Soil Stability and Filamentous Fungi

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Foreword

This dissertation is a cumulative work of manuscripts, either published or submitted, selected from my publication list. Therefore, this thesis is based on the following papers which are referred to by their Roman numerals. The bibliographic references cited through all chapters are listed together after Chapter 6.

I. Zheng W, Morris EK, Rillig MC. (2014). Ectomycorrhizal fungi in association with *Pinus sylvestris* seedlings promote soil aggregation and soil water repellency. Soil Biol Biochem **78**:326–331. doi:10.1016/j.soilbio.2014.07.015

II. Zheng W, Morris EK, Lehmann A, Rillig MC. Does soil water repellency promote soil water-stable aggregation? A meta-analysis (in preparation).

III. Zheng W, Lehmann A, Rillig MC. Enzyme activities, growth features and water related traits of saprotrophic fungi: studying saprotrophic fungi affecting soil formation and stability using a trait-based approach (in preparation)

Acknowledgments

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First, I am grateful to my supervisor Matthias Rillig for offering me the opportunity to study here and being tolerant to my quirks in all ways... I am equally grateful to my second supervisor Kathryn Morris, her kindness and support saved me.

Then, I have to say I am so lucky to be in such a vivid group. I have left the home city for 13 year and wandered from city to city, every time more further from my origin. Here in Berlin in the past 5 years I shared many happy and precious moments with my colleagues. They are not only supportive in many ways to help me in my research, but also open a door for me to know the world better.

I want to thank Kathryn Morris, Stefan Hempel, Stavros Veresoglou and Anika Lehmann for their statistical advices and comments. To Sabine Buchert, Gabriele Erzigkeit and Sabine Artelt for helping me in doing experiments. To Ulfah Mardhiah, Kriszta Valyi, Dongwei Wang, Tessa Camenzind, Jeannine Wehner and Stefanie Maaß.....There are too many of you who I don't list but cherish in my heart.

Finally, I want to express my gratitude for my families. Although you are 7406 km away from me, I know from the bottom of my heart that you are always there and support me no matter how stupid the things I am planning.

Special thanks to Anika and Zheng who continuously motivate me to finish my study by chocolate and hugs.

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Chapter 1

General introduction

Soil stability

Soil is the skin of the Earth where life happens (Chesworth, 2008). It is the reason why the Earth is such a vivid planet where our mankind can live. Thus, keeping the stability of soil is our primary task. As we know, a stable soil is a well-structured one, in which it consists of different size of aggregates (Yoder, 1936). These aggregates are clusters of particles that adhere to each other more strongly than to the surrounding particles (Kemper and Rosenau, 1986). Thus, keeping aggregates stability (AS) is the first concern of maintaining soil stability. When aggregates are formed, soil organic matter (SOM) plays the role of adhesion as binding agents. In return, aggregates keep SOM stable in soil that turn soil into the largest and most dynamic carbon pool on Earth (Post et al., 1982). A group of special SOM brings in a debated soil feature which is water-repellency.

Soil water-repellency has been documented ever since the 1940s (DeBano, 2000). It is a reduction in the rate of wetting and retention of water in soil caused by the presence of hydrophobic coatings on soil particles (DeBano, 2000; Hallett, 2007). Since then, publications related to SWR have increased exponentially (Dekker et al., 2005), because it is closely related to water waste in agriculture and erosion in post-fire ecosystems. Researchers began to look into the mechanisms of SWR formation in order to find effective remedies (Müller et al., 2011). Along with better understanding of SWR, the positive side of it was revealed (Sullivan, 1990). In natural condition, SWR usually happens after a severe drought (Hallett, 2007). When soil aggregates have low water content, they are vulnerable to rapid wetting (Zaher and Caron, 2008). In this condition SWR slows down the wetting process, consequently protects soil

aggregates and increases soil stability (Sullivan, 1990). The positive relationship between SWR and AS has shown in field and laboratory experiments in which SWR was either induced by fire or organic matter alteration (Annabi et al., 2007; Arcenegui et al., 2008; Badía-Villas et al., 2014; Bartoli and Dousset, 2011; Jordan et al., 2011; Mataix-Solera and Doerr, 2004; Neris et al., 2013; Vogelmann et al., 2013).

Filamentous soil fungi

As mentioned above, both SWR and AS originate from SOMs coating on surfaces of soil aggregates. Soil fungi, especially filamentous ones, are the main contributor in this process, because they explore a large volume of soil by filamentous growth. Mycelia of filamentous fungi can exude organic matter to bind adjacent particles while enmeshing particles though a network structure (Rillig and Mummey, 2006; Ritz and Young, 2004). At the same time, filamentous fungi are known to produce a group of hydrophilic proteins: hydrophobins that are important in fungal physiology and morphology (Linder et al., 2005; Wessels, 2000).

However, filamentous soil fungi, SWR, AS and SOM are only intimately connected in theory. In practice, they are separately studied in topics that should be integrated. Even though AS is the most intensively investigated topic, the experimental studies between AS and soil fungi are mainly biased towards arbuscular mycorrhizal fungi (AMF; forming a type of obligate symbiosis between roots and fungi). Ectomycorrhizal (EcM) fungi and saprotrophic fungi are equally important as AMF but rarely addressed (Rillig and Mummey, 2006; Ritz and Young, 2004). EcM fungi are the symbiosis association between higher plants and fungi; they can grow saprotrophically (Moore et al., 2011). Saprotrophic fungi as the decomposers widely exist in soil and recycle biological remains. Fungi may have shared mechanisms in SWR and AS, but the specialties of EcM and saprotrophic fungi should not be ignored (Moore et al., 2011).

