.50327765	0.006	0.44455377	0.023
B-29d	*UOF-29d*	0.22516814	0.049	0.08011827	0.649
B-29d	*UOF-62d*	0.33817306	0.018	0.11505899	0.319
B-62d	*T0*	0.64087025	0.012	0.82222613	0.011
B-62d	*RW-29d*	0.46074089	0.013	0.13345428	0.293
B-62d	*RW-62d*	0.3882874	0.019	0.61437646	0.010
B-62d	*UOF-29d*	0.2133311	0.039	0.19862897	0.136
B-62d	*UOF-62d*	0.23456174	0.048	0.12200246	0.328
T0	*RW-29d*	0.51125649	0.013	0.68927357	0.013
T0	*RW-62d*	0.60071894	0.01	0.86932213	0.010
T0	*UOF-29d*	0.5291089	0.013	0.68545907	0.009
T0	*UOF-62d*	0.53862886	0.015	0.73201336	0.009
RW-29d	*RW-62d*	0.26825561	0.009	0.58447117	0.014
RW-29d	*UOF-29d*	0.26153564	0.027	0.24079217	0.086
RW-29d	*UOF-62d*	0.2509688	0.029	0.11901786	0.349
RW-62d	*UOF-29d*	0.24465219	0.021	0.14088382	0.289
RW-62d	*UOF-62d*	0.19351802	0.063	0.49003040	0.021
UOF-29d	*UOF-62d*	0.1250903	0.265	0.13595526	0.319

Table S6: Results of pairwise PERMANOVA (Adonis) with a significance threshold of $p < 0.05$ of fungal Bray-Curtis dissimilarities for composition and function. Prefix T- Total samples; Prefix A- Active samples; Untreated soil (T0); Soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with facade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with facade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d).

		Composition		**Function**	
Group1	**Group2**	**R^2**	**p-Value**	**R^2**	**p-Value**
A-B-29d	*A-B-62d*	0.5390375	0.006	0.57782866	0.008
A-B-29d	*A-RW-29d*	0.1374999	0.182	0.23213533	0.066
A-B-29d	*A-RW-62d*	0.1327154	0.220	0.12086638	0.300
A-B-29d	*A-UOF-29d*	0.1353631	0.257	0.15443580	0.180
A-B-29d	*A-UOF-62d*	0.5181511	0.010	0.61324017	0.006
A-B-29d	*B-29d*	0.8071074	0.018	0.90188026	0.009
A-B-29d	*B-62d*	0.8123958	0.008	0.91249151	0.009
A-B-29d	*T0*	0.7828418	0.011	0.88092280	0.005
A-B-29d	*RW-29d*	0.7667687	0.007	0.88941848	0.007
A-B-29d	*RW-62d*	0.7885201	0.012	0.87465665	0.013
A-B-29d	*UOF-29d*	0.7954870	0.009	0.90073529	0.012
A-B-29d	*UOF-62d*	0.8162885	0.009	0.90282142	0.010
A-B-62d	*A-RW-29d*	0.3009869	0.009	0.40083078	0.010
A-B-62d	*A-RW-62d*	0.4953059	0.013	0.54231916	0.012
A-B-62d	*A-UOF-29d*	0.3270892	0.011	0.30685949	0.011
A-B-62d	*A-UOF-62d*	0.1189550	0.380	0.15224492	0.197
A-B-62d	*B-29d*	0.9219635	0.008	0.96786548	0.004
A-B-62d	*B-62d*	0.9259036	0.009	0.97509735	0.005
A-B-62d	*T0*	0.8903271	0.012	0.95356735	0.008
A-B-62d	*RW-29d*	0.8788065	0.010	0.95334695	0.008
A-B-62d	*RW-62d*	0.8962710	0.006	0.94275757	0.011
A-B-62d	*UOF-29d*	0.9084384	0.010	0.96518293	0.010
A-B-62d	*UOF-62d*	0.9242220	0.012	0.96798269	0.013
A-RW-29d	*A-RW-62d*	0.1600884	0.050	0.25364849	0.009
A-RW-29d	*A-UOF-29d*	0.1393168	0.109	0.16485776	0.101
A-RW-29d	*A-UOF-62d*	0.3044105	0.002	0.43014810	0.011
A-RW-29d	*B-29d*	0.6126520	0.011	0.64527770	0.008
A-RW-29d	*B-62d*	0.6158678	0.008	0.65691474	0.006
A-RW-29d	*T0*	0.5969602	0.013	0.62362070	0.009
A-RW-29d	*RW-29d*	0.5796439	0.010	0.64518169	0.003
A-RW-29d	*RW-62d*	0.5995163	0.012	0.62977662	0.006
A-RW-29d	*UOF-29d*	0.5965207	0.005	0.64595101	0.008
A-RW-29d	*UOF-62d*	0.6218075	0.008	0.64111596	0.010
A-RW-62d	*A-UOF-29d*	0.1666404	0.015	0.09823791	0.493
A-RW-62d	*A-UOF-62d*	0.4791355	0.007	0.56094761	0.006
A-RW-62d	*B-29d*	0.8275918	0.009	0.83508515	0.008
A-RW-62d	*B-62d*	0.8317845	0.008	0.84838771	0.006

