Adaptions of saprotrophic filamentous fungi to drought stress in soils: Hydraulic redistribution through mycelia networks and transcriptional responses

Dissertation

ZUR ERLANGUNG DES DOKTORGRADES

Dr. rer. nat.

DER FAKULTÄT FÜR BIOLOGIE, CHEMIE UND GEOWISSENSCHAFTEN

DER UNIVERSITÄT BAYREUTH

vorgelegt von:

Alexander Guhr, M.Sc. Molecular Ecology

geboren am 31.03.1987 in Erfurt

Die vorliegende Arbeit wurde in der Zeit von 01/2013 bis 07/2016 in Bayreuth am Lehrstuhl für Bodenökologie unter Betreuung von Herrn Professor Dr. Egbert Matzner angefertigt.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. Nat.).

Dissertation eingereicht am: 12.07.2016

Zulassung durch die Promotionskommission: 20.07.2016

Wissenschaftliches Kolloquium: 30.08.2016

Amtierender Dekan: Prof. Dr. Stefan Schuster

Prüfungsausschuss:

Prof. Dr.	Egbert Matzner	(Erstgutachter)
Prof. Dr.	Gerhard Gebauer	(Zweitgutachter)
Prof. Dr.	Berd Huwe	(Vorsitz)
PD. Dr.	Derek Peršoh	

“Fate always wins…

… at least, when people stick to the rules.”

Terry Pratchett

Summary

The desiccation of upper soil horizons is a common phenomenon leading to a decrease in microbial activity. Recent studies have shown that fungal communities are often less sensitive and better adapted to soil desiccation than bacterial communities. Mechanisms behind this observation and general drought responses of filamentous saprotrophic fungi are only scarcely analysed. One reason for better fungal adaptation to desiccation may be hydraulic redistribution (HR) of water from moist to dry soil zones along water potential gradients by mycelia networks.

The general goal of this thesis was to investigate the potential of saprotrophic fungi for HR in the non-differentiated mycelium of *Agaricus bisporus* and in the mycelial cord former *Schizophyllum commune*. In addition, the impact of HR on mineralisation of organic matter as well as N translocation within mycelia networks was determined. Further, this study aimed to analyse transcriptional and respiratory responses of *A. bisporus* exposed to drought stress and how they are impacted by the antioxidant riboflavin.

Fungal potential for HR, in comparison to capillary transport, and the impact of HR on C mineralisation and N translocation were assessed in mesocosms using labelling experiments. The mesocosms consisted of 2 chambers, filled with sandy soil and separated by a 2 mm air gap to prevent bulk flow of water. After 6 weeks of growth, chambers were desiccated to a water potential of about -9.5 MPa. Afterwards, chamber I was rewetted to field capacity while chamber II remained dry. One set of mesocosms was rewetted with deuterium labelled water and soil from chamber II was sampled over 3 d and analysed for ^{2}H abundance to quantify HR. A separate set of mesocosms was treated with labelled plant material in chamber II. In this case, CO_2 samples were extracted over 7 d and analysed for ^{13}C abundance to study the impact of HR on C mineralisation. Furthermore, enzyme activity on the soil surface of chamber II was analysed. N translocation was determined based on $\delta^{15}N$ values in soil of chamber I after 7 d.

Respiratory and transcriptional response of *A. bisporus* to drought stress and riboflavin were assessed in separate mesocosm experiments under drought or no drought conditions and with or without 50 µM riboflavin addition. Transcriptomic changes and hyphal riboflavin contents were assessed by high-density microarray hybridization and high performance liquid chromatography, respectively.

A. bisporus and *S. commune* redistributed water at a flow velocity of about 0.3 and 0.43 cm min^{-1}, respectively, per hyphae, resulting in a water potential increase of the bulk soil. The amount of transferred water was similar to capillary transport in sterile sandy soil. Fungal hyphae have the potential to overcome capillary barriers between dry and wet soil

compartments via HR. HR seems to partly compensate water deficiency, if water is available in other zones of the mycelia network. And HR is likely a mechanism behind higher drought resistance of soil fungi compared to bacteria. Further, HR is an underrated pathway of water transport in soils and may lead to a transfer of water to zones of high fungal activity.

While HR by *A. bisporus* strongly enhanced C mineralisation by 2800% and enzymatic activity by 250-350% in the dry soil compartment, HR by *S. commune* only slightly increased C mineralisation and enzyme activity within 7 d. In addition, *S. commune* translocated N towards the substrate for hyphal growth thereon, whereas *A. bisporus* translocated N within the mycelial network towards the wet soil. The impact of fungal HR on C mineralisation and N translocation in dry soils seems to be species specific and related to the resource usage strategy.

The transcriptional response of *A. bisporus* to drought or riboflavin was mainly based on factors regulating transcription, translation and growth. This was even stronger in combined treatments. Further, riboflavin induced several protective mechanisms, methylglyoxal (cytotoxic byproduct of glycolysis) detoxifying lactoylglutathione lyase being most pronounced. Drought increased riboflavin content in hyphae about 5 times, with or without riboflavin addition. Without riboflavin addition, fungal respiration decreased by more than 50% at a water potential of about -20 MPa. Respiration remained about 2-3 times higher with riboflavin addition. These data indicate a stress priming function and a prominent role of riboflavin in drought responses of *A. bisporus*.

In conclusion, saprotrophic fungi have the potential for HR. Yet, the impact of fungal HR on C mineralisation and N translocation in dry soils seems to be species specific. The relevance of HR on ecosystem scales may therefore strongly depend on fungal community structure. Drought stress seems to mainly trigger enhanced protein biosynthesis and growth in *A. bisporus* which may in turn stimulate network extension and water redistribution. Further, riboflavin supports drought tolerance in *A. bisporus*.

Zusammenfassung

Eine Austrocknung der oberen Bodenschichten ist ein häufig auftretendes Phänomen und führt zu einer Reduktion der mikrobiellen Aktivität. Pilz-Gesellschaften sind dabei oft besser an Bodenaustrocknungen angepasst als Bakterien-Gesellschaften. Zugrundeliegende Mechanismen und allgemeine Reaktionen von filamentösen saprotrophen Pilzen auf Trockenstress sind bisher nur ungenügend untersucht. Ein Mechanismus könnte die Umverteilung von Wasser von feuchten zu trockenen Bodenzonen entlang von Wasserpotentialgradienten durch das Myzel von saprotrophen Pilzen sein („hydraulic redistribution“, HR).

Diese Arbeit hatte zum Ziel, das Potential von saprotrophen Pilzen für HR am Beispiel des undifferenzierten Myzels von *Agaricus bisporus* sowie für komplexere Netzwerkarchitekturen von *Schizophyllum commune* zu untersuchen. Weiterhin sollte der Einfluss von HR auf die C-Mineralisation und die N-Translokation durch das Myzel untersucht werden. Darüber hinaus war es das Ziel transkriptionelle und respiratorische Reaktionen von *A. bisporus* auf Trockenstress und/oder Riboflavinzugabe zu analysieren.

Die Fähigkeit für HR im Vergleich zu kapillarem Wassertransport und der Einfluss (von HR) auf die Mineralisation und Translokation wurden in Mesokosmen mithilfe von Markierungsexperimenten untersucht. Die Mesokosmen bestanden aus 2 Kammern und waren mit einem sandigen Boden befüllt und durch eine Luftlücke von 2 mm getrennt um Massenfluss von Wasser zu unterbinden. Nach einer Wachstumsphase von 6 Wochen wurden beide Kammern auf ein Wasserpotential von ca. -9.5 MPa getrocknet. Anschließend wurde Kammer I wieder auf Feldkapazität gebracht, während Kammer II trocken blieb. Bei einem Set von Mesokosmen wurde Kammer I mit Deuterium markiertem Wasser bewässert und anschließend wurden über 3 Tage Bodenproben aus Kammer II genommen und auf ^{2}H-Gehalte analysiert.

Ein separates Mesokosmenset wurde in Kammer II mit markiertem Pflanzenmaterial behandelt. Über 7 Tage wurden CO_2-Proben genommen und auf den ^{13}C-Gehalt untersucht. Außerdem wurde die Enzymaktivität an der Oberfläche von Kammer II analysiert und die N-Translokation basierend auf den ^{15}N-Gehalten in Kammer I nach 7 Tagen berechnet. Die transkriptionellen und respiratorischen Reaktionen von *A. bisporus* auf Trockenstress und Riboflavin wurden in seperaten Mesokosmenexperimenten untersucht. Trockenstress wurde mit oder ohne Behandlung der Hyphen mit 50 µM Riboflavin initialisiert. Veränderungen des Transkriptoms wurden mithilfe von Microarray-Hybridisierung und Riboflavingehalte in den Hyphen hingegen mittels HPLC analysiert.

A. bisporus und *S. commune* verteilten Wasser mit einer Geschwindigkeit von 0,3 bzw. 0,43 cm min^{-1} um. Die Menge des transportierten Wassers war vergleichbar mit kapillarem Wassertransport in sterilem sandigem Boden. HR scheint Wassermängel ausgleichen zu können, wenn in anderen Bereichen des Myzels Wasser verfügbar ist. Daher ist HR wahrscheinlich mitverantwortlich für die höhere Resistenz von Pilzen gegenüber Trockenheit im Vergleich zu Bakterien. HR ist außerdem ein unterschätzter Wassertransportweg in Böden, der zu einem direkten Transfer in Zonen hoher pilzlicher Aktivität führen und kapillare Barrieren überbrücken kann.

HR durch *A. bisporus* führte zu einem Anstieg der Mineralisation um 2800% und der Enzymaktivität um 250-350% in der trockenen Kammer. Im Fall von *S. commune* dagegen stieg die Mineralisation und die Enzymaktivität in den 7 Tagen nur geringfügig an. Außerdem transportierte *S.commune* N in Richtung des Substrats und nutzte es für dessen Besiedlung, während *A. bisporus* N aus dem Substrat in Richtung von Kammer I transportierte. Der Einfluss von HR auf die Mineralisation und Translokation scheint daher artspezifisch und von der Ressourcennutzung bestimmt zu sein.

Die transkriptionelle Reaktion von *A. bisporus* auf Trockenstress oder Riboflavin beruhte vor allem auf Faktoren, welche die Transkription, Translation und das Wachstum regulieren. Diese Wirkung war noch stärker in kombinierten Behandlungen. Darüber hinaus induzierte Riboflavin verschiedene Schutzmechanismen, wobei Lactoylglutathione-lyase (Abbau von zytotoxischem Methylglycol) am stärksten ausgeprägt war. Ohne Riboflavinzugabe brach die Respiration unter -20 MPa um mehr als 50% ein. Mit Riboflavinzugabe verblieb die Respiration 2-3-mal höher. Die Ergebnisse deuten auf eine Funktion von Riboflavin in der Trockenstressantwort von *A. bisporus* hin.

Zusammenfassend zeigte diese Arbeit, dass Pilze das Potential für HR haben, wobei der Einfluss von HR auf die C-Mineralisation und N-Translokation artspezifisch zu sein scheint. Die Auswirkung von HR auf Ökosystemebene ist daher vermutlich stark von der Pilzgesellschaft abhängig. Trockenstress scheint vor allem zu einer erhöhten Proteinbiosynthese und Wachstumsrate in *A. bisporus* zu führen. Dies könnte zu einer Zunahme der Netzwerkausbreitung führen und die Umverteilung von Wasser begünstigen. Des Weiteren erhöht Riboflavin die Trockentoleranz von *A. bisporus*.

Table of contents

Summary ..... **I**
Zusammenfassung ..... **III**
Table of contents ..... **V**
List of tables ..... **IX**
Abbreviations ..... **X**
Synopsis **1**
1.1 Introduction **1**
1.1.1 Drought and terrestrial ecosystems 1
1.1.2 The fungal kingdom 2
1.1.3 Fungi and soil processes 3
1.1.4 Mycelia networks of fungi: dispersal and nutrient transport 3
1.1.5 Fungal adaptions to drought: hydraulic redistribution and molecular adaptions 6
1.2 Objectives **10**
1.3 Materials and methods **11**
1.3.1 Overview 11
1.3.2 HR by saprotrophic fungi (study I, study II) 11
1.3.3 Quantification of HR 12
1.3.4 Mineralisation of organic matter 12
1.3.5 Analysis of soil enzyme activity 13
1.3.6 Determination of fungal biomass and N translocation 13
1.3.7 Transcriptional response of *A. bisporus* to drought and riboflavin (study III) 14
1.3.8 Respiratory activity 14
1.3.9 Riboflavin extraction 14
1.3.10 Transcriptome analysis 15
1.3.11 Data analysis 15
1.4 Results and discussion **16**
1.4.1 Quantification of HR by saprotrophic fungi (study I) 16
1.4.2 Impact of HR on C mineralisation and N translocation (study II) 20
1.4.3 Respiratory and transcriptional responses of *A. bisporus* to drought (study III) 25
1.4.4 Impact of riboflavin on drought tolerance of A. bisporus (study III) 29
1.5. Conclusions and perspectives **33**
1.6 References **36**
Manuscripts **47**

2. Redistribution of soil water by a saprotrophic fungus enhances carbon mineralisation 48
2.1 Abstract 49
2.2 Significance 49
2.3 Introduction 50
2.4 Result and discussions 51
2.5 Materials and Methods 56
2.5.1 General Setup 56
2.5.2 Quantification of hydraulic redistribution 56
2.5.3 Mineralisation of organic matter 57
2.5.4 Analysis of soil enzyme activity 58
2.5.5 Data analysis 59
2.6 Acknowledgements 60
2.7 References 60
3. Effect of water redistribution by two distinct saprotrophic fungi on carbon mineralisation and nitrogen translocation in dry soil 63
3.1 Abstract 64
3.2. Introduction 65
3.3 Materials and Methods 67
3.3.1 Eperimental Setup 67
3.3.2 Quantification of HR 68
3.3.3 C mineralization and enzyme activity 68
3.3.4 Determination of fungal biomass and N translocation 70
3.3.5 Data analysis 70
3.4 Results 72
3.4.1 Water redistribution by S. commune 72
3.4.2 Impact of HR by S. commune on enzyme activities and C mineralisation 73
3.4.3 N translocation during HR 75
3.6 Acknowledgements 79
3.7 References 80
4. Vitamin B_2 (riboflavin) increases drought tolerance of Agaricus bisporus 84
4.1 Abstract 85
4.2 Introduction 86
4.3 Materials and Methods 88
4.3.1 Experimental setup 88
4.3.2 Respiratory activity 88

4.3.3 Riboflavin extraction 88
4.3.4 Transcriptome analysis 89
4.3.5 Data analysis 90
4.4 Results 92
4.4.1 Impact of drought stress on hyphae 92
4.4.2 Primary assessment of transcriptional responses 93
4.4.3 Transcriptional responses to application of one factor 95
4.4.4 Transcriptional responses to application of two factors 97
4.4.5 Assessment of transcriptional control of riboflavin biosynthesis, uptake and processing 100
4.5 Discussion 102
4.5.1 Impact of drought stress on respiratory activity and hyphal riboflavin content 102
4.5.2 Transcriptional responses to drought or riboflavin addition 102
4.5.3 Impact of riboflavin addition on drought responses 103
4.5.4 Conclusions 104
4.6 Acknowledgements 107
4.7 References 108
4.8 Supporting Information 113
Contributions to the included Manuscripts 152
Acknowledgements 153
Publications 154
Declarations 155

List of figures

Fig. 1.1: **Schematic design of mesocosms consisting of 2 compartments** **11**

Fig. 1.2: **Hydraulic redistribution by hyphae of *S. commune* or *A. bisporus*.** **16**

Fig. 1.3: **Hydraulic redistribution by *A. bisporus* and *S. commune* in relation to time and air gap distance.** **17**

Fig. 1.4: **Enzyme activity on the soil surface per g fungal dry weight of *A. bisporus* or *S. commune*.** **20**

Fig. 1.5: **Enzyme activity on the soil surface of chamber II of mesocosms inoculated with *A. bisporus* in relation to air gap distance.** **21**

Fig. 1.6: **Cumulative carbon mineralisation.** **22**

Fig. 1.7: **Model for the link between the impact of HR by saprotrophic fungi on carbon mineralisation and nitrogen translocation and the fungal resource strategy.** **23**

Fig. 1.8: **Total respiration of the whole mesocosms inoculated with *A. bisporus* or *S. commune.*** **24**

Fig. 1.9: **Relationship between respiration rates and water potential.** **25**

Fig. 1.10: **Hyphae of *A. bisporus* grown A) with or B) without 50 µM riboflavin addition.** **29**

Fig. 2.1: **Hydraulic redistribution by hyphae of *A. bisporus*.** **51**

Fig. 2.2: **Enzyme activity on the soil surface of chamber II of mesocosms inoculated with *A. bisporus*.** **53**

Fig. 2.3: **Spatial distribution of enzyme activities on the soil surface of chamber II of mesocosms inoculated with *A. bisporus*.** **54**

Fig. 2.4: **Cumulative carbon mineralisation II of mesocosms inoculated with *A. bisporus*.** **55**

Fig. 3.1: **Hydraulic redistribution by hyphae of *S. commune*.** **72**

Fig. 3.2: **Enzyme activity on the soil surface per g fungal dry weight of mesocosms inoculated with *S. commune*.** **73**

Fig. 3.3: **Total respiration, 7 days after irrigation of chamber I.** **74**

Fig. 3.4: **Nitrogen isotope composition of soil extracted from chamber I of mesocosms inoculated with *A. bisporus.*** **75**

Fig. 3.5: **Content of Nitrogen and Carbon in recollected plant samples from chamber II, 7 days after irrigation of chamber I.** **75**

Fig. 4.1: **Relationship between respiration rates and water potentials.** **92**

Fig. 4.2: **Hyphal riboflavin content 14 days after irrigation stop.** **93**

Fig. 4.3: **Overview over gene ontology term enrichment analyses.** **94**

Fig. 4.4: **Differential gene expression between each pair of samples.** **95**

List of tables

Tab. 1.1: Gene enrichment analyses of the 5 most strongly induced and repressed genes between DC and WC mesocosms with information about biological function, if available. 26

Tab. 1.2: Gene enrichment analyses of the 5 most strongly induced and repressed genes between DR and DC mesocosms with information about biological function, if available. 30

Tab. 3.1: Ergosterol content and fungal biomass in soil samples of mesocosms inoculated with *A. bisporus* or *S. commune.* 73

Tab. 4.1: Gene ontology term enrichment analyses between DC and WC mesocosms (most specific GO terms). 96

Tab. 4.2: Gene ontology term enrichment analyses between WR and WC mesocosms (most specific GO terms). 97

Tab. 4.3: Gene ontology term enrichment analyses between DR and DC mesocosms (most specific GO terms). 99

Tab. 4.4: Gene ontology term enrichment analyses between DR and WR mesocosms (most specific GO terms). 100

Tab. S4. 1: Sample layout used for two-colour hybridization of the microarray. 113

Tab. S4.2: Change in water potential and respiration rates over time in days after stop of irrigation. 114

Tab. S4.3: Complete list of Gene Ontology term enrichment analyses between DC and WC mesocosms. 115

Tab. S4.4: Complete list of Gene Ontology (GO) term enrichment analyses between WR and WC mesocosms. 121

Tab. S4.5: Complete list of gene enrichment analyses of induced and repressed genes between DC and WC mesocosms with information about biological function, if available. 127

Tab. S4.6: Complete list of gene enrichment analyses of induced and repressed genes in microarray experiments between WR and WC mesocosms with information about biological function, if available. 131

Tab. S4.7: Complete list of Gene Ontology term enrichment analyses between DR and DC mesocosms. 135

Tab. S4.8: Complete list of Gene Ontology term enrichment analyses between DR and WR mesocosms. 140

Tab. S4.9: Complete list of gene enrichment analyses of induced and repressed genes in microarray experiments between DR and DC mesocosms with information about biological function, if available. 146

Tab. S4.10: Complete list of gene enrichment analyses of induced and repressed genes in microarray experiments between DR and WR mesocosms with information about biological function, if available. 148

Tab. S4.11: Gene enrichment analyses of genes coding for proteins associated with riboflavin biosynthesis, riboflavin transport as well as riboflavin processing. 151

Abbreviations

4-MNG	4-Methylumbelliferyl N-acetyl-β-D-glucosaminide
4-MC	4-methylumbelliferyl β-D-cellobioside
ANOVA	analysis of variance
BCAA	branched-chain amino acids
BCAT	branched-chain-amino-acid transaminase
C	carbon
CDS	coding sequences
CRAT	carnitine O-acetyltransferase
d	days
DC	drought control treatment
DFG	German Science Foundation
DNA	deoxyribonucleic acid
DOE-JGI	department of energy joint genome institute
DR	drought riboflavin treatment
DW	dry weight
EIF5A	eukaryotic translation initiation factor 5A-1
FAD	flavin adenine dinucleotide
Fig	Figure
FMN	flavin mononucleotide
GEO	gene expression omnibus
GLO1	lactoylglutathione lyase
GO	gene ontology
HAT	histone acetyltransferase
HPLC	high performance liquid chromatography
HR	hydraulic redistribution
hrs	hours
KMO	kynurenine-3-monooxygenase
LMM	linear mixed effect models
LYCH	lucifer yellow carbohydrazide
Med21	subunit 21 of the mediator complex protein
MGO	methylglyoxal

mRNA	messenger RNA
MUF	4-methylumbelliferone
N	nitrogen
n	sample size
n.s.	not significant
p	significance
r^2	adjusted coefficient of determination
rRNA	ribosomal RNA
RNA	ribonucleic acids
RNA-Seq	RNA sequencing
ROS	reactive oxygen species
RP	ribosomal proteins
sec	seconds
SEM	standard error of means
SD	standard deviation
T&T	translation and transcription
Tab	Table
TBCE	alpha-tubulin folding cofactor E
UCHL	ubiquitin carboxyl-terminal hydrolase
WC	wet control treatment
WR	wet riboflavin treatment

Synopsis

1.1 Introduction

1.1.1 Drought and terrestrial ecosystems

Periods of drought and the resulting soil desiccation are important abiotic stressors affecting organisms and ecosystems (Schimel et al., 2007). Drought is the major environmental stressor negatively affecting agriculture worldwide (Yang et al., 2010). Climate models even predict an increased frequency and magnitude of extreme weather events, including droughts, in the future (IPCC, 2007). The increase in mean annual temperature and the accompanying variations in spatio-temporal distribution of precipitation may have considerable impact on terrestrial ecosystem functions and soil processes in many parts of the world.

The negative consequences to be expected, among others in central Europe, were exemplarily demonstrated in the extreme drought event during the summer months of 2003 (Ciais et al., 2005). Such events strongly decline plant productivity and increase mortality of soil associated organisms. Further, drought affects the soil structure, i.e., by disruption of aggregates and destabilising of macropores due to shrinking, and induces hydrophobicity on soil surfaces (Cosentino et al., 2006; Mataix-Solera et al., 2007). The extent of the latter depends on the contents of clay and organic matter as well as microbial biomass and community structure (Haynes and Swift, 1990; Denef et al., 2001; Peng et al., 2007). Together, these factors can have substantial negative effects on C and N mineralisation after soil rewetting (Borken and Matzner, 2009).

Drought also limits diffusion of organic and inorganic soluble components as well as the mobility of extracellular enzymes and microorganisms in general (Borken and Matzner, 2009). Further, soil desiccation is generally accompanied by a decrease in soil respiration (Borken et al., 2003; Muhr et al. 2010), soil enzyme activity (Toberman et al., 2008; Herzog et al., 2013) as well as N mineralisation (Chen et al., 2011). In general, low water potentials lead to a limitation of microbial activity in soils and, under extreme conditions, to total inhibition (Schimel et al., 2007; Manzoni et al., 2012). Therefore, drought events are suggested to potentiellay turn temperate forest soils into transient net carbon sinks due to a strong reduction of organic matter decomposition by microbial activity (Borken et al., 2006). Drought stress can also alter soil microbial community composition (e.g., Sheik et al., 2011; Yuste et al., 2011). Impact of drought on microorganisms is thereby depending on amplitude and duration of the event. Drought can be counterbalanced up to a species-specific water potential threshold (Schimel et al., 1999). Below this threshold, mortality strongly increases (Sparling and Ross,

1988; van Gestel et al., 1992, 1993) and some organisms respond with the formation of resting stages (Chen and Alexander, 1973).

Already, sessile organisms in soils are frequently confronted with drought stress and will potentially even be more so in the future. However, they have evolved a variety of stress resistance, avoidance (i.e., maintaining relatively stable cellular water potentials at low soil water potentials) and tolerance strategies (i.e., maintaining normal cell functions at low cellular water potentials) to survive and adapt to low water potentials (Chavez et al., 2002; Cruz de Carvalho, 2008). Generally, drought stress is induced when water uptake, e.g., from the soil, cannot compensate water loss by the organisms, e.g., due to respiration (Hillel, 2003). Organisms follow two general strategies to cope with drought stress: restriction of water loss and/or promotion of water uptake (Kozlowski and Pallardy, 2002). The first includes morphological and physiological adaptions like increasing cell wall and cuticle thickness, the latter includes growth adjustments leading to increased biomass production or development of deep root systems to access moist soil horizons (Alster et al., 2013; Allison et al., 2013; Osakabe et al., 2014). Further, organisms respond with the production and/or uptake of secondary metabolites to cope with negative side effects of drought like induced production of reactive oxygen species (ROS).

While plants have been intensively studied with regard to drought responses and adaptations (e.g., Ingram and Bartels, 1996; Chaves et al., 2002; Tang et al., 2008; Osakabe et al., 2014), information on drought stress responses in fungi is scarce even though they are highly relevant for many ecosystems.

1.1.2 The fungal kingdom

The fungal kingdom is one of to the most diverse group of organisms on earth with an estimation of 0.8 to 5.1 million species, ranging from yeasts to lichenised fungi and highly complex cord-forming filamentous fungi (Blackwell, 2011). They belong to the eukaryotes and hence their cell biology is in many ways similar to that of plants and animals. Unique cell features of the fungal kingdom are, among others, the chitinous cell walls, the Spitzenkörper, and the presence of ergosterol as a component of the fungal plasma membrane. Fungi are classified into six phyla: Ascomycota, Basidiomycota, Glomeromycota, Blastocladiomycota, Chytriomycota and Neocallimastigomyctoa (Watkinson et al., 2015). Especially fungi belonging to the Ascomycota, Basidiomycota and Glomeromycota are intensively studied and have high relevance for human society. Some of this species are of high economic importance as a source of chemicals (e.g., antibiotics, organic acids and vitamins) or as food (e.g., *Agaricus bisporus*

(LANGE) IMBACH, the common mushroom) and in food processing (e.g., tempeh, tapioca, various kinds of chees) but also as plant or animal pathogens (Sharma, 2005). Further, fungi are widely distributed in all ecosystems and fulfil a broad spectrum of integral ecosystem services (Dighton, 2003).

1.1.3 Fungi and soil processes

In general, fungi are important regulators of soil structure and aggregation, nutrient availability and distribution, plant primary production as well as organic matter decomposition and mineralisation processes (Dighton, 2003). In systems where lignocellulosic plant components build the main input, like in forest ecosystems, fungal activity can dominate the microbial community and high densities of hyphae in litter and soil can be found (up to 800 m g^{-1} soil, Söderström, 1979). Two major groups of fungi have particularly high impact on soil processes: plant symbiotic mycorrhizal fungi and saprobic fungi. Mycorrhizal fungi strongly affect plant productivity, for example by supporting nutrient acquisition, especially in nutrient poor ecosystems, or by providing protection against pathogens (van der Heijden et al., 2008). Saprotrophic fungi are major agents of the soil nutrient cycle, mediate litter decomposition and strongly effect soil respiration (Crowther et al., 2012). Especially filamentous fungi are optimally equipped for litter decomposition. They have developed specialised enzymatic pathways for the degradation of lignocellulose complexes, the major structural components of vascular plants (Rayner and Boddy, 1988; Worrall et al., 1997; de Boer et al., 2005). Further, their hyphal growth form facilitates the penetration of plant tissues and is an essential adaption to a life in soil habitats (de Boer et al., 2005).

1.1.4 Mycelia networks of fungi: dispersal and nutrient transport

In forest soils, saprotrophic fungi have to deal with a heterogeneous distribution of nutrients and water in space and time (Fricker et al, 2008). Input of new resources by plant litter happens sporadically, often randomly and usually patchily. The same holds true for lateral soil water gradients due to a heterogeneous distribution of throughfall (Shachnovich et al. 2008). Saprotrophic fungi had to develop foraging strategies to guarantee an efficient exploitation of the heterogeneously distributed resource patches. In this respect, they can be distinguished into two major groups: species with a ‘resource-unit-restricted’ or a ‘non-resource-unit-restricted’ foraging strategy (Boddy, 1999). The first group is only capable to disperse by spores. The latter group can also extend from one nutrient source to the next as mycelium. The spectrum ranges from species with non-differentiated mycelium, i.e., only expending by radial hyphal growth, up to highly complex mycelia network organisations like mycelial cords, i.e., aggregations of predominantly parallel and longitudinally aligned hyphae, and rhizomorphs.

A mycelia network is developed by building hyphal connections between different resource patches and those networks are known to constantly rebuild themselves depending on changes in substrate and water availability (Fricker et al. 2008; Boddy et al. 2009). The networks can in some cases be rather long-lived and spread out over large areas from several square meters to many hectares (e.g., Thompson and Boddy, 1983; Smith et al., 1992; Ferguson et al., 2003). One extreme example is a genet (i.e., genetically identical individuals) of *Armillaria ostoyae* (ROMAGN.) HERINK with an estimated age of 1900-8650 years and covering over 965 hectares (Smith et al., 1992). Within the same genet, parts of the mycelia network can get separated from each other as result of rebuilding or for example due to grazing but can also reconnect in case of re-joining (Fricker et al, 2008). The networks are continuously reorganised based on active outgrowth in search for new resources, hyphal branching or fusion as well as regression and recycling of network regions without resource connection (Boddy, 1999; Booddy et al., 2007; Fricker et al., 2008). The latter are often partly preserved, however, and serve a ´sit and wait´ strategy in case new resources arrive (Wood et al., 2006; Fricker et al. 2008). The extent of hyphal recycling correlates positively with the recalcitrant of the resource, extent of hyphal scattering and the resulting time needed to consume the resource (Heaton et al., 2016).

Resource contact triggers considerable morphological changes and leads to a remodelling of the network. Connections of the new resource with previously colonised sources are strengthened while the general radial extension is reduced (Bolten et al., 1991; Boddy, 1993; Boddy, 1999; Donelly and Boddy, 1997). In the process, high amounts of biomass can be reallocated and contact to the new resource can trigger uptake, storage and redistribution of nutrients within the network (Watkinson et al., 2006). Depending on size and quality of the successful colonised resources, network extension is started again after some time (Booddy et al., 2007). A range of fungal species, mainly found in the phylum Basidiomycota, can show even more complex mycelia network organisations like formation of cords or rhizomorphs. Mycelia cords form hubs from which further mycelium grows out to assimilate mineral nutrients and transfer it back to the main cord system (Boddy, 1993; Olsson et al., 2003; Boddy et al., 2007). Such cords are mostly formed in substrates with high carbon and low nitrogen content, meaning that hyphae are often the main nitrogen source in the system (Jennings and Watkinson, 1982).

Within the mycelia network, nutrients and water are translocated depending on concentration gradients from sources to sinks, not necessarily coupled to mycelial growth (Lindahl et al., 2001). Translocation of nutrients and water in hyphae can occur bi-directional, either from the nutrient source towards the base of the mycelia network or towards the growing front due to a

creation of nutrient sinks by production of biomass or by active transport to favour degradation conditions (Boberg et al., 2014; Frey et al., 2000; Tlalka et al., 2002; Jennings, 1987; Olsson and Gray, 1998; Tlalka et al., 2003, Cairney, 1992). Processes affecting dynamics and direction of nutrient transport within mycelia networks are still not completely understood and especially species foraging strategy could strongly impact the potential for nutrient redistribution (Watkinson et al., 2006).

The translocation of nutrients and water is most effective in cords and much faster than in non-differentiated mycelium (Wells et al., 1990; Boddy, 1993; Boddy and Watkinson, 1995; Lindahl et al., 2001; Cairney, 2005; Tlalka et al., 2008; Watkinson et al., 2006; Boberg et al., 2010). Distances for nutrient translocation in fungal cords can be >1 m (Boddy, 1993) and translocation rates can be quite high (up to > 25 cm h^{-1} in cords interconnecting resources; Wells and Boddy, 1990). Therefore, fungal cords and rhizomorphs are considered as major highways for water and nutrient transport through soil (e.g., Duddridge et al., 1980; Eamus et al., 1985, Jennings, 1987; Cairney, 2005). The best known example in this respect is the so called 'wood-wide-web', a comparison to the world-wide-web allegorising the importance of an underground fungal resource network accessible by plants. The concept was first descript by Simard et al. (1997) and concluded from a net carbon flow between ectomycorrhizal tree seedlings. To make the metaphor complete, Leake (2005) demonstrated that some achlorophyllous plants can 'hack' into the system and extract carbon without supplying anything in return.

Nutrient and water translocation are not limited to ectomycorrhizal species and can also be observed in saprotrophic fungi (e.g., Tlalka et al., 2008; Watkinson et al., 2006). The mechanism of translocation is similar in both cases and even an exchange among symbionts and saprobionts is possible (Lindahl et al., 1999; Read and Perez-Moreno, 2003; Fricker et al, 2008). In fact, ectomycorrhizal and saprotrophic species are often closely related and some mycorrhizal species show saprotrophic potential (Hibbett et al., 2000; Perez-Moreno and Read, 2000; Hibbett and Binder, 2002; Talbot et al., 2008; Lindahl and Tunlid, 2015). Yet, mycorrhizal and saprotrophic species are often spatially separated in soils and seem to play independent roles in organic matter decomposition: mycorrhizal fungi are mostly related to nitrogen mobilisation and saprotrophic species are more important for carbon mobilisation (Hobbie and Horton, 2007; Lindahl et al., 2007; Talbot et al., 2013; Lindahl and Tunlid, 2015). In any way, fungi have high impact on carbon sequestration into the soil, either directly from plant associations (Leake et al., 2004) or due to litter decomposition (Frey et al., 2003).

Since biological processes in general strongly depend on soil water potentials (Schimel et al., 2007), knowledge about responses and adaptions of fungi to drought are of high importance considering the predicted increase in extreme weather events. Especially saprotrophic fungi have to deal with low water potentials at regular intervals, considering that they seem to be dominant in the upper organic layers of soils (Lindahl et al., 2007) and that those layers are frequently exposed to desiccation, e.g., during summer drought events.

1.1.5 Fungal adaptions to drought: hydraulic redistribution and molecular adaptions

Considering the whole soil microbial activity in non-tropical climates, one can observe that fungal communities are often less sensitive and better adapted to drought events compared to bacterial communities (Alster et al., 2013; Allison et al., 2013; Bapiri et al., 2010; Sheik et al., 2011; Yuste et al., 2011). This is primarily based on the need of bacteria for a constant supply of water for activity and mobility (Greenwood, 1967). Further, fungi have strong cell walls which reduce water losses and the walls can even be enhanced by cross-linking of polymers and thickening under stress (Schimel et al., 2007). While this is also true for a range of bacteria (especially gram-postive bacteria), filamentous fungi may have a unique morphological advantage in their mycelial nature.

The hyphal growth form may be one major adaptation of filamentous fungi to soil desiccation. It allows for the redistribution of water over long distances through mycelia networks along soil water potential gradients from wet to dry soil areas. This process is called hydraulic redistribution (HR). It was first described for root systems of the shrub *Artemisia tridentata* NUTTALL where redistribution from deep moist to shallow dry layers was observed when stomatas were closed (Richards and Caldwell, 1987). By now, the mechanism was found in a wide range of plant species (Caldwell et al., 1998), from arid (e.g., Armas et al., 2012; Prieto et al., 2010) to mesic temperate (Emerman and Dawson, 1996; Kurz-Besson et al., 2006) and tropical environments (Moreira et al., 2003; Scholz et al., 2008). Redistribution can not only occur upwards, but also downwards and lateral. HR can improve survival and nutrition uptake under drought in a variety of ways, e.g., by extending life span and activity of fine roots, root hairs and associated microorganisms in dry surface soils (Bauerle et al., 2008; Querejeta et al., 2003), by improving ion mobility and diffusion to roots (Dawson, 1997) and by favouring degradation processes of organic matter (Aanderud and Richards, 2009; Hawkins et al., 2009; Armas et al., 2012). A redistribution of water along water potential gradients can also be accomplished along plant associated mycorrhizal networks (Egerton-Warburton et al., 2007; Querejeta et al., 2003; Plamboeck et al., 2007). Even a transfer between plants via mycorrhizal networks connecting the roots of donor and receiver plants, similar to the carbon exchange in

the ‘wood-wide-web‘, is possible (Egerton-Warburton et al. 2007). The redistributed water can also substantially increase water availability in the surrounding soil by leakage from root or hyphal tips and hence be available for other organisms as well (Querejeta et al., 2003; Domec et al., 2004). While water translocation was also documented for non-mycorrhizal fungi, it was mostly attributed to variations in osmotic potential and not studied in connection to other soil processes (Brownlee et al. 1983; Granlund et al., 1985; Thompson et al., 1985; Jennings, 1987). The ability of saprotrophic fungi for HR would provide a direct and fast connection between water and nutrient sources in soils which would be hardly accessible to bacteria. This could have enormous impact on decomposition and mineralisation processes under drought conditions. In addition, as described above for nutrient translocation, HR may be affected by fungal foraging strategy and is probably more effective through mycelial cords than through single hyphae. This might also modify the impact of HR on C mineralisation.

Molecular adaptions of fungi to drought are even more scarcely analysed and data is only available for few species. Based on available data, two major groups of gene targets seem to be commonly triggered in fungi in response to drought: targets associated to oxidative stress protection and transcriptional regulation. The first group is especially relevant since prolonged water deficit is generally accompanied by an excessive production of ROS and hence oxidative stress (Smirnoff, 1993). ROS are highly reactive and can cause cell death by uncontrolled oxidation of cellular components (Cruz de Carvalho, 2008; Mittler, 2002). Most organisms have evolved a large variety of enzymatic and non-enzymatic processes to diminish harmful ROS effects. The major players in response to ROS are the enzymes superoxide dismutase, glutathione peroxidase and catalase (Ingram and Bartels, 1996). In addition, metabolites like glutathione and some vitamins can also have high importance as antioxidants (Mittler, 2002). Development of effective protective mechanisms against ROS seem to be highly important for drought stress tolerance in fungal species, e.g., in lichens (Kranner et al., 2008).

Accordingly, drought stress induced activities of enzymatic antioxidants (superoxide dismutase, catalase and peroxidase) as well as contents of non-enzymatic antioxidants (ascorbic acid and glutathione) in fruit bodies of *Auricularia auricula-judae* (BULLIARD) SCHRÖTER (Ma et al., 2014). Further, transcriptome analysis of a drought-adapted fungus isolated from the desert lichen *Endocarpon pusillum* HEDWIG revealed that drought stress induced the expression of genes involved in vitamin B_6 biosynthesis (Wang et al., 2015). Up-regulation of a gene involved in vitamin B_6 biosynthesis in response to oxidative stress was also observed in the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (BLASZKOWSKI, WUBET, RENKER AND BUSCOT) WALKER AND SCHÜßLER (Benabdellah et al., 2009). Vitamin B6 is an essential co-

factor of enzymatic reactions mostly related to amino acid metabolism but is also an antioxidant with potential to protect against ROS (Bilski et al., 2000). Another vitamin with antioxidative potential is riboflavin (vitamin B_2). Riboflavin is primarily important as a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both are redox cofactors and play a major role in the electron acceptor chain in the mitochondria (Sandoval et al., 2008). Its ability as an electron acceptor makes it also suitable as an antioxidant (Ashoori and Saedisomeolia, 2014). In addition, FAD derived from riboflavin is essential for many enzymes, like glutathione reductase, which reduces H_2O_2 (Beutler, 1969; Gill and Tuteja, 2010). Furthermore, riboflavin seems to generally stimulate production of antioxidative components in plants (Mori and Sakurai, 1995; Taheri and Tarighi, 2010). The ability of riboflavin to counteract drought stress has already been described for tobacco plants, where low levels of riboflavin improved drought tolerance (Deng et al., 2014). Fungal riboflavin production has been reported to be linked to cellular stress (Boretsky et al., 2007; Schlösser et al., 2007). Hence, similar to plants, riboflavin may have an antioxidative function and a role in drought stress responses in filamentous fungi.

Furthermore, results by Raffaello et al. (2014) showed that in the wood decaying fungus *Heterobasidion annosum* (FRIES) BREFELD abiotic stress induced mainly gene expression of targets belonging to the antioxidative cytochrome P450. Cytochromes are another group of compounds with high antioxidative potential due to their high electron acceptor ability (Pereverzev et al., 2003).

In addition to ROS protection, drought seems to trigger mainly gene expression changes of factors regulating transcription and translation in fungi. The fungal symbiont of *E. pusillum* showed increased expression of several ribosomal proteins (Wang et al., 2015). As part of the ribosome they are responsible for protein biosynthesis but they are also considered to be important for the regulation and control of mRNA translation (Lindström, 2009). Several fungal specific transcription factors were differentially expressed in *H. annosum* in response to abiotic stress (Raffaello et al., 2014). In general, activity of such regulation factors is probably required for the further controlled expression of specific mRNAs in response to the stress. In addition, a general up-regulation of expression is likely also needed to counterbalance damage and loss of DNA, RNA and proteins due to drought stress (Wang et al., 2015).

Further drought response strategies seem to be the production of chaperones and transport proteins. Chaperones play an important role in dealing with cellular problems resulting from protein misfolding and damage under drought by either repairing or degrading them. Up-

regulation of corresponding gene targets were observed in the fungal symbiont of *E. pusillum* as well as *R. irregularis* (Estrada et al., 2013; Wang et al., 2015). In addition, enhanced expression of transport proteins in response to drought was observed in *H. annosum* with an up-regulation of transporters of the major facilitator superfamily 1 (MFS-1) and in *R. irregularis* with an induced expression of genes coding for aquaporins (Li et al., 2013; Raffaello et al., 2014). Aquaporins are present in a wide range of organisms. They channel water movement through biological membranes with a 10–100 times higher velocity than by diffusion alone and hence are important for osmoregulation (Agre et al., 2002). In addition, they are probably required for the symplastic water transport at the observed fast flow velocity in fungal mycelia networks. Associated genes were found in a variety of fungi, including *A. bisporus* (e.g., Morin et al., 2012).

Data on transcriptional responses of litter degrading filamentous fungi to drought are not available so far. Knowledge of the mechanisms behind drought stress response in this group would be of great interest to predict variations in soil biogeochemistry with regard to the predicted increase in number of drought events.

1.2 Objectives

This thesis aims to give new insights into physiological and molecular drought responses and adaptions of saprotrophic filamentous fungi in the prospect of soil processes. This will be processed in 3 case studies with the goals to (1) determine and quantify the potential for HR of the saprotrophic fungi *A. bisporus* with a non-differentiated mycelium and the mycelial cord-former *Schizophyllum commune* FRIES, (2) study the impact of fungal HR on C mineralisation and N translocation, and (3) analyse the general transcriptional responses of *A. bisporus* to drought stress and the impact of the antioxidant riboflavin on drought tolerance.

The following hypotheses were tested:

1. Saprotrophic fungi have the potential for HR.
2. HR triggers mineralisation of organic matter under drought conditions.
3. HR and its impact on C mineralisation as well N translocation in dry soils is linked to the fungal foraging strategy.
4. Transcriptional drought responses of the saprotrophic fungus *A. bisporus* are mainly based on mechanisms associated to oxidative stress protection and transcriptional regulation.
5. The antioxidant riboflavin improves the drought tolerance of *A. bisporus*.

1.3 Materials and methods

1.3.1 Overview

Mesocosm studies using labelling experiments were carried out to analyse the potential for HR of the saprotrophic fungi *A. bisporus* and *S. commune* (study I) and to study the impact of HR on C mineralisation and N translocation (study II).

The general transcriptional responses of the saprotrophic fungus *A. bisporus* to drought and the impact of the antioxidant riboflavin on drought tolerance were analysed in mesocosm experiments with an automated irrigation and with or without riboflavin addition (study III).

1.3.2 HR by saprotrophic fungi (study I, study II)

Experiments were carried out in mesocosms (adapted from Querejeta et al. (2003), Fig. 1.1) with 2 chambers made of Makrolon (à 6×20×15 cm), filled with a steam sterilised mixture (1:1 v/v) of loamy soil and quartz sand. The bulk density of the soil was 1.33 g cm^{-3}. The saturated hydraulic conductivity of the soil was 3.826 cm day^{-1} (with m= 1-1/n; van Genuchten et al., 1991). A 2 mm thick air gap between chambers prevented capillary flow of water and was stabilised by 2 stainless steel mesh screens (pore size: 160 µM). Fungal cultures of *A. bisporus* and *S. commune* were grown on malt extract peptone agar at 14°C. Chamber I was inoculated by placing a 1 cm^2 agar plate with fungal hyphae close to the air gap into the soil at a depth of approx. 2 cm.

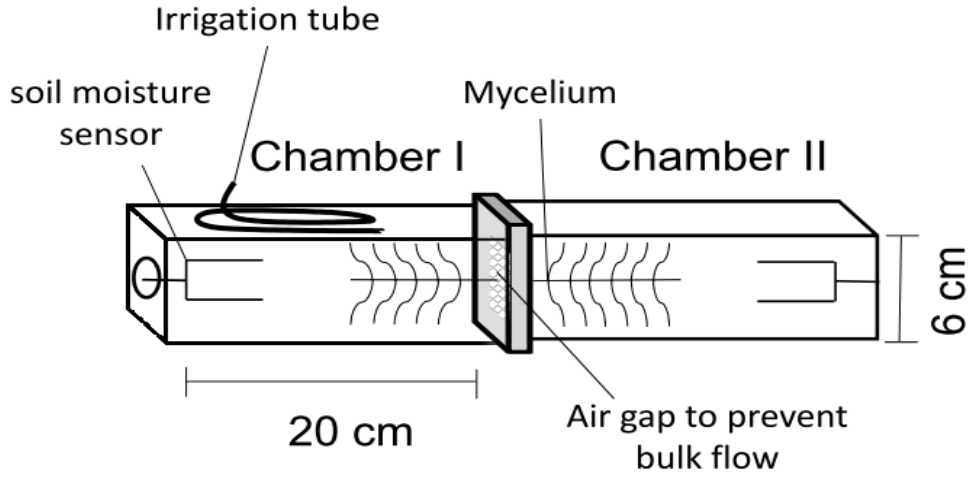


Fig. 1.1: Schematic design of mesocosms consisting of 2 compartments.

A. bisporus is one of the best studied filamentous fungal species, with a complete available genome (Morin et al., 2012). It is a secondary litter decomposer with an optimal C:N resource ratio for growth of 20:1 (Chang and Miles, 1986). *S. commune* is one of the most widespread fungi on earth and has the ability to form mycelial cords (Balaș and Tănase, 2012). *S. commune*

is adapted to high C:N ratios and a white-rot fungus of many tree species (Schmidt and Liese, 1980). Additionally, *S commune* grows on litter (Olsson and Gray, 1998; Seephueak et al., 2011) and is associated to the decomposition of roots (Zhang et al., 2009; Glen et al., 2014).

Mesocosms were kept at field capacity with a growth medium (Nazrul and YinBing, 2011) at +23°C for 6 weeks. Afterwards, soil in both chambers was desiccated to approx. -9.5 MPa for 6 to 8 weeks. Subsequently, only chamber I was rewetted to field capacity with tap water while chamber II of each unit remained dry. HR was prevented in the controls by cutting the hyphal bridges between the chambers prior to rewetting. The number of hyphae bridging the air gap was estimated by extrapolating hyphae from 10 openings. All cords were counted as well. Two experiments with separate sets of mesocosms were conducted to quantify HR and to determine the impact of HR on C mineralisation and N translocation between chambers.