Fungal traits in a trait-based approach

To reveal the immense potential of soil fungi in the role of AS and SWR, a trait-based approach have been proposed by many soil scientists (Aguilar-Trigueros et al., 2015; Powell et al., 2013; Rillig et al., 2014). This method is frequently used to unravel mechanisms and functioning on

a system level by exploring features on individual levels in evolutionary biology and community, ecosystem and microbial ecology (Ackerly and Cornwell, 2007; Garnier and Navas, 2012; Powell et al., 2013; Rillig et al., 2014). The trait-based approach has been well-established in understanding functions in plant ecology (Ackerly and Cornwell, 2007; Moles et al., 2005; Violle et al., 2007).

To initiate the practice of trait-based approaches in fungal ecology, we have to build up a fungal trait database to connect fungi and their environmental function. Since soil fungi, AS and SWR are three closely related aspects, exploring fungal traits responding in these processes will be a good start. AS and SWR build up along with mycelia exploring the soil environment. Therefore, we should identify how fungi grow (horizontally and vertically) and how they obtain nutrients by exuding enzymes, which are fundamental for their interaction with soil (Aguilar-Trigueros et al., 2015). We also include water repellency of fungal surface as Rillig et al. (2014) have suggested in a list of fungal traits for understanding soil aggregation. The chosen traits are not only for connecting fungi to their soil function but also for understanding their lifestyle in soil.

Thesis outline

The main objective of this dissertation is to explore the interrelationships among filamentous fungi (EcM fungi and saprotrophic fungi), soil aggregate stability (AS) and soil water repellency (SWR), which are further detailed in the following three topics:

1. effects of EcM fungi in association with *Pinus sylvestris* seedlings on soil aggregation and soil water repellency (chapter 2);
2. interaction between SWR and AS, along with variables affecting SWR (chapter 3);
3. and fungal traits of saprotrophic fungi associated with SWR and AS (chapter 4).

The topics were addressed in three chapters using an experimental approach and also through data mining. For **Chapter 2**, we conducted an experiment in a climate chamber, for **Chapter 4** we ran an experiment in a petri dish system and for **Chapter 3** we performed a meta-analysis using statistically synthesized published data (Borenstein et al., 2009).

Fungal effects on AS and SWR are biased towards arbuscular mycorrhizal fungi (another abundant group of soil fungi). Due to the interests in (1) the degree of EcM fungi promoting SWR and AS and (2) the potential role of EcM fungi in influencing SWR and AS, in **Chapter 2** we quantified how nine EcM fungi in association with *Pinus sylvestris* seedlings affected AS and SWR of a sandy loamy soil. In the experiment we used water drop penetration time to measure SWR, water stable aggregation and mean weight diameter for AS and other abiotic factors (soil pH, soil protein content).

In **Chapter 3**, we focused on the interaction between AS and SWR, since they are intuitively related but rarely tested in experiments. We intended to synthesize current findings on this topic and found 27 publications of 119 trials which reported AS and SWR quantitively. From the publications we also extracted relevant edaphic moderators and experimental moderators in order to identify what environmental factors influence SWR and what are the potential pitfalls of the ways experiments are run.

A trait-based approach is a method that brings together traits of organisms and their ecological functions. To apply this method in future studies on the relationship between soil fungal traits and AS and SWR, first, we need to build up a database on relevant fungal trait. Thus, in **Chapter 4**, traits of 31 saprotrophic fungi -which were isolated from the same grassland- were screened for traits on growth, enzyme and water: we measured colony extension rate (*Kr*), biomass density, enzyme activities (acid phosphatase, cellobiohydrolase, leucine aminopeptidase and laccase), mycelial water content and hydrophobicity of the fungal surface. In the experiment, the strains were grown in a petri dish unit on both agar and cellophane surface and harvested when they were in a linear growth phase. The effects of two factors: cellophane and growth age were tested, since we were interested in how labile the traits are. We also investigated (1) do traits have phylogenetic signals and (2) are there trade-offs in traits by phylogenetically independency contrasts.

Finally, we summarize our findings in **Chapter 5**.

Chapter 2

Ectomycorrhizal fungi in association with *Pinus sylvestris* seedlings promote soil aggregation and soil water repellency

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Abstract

Research on fungal effects on soil aggregation has been heavily biased towards arbuscular mycorrhiza. Even though ectomycorrhizal fungi are thought to be as important as arbuscular mycorrhizal fungi and saprotrophic fungi in contributing to soil structure, there are few experimental studies on this topic. Here we quantified how nine ectomycorrhizal fungi in association with *Pinus sylvestris* seedlings affected soil aggregation and soil water repellency of a sandy loamy soil. Water-stable aggregates (>0.25 mm diameter) increased for *Laccaria bicolor*, *Laccaria laccata*, *Lactarius theiogalus*, *Paxillus involutus* and *Suillus bovinus* by 6 - 12 %. Mean weight diameter (MWD) also increased, primarily in the 2-4 mm diameter size class. However, *Suillus granulatus* increased water-stable aggregates but not MWD, conversely *Rhizopogon roseolus* and *Suillus luteus* increased MWD but not water-stable aggregates. We also found *Lt. theiogalus*, *R. roseolus* and *S. luteus* promoted soil water repellency. Furthermore, hyphal length was weakly correlated with MWD ($R = 0.27$, *p-value* < 0.05), especially with aggregate mass in the 2-4 mm size class ($R = 0.32$, *p-value* < 0.05). However, we could not identify clear soil effects (soil pH, soil protein content) serving as explanation for either soil aggregation or soil water repellency. Thus, we conclude that interactions between fungi and soil structure are a species-dependent processes based on yet to be characterized fungal traits. Our results have added further evidence from direct experimentation that ectomycorrhizal fungi can contribute to soil aggregation.