A-RW-62d	*T0*	0.8001291	0.008	0.80584155	0.010
A-RW-62d	*RW-29d*	0.7879899	0.010	0.82789854	0.008
A-RW-62d	*RW-62d*	0.8064458	0.007	0.81576654	0.014
A-RW-62d	*UOF-29d*	0.8136374	0.010	0.83783540	0.008
A-RW-62d	*UOF-62d*	0.8339220	0.004	0.83752218	0.013
A-UOF-29d	*A-UOF-62d*	0.3108366	0.010	0.33244424	0.008
A-UOF-29d	*B-29d*	0.7180149	0.010	0.57541710	0.004
A-UOF-29d	*B-62d*	0.7221091	0.005	0.58722908	0.010
A-UOF-29d	*T0*	0.6941480	0.007	0.54581972	0.009
A-UOF-29d	*RW-29d*	0.6799997	0.010	0.57546503	0.006
A-UOF-29d	*RW-62d*	0.7022531	0.010	0.56300253	0.010
A-UOF-29d	*UOF-29d*	0.7052168	0.010	0.57762815	0.011
A-UOF-29d	*UOF-62d*	0.7275791	0.006	0.56947954	0.008
A-UOF-62d	*B-29d*	0.9183251	0.014	0.97295591	0.007
A-UOF-62d	*B-62d*	0.9222556	0.010	0.97995056	0.006
A-UOF-62d	*T0*	0.8863953	0.007	0.95959255	0.006
A-UOF-62d	*RW-29d*	0.8760194	0.009	0.95867772	0.010
A-UOF-62d	*RW-62d*	0.8924938	0.009	0.94816964	0.010
A-UOF-62d	*UOF-29d*	0.9047316	0.015	0.97016298	0.006
A-UOF-62d	*UOF-62d*	0.9212045	0.014	0.97309384	0.010
B-29d	*B-62d*	0.1397769	0.074	0.12466033	0.321
B-29d	*T0*	0.3466874	0.007	0.35602452	0.018
B-29d	*RW-29d*	0.1604909	0.009	0.13461128	0.272
B-29d	*RW-62d*	0.1240998	0.188	0.11890784	0.351
B-29d	*UOF-29d*	0.1558518	0.022	0.12013784	0.408
B-29d	*UOF-62d*	0.1917962	0.013	0.14066943	0.245
B-62d	*T0*	0.3414416	0.006	0.46973544	0.009
B-62d	*RW-29d*	0.1516923	0.008	0.09670934	0.533
B-62d	*RW-62d*	0.1384531	0.028	0.11428281	0.412
B-62d	*UOF-29d*	0.1360295	0.122	0.09577199	0.561
B-62d	*UOF-62d*	0.1881036	0.030	0.08715964	0.618
T0	*RW-29d*	0.2899742	0.008	0.42044193	0.012
T0	*RW-62d*	0.3000184	0.006	0.38549644	0.009
T0	*UOF-29d*	0.3614718	0.008	0.46940600	0.011
T0	*UOF-62d*	0.3687950	0.004	0.46844923	0.011
RW-29d	*RW-62d*	0.1678250	0.011	0.08406826	0.590
RW-29d	*UOF-29d*	0.1525185	0.014	0.13097998	0.236
RW-29d	*UOF-62d*	0.2196684	0.006	0.10699359	0.408
RW-62d	*UOF-29d*	0.1612495	0.019	0.13315250	0.345
RW-62d	*UOF-62d*	0.1184188	0.354	0.07619502	0.612
UOF-29d	*UOF-62d*	0.1753857	0.026	0.13519969	0.237