1.3.3 Quantification of HR

To quantify HR, chamber I was rewetted with deuterium-labelled water (3 atom% enrichment). In addition, lucifer yellow carbohydrazide (LYCH; 0.1% w/v) was added to the water as a marker for apoplastic translocation (Plamboeck et al., 2007). Mesocosms were then air-tightly closed and only opened for sampling of soil cores. Samples were taken from chamber II in 5, 10, and 15 cm distance to the air gap after 24, 48, and 72 hrs. Water was extracted from the soil by cryogenic vacuum extraction. Deuterium analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using thermal conversion/isotope-ratio mass-spectrometry (TC-IRMS; IRMS: delta V advantage, Thermo Fisher Scientific, Bremen, Germany; pyrolysis oven: TG pyrolysis oven HTO, HEKAtech, Wegberg, Germany; interface: ConFlo IV, Thermo Fisher Scientific, Bremen, Germany). The amount of redistributed water was calculated using a 2-end-member mixing model (Dawson et al., 2002). In total, 4 HR as well as 4 control mesocosms were inoculated with *A. bisporus* or *S. commune* and treated with deuterium labelled water. Further, 2 non-inoculated mesocosms were treated like above to measure capillary water transport in the soil along the water potential gradient. These mesocosms had no air gaps or mesh screens and were filled throughout with sterile homogenised soil. Water transport between chambers was calculated per contact surface (HR and control= 47.1 cm²; capillary transport= 60 cm²).

1.3.4 Mineralisation of organic matter

CO_2 efflux was used to estimate the impact of HR on mineralisation of organic matter under drought conditions. Double labelled plant material (*Triticum aestivum* L. green shoots; >97 atom% ^{13}C; >98 atom % ^{15}N; C: 39%; N: 3.8%; C/N ratio: 10; 5 ground samples of 20 mg each)

was placed on the soil surface of chamber II at regular intervals of 4 cm, shortly before rewetting chamber I. Mescosms were then closed air-tightly and were not opened for 7 days. CO_2 effluxes were hourly measured using the dynamic closed-chamber technique (Rochette et al., 1997, infrared gas analyser: LiCOR 820, Licor, U.S.A.). Mesocosms were purged with CO_2 free synthetic air between measurements to reduce CO_2 concentrations. In addition, the extracted air was collected every 12 hrs for $\delta^{13}C$ analysis to determine the mineralisation of the plant material in chamber II. ^{13}C analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using GC-Combustion-IRMS (GC: Trace GC 2000, CE Instruments, Milano, Italy; interface: GC-combustion III, Thermo Fisher Scientific, Bremen, Germany; IRMS: delta plus, Thermo Fisher Scientific, Bremen, Germany). In total, 5 or 6 HR mescosms with intact fungal connections as well as control mescosoms were inoculated with *A. bisporus* or *S. commune,* respectively, and treated with labelled plant material.

1.3.5 Analysis of soil enzyme activity

Soil enzyme activity was analysed using soil zymography (Spohn et al., 2013; Spohn and Kuzyakov, 2013). N-acetylglucosaminidase and cellobiohydrolase activities were analysed using 4-methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MNG) and 4-methylumbelliferyl β-D-cellobioside (4-MC), respectively. Polyamide membrane filters were saturated with 4-MNG or 4-MC and placed at 20°C in turn on top of a 1% agarose gel attached to the soil surface of chamber II, starting with 4-MNG (filters and gel: 4 stripes à 2x11 cm, placed in between the plant material samples). The membrane filters were extracted after 25 min for 4-MNG and 20 min for 4-MC and illuminated on a fluorescent transilluminator in the dark (wavelength: 355 nm, Desaga GmbH, Wiesloch, Germany). Enzyme activates were calculated as µg MUF released per mm² soil area and fungal biomass (g) within one hour.

1.3.6 Determination of fungal biomass and N translocation

Soil cores were taken and remains of the labelled plant material were quantitatively recollected after soil enzyme activity measurements. Fungal biomass was determined from soil cores by ergosterol extractions according to Djajakirana et al. (1996). Ergosterol detection was conducted by high performance liquid chromatography (HPLC, System Gold 125 Solvent Module, Beckman Coulter, Brea, U.S.A.; column: MZ Spherisorb ODS-2 C18, 150 x 3 mm, MZ Analysentechnik, Mainz, Germany) at a detection wavelength of 282 nm (System Gold 166 UV-Detector, Beckman Coulter). Ergosterol extractions were also performed for fungal culture samples and related to the fungal dry weight (*A. bisporus*: 195 µg ergosterol g^{-1} fungal DW; *S. commune*: 186 µg ergosterol g^{-1} fungal DW). Fungal biomass was expressed as g fungal DW per g soil DW.

Remaining soil samples were dried at 60°C for 48 hrs, ground in ball mills, and analysed for ^{13}C and ^{15}N isotope abundance. $\delta^{13}C$ as well as $\delta^{15}N$ analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using an elemental analyzer – isotope-ratio mass-spectrometer linkage (EA-IRMS; EA: NC 2500, CE Instruments, Milano, Italy; IRMS: delta plus, Thermo Fisher Scientific, Bremen, Germany; interface: ConFlo III, Thermo Fisher Scientific, Bremen Germany). Recollected plant samples were treated as described for soil samples and analysed for C and N contents with a CN analyser (Elementar Vario EL, Hanau, Germany).

1.3.7 Transcriptional response of *A. bisporus* to drought and riboflavin (study III)

Response of *A. bisporus* to drought stress and riboflavin was determined using gas-tight mesocosms (poly-acrylic cylinders, 17.1 x 9.5 cm, cf. Muhr et al., 2010) at +15°C. Five g of steam sterilised hay were inoculated by placing 1 cm² agar plates with *A. bisporus* on top. The mescosms were irrigated every 6 hrs with 2.5 ml for 3 or 5 weeks, depending on the treatment. Four treatments, each 4-fold replicated, were established: wet control (WC, irrigation with tap water), wet riboflavin (WR, irrigation with 50 µM riboflavin), drought control (DC, 3 weeks irrigation with tap water and desiccation for 2 weeks), drought riboflavin (DR, 3 weeks irrigation with 50 µM riboflavin and desiccation for 2 weeks).

1.3.8 Respiratory activity

CO_2 effluxes were measured using the dynamic closed-chamber technique. All mesocosms were ventilated with atmospheric air at 0.3 L min^{-1} between measurements. CO_2 concentrations were logged every 90 min over 60 secs in 10 sec intervals. Water potentials of the hay were calculated based on measured air humidity and temperature (SHT15, Sensirion AG, Switzerland) using the Magnus formula and the Kelvin equation (Magnus, 1844; Haurwitz, 1945). Day 10 was omitted since stable power supply could not be guaranteed on this day.

1.3.9 Riboflavin extraction

About 10 mg hyphal fresh weight were collected from all mescosms after 5 weeks. Hyphae were washed 3 times with 1 ml phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) and subsequently centrifuged for 5 min at 21.500 x g. The supernatant was discarded each time. Hyphae were then crushed 3 times on liquid nitrogen using pestles for microtubes and suspended in 200 µl 20% methanol. All subsamples were filtered using a 0.45 µM nylon syringe filter and combined. Hyphal riboflavin content was determined by HPLC (Agilent 1200 series, Agilent Technologies, CA, USA; column: MultoHigh 100 RP 18-5µ, 250 x 4 mm; pre-column: 20 x 4 mm, both CS-Chromatographie

Service GmbH, Langerwehe, Germany) at 30 °C. The mobile phase was 20 mM sodium acetate (pH 3) - 50% acetonitrile at 1 ml min^{-1}. Signals at 270 and 445 nm were recorded.

1.3.10 Transcriptome analysis

An independent experiment was conducted as described above (WC, WR, DC, and DR) to determine the transcriptional responses to drought stress and riboflavin addition. About 10 mg fresh weight of hyphae were collected 3 and 7 days after irrigation stop from each mesocosm, immediately shock frozen in liquid nitrogen and stored at -80 °C. Total RNA was extracted from hyphae using the RNeasy Microarray Tissue Kit (Qiagen GmbH, Hilden, Germany) and quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific). A high-density microarray was designed from annotated coding sequences of the *A. bisporus var bisporus* (H97) v2.0 release (Morin et al. 2012). Total RNA was labelled using the Low Input Quick Amp Labeling Kit (Agilent Technologies). Dye-swap experiments were included in the microarray design. Processed microarrays were scanned and quantified using a high-resolution microarray scanner and Agilent's feature extraction software (Agilent Technologies).

1.3.11 Data analysis

All statistical analyses and graphics were done using R 3.1.3 (R Developmental Core Team, 2015). Normality and homogeneity tested using Shapiro-Wilk-Test and Levene's-test, respectively. Analysis of Variance (ANOVA), followed by a Tukey-HSD-test as post-hoc test, was used to test for statistical differences among groups. Kruskal–Wallis tests with pair–wise Wilcox tests for posthoc comparisons were used, if conditions were not fulfilled.Variations in enzyme activities as well as C, N, $\delta^{13}C$ and $\delta^{15}N$ contents among treatments were analysed using linear mixed effect models (LMM) as implemented in the R package lme4 and likelihood ratio-tests (Faraway, 2005; Bates et al., 2015). Data values were ranked for LMM, if prerequisites were not fulfilled. One HR mesocosm with *S. commune* was excluded from all data analysis since soil water potential was far below -10 MPa when starting the experiment. Variations in riboflavin content were analysed using LMM as implemented in the R package nlme (Pinheiro et al., 2015). Pair–wise posthoc comparisons were conducted using the R package multcomp (Hothorn et al., 2008). Microarray normalisation and differential gene expression analysis was done with the R package limma (Smyth, 2005). The microarray data were deposited in NCBI's GEO archive under accession no. GSE73010. Variations in gene expression were only taken into consideration with a 2-fold change or higher (i.e., $\log_2$-values above 1 or below -1) and a p-value below 0.1. Gene targets were identified using available annotations from databases of Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes and InterPro.

1.4 Results and discussion

1.4.1 Quantification of HR by saprotrophic fungi (study I)

Both, *A. bisporus* as well as *S. commune* redistributed water from chamber I to chamber II. After 72 hrs, the amount of redistributed water was in both cases about 3 times higher in mesocosms with fungal connections than in the control mesocosms (Fig. 1.2). The amount of redistributed water by capillary water transport was similar to HR by *S. commune* but lower then by *A. bisporus*. The increase in water content within chamber II was generally higher in mesocosms inoculated with *A. bisporus* compared to *S. commune* with exception of the short distance area after 24 hrs (Fig. 1.3). In case of *A. bisporus*, variations in water content increase among HR and control mesocosms were visible after 48 hrs and pronounced after 72 hrs (Fig. 1.3a). In contrast, water content increase was continuously higher in HR compared to control mesocosms in case of *S. commune* (Fig. 1.3b). Further, increase in water content was higher with fungal HR compared to capillary transport in the short distance but similar or lower in the medium and long distance area to the air gap.

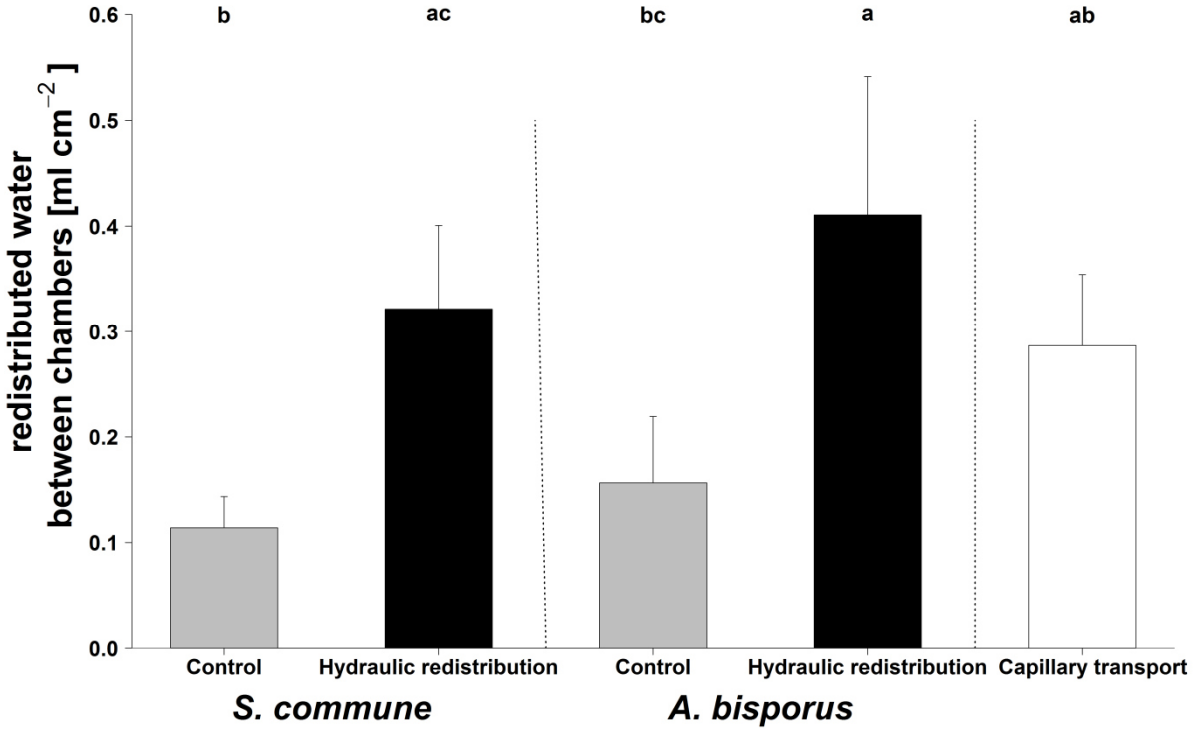


Fig. 1.2: Hydraulic redistribution (HR) by hyphae of *S. commune* or *A. bisporus*. Amount of water redistributed from chamber I to chamber II per contact surface, 72 hrs after the irrigation of chamber I. Calculation based on hydrogen stable isotope ratios. Black: active hydraulic redistribution (HR), grey: control with no fungal connection, white: soil without air gap and fungal inoculation. Mean + SD; n= 4 (control, HR), 2 (soil). Different letters indicate significant variances among groups.

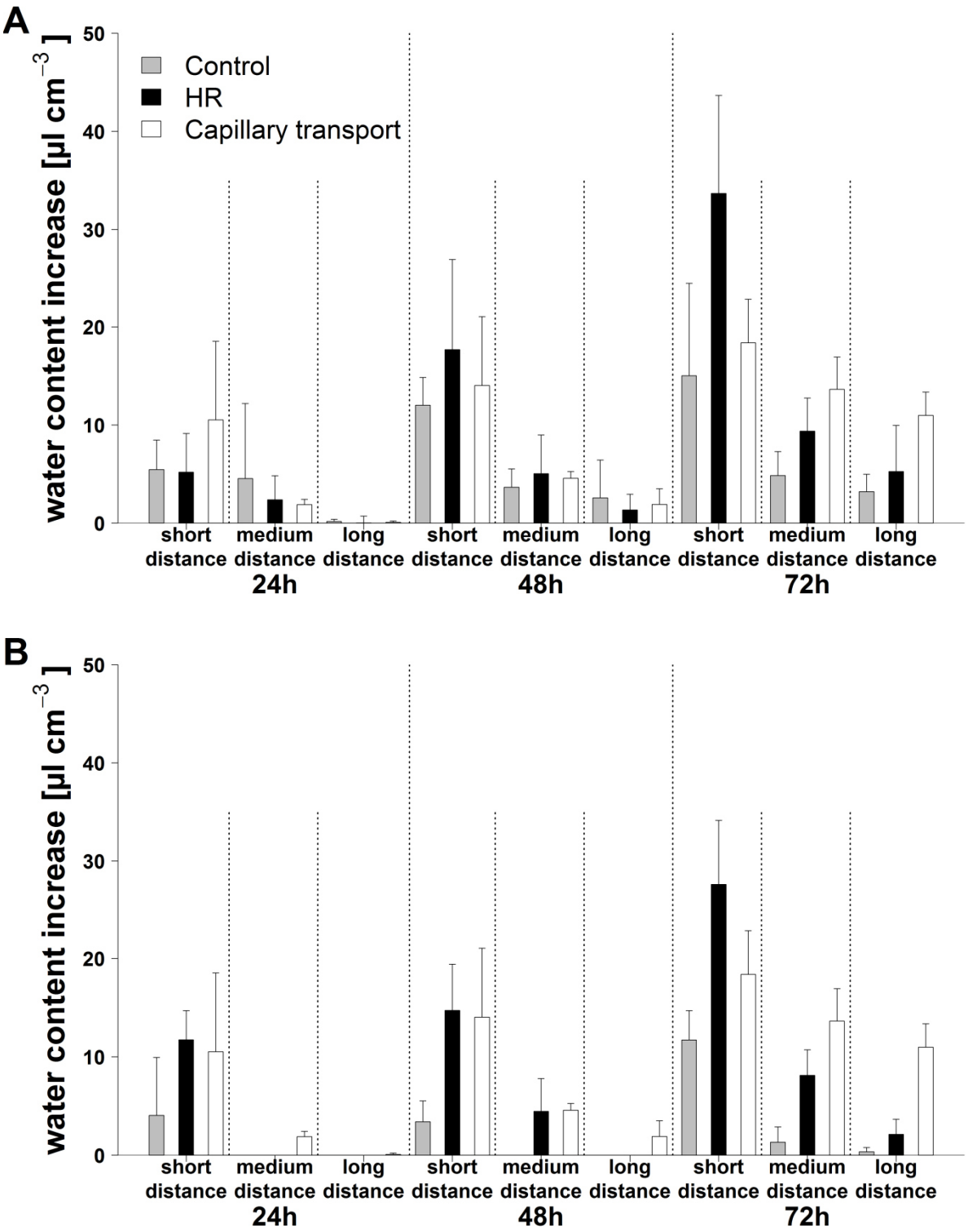


Fig. 1.3: Hydraulic redistribution (HR) by A) *A. bisporus* and B) *S. commune*. Water content increase in chamber II in relation to the air gap distance (short distance: 0-6.6 cm, medium distance: 6.6-13.2 cm, long distance: 13.2-20 cm), 24, 48 and 72 hrs after irrigation of chamber I. Calculation based on hydrogen stable isotope ratios. Black: active hydraulic redistribution (HR), grey: control with no fungal connection. Mean + SD; n= 4 (control, HR), 2 (soil).

Saprotrophic fungi clearly have the potential for HR. After 72 hrs, HR through hyphae resulted in an average water flux from chamber I to chamber II of 67 µl cm^{-2} day^{-1} with *A. bisporus* and 50 µl cm^{-2} day^{-1} with *S. commune* in comparison to 65 µl cm^{-2} day^{-1} by capillary water transport. The number of hyphae bridging the two chambers in the air gap was about 2300 cm^{-2} in case of *A. bisporus* and 880 cm^{-2} in case of *S. commune*, where in a few cases mycelia cords were observed (about 1-2 per mesocosm). An average flow velocity per hyphae was calculated of about 0.3 cm min^{-1} and 0.43 cm min^{-1}, respectively, using the Bernoulli's equation. The higher flow velocity in case of *S. commune* indicates that fungal HR is impacted by fungal foraging strategy and facilitated by mycelia cords.

Water transport by HR trough hyphae was similar to or in case of *A. bisporus* even higher then capillary water transport. Especially at the short distance HR clearly exceeded capillary transport. The amount of redistributed water can probably exceed soil capillary water transport in natural soils as well, considering the high hyphal densities in soils (Söderström, 1979) and the lower hydraulic conductivity of loamy and clayey soils in comaprsion to the here used sandy soil. HR by mycelia networks also has the potential to bridge capillary barriers in the soil, e.g., resulting from differences in pore volumes between horizons (Price and Whitehead, 2004). This could overcome a disrupted capillary rise from wet underlying horizons to upper dry organic layers during drought events. While saprotrophic fungi are often concentrated on the organic layers (Lindahl et al., 2007), the results show that even a short distance ingrowth into the deeper layer can result in substantial water redistribution at even higher rates than could be accomplished by capillary transport. Therefore, HR can strongly stimulate microbial activity in dry soils considering that organic layers have the highest microbial activity in soils and that biological processes depend on soil water potentials (Schimel et al., 2007; Šnajdr et al., 2008). Further, HR by saprotrophic fungi likely also contributes to a redistribution of water along lateral gradients induced by heterogeneous distribution of throughfall (Shachnovich et al., 2008).

The generally lower water content increase in control mesocosms inoculated with *S. commune* in comparison to *A. bisporus* might be based on variations in hydrophobicity. Mesocosms inoculated with *S. commune* showed considerable longer entrance times for water into the soil (in one case more than one hour). Droughts are known to induce hydrophobicity of soil surfaces which can be impacted by the microbial biomass (Haynes and Swift, 1990; Denef et al., 2001; Peng et al., 2007). Especially fungi are long known to potentially increase hydrophobicity (e.g., Shantz and Piemeisel, 1917; York and Canaway, 2000). Hydrophobins, small proteins forming hydrophobic coatings on surfaces, are considered as one major mechanism whereby fungi

increase soil hydrophobicity (Wessels, 1997; Wösten, 2001; Linder et al., 2005; Rillig, 2005). *S. commune* is especially known for the production of hydrophobins. They were first described in this species and can make up 10 % of the total protein reservoir (Wessels et al., 1991).

Analyses of the fluorescence staining with LYCH as a marker for apoplastic water translocation turned out to be not applicable. A development of a strong auto-fluorescence of *A. bisporus* was observed under drought at the same wavelengths as LYCH, which made it impossible to identify the fluorescence source.

1.4.2 Impact of HR on C mineralisation and N translocation (study II)

In general, *S. commune* showed significantly higher enzyme activity rates per g fungal biomass than *A. bisporus* (NAG= LMM: χ^2= 24.363, p<0.001; cellobiohydrolase= LMM: χ^2= 24.363, p<0.001; Fig. 1.4). The fungal biomass was thereby on average considerable lower in case of *S. commune* (7.3 mg fungal DW g^{-1} soil DW) compared to *A. bisporus* (41.5 mg fungal DW g^{-1} soil DW; see Tab. 3.1).

HR by *A. bisporus* increased enzyme activity on average by 350% for N-acetylglucosaminidase and by 250% for cellobiohydrolase compared to the controls (NAG= LMM: χ^2= 6.589, p=0.01; cellobiohydrolase= LMM: χ^2= 4.316, p=0.038). Enzyme activities decreased with increasing distance to the air gap when HR was active in *A. bisporus* (Fig. 1.5), while no such pattern was observed in the controls. HR by *S. commune* increased enzyme activity per g fungal biomass on average by 150% for NAG and by 50% for cellobiohydrolase compared to controls, but variations were not significant at the p<0.05 level (NAG= LMM: χ^2= 3.099, p= 0.078; cellobiohydrolase= LMM: χ^2= 1.694, p= 0.193).

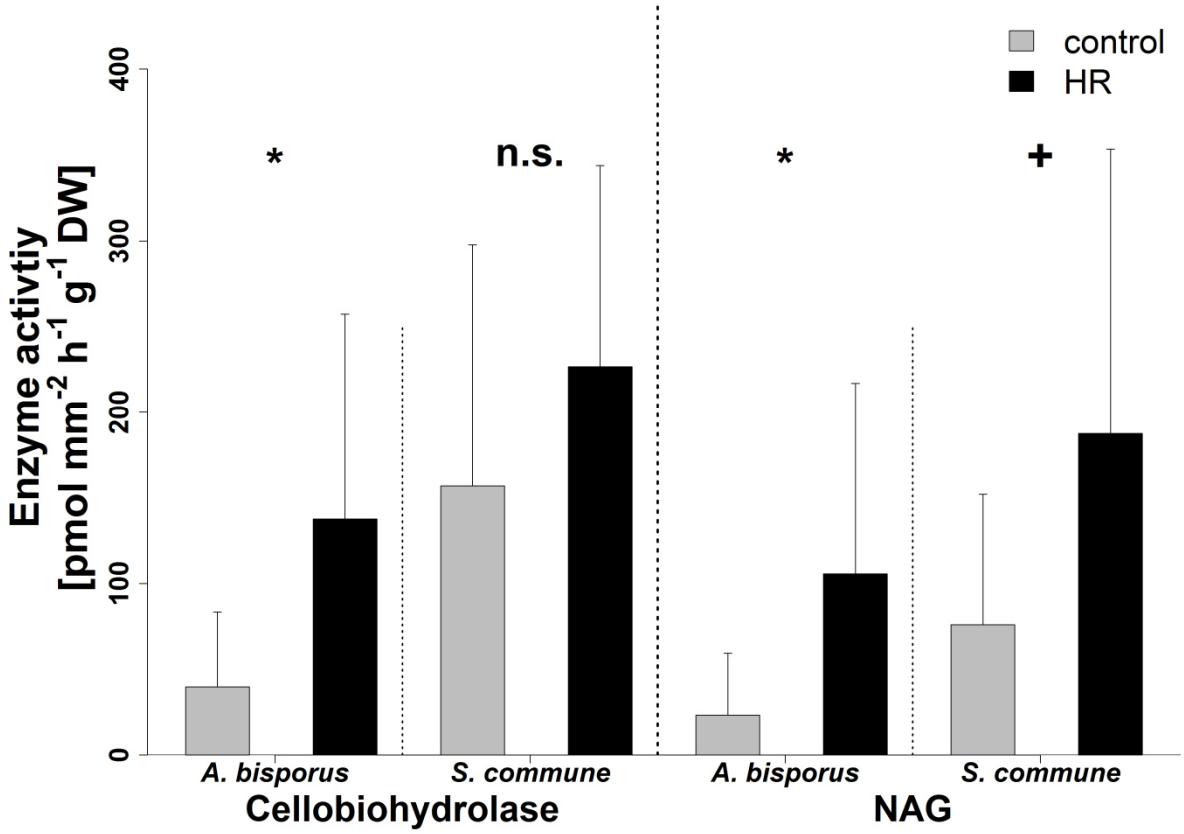


Fig. 1.4: Enzyme activity on the soil surface per g fungal dry weight of *A. bisporus* or *S. commune* (DW). Measured in chamber II, 7 days after irrigation of chamber I. Black: active hydraulic redistribution (HR), grey: control with no fungal connection, NAG= N-acetylglucosaminidase. Mean + SD; n= 6 (*S. commune*: Control), 5 (*A. bisporus*: Control + HR, *S. commune*: HR). *<0.05; + p<0.1; n.s.= not significant.

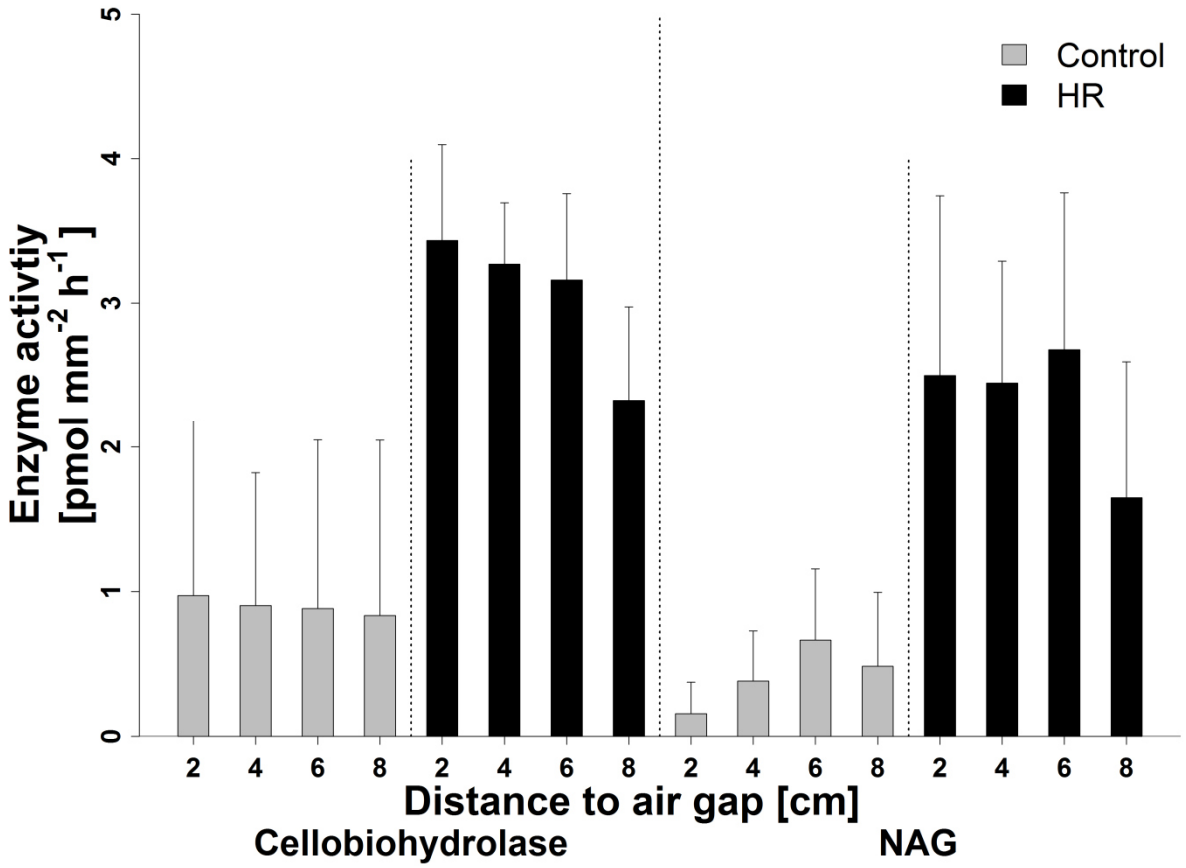


Fig. 1.5: Enzyme activity on the soil surface of chamber II of mesocosms inoculated with *A. bisporus*, 7 days after irrigation of chamber I. Black: active hydraulic redistribution (HR), grey: control with no fungal connection, NAG= N-acetylglucosaminidase. Mean + SD; n= 5.

HR by *A. bisporus* led to an increased C mineralisation of the labelled plant material that was apparent after 24 hrs and increased throughout the rest of the experiment (Fig. 1.6). After 168 hrs, the cumulative C mineralisation amounted to 59.7 g CO_2 kg^{-1} C with active HR and 2.1 g CO_2 kg^{-1} C in controls (Wilcoxon rank sum test: $W_{1,9}$=0, P<0.01). In contrast, no changes were found in the mineralisation of the ^{13}C labelled plant samples in the dry chambers between HR and controls in case of *S. commune* (cumulative C mineralisation after 168 hrs: HR= 8.7 g CO2 kg^{-1} C, control= 8.1 g CO2 kg^{-1} C; Wilcoxon rank sum test: $W_{1,10}$= 11, p= 0.537).

HR trough mycelia networks clearly has impact on soil processes. This is particularly noticeable in case of *A. bisporus* where HR can strongly enhance carbon mineralisation and enzymatic activity in dry soils. In addition, HR can at least partly compensates water deficiency, if water is available in other zones of the mycelia network. The strong increase in C mineralisation demonstrates the potential of HR by saprotrophic fungi to affect soil processes during drought events.

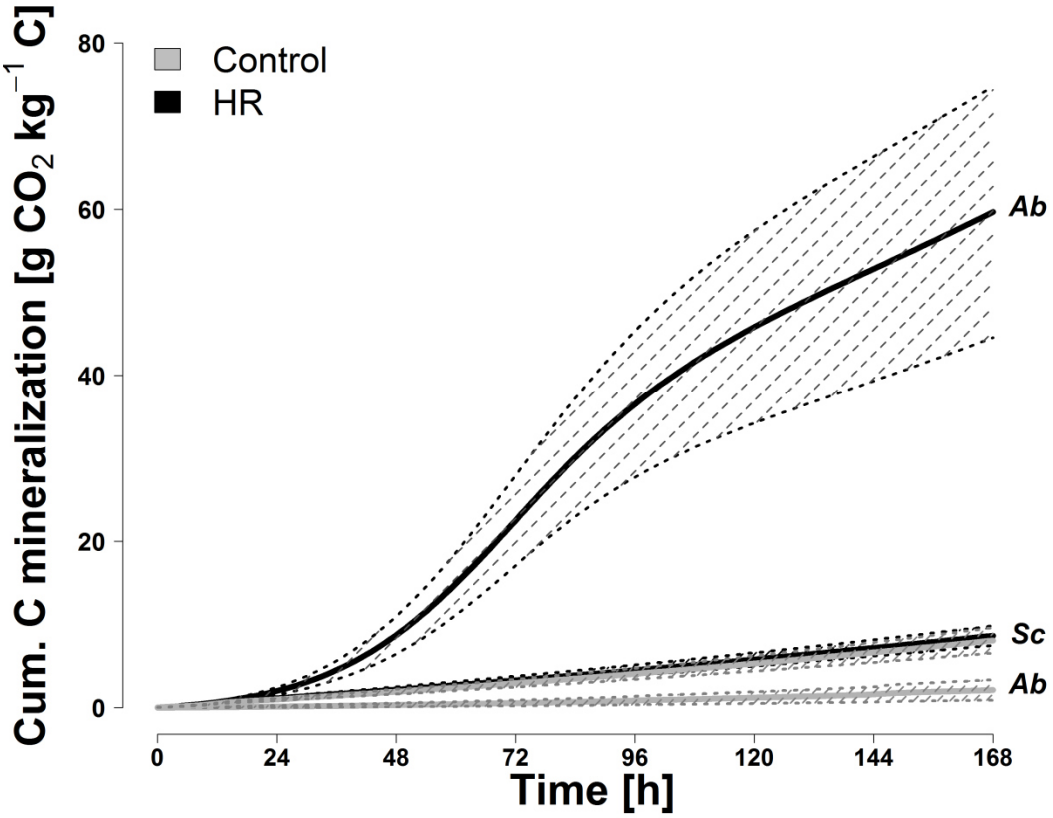


Fig. 1.6: Cumulative carbon mineralisation. Calculation based on ^{13}C-CO_2 efflux from labelled plant material in chamber II, following irrigation of chamber I. Black: active hydraulic redistribution (HR), grey: control with no fungal connection. *Ab*= *A. bisporus*, *Sc*= *S. commune*. n= 6 (*S. commune*: Control), 5 (*A. bisporus*: Control + HR, *S. commune*: HR), Solid lines= mean values, dashed lines= SEM).

However, how HR influences C and N turnover in soil appears to be species specific. The impact of HR on mineralisation of organic matter by *S. commune* was considerably lower than by *A. bisporus*, although *S. commune* showed the higher potential for HR. Further, only *A. bisporus* translocated N from chamber II to chamber I against the soil water gradient (LMM: χ^2= 6.233, p= 0.013; Fig. 3.4), accompanied by a simultaneous decrease in N content of the added plant material (N: LMM: χ^2= 4.297, p= 0.038; C: LMM: χ^2= 0.323, p= 0.570; Fig. 3.5). In contrast, N translocation towards chamber I was not observed for *S. commune* (LMM: χ^2= 0.468, p= 0.494). On the contrary, C and N contents even increased in the newly added plant material in result of hyphal growth thereon (N: LMM: χ^2= 3.209, p= 0.073; C: LMM: χ^2= 3.574, p= 0.059). Differences in impact of HR on C mineralisation and N translocation between the species might be related to the foraging strategy and especially the resource usage (see Fig. 1.7). In the plant kingdom, 2 major resource strategies can be distinguished: an acquisitive resource strategy, i.e., a rapid acquisition and depletion of resources, and a conservative resource strategy, i.e., conservation of resources within protected tissues and slow depletion rates (Díaz et al., 2004; Birhane et al., 2014). This trade-off between specialisations is

applicable for various resources (e.g., water, nutrients, and light) and seems to be mostly independent of environmental framework conditions (Díaz et al., 2004). The results suggest that the same may hold true for fungi.

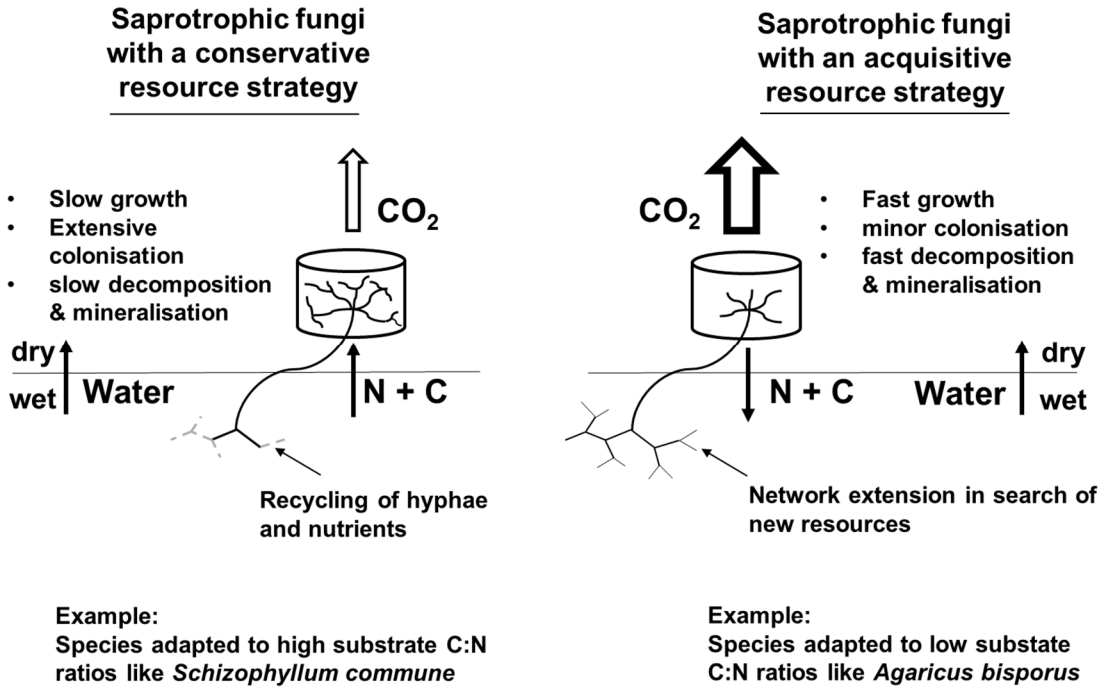


Fig. 1.7: Model for the link between the impact of HR by saprotrophic fungi on carbon mineralisation and nitrogen translocation and the fungal resource strategy.

A. bisporus has a low growth optimum C:N ratio of about 20:1 (Smith and Hayes, 1972; Chang and Miles, 1986) and also frequently colonises dung from herbivores (Kerrigan et al., 1998). Hence, the species is probably adapted to sources with relatively high amounts of easily accessible N and a fast relocation of N into other parts of the mycelia network to amplify network extension in search for new sources. Rapid network extension also requires high amounts of C for fast growth, which explains the strong enhancement of C mineralisation. Therefore, *A. bisporus* seems to follow an acquisitive resource strategy, resulting in a rapid acquisition and depletion of resources as well as fast network growth rates (cf. Díaz et al., 2004; Birhane et al., 2014).

In contrast, *S. commune* is adapted to substrates with high C:N ratios (Schmidt and Liese, 1980). Fungi invading such substrates are often adapted to slow degradation processes (Yang et al., 1980). They are also known to translocate nutrients towards new nutrient sources, either indirectly by growth or also directly by active transport to facilitate degradation (Boberg et al., 2014; Frey et al., 2000; Tlalka et al., 2002). Hence, total N and C content increases in the new source due to a redirection of nutrients and biomass towards the growing front of the mycelia network. *S. commune* seemed to be focused on the colonisation and conservation rather than on

mineralisation of the new source, independent of HR. Therefore, *S. commune* seems to follow a conservative resource strategy, leading to a conservation of resources by colonisation and consequently slow resource depletion and network growth rates (cf. Díaz et al., 2004; Birhane et al., 2013). This would be in accordance with the low C mineralisation rates of the labelled substrates in mesocosms inoculated with *S. commune*. Variations in C mineralisation and N translocation due to HR might only be visible over longer periods after a comprehensive colonisation of the resource.

However, a strong increase by 50% of total respiration with HR compared to controls in case of *S. commune* suggests that HR affects the biological activity of *S. commune* also on the short time scale (Wilcoxon rank sum test: $W_{1,10}$= 3, p= 0.03; Fig. 1.8). Variations seem thereby to be limited on the older parts of mycelial networks where recycling of hyphae is probably enhanced. Fungi are known to constantly rebuild their mycelial networks depending on substrate and water availability. Older hyphae are degraded for a transfer and reuse at the growing front (Fricker et al., 2008; Boddy et al., 2009). Since extent of hyphal recycling positively correlates with extent of hyphal scattering (Heaton et al., 2016), the lower fungal biomass of *S. commune* compared to *A. bisporus* might explain why an increase in total respiration was only visible in case of *S. commune*.

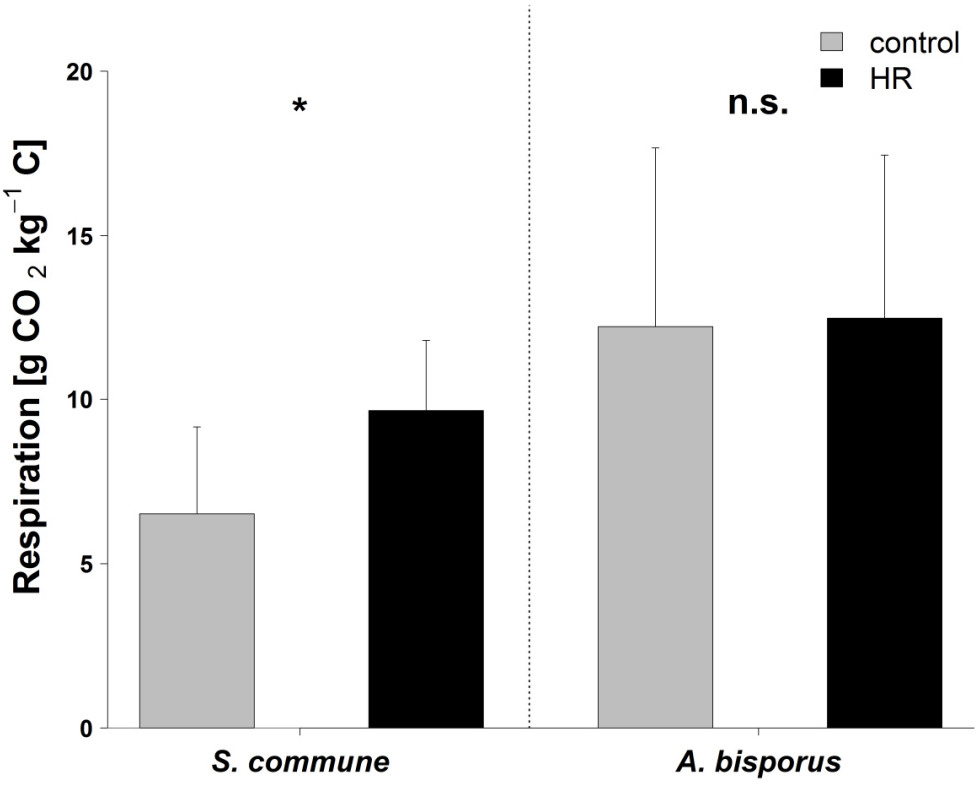


Fig. 1.8: Total respiration of the whole mesocosm inoculated with *A. bisporus* or *S. commune*, 7 days after irrigation of chamber I. Calculation based on CO_2 efflux. Black: active hydraulic redistribution (HR), grey: control with no fungal connection. Mean + SD; n= 6 (*S. commune*: Control), 5 (*A. bisporus*: Control + HR, *S. commune*: HR). * p<0.05, n.s.= not significant.

1.4.3 Respiratory and transcriptional responses of *A. bisporus* to drought (study III)

Respiration rates of drought-stressed hyphae of *A. bisporus* (DC) decreased sharply with decreasing water potentials (Fig. 1.9). Minimum respiration values were reached when water potential deceeded -40 MPa. This is comparable to thresholds found by Manzoni et al. (2012) of about -36 MPa for respiratory activity of litter decomposers. In result, an exponential relationship was found between water potential and respiration. Water potential and respiration rates remained relatively stable throughout the experiment in mesocosms without drought stress (cf. Tab. S4.1). Total hyphal RNA content per fungal dry weight was on day 3 on average higher in the drought stressed treatment (0.24 ± 0.09 µg mg^{-1} DW) than in the constantly wet treatment (0.11 ± 0.01 µg mg^{-1} DW). Further, an increase in biomass was visually observed in the initial days after the irrigation stop.

Transcriptome analysis revealed that in total 63 and 37 genes were differential expressed on day 3 and day 7, respectively, in response to drought stress treatment (DC vs. WC). The 5 genes with known function and either strongest induced or repressed expression were mostly regulators of transcription and translation or cell processes (Tab. 1.1).

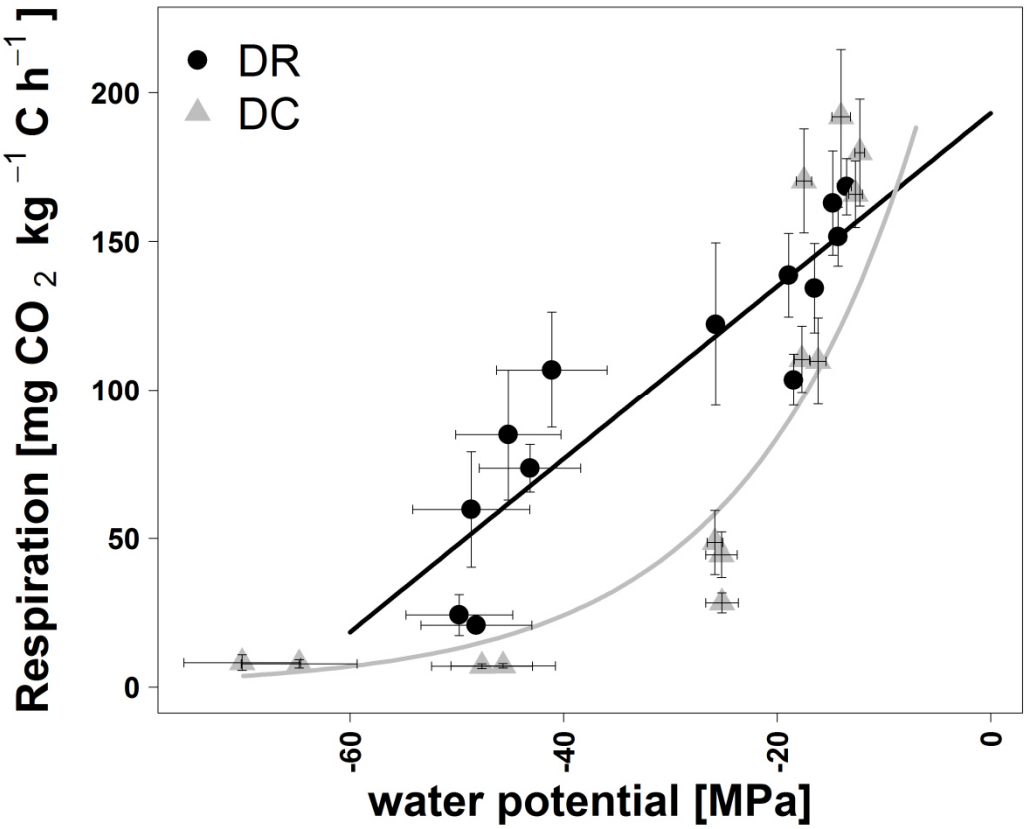


Fig. 1.9: Relationship between respiration rates [mg CO_2 kg^{-1} h^{-1}] and water potential [MPa] in mesocosms with (DR, y= 2.912*x+193.13, Spearman's rank correlation: r^2= 0.85, p<0.001) and without access to excessive riboflavin before drought stress treatment (DC, y= 291.13*$e^{0.0623*x}$, Spearman's rank correlation: r^2= 0.75, p<0.001). Mean ± SEM, n= 4.