Introduction

As important terrestrial mutualistic fungal groups, ectomycorrhizal (EcM) fungi and arbuscular mycorrhizal (AM) fungi have been intensively studied regarding their effects on plant growth, plant communities and ecosystem processes (Smith and Read, 2008). Nevertheless, concerning the relationship between soil fungi and soil aggregation, research has been predominantly focused on AM fungi (Rillig and Mummey, 2006; Tisdall and Oades, 1982), perhaps because of the widely accepted importance of this process in agroecosystems and grasslands, in which AM fungi predominate.

Despite this imbalance in research, several lines of evidence suggest that EcM fungi may be as important as AM fungi in improving soil structure. Firstly, filamentous soil fungi, including EcM fungi, saprophytic fungi and others should simply be able to influence soil structure by virtue of their hyphal growth habit, which could enmesh particles and bind them into soil aggregates (Rillig and Mummey, 2006; Ritz and Young, 2004; Six et al., 2004; Tisdall and Oades, 1982; Tisdall et al., 1997). Secondly, already Thornton et al. (1956) observed that mycelia aggregated sandy soil under *Pinus radiata*, and more recently Caesar-Tonthat et al. (2013) observed that soil aggregation increased in the zone adjacent to *Agaricus lilaceps* (an EcM fungus) fairy rings.

Thirdly, some limited results show that EcM fungi and saprotrophic fungi and their extracellular exudates promoted soil water-stable aggregates (WSA). For example, *Pisolithus tinctorius* (an EcM fungus) when colonizing *Fraxinus uhdei* increased WSA of the 0.5-1 mm diameter fraction of a sandy clay loam by 3% (Ambriz et al., 2010). Saprotrophically growing EcM fungi also promoted soil aggregation to different degrees (Graf and Gerber, 1997; Graf et al., 2006), and in an experiment using clay particles smaller than 2 mm, the EcM fungus *Hebeloma* sp. and the saprotrophic fungus *Rhizoctonia solani* both increased aggregation >50 µm significantly, with *Hebeloma* sp. having a smaller effect (Tisdall et al., 1997). In a non-sterile soil, unidentified saprotrophic fungi had a significant, positive effect on macroaggregate formation (Denef et al., 2001). Exuded extracellular mucilage from saprotrophic basidiomycete

fungi, likely polysaccharides, has been related to soil aggregate water stability (Caesar-Tonthat, 2002). Mucilage from Trichocomaceae (Ascomycota) showed similar results and 6 isolates of this group of fungi increased the mean weight diameter (MWD) of soil aggregates of inoculated soil by more than 12%, although the effect was transient and decreased after 3 weeks (Daynes et al., 2012). Thus, even though direct evidence for soil aggregation by symbiotically growing EcM fungi is very rare, it is highly likely that soil aggregate formation by fungal mycelia is a process mediated by many types of filamentous fungi, including EcM fungi.

Among fungal exudates, hydrophobic compounds may be especially important for soil aggregation. Some EcM fungal hyphae are hydrophobic, which presumably helps fungi transport nutrients and water while exploring larger distances in soil (Agerer, 2001; Unestam and Sun, 1995). They could produce hydrophobins, which are small hydrophobic proteins, having multiple functions in mycelium growth, fruiting body formation, and the alteration of surface polarity (Linder et al., 2005; Wessels, 2000; Wösten and Willey, 2000). These hydrophobic compounds are thought to have an additional function of affecting soil wettability and inducing soil water repellency (SWR) (Diehl, 2013; Hallett, 2007; Rillig, 2005). For example, in forests SWR under *Pinus* was closely related to fungal activities (Lozano et al., 2013).

There has been a rapid increase in research on SWR (Dekker et al., 2005), not only focused on the role of fungi, but also concerning the relationship between SWR and soil water stable aggregation (Bisdom et al., 1993; Vogelmann et al., 2013b). The proposed mechanism for this relationship is that increasingly hydrophobic soil organic matter (SOM) could prevent breakage of dry soil aggregates during rewetting, therefore creating more water-stable aggregates (Piccolo and Mbagwu, 1999; Six et al., 2004). It is not yet clear whether or not the fungal associated SOM contributing to SWR and soil aggregation are the same, but Rillig (2005) has proposed that hydrophobins may be involved in both processes. Thus, it is important to conceptualize soil aggregation and SWR as two processes simultaneously influenced by EcM fungi.

In this research, soil aggregation and SWR were studied as functions of EcM in order to understand relationships among EcM fungal mycelia, soil structure, and soil moisture. The objectives of this study were: (1) to test whether nine commonly studied EcM fungi in association with *Pinus sylvestris* seedlings were able to promote soil aggregation and SWR, (2) to test whether pH (a key factor in SWR; Diehl, 2013) or soil protein content were related to soil aggregation and SWR, and (3) to test whether SWR was related to soil aggregation.

Materials and methods

Soil, fungi and seedlings cultivation

Soil was collected from a meadow near an experimental field of Freie Universität Berlin. Soil at the site was an Albic Luvisol with the following properties: sand = 74%, silt = 18% and clay = 8%; 64% initial WSA; pH($CaCl_2$) = 7; 6.9 mg/100 g P (calcium–acetate–lactate); 5.0 mg/100 g K (calcium–acetate–lactate); 0.12% N (total); 1.87% C (total) (analyses conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany; and using a Euro EA C/N analyzer, HEKAtech GmbH, Wegberg, Germany) (Rillig et al., 2010). Soil was sieved (10 mm) to remove stones and roots. Following that, the soil was steamed at 80 °C (8 h) to eliminate fungi and soil animals. Soil was then air dried and sieved to pass a 4 mm sieve to remove smaller roots and twigs and to further homogenize the soil.