Table S7: Indicator species analysis of Total (Prefx T-) and active (Prefix A-) bacterial and fungal community compositions after eluate treatment, showing the Stat value and significance of each species and its function ordered after the Stat value. Prefix T- Total samples; Prefix A- Active samples; untreated soil (T0); soil treated with rainwater for 29 days (RW-29d) or 62 days (RW-62d); soil treated with façade eluates without biocides for 29 days (B-29d) or 62 days (B-62d); soil treated with façade eluates containing in-can preservatives and film preservatives for 29 days (UOF-29d) or 62 days (UOF-62d). $^{+}$unclassified members; $^{\prime}$ uncultured

	Treatment	Stat value	p-value	Significance	Taxa	Relative abundance [%]	Function
Bacteria	T0	0.950	0.0001	***	Caulobacteraceae^{+}	0.014	chemoheterotrophy
		0.859	0.0001	***	*Nostoc* PCC-73102	0.16	photoautotroph
		0.764	0.0001	***	*Pontibacter*	0.0	aerobic chemoheterotrophy, chemoheterotrophy
		0.722	0.0001	***	*Noviherbaspirillum*	0.03	denitrification
		0.707	0.0010	***	Thermoplasamatota^{+}	0.002	chemoheterotroph
		0.690	0.0007	***	*Saccharomonospora*	0.06	plant pathogen
		0.690	0.0003	***	Nocardiaceae^{+}	0.03	Pathogen

	0.662	0.0004	***	*Quadrisphaera*	0.011	NA
	0.645	0.041	**	Peptocoocaceae^{+}	0.004	fermentation
	0.577	0.0066	**	Luteimonas	0.37	chemoheterotrophy
	0.567	0.0262	*	Pedosphaera	0.33	chemoheterorophy
	0.512	0.0436	*	*Nostoc* PCC-7524	0.14	photoautotrophy
T-RW-29d	0.707	0.0005	***	Candidatus *Koribacter*	0	chemoheterotrophy
	0.640	0.0047	**	*Calditerricola*	0.001	chemoheterotrophy
	0.627	0.0021	**	*Verrucomicrobium*	0.002	chemoheterotrophy
T-RW-62d	0.647	0.0105	*	Ruminococcaceae‡	0.02	fermentation
T-B-29d	0.721	0.0011	**	*Lentimicrobium*	0	chemoorganotrophy
	0.597	0.0075	**	WCHB1-32	0.001	chemoorganotrophy
T-B-62d	0.716	0.0025	**	Deefgea	0.001	chemoheterotrophy

	0.524	0.0219	*	Micropepsaceae^{+}	0.18	chemoheterotrophy
T-UOF-29d	0.729	0.0001	***	*Ricketsiella*	0.0025	chemoheterotrophy
	0.716	0.0029	**	*Clostridium sensu stricto 6*	0.001	fermentation
	0.703	0.0033	**	*Ornithinibacter*	0.001	chemoheterotrophy
	0.604	0.0168	*	Woesearchaeales^{+}	0	fermentation
	0.604	0.0185	*	Myxococcaceae^{+}	0.015	chemoorganotrophy
	0.567	0.0298	*	Candidatus *Nitrotoga*	0	nitrification
T-UOF-62d	0.611	0.0091	**	Coleofasciculaceae^{+}	0.022	photoautotrophy
A-RW-29d	0.684	0.0016	**	*Rhizorhapis*	0	chemoheterotrophy
	0.617	0.0039	**	*Sediminibacterium*	0	chemoheterotrophy
	0.592	0.0100	**	Candidatus *Obscuribacter*	0	aerobic heterotrophy, fermentation
	0.563	0.0012	**	37-13	0.002	fermentation