Tab. 1.1: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of the five most strongly induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between DC (no riboflavin addition prior to drought stress) and WC (no drought stress and no riboflavin addition) mesocosms with information about biological function, if available. T&T = Regulation of translation and transcription.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the DC/WC microarray experiments							
Ribosomal protein S7	136259	T&T	Regulation of development and translation	3.70	<0.001	3.33	<0.001
Ribosomal protein L10	191238	T&T	Regulation of development and translation	2.48	<0.001	1.28	<0.001
Histone acetyltransferase	182197	T&T	Acetylation of amino acids on histone proteins	1.96	0.020	0.40	0.070
Subunit 21 of mediator complex protein	193554	T&T	Coactivator of the mediator complex, involved in regulation of transcription of nearly all RNA polymerase II-dependent genes	1.74	0.000	1.63	0.009
Ribosomal protein L7A/S12e	195860	T&T	Regulation of development and translation	1.61	<0.001	1.74	<0.001
Golgi pH regulator	188048	Regulation of cell processes	Required for functionality of the Golgi apparatus	1.81	0.037	0.11	0.446
CDC20	195164	Growth	Activation factor of the anaphase promoting complex	2.32	<0.001	0.03	0.720
MFS1	211434	Transporter	Transporter/transmembrane protein	1.03	<0.001	1.89	<0.001
MFS substrate transporter	114522	Transporter	Transporter/transmembrane protein	0.17	0.148	1.89	0.002
Cytochrome C	135048	Protection	Oxidative protection	1.14	<0.001	1.50	<0.001
Repressed genes of the DC/WC microarray experiments							
Ubiquitin	192160	Regulation of cell processes	Post-translational modifications, e.g., interaction with histones	-2.04	<0.001	-2.16	<0.001
Mitochondrial DNA polymerase gamma 1	114559	Regulation of cell processes	Involved in the replication of mitochondrial DNA	-1.89	<0.001	-1.19	<0.001
Histone H2B	133899	Regulation of cell processes	DNA binding	-0.03	0.770	-1.53	<0.001
Histones H3	139997	Regulation of cell processes	DNA binding	0.21	0.100	-1.23	<0.001
Carnitine O-acyltransferase	200564	Transporter	Fatty acid transport into mitochondria for β-oxidation	-1.85	<0.001	-1.52	<0.001
Ubiquitin thiolesterase	146556	Regulation of cell processes	Performs second step in the ubiquitination reaction	-1.32	<0.001	0.11	0.250

The main transcriptional response seemed to be based on genes coding for transcription and translation regulating factors with potentially epigenetic functions. This included the ribosomal protein (RP) genes S7 and L10. RPs are part of the protein translation machinery and hence an increased expression may promote protein biosynthesis (Lindström, 2009). Many RP have additional functions in various processes including target specific regulation of translation, e.g., by binding and protecting mRNAs at non-coding regions (Lindström, 2009; Bhavsar et al., 2010). For example, RPL10 is known to impact protein concentrations in relation to oxidative stress, e.g., of targets related to transcription, translation and DNA replication (Chiocchetti et al., 2014).

Further, nucleosome modifications are likely facilitated in response to drought by an induced expression of a gene coding for a histone acetyltransferase (HAT) gene and a repressed expression of genes coding for ubiquitin, an ubiquitin thiolesterase as well as histones H2B and H3. One of the manifold functions of ubiquitin is the stabilisation of nucleosomes by interaction with histones (Ciechanover, 2015). This increases DNA compaction and protection but decreases access of regulatory proteins (Mello and Almouzni, 2001; Chandrasekharan et al., 2009). HAT conduct the acetylation of internal lysine residues of histones, which neutralises the positive charge of lysine and leads to a modification of nucleosome structures (Kuo and Allis, 1998). These reversible nucleosome modifications affect the interaction of histones with other proteins and the DNA depending on the concentrations of free modifiers as well as the site of the modification (Zhang, 2003). They can lead to a reduction of DNA compaction and facilitate the access of regulatory proteins which tends to promote DNA replication and transcription but can also lead to gene silencing (Cao and Yan, 2012; Kim et al., 2015). The impact on transcription is often gene-specific, e.g., Kim et al. (2008) observed specific acetylation patterns on different drought stress-inducible genes in plants.

These differentially expressed genes may have partly promoted gene expression. This would be in accordance with the induced expression of a gene coding for subunit 21 of the mediator complex protein (Med21). Med21 is required for expression of nearly all RNA polymerase II-dependent genes in fungi (Hengartner et al., 1995). In addition, some genes show induced expression with the potential to stimulate cell proliferation and growth (e.g., coding for RPS7 and Cdc20). An orthuoulogе of RPS7 was found to be essential for growth and to stimulate growth by induced expression in fungi (Synetos et al., 1992). An orthoulogue of Cdc20 was found to be an activation factor of the anaphase promoting complex/ cyclosome and to promot progression through mitosis (Kramer et al., 2000).

A more direct link to protective mechanisms in response to drought stress was detectable with the induced expression of a gene coding for a cytochrome C and the repressed expression of a carnitine O-acetyltransferase (CRAT) gene. Cytochrome C is important for the electron transport chain in mitochondria and also known for its antioxidative potential (Pereverzev et al., 2003). CRAT take part in and also regulate the transfer of fatty acids into mitochondria for β-oxidation. Hence, its down-regulation probably reduced β-oxidation and the production of reactive oxygen species as a potential byproduct of β-oxidation (Chen et al., 2015).

Overall, protective mechanisms represented only a small part of the transcriptional response to drought but were related to oxidative stress. The main transcriptional response of *A. bisporus* was based on targets with the potential to increase transcription, translation and growth rates. Differential expression of genes belonging to the different groups may thereby have been interdependent since an increase in cell proliferation and growth leads to increasing demands for novel proteins. Hence, transcription of translation-related elements (e.g., RPs, rRNA) correlates strongly with growth rates (García-Martínez et al., 2015). Since non-coding RNA (e.g., rRNA) can make up to 60-75% of total transcripts during growth (García-Martínez *et al.* 2015), this may also partly explain the higher total extractable RNA concentrations in DC compared to WC.

Therefore, the main strategy of *A. bisporus* to cope with drought stress may be based on the promotion of water uptake by increased biomass production. Increases of hyphal length were also previously documented in field studies in response to drought (Allison et al., 2013; Alster et al., 2013). This may partially compensate the water deficit by accessing remaining water reservoirs and, as described above, by redistribution of water. An expansion of fungal networks in response to drought could thus strongly support HR along hyphal tissue. In turn, this could have considerable relevance on soil processes considering that HR can strongly enhance mineralisation under drought stress.

Interestingly, transcriptional response to either only drought (DC/WC) or riboflavin (WR/WC) strongly overlapped by over 50% (49 of a total of 92 genes, including all strongest induced genes, se also Fig. 4.3). This suggests that physiological responses to both factors are strongly connected. It could indicate a stress priming function of riboflavin, i.e., induction of basal defence responses leading to a faster and/or stronger activation of defence mechanisms in later stress events (e.g., Conrath et al., 2015).

1.4.4 Impact of riboflavin on drought tolerance of A. bisporus (study III)

A comparison of the observed hyphal auto-florescence in study I and II with fluorescence spectra of known fluorophores indicated riboflavin to be a potential match. A possible relationship between drought stress and riboflavin production in *A. bisporus* as well as the impact of riboflavin on physiological and transcriptional responses to drought was therefore analysed.

In DR treated mesocosms, respiration rates correlated linear with water potentials (see Fig. 1.9). Respiration rates were about 2-3 times higher compared to DC starting at the point when water potentials dropped below -20 MPa until the end of the experiment. Hence, riboflavin addition attenuated a drought induced respiration decrease and riboflavin addition had a positive impact on respiratory activity under similar or even lower water potentials. This is comparable to the observed effect of riboflavin in tobacco plants (Deng et al., 2014).

Riboflavin content in hyphae varied significantly among treatments after 14 days (LMM: $F_{1,15}$= 5.59; p= 0.014, cf. Fig. 4.2). Intracellular riboflavin content increased slightly with riboflavin irrigation, strongly under drought stress and was further increased in the combined treatment (mean values: WC= 0.9 mg g^{-1} DW; WR= 1.9 mg g^{-1} DW; DC= 6.1 mg g^{-1} DW; DR= 10.5 mg g^{-1} DW). Riboflavin is thereby strongly accumulated compared to the irrigation concentration (18.8 µg ml^{-1}) by up to 500 times, which could only be explained by induced uptake and/or biosynthesis. Riboflavin treatment also led to a yellow colouring of hyphae (Fig. 1.10).

Total hyphal RNA content per fungal dry weight was again on day 3 on average higher in the drought stressed DR treatment (4.46 ± 2.04 µg mg^{-1} DW) compared to the constantly wet WR treatment (0.41 ± 0.35 µg g^{-1} DW).

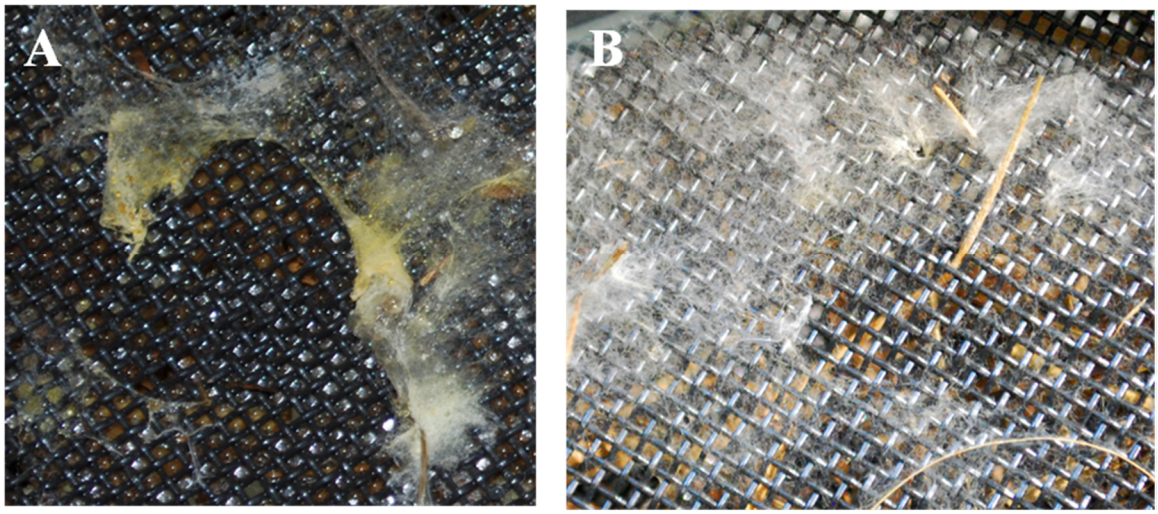


Fig. 1.10: Hyphae of *A. bisporus* grown A) with or B) without 50 µM riboflavin addition.

Tab. 1.2: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of the five most strongly induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between DR (with 50 µM riboflavin addition prior to drought stress) and DC (without riboflavin addition prior to drought stress) mesocosms with information about biological function, if available.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the DR/DC microarray experiments							
Phosphatidylglycerophosphatase GEP4, putitative	205009	Regulation of cell processes	Involved in the biosynthesis of cardiolipin (part of mitochondrial membranes)	1.63	0.001	2.42	<0.001
lactoylglutathione lyase	229618	Protection	Protection against methylglyoxal	1.23	0.180	2.26	0.030
Cytochrome C	135048	Protection	Oxidative protection	0.20	0.110	1.80	0.006
Branched-chain amino acid aminotransferase 1	190047	Protection	Detoxification of BCAA	1.19	0.002	0.57	0.060
alpha-tubulin folding cofactor E	63759	Growth	Involved in folding of alpha-tubulin	0.95	0.250	2.06	0.030
Glycoside hydrolase	153780	Metabolism	Hydrolysis of glycosidic bonds in complex sugars	0.33	0.340	1.63	<0.001
MFS-1	211434	Transporter	Transporter/transmembrane protein	1.76	<0.001	0.77	<0.001
NSF attachment protein	189575	Transporter	Involved in membrane fusion and transfer of membrane vesicles from one membrane compartment to another	1.21	<0.001	0.26	0.270
Repressed genes of the DR/DC microarray experiments							
Histone H3	139997	Regulation of cell processes	DNA binding	-2.05	<0.001	-2.84	<0.001
Histone H2B	133899	Regulation of cell processes	DNA binding	-1.25	0.030	-2.45	<0.001
NADH ubiquinone oxidoreductase	192620	Metabolism	Catalyses electron transfer from NADH to coenzyme Q10	-1.35	0.020	-0.14	0.790
Ubiquitin	133168	Regulation of cell processes	Post-translational modifications, e.g., interaction with histons	-1.15	0.020	0.63	0.150
Ebp2	193895	Translation and Transcription	RNA biosynthesis factor	-0.25	0.670	-1.06	0.090

In response to presence or absence of exogenous riboflavin and drought stress (DR/DC), 25 and 18 genes showed differential expression on day 3 and day 7, respectively. A strong differential gene expression was mostly observed for genes belonging to protective mechanisms and the regulation of transcription, cell processes as well as metabolism (Tab. 1.2). Especially the protective mechanisms may explain the observed better performance under drought conditions.

Most pronounced was a gene encoding for a lactoylglutathione lyase (GLO1), an enzyme that belongs to the glyoxalyses system. GLO1 is responsible for detoxification of methylglyoxal, a by-product of glycolysis with cytotoxic effects (Martins et al., 2001; Thornalley, 2003; Allaman et al., 2015). A generally increased metabolic activity in the early stages of drought stress response can lead to high methylglyoxal levels and hence, stress tolerance can depend on glyoxalase activity (Hossain et al., 2009). An additionally induced gene coded for a branched-chain-amino-acid transaminase (BCAT), which governs the degradation of branched-chain amino acids (BCAA). BCAA and the breakdown products (e.g., glutamate) are potentially cytotoxic (Eden and Benvenisty, 1999). Free BCAA pools are mostly affected by protein degradation, e.g., in response to protein stress damage (Di Martino et al., 2003). Accordingly, BCAA levels were shown to increase under stress conditions and BCAT expression to be elevated under drought stress in plants (Malatrasi et al., 2006). Therefore, BCAT were likely providing protection against increased BCAA levels under the drought stress conditions.

While these mechanisms might have safeguarded drought tolerance, genes coding for proteins belonging to other groups probably also affected the performance during drought. This included Gep4, a glycoside hydrolases and alpha-tubulin folding cofactor E (TBCE). Gep4 is involved in the biosynthesis of cardiolipin, an essential component of mitochondrial membranes with functions in mitochondrial energy metabolism, and is needed for efficient cell growth (Claypool et al., 2008; Osman et al., 2010). Glycoside hydrolases are responsible for the hydrolysis of complex sugars (including cellulose) and induced expression may stimulate organic matter decomposition (Langston et al., 2011). TBCE is one of four proteins essential for correct folding of tubulins and seems to stimulate vegetative growth in fungi in association with the other cofactors (Kortazar et al., 2007; Zhang et al., 2015). Further, TBCE seems to provide protection against misfolded protein stress by interaction with the proteasomes (Voloshin et al., 2010).

Based on the repressed expression rates of genes coding for histone H3 and H2B as well as ubiquitin, hyphae in DR treatments seemed to show even lower rates of histone ubiquitination and DNA compection. This may have facilitated the access of regulatory proteins and led to enhanced DNA replication as well as transcription rates (e.g., Chandrasekharan et al., 2009).

Overall, riboflavin addition stimulated even more strongly differential expression of genes regulating transcription and growth then drought treatment alone. In addition, survival chances were improved by induction of protective mechanisms. These factors together may explain the observed higher respiratory activity in comparison at the same levels of water deficiency.

1.5. Conclusions and perspectives

This thesis helps to close the knowledge gap on how filamentous saprotrophic fungi cope with low water potentials and why fungal communities are often better adapted to drought then bacterial communities. Furthermore, the results extend our knowledge on physiological and transcriptional adaptions and responses of saprotrophic filamentous fungi to drought stress.

Saprotrophic fungi clearly have the potential for HR. The amount of transferred water could thereby exceed capillary transport and led to an increase in water content of the surrounding soil. The extent of HR seemed to depend on the fungal foraging strategy and to be facilitated by mycelia cords. In any way, HR through mycelia networks seems to be one major adaption of filamentous fungi to drought with potentially high impact on soil processes and on surrounding organisms. Fungal HR is likely a mechanism behind the higher drought tolerance of soil fungal communities since it can partly compensate water deficiency, if water is available in other zones of the mycelia network. Further, fungal HR has the potential to overcome capillary barriers during drought events and is an underestimated pathway of water transport in soils.

Unfortunately, it was not possible to distinguish between apoplastic and symplastic way of water translocation through fungal hyphae. Fluorescence measurements after LYCH treatment turned out to be not suitable since hyphae developed a strong autofluorescence during drought stress. Another fluorescence marker or a different methodical approach might be necessary. Moreover, soil moisture sensors used in study I and II turned had a high failure rate. Therefore, these data were partially incomplete or not reliable and hence, were omitted from data evaluation. That is unfortunate because they could have helped to specify water content changes on the whole chamber scale. New generation sensors might be more suitable for this task. In addition, a change in adhesive material for the mesocosms may be appropriate for a further use since the glue seemed unstable on the long run and some mesocosms had to be reinitiated during reuse.

While saprotrophic fungi have the potential for HR, the impact of fungal HR on C mineralisation and N translocation in dry soils seemed to be species specific and related to the fungal foraging as well as resource usage strategy. Great variations are to be expected especially in terms of time scales between fast and slow growing species. Predictions of the impact of HR by saprotrophic fungi on processes in dry ecosystems may therefore strongly depend on fungal community structure and in any way need more data from a greater spectrum of species. Further, field experiments are necessary to evaluate the actual significance of saprotrophic fungal HR

under field conditions. An experimental approach needs to be developed for measuring fungal HR in the field. Inoculated chambers separated from the soil by a capillary barrier, equipped with an autonomous irrigation system and isotopic labelling might be suitable for this purpose.

In any case, drought stress may even stimulate the extent of fungal HR in soils since exposure to drought can strongly change gene expression of factors regulating and stimulating transcription, translation and growth as was shown in case of *A. bisporus*. A resulting increase in growth rate and mycelial network extension could in turn facilitate exploitation and redistributing of water from remaining reservoirs. Protective pathways represented only a small part of the transcriptional response to drought alone and were related to oxidative stress. While an increase in hyphal length was visually observed with decreasing water contents during the experiments, an indicator for fungal biomass was unfortunately not determined. A strong biomass increase was not expected in advance and all available hyphal material had to be immediately collected for RNA isolations resulting in a lack of samples for further analysis. Hence, a determination of biomass needs to be included in subsequent studies.

The strong overlap of transcriptional responses to either drought or riboflavin suggests a strong correlation of physiological responses and indicates a potential for stress priming of riboflavin. Treatment with riboflavin under drought stress even further stimulated transcription, translation and growth enhancing factors. Further, Riboflavin triggered a variety of underlying protective mechanisms supporting drought stress tolerance. Hence, *A. bisporus* clearly benefited from riboflavin addition under drought stress. In addition, drought stress led to an increase in hyphal riboflavin content. Therefore, riboflavin likely participates in drought responses of *A. bisporus*.

Based on the transcriptional changes (e.g., histone acetyltransferase, ubiquitin), it seems likely that drought stress responses in *A. bisporus* are partly regulated by epigenetic modifications of nucleosomes. Precise mechanisms of the epigenetic regulation remain unclear and require further attention. New methods could give an overview on the dynamics and extent of nucleosome modifications and may be suitable to provide a more precise picture on the nature and targets of stress response regulations (e.g., Hathaway et al., 2012). Furthermore, proteomic approaches could help to identify actual changes in protein levels and provide a more comprehensive picture on the dynamic changes in response to drought stress.

Further, it would be interesting to study memory effects of mild drought events and/or riboflavin on stronger and extended drought events later-on. Plants are already extensively studied with regard to stress priming and show faster and stronger activation of defense mechanisms in the primed state due to induced basal response mechanisms (e.g., Conrath et al.,

2015). Stress priming also has positive effects on survival of some microbial species with potential to impact microbial community structure (Andrade-Linares et al., 2016). Epigenetic mechanisms seem to be especially important for the regulation of stress priming (Conrath et al., 2015). Thus, *A. bisporus* appears to be a suitable candidate for studying stress priming in saprotrophic fungi, considering the observed induction of epigenetic regulations under drought stress.

1.6 References

Aanderud, Z.T., Richards, J.H., 2009. Hydraulic redistribution may stimulate decomposition. Biogeochemistry 95, 323–333.

Agre, P., King, L.S., Yasui, M., Guggino, W.B., Ottersen, O.P., Fujiyoshi, Y., Engel, A., Nielsen, S., 2002. Aquaporin water channels - from atomic structure to clinical medicine. J. Physiol. 542, 3–16.

Allaman, I., Bélanger, M., Magistretti, P.J., 2015. Methylglyoxal, the dark side of glycolysis. Front. Neurosci. 9, 23.

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, Jennifer B. H., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.

Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil. Biol. Biochem. 64, 68–79.

Andrade-Linares, D.R., Lehmann, A., Rillig, M.C., 2016. Microbial stress priming: a meta-analysis. Environ. Microbiol. 18, 1277–1288.

Armas, C., Kim, J., Bleby, T., Jackson, R., 2012. The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. Oecologia 168, 11–22.

Ashoori, M., Saedisomeolia, A., 2014. Riboflavin (vitamin B2) and oxidative stress: a review. Br. J. Nutr., 1–7.

Balaş, T., Tănase, C., 2012. Description of in vitro cultures for some spontaneous lignicolous basidiomycetes species. Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, s. II a. Biologie Vegetală 58, 19–29.

Bapiri, A., Bååth, E., Rousk, J., 2010. Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb. Ecol. 60, 419–428.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Soft. 67, 1–51.

Bauerle, T.L., Richards, J.H., Smart, D.R., Eissenstat, D.M., 2008. Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. Plant Cell Environ. 31, 177–186.

Benabdellah, K., Azcón-Aguilar, C., Valderas, A., Speziga, D., Fitzpatrick, T.B., Ferrol, N., 2009. GintPDX1 encodes a protein involved in vitamin B6 biosynthesis that is up-regulated by oxidative stress in the arbuscular mycorrhizal fungus *Glomus intraradices.* New Phytol. 184, 682–693.

Beutler, E., 1969. Effect of flavin compounds on glutathione reductase activity: in vivo and in vitro studies. J. Clin. Invest. 48, 1957–1966.

Bhavsar, R.B., Makley, L.N., Tsonis, P.A., 2010. The other lives of ribosomal proteins. Hum. Genomics 4, 327.

Bilski, P., Li, M.Y., Ehrenshaft, M., Daub, M.E., Chignell, C.F., 2000. Vitamin B_6 (Pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. Photochem. Photobiol. 71, 129–134.

Birhane, E., Sterck, F.J., Bongers, F., Kuyper, T.W., 2014. Arbuscular mycorrhizal impacts on competitive interactions between *Acacia etbaica* and *Boswellia papyrifera* seedlings under drought stress. J. Plant Ecol. 7, 298–308.

Blackwell, M., 2011. The fungi: 1, 2, 3 … 5.1 million species? Am. J. Bot. 98, 426–438.

Boberg, J.B., Finlay, R.D., Stenlid, J., Ekblad, A., Lindahl, B.D., 2014. Nitrogen and carbon reallocation in fungal mycelia during decomposition of boreal forest litter. PloS one 9, e92897.

Boberg, J.B., Finlay, R.D., Stenlid, J., Lindahl, B.D., 2010. Fungal C translocation restricts N-mineralisation in heterogeneous environments. Funct. Ecol. 24, 454–459.

Boddy, L., 1993. Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. Mycol. Res. 97, 641–655.

Boddy, L., Watkinson, S.C., 1995. Wood decomposition, higher fungi, and their role in nutrient redistribution. Can. J. Bot. 73, 1377–1383.

Boddy, L., 1999. Saprotrophic Cord-Forming Fungi: Meeting the Challenge of Heterogeneous Environments. Mycologia 91, 13–32.

Boddy, L., Jones, T.H., Gadd, G., Watkinson, S.C., Dyer, P.S., 2007. Mycelial responses in heterogeneous environments: parallels with macroorganisms. In: Gadd, G., Watkinson, S.C., Dyer, P.S. (Eds.), Fungi in the Environment. Cambridge University Press, Cambridge, pp. 112–140.

Boddy, L., Hynes, J., Bebber, D.P., Fricker, M.D., 2009. Saprotrophic cord systems: dispersal mechanisms in space and time. Mycoscience 50, 9–19.

Boretsky, Y.R., Protchenko, O.V., Prokopiv, T.M., Mukalov, I.O., Fedorovych, D.V., Sibirny, A.A., 2007. Mutations and environmental factors affecting regulation of riboflavin synthesis and iron assimilation also cause oxidative stress in the yeast *Pichia guilliermondii.* J. Basic Microbiol. 47, 371–377.

Borken, W., Davidson, E.A., Savage, K., Gaudinski, J., Trumbore, S.E., 2003. Drying and wetting effects on carbon dioxide release from organic horizons. Soil Sci. Soc. Am. J. 67, 1888.

Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Glob. Chang. Biol. 12, 177–193.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralisation and fluxes in soils. Glob. Chang. Biol. 15, 808–824.

Brownlee, C., Duddridge, J.A., Malibari, A., Read, D.J., 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71, 433–443.

Cairney, J.W.G., 1992. Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. Mycol. Res. 96, 135–141.

Cairney, J.W.G., 2005. Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. Mycol. Res. 109, 7–20.

Caldwell, M.M., Dawson, T.E., Richards, J.H., 1998. Hydraulic lift. Consequences of water efflux from the roots of plants. Springer, Berlin, Germany.

Cao, J., Yan, Q., 2012. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Front. Oncol. 2, 26.

Chandrasekharan, M.B., Huang, F., Sun, Z.-W., 2009. Ubiquitination of histone H2B regulates chromatin dynamics by enhancing nucleosome stability. Proc. Natl. Acad. Sci. U. S. A. 106, 16686–16691.

Chang, S.T., Miles, P.G., 1986. Mushroom technology. Mushroom Newslett. Tropics 6, 6–11.

Chavez, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P., Osorio, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. Ann. Bot. 89, 907–916.

Chen, H., Hao, G., Wang, L., Wang, H., Gu, Z., Liu, L., Zhang, H., Chen, W., Chen, Y.Q., 2015. Identification of a critical determinant that enables efficient fatty acid synthesis in oleaginous fungi. Sci. Rep. 5, 11247.

Chen, M., Alexander, M., 1973. Survival of soil bacteria during prolonged desiccation. Soil. Biol. Biochem. 5, 213–221.

Chen, Y.-T., Borken, W., Stange, C.F., Matzner, E., 2011. Effects of decreasing water potential on gross ammonification and nitrification in an acid coniferous forest soil. Soil. Biol. Biochem. 43, 333–338.

Chiocchetti, A.G., Haslinger, D., Boesch, M., Karl, T., Wiemann, S., Freitag, C.M., Poustka, F., Scheibe, B., Bauer, J.W., Hintner, H., Breitenbach, M., Kellermann, J., Lottspeich, F., Klauck, S.M., Breitenbach-Koller, L., 2014. Protein signatures of oxidative stress response in a patient specific cell line model for autism. Mol. Autism. 5, 10.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., Noblet, N. de, Friend, A.D., Friedlingstein, P., Grünwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533.

Ciechanover, A., 2015. The unravelling of the ubiquitin system. Nat. Rev. Mol. Cell. Biol. 16, 322–324.

Claypool, S.M., Oktay, Y., Boontheung, P., Loo, J.A., Koehler, C.M., 2008. Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. J. Cell. Biol. 182, 937–950.

Conrath, U., Beckers, Gerold J M, Langenbach, Caspar J G, Jaskiewicz, M.R., 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53, 97–119.

Cooper, R.A., 1984. Metabolism of methylglyoxal in microorganisms. Annu. Rev. Microbiol. 38, 49–68.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil. Biol. Biochem. 38, 2053–2062.

Crowther, T.W., Boddy, L., Hefin Jones, T., 2012. Functional and ecological consequences of saprotrophic fungus-grazer interactions. ISME J. 6, 1992–2001.

Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: Production, scavenging and signaling. Plant. Signal. Behav. 3, 156–165.

Dawson, T.E., 1997. Water loss from tree influences soil water nutrient status and plant performance. In: Flores, E.H., Lynch, J.P., Eissenstat, D. (Eds.), Radical biology: advances and perspectives on the function of plant roots. American Society of Plant Physiologists, Rockville, pp. 235–250.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. Annu. Rev. Ecol. Syst. 33, 507–559.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29, 795–811.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil. Biol. Biochem. 33, 1599–1611.

Deng, B., Jin, X., Yang, Y., Lin, Z., Zhang, Y., 2014. The regulatory role of riboflavin in the drought tolerance of tobacco plants depends on ROS production. Plant Growth. Regul. 72, 269–277.

Di Martino, C., Delfine, S., Pizzuto, R., Loreto, F., Fuggi, A., 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytol. 158, 455–463.

Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J., Jalili, A., Montserrat-Martí, G., Grime, J.P., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., Torres-Espuny, L. de, Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martínez, M.,

Romo-Díez, A., Shaw, S., Siavash, B., Villar-Salvador, P., Zak, M.R., 2004. The plant traits that drive ecosystems: Evidence from three continents. J. Veg. Sci. 15, 295–304.

Dighton, J., 2003. Fungi in Ecosystem Processes. CRC Press, New York.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299–304.

Domec, J.C., Warren, J.M., Meinzer, F.C., Brooks, J.R., Coulombe, R., 2004. Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. Oecologia 141, 7–16.

Donnelly, D.P., Boddy, L., 1997. Development of mycelial systems of *Stropharia caerulea* and *Phanerochaete velutina* on soil: effect of temperature and water potential. Mycol. Res. 101, 705–713.

Duddridge, J.A., Malibari, A., Read, D.J., 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287, 834–836.

Eamus, D., Thompson, W., Cairney, J.W.G., Jennings, D.H., 1985. Internal Structure and Hydraulic Conductivity of Basidiomycete Translocating Organs. J. Exp. Bot. 36, 1110–1116.

Eden, A., Benvenisty, N., 1999. Involvement of branched-chain amino acid aminotransferase (Bcat1/Eca39) in apoptosis. FEBS Lett. 457, 255–261.

Egerton-Warburton, L.M., Querejeta, J.I., Allen, M.F., 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. J. Exp. Bot. 58, 1473–1483.

Emerman, S.H., Dawson, T.E., 1996. Hydraulic lift and its influence on the water content of the rhizosphere. an example from sugar maple, Acer saccharum. Oecologia 108, 273–278.

Estrada, B., Barea, J.M., Aroca, R., Ruiz-Lozano, J.M., 2013. A native *Glomus intraradices* strain from a Mediterranean saline area exhibits salt tolerance and enhanced symbiotic efficiency with maize plants under salt stress conditions. Plant Soil 366, 333–349.

Faraway, J.J., 2005. Extending the linear model with R: Generalized linear, mixed effects and nonparametric regression models. CRC Press, Boaca Raton, London, New York.

Ferguson, B.A., Dreisbach, T.A., Parks, C.G., Filip, G.M., Schmitt, C.L., 2003. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Can. J. For. Res. 33, 612–623.

Frey, S.D., Elliott, E.T., Paustian, K., Peterson, G.A., 2000. Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. Soil. Biol. Biochem. 32, 689–698.

Frey, S.D., Six, J., Elliott, E.T., 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil–litter interface. Soil. Biol. Biochem. 35, 1001–1004.

Fricker, M.D., Bebber, D., Boddy, L., 2008. Mycelial networks: Structure and dynamics. In: Boddy, L., Frankland, J., van West, P. (Eds.), Ecology of Saprotrophic Basidiomycetes. Elsevier, London, pp. 3–18.

García-Martínez, J., Delgado-Ramos, L., Ayala, G., Pelechano, V., Medina, D.A., Carrasco, F., González, R., Andrés-León, E., Steinmetz, L., Warringer, J., Chávez, S., Pérez-Ortín, J.E., 2015. The cellular growth rate controls overall mRNA turnover, and modulates either transcription or degradation rates of particular gene regulons. Nucleic Acids Res. 44, 3643-3658.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.

Glen, M., Yuskianti, V., Puspitasari, D., Francis, A., Agustini, L., Rimbawanto, A., Indrayadi, H., Gafur, A., Mohammed, C.L., Woodward, S., 2014. Identification of basidiomycete fungi in Indonesian hardwood plantations by DNA barcoding. For. Path. 44, 496–508.

Granlund, H.I., Jennings, D.H., Thompson, W., 1985. Translocation of solutes along rhizomorphs of *Armillaria mellea.* Trans. Br. Mycol. Soc. 84, 111–119.

Greenwood, D.J., 1967. Studies on oxygen transport through mustard seedlings (*Sinapis alba* L.). New Phytol. 66, 597–606.

Hathaway, N.A., Bell, O., Hodges, C., Miller, E.L., Neel, D.S., Crabtree, G.R., 2012. Dynamics and memory of heterochromatin in living cells. Cell 149, 1447–1460.

Haurwitz, B., 1945. Insolation in relation to cloudiness and cloud density. J. Meteor. 2, 154–166.

Hawkins, H., Hettasch, H., West, A.G., Cramer, M.D., 2009. Hydraulic redistribution by *Protea* 'Sylvia' (Proteaceae) facilitates soil water replenishment and water acquisition by an understorey grass and shrub. Funct. Plant Biol. 36, 752–760.

Haynes, R.J., Swift, R.S., 1990. Stability of soil aggregates in relation to organic constituents and soil water content. J. Soil Sci. 41, 73–83.

Heaton, L.L.M., Jones, N.S., Fricker, M.D., 2016. Energetic Constraints on Fungal Growth. Am. Nat. 187, E27-40.

Hengartner, C.J., Thompson, C.M., Zhang, J., Chao, D.M., Liao, S.M., Koleske, A.J., Okamura, S., Young, R.A., 1995. Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. 9, 897–910.

Herzog, C., Peter, M., Pritsch, K., Günthardt-Goerg, M.S., Egli, S., 2013. Drought and air warming affects abundance and exoenzyme profiles of *Cenococcum geophilum* associated with *Quercus robur, Q. petraea and Q. pubescens.* Plant Biol. 15, 230–237.

Hibbett, D.S., Gilbert, L.B., Donoghue, M.J., 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407, 506–508.

Hibbett, D.S., Binder, M., 2002. Evolution of complex fruiting-body morphologies in homobasidiomycetes. Proc. Biol. Sci. 269, 1963–1969.

Hillel, D., 2003. Introduction to Environmental Soil Physics. Elsevier Academic Press, Amsterdam.

Hobbie, E.A., Horton, T.R., 2007. Evidence that saprotrophic fungi mobilise carbon and mycorrhizal fungi mobilise nitrogen during litter decomposition. New Phytol. 173, 447–449.

Hossain, M.A., Hossain, M.Z., Fujita, M., 2009. Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. Aust. J. Crop Sci. 3, 53–64.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Ingram, J., Bartels, D., 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 377–403.

IPCC, 2007. Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC. Cambridge University Press.

Jennings, L., Watkinson, S.C., 1982. Structure and development of mycelial strands in *Serpula lacrimans.* Trans. Br. Mycol. Soc. 78, 465–474.

Jennings, D.H., 1987. Translocation of solutes in fungi. Biol. Rev. 62, 215–243.

Kerrigan, R.W., Carvalho, D.B., Horgen, P.A., Anderson, J.B., 1998. The indigenous coastal Californian population of the mushroom *Agaricus bisporus*, a cultivated species, may be at risk of extinction. Mol. Ecol. 7, 35–45.

Kim, J.-M., To, T.K., Ishida, J., Morosawa, T., Kawashima, M., Matsui, A., Toyoda, T., Kimura, H., Shinozaki, K., Seki, M., 2008. Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana.* Plant Cell Physiol. 49, 1580–1588.

Kortazar, D., Fanarraga, M.L., Carranza, G., Bellido, J., Villegas, J.C., Avila, J., Zabala, J.C., 2007. Role of cofactors B (TBCB) and E (TBCE) in tubulin heterodimer dissociation. Exp. Cell Res. 313, 425–436.

Kozlowski, T.T., Pallardy, S.G., 2002. Acclimation and Adaptive Responses of Woody Plants to Environmental Stresses. Bot. Rev. 68, 270–334.

Kramer, E.R., Scheuringer, N., Podtelejnikov, A.V., Mann, M., Peters, J.-M., 2000. Mitotic regulation of the APC activator proteins CDC20 and CDH1. Mol. Biol. Cell 11, 1555–1569.

Kranner, I., Beckett, R., Hochman, A., Nash, T.H., 2008. Desiccation-Tolerance in Lichens: A Review. Bryologist 111, 576–593.

Kuo, M.-H., Allis, C.D., 1998. Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20, 615–626.

Kurz-Besson, C., Otieno, D., Lobo do Vale, R., Siegwolf, R., Schmidt, M., Herd, A., Nogueira, C., David, T., David, J., Tenhunen, J., Pereira, J., Chaves, M., 2006. Hydraulic Lift in Cork Oak Trees in a Savannah-Type Mediterranean Ecosystem and its Contribution to the Local Water Balance. Plant Soil 282, 361–378.

Langston, J.A., Shaghasi, T., Abbate, E., Xu, F., Vlasenko, E., Sweeney, M.D., 2011. Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61. Appl. Environ. Microbiol. 77, 7007–7015.

Leake, J., Johnson, D., Donnelly, D., Muckle, G., Boddy, L., Read, D., 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can. J. Bot. 82, 1016–1045.

Leake, J.R., 2005. Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the plant myth. Mycologist 19, 113.

Li, T., Hu, Y.-J., Hao, Z.-P., Li, H., Wang, Y.-S., Chen, B.-D., 2013. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices.* New Phytol. 197, 617–630.

Lindahl, B.D., Stenlid, J., Olsson, S., Finlay, R., 1999. Translocation of ^{32}P between interacting mycelia of a wood-decomposing fungus and ectomycorrhizal fungi in microcosm systems. New Phytol. 144, 183–193.

Lindahl, B.D., Finlay, R., Olsson, S., 2001. Simultaneous, bidirectional translocation of ^{32}P and ^{33}P between wood blocks connected by mycelial cords of *Hypholoma fasciculare.* New Phytol. 150, 189–194.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. 173, 611–620.

Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. l. New Phytol. 205, 1443–1447.

Linder, M.B., Szilvay, G.R., Nakari-Setälä, T., Penttilä, M.E., 2005. Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbial. Rev. 29, 877–896.

Lindström, M.S., 2009. Emerging functions of ribosomal proteins in gene-specific transcription and translation. Biochem. Biophys. Res. Commun. 379, 167–170.

Ma, H., Xu, X., Feng, L., 2014. Responses of antioxidant defenses and membrane damage to drought stress in fruit bodies of *Auricularia auricula-judae.* World J. Microbiol. Biotechnol. 30, 119–124.

Magnus, G., 1844. Versuche uber die Spannkrafte des Wasserdampfes. Ann. Phys. 61, 225–248.

Malatrasi, M., Corradi, M., Svensson, J.T., Close, T.J., Gulli, M., Marmiroli, N., 2006. A branched-chain amino acid aminotransferase gene isolated from *Hordeum vulgare* is differentially regulated by drought stress. Theor. Appl. Genet. 113, 965–976.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938.

Martins, A.M., Cordeiro, C.A., Ponces Freire, A.M., 2001. In situ analysis of methylglyoxal metabolism in *Saccharomyces cerevisiae.* FEBS Lett. 499, 41–44.

Mataix-Solera, J., Arcenegui, V., Guerrero, C., Mayoral, A.M., Morales, J., González, J., García-Orenes, F., Gómez, I., 2007. Water repellency under different plant species in a calcareous forest soil in a semiarid Mediterranean environment. Hydrol. Process. 21, 2300–2309.

Mello, J.A., Almouzni, G., 2001. The ins and outs of nucleosome assembly. Curr. Opin. Genet. Dev. 11, 136–141.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

Moreira, M.Z., Scholz, F.G., Bucci, S.J., Sternberg, L.S., Goldstein, G., Meinzer, F.C., Franco, A.C., 2003. Hydraulic lift in a neotropical savanna. Funct. Ecol. 17, 573–581.

Mori, T., Sakurai, M., 1995. Effects of riboflavin and increased sucrose on anthocyanin production in suspended strawberry cell cultures. Plant Sci. 110, 147–153.

Morin, E., Kohler, A., Baker, A.R., Foulongne-Oriol, M., Lombard, V., Nagy, L.G., Ohm, R.A., Patyshakuliyeva, A., Brun, A., Aerts, A.L., Bailey, A.M., Billette, C., Coutinho, P.M., Deakin, G., Doddapaneni, H., Floudas, D., Grimwood, J., Hildén, K., Kües, U., Labutti, K.M., Lapidus, A., Lindquist, E.A., Lucas, S.M., Murat, C., Riley, R.W., Salamov, A.A., Schmutz, J., Subramanian, V., Wösten, Han A B, Xu, J., Eastwood, D.C., Foster, G.D., Sonnenberg, Anton S M, Cullen, D., de Vries, Ronald P, Lundell, T., Hibbett, D.S., Henrissat, B., Burton, K.S., Kerrigan, R.W., Challen, M.P., Grigoriev, I.V., Martin, F., 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc. Natl. Acad. Sci. U. S. A. 109, 17501–17506.

Muhr, J., Franke, J., Borken, W., 2010. Drying–rewetting events reduce C and N losses from a Norway spruce forest floor. Soil. Biol. Biochem. 42, 1303–1312.

Nazrul, M.I., YinBing, B., 2011. Differentiation of homokaryons and heterokaryons of *Agaricus bisporus* with inter-simple sequence repeat markers. Microbiol. Res. 166, 226–236.

Okuda, S., Nishiyama, N., Saito, H., Katsuki, H., 1998. 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J. Neurochem. 70, 299–307.

Olsson, P.A., Jakobsen, I., Wallander, H., 2003. Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. In: Baldwin, I.T., Caldwell, M.M., Heldmaier, G., Lange, O.L., Mooney, H.A., Schulze, E.-D., Sommer, U., van der Heijden,Marcel G. A, Sanders, I.R. (Eds.), Mycorrhizal ecology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 93–115.

Olsson, S., Gray, S.N., 1998. Patterns and dynamics of ^{32}P-phosphate and labelled 2-aminoisobutyric acid (^{14}C-AIB) translocation in intact basidiomycete mycelia. FEMS Microbiol. Ecol. 26, 109–120.

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S.P., 2014. Response of plants to water stress. Front. Plant Sci. 5, 86.

Osman, C., Haag, M., Wieland, F.T., Brügger, B., Langer, T., 2010. A mitochondrial phosphatase required for cardiolipin biosynthesis: the PGP phosphatase Gep4. EMBO J. 29, 1976–1987.

Peng, X., Horn, R., Smucker, A., 2007. Pore Shrinkage Dependency of Inorganic and Organic Soils on Wetting and Drying Cycles. Soil Sci. Soc. Am. J. 71, 1095.

Pereverzev, M.O., Vygodina, T.V., Konstantinov, A.A., Skulachev, V.P., 2003. Cytochrome c, an ideal antioxidant. Biochm. Soc. Trans. 31, 1312–1315.

Perez-Moreno, J., Read, D.J., 2000. Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. 145, 301–309.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and the R Development Core Team, 2015. nlme: Linear and nonlinear mixed effects models.

Plamboeck, A.H., Dawson, T.E., Egerton-Warburton, L.M., North, M., Bruns, T.D., Querejeta, J.I., 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. Mycorrhiza 17, 439–447.

Price, J.S., Whitehead, G.S., 2004. The influence of past and present hydrological conditions on *Sphagnum* recolonisation and succession in a block-cut bog, Québec. Hydrol. Process. 18, 315–328.

Prieto, I., Kikvidze, Z., Pugnaire, F., 2010. Hydraulic lift. soil processes and transpiration in the Mediterranean leguminous shrub *Retama sphaerocarpa* (L.) Boiss. Plant Soil 329, 447–456.

Querejeta, J.I., Egerton-Warburton, L.M., Allen, M.F., 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134, 65.

Raffaello, T., Chen, H., Kohler, A., Asiegbu, F.O., 2014. Transcriptomic profiles of *Heterobasidion annosum* under abiotic stresses and during saprotrophic growth in bark, sapwood and heartwood. Environ. Microbiol. 16, 1654–1667.

Ray, S., Dutta, S., Halder, J., Ray, M., 1994. Inhibition of electron flow through complex I of the mitochondrial respiratory chain of Ehrlich ascites carcinoma cells by methylglyoxal. Biochem. J. 303, 69–72.

Rayner, A.D.M., Boddy, L., 1988. Fungal decomposition of wood. Its biology and ecology. John Wiley & Sons Ltd, New York.

Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? New Phytol. 157, 475–492.

Richards, J.H., Caldwell, M.M., 1987. Hydraulic lift. Substantial nocturnal water transport between soil layers by Artemisia tridentata roots. Oecologia 73, 486–489.

Rillig, M.C., 2005. A connection between fungal hydrophobins and soil water repellency? Pedobiologia 49, 395–399.

Rochette, P., Ellert, B., Gregorich, E.G., Desjardins, R.L., Pattey, E., Lessard, R., Johnson, B.G., 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. Can. J. Soil Sci. 77, 195–203.

Sandoval, F.J., Zhang, Y., Roje, S., 2008. Flavin nucleotide metabolism in plants: monofunctional enzymes synthesize fad in plastids. J. Biol. Chem. 283, 30890–30900.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil. Biol. Biochem. 31, 831–838.

Schlösser, T., Wiesenburg, A., Gätgens, C., Funke, A., Viets, U., Vijayalakshmi, S., Nieland, S., Stahmann, K.-P., 2007. Growth stress triggers riboflavin overproduction in *Ashbya gossypii.* Appl. Microbiol. Biotechnol. 76, 569–578.

Schmidt, O., Liese, W., 1980. Variability of Wood Degrading Enzymes of *Schizophyllum commune.* Holzforschung 34, 67–72.

Scholz, F.G., Bucci, S.J., Goldstein, G., Moreira, M.Z., Meinzer, F.C., Domec, J.C., Villalobos-Vega, R., Franco, A.C., Miralles-Wilhelm, F., 2008. Biophysical and life-history determinants of hydraulic lift in Neotropical savanna trees. Funct. Ecol. 22, 773–786.

Seephueak, P., Phongpaichit, S., Hyde, K.D., Petcharat V, 2011. Diversity of saprobic fungi on decaying branch litter of the rubber tree (*Hevea brasiliensis*). Mycosphere 2, 307–330.

Shachnovich, Y., Berliner, P.R., Bar, P., 2008. Rainfall interception and spatial distribution of throughfall in a pine forest planted in an arid zone. J. Hydrol. 349, 168–177.

Shantz, H.L., Piemeisel, R.L., 1917. Fungus fairy rings in eastern Colorado and their effect on vegetation. J. Agricult. Res. 11, 191–245.

Sharma, P.D., 2005. The Fungi. Rastogi Publications, Meerut.
Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of warming and drought on grassland microbial communities. ISME J. 5, 1692–1700.
Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., Molina, R., 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388, 579–582.
Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125, 27–58.
Smith, J.F., Hayes, W.A., 1972. Use of autoclaved substrates in nutrional investigations on the cultivated mushroom. Mushroom Sci. 8, 355–362.
Smith, M.L., Bruhn, J.N., Anderson, J.B., 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356, 428–431.
Smyth, G.K., 2005. limma: Linear Models for Microarray Data. In: Gentleman, R., Carey, V., Huber, W., Irizarry, R., Dudoit, S. (Eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Springer New York, pp. 397-420.
Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil. Soil. Biol. Biochem. 40, 2068–2075.
Söderström, B.E., 1979. Seasonal fluctuations of active fungal biomass in horizons of a podzolized pine-forest soil in central Sweden. Soil. Biol. Biochem. 11, 149–154.
Sparling, G.P., Ross, D.J., 1988. Microbial contributions to the increased nitrogen mineralisation after air-drying of soils. Plant Soil 105, 163–167.
Spohn, M., Carminati, A., Kuzyakov, Y., 2013. Soil zymography – A novel in situ method for mapping distribution of enzyme activity in soil. Soil. Biol. Biochem. 58, 275–280.
Spohn, M., Kuzyakov, Y., 2013. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with ^{14}C imaging. Soil. Biol. Biochem. 67, 106–113.
Steinberg, G., Schliwa, M., 1995. The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties. Mol. Biol. Cell 6, 1605–1618.
Synetos, D., Dabeva, M.D., Warner, J.R., 1992. The yeast ribosomal protein S7 and its genes. J. Biol. Chem. 267, 3008–3013.
Taheri, P., Tarighi, S., 2010. Riboflavin induces resistance in rice against *Rhizoctonia solani* via jasmonate-mediated priming of phenylpropanoid pathway. J. Plant Physiol. 167, 201–208.
Talbot, J.M., Allison, S.D., Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct. Ecol. 22, 955–963.
Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil. Biol. Biochem. 57, 282–291.
Tang, L., Kim, M.D., Yang, K.-S., Kwon, S.-Y., Kim, S.-H., Kim, J.-S., Yun, D.-J., Kwak, S.-S., Lee, H.-S., 2008. Enhanced tolerance of transgenic potato plants overexpressing nucleoside diphosphate kinase 2 against multiple environmental stresses. Transgenic Res. 17, 705–715.
Thompson, W., Boddy, L., 1983. Decomposition of suppressed oak trees in even-aged plantations. II. Colonisation of tree roots by cord- and rhizomorph-producing basidiomycetes. New Phytol. 93, 277–291.
Thompson, W., Eamus, D., Jennings, D.H., 1985. Water flux through mycelium of *Serpula lacrimans.* Trans. Br. Mycol. Soc. 84, 601–608.
Thornalley, P.J., 2003. Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation. Biochm. Soc. Trans. 31, 1343–1348.

Tlalka, M., Hensman, D., Darrah, P.R., Watkinson, S.C., Fricker, M.D., 2003. Noncircadian oscillations in amino acid transport have complementary profiles in assimilatory and foraging hyphae of *Phanerochaete velutina.* New Phytol. 158, 325–335.