Nine ectomycorrhizal fungal isolates from five families (Table 2-1) were chosen to test the effects of EcM fungi on soil aggregation. Six of them were kindly provided by Prof. A. Polle (Georg-August University Göttingen), the others were obtained from the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (http://www.cbs.knaw.nl/). These EcM fungi were selected because of their availability and record of intensive study. Scots pine, *Pinus sylvestris*, was chosen as the host plant because it can be colonized by all our EcM fungi (Colpaert and Laere, 1996; Colpaert et al., 1992). Commercial seeds were from Forstsaatgut-Beratungsstelle (Münster, Germany).

Modified Melin-Norkrans' (MMN) medium (Kottke et al., 1987) was used to cultivate EcM fungi. In order to prevent mycelia from growing inside the agar a sheet of cellophane was autoclaved in water at 121°C for 5 min, and then placed on the surface of agar (Cassago et al., 2002). Afterwards two mycelium plugs were placed on the cellophane and grown at 25°C for 3 weeks to produce inocula. In the control, only a sheet of sterilized cellophane was added without fungal plugs. Scots pine seeds were surface sterilized in 30% H_2O_2 for 15min, rinsed thoroughly, soaked in water for 4 hours, and then added to Petri dishes with 1% water agar

sealed with parafilm to maintain moisture and sterility during germination. Ten days later 200 germinated seeds were sown into 10 boxes (16cm×11cm×6cm, L×W×H) with a sterilized perlite and vermiculite mixture (1:1); each box had 20 seedlings. Seedlings were watered by tap water twice a week. After 4 weeks pre-growth in the climate chamber (75% humidity; 24/16°C, day/night temperature), the seedlings were inoculated.

Table 2-1: Species and families of ectomycorrhizal (EcM) fungal isolates used in this study

Family	Species
Hydnagiacea	*Laccaria bicolor*
	Laccaria laccata
Russulaceae	*Lactarius rufus*
	Lactarius theiogalus
Paxillaceae	*Paxillus involutus*
Rhiopogonaceae	*Rhizopogon roseolus*
Suillaceae	*Suillus bovinus*
	Suillus granulatus
	Suillus luteus

Inoculation and growth

Roots of seedlings were inoculated with EcM fungi using a “sandwich” method (Colpaert and Laere, 1996). Briefly, roots of a seedling were spread on a piece of cellophane with mycelia (treatment) or without (control), keeping roots in contact with inocula in case of treatment, covering them with two pieces of filter paper (Rotilabo-Rundfilter, Typ111A) soaked in modified MMN liquid medium, and then inoculated for 5 days. Seedlings were randomly assigned to treatments, at which point seedling fresh weight was recorded (before inoculation) for subsequent use of this measure as a covariate. Then each seedling was transplanted into a bleached plastic pot (1 L, 13cm x 14cm, WxH) with 800 g prepared soil. In all, there were nine fungal treatments and one control, replicated 8 times, for a total of 80 pots. Inoculated seedlings

were placed in a climate chamber (75% humidity; 24/16°C, day/night temperature) at random positions, and watered 3 times per week for 12 weeks until harvest.

Harvest and measurements

The seedlings were separated into roots and shoots. The harvested roots were analyzed by WinRhizo Pro 2007d (Regent Instruments Inc., Quebec City, Canada) to obtain root length and the number of root tips. Shoots and thoroughly washed roots were oven dried at 45°C to measure dry weights. Root were initially examined under the dissecting microscope (10 ×) for the presence of mycorrhizal structures, where mycelia ramifications and color changes of root tips were considered to be evidence of EcM root colonization. If there was no obvious morphological change observed, we used a staining method adapted from Vierheilig *et al.* (1998) to check for EcM colonization with a compound microscope (200 ×). For this, six root segments, each 2 cm of lateral root, were cleared in 10% KOH (90°C for 90 min), bleached with H_2O_2, acidified with HCl, and then stained with 5% ink-vinegar stain. Air dried soil passed through a 4 mm sieve to break up big lumps, soil was all collected and used to measure the following variables.

Hyphal length in the soil was determined according to Jakobsen et al. (1992). Briefly, 100 ml distilled water and 12 ml sodium hexametaphosphate solution (35 g l^{-1}) were added to 4.0 g of soil, and shaken end-over-end for 30 s. After allowing sedimentation for 30 min, the contents were poured onto a 38 μm sieve. The material on the sieve was transferred to an Erlenmeyer flask. 200 ml distilled water were added and the flask was gently shaken for 5 s. After allowing the material to settle for 1 min, 1 ml was taken from the solution, filtered through a 0.45 μm filter (Millipore), and stained with Trypan blue (0.05%). The filter disk was transferred to a glass slide, and hyphae were quantified at 200× using a line-intersect method. Because there is no way to differentiate between EcM and saprobic fungi, all hyphal structures were counted, with the idea that an increase in hyphal production would be revealed by comparison with the control.