0.556	0.0005	***	Obscuribacteraceae	0	aerobic heterotrophy, fermentation
0.540	0.0005	***	Lineage IV	0	fermentation
0.520	0.0230	*	*Pelomonas*	0.031	chemoorganotrophy
0.505	0.0005	***	*Pseudoalteromonas*	46,66	chemoheterotrophy
0.500	0.0413	*	*Undibacterium*	0	chemoheterotrophy
0.498	0.0007	***	*Curvibacter*	0.023	chemoheterotrophy
0.497	0.0333	*	Aphaproteobacteria$^+$	0.022	chemoheterotrophy
0.496	0.0021	***	*Acidovorax*	0.032	chemoorganotrophy
0.496	0.0320	*	Alcaligenaceae$^+$	0.008	chemoorganotrophy
0.492	0.0024	**	*Halomonas*	0	aerobic heterotrophy, fermentation
0.476	0.0032	**	*Nesterenkonia*	0.015	fermentation

	0.473	0.0302	*	Caulobacteraceae$^+$	0.198	aerobic chemoheterotrophy, chemoheterotrophy
	0.469	0.0381	*	Bacilli$^{'}$	0.014	aerobic chemoheterotrophy, chemoheterotrophy
A-RW-62d	0.645	0.0039	**	Cytophagales$^+$	0.03	chemoorganotrophy
	0.631	0.0060	**	*Dyadobacter*	0.008	chemoorganotrophy
	0.590	0.0126	*	Silvanigrellaceae$^+$	0.021	chemoorganotrophy
	0.584	0.0060	**	A0839	0	NA
	0.582	0.0036	**	*Novosphingobium*	0.001	aromatic compound degradation, chemoheterotrophy
A-B-29d	0.736	0.0002	***	Kineosporiaceae$^+$	0.003	chemoorganotrophy

	0.566	0.0077	**	*Comamonas*	0.031	chemoorganotrophy
A-B-62d	0.907	0.0001	***	Devosiaceae$^{'}$	0.435	chemoheterotrophy
	0.893	0.0001	***	Micrococcales^{+}	4.208	chemoorganotrophy
	0.890	0.0001	***	*Bervundimonas*	0	chemoheterotrophy
	0.862	0.0001	***	*Oerskovia*	0	Fermentation, chemoheterotrophy
	0.686	0.0004	***	*Arsenicitalea*	0.003	chemoheterotrophy
	0.639	0.0024	**	*Achromobacter*	0.001	chemoorganotrophy
	0.550	0.0106	*	*Chryseobacterium*	0	chemoorganotrophy
A-UOF-29d	0.717	0.0003	***	*Demequina*	0.049	chemoorganotrophy
	0.614	0.0002	***	*Janthinobacterium*	0.013	chemoorganotrophy
A-UOF-62d	0.746	0.0002	***	*Glutamicibacter*	0	chemoorganotrophy
	0.635	0.0001	***	*Paracoccus*	0	chemoorganotrophy

		0.628	0.0005	***	*Taibaiella*	0.001	nitrate reduction
		0.569	0.0057	**	*Cellulosimicrobium*	0	fermentation
Fungi	T0	0.956	0.0001	***	*Rhodotorula*	1.84	unspecified saprotroph
		0.926	0.0001	***	*Fimicolochytrium*	0.36	pollen saprotroph
		0.845	0.0001	***	*Powellomyces*	0.23	pollen saprotroph
		0.798	0.0001	***	Chlamydomonales$^+$	0.90	photoautotrophic
		0.690	0.0002	***	*Arthographis*	0.36	unspecified saprotroph
		0.647	0.0041	***	*Malbranchea*	0.028	soil saprotroph
		0.576	0.0102	*	*Dictyosporium*	0.065	litter saprotroph
		0.570	0.0040	**	*Pyrenochaetopsis*	0.488	wood saprotroph
	T-RW-29d	0.915	0.0001	***	*Pseudoneoconiothyrium*	0.267	wood saprotroph
		0.575	0.0044	**	Didymellaceae$^+$	4.24	plant pathogen