Tlalka, M., Watkinson, S.C., Darrah, P.R., Fricker, M.D., 2002. Continuous imaging of amino-acid translocation in intact mycelia of *Phanerochaete velutina* reveals rapid, pulsatile fluxes. New Phytol. 153, 173–184.

Tlalka, M., Fricker, M., Watkinson, S., 2008. Imaging of long-distance alpha-aminoisobutyric acid translocation dynamics during resource capture by Serpula lacrymans. Appl. Environ. Microbiol. 74, 2700–2708.

Toberman, H., Freeman, C., Evans, C., Fenner, N., Artz, R.R.E., 2008. Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland *Calluna* heathland soil. FEMS Microbiol. Ecol. 66, 426–436.

van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310.

van Genuchten, M Th, Leij, F.J., Yates, SR, 1991. The RETC code for quantifying the hydraulic functions of unsaturated soils. EPA Report 600/2-91/065, U.S. Salinity Laboratory, USDA-ARS, Riverside.

van Gestel, M., Ladd, J.N., Amato, M., 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. Soil. Biol. Biochem. 24, 103–111.

van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: The fate of fast- and slow-growing microorganisms in soils from different climates. Soil. Biol. Biochem. 25, 109–123.

Voloshin, O., Gocheva, Y., Gutnick, M., Movshovich, N., Bakhrat, A., Baranes-Bachar, K., Bar-Zvi, D., Parvari, R., Gheber, L., Raveh, D., 2010. Tubulin chaperone E binds microtubules and proteasomes and protects against misfolded protein stress. Cell Mol. Life Sci. 67, 2025–2038.

Wang, Y., Zhang, X., Zhou, Q., Zhang, X., Wei, J., 2015. Comparative transcriptome analysis of the lichen-forming fungus *Endocarpon pusillum* elucidates its drought adaptation mechanisms. Sci. China Life Sci. 58, 89–100.

Watkinson, S.C., Boddy, L., Money, N., 2015. The Fungi. Elsevier Science, Oxford.

Watkinson, S., Bebber, D., Darrah, P., Fricker, M.D., Tlalka, M., Boddy, L., Gadd, G.M., 2006. The role of wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: Gadd, G.M. (Ed.), Fungi in Biogeochemical Cycles. Cambridge University Press, Cambridge, pp. 151–181.

Wells, J.M., Boddy, L., 1990. Wood decay, and phosphorus and fungal biomass allocation, in mycelial cord systems. New Phytol. 116, 285–295.

Wells, J.M., Hughes, C., Boddy, L., 1990. The fate of soil-derived phosphorus in mycelial cord systems of *Phanerochaete velutina* and *Phallus impudicus.* New Phytol. 114, 595–606.

Wessels, J.G.H., de Vries, O. M.H., Asgeirsdottir, S.A., Schuren, F., 1991. Hydrophobin Genes Involved in Formation of Aerial Hyphae and Fruit Bodies in Schizophyllum. Plant Cell 3, 793–799.

Wessels, J.G.H., 1997. Hydrophobins: Proteins that Change the Nature of the Fungal Surface. Adv. Microb. 38, 1–45.

Wood, J., Tordoff, G.M., Jones, T.H., Boddy, L., 2006. Reorganization of mycelial networks of *Phanerochaete velutina* in response to new woody resources and collembola (*Folsomia candida*) grazing. Mycol. Res. 110, 985–993.

Worrall, J.J., Anagnost, S.E., Zabel, R.A., 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89, 199–219.

Wösten, H.A., 2001. Hydrophobins: multipurpose proteins. Annu. Rev. Microbiol. 55, 625–646.

Yang, H.H., Effland, M.J., Kirk, T.K., 1980. Factors influencing fungal degradation of lignin in a representative lignocellulosic, thermomechanical pulp. Biotechnol. Bioeng. 22, 65–77.

Yang, S., Vanderbeld, B., Wan, J., Huang, Y., 2010. Narrowing Down the Targets: Towards Successful Genetic Engineering of Drought-Tolerant Crops. Mol. Plant 3, 469–490.

York, C., Canaway, P., 2000. Water repellent soils as they occur on UK golf greens. J. Hydrol. 231-232, 126–133.

Yuste, J., Peñuelas, J., Estijarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486.

Zhang, Y., 2003. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev. 17, 2733–2740.

Zhang, C., Yin, L., Dai, S., 2009. Diversity of root-associated fungal endophytes in *Rhododendron fortunei* in subtropical forests of China. Mycorrhiza 19, 417–423.

Zhang, X., Chen, X., Jiang, J., Yu, M., Yin, Y., Ma, Z., 2015. The tubulin cofactor A is involved in hyphal growth, conidiation and cold sensitivity in Fusarium asiaticum. BMC Microbiol. 15, 35.

Manuscripts

2. Redistribution of soil water by a saprotrophic fungus enhances carbon mineralisation

Authors: Alexander Guhr[a], Werner Borken[a], Marie Spohn[a] and Egbert Matzner[a]

[a]Department of Soil Ecology, BayCEER, University of Bayreuth, Dr.-Hans-Frisch-Straße 1-3, 95448 Bayreuth

Keywords: saprotrophic fungi, hydraulic redistribution, drought, carbon mineralisation

Published as:

Alexander Guhr, Werner Borken, Marie Spohn and Egbert Matzner (2015). Redistribution of soil water by a saprotrophic fungus enhances carbon mineralisation. *Proceedings of the National Academy of Sciences of the United States of America* 112, 14647–14651. DOI: 10.1073/pnas.1514435112.

2.1 Abstract

The desiccation of upper soil horizons is a common phenomenon leading to a decrease of soil microbial activity and mineralisation. Recent studies have shown that fungal communities and fungal-based food webs are less sensitive and better adapted to soil desiccation than bacterial based food webs. One reason for better fungal adaptation to soil desiccation may be hydraulic redistribution of water by mycelia networks. Here, we show that a saprotrophic fungus (*Agaricus bisporus*) redistributes water from moist (-0.03 MPa) into dry (-9.5 MPa) soil at about 0.3 cm min^{-1} in single hyphae, resulting in an increase in soil water potential after 72 hrs. The increase in soil moisture by hydraulic redistribution significantly enhanced carbon mineralisation by 2800% and enzymatic activity by 250-350% in the previously dry soil compartment within 168 hrs. Our results demonstrate that hydraulic redistribution can partly compensate water deficiency if water is available in other zones of the mycelia network. It is likely a mechanism behind higher drought resistance of soil fungi compared to bacteria. Moreover, hydraulic redistribution by saprotrophic fungi is an underrated pathway of water transport in soils and may lead to a transfer of water to zones of high fungal activity.

2.2 Significance

This work shows a mechanism behind the observed higher drought resistance of soil fungi compared to bacteria. It also demonstrates the relevance of hydraulic redistribution by saprotrophic fungi for ecosystem ecology by influencing the carbon and water cycle in soils and terrestrial ecosystems under drought conditions. Furthermore, we documented a so far underrated pathway of water in desiccated soils.

2.3 Introduction

Drought is one of the most important and also frequent abiotic stresses in terrestrial ecosystems (1). With respect to soil processes, soil desiccation limits microbial activity, decreases soil enzyme activity (2), soil respiration (3, 4) and N mineralisation (5). In addition, drought can also alter soil microbial community composition (2, 6).

During desiccation, soil fungal communities and fungal based food webs are better adapted to drought than bacteria communities and bacteria based food webs (7, 8). Bacteria are more strongly retarded than fungi (9) since bacterial activity needs a constant supply of water (10). One reason for the better adaptation of fungi compared to bacteria at low soil water potentials is seen in their strong cell walls, preventing water losses (1). The strength of fungal cell walls can even be enhanced by cross-linking of polymers and thickening under stress. Another reason for the better adaptation of fungi to soil desiccation might be hydraulic redistribution of water by mycelia networks. Hydraulic redistribution is defined as the passive transport of water in soils through organisms along a gradient in soil water potential and was first observed for plant roots (11). Hydraulic redistribution through plant roots was shown to improve plant survival and nutrient uptake by extending the life span and activity of roots (12) and by favouring mineralisation of soil organic matter (13). Mycorrhiza fungal hyphae can also relocate water along gradients in soil water potential (12, 14, 15, 16). In addition, some studies reported the transport of nutrients and water over larger distances (>1 m) by saprotrophic fungal hyphae in non-soil systems. The water transport in hyphae was attributed to gradients in osmotic potentials (17, 18, 19, 20, 21). Further, water leakage from hyphae into dry growth medium was observed (22).

Saprotrophic fungi are main regulators of soil nutrient cycling, litter decomposition and soil respiration due to their specific enzymatic activities (23, 24) and due to the high density of hyphae in soil (up to 800 m g^{-1} soil, 25) especially in the litter layers. The ability of saprotrophic fungi for hydraulic redistribution would provide a direct and fast connection between water and nutrient sources in soils which would be hardly accessible to bacteria. This could have enormous impact on decomposition processes under drought conditions.

Here, we show the potential of the saprotrophic fungus *Agaricus bisporus* for hydraulic redistribution and impact of hydraulic redistribution on carbon mineralisation in a desiccated soil.

2.4 Result and discussions

We analysed hydraulic redistribution using 4–5 replicates of two-chamber units filled with homogenised mineral soil. The single chambers of each unit were separated by a 2 mm air gap to prevent bulk flow of water (Fig. 1.1). After inoculation and the establishment of hyphal bridges through the air gap between the two chambers, the soil in both chambers was desiccated to a soil water potential of about -9.5 MPa. Thereafter, only chamber I was rewetted to field capacity (-0.03 MPa) while chamber II of each unit remained dry. Hydraulic redistribution was prevented in the controls by cutting the hyphal bridges between the two chambers. Volumetric water contents, soil water potential and deuterium-labelling were used for quantification of hydraulic redistribution.

72 hours after rewetting of chamber I with deuterium labelled water, the volumetric soil water content of chamber II increased on average by about 0.02 cm^3 H_2O cm^{-3} soil. The small increase of 0.006 cm^3 H_2O cm^{-3} soil in chamber II of the controls can be attributed to diffusion of gaseous water through the air gap. Deuterium signatures of redistributed soil water supported the rates of hydraulic redistribution by *A. bisporus*: After 72 hours the amount of redistributed water from chamber I to chamber II was three times higher with hydraulic redistribution than in the controls (Fig. 2.1). This was accompanied by an increase in water potential of the bulk soil in chamber II from -9.5 to -6.9 MPa with hydraulic redistribution while in the controls soil water potential only increased from -9.2 to -8.2 MPa.

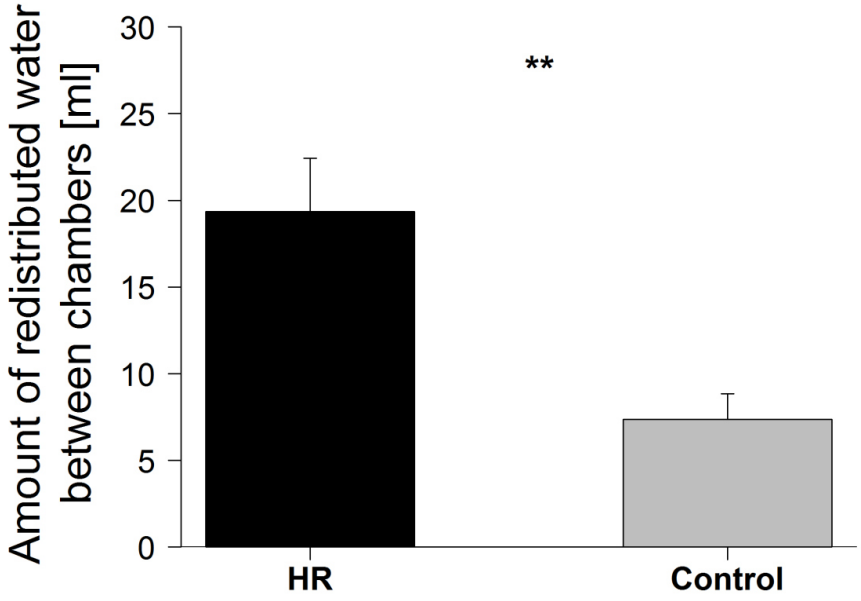


Fig. 2.1: Hydraulic redistribution (HR) by hyphae. Amount of water redistributed from chamber I to chamber II 72 h after the irrigation of chamber I. Calculation based on hydrogen stable isotope ratios. Black: active HR, grey: control with no fungal connection (Mean + SEM, n= 4, Mann–Whitney U test: $U_{1,7}$= 0, **= p<0.01).

After 72 hrs, hydraulic redistribution through hyphae had resulted in an average water flux of 67 µl cm^{-2} day^{-1} between both chambers. The number of hyphae bridging the two chambers in the air gap was about 2300 cm^{-2}. The total water flux between the chambers corresponds to a specific water flux of about 0.03 µl day^{-1} through a single hyphae. A flow velocity of about 0.3 cm min^{-1} in single hyphae was calculated using the Bernoulli's equation for the central cell lumen of hyphae (outer diameter: 4.4 µM, central cell lumen approximately 2/3 of the total hyphal diameter, 26). This corresponds to flow velocities observed for the central cell lumen in AM mycorrhizal hyphae (0.3 cm min^{-1}, 27).

In general, passive mass flow is considered as the main mechanism of water transport in fungal hyphae (28), but active transport mechanisms for water might also be involved, like cytoplasmic streaming (with velocities of 0.03–0.05 cm min^{-1} in hyphae, 29), vesicles moved by motor proteins (up to 0.02 cm min^{-1}, 30), and vacuolar pathways (about 0.005 cm min^{-1}, 31). Given these flow velocities, active transport cannot be the reason for the observed high velocities, and thus, water redistribution is driven by passive mass flow along the soil water potential gradient. The mass flow of water can be apoplastic as well as symplastic. Symplastic water transport at such flow velocities requires the presence of aquaporins. Those are likely part of the cell membrane in *A. bisporus* as the encoding genes were identified (32).

Amount and velocity of hydraulic redistribution by saprotrophic fungi might even be higher under natural soil conditions than in our experiment. First, hyphal density in the chamber experiment was small compared to natural soils. From the number of hyphae in the air gap and an estimated tortuosity factor of 2 for hyphal length in soil pores, a total hyphal length of about 30 m g^{-1} soil DW close to the air gap was calculated. In natural soils hyphal length of up to 800 m g^{-1} soil are reported (25). Second, the potential for hydraulic redistribution is likely larger for fungal species that form more complex mycelial structures, such as rhizomorphs or cords, i.e., aggregations of longitudinal aligned hyphae. While *A. bisporus* is capable to form such cords, only single hyphae were found in our experiment. Cord forming fungi were found to be very effective in translocating nutrients (20, 21, 33, 34). The transport of nutrients in cords is much faster than in non-differentiated mycelium (28) and the distances for nutrient translocation in fungal cords can be >1 m (21). After localization and identification of substrates in the soil, cord connections are strengthened to exploit the substrate while other parts of the mycelium regress (35, 36), preferentially directing water to the substrate. Hence, as with nutrient translocation, hydraulic redistribution through fungal cords and especially rhizomorphs is probably more effective than through single hyphae.

In an additional experiment, we determined the effect of hydraulic redistribution on carbon mineralisation in desiccated soils by measuring soil enzyme activities and mineralisation of ^{13}C labelled plant material. Soil zymography allowed us to measure enzyme activity *in situ* under different soil water contents, thus showing the effect of soil moisture on enzyme activity. Hydraulic redistribution by hyphae increased enzyme activity on average by 350% for N-acetylglucosaminidase and by 250% for cellobiohydrolase compared to the controls (Fig. 2.2). Enzyme activities decreased with increasing distance to the air gap in chamber II when hydraulic redistribution was active (Fig. 2.3), while no such pattern was observed in the controls. Similar relations of soil enzyme activities were observed in soils with and without irrigation (2, 37), emphasizing the significance of hydraulic redistribution by fungi.

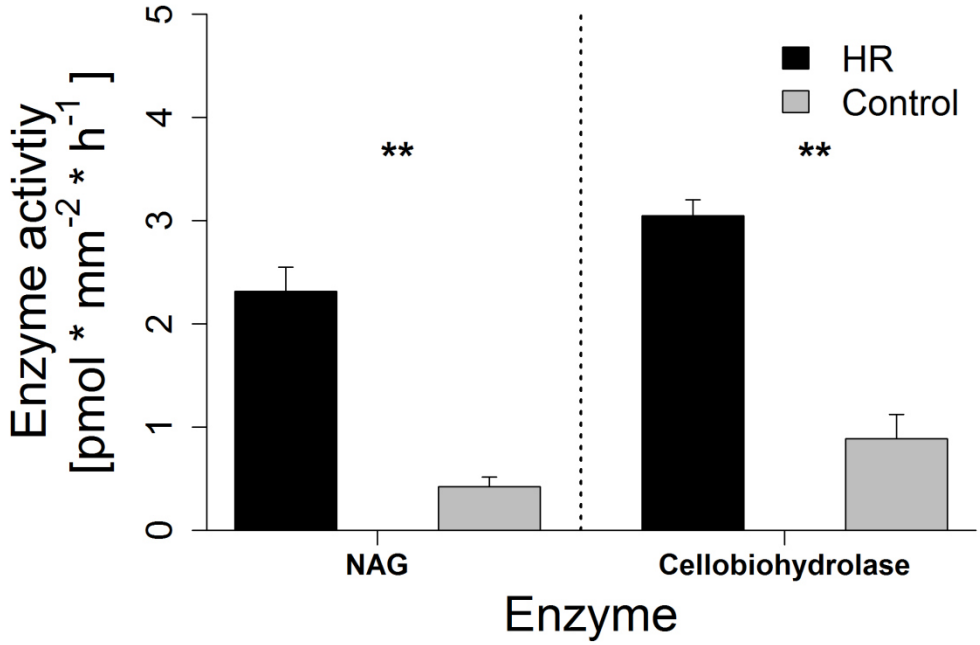


Fig. 2.2: Enzyme activity on the soil surface. Measured in chamber II, 7 days after irrigation of chamber I. Black: active HR, grey: control with no fungal connection (Mean + SEM, n= 5, chitinase: lme: $F_{1;39}$= 20.30, **= p<0.01, cellobiohydrolase: lme: $F_{1,39}$= 15.91, **= p<0.01).

Hydraulic redistribution led to an enrichment in ^{13}C of the respired CO_2 that was apparent already after 24 hrs and increased throughout the rest of the experiment. After 168 hrs, the cumulative C mineralisation amounted to 59.7 g CO_2 kg^{-1} C with active hydraulic redistribution and 2.1 g CO_2 kg^{-1} C in controls (Mann–Whitney U test: $U_{1,9}$= 0, P<0.01, Fig. 2.4). This relation is similar to variations in C mineralisation rates between dry and rewetted litter in forest soils (0.11 and 137 mg CO_2 kg^{-1} C hr^{-1}, respectively, 38) which again illustrates the significance of hydraulic redistribution for mineralisation.

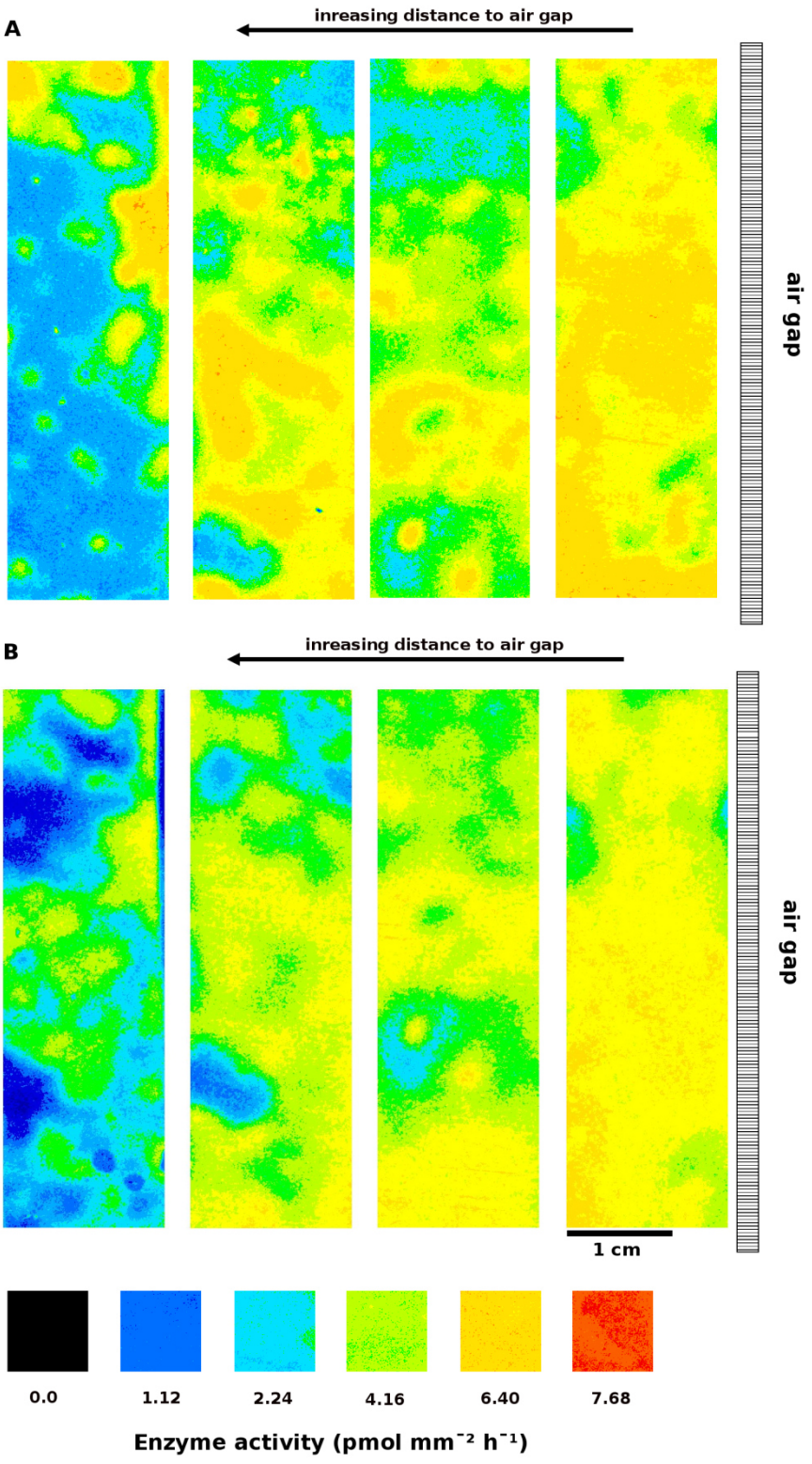


Fig. 2.3: Spatial distribution of enzyme activity on the soil surface. A.) Cellobiohydrolase and B.) N-acetylglucosaminidase in chamber II of one mesocosm with active HR, 7 days after irrigation of chamber I. The calibration line for the enzyme activity is presented at the bottom.

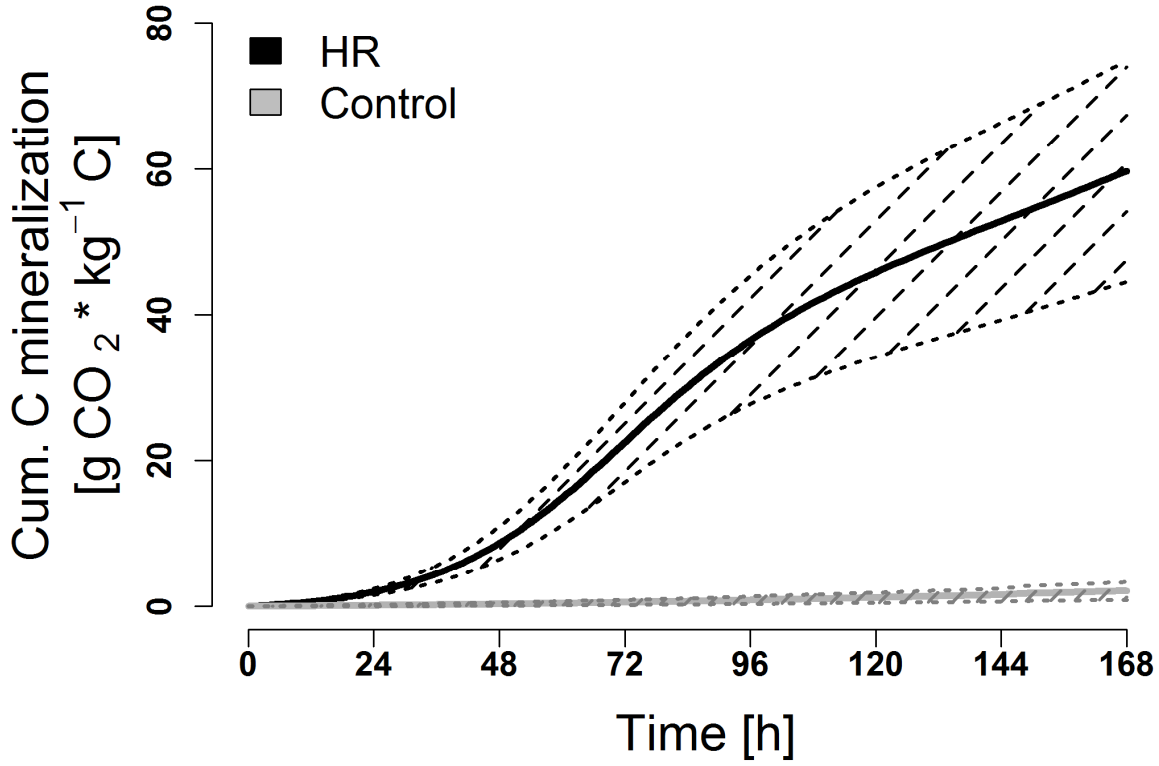


Fig. 2.4: Cumulative carbon mineralisation. Calculation based on ^{13}C-CO_2 efflux from labelled plant material in chamber II, following irrigation of chamber I. Black: active HR, grey: control with no fungal connection (Solid lines= mean values, dashed lines= SEM, n= 5).

Therefore, saprotrophic fungi not only have the capability of hydraulic redistribution but can also partly compensate unfavourable soil moisture conditions in desiccated soil as long as water is available in other zones of the mycelial network, like in deeper soil horizons. Under drought conditions a descending desiccation from the substrate rich upper soil layers to the subsoil develops. The leakage of water from the hyphae into the surrounding soil is concentrated to hyphal tips (39), and hyphal tips are concentrated in the growing part of the mycelium close to the substrate (35). Water redistribution through mycelium, bypassing capillary transport through soil pores, has probably been underrated as a pathway for water movement in desiccated soils. Hydraulic redistribution is of particular relevance for bridging capillary barriers. Overall, hydraulic redistribution likely leads to a transfer of water to hotspots of fungal activity with preferential wetting of the surrounding substrate. It enables high fungal enzymatic activity in the growth zone even under low soil water potentials. Hence, hydraulic redistribution likely is a mechanism behind the higher resistance of soil fungi compared to bacteria to soil desiccation.

2.5 Materials and Methods

2.5.1 General Setup

Experiments were carried out in mesocosms (adapted from 39) represented by two chambers (a 6×20×15 cm), filled with a steam sterilised mixture (2:1:1 v/v/v) of loamy soil (17% clay; 76% silt; 7% sand) and medium coarse quartz sand (Dorsilit 8, particle size range: 0.3-0.8 mm) as well as coarse quartz sand (Dorsilit 7, 0.6-1.2 mm, Dorfner GmbH & Co., Hirschau, Germany). The two chambers made of Makrolon (Bayer AG, Leverkusen, Germany) had openings on the sides facing each other, which were covered by two 160 µM pore size stainless steel mesh screens. A 2 mm thick air gap between both chambers prevented capillary flow of water and was stabilised by 2 additional perforated stainless steel mesh screens with 2 mm pore size. Chamber tops were removable but were air-tight if closed. The soil surface was compressed slightly to obtain a uniformly flat surface. Fungal cultures (DSM No. 3056: *Agaricus bisporus* (LANGE) IMBACH) were received from the Leibniz Institute DSMZ (Braunschweig, Germany) and grown on MEPA (malt extract peptone agar) at 14°C. The soil of chamber I was inoculated by placing a 1 cm^2 agar plate with fungal hyphae into the substrate close to the air gap at a depth of approx. 2 cm. *A. bisporus* was chosen since its one of the best studied filamentous fungal species, with a complete available genome, that shows a fast growth rate. Both chambers were maintained at 23°C and irrigated regularly to field capacity with a liquid fungal growth medium (2% glucose, 0.2% peptone, 0.2% yeast extract, 0.1% K_2HPO_4, 0.46% KH_2PO_4, and 0.05% $MgSO_4$, 40) for 6 weeks. The chamber tops were kept open, but were covered with glass microfiber filter paper (Grade 934-AH, Whatman Ltd, Maidstone, United Kingdom) during the growth phase to facilitate air exchange and avoid contaminations. Volumetric water content was controlled continuously using soil moisture sensors monitoring the dielectric constant of the media (ECH_2O-10 moisture sensor, Decagon Devices Inc., Pullman WA, U.S.A.).

2.5.2 Quantification of hydraulic redistribution

After desiccation for 6 weeks at 23°C to a soil water potential of approximately -9.5 MPa in both chambers, chamber I was rewetted to field capacity (-0.03 MPa) with deuterium-labelled water (3 at% deuterium enrichment, ROTH GmbH + Co. KG, Karlsruhe, Germany). Mescosms were then closed air-tight and only opened for sampling of soil cores. Soil cores of chamber II were destructively sampled 72 hrs after irrigation of chamber I. Water for isotope analyses was extracted from soil samples by cryogenic vacuum extraction (41). Hydrogen stable isotope analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using thermal conversion/isotope-ratio mass-spectrometry (TC-IRMS; isotope mass

spectrometer: delta V advantage, Thermo Fisher Scientific, Bremen, Germany; pyrolysis oven: TG pyrolysis oven HTO, HEKAtech, Wegberg, Germany; interface: ConFlo IV, Thermo Fisher Scientific, Bremen, Germany).

In addition, soil water potential was measured on collected soil samples (4 cm diameter, 0.5 cm thickness) using the chilled mirror dewpoint method (WP4-T, Decagon Devices Inc., Pullman WA, U.S.A., 42).

Controls were established by mesocosms treated in the same way as described above, but the hyphal connections between the chambers in the air gap were severed by cutting with a thin stainless-steel wire before irrigating chamber I. In total, four mescosms with intact fungal connections as well as four control mescosoms were treated with deuterium labelled water.

2.5.3 Mineralisation of organic matter

CO_2 efflux from the soil is an indicator of the general activity of soil microorganisms and was therefore used to estimate the impact of hydraulic redistribution on mineralisation of organic matter under drought conditions. The use of ^{13}C labelled plant material (*Triticum aestivum* L. green shoots; >97 atom% ^{13}C; C/N ratio: 15; IsoLife, Wageningen, The Netherlands) enabled us to trace the origin of collected CO_2. Labelled plant material (5 ground samples of 20 mg each) were placed at regular intervals of 4 cm from the mesh screen on the soil surface of the non-irrigated chamber II, shortly before rewetting chamber I. Mescosms were then closed air-tight and were not opened for 7 days. CO_2 effluxes were regularly measured for 7 days at 20°C using the dynamic closed-chamber technique (43). CO_2 concentrations in the mesocosms headspace (1.2 L) were measured every 6 minutes for 30 minutes periods for the first 24 hrs with an infrared gas analyser (LiCOR 820, Licor, U.S.A.). Beginning with the second day of experiments, CO_2 concentrations were measured for 48 minutes periods. Soil CO_2 effluxes were calculated from the slope of the linear regression between CO_2 concentration and incubation time. An alternative air path flow was opened at the end of each measurement cycle for 30 min on the first day of measurements and subsequently for 12 min to flush the system with CO_2 free synthetic air and reduce the CO_2 concentration. In addition, the extracted air was collected every 12 hrs for further analysis of ^{13}C isotope contents to determine the percentage of decomposed plant material in chamber II. Extracted air was stored in 10 ml butyl rubber septum-capped vials by flushing for 90 s. Septa were heated at 105°C for 12 hrs prior to vial closing to prolong stability of CO_2 isotope composition (44). Vials were flushed with N_2 for 90 sec prior usage to remove environmental CO_2. ^{13}C-CO_2 efflux for the whole measurement cycle was interpolated from measured ^{13}C-CO_2 efflux values using a Gaussian function extended with a linear term to

adjust for divergences from a normality distribution. Function fitness was optimised using Solver (Microsoft cooperation, Redmond, U.S.A.).

$\delta^{13}C$ analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using using GC-Combustion-IRMS (GC: Trace GC 2000, CE Instruments, Milano, Italy; interface: GC-combustion III, Thermo Fisher Scientific, Bremen, Germany; IRMS: delta plus, Thermo Fisher Scientific, Bremen, Germany). In total, five mescosms with intact fungal connections as well as five control mescosoms were treated with labelled plant material.

2.5.4 Analysis of soil enzyme activity

The impact of hydraulic redistribution on soil enzyme activity was analysed using soil zymography (45 modified by 46). This *in situ* method allows for analysis of the two-dimensional distribution of enzyme activities in soil with high spatial resolution and under different water contents in contrast to more traditional methods that are based on the determination of enzyme activity in solution. Hence, this provided a comprehensive picture of the allocation of redistributed water in chamber II and the resulting influence on enzyme activities. In addition, the study of enzymatic activities provides functional information on specific aspects of organic matter mineralisation and can therefore support the results of CO_2 efflux measurements. N-acetylglucosaminidase and cellobiohydrolase activity were analysed using the artificial substrates 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MNG) and 4-methylumbelliferyl β-D-cellobioside (4-MC, both Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), respectively. The fluorogenic 4-methylumbelliferone (MUF) is released from 4-MNG and 4-MC due to hydrolytic cleavage in presence of compatible enzymes. In soils, the activity of chitinase is considered as a good indicator of fungal biomass and activity (47, 48).

A 1% agarose gel (size of 0.1×12.0×11.0 cm) was cast in systems usually used for vertical gel-electrophoresis (Biometra GmbH, Göttingen, Germany). The gel was sliced in 4 parts à 2x11 cm and all 4 parts were attached to the soil surface of the non-irrigated chamber II in the space between the labelled plant material samples. Polyamide membrane filters (0.45 µM pore size, Sartolon, Sartorius AG, Göttingen, Germany) were sliced in 10 parts à 2x11 cm. Half of the slices were saturated with a 4-MNG solution or 4-MC solution (25% w/v, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), respectively. 4 slices of each group were placed in turn on top of the gel slices, starting with the 4-MNG group. The membrane filter was extracted after an incubation time of 25 min at 20°C for 4-MNG and 20 min for 4-MC and illuminated on a fluorescent transilluminator in the dark (wavelength: 355 nm, Desaga GmbH, Wiesloch,

Germany). Pictures were taken with a digital camera (Nikon D3100) and analysed in comparison to controls without hydraulic redistribution. To adjust for differences in exposure time, which are necessary to avoid overexposure at high activities and loss of details at low activities, one filter slice was not incubated on the soil but photographed together with the others and served as a standard of zero activity. A calibration line was prepared from membranes soaked in different solutions of MUF concentrations (0, 35, 70, 130, 200 µM). These calibration membranes were cut into strips of 2 cm, and photographed under the UV light in the same way as the zymogram membranes. The amount of MUF on an area basis was calculated from the volume of solution taken up by the membrane and by the size of the membrane.

Image processing and analysis were done using the open source software imageJ 1.46r (Wayne Rasband, National institutes of health, U.S.A., 2014). The digital images were transformed to 8-bit, i.e., greyscale images. To illustrate the results, the values of the greyscale image were depicted in false color. The linear correlation between the MUF concentration and the mean of greyscale in an area of 4 cm^2 of each calibration gel were calculated using the software R. A segment à 1.5 x 7.5 cm with no visible disturbance was selected from the soil zymograms and mean values of the greyscale were measured. Values were standardised based on the difference between the standards of zero activity and the calibration membrane with 0 µM MUF concentration. Values were expressed as µg MUF per hour and mm^2.

2.5.5 Data analysis

All statistical analyses and graphics were done using R 3.1.0 (R Developmental Core Team, 2014). Normality and homogeneity of the data were tested using Shapiro-Wilk-Test and Levene's-test, respectively. Enzyme activities were analysed using linear mixed effect models as implemented in the R package nlme (49). The sample origin from the different chambers was added as a random factor into the model to adjust for random variances among chambers. For pair–wise posthoc comparisons general linear hypotheses based on Tukey all–pair comparisons were conducted, using the R package multcomp (50).

Kruskal–Wallis test with pair–wise Wilcox tests for posthoc comparisons were used if data was not normal and/or variances were not homogeneously distributed.

2.6 Acknowledgements

We thank C. Werner and M. Dubbert for their support for the cryogenic vacuum extraction of soil samples. We also thank G. Rambold and the mycology department of the University of Bayreuth for help with and usage of facilities for treating and storing fungal cultures. We further thank B. Gilfedder for critical feedback and discussions. Furthermore, we thank the Central Isotopic Laboratory of the Bayreuth Center of Ecology and Environmental Research (BayCEER) for the stable isotope analyses. This study was supported by DFG grant (DFG-MA1089/23-1).

2.7 References

1. Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88:1386–1394.
2. Toberman H, Freeman C, Evans C, Fenner N, Artz RRE (2008) Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland *Calluna* heathland soil. *FEMS Microbiol. Ecol.* 66:426–436.
3. Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralisation and fluxes in soils. *Glob. Chang. Biol.* 15:808–824.
4. Muhr J, Franke J, Borken W (2010) Drying–rewetting events reduce C and N losses from a Norway spruce forest floor. *Soil Biol. Biochem.* 42:1303–1312.
5. Chen Y, Borken W, Stange CF, Matzner E (2011) Effects of decreasing water potential on gross ammonification and nitrification in an acid coniferous forest soil. *Soil Biol. Biochem.* 43:333–338.
6. Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J.* 7:2229–2241.
7. Vries FT *et al. (*2012) Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Chang.* 2:276–280.
8. Six J (2012) Soil science: Fungal friends against drought. *Nat. Clim. Chang.* 2:234–235.
9. Griffin DM (1969) Soil Water in the Ecology of Fungi. *Annu. Rev. Phytopathol.* 7:289–310.
10. Greenwood DJ (1967) Studies on oxygen transport through mustard seedlings (*Sinapis alba* L.). *New Phytol.* 66:597–606.
11. Richards JH, Caldwell MM (1987) Hydraulic lift. Substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* 73:486–489.
12. Querejeta JI, Egerton-Warburton LM, Allen MF (2003) Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* 134:55–64.
13. Armas C, Kim J, Bleby T, Jackson R (2012) The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. *Oecologia* 168:11–22.
14. Duddridge JA, Malibari A, Read DJ (1980) Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* 287:834–836.

15. Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant Soil* 71:433–443.

16. Egerton-Warburton LM, Querejeta JI, Allen MF (2007) Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *J. Exp. Bot.* 58:1473–1483.

17. Brownlee C, Jennings DH (1982) Long distance translocation in *Serpula lacrimans*: Velocity estimates and the continuous monitoring of induced perturbations. *Trans. Br. Mycol. Soc.* 79:143–148.

18. Granlund HI, Jennings DH, Thompson W (1985) Translocation of solutes along rhizomorphs of *Armillaria mellea*. *Trans. Br. Mycol. Soc.* 84:111–119.

19. Thompson W, Eamus D, Jennings DH (1985) Water flux through mycelium of *Serpula lacrimans*. *Trans. Br. Mycol. Soc.* 84:601–608.

20. Jennings DH (1987) Translocation of solutes in fungi. *Biol. Rev.* 62:215–243.

21. Boddy L (1993) Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. *Mycol. Res.* 97:641–655.

22. Clarke RW, Jennings DH, Coggins CR (1980) Growth of *Serpula lacrimans* in relation to water potential of substrate. *Trans. Br. Mycol. Soc.* 75:271–280.

23. Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 36:191–218.

24. Crowther TW, Boddy L, Hefin Jones T (2012) Functional and ecological consequences of saprotrophic fungus-grazer interactions. *ISME J.* 6:1992–2001.

25. Söderström BE (1979) Seasonal fluctuations of active fungal biomass in horizons of a podzolized pine-forest soil in central Sweden. *Soil Biol. Biochem.* 11:149–154.

26. Sanders FE, Tinker PB (1973) Phosphate flow into mycorrhizal roots. *Pestic. Sci.* 4:385–395.

27. Ruth B, Khalvati M, Schmidhalter U (2011) Quantification of mycorrhizal water uptake via high-resolution on-line water content sensors. *Plant Soil* 342:459–468.

28. Cairney JWG (2005) Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. *Mycol. Res.* 109:7–20.

29. Marks GC, Kozlowski TT, eds (1973) *Ectomycorrhizae: Their Ecology and Physiology* (Academic Press, New York).

30. Steinberg G, Schliwa M (1995) The Neurospora organelle motor: a distant relative of conventional kinesin with unconventional properties. *Mol. Biol. Cell* 6:1605–1618.

31. Darrah PR, Tlalka M, Ashford A, Watkinson SC, Fricker MD (2006) The vacuole system is a significant intracellular pathway for longitudinal solute transport in basidiomycete fungi. *Eukaryotic Cell* 5:1111–1125.

32. Nehls U, Dietz S (2014) Fungal aquaporins: cellular functions and ecophysiological perspectives. *Appl. Microbiol. Biotechnol.* 98:8835–8851.

33. Boddy L, Watkinson SC (1995) Wood decomposition, higher fungi, and their role in nutrient redistribution. *Can. J. Bot.* 73:1377–1383.

34. Boberg JB., Finlay RD, Stenlid J, Lindahl BD (2010) Fungal C translocation restricts N-mineralisation in heterogeneous environments. *Funct. Ecol.* 24:454–459.

35. Fricker MD, Bebber D, Boddy L (2008) in *Ecology of Saprotrophic Basidiomycetes.* (Elsevier), pp 3–18.

36. Boddy L, Hynes J, Bebber DP, Fricker MD (2009) Saprotrophic cord systems: dispersal mechanisms in space and time. *Mycoscience* 50:9–19.

37. Sardans J, Peñuelas J (2005) Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biol. Biochem.* 37:455–461.

38. Kelliher F, Ross D, Law B, Baldocchi D, Rodda N (2004) Limitations to carbon mineralisation in litter and mineral soil of young and old ponderosa pine forests. *For. Ecol. Manage.* 191:201–213.

39. Plamboeck AH. *et al. (*2007) Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza* 17:439–447.

40. Nazrul MI, YinBing B (2011) Differentiation of homokaryons and heterokaryons of *Agaricus bisporus* with inter-simple sequence repeat markers. *Microbiol. Res.* 166:226–236.

41. Dalton FN (1988) Plant root water extraction studies using stable isotopes. *Plant Soil* 111:217-221.

42. Gee GW, Campbell MD, Campbell GS, Campbell JH (1992) Rapid measurement of low soil water potentials using a water activity meter. *Soil Sci. Soc. Am. J.* 56:1068–1070.

43. Rochette P *et al. (*1997) Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. *Can. J. Soil Sci.* 77:195–203.

44. Midwood AJ. *et al. (*2006) Collection and storage of CO_2 for ^{13}C analysis: An application to separate soil CO_2 efflux into root- and soil-derived components. *Rapid Commun. Mass Spectrom.* 20:3379–3384.

45. Spohn M, Carminati A, Kuzyakov Y (2013) Soil zymography – A novel in situ method for mapping distribution of enzyme activity in soil. *Soil Biol. Biochem.* 58:275–280.

46. Spohn M, & Kuzyakov Y (2013) Distribution of microbial-and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation–Coupling soil zymography with ^{14}C imaging. *Soil Biol. Biochem.* 67: 106-113.

47. Reeslev M, Miller M, Nielsen KF (2003) Quantifying mold biomass on gypsum board: Comparison of ergosterol and beta-N-Acetylhexosaminidase as mold biomass parameters. *Appl. Environ. Microbiol.* 69:3996–3998.

48. Andersson M, Kjøller A, Struwe S (2004) Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. *Soil Biol. Biochem.* 36:1527–1537.

49. Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team (2015) *nlme: Linear and nonlinear mixed effects models.*

50. Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom. Z.* 50:346–363.

3. Effect of water redistribution by two distinct saprotrophic fungi on carbon mineralisation and nitrogen translocation in dry soil

Authors: Alexander Guhr[a], Carlo Marzini[a], Werner Borken[a], Christian Poll[b], and Egbert Matzner[a]

[a]Department of Soil Ecology, BayCEER, University of Bayreuth, Dr.-Hans-Frisch-Straße 1-3, 95448 Bayreuth

[b]Soil Biology Section, Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70593 Stuttgart

Keywords: saprotrophic fungi, hydraulic redistribution, drought, carbon mineralisation, nitrogen translocation, foraging strategy

Published as:

Alexander Guhr, Carlo Marzini, Werner Borken, Christian Poll and Egbert Matzner (2016) Effect of water redistribution by two distinct saprotrophic fungi on carbon mineralisation and nitrogen translocation in dry soil. *Soil Biology and Biochemistry* 103, 380–387. DOI: 10.1016/j.soilbio.2016.09.009.

3.1 Abstract

Hydraulic redistribution (HR) of water from wet to dry soil compartments by non-differentiated mycelium was recently shown for the saprotrophic fungus *Agaricus bisporus*. The redistributed water triggered the carbon (C) mineralization in the dry soil. The potential of other saprotrophic fungal species and their mycelia networks for HR in soils is unknown. Here, we tested the potential for HR of the mycelial cord forming species *Schizophyllum commune,* compared it to capillary water transport in a sandy soil and assessed the impact of HR on C mineralization and enzyme activities in mesocosm experiments with dry and wet soil compartments using labeled water (^{2}H) and labeled organic substrate (^{13}C, ^{15}N). Further, we determined nitrogen (N) translocation between the soil compartments by the mycelium of *S. commune* and *A. bisporus*. The flow velocity of redistributed water in single hyphae of *S. commune* was about 0.43 cm min^{-1} which is 1.5-2 times higher than in hyphae of *A. bisporus*, suggesting that cords enhance fungal HR. The amount of redistributed water was similar to capillary transport in the sterile sandy soil. Despite greater potential for HR, *S. commune* only slightly increased C mineralization and enzyme activity in the dry soil within 7 days. *S. commune* translocated N towards the organic substrate in the dry soil and used it for hyphal growth whereas *A. bisporus* redistributed N within the mycelial network towards the wet soil. Our results suggest that fungal hyphae have the potential to overcome capillary barriers between dry and wet soil compartments via HR and that the impact of fungal HR on C mineralization and N translocation is related to the foraging strategy and the resource usage of the fungus species.

3.2. Introduction

Periods of drought and the resulting soil desiccation are important abiotic stressors negatively affecting soil organisms and ecosystems (Schimel et al., 2007). Low water potentials lead to a limitation, or under extreme conditions, even to a total inhibition of microbial activity in soils (Borken and Matzner, 2009; Manzoni et al., 2012). Drought stress can also alter microbial community composition, with fungal communities in non-tropical climate systems usually being less sensitive and better adapted compared to bacterial communities (Bapiri et al., 2010; Sheik et al., 2011; Yuste et al., 2011; Allison et al., 2013; Alster et al., 2013).

A shift of the bacteria to fungi ratio in soils due to water stress results from the need of many bacteria for a constant water supply (Greenwood, 1967). The potential of mycelia networks for hydraulic redistribution (HR), i.e., water transport along soil water potential gradients, might be a mechanism for the higher resistance of some fungi to drought. HR from wet to dry soil compartments has been shown for plant roots and mycelia networks of mycorrhizal fungi as well as for a saprotrophic fungus (Thompson et al., 1985; Jennings, 1987; Dawson, 1993; Querejeta et al., 2003; Guhr et al., 2015). The redistributed water in hyphae and roots can increase the water content in the soil by leakage from root or hyphal tips (Querejeta et al., 2003; Domec et al., 2004). Further, as recently shown for the saprotrophic fungus *Agaricus bisporus* (Lange) Imbach, HR by non-differentiated mycelium can enhance extracellular enzyme activity and the mineralization of soil organic matter in dry soil (Guhr et al., 2015).

It is, however, not clear if HR is a general trait of saprotrophic fungi and how the redistributed water is used by the fungi. The translocation of water and nutrients is known to vary among fungal species with different foraging strategies (Cairney, 1992; Cairney, 2005). The same may hold true for the effect of HR on organic matter mineralization.