For the pH ($CaCl_2$) measurement, 3.0 g soil was added to 15 mL 0.01 M $CaCl_2$, and stirred until a stable readout was reached. The total Bradford-reactive soil protein content was measured (Bradford, 1976) after extracting soil for 4 hours in citrate buffer (50 mM) Stacked sieves (53 µm, 212 µm, 0.5 mm, 1.0 mm, and 2.0 mm) were used to measure the soil aggregate distribution (starting mass of 50 g soil) and the mean weight diameter (MWD; Kemper and Rosenau, 1986). MWD was calculated using $MWD = (3\ mm * W_{2.0}) + (1.5\ mm * W_1) + (0.75\ mm * W_{0.5}) + (0.356\ mm * W_{0.212}) + (0.1325\ mm * W_{0.053})$, where W is the coarse matter corrected proportion of aggregates in each size class. Water-stable aggregates were determined according to Kemper and Rosenau (1986). Briefly, 4.0 g of dried soil were re-wetted with distilled water by capillary action on a 250 µm sieve. The wet soil was sieved in a wet-sieving machine (Eijkelkamp, The Netherlands) for 5 min. Subsequently, the water-stable aggregates were gently crushed to pass the sieve. After drying at 80 °C the mass of the water-stable aggregate fraction, of the coarse matter (>250 µm) and of the soil matter <250 µm was determined gravimetrically. The proportion of aggregated soil was corrected for coarse matter. Water drop penetration time (WDPT) was measured to determine soil water repellency (SWR; Hallett, 2007). Soil dried at room temperature was distributed on a plastic tray in a layer of approximately 2 cm. 8 water drops (8 µl each) were placed on the soil, and the time until the drop infiltrated was recorded separately for each drop, and then averaged per sample. Water-ethanol series dilutions were used to measure the water repellency of EcM fungal mycelium, with a method adapted from Dekker and Ritsema (1994). A series of aqueous ethanol solutions were prepared in 5% increments starting from 5% to 100% ethanol. A droplet (8 µL) of ethanol solution was applied on the inner zone of the mycelial mat grown on modified MMN agar. If the time interval of droplet infiltration was less than 5 s, the percentage of ethanol solution was recorded. Three fungal cultures were tested per isolate, and for each fungal culture three droplets were applied.

Statistical analysis

Data were transformed as needed to meet parametric statistical assumptions; however, non-transformed values are reported in the figures and tables. Linear models were used to carry out analysis of variance, analysis of covariance and correlations of our single factor experiment. Firstly, EcM fungal treatment effects on shoot biomass, root biomass, root length, hyphal length, water-stable aggregates, water drop penetration time, soil pH and soil protein content were checked, with starting seedling biomass as a covariate. Secondly, EcM fungal treatment on the soil size classes was tested by MANOVA combined with univariate tests on each size class to test for any difference between the EcM fungal treatment and the control. Thirdly, a pairwise correlation between root length, hyphal length, WSA, MWD, WDPT, soil pH, soil protein content and soil classes of 2-4 mm was applied. All analyses were performed with R software v. 302 (R Core Team, 2014).

Results

Growth of fungi and plant

Since some plants died during the experiment, only 68 samples were harvested rather than 80. All surviving plants were colonized by EcM fungi, except the controls. Meanwhile in all fungal addition treatments hyphal length in soil increased significantly by 34% to 61% compared to the non-inoculated control (Fig. 2-1) and the pre-experiment hyphal length of 9.0 m g^{-1}. Fungal treatments had no growth effect on pine seedlings; only *L. laccata* decreased biomass and root length (Table 2-2). The number of root tips was congruent with root data (data not shown). No significant difference was found in root volume, which was 0.13 ± 0.08 cm^3 (mean of all treatments and control ± SD).