	0.548	0.0119	*	*Psathyrella*	0.15	wood saprotroph
T-RW-62d	0.61	0.0047	**	*Scutellinia*	0	wood saprotroph
T-B-26d	0.640	0.0016	**	*Phialocephala*	0.218	soil saprotroph
	0.598	0.0013	**	*Trichophyton*	0.009	animal parasite
	0.578	0.0154	*	*Sarocladium*	0.01	plant pathogen
	0.505	0.0278	*	*Alatospora*	0.037	litter saprotroph
T-B-62d	0.688	0.0007	***	*Parathyridaria*	0	litter saprotroph
	0.619	0.0007	***	*Dothiorella*	0.003	plant pathogen

	0.602	0.0004	***	Ramalinaceae$^+$	0	symbiotic
	0.574	0.0037	**	Myrmecridiales$^+$	0	insect parasite
T-UOF-29d	0.827	0.0001	***	*Fusariella*	0.007	plant pathogen
	0.786	0.0002	***	*Hysterium*	0.01	wood saprotroph
	0.647	0.0038	**	Hysteriaceae$^+$	0.0004	wood saprotroph
	0.610	0.0081	**	*Sarea*	0.003	unspecified saprotroph
	0.586	0.0034	**	Hypocreales$^+$	0.129	wood saprotroph

	0.508	0.0291	*	*Lophodermium*	0	plant pathogen
T-UOF-62d	0.841	0.0003	***	*Pleurophoma*	0.229	plant pathogen
	0.733	0.0037	**	Dermateceae$^+$	0.145	litter saprotroph
	0.664	0.004	***	*Acaulium*	0.032	animal parasite
	0.640	0.0014	**	Sordariales$^+$	0.24	dung saprotroph
	0.608	0.0034	**	*Cephaliophora*	0.06	soil saprotroph
	0.516	0.0124	*	*Endoconidiphora*	0.05	plant pathogen
	0.474	0.0109	*	Botryosphaeriales$^+$	0.128	plant pathogen
A-RW-29d	0.669	0.0035	**	*Saccharomyces*	0.98	nectar/tap saprotroph
	0.557	0.0001	***	GS02$^+$	3.89	NA

A-RW-62d	0.684	0.0006	***	*Taxus*	0.21	NA
A-B-29d	0.488	0.0436	*	*Chlorococcum*	1.42	photoautotrophic
A-B-62d	0.778	0.0002	***	Rhizophydiales^{+}	0	algal parasite
	0.748	0.0001	***	*Rhizophydium*	0.972	algal parasite
A-UOF-29d	0.516	0.0263	*	*Trebouxia*	0.35	photoautotrophic
	0.503	0.0401	*	*Operculomyces*	0	pollen saprotroph
A-UOF-62d	0.933	0.0001	***	Eukaryota kgd *incertae sedis*	0.088	NA

Signif. Codes: 0 '***'; 0.001'**'; 0.01 '

Table S8: Omitted species analysis of Total (Prefix T-) and Active (Prefix A-) bacterial and fungal communities following eluate treatment. The following table presents the genera that are uniquely present in either T0 or RW-treated samples and absent in all facade eluate-treated samples. This analysis distinguishes between total (T-) and active (A-) microbial community compositions, identifying genera that are exclusively associated with specific eluate treatments. Prefix T- Total samples; Prefix A- Active samples; untreated soil (T0); soil treated with rainwater RW; $^{+}$unclassified members; ^{ʿ} uncultured.