Filamentous fungi have developed different foraging strategies by differentiation of mycelia network organizations. Some species only expend by radial growth of hyphae while others form complex mycelial network organizations like mycelial cords, i.e., aggregations of predominantly parallel and longitudinally aligned hyphae, and rhizomorphs (Boddy, 1999). The translocation of nutrients and water is more effective in mycelial cords and rhizomorphs than in non-differentiated mycelium (e.g., Lindahl et al., 2001; Cairney, 2005; Watkinson et al., 2006). Distances for nutrient translocation in fungal cords can be >1 m (Boddy, 1993) and translocation velocity can be > 25 cm h^{-1} in cords (Wells and Boddy, 1990). Therefore, fungal cords and rhizomorphs are considered as “highways” for water and nutrient transport in soils

(e.g., Jennings, 1987; Cairney, 2005). As with nutrient translocation, HR through fungal cords is probably more effective than through single hyphae.

The interaction of HR by mycelia networks and N translocation is yet unknown. In general, transport of nutrients and water in hyphae can occur bi-directionally, either towards the growing front or towards the base of the mycelial network (Cairney, 1992). The transport is generally considered to occur from sources to sinks (Lindahl et al., 2001). Therefore, nutrients are mainly translocated from nutrient sources at the growing front towards the base (Cairney, 1992). However, transfer of nutrients can also occur towards the growing front supporting the production of biomass (Jennings, 1987; Olsson and Gray, 1998; Tlalka et al., 2003). Further, saprotrophic fungi that decompose substrates with high C:N ratios are known to translocate N towards new C sources (Boberg et al., 2014; Frey et al., 2000; Tlalka et al., 2002). Thus, the fungal foraging strategy may have an effect on the amount and direction of N translocation within the mycelia network (Watkinson et al., 2006).

A saprotrophic fungal species of interest for studying HR by fungi is *Schizophyllum commune* Fries, as this species has the ability to form mycelial cords (Balaș and Tănase, 2012). *S. commune* is one of the most widespread fungi on earth (Ohm et al., 2010), adapted to substrates with high C:N ratios and known as a wood decomposing fungus causing white rot in woody debris (Schmidt and Liese, 1980). Additionally, *S. commune* grows on aboveground litter (Olsson and Gray, 1998; Seephueak et al., 2011), dead roots (Zhang et al., 2009; Glen et al., 2014) and was also found in mineral soils (Varghese, 1972; Neuhauser et al., 2009). Its genome includes a high number of genes coding for enzymes needed for degradation of lignocellulosic compounds (Ohm et al., 2010). The wide distribution and the high potential for decomposition of plant derived lignocellulosic components makes this species a suitable candidate for studying HR and nutrient translocation.

Here, we measured water translocation rates by HR of *S. commune* and compared the rates to capillary transport of a sandy soil. We further analyzed the impact of HR by *S. commune* on C mineralization and enzyme activities in dry soil-litter systems. Furthermore, we assessed the translocation of N within the mycelia networks of *S. commune* and *A. bisporus* in a mesocosm system with a strong gradient in soil water potential.

3.3 Materials and Methods

3.3.1 Eperimental Setup

Experiments were carried out in mesocosms as described in Guhr et al. (2015). Briefly, mesocosms consisted of 2-chamber units (6×20×15 cm) filled with a homogenized and steam sterilized mineral soil (2:1:1 v/v/v mixture of loamy soil (17% clay; 76% silt; 7% sand), medium coarse quartz sand (Dorsilit 8, particle size range: 0.3-0.8 mm) and coarse quartz sand (Dorsilit 7, 0.6-1.2 mm, both Dorfner GmbH & Co., Hirschau, Germany). The bulk density of the soil was adjusted to 1.33 g cm^{-3}. Soil hydraulic parameters according to the van Genuchten model (with m= 1-1/n; van Genuchten et al. 1991) were: residual water content = 0.078 m^3 m^{-3}; saturation water content = 0.41 m^3 m^{-3}; saturated hydraulic conductivity = 3.83 cm day^{-1}. The chambers of a mesocosm were separated by a 2 mm air gap (hydraulic barrier) to prevent capillary water flow. The air gap was stabilized by 2 stainless steel mesh screens on each side (pore-size: 160 µm).

Soil of chamber I was inoculated with *S. commune* (provided by the Department of Mycology at the University of Bayreuth) or *A. bisporus* (DSM No. 3056, from the Leibniz Institute DSMZ) by placing a 1 cm^2 agar plate with fungal hyphae close to the air gap at approx. 2 cm depth. The mesocosms were maintained at 23°C and irrigated regularly to field capacity with a liquid fungal growth medium (2% glucose, 0.2% peptone, 0.2% yeast extract, 0.1% K_2HPO_4, 0.46% KH_2PO_4, and 0.05% $MgSO_4$, Nazrul and YinBing, 2011) for 6 weeks to generate hyphal growth in both chambers. Afterwards, the soil of all mesocosms (both chambers) was desiccated for 6 to 8 weeks to a soil water potential of about -9.5 MPa. Volumetric water contents were measured continuously using soil moisture sensors (ECH_2O-10 moisture sensor, Decagon Devices Inc., Pullman WA, U.S.A.).

After desiccation, only chamber I was rewetted to field capacity (-0.03 MPa) while chamber II of each unit remained dry. These water potentials (-0.03 MPa and -9.5 MPa) were adjusted to achieve a strong water potential gradient between the chambers at moderate water stress for the fungi (cf. Dix, 1984; Manzoni et al., 2012). The number of hyphae bridging the air gap was estimated after each experiment by counting hyphae in 10 randomly chosen openings on the mesh screen. The mean value of all counts was then extrapolated to the whole area of the air gap (25 openings per cm^2, cross-sectional area of all openings at the soil contact area: 47.1 cm^2). Further, all visible cords were counted as well.

Two experiments were conducted with both fungi to quantify (I) HR (n=4 for each species), and (II) C mineralization and N translocation (n=5 *A. bisporus*; n=6 *S. commune*) between the

chambers of the mesocosms. In the controls (n= 4-6 as in the treatments, respectively), HR was prevented by cutting the hyphal bridges between the 2 chambers with a thin stainless-steel wire prior to rewetting. Additionally, 2 non-inoculated mesocosms without fungal hyphae were prepared to measure capillary water transport from chamber I to chamber II along the water potential gradient. These mesocosms had no air gaps or mesh screens and were filled throughout with homogenized mineral soil.

3.3.2 Quantification of HR

To quantify HR and capillary water transport, chamber I was rewetted with deuterium-labeled water (3 atom% deuterium enrichment, ROTH GmbH + Co. KG, Karlsruhe, Germany). Mesocosms were then closed air-tight and only opened for destructive sampling of soil cores from chamber II 72 h after irrigation of chamber I. About 50 g soil dry weight was taken at each of 3 distance points from the air gap (5, 10 and 15 cm). Soil water potential was measured using the dewpoint method (WP4-T, Decagon Devices Inc., Pullman WA, U.S.A.). Water for deuterium analysis was extracted by cryogenic vacuum extraction. Deuterium analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using thermal conversion/isotope-ratio mass-spectrometry (TC-IRMS; IRMS: delta V advantage, Thermo Fisher Scientific, Bremen, Germany; pyrolysis oven: TG pyrolysis oven HTO, HEKAtech, Wegberg, Germany; interface: ConFlo IV, Thermo Fisher Scientific, Bremen, Germany). Values are reported in conventional delta notation, defined as ‰ deviation from a reference standard (VSMOW: Vienna standard mean ocean water). The amount of redistributed water from chamber I to chamber II was calculated using a 2-end-member linear mixing model for all distances and summed up for the whole chamber (Dawson et al., 2002). Water transport between chambers was calculated per cm² contact surface (HR and Control= 47.1 cm²; capillary water transport= 60 cm²).

3.3.3 C mineralization and enzyme activity

The effect of HR on C mineralization was studied by using ^{13}C labeled plant material and by measuring the $^{13}CO_2$ efflux. Five portions à 20 mg of uniformly double-labeled and ground plant material (green shoots of *Triticum aestivum* L.; >97 atom% ^{13}C; >98 atom % ^{15}N; C: 39%; N: 3.8%; C/N ratio: 10; IsoLife, Wageningen, The Netherlands) were placed on the soil surface in the middle of chamber II at 2, 6, 10, 14 and 18 cm distance from the air gap, shortly before rewetting of chamber I. Mesocosms were then air-tightly closed and not opened for 7 days. CO_2 effluxes were continuously measured every hour for 7 days at 20°C using the dynamic closed chamber technique (cf. Muhr et al., 2008; infrared gas analyzer: LiCOR 820, Licor, USA). Soil

CO_2 effluxes were calculated from the slope of the linear regression between CO_2 concentration and incubation time. Headspace of mesocosms (1.2 L) was flushed with CO_2 free synthetic air between the measurements to avoid oversaturation of CO_2 concentrations.

Gas samples for $^{13}CO_2$ isotope analysis were collected every 12 h at the beginning of the flushing procedure by filling 10 ml butyl rubber septum-capped glass vials. ^{13}C analyses were conducted at the Laboratory for Isotopic-Biogeochemistry (University of Bayreuth) using GC-Combustion-IRMS (GC: Trace GC 2000, CE Instruments, Milano, Italy; interface: GC-combustion III, Thermo Fisher Scientific, Bremen, Germany; IRMS: delta plus, Thermo Fisher Scientific, Bremen, Germany). Mineralization of labelled plant material in chamber II was estimated from $^{13}CO_2$ efflux. For this purpose, $^{13}CO_2$ abundance was interpolated for the whole 7-day incubation period using a Gaussian function extended with a linear term to adjust for divergences from a normal distribution. Function fitness was optimized using Solver (Microsoft cooperation, Redmond, U.S.A.).

The impact of HR on soil enzyme activity in chamber II was analyzed using soil zymography after reopening the mesocosms at the end of the 7 days (Spohn et al., 2013; modified by Spohn and Kuzyakov, 2013). This allowed the analysis of enzyme activities under different water contents in contrast to traditional methods based on the determination of enzyme activity in solution. N-acetylglucosaminidase (NAG) and cellobiohydrolase activity was determined using the artificial substrates 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MNG) and 4-methylumbelliferyl β-D-cellobioside (4-MC, both Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), respectively. The fluorogenic 4-methylumbelliferone (MUF) is released from 4-MNG and 4-MC due to hydrolytic cleavage in presence of compatible enzymes. Polyamide membrane filters were saturated with 4-MNG or 4-MC and placed in turn on top of a 1% agarose gel attached to the soil surface of the non-irrigated chamber II, starting with 4-MNG (filters and gel: 4 stripes à 2x11 cm, placed in between the plant material samples). The membrane filters were extracted after 25 min at 20°C for 4-MNG and 20 min for 4-MC, illuminated on a fluorescent transilluminator in the dark (wavelength: 355 nm, Desaga GmbH, Wiesloch, Germany) and photographed (Nikon D3100). A segment à 1.5x7.5 cm with no visible disturbance on the digital image was selected and mean values of the grayscale were measured with imageJ 1.46r (Wayne Rasband, National Institutes of Health, USA, 2014). Enzyme activates were calculated based on a calibration curve of different MUF concentrations (0, 35, 70, 130, 200 µM on 2 cm² membranes, measured as described above) and expressed as pmol MUF per mm² soil area, fungal biomass (g) and hour (h).Values were standardized based on

the difference between a control filter slice (no incubation on the soil and photographed with the others) and the calibration membrane with 0 µM MUF concentration to adjust for differences in exposure time.

3.3.4 Determination of fungal biomass and N translocation

Two soil cores were taken at each of 3 distance points from the air gap (5, 10 and 15 cm) in chamber I and II after completing measurements of enzyme activity. In addition, remains of the added labeled plant material were quantitatively recollected. All samples were immediately frozen and stored at -25°C until further use to determine fungal biomass, C and N translocation.

Soil fungal biomass was determined by ergosterol, as ergosterol is highly correlated to living fungal biomass (Davis and Lamar, 1992). Ergosterol was extracted from soil in ethanol according to Djajakirana et al., (1996) and then measured with high performance liquid chromatography (HPLC, System Gold 125 Solvent Module, Beckman Coulter, Brea, U.S.A.; column: MZ Spherisorb ODS-2 C18, 150 x 3 mm, MZ Analysentechnik, Germany) at a detection wavelength of 282 nm (System Gold 166 UV-Detector, Beckman Coulter, Brea, U.S.A.). Since ergosterol contents can vary among fungal species (Newell et al., 1987), ergosterol was also extracted from pure fungal cultures of *S. commune* and *A. bisporus* and correlated to fungal dry weight (*A. bisporus*: 195 µg ergosterol g^{-1} fungal DW; *S. commune*: 186 µg ergosterol g^{-1} fungal DW). Results were expressed as g fungal DW per g soil DW and averaged for each chamber.

Carbon and N translocation by fungal hyphae was assessed by stable isotope analysis of soil samples from chamber I and II. Soil samples were defrosted, dried at 60°C for 48 h, ground in ball mills, and analyzed for ^{13}C and ^{15}N isotope signature using an elemental analyzer – isotope-ratio mass-spectrometer linkage (EA-IRMS; EA: NC 2500, CE Instruments, Milano, Italy; IRMS: delta plus, Thermo Fisher Scientific, Bremen, Germany; interface: ConFlo III, Thermo Fisher Scientific, Bremen Germany). Carbon and N translocation from the labeled plant material to chamber I was calculated by a 2-end-member linear mixing model (Dawson et al., 2002).

Plant residues were measured as described for soil samples and subsequently analyzed for C and N contents with a CN analyzer (Elementar Vario EL, Hanau, Germany).

3.3.5 Data analysis

All statistical analyzes and graphics were done using R 3.1.0 (R Developmental Core Team, 2014). Normality and homogeneity of the data were tested using the Shapiro-Wilk-Test and

Levene's-test, respectively. Analysis of variance (ANOVA), followed by a Tukey-HSD-test as post-hoc test, was used to test for statistical differences among groups. Kruskal–Wallis tests with pair–wise Wilcox tests for post-hoc comparisons were used if data are not normal and/or variances were not homogeneously distributed.

Enzyme activities as well as C, N, $\delta^{13}C$ and $\delta^{15}N$ contents were analyzed using linear mixed effect models (LMM) as implemented in the R package lme4 and likelihood ratio-tests (Faraway 2005; Bates et al., 2015). Data values were ranked for LMM, if data were not normal and/or variances were not homogeneously distributed. Treatment types were added as fixed effects into the model. The different mesocosms as well as position within the chambers were added as random factors to account for random variances among mesocosms.

3.4 Results

3.4.1 Water redistribution by S. commune

After 72 h, the amount of redistributed water from chamber I to chamber II with HR by *S. commune* was about 3 times higher than in the controls and in a similar range to HR by *A. bisporus* (Fig. 3.1). The amount of redistributed water by capillary water transport was similar to HR by *S. commune* and on average lower than HR by *A. bisporus*. HR through hyphae of *S. commune* resulted in an average water flux from chamber I to chamber II of 50 µl cm^{-2} day^{-1} in comparison to 65 µl cm^{-2} day^{-1} by capillary water transport. The number of hyphae by *S. commune* bridging the air gap between the 2 chambers was about 880 cm^{-2} and in a few cases mycelia cords in the air gaps were observed (about 1-2 per mesocosm). The total water flux between the wet and dry chambers corresponded to a specific water flux of about 0.06 µl day^{-1} in single hyphae. An average flow velocity of about 0.43 cm min^{-1} in single hyphae was calculated using the Bernoulli's equation for the central cell lumen of hyphae (mean outer diameter= 5.3 µm).

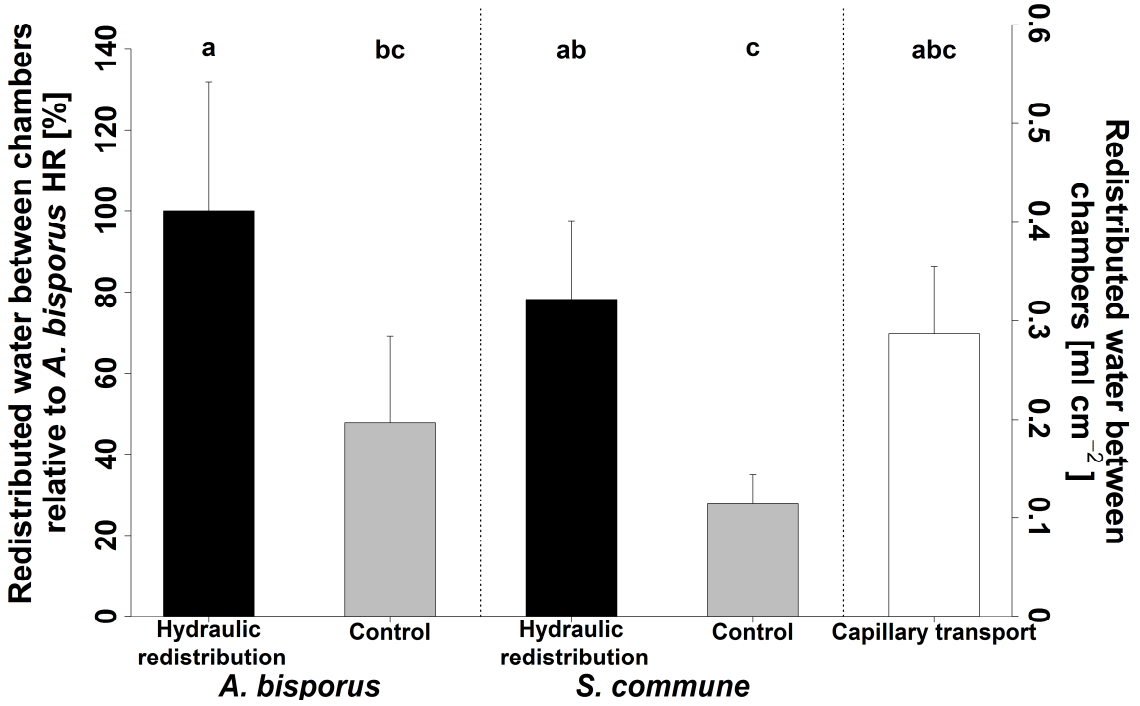


Fig. 3.1: Redistributed water from chamber I to chamber II within 72 h after irrigation of chamber I. The left y-axis shows the hydraulic redistribution (HR) of treatments expressed as relative percentages based on the HR treatment with *A. bisporus* (100%) and right y-axis shows those values expressed as total amounts per contact surface. Calculation based on hydrogen stable isotope ratios. Black: active HR, gray: control with disrupted fungal connections, white: soil without air gap and fungal inoculation. ANOVA: $F_{4,13}$= 6.76, p=0.004. Mean + SD; SD based on percent values; n= 4 (control, HR), 2 (soil). Different letters indicate significant differences among treatments.

3.4.2 Impact of HR by S. commune on enzyme activities and C mineralisation

Ergosterol contents and fungal biomass were lower in mesocosms inoculated with *S. commune* than in those inoculated with *A. bisporus* (Tab. 3.1).

Tab. 3.1: Ergosterol content and fungal biomass in soil samples of chamber I and II inoculated with *A. bisporus* or *S. commune*, 7 days after exposing labelled plant material on the soil surface of chamber II and irrigation of chamber I. HR: active hydraulic redistribution, control: controls with no fungal connection. Mean ± SD.

Treatment	Species	Chamber	Ergosterol [µg g^{-1} soil DW]		Fungal biomass [mg DW g^{-1} soil DW]	
Control	*S. commune*	I	5.93	±2.26	6.65	±2.38
HR	*S. commune*	I	7.62	±2.67	8.50	±3.14
Control	*S. commune*	II	6.36	±4.81	6.73	±5.13
HR	*S. commune*	II	9.20	±7.49	9.82	±8.02
Control	*A. bisporus*	I	29.84	±27.51	27.95	±24.87
HR	*A. bisporus*	I	25.17	±13.81	25.31	±14.14
Control	*A. bisporus*	II	37.08	±21.86	36.45	±21.69
HR	*A. bisporus*	II	46.57	±38.93	46.46	±39.80

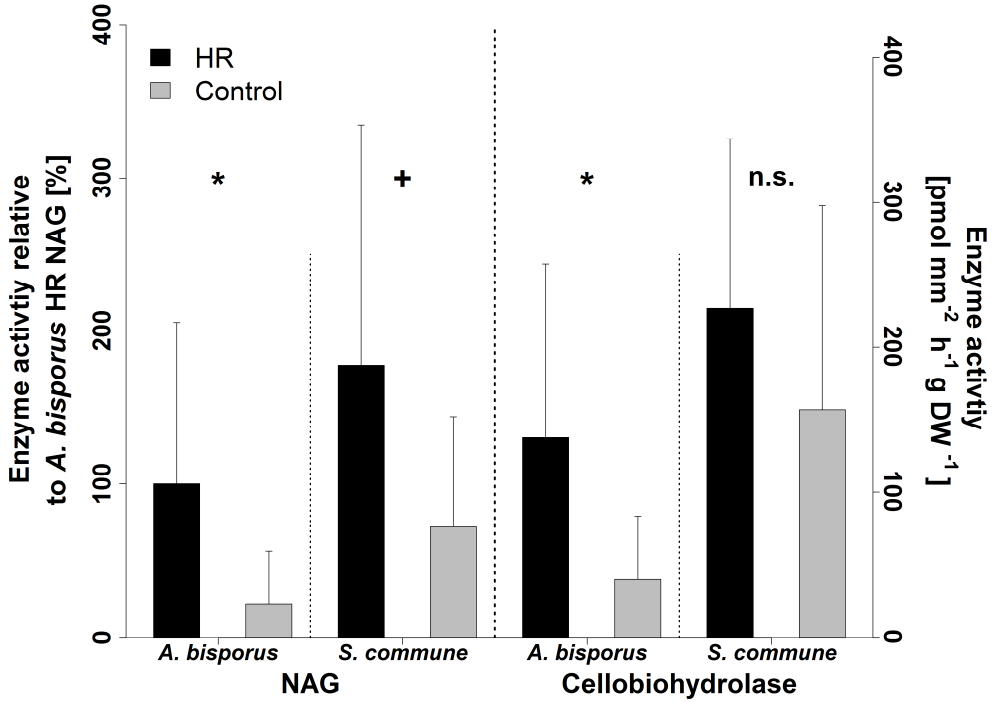


Fig. 3.2: Enzyme activity on the soil surface of chamber II, 7 days after irrigation of chamber I. The left y-axis shows the enzyme activities as relative percentages based on the N-acetylglucosaminidase (NAG) activity of the hydraulic redistribution (HR) treatment with *A. bisporus* (100%) and right y-axis shows those values expressed as total amounts per g fungal dry weight. Black: active HR, gray: control with disrupted fungal connection. *S. commune*= cellobiohydrolase= LMM: χ^2= 1.694, p= 0.193; NAG= LMM: χ^2= 3.099, p= 0.078. *A. bisporus*= cellobiohydrolase= LMM: χ^2= 4.305, p= 0.038; NAG= LMM: χ^2= 6.507, p= 0.011. Mean + SD; SD based on percent values; n= 6 (*S.commune* control), 5 (all other treatments). * p<0.05; + p<0.1; n.s.= not significant.

Enzyme activities per gram fungal biomass were higher for *S. commune* than for *A. bisporus* (NAG= LMM: χ^2= 10.539, p= 0.001; cellobiohydrolase= LMM: χ^2= 21.446, p<0.001). HR by hyphae of *S. commune* increased enzyme activity per g fungal biomass on average by 150% for NAG and by 50% for cellobiohydrolase compared to controls, but because of the large variability the increases in enzyme activity were not significant at the p<0.05 level (Fig. 3.2).

Total CO2 efflux of the whole mesocosms increased by 50% with HR by *S. commune* compared to the controls (Fig. 3.3). However, no changes were found in the ^{13}C signature of CO2 between HR and controls. (Fig. 3.4). An opposite pattern was found for *A. bisporus*, i.e., no effect on CO2 efflux, but significant increase in $^{13}CO2$ efflux by mineralization of added plant material in the HR treatment.

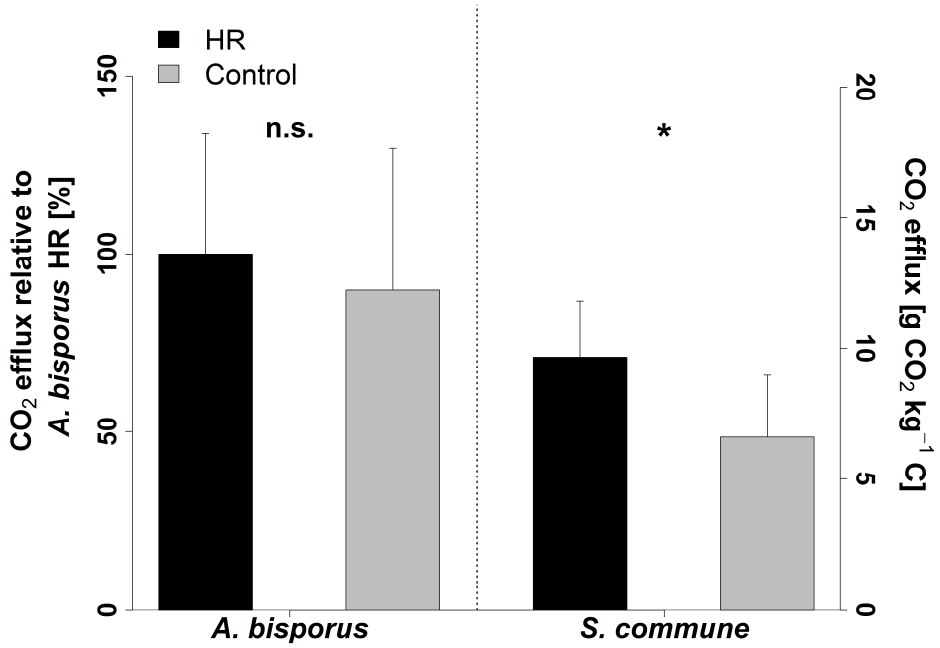


Fig. 3.3: CO_2 efflux of the whole mesocosms within 7 days after irrigation of chamber I. The left y-axis shows the values expressed as relative percentages based on the HR treatment with *A. bisporus* (100%) and right y-axis shows those values expressed as total amounts. Black: active HR, gray: control with disrupted fungal connection. *S. commune*= Wilcoxon rank sum test: $W_{1,10}$= 3, p= 0.03; *A. bisporus*= Wilcoxon rank sum test: $W_{1,9}$= 12, p= 1 Mean + SD; SD based on percent values; n= 6 (*S.commune* control), 5 (all other treatments). * p<0.05; n.s.= not significant.

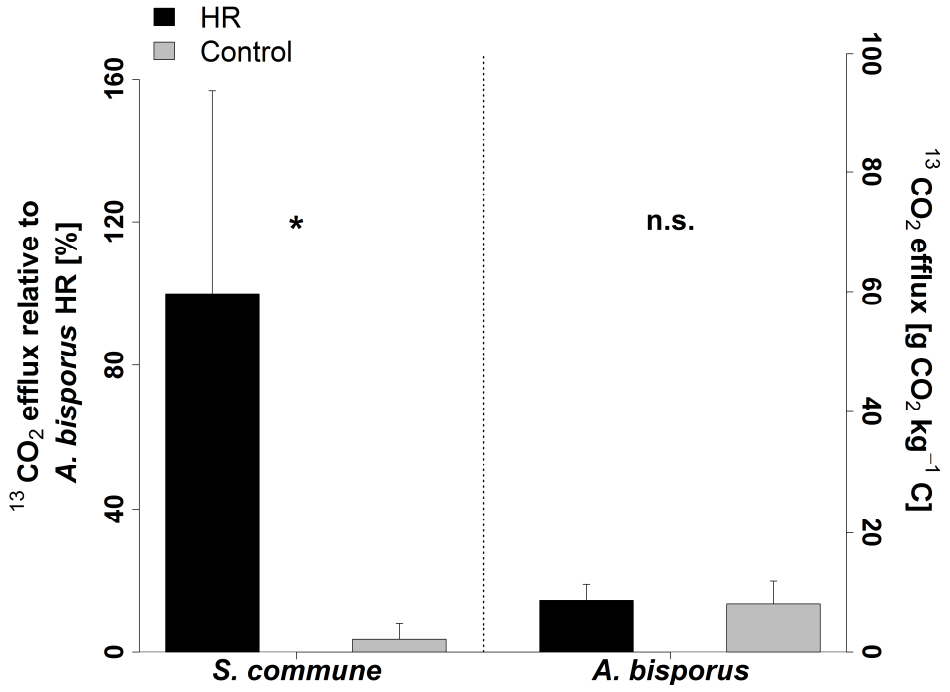


Fig. 3.4: $^{13}CO_2$ efflux through the mineralization of the labeled plant material within 7 days after irrigation of chamber I. The left y-axis shows the values expressed as relative percentages based on the HR treatment with *A. bisporus* (100%) and right y-axis shows those values expressed as total amounts. Black: active HR, gray: control with disrupted fungal connection. *S. commune*= Wilcoxon rank sum test: $W_{1,10}$= 11, p= 0.537. *A. bisporus*= Wilcoxon rank sum test: $W_{1,9}$= 0, p= 0.008. Mean + SD; SD based on percent values; n= 6 (*S.commune* control), 5 (all other treatments); * p<0.05; n.s.= not significant.

3.4.3 N translocation during HR

In case of *A. bisporus*, $\delta^{15}N$ values were significantly increased in chamber I after 7 days with HR compared to controls (LMM: χ^2= 6.233, p= 0.013). After subtracting the controls, 25 µg N from the labeled plant material were transferred from chamber II to chamber I after 7 days (of 3800 µg plant material N). This corresponded to a specific translocation rate of about 170 µg N cm^{-2} hyphae d^{-1} (number of hyphae bridging the 2 chambers: about 1500 cm^{-1}, hyphal diameter: 4.45 µm). In addition, the N content of the recollected plant material decreased in mesocosms with HR by *A. bisporus* compared to controls (Fig. 3.5).

For *S. commune* there was no difference between treatments and controls with respect to $\delta^{15}N$ values in chamber I. In contrast, increased N and C contents were determined in recollected labeled plant material from HR mesocosms compared to controls (Fig. 3.5). $\delta^{13}C$ values did not differ between treatments in chamber I for both fungi.

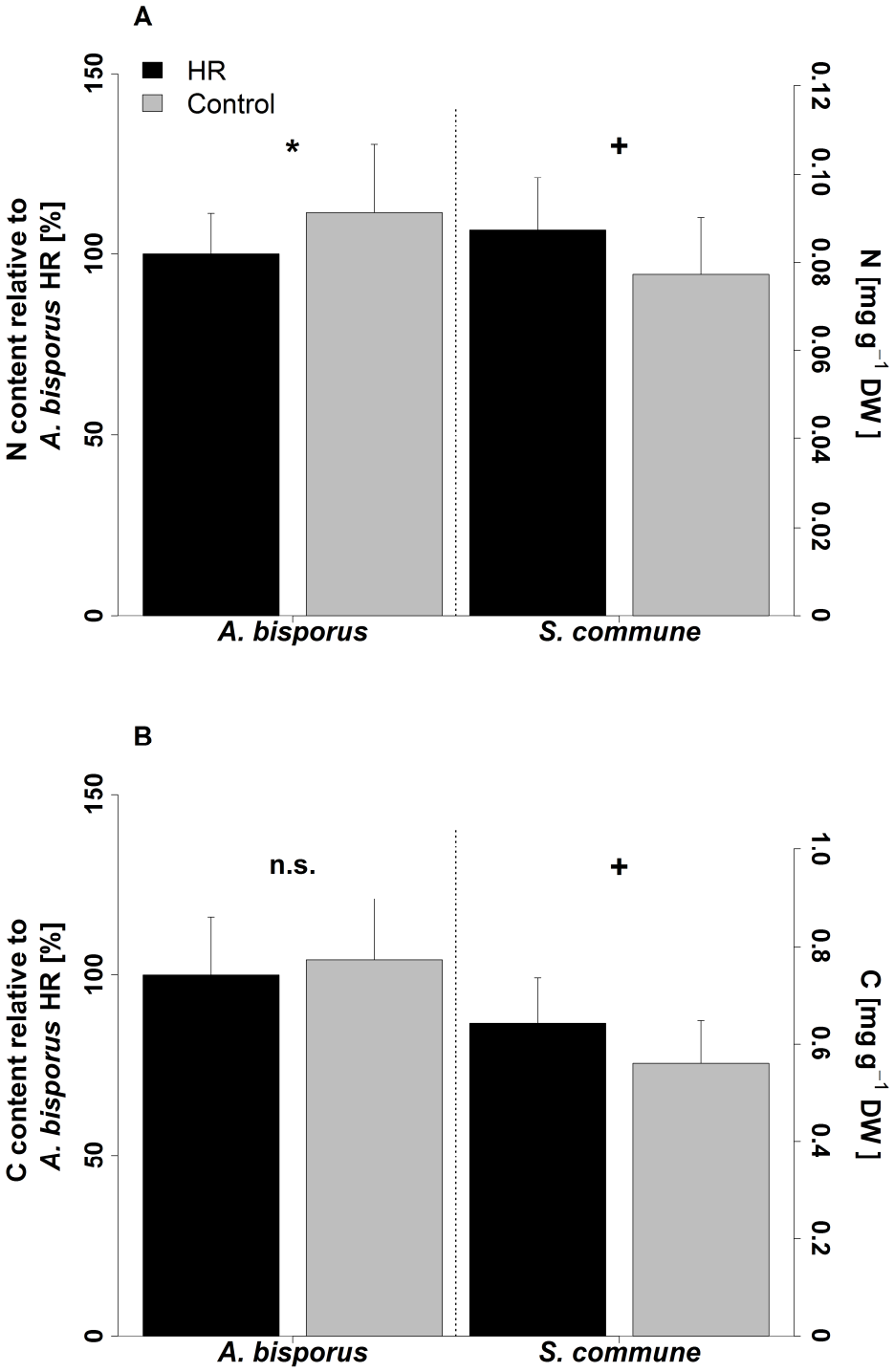


Fig. 3.5: Content of A) Nitrogen (N) and B) Carbon (C) in recollected plant material from chamber II. The left y-axis shows the values expressed as relative percentages based on the HR treatment with *A. bisporus* (100%) and right y-axis shows those values expressed as total amounts. Black: active HR, gray: control with disrupted fungal connection. N= *A. bisporus*: LMM: χ^2= 4.297, p= 0.038; *S. commune*: LMM: χ^2= 3.209, p= 0.073. C= *A. bisporus*: LMM: χ^2= 0.323, p= 0.57; *S. commune*: LMM: χ^2= 3.574, p= 0.059. Mean + SD; SD based on percent values; n= 6 (*S.commune* control), 5 (all other treatments). * p<0.05; + p<0.1; n.s.= not significant.

3.5 Discussion

This study documents, that *S. commune* has the ability for HR along a soil water potential gradient. Hence, HR seems common in a range of higher basidiomycetes, including mycorrhizal as well as saprotrophic species (Thompson et al., 1985; Jennings, 1987; Querejeta et al., 2003; Guhr et al., 2015). The specific water flux through hyphae, or the flow velocity in hyphae was 1.5-2 times higher in S. *commune* than in *A. bisporus* (Guhr et al., 2015), which indicates that fungal HR is impacted by mycelia network organization. The formation of mycelia cords with aggregations of predominantly parallel, longitudinally aligned hyphae (cf. Lindahl et al., 2001; Cairney, 2005; Watkinson et al., 2006) may have supported the higher flow rates in *S. commune*.

HR by hyphae was similar to soil capillary water transport. The amount of redistributed water might even exceed soil capillary water transport considering the high hyphal density reported for natural soils (Söderström, 1979) and the high hydraulic conductivity of the sandy soil used in this study in comparison to loamy and clayey soils. In dry soils, HR by saprotrophic fungi may therefore have a significant impact on biological processes that are dependent on soil water potentials. Drought events lead to desiccation of the top soil while soil water availability remains higher in the underlying soil horizons. In forest soils, the transition between the organic layers with their larger pore volume and the mineral soil can act as barrier for capillary rise under dry conditions (Price and Whitehead, 2004). This reduces the water availability for microorganisms in the organic layer during desiccation. Mycelia networks bridging the mineral soil and the organic layer therefore have the potential to overcome capillary barriers and increase water availability in organic layers during drought events. This could strongly stimulate soil microbial activity, because organic layers have the highest biological activity and microbial biomass in forest soils (e.g., Šnajdr et al., 2008). Further, HR by saprotrophic fungi likely also contributes to a lateral redistribution of water within organic layers if lateral gradients in soil water potential occur. Thereby, HR could partly overcome drought stress in forest floors induced by heterogeneous distribution of throughfall (Shachnovich et al., 2008).

The impact of HR on mineralization of organic matter by *S. commune* was considerably lower than for *A. bisporus* (Guhr et al., 2015). Further, only *A. bisporus* translocated N from chamber II to chamber I, even against the strong soil water gradient, followed by a decrease of the N content in the added plant material. N translocation rates in hyphae of *A. bisporus* were far lower than estimated N translocation rates in hyphae of mycorrhizal fungi towards host plants (about 21 mg N cm^{-2} hyphae d^{-1}, Ames et al., 1983). The specific N translocation rates in *A. bisporus* hyphae in our study may be low considering that the labelled plant material only

constituted about 0.4% to total N in chamber II and was concentrated on a few spots. In contrast, N transport was not observed for *S. commune* and C and N contents even increased in the added plant material as a result of hyphal growth. The differences in C mineralization and N translocation between *S. commune* and *A. bisporus* might be explained by their foraging strategies and resource usage. *A. bisporus* has its optimum for growth at rather low substrate C:N ratios of about 20 (Smith and Hayes, 1972; Chang and Miles, 1986). Since *A. bisporus* also frequently colonizes dung from herbivores (Kerrigan et al., 1998), the species seems adapted to relatively high amounts of easily accessible N. Fast transfer of N from a fresh nutrient source into other parts of the mycelia network to extent the network could therefore be common for fungi with a similar foraging strategy. Rapid network extension also requires high amounts of C, which explains the strong increase of the $^{13}CO_2$ efflux through the mineralization of the labeled substrate in the *A. bisporus* HR treatment.

In contrast to *A. bisporus, S. commune* is adapted to substrates with high C:N ratios (Schmidt and Liese, 1980). Fungi adapted to sources with high C:N ratios are known to translocate nutrients towards new sources, either indirectly by growth or directly by active transport (Boberg et al., 2014; Frey et al., 2000; Tlalka et al., 2002). Hence, total N and C content might increase in the new source due to a redirection of nutrients by the mycelia network as supported by our study (Jennings, 1987; Olsson and Gray, 1998; Tlalka et al., 2003). The foraging strategy of *S. commune* seems to focus on the colonization rather than on the mineralization of the new source, independent of HR. Accordingly, in our study visually detectable growth of hyphae on the labeled plant material was observed in control mesocosms with *S. commune,* but not for *A. bisporus*. This is in accordance with the low $^{13}CO_2$ efflux through the mineralization of the labeled substrates in mesocosms with *S. commune*. Extensive mineralization of substrate by fungi adapted to high C:N ratios probably only starts after comprehensive colonization of the resource. Mineralization and translocation of N due to HR might therefore be only visible after longer time periods for fungi adapted to high C:N ratios (like *S. commune*) (Yang et al., 1980). *S. commune* is known to withstand relatively high levels of desiccation with growth even up to -6 MPa (Dix, 1984; Ohm et al., 2010). Therefore, HR for might for this species not be as crucial as for *A. bisporus*. The better adaptation of *S. commune* to drought is in accordance with the generally higher enzyme activities per gram fungal biomass of in comparison to *A. bisporus.* Further, a significant increase of total CO_2 efflux with HR was observed fo *S. commune* but not for *A. bisporus*. This increase of CO_2 efflux is probably based on reorganization and degradation of older hyphae, since fungi are known to constantly rebuild the mycelium depending on substrate and water availability (Fricker et al., 2008; Boddy et al., 2009).

In conclusion, HR is likely common in a broad spectrum of higher basidiomycetes, including mycorrhizal as well as saprotrophic species. The amount to HR is comparable to capillary water flow in sandy soils and HR is especially of relevance if considering its potential to overcome capillary barriers. Impact of HR on decomposition and mineralization of organic material as well as way of N translocation within the mycelia network seem to depend on the foraging strategy and the resource usage of the fungi.

3.6 Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft Grant [DFG-MA1089/23-1]. We thank C. Werner, M. Dubbert and I. Thaufelder for support with the cryogenic vacuum extraction of soil samples and K. Söllner for C and N analysis. We also thank G. Rambold for providing fungal cultures of *S. commune* and access to facilities for treating and storing fungal cultures. We further thank M. Spohn for the introduction to the soil zymography method. Furthermore, we thank the Central Isotopic Laboratory of the Bayreuth Center of Ecology and Environmental Research (BayCEER) for the stable isotope analyses.

3.7 References

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, Jennifer B. H., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.

Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil. Biol. Biochem. 64, 68–79.

Ames, R.N., Reid, C.P.P., Porter, L.K., Cambardella, C., 1983. Hyphal uptake and transport of nitrogen from two ^{15}N-labeled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol. 95, 381–396.

Balaş, T., Tănase, C., 2012. Description of in vitro cultures for some spontaneous lignicolous basidiomycetes species. Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, s. II a. Biologie Vegetală 58, 19–29.

Bapiri, A., Bååth, E., Rousk, J., 2010. Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb. Ecol. 60, 419–428.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Soft. 67, 1–51.

Boberg, J.B., Finlay, R.D., Stenlid, J., Ekblad, A., Lindahl, B.D., 2014. Nitrogen and carbon reallocation in fungal mycelia during decomposition of boreal forest litter. PloS one 9, e92897.

Boddy, L., 1993. Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. Mycol. Res. 97, 641–655.

Boddy, L., 1999. Saprotrophic Cord-Forming Fungi: Meeting the Challenge of Heterogeneous Environments. Mycologia 91, 13–32.

Boddy, L., Hynes, J., Bebber, D.P., Fricker, M.D., 2009. Saprotrophic cord systems: dispersal mechanisms in space and time. Mycoscience 50, 9–19.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob. Chang. Biol. 15, 808–824.

Cairney, J.W.G., 1992. Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. Mycol. Res. 96, 135–141.

Cairney, J.W.G., 2005. Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. Mycol. Res. 109, 7–20.

Chang, S.T., Miles, P.G., 1986. Mushroom technology. Mushroom Newslett. Tropics 6, 6–11.

Davis, M.W., Lamar, R.T., 1992. Evaluation of methods to extract ergosterol for quantitation of soil fungal biomass. Soil. Biol. Biochem. 24, 189–198.

Dawson, T.E., 1993. Hydraulic lift and water use by plants. implications for water balance, performance and plant-plant interactions. Oecologia 95, 565–574.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable Isotopes in Plant Ecology. Annu. Rev. Ecol. Syst. 33, 507–559.

Dix, N.J., 1984. Minimum water potentials for growth of some litter-decomposing agarics and other basidiomycetes. Trans. Br. Mycol. Soc. 83, 152–153.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299–304.

Domec, J.-C., Warren, J.M., Meinzer, F.C., Brooks, J.R., Coulombe, R., 2004. Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. Oecologia 141, 7–16.

Faraway, J.J., 2005. Extending the linear model with R: Generalized linear, mixed effects and nonparametric regression models. CRC Press, Boaca Raton, London, New York.

Frey, S.D., Elliott, E.T., Paustian, K., Peterson, G.A., 2000. Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. Soil. Biol. Biochem. 32, 689–698.

Fricker, M.D., Bebber, D., Boddy, L., 2008. Mycelial networks: Structure and dynamics. In: Boddy, L., Frankland, J., van West, P. (Eds.), Ecology of Saprotrophic Basidiomycetes. Elsevier, London, pp. 3–18.

Glen, M., Yuskianti, V., Puspitasari, D., Francis, A., Agustini, L., Rimbawanto, A., Indrayadi, H., Gafur, A., Mohammed, C.L., Woodward, S., 2014. Identification of basidiomycete fungi in Indonesian hardwood plantations by DNA barcoding. For. Path. 44, 496–508.

Greenwood, D.J., 1967. Studies on oxygen transport through mustard seedlings (*Sinapis alba* L.). New Phytol. 66, 597–606.

Guhr, A., Borken, W., Spohn, M., Matzner, E., 2015. Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. Proc. Natl. Acad. Sci. U. S. A. 112, 14647–14651.

Jennings, D.H., 1987. Translocation of solutes in fungi. Biol. Rev. 62, 215–243.

Kerrigan, R.W., Carvalho, D.B., Horgen, P.A., Anderson, J.B., 1998. The indigenous coastal Californian population of the mushroom *Agaricus bisporus*, a cultivated species, may be at risk of extinction. Mol. Ecol. 7, 35–45.

Lindahl, B., Finlay, R., Olsson, S., 2001. Simultaneous, bidirectional translocation of ^{32}P and ^{33}P between wood blocks connected by mycelial cords of *Hypholoma fasciculare*. New Phytol. 150, 189–194.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938.

Muhr, J., Goldberg, S.D., Borken, W., Gebauer, G., 2008. Repeated drying-rewetting cycles and their effects on the emission of CO_2, N_2O, NO, and CH_4 in a forest soil. J. Plant Nutr. Soil Sci. 171, 719–728.

Nazrul, M.I., YinBing, B., 2011. Differentiation of homokaryons and heterokaryons of Agaricus bisporus with inter-simple sequence repeat markers. Microbiol. Res. 166, 226–236.

Neuhauser, S., Huber, L., Kirchmair, M., 2009. A DNA based method to detect the grapevine root-rotting fungus *Roesleria subterranea* in soil and root samples. Phytopathol. Mediterr. 48, 59–72.

Newell, S.Y., Miller, J. David, Fallon, R.D., 1987. Ergosterol content of salt-marsh fungi: Effect of growth conditions and mycelial age. Mycologia 79, 688–695.

Ohm, R.A., de Jong, Jan F, Lugones, L.G., Aerts, A., Kothe, E., Stajich, J.E., de Vries, Ronald P, Record, E., Levasseur, A., Baker, S.E., Bartholomew, K.A., Coutinho, P.M., Erdmann, S., Fowler, T.J., Gathman, A.C., Lombard, V., Henrissat, B., Knabe, N., Kües, U., Lilly,

W.W., Lindquist, E., Lucas, S., Magnuson, J.K., Piumi, F., Raudaskoski, M., Salamov, A., Schmutz, J., Schwarze, Francis W M R, vanKuyk, P.A., Horton, J.S., Grigoriev, I.V., Wösten, Han A B, 2010. Genome sequence of the model mushroom *Schizophyllum commune.* Nat. Biotechnol. 28, 957–963.

Olsson, S., Gray, S.N., 1998. Patterns and dynamics of ^{32}P-phosphate and labeled 2-aminoisobutyric acid (^{14}C-AIB) translocation in intact basidiomycete mycelia. FEMS Microbiol. Ecol. 26, 109–120.

Price, J.S., Whitehead, G.S., 2004. The influence of past and present hydrological conditions on *Sphagnum* recolonization and succession in a block-cut bog, Québec. Hydrol. Process. 18, 315–328.

Querejeta, J.I., Egerton-Warburton, L.M., Allen, M.F., 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134, 65.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schmidt, O., Liese, W., 1980. Variability of Wood Degrading Enzymes of *Schizophyllum commune.* Holzforschung 34, 67–72.

Seephueak, P., Phongpaichit, S., Hyde, K.D., Petcharat V, 2011. Diversity of saprobic fungi on decaying branch litter of the rubber tree (*Hevea brasiliensis*). Mycosphere 2, 307–330.

Shachnovich, Y., Berliner, P.R., Bar, P., 2008. Rainfall interception and spatial distribution of throughfall in a pine forest planted in an arid zone. J. Hydrol. 349, 168–177.

Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of warming and drought on grassland microbial communities. ISME J. 5, 1692–1700.

Smith, J.F., Hayes, W.A., 1972. Use of autoclaved substrates in nutrional investigations on the cultivated mushroom. Mushroom Sci. 8, 355–362.

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil. Soil. Biol. Biochem. 40, 2068–2075.

Söderström, B.E., 1979. Seasonal fluctuations of active fungal biomass in horizons of a podzolized pine-forest soil in central Sweden. Soil. Biol. Biochem. 11, 149–154.

Spohn, M., Carminati, A., Kuzyakov, Y., 2013. Soil zymography – A novel in situ method for mapping distribution of enzyme activity in soil. Soil. Biol. Biochem. 58, 275–280.

Spohn, M., Kuzyakov, Y., 2013. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with ^{14}C imaging. Soil. Biol. Biochem. 67, 106–113.

Thompson, W., Eamus, D., Jennings, D.H., 1985. Water flux through mycelium of *Serpula lacrimans.* Trans. Br. Mycol. Soc. 84, 601–608.

Tlalka, M., Watkinson, S.C., Darrah, P.R., Fricker, M.D., 2002. Continuous imaging of amino-acid translocation in intact mycelia of *Phanerochaete velutina* reveals rapid, pulsatile fluxes. New Phytol. 153, 173–184.