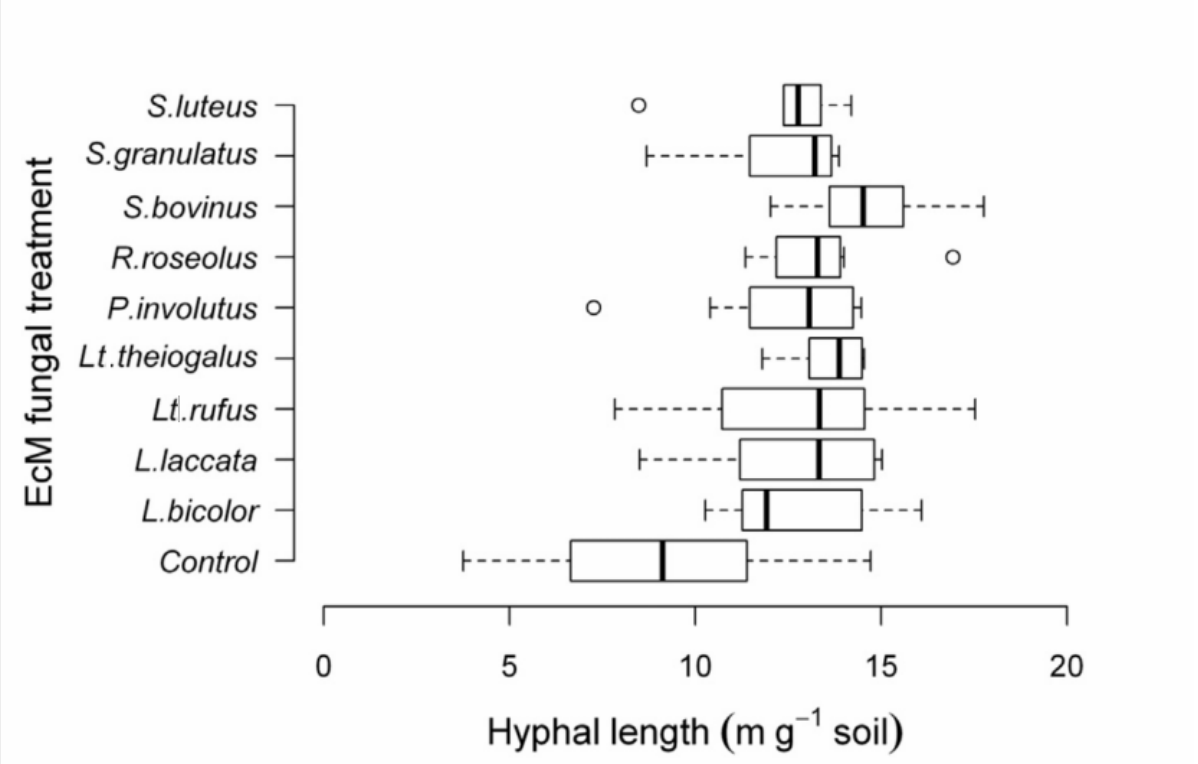


Fig. 2-1: Hyphal length in soil accumulated after treated with EcM fungi for 12 weeks.

Table 2-2: Effects of EcM fungi on shoot biomass, root biomass and root length of pine seedlings (standard deviation of the mean in brackets). Means in bold differ significantly (*p-value* < 0.05) from control in each column.

Treatments	Shoot biomass DW (mg)	Root biomass DW (mg)	Root length (m)
L.bicolor	98.61(36.21)	58.64(23.94)	1.96(0.85)
L.laccata	78.59(24.31)	51.73(15.73)	1.50(0.55)
L.rufus	162.17(88.48)	103.13(71.59)	3.31(1.98)
Lt.theiogalus	93.07(46.36)	52.49(25.77)	1.78(1.00)
P.involutus	88.75(22.35)	56.88(18.74)	1.70(0.39)
R.roseolus	98.05(33.56)	73.28(20.84)	2.10(0.59)
S.bovinus	113.49(43.75)	86.15(32.57)	2.48(1.01)
S.granulatus	69.95(14.81)	51.25(16.64)	1.35(0.34)
S. luteus	95.53(22.66)	56.10(17.92)	1.57(0.76)
Control	107.14(33.22)	90.60(44.88)	2.08(0.84)

DW: dry weight

Soil features under the influence of mycorrhizae

After 12 weeks of growth, WSA was significantly increased by 6 to 12% compared to the control for 6 of our isolates. The other three isolates showed a similar trend, but effects were not significant. MWD increased significantly for seven of the nine tested isolates (Table 2-3). Overall, WSA and MWD were weakly correlated (R = 0.30, *p-value* < 0.05); *R. roseolus* and *S. luteus* increased MWD without changes in WSA, and conversely, *S. granulatus* increased WSA without changes in MWD. Our MANOVA was significant (Pillai = 1.68; *p-value* < 0.001), and so we proceeded with univariate tests for individual size classes. The most pronounced changes occurred in size class 2-4 mm, with eight of the nine isolates causing significant increases, from 3% greater than initial values in the control to a maximum of 14% in the treatment with *Lt. theiogalus* (Fig. 2-2). Soil size classes from 500 µm to 2 mm did not change significantly compared to the control in any of the treatments. We observed significant

decreases in size classes <53 µm, 53-212 µm and 212-500 µm, only for isolates where the 2-4 mm size class increased.

For five isolates (*Lt. theiogalus, P. involutus, R. roseolus, S. bovinus, S. luteus*) there was a significant increase in soil pH, however the changes were minimal compared to the control. (Table 2-3). The soil protein content did not change, without any obvious trends compared to the control (Table 2-3).