	Bacteria	**Funig**
T0	*Anabaena* PCC-7122 Bacteroidota^{+} Geobacteraceae^{+} mle1-8 *Nodularia* PCC-9350 Plot4-2H12 Thermomicrobiales^{+}	Branch06 $^{+}$
RW	Acidobacteriota^{+} *Azospira* *Cellvibrio* *Clostridia UCG-014* Desulfotomaculales^{+} *Dojkabacteria* E1B-B3-114 *Listeria* *Rubrobacter*	*Arrhenia* Botryosphaeriaceae $^{+}$ *Dioszegia* *Liberomyces* *Pochonia* *Rhizophlyctis*
A-RW	*Alicyclobacillus* *Asticcacaulis*	*Thelephora* Ulmaceae $^{+}$

	Babeliales	
	Bathyarchaeia	
	Candidimonas	
	DEV007	
	Dietzia	
	Gordonia	
	Idiomarina	
	Leuconostoc	
	Neisseriaceae^{+}	
	Pleomorphomonas	
	Propionicicella	
	Proteobacteria^{+}	
	Rhodospirillaceaeʽ	
	Runella	
	Saccharimonadaceae^{+}	
	Saccharopolyspora	
	Silvanigrellaceae^{+}	
	Syntrophobacter	

4 List of publications in peer-reviewed journals

2025

Reiß, F., Kiefer, N., Tanunchai B., Reiß, P., Kalkhof, S., & Noll, M. (2026). Continuous intake of facade eluates affects active and total soil microbiome. *Biology and Fertility of Soils*, 62, 75–95. doi: 10.1007/s00374-025-01955-9 (IF 2024 = 5.6)

Reiß, F., Kiefer, N., Reiß, P., Kalkhof, S., & Noll, M. (2025). Facade eluates affect active and total soil microbiome. *Environmental Pollution*, 364, 125242. doi: 10.1016/j.envpol.2024.125242. (IF 2023 = 7.3)

Reiß, F., Noll, M. Das aktive Mikrobiom als ökotoxikologischer Indikator in Umweltproben. *Biospektrum*, 31, 63-65 (2025). doi: 10.1007/s12268-025-2384-1 (IF 2024 = 0.104)

2024

Reiß, F., Kiefer, N., Purahong, W., Borken, W., Kalkhof, S., & Noll, M. (2024). Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials. *Science of the Total Environment*, 912, 168689. doi: 10.1016/j.scitotenv.2023.168689. (IF 2022 = 9.8)

Kiefer, N., Nichterlein, M., **Reiß, F.**, Runge, M., Biermann, U., Wieland, T., Noll, M., & Kalkhof, S. (2024). Eluates from facades at the beginning of their service time affect aquatic and sediment organisms. *Science of the Total Environment*, 906, 167531. doi: 10.1016/j.scitotenv.2023.167531. (IF 2023 = 8.2)

2023

Reiß, F., Schumann, A., Sohl, L., Thamm, M., Scheiner, R., & Noll, M. (2023). Fungicides and insecticides alter the microbial community on the cuticle of honey bees. *Frontiers in Microbiology*, 14, 1271498. doi: 10.3389/fmicb.2023.1271498. (IF 2021 = 6.064)

Thamm, M.*, **Reiß, F.***, Sohl, L., Gabel, M., Noll, M., & Scheiner, R. (2023). Cuticular microbiome composition of *Osmia bicornis* is different to *Apis mellifera* honeybees. *Microorganisms*, 11, 2780. doi: 10.3390/microorganisms11222780. (IF 2023 = 4.1) *These authors contributed equally to this work.

2022

Olliges, E.*, Stroppe, S.*, Haile, A., Reiß, F., Malhis, M., Funke, S. A. & Meissner, K., (2022). Open-Label Placebo Administration Decreases Pain in Elderly Patients With Symptomatic Knee Osteoarthritis – A Randomized Controlled Trial. *Frontiers in Psychiatry*, 13, 853497. doi: 10.3389/fpsyt.2022.853497 (IF 2022 = 3.2) *These authors contributed equally to this work.