Tlalka, M., Hensman, D., Darrah, P.R., Watkinson, S.C., Fricker, M.D., 2003. Noncircadian oscillations in amino acid transport have complementary profiles in assimilatory and foraging hyphae of *Phanerochaete velutina.* New Phytol. 158, 325–335.

Van Genuchten, M.T., Leij, F.J., Yates, S.R., 1991. The RETC code for quantifying the hydraulic functions of unsaturated soils. EPA Report 600/2-91/065, U.S. Salinity Laboratory, USDA-ARS, Riverside.

Varghese, G., 1972. Soil microflora of plantations and natural rain forest of West Malaysia. Mycopathol. Mycol. Appl. 48, 43–61.

Watkinson, S., Bebber, D., Darrah, P., Fricker, M.D., Tlalka, M., Boddy, L., Gadd, G.M., 2006. The role of wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: Gadd, G.M. (Ed.), Fungi in Biogeochemical Cycles. Cambridge University Press, Cambridge, pp. 151–181.

Wells, J.M., Boddy, L., 1990. Wood decay, and phosphorus and fungal biomass allocation, in mycelial cord systems. New Phytol. 116, 285–295.

Yang, H.H., Effland, M.J., Kirk, T.K., 1980. Factors influencing fungal degradation of lignin in a representative lignocellulosic, thermomechanical pulp. Biotechnol. Bioeng. 22, 65–77.

Yuste, J., Peñuelas, J., Estijarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486.

Zhang, C., Yin, L., Dai, S., 2009. Diversity of root-associated fungal endophytes in *Rhododendron fortunei* in subtropical forests of China. Mycorrhiza 19, 417–423.

4. Vitamin B_2 (riboflavin) increases drought tolerance of *Agaricus bisporus*

Authors: Alexander Guhr[a], Marcus A. Horn[b] & Alfons R. Weig[c]

[a]Department of Soil Ecology, BayCEER, University of Bayreuth, Dr. Hans-Frisch-Straße 1-3, 95448 Bayreuth

[b]Institute of Microbiology, Leibniz University Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany

[c]Genomics & Bioinformatics, BayCEER, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth

Keywords: saprotrophic filamentous fungi, *Agaricus bisporus*, drought stress, microarray, differential gene expression, riboflavin

In preparation.

4.1 Abstract

Drought is a stressor for many soil inhabiting organisms. While plants have been extensively investigated for drought adaptive mechanisms, little information is available for fungi. Antioxidants are especially relevant, since desiccation is accompanied by an excessive intracellular production of reactive oxygen species. Riboflavin (vitamin B_2) is an antioxidant regulating drought tolerance in plants. A similar function may exist in fungi. Here, we examined the respiratory and transcriptional response of *Agaricus bisporus* to drought and the impact of riboflavin. Mesocosm experiments with 4 groups were established: hyphae were treated with or without 50 µM riboflavin under drought or no drought conditions. Drought increased riboflavin content in hyphae about 5 times with, but also without riboflavin addition. Without riboflavin addition, fungal respiration decreased by more than 50% at water potentials of about -20 MPa. With riboflavin addition, respiration remained about 2-3 times higher. The transcriptional response to only drought or riboflavin strongly overlapped and was mainly based on factors regulating transcription and translation. This was even stronger in combined treatments. Riboflavin induced protective mechanisms in drought stressed hyphae. Most pronounced was the methylglyoxal (cytotoxic by-product of glycolysis) detoxifying lactoylglutathione lyase. Thus, our data suggests a stress priming function and a prominent role of riboflavin in drought responses of *A. bisporus*.

4.2 Introduction

Periods of drought and the resulting soil desiccation are important abiotic stressors negatively affecting organisms and ecosystems (Schimel et al. 2007) as well as agricultural yields worldwide (Yang et al. 2010). Soil desiccation is accompanied by a decrease in soil respiration (Borken et al. 2003; Muhr et al. 2010) and decreased soil enzyme activity (Herzog et al. 2013). In general, low water potentials lead to a limitation, or under extreme conditions, even to a total inhibition of microbial activity in soils (Manzoni et al. 2012). Drought stress can also alter microbial community composition, whereby fungal communities are often less sensitive compared to bacterial communities (Sheik et al. 2011; Yuste et al. 2011).

Sessile organisms are confronted with drought stress but have evolved a variety of stress avoidance and tolerance strategies to survive and adapt to low water potentials (Chavez et al. 2002). Strategies include growth adjustments like development of deep root systems to access moist soil horizons, but also morphological and physiological adaptions like increasing cell wall and cuticle thickness or production of antioxidants (Chavez et al. 2002; Osakabe et al. 2014). The latter is of great importance since prolonged water deficit is generally accompanied by an excessive production of reactive oxygen species (ROS) and hence oxidative stress (Smirnoff 1993). ROS can cause cell death by uncontrolled oxidation of cellular components (Mittler 2002). Most organisms have evolved a large variety of enzymatic and non-enzymatic processes to diminish harmful ROS effects. The major players in response to ROS are superoxide dismutase, glutathione peroxidase and catalase (Ingram and Bartels 1996). Metabolites like glutathione and riboflavin (vitamin B2) can also act as antioxidants (Mittler 2002).

Riboflavin is primarily important as a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), both are redox cofactors, e.g., of the electron transport chain in the mitochondria (Sandoval et al. 2008). Its ability as an electron acceptor makes it also suitable as an antioxidant (Ashoori and Saedisomeolia 2014). In addition, FAD derived from riboflavin is essential for many enzymes, like glutathione reductase, which reduce H2O2 (Beutler 1969). Furthermore, riboflavin seems to generally stimulate production of antioxidative components in plants (Mori and Sakurai 1995). Riboflavin may also have a stress priming function in plants, i.e., induction of basal defense responses leading to a faster and/or stronger activation of defense mechanisms in later stress events (Taheri and Tarighi 2010). The ability of riboflavin to counteract drought stress has already been described for tobacco plants, where low levels of

riboflavin increased drought tolerance (Deng et al. 2014). Similar to plants, an antioxidative function of riboflavin may exist in filamentous fungi.

While molecular mechanisms of drought adaptations have been intensively studied in plants (e.g., (Ingram and Bartels 1996; Chavez et al. 2002; Osakabe et al. 2014), information on physiological drought stress responses in filamentous fungi is scarce. Next-generation sequencing technologies provided sequences of whole-genomes and transcriptomes for many organisms (Grigoriev et al. 2014), which now enable the study of molecular mechanisms of drought adaptations for so far neglected species. Saprotrophic filamentous fungi can be of great interest in this respect. First, they belong to the main regulators of soil nutrient cycling, litter decomposition, and soil respiration (Hättenschwiler et al. 2005; Crowther et al. 2012) and their hyphae can contribute significantly to the soil biomass (up to 6 mg g-1 soil; (Baldrian et al. 2013). Second, fruiting bodies of many species are edible and industrially cultivated with great economical relevance (Chang and Miles 1986). Thus, knowledge of the mechanisms regulating drought stress response is of great interest for modelling fungal impact on soil biogeochemistry with regard to increasing numbers of drought events and to optimize cultivation procedures.

Thus, we analyzed transcriptional and respiratory responses of Agaricus bisporus to drought stress in the absence and presence of riboflavin. A. bisporus is a saprophytic, filamentous, cord-forming fungus and a useful model organism for soil nutrient cycling due to its natural habitat in humic-rich environments (Burton et al. 1997; Morin et al. 2012). The annotated genome of A. bisporus (Agaricus bisporus var. bisporus (H97) v2.0; http://genome.jgi-psf.org/programs/fungi/index.jsf; (Morin et al. 2012) allowed us to study transcriptomic changes by high-density microarray hybridization and to identify mechanisms underlying drought stress adaptations and the role of riboflavin in drought stress response.

4.3 Materials and Methods

4.3.1 Experimental setup

To determine the relationship between riboflavin content in hyphae and drought stress resistance of *Agaricus bisporus* LANGE (IMBACH), experiments were conducted in gas-tight mesocosms (poly-acrylic cylinders and lids, 17.1 cm diameter, 9.5 cm height, for a complete description cf. Muhr et al. 2010) placed in a +15°C climate chamber. 5 g of steam sterilised hay were inoculated with *A. bisporus* (DSM No. 3056) by placing a 1 cm² agar plate (malt extract peptone agar) with fungal hyphae on top of the hay. The mesocosms were irrigated every 6 hours with 2.5 ml for 3 or 5 weeks, depending on the treatment. Excessive water was discharged over an opening in the bottom of the mesocosms through a gas-tight siphon.

Four treatments, each four-fold replicated, were established:

Wet control (WC): irrigation without riboflavin for 5 weeks

Wet riboflavin (WR): irrigation with 50 µM riboflavin for 5 weeks

Drought control (DC): 3 weeks irrigation without riboflavin and subsequent desiccation for 2 weeks

Drought riboflavin (DR): 3 weeks irrigation with 50 µM riboflavin and subsequent desiccation for 2 weeks

4.3.2 Respiratory activity

Respiratory activity was estimated by monitoring the CO_2 efflux of the mesocosms using the dynamic closed-chamber technique (cf. Rochette et al. 1997; infrared gas analyzer: LI-820, LI-COR, Nebraska). To avoid continuous increase of gas concentrations and consumption of oxygen, all mesocosms were continuously ventilated with a stream of atmospheric air at a rate of 0.3 L min^{-1} between CO_2 measurements. Measurement cycles for each mesocosm started with a 240 s initializing phase to guarantee a good ventilation of the system prior to a 60 s measurement phase of CO_2 production. CO_2 concentration increase was logged automatically in 10 s intervals. CO_2 efflux was calculated from the slope of the linear regression between CO_2 concentration and incubation time. The infrared gas analyzer was purged for 30 s after each measurement cycle to avoid cross-contaminations between mesocosms. The measurement cycle was repeated about 16 times per day for each mesocosms. Day 10 was excluded from measurements since stable power supply could not be guaranteed on this day. Respiration rates were expressed as mg CO_2 per kg substrate and hour.

In addition, relative air humidity and temperature was determined during the measurement phase using a digital humidity and temperature sensor (SHT15, Sensirion AG, Switzerland). Based on the measured vapor pressure, saturated vapor pressure, and dew point temperature, the water potentials of the hay were calculated using the Magnus formula and the Kelvin equation (Magnus 1844; Haurwitz 1945).

4.3.3 Riboflavin extraction

About 10 mg fresh weight of hyphae were collected from all mesocosms after 5 weeks of experiment, shock frozen in liquid nitrogen and stored in 1.5 ml microtubes at -25 C until further analyzes.

Hyphae were washed three times with 1 ml PBS (phosphate buffered saline: 137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) and subsequently centrifuged for 5 min at 15000 rpm (Himac CT15E, Hitachi Koki Co. Ltd, Japan). The supernatant was discarded each time and samples were stored in the dark at room temperature overnight. Hyphae were then crushed three times on liquid nitrogen using pestles for microtubes and suspended in 200 µl 20% methanol. Subsamples were filtered using a 0.45 µM nylon syringe filter (25 mm diameter, Roth GmbH + Co. KG, Germany) and combined into colored auto sampler vials.

Analytical methods

Riboflavin was determined by high performance liquid chromatography (HPLC; Agilent 1200 series, Agilent Technologies, CA, USA) equipped with a 1200 Series diode-array-detector, quaternary pump, vacuum degasser, standard autosampler, column oven, and a MultoHigh 100 RP 18-5µ column (250 x 4 mm) with pre-column (20 x 4 mm) [both CS-Chromatographie Service GmbH, Langerwehe, Germany)] at 30 °C. The injection volume was 50 µl, and the mobile phase was 20 mM sodium acetate (pH 3) - 50% acetonitrile at 1 ml min^{-1}. Online spectra from 220 to 700 nm were used to confirm peak purity, and the signal at 367 nm was used for quantification with external standards. Signals at 270 and 445 nm were recorded and used for peak purity evaluation. Riboflavin was quantified based on external standard series of different riboflavin concentrations (0.1, 1, 10, 50, and 100 µM) and expressed as mg riboflavin per g dry weight.

4.3.4 Transcriptome analysis

To determine differential gene expression in response to drought stress and riboflavin addition an independent experiment was conducted as described above (WC, WR, DC, DR; see experimental setup). In this case, samples were collected at 2 time points starting three days after end of irrigation (t_1, short term responses) and 7 days after end of irrigation (t_2, long term

responses). These days were chosen since preliminary test showed beginning variations among groups in respiration rates at day 3 and pronounced variations at day 7. At each time point about 10 mg fresh weight of hyphae were collected from each mesocosm and immediately shock frozen in liquid nitrogen and stored at -80 °C until further analyses.

RNA isolation and microarray hybridization experiments

Total RNA was extracted from hyphae tissue using the RNeasy Microarray Tissue Kit (Qiagen GmbH, Hilden, Germany) as recommended by the manufacturer. The only modification was that the initial cell lysis step was facilitated by homogenization of the hyphae at 5 m/sec. for 40 sec. using 0.5 mm glass beads in a benchtop homogeniser (Fastprep-24, MP Biomedicals, Heidelberg, Germany). Total RNA in hyphae was quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Dreieich, Germany).

A high-density microarray was designed from annotated coding sequences (CDS) of the *A. bisporus* var *bisporus* (H97) v2.0 release available via the MycoCosm portal at the Joint Genome Institute (http://genome.jgi-psf.org/programs/fungi/index.jsf; Morin et al. 2012). CDS were uploaded to Agilent's eArray portal (https://earray.chem.agilent.com/earray/) and 60mer oligonucleotides were designed for an 8x 15k microarray layout; 10,413 microarray probes were designed from 10,438 annotated CDS using the default probe design settings. Unused features of the 15k array were filled with randomly selected *Agaricus* CDS probes in addition to Agilent's control probes. The *A. bisporus* microarray was ordered from Agilent (Agilent Technologies, Santa Clara, CA, USA) and will be available under design ID 071199. For microarray hybridization, 150 ng of total RNA of each sample were used and labelled using the Low Input Quick Amp Labeling Kit as recommended by the manufacturer (manual G4140-90050, Agilent Technologies). Dye-swap experiments were included in the microarray design as indicated in Supplemantary Tab. S4.1. The hybridization experiments were performed as recommended in the Two-Color Microarray-Based Gene Expression Analysis protocol (manual G4140-90050, Agilent Technologies). Processed microarrays were scanned using a high-resolution microarray scanner (Agilent Technologies) and spot intensities were quantified using Agilent's feature extraction software.

4.3.5 Data analysis

Statistical analysis and graphics were done using R 3.1.3 (R Core Team, 2015). Normality and homogeneity of the data was tested using Shapiro-Wilk-Test and Levene's-test, respectively. Kruskal–Wallis test with pair–wise Wilcox tests for posthoc comparisons were used if data was not normal and/or variances were not homogeneously distributed. Variations in riboflavin

content among treatments were analysed using linear mixed effect models (LMM) as implemented in the R package nlme (Pinheiro et al. 2015). The mesocosms atmospheric air influx distribution unit were added as a random factor into the model to adjust for potential random variances among them. Data values were ranked for LMM since they were not normal distributed. For pair–wise posthoc comparisons, general linear hypotheses based on Tukey all–pair comparisons were conducted using the R package multcomp (Hothorn et al. 2008).

Microarray normalization and differential gene expression analysis was done using R and the limma package (Smyth 2005). Shortly, median spot intensities were corrected for local background using the 'normexp' method, normalised within arrays by the 'loess' method, and normalised between arrays using the 'aquantile' method. In order to compare expression levels of genes across all eight subarrays present on the 8x 15k microarray slide, the 'Separate Channel Analysis of Two-Color Data' of the limma user's guide (chapter 12; revision June, 17, 2014) was followed. The microarray data were deposited in NCBI's GEO archive under accession no. GSE73010 (http://www.ncbi.nlm.nih.gov/geo/).

Variation in gene expression between treatments was analysed but only genes with an induced (positive-log-ratio values) or repressed (negative-log-ratio values) $\log_2$-value above 1 or below -1, respectively and a p-value below 0.1 were taken into consideration. Gene targets were identified if possible by using available annotations from databases of Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and InterPro or by blasting sequences using UniProt (The UniProt Consortium 2014).

GO enrichment analyses were performed using Blast2GO (Conesa et al. 2005). The entire *A. bisporus* GO annotation available from DOE-JGI was loaded as the reference annotation in Blast2GO and GO enrichment analyses were conducted using Fisher's exact t-test (one-tailed, p-value <0.05) independently with each of the significantly induced and repressed gene lists.

4.4 Results

4.4.1 Impact of drought stress on hyphae

Respiration rates remained relatively stable throughout the experiment in mesocosms without drought stress (mean respiration= WR: 170.40 ± 23.14 mg CO_2 kg^{-1} C h^{-1}; WC: 171.53 ± 28.73 mg CO_2 kg^{-1} C h^{-1}, Supplemantary Tab. S4.2). Respiration of drought-stressed controls (DC) decreased sharply with decreasing water potentials and reached minimum values when water potential reached about -40 MPa (Fig. 4.1). Afterwards respiration decreased only slightly over time. In result, an exponential relationship was found between water potential and respiration in DC mesocosms. In mesocosms treated with riboflavin and drought (DR), the respiration rate decrease was less steep and a linear relationship between water potential and respiration was found. In result, respirations rates were higher in DR compared to DC starting when water potentials dropped below values of about -20 MPa until the end of the experiment.

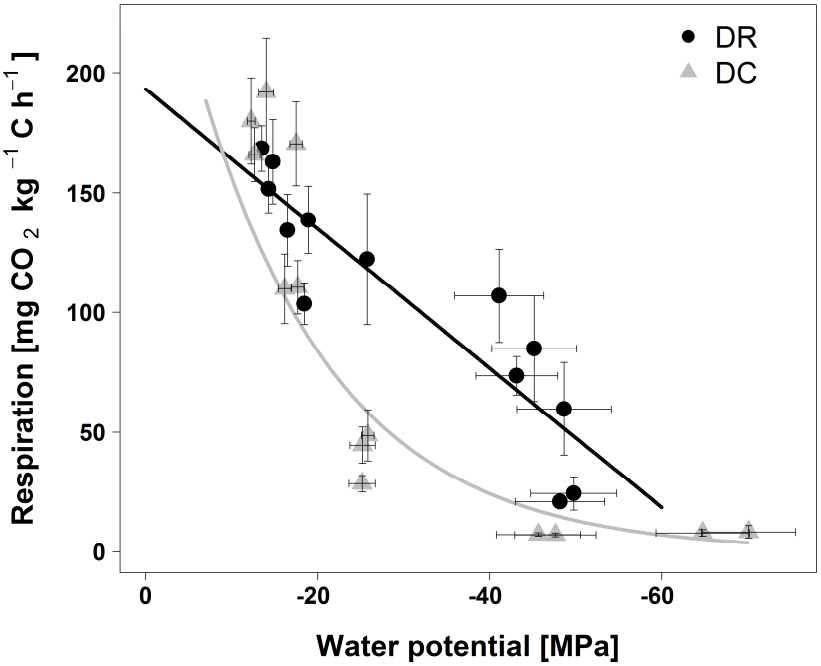


Fig. 4.1: Relationship between respiration rates [mg CO_2 kg^{-1} h^{-1}] and water potential [MPa] in mesocosms with (DR, y= 2.912*x+193.13, Spearman's rank correlation: r^2= 0.85, p<0.001) and without access to excessive riboflavin before drought stress treatment (DC, y= 291.13*$e^{0.0623*x}$, Spearman's rank correlation: r^2= 0.75, p<0.001). Mean ± SEM, n= 4.

Hyphal riboflavin content was slightly increased in irrigated samples (WR/WC: 2.1 fold) by the addition of riboflavin to the irrigation water (Fig. 4.2). A much stronger increase in hyphal riboflavin was observed in drought-stressed hyphae on day 14 in conventional (DC compared to WC: 6.8 fold) and riboflavin-supplemented experiments (DR compared to WR: 5.5 fold). The elevated concentration of riboflavin in hyphae of the riboflavin-supplemented experiments could only be explained by subcellular sequestration processes and/or (active) uptake processes.

The riboflavin content of the irrigation water (18.8 µg ml^{-1}) was considerably lower than the hyphal riboflavin content even under well-watered conditions. Riboflavin treatment also lead to a visible yellow coloring of hyphae.

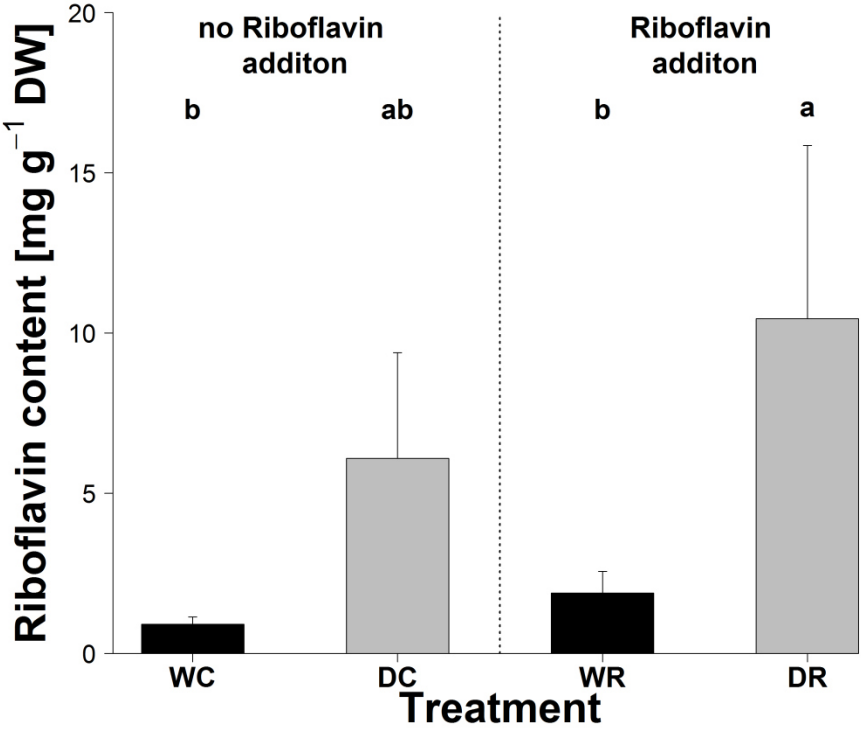
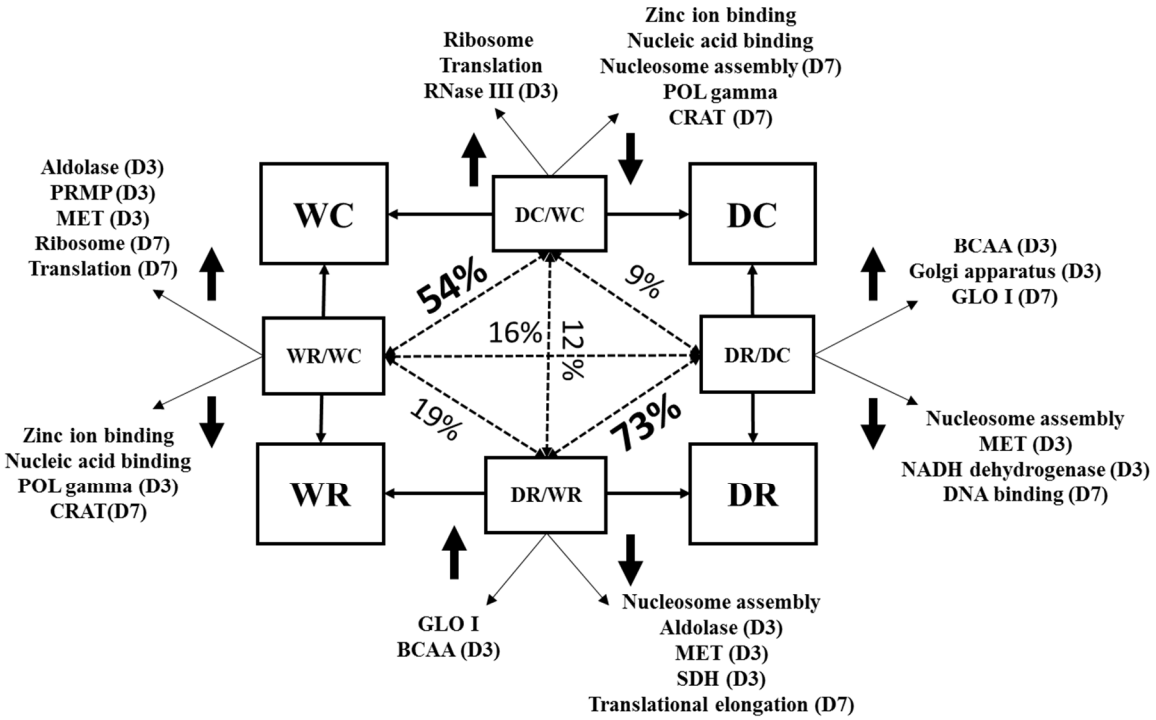


Fig. 4.2: Hyphal riboflavin content [mg g^{-1} hyphal DW] 14 days after irrigation stop. DR= 50 µM riboflavin addition prior to drought stress; WR= 50 µM riboflavin addition, no drought stress; DC= no riboflavin addition prior to drought stress; WC= no riboflavin addition, no drought stress. Different letters indicate significant differences among groups. LMM: $F_{1,15} = 5.59$; p= 0.014. Mean ± SE, n= 4.

Total (extractable) hyphal RNA content per fungal dry weight was on day 3 on average higher in the drought stress treatments (DC= 0.24 ± 0.09 µg mg^{-1} DW; DR= 4.46 ± 2.04 µg mg^{-1} DW) than in the constantly wet treatments (WC= 0.11 ± 0.01 µg mg^{-1} DW; WR= 0.41 ± 0.35 mg g^{-1} DW).

4.4.2 Primary assessment of transcriptional responses

The two experimental variables (riboflavin addition and drought stress) were analyzed at two time points (3 and 7 days after start of the experiments) using replicate samples, resulting in the analysis of 8 independent samples for microarray hybridization experiments. Statistical analyses allowed us to identify differentially regulated genes upon the two experimental contrasts: drought stressed in comparison to non-drought stressed hyphae (DC/WC and DR/WR) and riboflavin-supplemented in comparison to non-riboflavin-supplemented hyphae (WR/WC and DR/DC), respectively (for overview see Fig. 4.3). Differences between the two time points were displayed but not further analyzed since considerable overlap of induced and repressed target groups were observed between day 3 and 7, respectively.



Tab. 4.3: Overview over gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for most specific GO terms up to P-value 0.01; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. DR= no riboflavin addition prior to drought stress; WR= no drought stress, 50 µM riboflavin addition; DC= no riboflavin addition prior to drought stress; WC= no riboflavin addition, no drought stress. GLO1= lactoylglutathione lyase activity; BCAA= branched-chain-amino-acid transaminase activity; CRAT= carnitine O-acetyltransferase activity; MET= mitochondrial electron transport; PRMP= purine ribonucleoside/ribonucleotide metabolic process; SDH= succinate dehydrogenase activity; POL gamma= gamma DNA polymerase complex; Aldolase= Fructose-bisphosphate aldolase; RNase III= bidentate ribonuclease III activity.

Differentially regulated genes considerably overlapped by 54% (49 of 92 genes) between only drought application (DC/WC) and only riboflavin addition (WR/WC) (Fig. 4.3). On the other hand, an even larger overlap of differentially regulated genes of 73% (33 of 45 genes) was observed, if both factors were applied: drought application to riboflavin-supplemented mycelia (DR/WR), or riboflavin addition to drought-stressed mycelia (DR/DC). In contrast, differentially regulated genes of one-factor and two-factor contrasts (either drought or riboflavin versus drought plus riboflavin) did hardly overlap indicating that transcriptional responses to drought and riboflavin treatments are different from responses to only one of these factors alone.

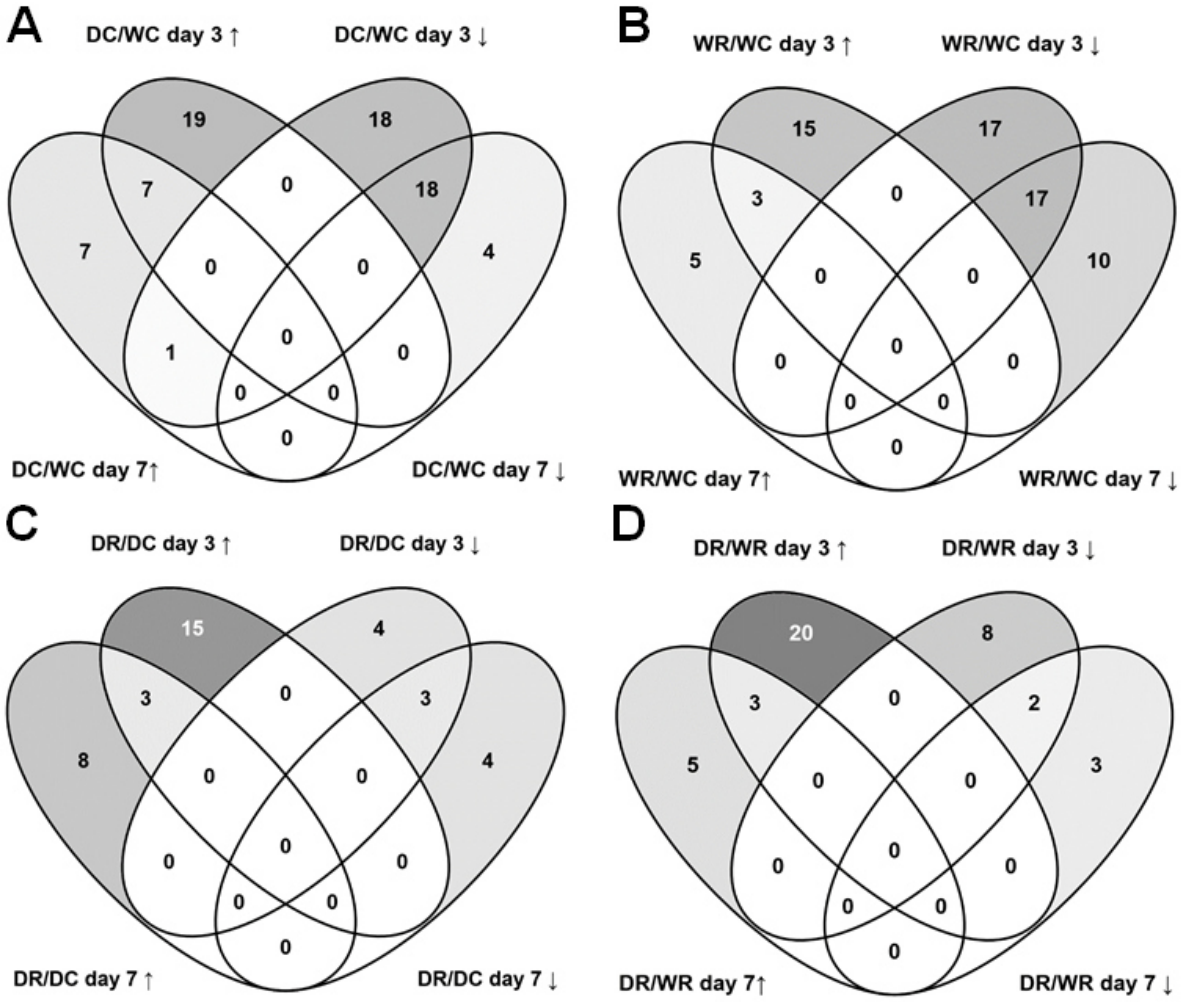


Fig. 4.4: Differential expression between each pair of samples. Venn diagrams showing unique and shared genes between (A) DC and WC mescosms, (B) DR and DC, (C) DR and WR mescosms, and (D) WR and WC mescosms of induced (↑) and repressed (↓) genes in microarrays on day 3 and 7 after irrigation stop. Overlapping genes were identified using VENNY 2.1 (Oliveros 2015). DR= 50 µM riboflavin addition prior to drought stress; WR= 50 µM riboflavin addition, no drought stress; DC= no riboflavin addition prior to drought stress; WC= no riboflavin addition, no drought stress.

4.4.3 Transcriptional responses to application of one factor

In total, 63 and 37 genes showed differential expression on day 3 and day 7 in response to only drought stress (DC/WC), respectively (Fig. 4.4A). In response to exogenous riboflavin addition without drought stress (WR/WC), 52 and 35 genes showed differential expression on day 3 and day 7, respectively (FIG. 4.4B).

GO enrichment analyses revealed that expression of genes related to ribosomes and protein translation were significantly induced in response to either drought or exogenous riboflavin addition compared to wet controls (Tab. 4.1; Tab. 4.2; Supplemantary Tab. S4.3; Supplemantary Tab. S4.4). Enriched-GO terms within induced genes found only in the DC/WC treatment were related to bidentate ribonuclease III activity and in the WR/WC to fructose-

bisphosphate aldolase activity, generation of precursor metabolites and energy, cellular component biogenesis and mitochondrial electron transport.

Tab. 4.1: Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for most specific GO terms up to p-value 0.01; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DC= no riboflavin addition prior to drought stress; WC= no drought stress, no riboflavin addition.

GO-ID	Term	Category	p-Value	Genes
Enriched-GO terms found within induced genes between DC and WC on day 3				
GO:0015935	small ribosomal subunit	C	0.010	1
Enriched-GO terms found within repressed genes between DC and WC on day 3				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	12
GO:0000786	Nucleosome	C	0.001	2
GO:0006334	nucleosome assembly	P	0.001	2
GO:0005760	gamma DNA polymerase complex	C	0.003	1
GO:0004092	carnitine O-acetyltransferase activity	F	0.009	1
Enriched-GO terms found within induced genes between DC and WC on day 7				
GO:0005840	Ribosome	C	<0.001	6
GO:0003735	structural constituent of ribosome	F	<0.001	6
GO:0006412	Translation	P	<0.001	6
GO:0004502	kynurenine 3-monooxygenase activity	F	0.002	1
Enriched-GO terms found within repressed genes between DC and WC on day on day 7				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	12
GO:0000786	Nucleosome	C	0.001	2
GO:0006334	nucleosome assembly	P	0.001	2
GO:0005760	gamma DNA polymerase complex	C	0.003	1
GO:0004092	carnitine O-acetyltransferase activity	F	0.009	1

Further analyses revealed differential gene expressions of a variety of specific gene targets (Supplemantary Tab. S4.5; Supplemantary Tab. S4.6). Genes with known function and induced expression were in both cases mostly related to the regulation of translation and transcription. This included genes coding for the ribosomal proteins S7, L10 and S11 as well as histone acetyltransferase, subunit 21 of mediator complex protein, nuclear cap-binding protein subunit 3 and an ubiquitin carboxyl-terminal hydrolase. Further, genes coding for cytochrome C and a general substrate transporter of the major facilitator superfamily showed induced expression in both cases. Genes showing induced expression only within the DC/WC treatment were mostly also in relation to regulation of translation, transcription and cell processes and coded for a

ribonuclease III, the Golgi pH regulator, Cdc20, the ribosomal proteins L7A/S12e, L14b/L23e, L40e and L18ae as well as a long-chain-fatty-acid--CoA ligase. Histone H3, a NADH ubiquinone oxidoreductase and a fructose 1,6-bisphosphate aldolase showed only induced expression in the WR/WC treatment.

Tab. 4.2: Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for most specific GO terms up to P-value 0.01; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DC= no riboflavin addition prior to drought stress; DR= 50 µM riboflavin addition prior to drought stress.

GO-ID	Term	Category	P-Value	Genes
Enriched-GO terms found within induced genes between WR and WC on day 3				
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	4
GO:0006091	generation of precursor metabolites and energy	P	0.002	2
GO:0046034	ATP metabolic process	P	0.002	2
GO:0004332	fructose-bisphosphate aldolase activity	F	0.004	1
GO:0032991	macromolecular complex	C	0.006	4
GO:0044446	intracellular organelle part	C	0.007	3
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P	0.007	1
Enriched-GO terms found within repressed genes between WR and WC on day 3				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	11
GO:0005760	gamma DNA polymerase complex	C	0.004	1
GO:0051082	unfolded protein binding	F	0.008	2
Enriched-GO terms found within induced genes between WR and WC on day 7				
GO:0003735	structural constituent of ribosome	F	<0.001	3
GO:0005840	ribosome	C	<0.001	3
GO:0006412	translation	P	0.001	3
GO:0044085	cellular component biogenesis	P	0.004	2
Enriched-GO terms found within repressed genes between WR and WC on day 7				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	9
GO:0004092	carnitine O-acetyltransferase activity	F	0.009	1

In contrast, gene expressions were significantly repressed for genes related to the GO terms zinc ion binding and nucleic acid binding as well as the gamma DNA polymerase complex, and carnitine O-acetyltransferase activity in DC as well as WR compared to WC. Enriched-GO terms within repressed genes found only in the DC/WC treatment were related to nucleosome as well as nucleosome assembly and in the WR/WC treatment to unfolded protein binding.

A strong repression was in both cases detectable for specific gene targets coding for ubiquitin, ubiquitin thiolesterase, carnitine O-acetyltransferase, GC-rich sequence DNA-binding factor, prefoldin, thymocyte nuclear protein 1 and mitochondrial DNA polymerase gamma 1. Unique in the DC/WC treatment was a repressed expressions of genes coding for Histone H3 and H2B, an ubiquitin-conjugating enzyme, and a DNA mismatch repair protein. Genes coding for an alpha-tubulin folding cofactor E, a glycoside hydrolase, the phosphatidyl-glycero-phosphatase GEP4 and a zinc/iron permease showed only repressed expression in the WR/WC treatment.

4.4.4 Transcriptional responses to application of two factors

In response to presence or absence of exogenous riboflavin and drought stress (DR/DC), 25 and 18 genes showed differential expression on day 3 and day 7, respectively (Fig. 4.4C). Comparison of the two treatments with exogenous riboflavin addition (DR/WR) revealed that in response to drought stress 33 and 13 genes showed differential expression on day 3 and day 7, respectively (Fig. 4.4D).

GO enrichment analyses revealed that in both cases expression of genes related to branched-chain-amino-acid transaminases activity and lactoylglutathione lyase activity was significantly increased (Tab. 4.3; Tab. 4.4; Supplemantary Tab. S4.7; Supplemantary Tab. S4.8). Enriched-GO terms within induced genes found only in the DR/DC treatment were related to the Golgi apparatus.

Expression analysis of specific gene targets revealed in both cases a strong induction of genes coding for the known detoxification and protective enzymes/proteins lactoylglutathione lyase, a branched-chain-amino-acid transaminase and cytochrome C (Supplemantary Tab. S4.9; Supplemantary Tab. S4.10). Further genes with known function and induced gene expression in both cases coded for Mra1, the cell wall synthesis protein Knh1, Gep4, various transport proteins, a glycoside hydrolases and alpha-tubulin folding cofactor E. Unique to the DR/DC treatment was an induced expression of a gene coding for the eukaryotic translation initiation factor 5A-1 (EIF5A). The gene coding for the subunit 21 of the mediator complex protein showed only in the DR/WR treatment induced expression.

In contrast, gene expressions were significantly repressed of genes related to the GO terms nucleosome, nucleosome assembly and mitochondrial electron transport in the DR/DC as well as the DR/WR treatment. Enriched-GO terms within repressed genes found only in the DR/DC treatment were related to NADH dehydrogenase activity and in the DR/WR treatment to fructose-bisphosphate aldolase activity, succinate dehydrogenase activity and translational elongation.

A gene expression repression was in both cases detectable for specific genes coding for Histones H3 and H2B, ubiquitin and NADH ubiquinone oxidoreductase. Unique to the DR/DC treatment was a repressed expression of genes coding for Ebp2 and to the DR/WR treatment of succinate dehydrogenase and fructose 1,6-bisphosphate aldolase as well as the ribosomal proteins L10 and L14b/L23e.

Tab. 4.3: Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for most specific GO terms up to p-value 0.01; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DR= no riboflavin addition prior to drought stress; WR= no drought stress, 50 µM riboflavin addition.

GO-ID	Term	Category	p-Value	Genes
Enriched-GO terms found within induced genes between DR and DC on day 3				
GO:0004084	branched-chain-amino-acid transaminase activity	F	0.001	1
GO:0005794	Golgi apparatus	C	0.007	1
GO:0009081	branched-chain amino acid metabolic process	P	0.008	1
Enriched-GO terms found within repressed genes between DR and DC on day 3				
GO:0000786	Nucleosome	C	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P	0.004	1
GO:0008137	NADH dehydrogenase (ubiquinone) activity	F	0.010	1
Enriched-GO terms found within induced genes between DR and DC on day 7				
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
Enriched-GO terms found within repressed genes between DR and DC on day 7				
GO:0000786	Nucleosome	C	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0003677	DNA binding	F	0.004	2
GO:0005634	Nucleus	C	0.006	2

Tab. 4.4: Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for most specific GO terms up to p-value 0.01; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. WR= no drought stress, 50 µM riboflavin addition; WC= no drought stress, no riboflavin addition.

GO-ID	Term	Category	p-Value	Genes
Enriched-GO terms found within induced genes between DR and WR on day 3				
GO:0004084	branched-chain-amino-acid transaminase activity	F	0.002	1
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
Enriched-GO terms found within repressed genes between DR and WR on day 3				
GO:0009987	nucleosome	C	<0.001	2
GO:0000786	nucleosome assembly	P	<0.001	2
GO:0006334	fructose-bisphosphate aldolase activity	F	0.003	1
GO:0004332	mitochondrial electron transport, NADH to ubiquinone	P	0.006	1
GO:0006120	succinate dehydrogenase (ubiquinone) activity	F	0.008	1
Enriched-GO terms found within induced genes between DR and WR on day 7				
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
Enriched-GO terms found within repressed genes between DR and WR on day 7				
GO:0000786	nucleosome	C	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0006414	translational elongation	P	0.007	1

4.4.5 Assessment of transcriptional control of riboflavin biosynthesis, uptake and processing

Special regard in analysis of transcriptional response of specific gene targets was placed on enzymes associated to production, uptake and transformation of riboflavin. We analyzed annotated genes coding for enzymes of the riboflavin biosynthesis pathway (RIB3= 3,4-dihydroxy-2-butanone 4-phosphate synthase, Protein ID: 143682; RIB4= 6,7-dimethyl-8-ribityllumazine synthase, Protein ID: 190326; RIB5=riboflavin synthases, final step in riboflavin production, Protein ID: 182135) as well as best hits for the remaining biosynthesis steps after blasting annotated genes (Gene ID's: 852247; 854088; 852450) from *Saccharomyces cerevisiae* (RIB1= GTP cyclohydrolase II, Protein ID: 191759; RIB2= Diaminohydroxyphosphoribosylaminopyrimidine deaminase, Protein ID's: 185612, 190437; RIB7= 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate reductase, Protein ID: 213834). We further analyzed expression of genes coding for enzymes responsible for riboflavin processing (riboflavin kinase= Protein ID: 202343 and FAD synthetase= Protein ID:

194855) as well as a gen assumed to encode for the riboflavin plasma membrane transporter Mch5 (best hit after blasting annotated gene ID 19293338 from the basidiomycete *Moniliophthora roreri*, Protein ID: 190143). Variations in gene expression of these targets were relatively small between all treatments and in no case above the 2-fold threshold (Supplemantary Tab. S4.11).

4.5 Discussion

This study extends our knowledge on respiratory and transcriptional responses of saprotrophic filamentous fungi to drought stress and shows the impact of riboflavin on drought tolerance in fungi.

4.5.1 Impact of drought stress on respiratory activity and hyphal riboflavin content

Respiration of non-riboflavin treated hyphae decreased sharply with decreasing water potentials and reached minimum values around -40 MPa. This is comparable to thresholds found by Manzoni et al. (2012) of about -36 MPa for respiratory activity of litter decomposers. Riboflavin addition attenuated the drought induced respiration decrease. Respiration remained about 2-3 times higher than without riboflavin below water potentials of about -20 MPa. Hence, riboflavin addition had a positive impact on respiratory activity of *A. bisporus* under similar or even lower water potentials. This is comparable to the observed effect of riboflavin in tobacco plants (Deng et al. 2014).

Intracellular riboflavin content increased under drought stress and was further increased with exogenous riboflavin added to the irrigation water. Riboflavin was thereby strongly accumulated in hyphae (10.5 mg g^{-1} DW) compared to the irrigation water concentration (18.8 µg ml^{-1}) by up to 500 times. Homologous to proteins from *Saccharomyces cerevisiae* associated with riboflavin biosynthesis (RIB enzymes), transport (Mch5) and processing (riboflavin kinase and FAD synthetase) showed no significant variations in expression and could not explain the increased riboflavin content (Supplemantary Tab. S4.11). Other regulatory mechanisms than transcriptional control are potentially of importance for the intracellular riboflavin content. However, the regulation of riboflavin biosynthesis in fungi is still only partly understood (Abbas and Sibirny 2011). In riboflavin overproducing strains of *Eremeothecium ashbyii* and *Ashbya gossypii*, riboflavin production is linked to a derepression of biosynthesis enzymes during nutritional stress (Koltun et al. 1983; Schlösser et al. 2007). In addition, riboflavin production in *Pichia guilliermondii* is linked to increased cellular contents of reactive malondialdyhyde due to oxidative stress (Protchenko et al. 1999). Riboflavin biosynthesis is also regulated by metal ions and iron deficiency is known to stimulate riboflavin biosynthesis but underlying mechanisms are unknown (Fischer and Bacher 2005; Boretsky et al. 2007). Therefore, the processes regulating intracellular riboflavin contents in fungi require further attention. A proteomic approach might be helpful in case of *A. bisporus*.

4.5.2 Transcriptional responses to drought or riboflavin addition

The transcriptional response of *A. bisporus* to either only drought or riboflavin did strongly overlap. This suggests that physiological responses to both treatments are connected. It could indicate a stress priming function of riboflavin in fungi. Responses were mainly based on differential expression of genes with importance for the regulation of transcription and translation and potentially epigenetic functions. Yet, precise mechanisms and targets are only partly understood in all cases (cf. (Lindström 2009; Cao and Yan 2012; Kim et al. 2015). Particularly noticeable was the strong increase in expression of genes coding for various ribosomal proteins (RP). Since they are part of the translation machinery, an effect on mRNA-specific protein synthesis may result (Lindström 2009; Xue and Barna 2012). Many RP have additional functions in various processes, including target specific regulation of translation, e.g., by binding and protecting mRNAs at non-coding regions (Lindström 2009; Bhavsar et al. 2010). For example, RPL10 affected concentrations of proteins related to transcription, translation and DNA replication as well as redox metabolism in *S. cerevisiae* in association to oxidative stress (Chiocchetti et al. 2014).

Further, nucleosome modifications were likely facilitated by the induced expression of genes coding for a histone acetyltransferase (HAT) and an ubiquitin carboxyl-terminal hydrolase (UCHL) as well as the repressed expression of ubiquitin and an ubiquitin thiolesterase. The ubiquitin system has manifold functions in regulation of cell processes (Ciechanover 2015). One is the stabilization of nucleosomes by interaction with histones, which increases DNA compaction and protection but decreases access of regulatory proteins (Mello and Almouzni 2001; Chandrasekharan et al. 2009). UCHLs cleave ubiquitin from targets and hence reverse the ubiquitinylation process (Ventii and Wilkinson 2008). HATs take part in the regulation of gene expression via the acetylation of internal lysine residues of histones, which neutralizes the positive charge and modifies nucleosome structures (Kuo and Allis 1998). These reversible modifications affect the interaction of histones with other proteins and the DNA depending on the concentrations of free modifiers as well as the modification site (Zhang 2003). The modifications can facilitate the access of regulatory proteins to the DNA which tends to induce transcription but can also lead to gene silencing (Cao and Yan 2012; Kim et al. 2015). The impact on transcription is often gene-specific, e.g., Kim et al. (2015) observed specific acetylation patterns for drought stress-inducible genes, including various transcription factors, in plants.

The above stated differentially expressed genes may have partially promoted gene expression. This would be in accordance with the induced expression of the gene coding for subunit 21 of the mediator complex protein (Med21). Med21 is required for expression of nearly all RNA polymerase II-dependent genes in fungi (Hengartner et al. 1995). In addition, orthologues genes of RPS7 were shown to stimulate cell proliferation and growth in *S. cerevisiae* (Synetos et al. 1992) and orthologues of Cdc20 can regulate and promote progression through mitosis (Kramer et al. 2000). Differential expression of all genes may have been interdependent since an increase in cell proliferation and growth increases demands for novel proteins. Hence, gene expression of translation related elements (including rRNA) correlates strongly with growth rates (García-Martínez et al. 2015). Since non-coding RNA (e.g., rRNA) can make up to 60-75% of total transcripts during growth (Warner 1999; García-Martínez et al. 2015), this may also partly explain the higher total extractable RNA concentrations in DC compared to WC.