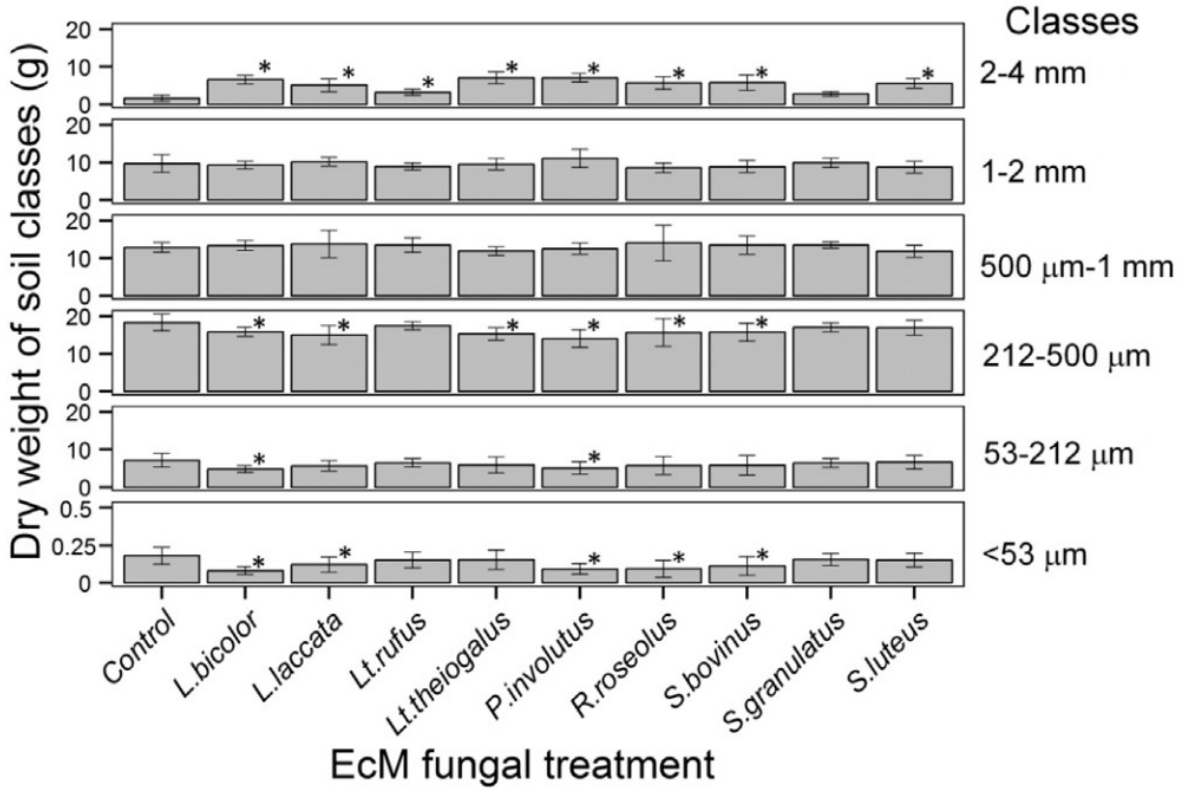


Fig. 2-2: Size distribution of soil aggregates under the influence of EcM for 12 weeks. Bars with asterisk differ significantly (*p-value* < 0.05) from the control.

Hydrophobic feature of isolates and soil water repellency

The hydrophobic features of mycelia growing asymbiotically on plates (without host plant) and soil water repellency of soil samples are shown in Table 4. Out of nine isolates, six were hydrophobic, with alcohol percentages ranging from 35% to 85%. Soil treated with isolates for which we found no evidence of mycelium hydrophobicity also showed no increase of WDPT. However, not all isolates characterized as hydrophobic led to significantly increased soil

WDPT. For example, *S. bovinus* and *S. granulatus*, even though they were the most hydrophobic isolates used in this experiment, did not increase WDPT.

Table 2-3: Soil parameters (WSA, MWD, WDPT, soil pH and soil protein content) and fungal mycelium hydrpobicity (standard deviation of the mean in brackets). Means in bold differ significantly (*p-value* < 0.05) from control in each column.

Treatments	WSA (%)	MWD	WDPT (s)	Soil pH	Soil protein content (mg g^{-1})	Mycelium hydrophobicity (as alcohol percentage)
L.bicolor	**68.04(4.38)**	**1.33(0.10)**	2(1)	6.87(0.04)	0.76(0.13)	0 (0)
L.laccata	**68.35(4.55)**	**1.24(0.09)**	5(2)	6.82(0.03)	0.85(0.04)	0 (0)
L.rufus	66.15(4.26)	1.07(0.08)	4(1)	6.83(0.03)	0.82(0.07)	0 (0)
Lt.theiogalus	**71.28(3.02)**	**1.34(0.18)**	**11(11)**	**6.84(0.03)**	0.85(0.04)	36.3(2.2)
P.involutus	**68.24(3.10)**	**1.41(0.13)**	3(1)	**6.86(0.03)**	0.76(0.06)	65.0(0)
R.roseolus	65.29(3.59)	**1.24(0.18)**	**10(8)**	6.85(0.04)	0.81(0.13)	72.5(1.8)
S.bovinus	**71.19(4.72)**	**1.25(0.21)**	3(1)	**6.86(0.03)**	0.75(0.07)	76.9(2.1)
S.granulatus	**70.19(4.74)**	1.07(0.09)	4(1)	6.81(0.02)	0.81(0.03)	83.1(2.1)
S. luteus	65.38(3.48)	**1.21(0.15)**	**9(8)**	**6.85(0.03)**	0.84(0.07)	84.9(1.6)
Control	63.92(2.84)	0.96(0.14)	3(1)	6.81(0.02)	0.79(0.09)	NA

NA: not available

The relationship between soil features and EcM associations

Root length was correlated with WDPT (R = 0.27, *p-value* < 0.05), but not WSA and MWD. Hyphal length was only weakly correlated with MWD (R = 0.27, *p-value* < 0.05), but not with WSA or WDPT. The correlation was more clear between hyphal length and aggregate mass in the 2-4 mm size class (R = 0.32, *p-value* < 0.05).

Discussion

In our greenhouse experiment using nine EcM fungal isolates we show that EcM associations significantly increased water-stable soil aggregation, with most pronounced changes occurring in macroaggregates in the 2-4 mm diameter size class. We cannot draw the general conclusion that EcM fungi enhance soil aggregation by only testing 9 isolates with a single host plant, but our results highlight the potential importance of EcM in soil aggregation and that this function of EcM deserves further study. We also show for the first time that three EcM isolates in association with pine seedlings induced SWR, as measured by WDPT. This extends our knowledge on fungi causing SWR, which has been shown previously also for AM fungi (Rillig et al., 2010), Ascomycetes (Chau et al., 2012) and Basidiomycetes (Spohn and Rillig, 2012). While it was no surprise that EcM fungi could induce WSA or SWR, it was interesting how WSA and SWR varied with different EcM fungi.

A third of our isolates were hydrophilic and the rest hydrophobic to some degree. Only hydrophobic fungal isolates induced SWR. The hydrophobicity of fungal hyphae may differ under different cultivation conditions, but our results are congruent with previous findings (Agerer, 2001; Unestam and Sun, 1995; Unestam, 1991). Not all hydrophobic isolates increased SWR indicating that mycelium hydrophobicity - at least as measured under our culture conditions - is a necessary but not a sufficient condition for SWR. Why did hydrophobic mycelium not always induce soil hydrophobicity in our study? Possible reasons may include inefficient coating and coverage of soil particles with hydrophobicity-inducing substances, either due to the mycelium growth form (e.g., branching, relative amount of thinner hyphae, degree of surface coverage) or due to rate of release of the substance into the soil. Data we collected here did not provide clues; for example our soil protein measurement did not provide additional information. Future studies should thus be specifically aimed at testing some of these ideas.