2021

Reiß, F.*, Kiefer, N.*, Noll, M., Kalkhof, S., 2021. Application, release, ecotoxicological assessment of biocide in building materials and its soil microbial response. *Ecotoxicology and Environmental Safety*. doi: 10.1016/j.ecoenv.2021.112707 (IF 2022 = 7.129) *These authors contributed equally to this work.

5 List of conference abstracts / presentations

2025

Reiß, F., Kiefer, N., Kalkhof, S., & **Noll, M.** (2025). Facade eluates affect active and total soil microbiome. Short lecture, VAAM, Germany, Bochum.

2023

Reiß, F., Kiefer, N., Kalkhof, S., & Noll, M. (2023). Soil microbial composition directly respond to the presence of biocides from building materials. Poster presentation, BayCEER Workshop 2023, Germany, Bayreuth.

Kiefer, N., Reiß, F., Noll, M., & Kalkhof, S. (2023). Biozide aus Fassaden: Zusammenfassung der ökotoxikologischen, mikrobiologischen, biozidanalytischen Relevanz sowie Modellierung des Verhaltens im Boden. Platform presentation, Projekttreffen "Beregnete Fassaden". Germany, Frankfurt.

Reiß, F., Kiefer, N., Kalkhof, S., & **Noll, M.** (2023). Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials. Short lecture 15, VAAM, Germany, Göttingen.

Reiß, F., Kiefer, N., Kalkhof, S., & Noll, M. (2023). Biocides containing facade eluates alter soil microbial community composition and activity. Platform presentation, SETAC Europe 2023, Dublin, Ireland.

Reiß, F., Schuhmann, A., Sohl, L., Thamm, M., Scheiner, R., & Noll, M. (2023). Fungicides and insecticides can alter the microbial community on the cuticle of honeybees. Poster presentation, SETAC Europe 2023, Dublin, Ireland.

Kiefer, N., Reiß, F., Klein, J., Klein, M., Noll, M., & Kalkhof, S. (2023). Measurement and simulation of distribution, degradation, maximal soil concentrations and ecotoxic effects of biocides being released from building facades. Poster presentation, SETAC Europe 2023, Dublin, Ireland.

2022

Kiefer, N., Reiß, F., Klein, J., Klein, M., Biermann, U., Noll, M., & Kalkhof, S. (2022). Measurement and simulation of distribution, degradation, maximal soil concentrations and ecotoxic effects of biocides being released from building facades. Poster presentation, SETAC Europe 2022, Copenhagen, Denmark.

Reiß, F., Kiefer, N., Kalkhof, S., & Noll, M. (2022). Soil microbial composition and replication directly respond to the presence of biocides from building materials. Poster presentation, SETAC Europe 2022, Copenhagen, Denmark.

Kalkhof, S., Kiefer, N., Nichterlein, M., Reiß, F., Ciok, M., Befolo, O., Nouri, N., Grubert, J., Flechsig, G.-U., & Noll, M. (2022). Release and distribution of biocides from facades and their ecotoxicological effect on soil organisms. Abschlusskolloquium "Entwicklung eines Modells zur Bewertung der Umwelteigenschaften üblicher Putze und Mörtel im Außenbereich", Valley (IBP Holzkirchen).

Noll, M., Reiß, F., Kiefer, N., & Kalkhof, S. (2022). Application and release of biocide in building materials and its soil microbial response. Online presentation, Seminar in Focus Area Environment - Activity field "Environmental pollutants" & Priority Theme "Microbiologically influenced corrosion (MIC)".

2021

Kiefer, N., Reiß, F., Wittmann, E.-M., Noll, M., & Kalkhof, S. (2021). Update des Coburger BayÖkoTox-Projektes: Beeinflussung der mikrobiellen Diversität durch fassadenrelevante Biozide und Biozidmischung. Online presentation, Projekttreffen "Beregnete Fassaden".

2020

Kiefer, N., Reiß, F., Wittmann, E.-M., Noll, M., & Kalkhof, S. (2020). Forschungsprojekt BayÖkotox. Online presentation, Projekttreffen "Beregnete Fassaden".

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