Overall, the transcriptional responses observed in this study may lead to an increase in growth. An increase of hyphal lengths has been documented in field studies in response to drought (Allison et al. 2013; Alster et al. 2013). This could in turn partially compensate a water deficit by accessing remaining water reservoirs. Hydraulic redistribution (HR) from wet to dry soil areas along soil water gradients has been shown for plant root systems and fungal networks (Dawson 1993; Querejeta et al. 2003; Guhr et al. 2015). HR can improve survival and activity under drought stress, for example by prevention of air-induced embolism (Domec et al. 2004). Further, HR has been shown to strongly enhance organic matter decomposition by *A. bisporus* in dry soils (Guhr et al. 2015). Mycelia network extension in response to drought could thus strongly support HR along hyphal tissue.

4.5.3 Impact of riboflavin addition on drought responses

The transcriptional response of *A. bisporus* to drought and riboflavin treatment was highly similar independent of the order the treatments were theoretically applied (first riboflavin and then drought, i.e., DR/WR, or vice versa, i.e., DR/DC), suggesting a good reproducibility of results. The response was mostly based on transcription, translation and growth regulating factors as well as proteins involved in protective mechanisms.

Especially the last group probably explains the performance of DR under drought conditions. This included cytochrome C which is important for the electron transport chain and is known for its antioxidative potential (Pereverzev et al. 2003). Most pronounced was the induced expression of a gene coding for a lactoylglutathione lyase, an enzyme of the glyoxalyses system. It is responsible for detoxification of methylglyoxal (MGO) and other reactive aldehydes, which

are by-products of different metabolic pathways and cause negative effects like cell proliferation inhibition or protein damage (Martins et al. 2001; Thornalley 2003). MGO mainly results from triose-phosphate isomerase activity as well as the non-enzymatic isomerisation of unstable triose phosphates in consequence of glycolysis but also from acetol dehydrogenase activity and aminoacetone oxidation (Cooper 1984; Richard 1993; Allaman et al. 2015). High contents of MGO in cells were reported under drought stress, most likely due to increased metabolic activity in early stages of stress response, and an increased glyoxalase activity induces stress tolerance in plants (Hossain et al. 2009). Hence, riboflavin could help to increase MGO tolerance as described in Tarwadi and Agte (2011).

A gene coding for a branched-chain-amino-acid transaminase (BCAT), which are essential for the degradation of branched-chain amino acids (BCAA: leucine, isoleucine, and valine), showed increased expression as well. This may provide an alternative carbon source but BCAA and especially the breakdown products (α-keto-acids and glutamate) are potentially cytotoxic (Eden and Benvenisty 1999). Free BCAA pools are mostly affected by protein degradation, e.g., in response to protein damage during stress (Singh and Shaner 1995; Di Martino et al. 2003). Accordingly, BCAA levels were shown to increase under stress conditions and BCAT expression to be elevated under drought stress in plants (Malatrasi et al. 2006). Therefore, BCAT was most likely mediating BCAA detoxification under the prevailing drought stress conditions.

Moreover, riboflavin addition to drought stressed hyphae seemed to further trigger expressions of genes with the potential to impact transcription, translation and growth (e.g., Mra1 and Knh1). Mra1 is essential for fungal growth as part of the Ras1 signal pathway and can compensate Ras1 deficit by overexpression (Hakuno et al. 1996). Knh1 is involved in the biosynthesis of β-1,6-glucan, an essential part of fungal cell walls and novel synthesis is required during growth (Lesage and Bussey 2006). Based on the GO enrichments and the repressed expression of genes coding for ubiquitin as well as histones H3 and H2B, DR may have shown even lower levels of histone ubiquitination and DNA compaction. This may have facilitated the access of regulatory proteins to the DNA and strongly affected DNA replication and transcription. In addition, a gene coding for EIF5A showed increased expression in the DR/DC treatment. EIF5A is a translational elongation factor and essential for cell proliferation (Park et al. 1997). EIF5A also partly regulates environmental stress responses by changing translation patterns and promoting stress granule assembly (Li et al. 2010; Henderson and

Hershey 2011). Together, differentially expressed genes of this group could even further stimulate transcription as well as growth to promote water uptake.

4.5.4 Conclusions

The main transcriptional response of *A. bisporus* to either drought or riboflavin seemed to be based on differential transcription and increased translation and growth (e.g., various ribosomal proteins, HAT, ubiquitinylation elements). In particular, differentially expressed genes related to chromatin modifications suggest that epigenetic regulations could have occurred, but this aspect was outside the scope of the presented work and requires further attention. The strong overlap between drought and riboflavin treatment suggests a strong correlation of physiological responses and indicates a potential for stress priming of riboflavin.

Treatment with riboflavin under drought stress seemed to further impact transcription, translation and growth by inducing expression of additional genes (e.g., coding for Mra1, Knh1, EIF5A) as well as an even stronger differential expression of some targets (e.g., ubiquitin). In addition, Riboflavin triggered protective mechanisms supporting drought stress tolerance, the MGO detoxifying lactoylglutathione lyase being most pronounced. Hence, *A. bisporus* clearly benefited from riboflavin addition under drought and riboflavin attenuated the drought induced respiration decrease even under low water potentials. Drought stress also led to an increase in hyphal riboflavin content. Therefore, riboflavin likely has a role in drought responses of *A. bisporus*.

4.6 Acknowledgements

We thank G. Rambold and T. Leistner for access to lab facilities as well as help with the riboflavin extraction. We also thank M. Hochholzer, K. Söllner, A. Dallinger and R. Mertel for help with laboratory work. We further thank W. Borken and E. Matzner for critical feedback and discussions.

This work was supported by Deutsche Forschungsgemeinschaft Grant (DFG-MA1089/23-1).

4.7 References

Abbas CA, Sibirny AA. 2011. Genetic control of biosynthesis and transport of riboflavin and flavin nucleotides and construction of robust biotechnological producers. Microbiol Mol Biol Rev 75: 321–360.

Allaman I, Bélanger M, Magistretti PJ. 2015. Methylglyoxal, the dark side of glycolysis. Front Neurosci 9: 23.

Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, Martiny, Jennifer B. H. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94: 714–725.

Alster CJ, German DP, Lu Y, Allison SD. 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol Biochem 64: 68–79.

Ashoori M, Saedisomeolia A. 2014. Riboflavin (vitamin B2) and oxidative stress: a review. Br J Nutr 111: 1–7.

Baldrian P, Větrovský T, Cajthaml T, Dobiášová P, Petránková M, Šnajdr J, Eichlerová I. 2013. Estimation of fungal biomass in forest litter and soil. Fungal Ecol 6: 1–11.

Beutler E. 1969. Effect of flavin compounds on glutathione reductase activity: in vivo and in vitro studies. J Clin Invest 48: 1957–1966.

Bhavsar RB, Makley LN, Tsonis PA. 2010. The other lives of ribosomal proteins. Hum Genomics 4: 327.

Boretsky YR, Protchenko OV, Prokopiv TM, Mukalov IO, Fedorovych DV, Sibirny AA. 2007. Mutations and environmental factors affecting regulation of riboflavin synthesis and iron assimilation also cause oxidative stress in the yeast Pichia guilliermondii. J Basic Microbiol 47: 371–377.

Borken W, Davidson EA, Savage K, Gaudinski J, Trumbore SE. 2003. Drying and wetting effects on carbon dioxide release from organic horizons. Soil Sci Soc Am J 67: 1888.

Burton KS, Smith JF, Wood DA, Thurston CF. 1997. Extracellular proteinases from the mycelium of the cultivated mushroom Agaricus bisporus. Mycol Res 101: 1341–1347.

Cao J, Yan Q. 2012. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Front Oncol 2: 26.

Chandrasekharan MB, Huang F, Sun Z-W. 2009. Ubiquitination of histone H2B regulates chromatin dynamics by enhancing nucleosome stability. Proc Natl Acad Sci U S A 106: 16686–16691.

Chang ST, Miles PG. 1986. Mushroom technology. Mushroom Newslett Tropics 6. 6–11.

Chavez MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CP, Osorio ML, Carvalho I, Faria T, Pinheiro C. 2002. How plants cope with water stress in the field? Photosynthesis and growth. Ann Bot 89: 907–916.

Chiocchetti AG, Haslinger D, Boesch M, Karl T, Wiemann S, Freitag CM, Poustka F, Scheibe B, Bauer JW, Hintner H, Breitenbach M, Kellermann J, Lottspeich F, Klauck SM, Breitenbach-Koller L. 2014. Protein signatures of oxidative stress response in a patient specific cell line model for autism. Mol Autism 5: 10.

Ciechanover A. 2015. The unravelling of the ubiquitin system. Nat Rev Mol Cell Biol 16. 322–324.

Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674–3676.

Cooper RA. 1984. Metabolism of methylglyoxal in microorganisms. Annu Rev Microbiol 38. 49–68.

Crowther TW, Boddy L, Hefin Jones T. 2012. Functional and ecological consequences of saprotrophic fungus-grazer interactions. ISME J 6: 1992–2001.

Dawson TE. 1993. Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant interactions. Oecologia 95: 565–574.

Deng B, Jin X, Yang Y, Lin Z, Zhang Y. 2014. The regulatory role of riboflavin in the drought tolerance of tobacco plants depends on ROS production. Plant Growth Regul 72: 269–277.

Di Martino C, Delfine S, Pizzuto R, Loreto F, Fuggi A. 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytol 158: 455–463.

Domec J-C, Warren JM, Meinzer FC, Brooks JR, Coulombe R. 2004. Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. Oecologia 141: 7–16.

Eden A, Benvenisty N. 1999. Involvement of branched-chain amino acid aminotransferase (Bcat1/Eca39) in apoptosis. FEBS Lett 457: 255–261.

Fischer M, Bacher A. 2005. Biosynthesis of flavocoenzymes. Nat Prod Rep 22: 324–350.

García-Martínez J, Delgado-Ramos L, Ayala G, Pelechano V, Medina DA, Carrasco F, González R, Andrés-León E, Steinmetz L, Warringer J, Chávez S, Pérez-Ortín JE. 2015. The cellular growth rate controls overall mRNA turnover, and modulates either transcription or degradation rates of particular gene regulons. Nucleic Acids Res 44: 3643–3658.

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I. 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 42: D699-704.

Guhr A, Borken W, Spohn M, Matzner E. 2015. Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. Proc Natl Acad Sci U S A 112: 14647–14651.

Hakuno F, Hughes DA, Yamamoto M. 1996. The Schizosaccharomyces pombe mra1 gene, which is required for cell growth and mating, can suppress the mating inefficiency caused by a deficit in the Ras1 activity. Genes Cells 1: 303–315.

Hättenschwiler S, Tiunov AV, Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36: 191–218.

Haurwitz B. 1945. Insolation in relation to cloudiness and cloud density. J Meteor 2. 154–166.

Henderson A, Hershey JW. 2011. Eukaryotic translation initiation factor (eIF) 5A stimulates protein synthesis in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 108: 6415–6419.

Hengartner CJ, Thompson CM, Zhang J, Chao DM, Liao SM, Koleske AJ, Okamura S, Young RA. 1995. Association of an activator with an RNA polymerase II holoenzyme. Genes Dev 9: 897–910.

Herzog C, Peter M, Pritsch K, Günthardt-Goerg MS, Egli S. 2013. Drought and air warming affects abundance and exoenzyme profiles of Cenococcum geophilum associated with Quercus robur, Q. petraea and Q. pubescens. Plant Biol 15: 230–237.

Hossain MA, Hossain MZ, Fujita M. 2009. Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. Aust J Crop Sci 3: 53–64.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. Biom J 50: 346–363.

Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol 47: 377–403.

Kim J-M, Sasaki T, Ueda M, Sako K, Seki M. 2015. Chromatin changes in response to drought, salinity, heat, and cold stresses in plants. Front Plant Sci 6: 114.

Koltun LV, Shavlovskiĭ GM, Kashchenko VE, Trach VM. 1983. Changes in the enzyme activity of flavinogenesis in the process of culturing the fungus Eremothecium ashbyii. Mikrobiologiia 53: 43–47.

Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters J-M. 2000. Mitotic regulation of the APC activator proteins CDC20 and CDH1. Mol Biol Cell 11: 1555–1569.

Kuo M-H, Allis CD. 1998. Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20: 615–626.

Lesage G, Bussey H. 2006. Cell wall assembly in Saccharomyces cerevisiae. Microbiol Mol Biol Rev 70: 317–343.

Li CH, Ohn T, Ivanov P, Tisdale S, Anderson P. 2010. eIF5A promotes translation elongation, polysome disassembly and stress granule assembly. PLoS ONE 5: e9942.

Lindström MS. 2009. Emerging functions of ribosomal proteins in gene-specific transcription and translation. Biochem Biophys Res Commun 379: 167–170.

Magnus G. 1844. Versuche uber die Spannkrafte des Wasserdampfes. Ann Phys 61. 225–248.

Malatrasi M, Corradi M, Svensson JT, Close TJ, Gulli M, Marmiroli N. 2006. A branched-chain amino acid aminotransferase gene isolated from Hordeum vulgare is differentially regulated by drought stress. Theor Appl Genet 113: 965–976.

Manzoni S, Schimel JP, Porporato A. 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93: 930–938.

Martins AMT, Cordeiro CAA, Ponces Freire AMJ. 2001. In situ analysis of methylglyoxal metabolism in Saccharomyces cerevisiae. FEBS Lett 499: 41–44.

Mello JA, Almouzni G. 2001. The ins and outs of nucleosome assembly. Curr opin Genet Dev 11: 136–141.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7. 405–410.

Mori T, Sakurai M. 1995. Effects of riboflavin and increased sucrose on anthocyanin production in suspended strawberry cell cultures. Plant Sci 110: 147–153.

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, Bailey AM, Billette C, Coutinho PM, Deakin G, Doddapaneni H, Floudas D, Grimwood J, Hildén K, Kües U, Labutti KM, Lapidus A, Lindquist EA, Lucas SM, Murat C, Riley RW, Salamov AA, Schmutz J, Subramanian V, Wösten, Han A B, Xu J, Eastwood DC, Foster GD, Sonnenberg, Anton S M, Cullen D, de Vries, Ronald P, Lundell T, Hibbett DS, Henrissat B, Burton KS, Kerrigan RW, Challen MP, Grigoriev IV, Martin F. 2012. Genome sequence of the button mushroom Agaricus

bisporus reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl Acad Sci U S A 109: 17501–17506.

Muhr J, Franke J, Borken W. 2010. Drying–rewetting events reduce C and N losses from a Norway spruce forest floor. Soil Biol Biochem 42: 1303–1312.

Oliveros JC. 2015. Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Osakabe Y, Osakabe K, Shinozaki K, Tran L-SP. 2014. Response of plants to water stress. Front Plant Sci 5: 86.

Park MH, Lee YB, Joe YA. 1997. Hypusine is essential for eukaryotic cell proliferation. Biol Signals 6: 115–123.

Pereverzev MO, Vygodina TV, Konstantinov AA, Skulachev VP. 2003. Cytochrome c, an ideal antioxidant. Biochm Soc Trans 31: 1312–1315.

Pinheiro J, Bates D, DebRoy S, Sarkar, D. and the R Development Core Team. 2015. nlme: Linear and nonlinear mixed effects models.

Protchenko OV, Boretsky Y, Romanyuk TM, Fedorovych DV. 1999. Oversynthesis of riboflavin by yeast Pichia guilliermondii in response to oxidative stress. Ukr Biokhim Zh 72: 19–23.

Querejeta JI, Egerton-Warburton LM, Allen MF. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134: 55–65.

Richard JP. 1993. Mechanism for the formation of methylglyoxal from triosephosphates. Biochm Soc Trans 21: 549–553.

Rochette P, Ellert B, Gregorich EG, Desjardins RL, Pattey E, Lessard R, Johnson BG. 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. Can J Soil Sci 77: 195–203.

Sandoval FJ, Zhang Y, Roje S. 2008. Flavin nucleotide metabolism in plants: monofunctional enzymes synthesize fad in plastids. J Biol Chem 283: 30890–30900.

Schimel J, Balser TC, Wallenstein M. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88: 1386–1394.

Schlösser T, Wiesenburg A, Gätgens C, Funke A, Viets U, Vijayalakshmi S, Nieland S, Stahmann K-P. 2007. Growth stress triggers riboflavin overproduction in Ashbya gossypii. Appl Microbiol Biotechnol 76: 569–578.

Sheik CS, Beasley WH, Elshahed MS, Zhou X, Luo Y, Krumholz LR. 2011. Effect of warming and drought on grassland microbial communities. ISME J 5: 1692–1700.

Singh BK, Shaner DL. 1995. Biosynthesis of branched chain amino acids: from test tube to field. Plant Cell 7: 935–944.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125: 27–58.

Smyth GK. 2005. limma: Linear Models for Microarray Data. In: Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S, eds. Bioinformatics and computational biology solutions using R and Bioconductor. New York: Springer. p 397–420.

Synetos D, Dabeva MD, Warner JR. 1992. The yeast ribosomal protein S7 and its genes. J Biol Chem 267: 3008–3013.

Taheri P, Tarighi S. 2010. Riboflavin induces resistance in rice against Rhizoctonia solani via jasmonate-mediated priming of phenylpropanoid pathway. J Plant Physiol 167: 201–208.

Tarwadi KV, Agte VV. 2011. Effect of micronutrients on methylglyoxal-mediated in vitro glycation of albumin. Biol Trace Elem Res 143: 717–725.

The UniProt Consortium. 2015. UniProt: a hub for protein information. Nucleic Acids Res 43: D204-12.

Thornalley PJ. 2003. Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation. Biochm Soc Trans 31: 1343–1348.

Ventii KH, Wilkinson KD. 2008. Protein partners of deubiquitinating enzymes. Biochem J 414: 161–175.

Warner JR. 1999. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24. 437–440.

Xue S, Barna M. 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. Nat Rev Mol Cell Biol 13: 355–369.

Yang S, Vanderbeld B, Wan J, Huang Y. 2010. Narrowing Down the Targets: Towards Successful Genetic Engineering of Drought-Tolerant Crops. Mol Plant 3: 469–490.

Yuste J, Peñuelas J, Estijarte M, Garcia-Mas J, Mattana S, Ogaya R, Pujol M, Sardans J. 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob Chang Biol 17: 1475–1486.

Zhang Y. 2003. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev 17: 2733–2740.

4.8 Supporting Information

Tab. S4.1: Sample layout used for two-colour hybridization of the 8x15k microarray.

Subarray	Cy3 channel	Cy5 channel
1-1	DC (t_1)	WC (t_1)
1-2	WC (t_1)	DC (t_1)
1-3	DC (t_2)	WC (t_2)
1-4	WC (t_2)	DC (t_2)
2-1	DR (t_1)	WR (t_1)
2-2	WR (t_1)	DR (t_1)
2-3	DR (t_2)	WR (t_2)
2-4	WR (t_2)	DR (t_2)

Tab. S4.2: Change in water potential [MPa] and respiration rates [mg CO_2 kg^{-1} h^{-1}] over time in days after stop of irrigation. DR= 50 µM riboflavin addition prior to drought stress; DC= no riboflavin addition prior to drought stress; WR= 50 µM riboflavin addition, no drought stress; WC= no riboflavin addition, no drought stress. Asterisks indicate sampling dates for microarray/HPLC measurements Mean ± SEM, n =4.

	WR		WC		DR		DC	
Day	**Water potential [MPa]**	**Respiration [mg CO_2-C kg^{-1} h^{-1}]**	**Water potential [MPa]**	**Respiration [mg CO_2-C kg^{-1} h^{-1}]**	**Water potential [MPa]**	**Respiration [mg CO_2-C kg^{-1} h^{-1}]**	**Water potential [MPa]**	**Respiration [mg CO_2-C kg^{-1} h^{-1}]**
1	-12.46 ± 0.77	155.4 ± 18.18	-14.48 ± 0.65	146.81 ± 17.37	-14.78 ± 0.8	162.91 ± 17.49	-14.10 ± 0.88	191.93 ± 22.61
2	-18.17 ± 0.73	173.65 ± 18.92	-18.43 ± 0.79	149.09 ± 16.25	-18.92 ± 0.67	138.7 ± 14.05	-18.01 ± 0.73	170.38 ± 17.5
3	-12.36 ± 0.82	190.14 ± 14.47	-13.09 ± 0.73	194.85 ± 20.83	-14.28 ± 0.76	151.64 ± 9.87	-12.76 ± 0.67	165.89 ± 11.16
4	-10.62 ± 0.49	206.28 ± 9.28	-11.01 ± 0.49	216.45 ± 27.31	-13.49 ± 0.43	168.41 ± 9.46	-12.38 ± 0.47	179.89 ± 17.97
5	-13.75 ± 0.71	163.31 ± 14.79	-15.69 ± 0.70	138.88 ± 16.02	-16.49 ± 0.49	134.28 ± 15.03	-16.16 ± 0.74	109.75 ± 14.58
6	-14.41 ± 0.72	139.44 ± 12.34	-14.88 ± 0.69	197.93 ± 39.89	-18.44 ± 0.59	103.44 ± 8.67	-17.57 ± 0.69	110.39 ± 11.1
7	-10.72 ± 0.54	122.69 ± 18.25	-16.03 ± 0.87	133.7 ± 27.55	-25.75 ± 0.7	122.14 ± 27.38	-25.82 ± 0.74	48.58 ± 10.79
8	-15.33 ± 1.08	176.17 ± 20.17	-15.66 ± 1.07	191.97 ± 30.53	-41.1 ± 5.18	106.82 ± 19.48	-25.21 ± 1.48	44.43 ± 7.67
9	-10.77 ± 0.54	181.84 ± 5.46	-11.64 ± 0.58	170.16 ± 7.22	-45.18 ± 4.94	84.81 ± 22.00	-25.01 ± 1.72	28.28 ± 3.35
11	-16.22 ± 0.89	164.17 ± 15.1	-16.14 ± 0.85	152.51 ± 19.46	-43.14 ± 4.75	73.53 ± 8.05	-41.41 ± 4.18	7.1 ± 0.75
12	-12.52 ± 0.55	167.36 ± 8.66	-17.84 ± 1.00	162.48 ± 32.76	-48.65 ± 5.48	59.66 ± 19.44	-47.64 ± 4.72	7.03 ± 0.80
13	-12.52 ± 0.55	202.21 ± 27.07	-11.66 ± 0.46	158.76 ± 5.26	-48.17 ± 5.19	20.78 ± 2.66	-61.50 ± 5.43	7.8 ± 1.4
14	-19.25 ± 0.37	172.49 ± 23.28	-20.31 ± 1.10	216.34 ± 41.85	-49.77 ± 5.00	24.18 ± 6.91	-64.76 ± 5.43	8.19 ± 2.62

Indirect method used to measure water potentials in this experiment (digital humidity and temperature sensor), which was chosen to avoid disturbance of mesocosms, does not provide accurate values below -10 MPa. Values for wet treatments were subsequently redetermined under equal conditions using the chilled mirror dewpoint method (WP4-T, Decagon Devices Inc., Pullman WA, U.S.A.) and showed stable values over 14 days in WR (-0.21 ± 0.15 MPa) and WC (-0.21 ± 0.15 MPa).

Tab. S4.3: Complete list of Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for GO terms up to P-value 0.05; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DC= no riboflavin addition prior to drought stress; WC= no drought stress, no riboflavin addition.

GO-ID	Term	Category	P-Value	Genes
Enriched-GO terms found within induced genes between DC and WC on day 3				
GO:0043228	non-membrane-bounded organelle	C	<0.001	5
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	5
GO:0005840	ribosome	C	<0.001	4
GO:0003735	structural constituent of ribosome	F	<0.001	4
GO:1990904	ribonucleoprotein complex	C	<0.001	4
GO:0030529	intracellular ribonucleoprotein complex	C	<0.001	4
GO:0005198	structural molecule activity	F	<0.001	4
GO:0006412	translation	P	<0.001	4
GO:0043043	peptide biosynthetic process	P	<0.001	4
GO:0006518	peptide metabolic process	P	<0.001	4
GO:0043604	amide biosynthetic process	P	0.001	4
GO:0043603	cellular amide metabolic process	P	0.001	4
GO:0032991	macromolecular complex	C	0.001	5
GO:0044444	cytoplasmic part	C	0.002	4
GO:0010467	gene expression	P	0.002	5
GO:0044267	cellular protein metabolic process	P	0.003	5
GO:1901566	organonitrogen compound biosynthetic process	P	0.004	4
GO:0016443	bidentate ribonuclease III activity	F	0.006	1
GO:0043229	intracellular organelle	C	0.006	5
GO:0043226	organelle	C	0.006	5
GO:0005737	cytoplasm	C	0.007	4
GO:0044260	cellular macromolecule metabolic process	P	0.008	6
GO:1901564	organonitrogen compound metabolic process	P	0.010	4
GO:0019538	protein metabolic process	P	0.012	5
GO:0034645	cellular macromolecule biosynthetic process	P	0.014	4
	macromolecule biosynthetic process	P	0.015	4

GO-ID	Term	Category	P-Value	Genes
GO:0004525	ribonuclease III activity	F	0.018	1
GO:0032296	double-stranded RNA-specific ribonuclease activity	F	0.018	1
GO:0044271	cellular nitrogen compound biosynthetic process	P	0.019	4
GO:0070035	purine NTP-dependent helicase activity	F	0.019	2
GO:0008026	ATP-dependent helicase activity	F	0.019	2
GO:0015935	small ribosomal subunit	C	0.020	1
GO:0006414	translational elongation	P	0.022	1
GO:0004386	helicase activity	F	0.022	2
GO:0043170	macromolecule metabolic process	P	0.022	6
GO:0044424	intracellular part	C	0.025	5
GO:0007018	microtubule-based movement	P	0.027	1
GO:0006928	movement of cell or subcellular component	P	0.029	1
GO:0006807	nitrogen compound metabolic process	P	0.030	5
GO:0009987	cellular process	P	0.032	7
GO:0003777	microtubule motor activity	F	0.035	1
GO:0044391	ribosomal subunit	C	0.035	1
GO:0005875	microtubule associated complex	C	0.037	1
GO:0044237	cellular metabolic process	P	0.042	6
GO:0004221	obsolete ubiquitin thiolesterase activity	F	0.043	1
GO:0044249	cellular biosynthetic process	P	0.044	4
GO:0007017	microtubule-based process	P	0.045	1
GO:0042254	ribosome biogenesis	P	0.047	1
GO:0022613	ribonucleoprotein complex biogenesis	P	0.047	1
GO:1901576	organic substance biosynthetic process	P	0.047	4
GO:0003774	motor activity	F	0.048	1
GO:0042623	ATPase activity, coupled	F	0.049	2
Enriched-GO terms found within repressed genes between DC and WC on day 3				
GO:0008270	zinc ion binding	F	<0.001	11
GO:0046914	transition metal ion binding	F	<0.001	11
GO:0003676	nucleic acid binding	F	<0.001	12

GO-ID	Term	Category	P-Value	Genes
GO:0046872	metal ion binding	F	<0.001	11
GO:0043169	cation binding	F	<0.001	11
GO:0005760	gamma DNA polymerase complex	C	0.005	1
GO:0004502	kynurenine 3-monooxygenase activity	F	0.005	1
GO:0042575	DNA polymerase complex	C	0.005	1
GO:0043167	ion binding	F	0.009	13
GO:0051082	unfolded protein binding	F	0.012	2
GO:0004092	carnitine O-acetyltransferase activity	F	0.014	1
GO:0045735	nutrient reservoir activity	F	0.014	1
GO:1901363	heterocyclic compound binding	F	0.021	13
GO:0097159	organic cyclic compound binding	F	0.021	13
GO:0004629	phospholipase C activity	F	0.023	1
GO:0016272	prefoldin complex	C	0.027	1
GO:0006457	protein folding	P	0.028	2
GO:0005488	binding	F	0.032	17
GO:0003690	double-stranded DNA binding	F	0.036	1
GO:0030983	mismatched DNA binding	F	0.036	1
GO:0006298	mismatch repair	P	0.036	1
GO:0005759	mitochondrial matrix	C	0.045	1
GO:0004197	cysteine-type endopeptidase activity	F	0.049	1
Enriched-GO terms found within induced genes between DC and WC on day 7				
GO:0005840	ribosome	C	<0.001	6
GO:0003735	structural constituent of ribosome	F	<0.001	6
GO:1990904	ribonucleoprotein complex	C	<0.001	6
GO:0030529	intracellular ribonucleoprotein complex	C	<0.001	6
GO:0005198	structural molecule activity	F	<0.001	6
GO:0006412	translation	P	<0.001	6
GO:0043043	peptide biosynthetic process	P	<0.001	6
GO:0006518	peptide metabolic process	P	<0.001	6
GO:0043604	amide biosynthetic process	P	<0.001	6

GO-ID	Term	Category	P-Value	Genes
GO:0043228	non-membrane-bounded organelle	C	<0.001	6
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	6
GO:0043603	cellular amide metabolic process	P	<0.001	6
GO:0044444	cytoplasmic part	C	<0.001	6
GO:1901566	organonitrogen compound biosynthetic process	P	<0.001	6
GO:0044267	cellular protein metabolic process	P	<0.001	7
GO:0005737	cytoplasm	C	<0.001	6
GO:0032991	macromolecular complex	C	<0.001	6
GO:1901564	organonitrogen compound metabolic process	P	<0.001	6
GO:0034645	cellular macromolecule biosynthetic process	P	0.001	6
GO:0009059	macromolecule biosynthetic process	P	0.001	6
GO:0010467	gene expression	P	0.001	6
GO:0019538	protein metabolic process	P	0.001	7
GO:0044271	cellular nitrogen compound biosynthetic process	P	0.001	6
GO:0004502	kynurenine 3-monooxygenase activity	F	0.002	1
GO:0043229	intracellular organelle	C	0.003	6
GO:0043226	organelle	C	0.003	6
GO:0044249	cellular biosynthetic process	P	0.004	6
GO:1901576	organic substance biosynthetic process	P	0.004	6
GO:0044260	cellular macromolecule metabolic process	P	0.005	7
GO:0009058	biosynthetic process	P	0.005	6
GO:0034641	cellular nitrogen compound metabolic process	P	0.009	6
GO:0044424	intracellular part	C	0.014	6
GO:0043170	macromolecule metabolic process	P	0.016	7
GO:0015645	fatty acid ligase activity	F	0.017	1
GO:0004467	long-chain fatty acid-CoA ligase activity	F	0.017	1
GO:0031956	medium-chain fatty acid-CoA ligase activity	F	0.017	1
GO:0031957	very long-chain fatty acid-CoA ligase activity	F	0.017	1
GO:0031955	short-chain fatty acid-CoA ligase activity	F	0.017	1
GO:0006807	nitrogen compound metabolic process	P	0.017	6

GO-ID	Term	Category	P-Value	Genes
GO:0015935	small ribosomal subunit	C	0.024	1
GO:0006414	translational elongation	P	0.026	1
GO:0044237	cellular metabolic process	P	0.034	7
GO:0044391	ribosomal subunit	C	0.042	1
GO:0005622	intracellular	C	0.046	6
Enriched-GO terms found within repressed genes between DC and WC on day on day 7				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	12
GO:0046914	transition metal ion binding	F	<0.001	10
GO:0046872	metal ion binding	F	<0.001	10
GO:0043169	cation binding	F	<0.001	10
GO:1901363	heterocyclic compound binding	F	<0.001	12
GO:0097159	organic cyclic compound binding	F	<0.001	12
GO:0044815	DNA packaging complex	C	0.001	2
GO:0000786	nucleosome	C	0.001	2
GO:0032993	protein-DNA complex	C	0.001	2
GO:0065004	protein-DNA complex assembly	P	0.001	2
GO:0034728	nucleosome organization	P	0.001	2
GO:0031497	chromatin assembly	P	0.001	2
GO:0006334	nucleosome assembly	P	0.001	2
GO:0071824	protein-DNA complex subunit organization	P	0.001	2
GO:0006323	DNA packaging	P	0.002	2
GO:0071103	DNA conformation change	P	0.003	2
GO:0005760	gamma DNA polymerase complex	C	0.003	1
GO:0042575	DNA polymerase complex	C	0.003	1
GO:0006333	chromatin assembly or disassembly	P	0.003	2
GO:0000785	chromatin	C	0.004	2
GO:0043167	ion binding	F	0.005	10
GO:0034622	cellular macromolecular complex assembly	P	0.006	2
GO:0005488	binding	F	0.007	13

GO-ID	Term	Category	P-Value	Genes
GO:0044427	chromosomal part	C	0.007	2
GO:0006325	chromatin organization	P	0.007	2
GO:0004092	carnitine O-acetyltransferase activity	F	0.009	1
GO:0051276	chromosome organization	P	0.012	2
GO:0005694	chromosome	C	0.014	2
GO:0006461	protein complex assembly	P	0.014	2
GO:0070271	protein complex biogenesis	P	0.014	2
GO:0065003	macromolecular complex assembly	P	0.015	2
GO:0071822	protein complex subunit organization	P	0.016	2
GO:0022607	cellular component assembly	P	0.017	2
GO:0044085	cellular component biogenesis	P	0.028	2
GO:0005759	mitochondrial matrix	C	0.029	1
GO:0044446	intracellular organelle part	C	0.030	3
GO:0044422	organelle part	C	0.030	3
GO:0043933	macromolecular complex subunit organization	P	0.031	2
GO:0006996	organelle organization	P	0.034	2
GO:0098798	mitochondrial protein complex	C	0.035	1
GO:0016406	carnitine O-acyltransferase activity	F	0.041	1
GO:0016413	O-acetyltransferase activity	F	0.049	1

Tab. S4.4: Complete list of Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for GO terms up to P-value 0.05; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. WR= no drought stress, 50 µM riboflavin addition; WC= no drought stress, no riboflavin addition.

GO-ID	Term	Category	P-Value	Genes
Enriched-GO terms found within induced genes between WR and WC on day 3				
GO:0043228	non-membrane-bounded organelle	C	<0.001	4
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	4
GO:0006091	generation of precursor metabolites and energy	P	0.002	2
GO:0046034	ATP metabolic process	P	0.002	2
GO:0009205	purine ribonucleoside triphosphate metabolic process	P	0.002	2
GO:0009144	purine nucleoside triphosphate metabolic process	P	0.002	2
GO:0009199	ribonucleoside triphosphate metabolic process	P	0.002	2
GO:0009141	nucleoside triphosphate metabolic process	P	0.003	2
GO:0042278	purine nucleoside metabolic process	P	0.003	2
GO:0046128	purine ribonucleoside metabolic process	P	0.003	2
GO:0004332	fructose-bisphosphate aldolase activity	F	0.004	1
GO:0009119	ribonucleoside metabolic process	P	0.004	2
GO:0009259	ribonucleotide metabolic process	P	0.004	2
GO:0009126	purine nucleoside monophosphate metabolic process	P	0.004	2
GO:0009150	purine ribonucleotide metabolic process	P	0.004	2
GO:0009167	purine ribonucleoside monophosphate metabolic process	P	0.004	2
GO:0006163	purine nucleotide metabolic process	P	0.005	2
GO:1901657	glycosyl compound metabolic process	P	0.005	2
GO:0009116	nucleoside metabolic process	P	0.005	2
GO:0009161	ribonucleoside monophosphate metabolic process	P	0.005	2
GO:0009123	nucleoside monophosphate metabolic process	P	0.005	2
GO:0072521	purine-containing compound metabolic process	P	0.005	2
GO:0019693	ribose phosphate metabolic process	P	0.005	2
GO:1901564	organonitrogen compound metabolic process	P	0.006	4
GO:0044446	intracellular organelle part	C	0.007	3
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P	0.007	1

GO-ID	Term	Category	P-Value	Genes
GO:0042775	mitochondrial ATP synthesis coupled electron transport	P	0.009	1
GO:0044085	cellular component biogenesis	P	0.010	2
GO:0016832	aldehyde-lyase activity	F	0.011	1
GO:0042773	ATP synthesis coupled electron transport	P	0.011	1
GO:0022904	respiratory electron transport chain	P	0.011	1
GO:0022900	electron transport chain	P	0.011	1
GO:0006119	oxidative phosphorylation	P	0.011	1
GO:0006753	nucleoside phosphate metabolic process	P	0.011	2
GO:0055086	nucleobase-containing small molecule metabolic process	P	0.014	2
GO:0009987	cellular process	P	0.014	7
GO:0005840	ribosome	C	0.016	2
GO:0003735	structural constituent of ribosome	F	0.016	2
GO:0008137	NADH dehydrogenase (ubiquinone) activity	F	0.018	1
GO:0015935	small ribosomal subunit	C	0.018	1
GO:0050136	NADH dehydrogenase (quinone) activity	F	0.018	1
GO:1901575	organic substance catabolic process	P	0.018	2
GO:1901135	carbohydrate derivative metabolic process	P	0.019	2
GO:0006414	translational elongation	P	0.019	1
GO:0009056	catabolic process	P	0.020	2
GO:0019637	organophosphate metabolic process	P	0.021	2
GO:0003954	NADH dehydrogenase activity	F	0.023	1
GO:0043229	intracellular organelle	C	0.023	4
GO:0007018	microtubule-based movement	P	0.025	1
GO:0006757	ATP generation from ADP	P	0.026	1
GO:0006165	nucleoside diphosphate phosphorylation	P	0.026	1
GO:0046031	ADP metabolic process	P	0.026	1
GO:0046939	nucleotide phosphorylation	P	0.026	1
GO:0006096	glycolytic process	P	0.026	1
GO:0009135	purine nucleoside diphosphate metabolic process	P	0.026	1
GO:0006928	movement of cell or subcellular component	P	0.026	1

GO-ID	Term	Category	P-Value	Genes
GO:0009185	ribonucleoside diphosphate metabolic process	P	0.026	1
GO:1990904	ribonucleoprotein complex	C	0.027	2
GO:0030529	intracellular ribonucleoprotein complex	C	0.027	2
GO:0005198	structural molecule activity	F	0.028	2
GO:0006090	pyruvate metabolic process	P	0.028	1
GO:0045333	cellular respiration	P	0.030	1
GO:0044724	single-organism carbohydrate catabolic process	P	0.030	1
GO:0044815	DNA packaging complex	C	0.032	1
GO:0003777	microtubule motor activity	F	0.032	1
GO:0000786	nucleosome	C	0.032	1
GO:0009132	nucleoside diphosphate metabolic process	P	0.032	1
GO:0044391	ribosomal subunit	C	0.032	1
GO:0032993	protein-DNA complex	C	0.033	1
GO:0065004	protein-DNA complex assembly	P	0.033	1
GO:0034728	nucleosome organization	P	0.033	1
GO:0016655	oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	F	0.033	1
GO:0031497	chromatin assembly	P	0.033	1
GO:0005875	microtubule associated complex	C	0.033	1
GO:0006334	nucleosome assembly	P	0.033	1
GO:0071824	protein-DNA complex subunit organization	P	0.033	1
GO:0006323	DNA packaging	P	0.035	1
GO:0071840	cellular component organization or biogenesis	P	0.037	2
GO:0016052	carbohydrate catabolic process	P	0.039	1
GO:0004221	obsolete ubiquitin thiolesterase activity	F	0.039	1
GO:0007017	microtubule-based process	P	0.040	1
GO:0055114	oxidation-reduction process	P	0.040	1
GO:0042254	ribosome biogenesis	P	0.042	1
GO:0022613	ribonucleoprotein complex biogenesis	P	0.042	1
GO:0046496	nicotinamide nucleotide metabolic process	P	0.044	1
GO:0003774	motor activity	F	0.044	1

GO-ID	Term	Category	P-Value	Genes
GO:0019362	pyridine nucleotide metabolic process	P	0.045	1
GO:0006412	translation	P	0.048	2
GO:0043043	peptide biosynthetic process	P	0.049	2
Enriched-GO terms found within repressed genes between WR and WC on day 3				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0046914	transition metal ion binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	11
GO:0046872	metal ion binding	F	<0.001	10
GO:0043169	cation binding	F	<0.001	10
GO:0005760	gamma DNA polymerase complex	C	0.004	1
GO:0042575	DNA polymerase complex	C	0.004	1
GO:0051082	unfolded protein binding	F	0.008	2
GO:1901363	heterocyclic compound binding	F	0.008	12
GO:0004092	carnitine O-acetyltransferase activity	F	0.011	1
GO:0043167	ion binding	F	0.013	11
GO:0005488	binding	F	0.017	15
GO:0004629	phospholipase C activity	F	0.019	1
GO:0006457	protein folding	P	0.020	2
GO:0016272	prefoldin complex	C	0.022	1
GO:0005759	mitochondrial matrix	C	0.037	1
GO:0098798	mitochondrial protein complex	C	0.044	1
Enriched-GO terms found within induced genes between WR and WC on day 7				
GO:0043228	non-membrane-bounded organelle	C	<0.001	4
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	4
GO:0005840	ribosome	C	<0.001	3
GO:0003735	structural constituent of ribosome	F	<0.001	3
GO:1990904	ribonucleoprotein complex	C	<0.001	3
GO:0030529	intracellular ribonucleoprotein complex	C	<0.001	3
GO:0005198	structural molecule activity	F	0.000	3
GO:0032991	macromolecular complex	C	0.001	4

GO-ID	Term	Category	P-Value	Genes
GO:0043043	peptide biosynthetic process	P	0.001	3
GO:0006518	peptide metabolic process	P	0.001	3
GO:0043604	amide biosynthetic process	P	0.001	3
GO:0043603	cellular amide metabolic process	P	0.002	3
GO:0044444	cytoplasmic part	C	0.003	3
GO:0043229	intracellular organelle	C	0.004	4
GO:0043226	organelle	C	0.004	4
GO:0044085	cellular component biogenesis	P	0.004	2
GO:1901566	organonitrogen compound biosynthetic process	P	0.006	3
GO:0015935	small ribosomal subunit	C	0.012	1
GO:1901564	organonitrogen compound metabolic process	P	0.013	3
GO:0006414	translational elongation	P	0.013	1
GO:0044424	intracellular part	C	0.014	4
GO:0071840	cellular component organization or biogenesis	P	0.017	2
GO:0034645	cellular macromolecule biosynthetic process	P	0.017	3
GO:0009059	macromolecule biosynthetic process	P	0.018	3
GO:0010467	gene expression	P	0.020	3
GO:0044815	DNA packaging complex	C	0.021	1
GO:0000786	nucleosome	C	0.021	1
GO:0044391	ribosomal subunit	C	0.021	1
GO:0044267	cellular protein metabolic process	P	0.021	3
GO:0044271	cellular nitrogen compound biosynthetic process	P	0.021	3
GO:0032993	protein-DNA complex	C	0.022	1
GO:0065004	protein-DNA complex assembly	P	0.022	1
GO:0034728	nucleosome organization	P	0.022	1
GO:0031497	chromatin assembly	P	0.022	1
GO:0006334	nucleosome assembly	P	0.022	1
GO:0071824	protein-DNA complex subunit organization	P	0.022	1
GO:0006323	DNA packaging	P	0.024	1
GO:0042254	ribosome biogenesis	P	0.028	1

GO-ID	Term	Category	P-Value	Genes
GO:0044446	intracellular organelle part	C	0.029	2
GO:0044422	organelle part	C	0.029	2
GO:0071103	DNA conformation change	P	0.030	1
GO:0005622	intracellular	C	0.033	4
GO:0006333	chromatin assembly or disassembly	P	0.035	1
GO:0005623	cell	C	0.037	4
GO:0000785	chromatin	C	0.039	1
GO:0044249	cellular biosynthetic process	P	0.043	3
GO:1901576	organic substance biosynthetic process	P	0.045	3
GO:0034622	cellular macromolecular complex assembly	P	0.048	1
GO:0044427	chromosomal part	C	0.050	1
Enriched-GO terms found within repressed genes between WR and WC on day 7				
GO:0008270	zinc ion binding	F	<0.001	10
GO:0046914	transition metal ion binding	F	<0.001	10
GO:0046872	metal ion binding	F	<0.001	10
GO:0043169	cation binding	F	<0.001	10
GO:0003676	nucleic acid binding	F	<0.001	9
GO:0043167	ion binding	F	0.009	10
GO:0004092	carnitine O-acetyltransferase activity	F	0.009	1
GO:0016747	transferase activity, transferring acyl groups other than amino-acyl groups	F	0.043	2
GO:0016406	carnitine O-acyltransferase activity	F	0.043	1

Tab. S4.5: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between DC (no riboflavin addition prior to drought stress) and WC (no drought stress, no riboflavin addition) mesocosms with information about biological function, if available.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the DC/WC microarray experiments							
Ribosomal protein S7	136259	Translation & Transcription	Regulation of development & translation	3.70	<0.001	3.33	<0.001
Ribosomal protein L10	191238	Translation & Transcription	Regulation of development & translation	2.48	<0.001	1.28	<0.001
Histone acetyltransferase	182197	Translation & Transcription	acetylation of conserved lysine amino acids on histone proteins	1.96	0.020	0.40	0.070
Ribosomal protein L7A/S12e	195860	Translation & Transcription	Regulation of development & translation	1.61	<0.001	1.74	<0.001
Subunit 21 of mediator complex protein	193554	Translation & Transcription	coactivator of the mediator complex, involved transcription regulation of nearly all RNA polymerase II-dependent genes	1.74	0.000	1.63	0.009
Nuclear cap-binding protein subunit 3	177555	Translation & Transcription	binding and processing of mRNA	1.46	0.031	-0.06	0.542
Ribosomal protein L14b/L23e	195355	Translation & Transcription	Regulation of development & translation	1.11	<0.001	1.40	<0.001
Ribosomal protein S11	195415	Translation & Transcription	Regulation of development & translation	0.26	0.049	1.21	0.010
Ribonuclease III	209093	Translation & Transcription	binds and cleaves double-stranded RNA	1.17	0.030	0.13	0.240
Ribosomal Protein L40e	133168	Translation & Transcription	Regulation of development & translation	-0.18	0.170	1.13	<0.001
Ribosomal protein L18ae	194625	Translation & Transcription	Regulation of development & translation	0.49	0.004	1.10	<0.001
Golgi pH regulator	188048	regulation of cell processes	required for acidification and functions of the Golgi apparatus	1.81	0.037	0.11	0.446
Ubiquitin carboxyl-terminal hydrolase	195095	regulation of cell processes	deubiquitinating enzyme, reversing ubiquitination process	1.26	0.030	0.12	0.200

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
putative CDC20	195164	Growth	Anaphase promoting complex	2.32	<0.001	0.03	0.720
MFS-1	211434	Transporter	membrane transporter	1.03	<0.001	1.89	<0.001
MFS general substrate transporter	114522	Transporter	transporter/transmembrane protein	0.17	0.148	1.89	0.002
Cytochrome C	135048	Protection	oxidative protection	1.14	<0.001	1.50	<0.001
Long-chain-fatty-acid--CoA ligase	186655	Protection	protection against high LCFA exposure	0.07	0.560	1.01	0.020
Kynurenine 3-monooxygenase	64309	Metabolic	takes part in degradation of tryptophan	-1.36	0.090	1.31	0.040
Uncharacterized protein	139182	unknown	unknown	2.10	0.013	0.15	0.135
Uncharacterized protein	136293	unknown	unknown	0.10	0.384	2.07	0.007
Uncharacterized protein	191519	unknown	unknown	1.72	0.011	0.13	0.192
Uncharacterized protein	191142	unknown	unknown	1.68	0.030	-0.07	0.519
Putative transmembrane protein	139015	unknown	unknown	1.66	0.016	0.05	0.600
Putative transmembrane protein	194530	unknown	unknown	1.42	0.025	-0.07	0.500
Uncharacterized protein	194064	unknown	unknown	0.20	0.226	1.26	<0.001
Uncharacterized protein	75571	unknown	unknown	1.25	<0.001	-0.59	<0.001
Uncharacterized protein	77212	unknown	unknown	1.16	<0.001	-0.54	<0.001
BTB/POZ domain protein	187819	unknown	unknown	1.14	<0.001	1.23	0.010
N-terminal C2 domain protein	121081	unknown	unknown	1.11	0.021	0.28	0.049
F-box-like domain protein	123174	unknown	unknown	1.06	0.024	-0.05	0.560
Uncharacterized protein	180930	unknown	unknown	1.05	0.041	-0.10	0.402
Repressed genes of the DC/WC microarray experiments							
Ubiquitin	192160	regulation of cell processes	post-translational modifications, interacts also with histones	-2.04	<0.001	-2.16	<0.001
Mitochondrial DNA polymerase gamma 1	114559	regulation of cell processes	Involved in the replication of mitochondrial DNA	-1.89	<0.001	-1.19	<0.001

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Serine/threonine-specific protein kinase	188752	regulation of cell processes	unknown	-1.27	<0.001	-0.63	<0.001
Histones H3	139997	regulation of cell processes	DNA binding	0.21	0.100	-1.23	<0.001
Prefoldin, subunit 2	139452	regulation of cell processes	takes part in protein folding	-1.19	<0.001	-0.56	<0.001
Ubiquitin-conjugating enzyme	190501	regulation of cell processes	regulation of Ubiquitinylation	-1.18	<0.001	-0.72	<0.001
Thymocyte nuclear protein 1	122567	regulation of cell processes	may be involved in the induction of apoptosis	-1.17	0.001	-0.49	<0.001
DNA mismatch repair protein - MLH2/PMS1/Pms2 family	122323	regulation of cell processes	repair of signal base mispairs in DNA replication, non-essential accessory factor	-1.03	<0.001	-0.58	<0.001
Gc-rich sequence dna-binding factor-like protein	179259	Translation & Transcription	Transcriptional factor binding to the GC-rich sequences	-1.18	0.002	-0.81	<0.001
Kynurenine 3-monooxygenase	64309	Metabolic	takes part in degradation of tryptophan	-1.36	0.090	1.31	0.040
Carnitine O-acyltransferase	200564	Transporter	fatty acid transport into mitochondria for β-oxidation	-1.85	<0.001	-1.52	<0.001
Uncharacterized protein	121746	unknown	unknown	-3.30	0.002	-3.63	<0.001
Protein with zinc finger domain	113897	unknown	unknown	0.65	0.010	-1.16	<0.001
Protein with CCHC-type zinc finger domain	114663	unknown	unknown	-2.20	0.003	-2.66	<0.001
Protein with CCHC-type zinc finger domain	114687	unknown	unknown	-2.30	0.003	-2.65	<0.001
Protein with CCHC-type zinc finger domain	115378	unknown	unknown	-2.06	0.003	-2.49	<0.001
Protein with CCHC-type zinc finger domain	116162	unknown	unknown	-2.41	0.004	-2.83	<0.001
Protein with CCHC-type zinc finger domain	120132	unknown	unknown	-2.72	0.005	-3.11	<0.001

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Uncharacterized protein	194860	unknown	unknown	-2.77	0.006	-3.37	<0.001
Uncharacterized protein	146400	unknown	unknown	-2.41	0.004	-2.88	<0.001
Protein kinase	123418	unknown	unknown	-2.24	0.003	-2.77	<0.001
Uncharacterized protein	120331	unknown	unknown	-1.64	0.008	-1.99	<0.001
Leucine-rich repeat domain protein	117621	unknown	unknown	-1.83	0.005	-1.93	<0.001
Uncharacterized protein	206268	unknown	unknown	-1.65	0.001	-0.69	<0.001
Uncharacterized protein	121696	unknown	unknown	-1.62	0.009	-0.93	<0.001
Protein with DnaJ-domain	190689	unknown	unknown	-1.44	<0.001	-0.37	0.020
Uncharacterized protein	117512	unknown	unknown	0.46	0.083	-1.43	0.013
Ring finger domain protein	197138	unknown	unknown	-1.34	0.004	-0.54	0.001
Putative transmembrane protein	205303	unknown	unknown	-1.27	0.083	0.35	0.015
Uncharacterized protein	136268	unknown	unknown	-1.23	0.000	-0.01	0.887
PLC-like phosphodiesterase	122567	unknown	unknown	-1.17	<0.001	-0.49	<0.001
Protein with phosphatidylinositol-specific region	200290	unknown	unknown	-1.16	<0.001	-0.14	0.140
Protein with Cupin-like domain	191066	unknown	unknown	-1.09	0.010	-0.65	<0.001
FYVE-type domain protein	176255	unknown	unknown	-1.05	0.002	-0.43	0.001
DUF3245 family protein	117282	unknown	unknown	-1.05	0.001	-0.26	0.019
Uncharacterized protein	209983	unknown	unknown	-1.01	<0.001	0.20	0.148

Tab. S4.6: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between WR (no drought stress, 50 µM riboflavin addition) and WC (no drought stress, no riboflavin addition) mesocosms with information about biological function, if available.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the WR/WC microarray experiments							
Kinesin-like protein	229385	Growth	establishment of spindle bipolarity, cellular organization in late mitosis	1.36	0.065	0.02	0.980
NADH ubiquinone oxidoreductase	192620	Metabolism	catalyses electron transfer from NADH to coenzyme Q10	2.08	0.015	0.04	0.960
Cytochrome C	135048	Protection	oxidative protection	1.34	0.097	-0.19	0.800
Histones H3	139997	regulation of cell processes	DNA binding	1.13	0.005	1.36	<0.001
Ubiquitin carboxyl-terminal hydrolase	195095	regulation of cell processes	deubiquitinating enzyme, reversing ubiquitination process	1.21	0.092	-0.14	0.840
Ribosomal protein S7	136259	Translation & Transcription	Regulation of development & translation	5.33	0.050	4.21	0.090
Nuclear cap-binding protein subunit 3	177555	Translation & Transcription	binding and processing of mRNA	1.45	0.092	-0.21	0.790
Histone acetyltransferase	182197	Translation & Transcription	Acetylation of conserved lysine amino acids on histone proteins	1.78	0.105	-0.08	0.940
Ribosomal protein L10	191238	Translation & Transcription	Regulation of development & translation	4.95	0.014	3.81	0.080
Subunit 21 of mediator complex protein	193554	Translation & Transcription	coactivator of the mediator complex, involved transcription regulation of nearly all RNA polymerase II-dependent genes	0.02	0.895	2.16	0.080
Ribosomal protein S11	195415	Translation & Transcription	Regulation of development & translation	0.05	0.778	1.75	0.020
MFS general substrate transporter	114522	Transporter	transporter/transmembrane protein	-0.63	0.422	1.61	0.060

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
N-terminal C2 domain protein	121081	unknown	unknown	1.15	0.065	0.37	0.510
Uncharacterized protein	136293	unknown	unknown	0.07	0.948	2.04	0.070
Putative transmembrane protein	139015	unknown	unknown	1.68	0.058	0.24	0.770
Uncharacterized protein	139182	unknown	unknown	1.99	0.063	0.00	1.000
Uncharacterized protein	180930	unknown	unknown	1.05	0.060	0.00	1.000
Uncharacterized protein	190938	unknown	unknown	1.59	0.024	0.24	0.600
Uncharacterized protein	191142	unknown	unknown	1.58	0.098	0.09	0.910
Uncharacterized protein	191519	unknown	unknown	1.77	0.043	0.18	0.820
Putative transmembrane protein	194530	unknown	unknown	1.27	0.096	0.04	0.960
Protein with MYND-type zinc finger domain	211603	unknown	unknown	-0.05	0.908	1.01	0.050
Repressed genes of the WR/WC microarray experiments							
Alpha-tubulin folding cofactor E	63759	Growth	Involved in folding of alpha-tubulin	-1.14	0.326	-2.10	0.090
Glycoside hydrolase	153780	Metabolism	hydrolysis of glycosidic bonds in complex sugars, including degradation of biomass such as cellulose	-0.08	0.872	-1.60	0.010
Mitochondrial DNA polymerase gamma 1	114559	regulation of cell processes	Involved in the replication of mitochondrial DNA	-1.93	0.014	-1.08	0.120
Ubiquitin thiolesterase	146556	regulation of cell processes	performs second step in the ubiquitination reaction	-1.17	0.001	-0.84	0.010
Ubiquitin	192160	regulation of cell processes	post-translational modifications, interacts also with histones	-2.00	0.015	-1.69	0.030
Phosphatidylglycerophosphatase GEP4, putitative	205009	regulation of cell processes	involved in the biosynthesis of cardiolipin and cellular energy metabolism	-0.90	0.092	-2.25	<0.001

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Prefoldin beta-like protein	139452	regulation of cell processes	takes part in protein folding	-1.08	0.019	-0.46	0.250
SAM domain containing protein	117817	Signal transduction	Signal transduction mechanisms	-0.57	0.075	-1.02	0.010
Serine/threonine-specific protein kinase	188752	regulation of cell processes	unknown	-1.13	0.011	-0.75	0.060
Gc-rich sequence dna-binding factor-like protein	179259	Translation & Transcription	Transcriptional factor binding to the GC-rich sequences, represses transcription.	-1.11	0.009	-0.82	0.040
Monocarboxylate transporter	75392	Transporter	transporter/transmembrane protein	-1.48	0.026	-0.65	0.250
Zinc/iron permease	193646	Transporter	Fe^{2+}/Zn^{2+} regulated transporter	-0.87	0.147	-1.08	0.080
Carnitine O-acyltransferase	200564	Transporter	fatty acid transport into mitochondria for β-oxidation	-1.85	0.012	-1.18	0.070
Glycoside hydrolase family 44 protein	64785	unknown	unknown	-0.61	0.286	-1.11	0.070
Uncharacterized protein	75612	unknown	unknown	-1.87	0.066	0.04	0.730
Protein with zinc finger domain	113897	unknown	unknown	-0.07	0.922	-2.35	0.010
Protein with CCHC-type zinc finger domain	114629	unknown	unknown	-2.17	0.015	-2.03	0.020
Protein with CCHC-type zinc finger domain	114663	unknown	unknown	-2.33	0.013	-1.98	0.030
Protein with CCHC-type zinc finger domain	114687	unknown	unknown	-2.31	0.015	-2.02	0.030
Protein with CCHC-type zinc finger domain	115378	unknown	unknown	-2.10	0.013	-1.78	0.030
Protein with CCHC-type zinc finger domain	116162	unknown	unknown	-2.42	0.025	-2.24	0.030
Leucine-rich repeat domain protein	117621	unknown	unknown	-1.84	0.022	-1.44	0.060

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Uncharacterized protein	120331	unknown	unknown	-2.16	0.018	-2.67	0.010
Protein with CCHC-type zinc finger domain	121409	unknown	unknown	-2.37	0.025	-2.36	0.030
Uncharacterized protein	121696	unknown	unknown	-1.60	0.041	-1.05	0.160
Uncharacterized protein	121746	unknown	unknown	-3.72	0.010	-3.05	0.040
Protein with CCHC-type zinc finger domain	123048	unknown	unknown	-2.97	0.027	-3.11	0.020
Protein with CCHC-type zinc finger domain	123230	unknown	unknown	-2.54	0.042	-2.28	0.060
Protein kinase	123418	unknown	unknown	-2.29	0.015	-2.18	0.020
Uncharacterized protein	146400	unknown	unknown	-2.53	0.016	-2.25	0.030
Uncharacterized protein	186837	unknown	unknown	-1.60	0.004	-0.22	0.610
Uncharacterized protein	190197	unknown	unknown	-1.57	0.012	-0.32	0.560
Protein with DnaJ-domain	190689	unknown	unknown	-1.07	0.026	-0.43	0.190
Uncharacterized protein	194860	unknown	unknown	-2.65	0.013	-2.67	0.010
Uncharacterized protein	195960	unknown	unknown	-1.89	0.005	-0.18	0.740
Ring finger domain protein	197138	unknown	unknown	-1.30	0.023	-0.41	0.410
Protein with phosphatidylinositol-specific region	200290	unknown	unknown	-1.27	<0.001	-0.07	0.750
Uncharacterized protein	206268	unknown	unknown	-1.60	0.009	-0.79	0.140
dehydrogenase/reductase family protein	206800	unknown	unknown	-0.42	0.231	-1.23	0.010
Uncharacterized protein	209983	unknown	unknown	-1.48	<0.001	0.21	0.450
Putative transmembrane protein	213298	unknown	unknown	-0.57	0.138	-1.19	0.010
Protein with arrestin domain	175794	unknown	unknown	-0.65	0.321	-1.32	0.060

Tab. S4.7: Complete list of Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for GO terms up to P-value 0.05; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DC= no riboflavin addition prior to drought stress; DR= 50 µM riboflavin addition prior to drought stress.

GO-ID	Term	Category	P-Value	Genes
Enriched-GO terms found within induced genes between DR and DC on day 3				
GO:0004084	branched-chain-amino-acid transaminase activity	F	0.001	1
GO:0005794	Golgi apparatus	C	0.007	1
GO:0009081	branched-chain amino acid metabolic process	P	0.008	1
GO:0008483	transaminase activity	F	0.011	1
GO:0016769	transferase activity, transferring nitrogenous groups	F	0.014	1
GO:0005783	endoplasmic reticulum	C	0.020	1
GO:0043227	membrane-bounded organelle	C	0.027	2
GO:0043231	intracellular membrane-bounded organelle	C	0.027	2
GO:0012505	endomembrane system	C	0.037	1
GO:0006886	intracellular protein transport	P	0.047	1
GO:0034613	cellular protein localization	P	0.048	1
GO:0070727	cellular macromolecule localization	P	0.048	1
GO:0043229	intracellular organelle	C	0.049	2
GO:0043226	organelle	C	0.049	2
Enriched-GO terms found within repressed genes between DR and DC on day 3				
GO:0044815	DNA packaging complex	C	<0.001	2
GO:0000786	nucleosome	C	<0.001	2
GO:0032993	protein-DNA complex	C	<0.001	2
GO:0065004	protein-DNA complex assembly	P	<0.001	2
GO:0034728	nucleosome organization	P	<0.001	2
GO:0031497	chromatin assembly	P	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0071824	protein-DNA complex subunit organization	P	<0.001	2

GO-ID	Term	Category	P-Value	Genes
GO:0006323	DNA packaging	P	<0.001	2
GO:0071103	DNA conformation change	P	<0.001	2
GO:0006333	chromatin assembly or disassembly	P	<0.001	2
GO:0000785	chromatin	C	<0.001	2
GO:0034622	cellular macromolecular complex assembly	P	0.001	2
GO:0044427	chromosomal part	C	0.001	2
GO:0006325	chromatin organization	P	0.001	2
GO:0051276	chromosome organization	P	0.001	2
GO:0005694	chromosome	C	0.001	2
GO:0006461	protein complex assembly	P	0.001	2
GO:0070271	protein complex biogenesis	P	0.001	2
GO:0065003	macromolecular complex assembly	P	0.002	2
GO:0071822	protein complex subunit organization	P	0.002	2
GO:0022607	cellular component assembly	P	0.002	2
GO:0044085	cellular component biogenesis	P	0.003	2
GO:0043933	macromolecular complex subunit organization	P	0.003	2
GO:0006996	organelle organization	P	0.004	2
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P	0.004	1
GO:0042775	mitochondrial ATP synthesis coupled electron transport	P	0.005	1
GO:0042773	ATP synthesis coupled electron transport	P	0.006	1
GO:0022904	respiratory electron transport chain	P	0.006	1
GO:0022900	electron transport chain	P	0.006	1
GO:0006119	oxidative phosphorylation	P	0.006	1
GO:0009987	cellular process	P	0.006	5
GO:0016043	cellular component organization	P	0.009	2
GO:0008137	NADH dehydrogenase (ubiquinone) activity	F	0.010	1
GO:0050136	NADH dehydrogenase (quinone) activity	F	0.010	1

GO-ID	Term	Category	P-Value	Genes
GO:0003954	NADH dehydrogenase activity	F	0.013	1
GO:0045333	cellular respiration	P	0.017	1
GO:0043228	non-membrane-bounded organelle	C	0.018	2
GO:0043232	intracellular non-membrane-bounded organelle	C	0.018	2
GO:0016655	oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	F	0.019	1
GO:0015980	energy derivation by oxidation of organic compounds	P	0.019	1
GO:0044446	intracellular organelle part	C	0.020	2
GO:0044422	organelle part	C	0.020	2
GO:0055114	oxidation-reduction process	P	0.023	1
GO:0043234	protein complex	C	0.032	2
GO:0006091	generation of precursor metabolites and energy	P	0.033	1
GO:0031072	heat shock protein binding	F	0.033	1
GO:0051082	unfolded protein binding	F	0.036	1
GO:0016651	oxidoreductase activity, acting on NAD(P)H	F	0.038	1
GO:0003677	DNA binding	F	0.039	2
GO:0046034	ATP metabolic process	P	0.040	1
GO:0009205	purine ribonucleoside triphosphate metabolic process	P	0.042	1
GO:0009144	purine nucleoside triphosphate metabolic process	P	0.042	1
GO:0009199	ribonucleoside triphosphate metabolic process	P	0.042	1
GO:0009141	nucleoside triphosphate metabolic process	P	0.044	1
GO:0042278	purine nucleoside metabolic process	P	0.049	1
GO:0046128	purine ribonucleoside metabolic process	P	0.049	1
GO:0005634	nucleus	C	0.049	2
Enriched-GO terms found within induced genes between DR and DC on day 7				
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
GO:0016846	carbon-sulfur lyase activity	F	0.008	1

GO-ID	Term	Category	P-Value	Genes
GO:0005975	carbohydrate metabolic process	P	0.010	2
GO:0009055	electron carrier activity	F	0.024	1
Enriched-GO terms found within repressed genes between DR and DC on day 7				
GO:0044815	DNA packaging complex	C	<0.001	2
GO:0000786	nucleosome	C	<0.001	2
GO:0032993	protein-DNA complex	C	<0.001	2
GO:0065004	protein-DNA complex assembly	P	<0.001	2
GO:0034728	nucleosome organization	P	<0.001	2
GO:0031497	chromatin assembly	P	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0071824	protein-DNA complex subunit organization	P	<0.001	2
GO:0006323	DNA packaging	P	<0.001	2
GO:0071103	DNA conformation change	P	<0.001	2
GO:0006333	chromatin assembly or disassembly	P	<0.001	2
GO:0000785	chromatin	C	<0.001	2
GO:0034622	cellular macromolecular complex assembly	P	<0.001	2
GO:0044427	chromosomal part	C	<0.001	2
GO:0006325	chromatin organization	P	<0.001	2
GO:0051276	chromosome organization	P	<0.001	2
GO:0005694	chromosome	C	<0.001	2
GO:0006461	protein complex assembly	P	<0.001	2
GO:0070271	protein complex biogenesis	P	<0.001	2
GO:0065003	macromolecular complex assembly	P	<0.001	2
GO:0071822	protein complex subunit organization	P	<0.001	2
GO:0022607	cellular component assembly	P	<0.001	2
GO:0044085	cellular component biogenesis	P	<0.001	2
GO:0043933	macromolecular complex subunit organization	P	<0.001	2

GO-ID	Term	Category	P-Value	Genes
GO:0006996	organelle organization	P	<0.001	2
GO:0016043	cellular component organization	P	0.001	2
GO:0071840	cellular component organization or biogenesis	P	0.001	2
GO:0043228	non-membrane-bounded organelle	C	0.002	2
GO:0043232	intracellular non-membrane-bounded organelle	C	0.002	2
GO:0044446	intracellular organelle part	C	0.002	2
GO:0044422	organelle part	C	0.002	2
GO:0043234	protein complex	C	0.004	2
GO:0003677	DNA binding	F	0.004	2
GO:0005634	nucleus	C	0.006	2
GO:0032991	macromolecular complex	C	0.008	2
GO:0043227	membrane-bounded organelle	C	0.010	2
GO:0043231	intracellular membrane-bounded organelle	C	0.010	2
GO:0043229	intracellular organelle	C	0.018	2
GO:0043226	organelle	C	0.018	2
GO:0003676	nucleic acid binding	F	0.027	2
GO:0044424	intracellular part	C	0.035	2

Tab. S4.8: Complete list of Gene Ontology (GO) term enrichment analyses. Significantly enriched-GO terms identified in differentially expressed genes (shown for GO terms up to P-value 0.05; Fisher's exact t-test) based on the full GO annotation dataset of *A. bisporus* gene products. GO category abbreviations: F (molecular function), P (biological process), C (cellular component); Genes: number of differentially transcribed genes annotated with the GO term. DR= no riboflavin addition prior to drought stress; WR= no drought stress, 50 µM riboflavin addition.

GO-ID	Term	Category	P-Value	Genes
Enriched-GO terms found within induced genes between DR and WR on day 3				
GO:0004084	branched-chain-amino-acid transaminase activity	F	0.002	1
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
GO:0016846	carbon-sulfur lyase activity	F	0.012	1
GO:0005794	Golgi apparatus	C	0.014	1
GO:0009081	branched-chain amino acid metabolic process	P	0.015	1
GO:0008483	transaminase activity	F	0.021	1
GO:0016769	transferase activity, transferring nitrogenous groups	F	0.028	1
GO:0046873	metal ion transmembrane transporter activity	F	0.029	1
GO:0030001	metal ion transport	P	0.034	1
GO:0005783	endoplasmic reticulum	C	0.040	1
Enriched-GO terms found within repressed genes between DR and WR on day 3				
GO:0006091	generation of precursor metabolites and energy	P	<0.001	3
GO:0045333	cellular respiration	P	<0.001	2
GO:0009987	cellular process	P	<0.001	8
GO:0044815	DNA packaging complex	C	<0.001	2
GO:0000786	nucleosome	C	<0.001	2
GO:0032993	protein-DNA complex	C	<0.001	2
GO:0065004	protein-DNA complex assembly	P	<0.001	2
GO:0034728	nucleosome organization	P	<0.001	2
GO:0031497	chromatin assembly	P	<0.001	2
GO:0015980	energy derivation by oxidation of organic compounds	P	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0071824	protein-DNA complex subunit organization	P	<0.001	2
GO:0006323	DNA packaging	P	<0.001	2
GO:0055114	oxidation-reduction process	P	0.001	2
GO:0071103	DNA conformation change	P	0.001	2

GO-ID	Term	Category	P-Value	Genes
GO:0006333	chromatin assembly or disassembly	P	0.001	2
GO:0000785	chromatin	C	0.001	2
GO:0034622	cellular macromolecular complex assembly	P	0.002	2
GO:0046034	ATP metabolic process	P	0.002	2
GO:0009205	purine ribonucleoside triphosphate metabolic process	P	0.002	2
GO:0044427	chromosomal part	C	0.002	2
GO:0009144	purine nucleoside triphosphate metabolic process	P	0.002	2
GO:0009199	ribonucleoside triphosphate metabolic process	P	0.002	2
GO:0006325	chromatin organization	P	0.002	2
GO:0009141	nucleoside triphosphate metabolic process	P	0.002	2
GO:0042278	purine nucleoside metabolic process	P	0.003	2
GO:0046128	purine ribonucleoside metabolic process	P	0.003	2
GO:0009119	ribonucleoside metabolic process	P	0.003	2
GO:0004332	fructose-bisphosphate aldolase activity	F	0.003	1
GO:0051276	chromosome organization	P	0.003	2
GO:0009259	ribonucleotide metabolic process	P	0.003	2
GO:0009126	purine nucleoside monophosphate metabolic process	P	0.003	2
GO:0009150	purine ribonucleotide metabolic process	P	0.003	2
GO:0009167	purine ribonucleoside monophosphate metabolic process	P	0.003	2
GO:0006163	purine nucleotide metabolic process	P	0.004	2
GO:1901657	glycosyl compound metabolic process	P	0.004	2
GO:0009116	nucleoside metabolic process	P	0.004	2
GO:0009161	ribonucleoside monophosphate metabolic process	P	0.004	2
GO:0009123	nucleoside monophosphate metabolic process	P	0.004	2
GO:0005694	chromosome	C	0.004	2
GO:0072521	purine-containing compound metabolic process	P	0.004	2
GO:0006461	protein complex assembly	P	0.004	2
GO:0019693	ribose phosphate metabolic process	P	0.004	2
GO:0070271	protein complex biogenesis	P	0.004	2
GO:0043228	non-membrane-bounded organelle	C	0.004	3

GO-ID	Term	Category	P-Value	Genes
GO:0043232	intracellular non-membrane-bounded organelle	C	0.004	3
GO:0065003	macromolecular complex assembly	P	0.004	2
GO:0071822	protein complex subunit organization	P	0.005	2
GO:0022607	cellular component assembly	P	0.005	2
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	P	0.006	1
GO:0009117	nucleotide metabolic process	P	0.007	2
GO:0008177	succinate dehydrogenase (ubiquinone) activity	F	0.008	1
GO:0042775	mitochondrial ATP synthesis coupled electron transport	P	0.008	1
GO:0044085	cellular component biogenesis	P	0.008	2
GO:0006753	nucleoside phosphate metabolic process	P	0.009	2
GO:0043933	macromolecular complex subunit organization	P	0.009	2
GO:0016832	aldehyde-lyase activity	F	0.009	1
GO:0042773	ATP synthesis coupled electron transport	P	0.009	1
GO:0022904	respiratory electron transport chain	P	0.009	1
GO:0022900	electron transport chain	P	0.009	1
GO:0006119	oxidative phosphorylation	P	0.009	1
GO:0006996	organelle organization	P	0.010	2
GO:0055086	nucleobase-containing small molecule metabolic process	P	0.011	2
GO:0016635	oxidoreductase activity, acting on the CH-CH group of donors, quinone or related compound as acceptor	F	0.011	1
GO:0000104	succinate dehydrogenase activity	F	0.011	1
GO:1901135	carbohydrate derivative metabolic process	P	0.015	2
GO:0008137	NADH dehydrogenase (ubiquinone) activity	F	0.016	1
GO:0050136	NADH dehydrogenase (quinone) activity	F	0.016	1
GO:0019637	organophosphate metabolic process	P	0.016	2
GO:0044281	small molecule metabolic process	P	0.017	3
GO:0072350	tricarboxylic acid metabolic process	P	0.017	1
GO:0009060	aerobic respiration	P	0.017	1
GO:0006101	citrate metabolic process	P	0.017	1
GO:0006099	tricarboxylic acid cycle	P	0.017	1
GO:0003954	NADH dehydrogenase activity	F	0.020	1

GO-ID	Term	Category	P-Value	Genes
GO:0016043	cellular component organization	P	0.023	2
GO:0006757	ATP generation from ADP	P	0.024	1
GO:0006165	nucleoside diphosphate phosphorylation	P	0.024	1
GO:0046031	ADP metabolic process	P	0.024	1
GO:0046939	nucleotide phosphorylation	P	0.024	1
GO:0006096	glycolytic process	P	0.024	1
GO:0009135	purine nucleoside diphosphate metabolic process	P	0.024	1
GO:0009179	purine ribonucleoside diphosphate metabolic process	P	0.024	1
GO:0009185	ribonucleoside diphosphate metabolic process	P	0.024	1
GO:0006090	pyruvate metabolic process	P	0.025	1
GO:0044724	single-organism carbohydrate catabolic process	P	0.027	1
GO:0009132	nucleoside diphosphate metabolic process	P	0.028	1
GO:0016655	oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor	F	0.030	1
GO:0071840	cellular component organization or biogenesis	P	0.030	2
GO:0032991	macromolecular complex	C	0.030	3
GO:1901564	organonitrogen compound metabolic process	P	0.031	3
GO:0016052	carbohydrate catabolic process	P	0.034	1
GO:0046496	nicotinamide nucleotide metabolic process	P	0.039	1
GO:0019362	pyridine nucleotide metabolic process	P	0.040	1
GO:0019752	carboxylic acid metabolic process	P	0.041	2
GO:0043436	oxoacid metabolic process	P	0.041	2
GO:0016310	phosphorylation	P	0.042	2
GO:0006082	organic acid metabolic process	P	0.042	2
GO:0006733	oxidoreduction coenzyme metabolic process	P	0.045	1
GO:0072524	pyridine-containing compound metabolic process	P	0.045	1
Enriched-GO terms found within induced genes between DR and WR on day on day 7				
GO:0004462	lactoylglutathione lyase activity	F	0.002	1
GO:0016846	carbon-sulfur lyase activity	F	0.008	1
GO:0005975	carbohydrate metabolic process	P	0.010	2
GO:0009055	electron carrier activity	F	0.024	1

GO-ID	Term	Category	P-Value	Genes
	Enriched-GO terms found within repressed genes between DR and WR on day 7			
GO:0044085	cellular component biogenesis	P	<0.001	3
GO:0044815	DNA packaging complex	C	<0.001	2
GO:0000786	nucleosome	C	<0.001	2
GO:0032993	protein-DNA complex	C	<0.001	2
GO:0065004	protein-DNA complex assembly	P	<0.001	2
GO:0034728	nucleosome organization	P	<0.001	2
GO:0031497	chromatin assembly	P	<0.001	2
GO:0006334	nucleosome assembly	P	<0.001	2
GO:0071824	protein-DNA complex subunit organization	P	<0.001	2
GO:0071840	cellular component organization or biogenesis	P	<0.001	3
GO:0006323	DNA packaging	P	<0.001	2
GO:0071103	DNA conformation change	P	<0.001	2
GO:0043228	non-membrane-bounded organelle	C	<0.001	3
GO:0043232	intracellular non-membrane-bounded organelle	C	<0.001	3
GO:0006333	chromatin assembly or disassembly	P	<0.001	2
GO:0000785	chromatin	C	<0.001	2
GO:0034622	cellular macromolecular complex assembly	P	<0.001	2
GO:0044427	chromosomal part	C	<0.001	2
GO:0006325	chromatin organization	P	<0.001	2
GO:0051276	chromosome organization	P	<0.001	2
GO:0005694	chromosome	C	<0.001	2
GO:0006461	protein complex assembly	P	<0.001	2
GO:0070271	protein complex biogenesis	P	<0.001	2
GO:0065003	macromolecular complex assembly	P	<0.001	2
GO:0071822	protein complex subunit organization	P	0.001	2
GO:0022607	cellular component assembly	P	0.001	2
GO:0032991	macromolecular complex	C	0.001	3
GO:0043933	macromolecular complex subunit organization	P	0.001	2
GO:0006996	organelle organization	P	0.001	2

GO-ID	Term	Category	P-Value	Genes
GO:0043229	intracellular organelle	C	0.002	3
GO:0043226	organelle	C	0.002	3
GO:0016043	cellular component organization	P	0.003	2
GO:0044446	intracellular organelle part	C	0.006	2
GO:0044422	organelle part	C	0.006	2
GO:0006414	translational elongation	P	0.007	1
GO:0044424	intracellular part	C	0.007	3
GO:0043234	protein complex	C	0.010	2
GO:0003677	DNA binding	F	0.013	2
GO:0005622	intracellular	C	0.014	3
GO:0042254	ribosome biogenesis	P	0.014	1
GO:0022613	ribonucleoprotein complex biogenesis	P	0.014	1
GO:0044464	cell part	C	0.015	3
GO:0005623	cell	C	0.015	3
GO:0005634	nucleus	C	0.016	2
GO:0043227	membrane-bounded organelle	C	0.027	2
GO:0043231	intracellular membrane-bounded organelle	C	0.027	2
GO:0009987	cellular process	P	0.048	3

Tab. S4.9: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between DR (with 50 µM riboflavin addition prior to drought stress) and DC (without riboflavin addition prior to drought stress) mesocosms with information about biological function, if available.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the DR/DC microarray experiments							
Glyoxalase I	229618	Protection	protection against methylglyoxal	1.23	0.180	2.26	0.030
Cytochrome C	135048	Protection	oxidative protection	0.20	0.110	1.80	0.006
Aminotransferase (class IV)	190047	Protection	Branched-chain amino acid aminotransferase 1, detoxification by degradation of BCAA	1.19	0.002	0.57	0.060
MFS-1	211434	Transporter	membrane transporter (potentially phosphate)	1.76	<0.001	0.77	<0.001
NSF attachment protein	189575	Transporter	involved in membrane fusion and transfer of membrane vesicles from one membrane compartment to another	1.21	<0.001	0.26	0.270
Monocarboxylate transporter	75392	Transporter	transporter/transmembrane protein	1.14	0.010	0.20	0.610
Zinc/iron permease	193646	Transporter	Fe^{2+}/Zn^{2+} regulated transporter	1.00	0.030	0.46	0.260
EIF5A	195151	Translation & Transcription	Translation initiation factor, involved in translation elongation, actin dynamics, cell cycle progression, stress response and maintenance of cell wall integrity	0.84	<0.001	1.00	<0.001
Phosphatidylglycerophosphatase GEP4, putitative	205009	regulation of cell processes	involved in the biosynthesis of cardiolipin (part of mitochondrial membranes) and cellular energy metabolism	1.63	0.001	2.42	<0.001
Cell wall synthesis protein KNH1	194933	regulation of cell processes	biosynthesis of Beta-1,6-glucan	1.09	<0.001	1.07	<0.001
SAM domain containing protein	117817	Signal transduction	Signal transduction mechanisms	0.90	<0.001	1.22	<0.001
Glycoside hydrolase	153780	Metabolism	hydrolysis of glycosidic bonds in complex sugars, including cellulose	0.33	0.340	1.63	<0.001
Alpha-tubulin folding cofactor E	63759	Growth	Involved in folding of alpa-tubulin	0.95	0.250	2.06	0.030
Mra1	67862	Growth	downstream factor of Ras1, essential for cell growth, required for the promotion of mating	1.13	<0.001	0.73	0.010

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Uncharacterized protein	77212	unknown	unknown	2.03	0.003	0.71	0.190
Uncharacterized protein	195960	unknown	unknown	2.00	<0.001	0.21	0.580
Uncharacterized protein	190197	unknown	unknown	1.95	0.001	0.27	0.479
Uncharacterized protein	194064	unknown	unknown	0.51	0.119	1.92	<0.001
Uncharacterized protein	75612	unknown	unknown	1.87	0.016	-0.04	0.564
Uncharacterized protein	186837	unknown	unknown	1.85	<0.001	0.27	0.382
Uncharacterized protein	187811	unknown	unknown	1.68	0.063	1.68	0.063
Uncharacterized protein	75571	unknown	unknown	1.51	<0.001	-0.36	0.248
Uncharacterized protein	193661	unknown	unknown	-0.01	0.971	1.06	0.009
Uncharacterized protein	195064	unknown	unknown	1.03	<0.001	0.28	0.255
Leucine-rich repeat domain protein	121851	unknown	unknown	1.01	<0.001	0.62	0.002
Repressed genes of the DR/DC microarray experiments							
Histone H3	139997	regulation of cell processes	DNA binding	-2.05	<0.001	-2.84	<0.001
Histone H2B	133899	regulation of cell processes	DNA binding	-1.25	0.030	-2.45	<0.001
Ubiquitin	133168	regulation of cell processes	post-translational modifications, interacts also with histones	-1.15	0.020	0.63	0.150
Ebp2	193895	Translation & Transcription	RNA biosynthesis factor, required for efficient nuclear division	-0.25	0.670	-1.06	0.090
NADH ubiquinone oxidoreductase	192620	Metabolism	catalyses electron transfer from NADH to coenzyme Q10	-1.35	0.020	-0.14	0.790
Uncharacterized protein	190938	unknown	unknown	-1.07	0.008	-0.17	0.696
DUF2462 family protein	72803	unknown	unknown	-1.32	0.042	-1.06	0.080
Uncharacterized protein	117512	unknown	unknown	0.26	0.793	-1.82	0.087
Transmembrane protein	55578	unknown	unknown	-0.23	0.665	-1.37	0.025
Uncharacterized protein	196091	unknown	unknown	-0.24	0.612	-1.03	0.050
Protein with DnaJ-domain	196227	unknown	unknown	-1.05	0.067	-0.29	0.580

Tab. S4.10: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of induced and repressed genes in microarray experiments on day 3 and 7 after irrigation stop between DR (with 50 µM riboflavin addition prior to drought stress) and WR (no drought stress, 50 µM riboflavin addition) mesocosms with information about biological function, if available.

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Induced genes of the DR/WR microarray experiments							
Alpa-tubulin folding cofactor E	63759	Growth	Involved in folding of Alpha-tubulin	0.98	0.079	2.09	0.054
Mra1	67862	Growth	downstream factor of Ras1, essential for cell growth, required for the promotion of mating	1.15	0.001	0.66	0.002
Glycoside hydrolase	153780	Metabolism	hydrolysis of glycosidic bonds in complex sugars, including degradation of biomass such as cellulose	0.36	0.401	1.52	<0.001
Cytochrome C	135048	Protection	oxidative protection	-0.03	0.887	1.99	0.026
Aminotransferase (class IV)	190047	Protection	Branched-chain amino acid aminotransferase 1, detoxification by degradation of BCAA	1.2	<0.001	0.54	0.005
Glyoxalase I	229618	Protection	protection against methylglyoxal	1.25	0.046	2.29	0.055
Cell wall synthesis protein KNH1	194933	regulation of cell processes	biosynthesis of Beta-1,6-glucan	1.19	0.047	2.29	0.055
Phosphatidylglycerophosphatase GEP4, putitative	205009	regulation of cell processes	involved in the biosynthesis of cardiolipin (part of mitochondrial membranes), cellular energy metabolism	1.69	0.001	2.53	<0.001
SAM domain containing protein	117817	Signal transduction	Signal transduction mechanisms	0.95	<0.001	1.11	0.001
Subunit 21 of mediator complex protein	193554	Translation & Transcription	coactivator of the mediator complex, involved transcription regulation of nearly all RNA polymerase II-dependent genes	1.16	0.040	-0.32	0.010
Monocarboxylate transporter	75392	Transporter	transporter/transmembrane protein	1.07	0.012	0.17	0.245

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Zinc/iron permease	193646	Transporter	Fe^{2+}/Zn^{2+} regulated transporter	1	0.009	0.44	0.198
MFS-1	211434	Transporter	membrane transporter	1.71	<0.001	0.72	0.001
Uncharacterized protein	75571	unknown	unknown	1.8	0.001	-0.3	0.030
Uncharacterized protein	75612	unknown	unknown	1.26	0.062	0.13	0.261
Uncharacterized protein	77212	unknown	unknown	2.12	0.007	0.76	0.099
Uncharacterized protein	121851	unknown	unknown	1.06	<0.001	0.56	0.010
Uncharacterized protein	144976	unknown	unknown	2.24	<0.001	0.64	0.077
Uncharacterized protein	186837	unknown	unknown	1.9	<0.001	0.25	0.440
Uncharacterized protein	187811	unknown	unknown	1.24	0.045	1.26	0.056
Uncharacterized protein	190197	unknown	unknown	1.96	<0.001	0.24	0.563
Uncharacterized protein	194064	unknown	unknown	0.51	0.003	1.87	<0.001
Uncharacterized protein	195064	unknown	unknown	1.07	<0.001	0.26	0.111
Uncharacterized protein	195960	unknown	unknown	1.99	<0.001	0.18	0.639
Ribonuclease H-like protein	75272	Translation & Transcription	binds and cleaves double-stranded RNA	1.03	<0.001	0.56	0.002
Uncharacterized protein	75525	unknown	unknown	1.03	<0.001	-0.12	0.430
Uncharacterized protein	76138	unknown	unknown	1.31	0.059	0.6	0.559
Repressed genes of the DR/WR microarray experiments							
Histone H3	139997	regulation of cell processes	DNA binding	-2.31	<0.001	-3.05	<0.001
Histone H2B	133899	regulation of cell processes	DNA binding	-1.27	0.036	-2.71	<0.001
Ubiquitin	133168	regulation of cell processes	post-translational modifications, interacts also with histones	-1.18	0.001	0.62	0.084
NADH ubiquinone oxidoreductase	192620	Metabolism	catalyses electron transfer from NADH to coenzyme Q10	-1.31	0.043	-0.12	0.391
succinate dehydrogenase	190526	Metabolism	Enzyme of the citric acid cycle and the electron transport chain	-1.2	0.050	-0.87	0.235
Fructose 1,6-bisphosphate aldolase	192128	Metabolism	Enzyme of Glycolysis	-1.17	0.036	0.43	0.019

Encoding Protein	Protein ID	Group	Biological function	day 3		day 7	
				Log_2-ratio	p	Log_2-ratio	p
Ribosomal protein L10	191238	Translation & Transcription	Regulation of development & translation	-1.31	0.291	-2.17	0.054
Uncharacterized protein	190938	unknown	unknown	-1.1	0.019	-0.16	0.135
DUF2462 family protein	72803	unknown	unknown	-1.26	0.055	-0.95	0.030
Protein with DnaJ-domain	196227	unknown	unknown	-1.08	0.003	-0.27	0.563
Transmembrane protein	55578	unknown	unknown	-0.24	0.470	-1.44	0.045
Uncharacterized protein	117512	unknown	unknown	0.18	0.734	-1.89	0.066

Tab. S4.11: Gene enrichment analyses (Log_2-ratio distribution and p-Values) of genes coding for proteins associated with riboflavin biosynthesis (RIB1, RIB2, RIB3, RIB4, RIB5 and RIB7), riboflavin transport (Mch5) as well as riboflavin processing (riboflavin kinase and FAD synthetase) in microarray experiments on day 3 and 7 between treatments. WR= no drought stress, 50 µM riboflavin addition; WC= no drought stress, no riboflavin addition; DR= no riboflavin addition prior to drought stress; DC= no riboflavin addition prior to drought stress.

	Encoding Protein	RIB1	RIB2	RIB2	RIB3	RIB4	RIB5	RIB7	riboflavin kinase	FAD synthetase	Mch5
	Protein ID	**191759**	**185612**	**190437**	**143682**	**190326**	**182135**	**213834**	**202343**	**194855**	**190143**
	DC/WC microarray experiments										
day 3	**Log_2-ratio**	0.15	0.10	0.08	-0.01	0.09	0.14	-0.01	0.02	-0.12	0.28
	P-Value	0.178	0.405	0.442	0.944	0.433	0.227	0.951	0.901	0.279	0.028
day 7	**Log_2-ratio**	-0.07	0.03	-0.13	0.02	-0.16	-0.24	0.00	0.01	0.09	0.19
	P-Value	0.460	0.720	0.184	0.805	0.114	0.026	0.987	0.914	0.320	0.083
	DR/DC microarray experiments										
day 3	**Log_2-ratio**	-0.14	0.03	-0.12	0.03	0.17	-0.07	0.13	-0.07	0.14	-0.31
	P-Value	0.152	0.808	0.172	0.739	0.130	0.412	0.133	0.602	0.120	0.018
day 7	**Log_2-ratio**	0.08	0.13	0.17	-0.16	-0.14	0.19	0.02	-0.09	-0.16	-0.32
	P-Value	0.382	0.238	0.067	0.138	0.209	0.052	0.804	0.517	0.066	0.014
	DR/WR microarray experiments										
day 3	**Log_2-ratio**	-0.14	0.03	-0.12	0.03	0.18	-0.07	0.13	-0.07	0.14	-0.31
	P-Value	0.218	0.820	0.233	0.775	0.094	0.461	0.206	0.500	0.182	0.030
day 7	**Log_2-ratio**	0.08	0.12	0.16	-0.15	-0.12	0.18	0.02	-0.08	-0.15	-0.29
	P-Value	0.408	0.215	0.121	0.169	0.257	0.094	0.833	0.436	0.130	0.022
	WR/WC microarray experiments										
day 3	**Log_2-ratio**	0.27	0.07	0.19	-0.04	-0.11	0.19	-0.14	0.09	-0.25	0.56
	P-Value	0.057	0.651	0.137	0.761	0.475	0.145	0.245	0.652	0.060	0.005
day 7	**Log_2-ratio**	-0.09	-0.08	-0.31	0.18	-0.11	-0.45	-0.02	0.09	0.26	-0.10
	P-Value	0.499	0.575	0.027	0.223	0.347	0.004	0.866	0.623	0.041	0.620

Contributions to the included manuscripts

Manuscript 1: Redistribution of soil water by a saprotrophic fungus enhances carbon mineralisation. Alexander Guhr, Werner Borken, Marie Spohn and Egbert Matzner. Published in *Proceedings of the National Academy of Sciences of the United States of America* (2015) 112, 14647–14651.

A. Guhr	65%	research design, laboratory works, data analysis, manuscript preparation
W. Borken	10%	discussion of results, manuscript preparation
M. Spohn	5%	discussion of results
E. Matzner	20%	research design, discussion of results, manuscript preparation

Manuscript 2: Effect of water redistribution by two distinct saprotrophic fungi on carbon mineralisation and nitrogen translocation in dry soil. Alexander Guhr, Carlo Marzini, Werner Borken, Christian Poll and Egbert Matzner. Published in *Soil Biology and Biochemistry* (2016) 103, 380–387.

A. Guhr	55%	research design, laboratory works, data analysis, manuscript preparation
C. Marzini	20%	laboratory works
W. Borken	10%	discussion of results, manuscript preparation
C. Poll	5%	manuscript preparation
E. Matzner	10%	research design, discussion of results, manuscript preparation

Manuscript 3: Vitamin B2 (riboflavin) increases drought tolerance of *Agaricus bisporus*. Alexander Guhr, Marcus A. Horn and Alfons R. Weig. In preparation.

A. Guhr	65%	research design, laboratory works, data analysis, manuscript preparation
M. Horn	10%	laboratory works, manuscript preparation
A. Weig	25%	research design, laboratory works, discussion of results, manuscript preparation

Acknowledgements

My special thanks goes to Prof. Matzner for the opportunity to work on this project and for providing help and advice during all stages of my doctor thesis. I am grateful for the confidence he had in me and the freedom he gave me for developing my own ideas and experiments. I also thank Prof. Werner Borken for his support and helpful discussions. My thank goes also to the DFG for providing financial support for this research.

Further, I'm very grateful for the straitforword and uncomplicated support of all staff members of the soil ecology department, a way to oft underestimated resource: Uwe Hell for his technical support in all aspects of work and life, Karin Söllner for here experienced assistance in laboratory work and Ingeborg Vogler for her comprehensive administrative help. I also appreciated the support by Andrej Einhorn, Oliver Archner, Gerhard Küfner and Gerhard Müller as well as Peter Schmid and his staff on technical issues.

I'm also very grateful to PD. Dr. AlfonsWeig for his support in planning and evaluating study III. In addition, I thank Prof. Gerhard Rambold, Prof. Christane Werner and Dr. Mareen Dubbert as well as Prof. Marcus Horn and Dr. Anja Dallinger for the uncomplicated accesse to and help with technical equipment unavailable at our department. Of course I'm also very grateful for the respective technical support by Tina Leistner, Ilse Thaufelder and Ralf Martens. In general, I appreciated the uncomplicated cooperations within the Bayreuth Center of Ecology and Environmental Research (BayCEER).

Further, I want to thanks Prof. Gerhard Rambold for his guidance to the field of mycology as well as for his help and informative discussions.

I greatly appreciated the discussions with all my PhD colleagues: Dr. Beate Bleicher, Dr. Inken Krüger, Dr. Marianne Schütt, Dr. Mi-Hee Lee, Mai Van Dinh, Christine Heuck, and Emanuell Brückner. Special thanks goes to Dr. Andreas Bantle for the late evening discussions about R and the world. I want to also thank Carlo Marzini for his contribution to this work in the course of his master thesis.

Furthermore, I thank Prof. Gerhard Gebauer for his great helpfulness and him as well as the staff of the Central Isotopic Laboratory of the BayCEER, espacially Isolde Baumann and Petra Eckert, for the stable isotope analyses and access to the isotopic laboratory.

Finally, I want to thank my parents for their support and I want to espacially thank my love Nathalie Nida Moske for her patience and encouragement and most of all for helping me to keep in touch with reality.

Publications

Alexander Guhr, Werner Borken, Marie Spohn and Egbert Matzner (2015). Redistribution of soil water by a saprotrophic fungus enhances carbon mineralisation. *Proceedings of the National Academy of Sciences of the United States of America* 112, 14647–14651. DOI: 10.1073/pnas.1514435112.

Alexander Guhr, Carlo Marzini, Werner Borken, Christian Poll and Egbert Matzner (2016) Effect of water redistribution by two distinct saprotrophic fungi on carbon mineralisation and nitrogen translocation in dry soil. *Soil Biology and Biochemistry* 103, 380–387. DOI: 10.1016/j.soilbio.2016.09.009.

Alexander Guhr, Marcus A. Horn and Alfons R. Weig (2016). Vitamin B2 (riboflavin) increases drought tolerance of *Agaricus bisporus*. In preperation.

Declarations

(§ 5 Nr. 4 PromO)

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

(§ 8 S. 2 Nr.5 PromO)

Hiermit erkläre ich mich damit einverstanden, dass die elektronische Fassung meiner Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung hinsichtlich der eigenständigen Anfertigung der Dissertation unterzogen werden kann.

(§ 8 S. 2 Nr. 7 PromO)

Hiermit erkläre ich eidesstattlich, dass ich die Dissertation selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

(§ 8 S. 2 Nr. 8 PromO)

Ich habe die Dissertation nicht bereits zur Erlangung eines akademischen Grades anderweitig eingereicht und habe auch nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden.

(§ 8 S. 2 Nr. 9 PromO)

Hiermit erkläre ich, dass ich keine Hilfe von gewerblichen Promotionsberatern bzw. -vermittlern in Anspruch genommen habe und auch künftig nicht nehmen werde.

Bayreuth, den .....

Datum, Unterschrift