We assume EcM fungi follow the general mechanism by which fungi are hypothesized to affect soil aggregation: by gluing and enmeshing particles together (Rillig and Mummey, 2006; Six

et al., 2004). Our results show that irrespective of fungal exudates being hydrophobic or hydrophilic they could be binding agents for aggregates, sometimes creating water-stable aggregates, or not, as was the case for aggregates formed by *R. roseolus* and *S. luteus*. If fungal exudates as binding agents of soil are hydrophobic, fungi could promote WSA and SWR at the same time. SWR reduces the water penetration speed and protects aggregates from slaking, thus increasing aggregate water stability (Six et al., 2004). However, clearly non-hydrophobic mycelia could also produce aggregates, so increased SWR is not the only mechanistic pathway leading to stable soil aggregates produced by EcM fungi.

With our design it was not possible to disentangle the contributions of fungi, host plant and their interaction, which are all related to soil aggregation (Piotrowski et al., 2004; Rillig and Mummey, 2006; Six et al., 2004; Tisdall and Oades, 1982). Graf and Frei (2013) reported that with an EcM fungi-related increase in WSA there was also a promotion of plant growth. However, the effects of roots were likely negligible in our case (correlation between root length and WSA or WDPT was not significant). On the one hand, the pine seedling produced fine roots (average diameter was 0.3mm) of about 0.13 cm^3 in volume in 1 L pots. Thus, potential direct root effects were minimized by overall limited contact between soil and the root system in our pots. On the other hand, rarely (except for one fungus) did EcM inoculation cause morphological changes in roots in terms of root length or tip numbers. Thus, here EcM fungi hyphae likely were the major causal agent affecting changes in soil aggregation. However, hyphal length in our experiments was neither correlated with WSA nor WDPT. This is a finding similar to Piotrowski et al. (2004), who also found no such correlation when comparing the effects of five AM fungal species on soil aggregation.

Recent evidence suggests that mycelium components of EcM fungi may play a major role in the formation of stable N and C in SOM (Clemmensen et al., 2013; Treseder and Holden, 2013), which highlights the need to include mycorrhizal effects in models of global soil C stores. While there is increasing interest in EcM fungi in the context of soil C storage, our knowledge about fungal traits is quite deficient. Aspects of fungal hydrophobicity, for example, should be

incorporated in studies on soil C storage, since highly aggregated and repellent soils can lead to soil C protection and storage (Piccolo and Mbagwu, 1999; Six et al., 2004).We therefore suggest using a fungal trait-based approach to address the question of EcM-fungal mediated soil aggregation and C storage, as advocated for other fungal ecology questions recently (Aguilar-Trigueros et al., 2014), especially since hyphal length alone does not explain differences. Pertinent traits to include are quality and quantity of hyphal exudation and mycelial morphology (thickness, branching behavior and exploration type), because they may better approximate functional aspects of the mycelium with respect to soil aggregation.

Chapter 3

Does soil water repellency promote water-stable soil aggregation? A meta-analysis

Abstract

Soil water repellency (SWR) is a widely observed phenomenon where water is retained on the soil surface for a time before soaking in. High SWR not only causes problems in agriculture for prolonged irrigation and waste of water, but also might benefit soil by enhancing aggregate stability in post-fire, arid and seasonally dry ecosystems. For a better understanding of the mechanisms behind SWR, we performed a random-effects meta-analysis of 119 experiments from 27 publications, measuring aggregate stability (AS), the content of soil organic carbon and soil water repellency. We also explored how effects were moderated by soil pH, soil sand content, treatments (fire, microbe and organic matter), experimental setting (laboratory or field), plant type, sampling depth, sample drying methods, and SWR measurement (contact angle, water drop penetration time, repellency index and molarity of ethanol droplet). SWR, AS and soil organic carbon (SOC) were correlated positively, as were soil pH (4-8) and soil sand content. Experimental moderators did not affect SWR. We found that when soil was treated by fire the interaction between SWR and AS was different from other treatments, suggesting the presence of a different underlying mechanism. Two data issues were identified: (1) the effects of soil microbes and soil fauna were generally less well studied; and (2) the measuring conditions of SWR, especially soil water content, were not reported which caused problems in study comparisons. Our results emphasize the importance of integrating SWR and AS in soil research as interacting soil processes that influence soil stability and functions.

Introduction

Soil water repellency

Observations of soil water repellency (SWR) have been recorded as early as from the 1940s (DeBano, 2000). SWR is a phenomenon of retention of water on the soil surface rather than penetration (DeBano, 2000; Hallett, 2007). In the early stages of research, by summarizing the conditions for SWR, soil scientists began to associate this process with dry and wet cycles, fire, specific plant cover (Keizer et al., 2005b; Peng et al., 2003; Schnabel et al., 2013), fungi (York and Canaway, 2000), soil pH (Terashima et al., 2004; Wallis and Horne, 1992) and sandy soil texture (McHale et al., 2005; Nadav et al., 2011). At the same time, organic matter - especially due to its hydrophobic nature - is believed to be the origin of SWR. However, with the accumulation of data, the causes of SWR turned out to be more complex. Dry and wet cycles are not always needed to promote SWR (Peng et al., 2011); fire depending on its temperature can either break down SWR or promote SWR (Fox et al., 2007; Granged et al., 2011; Malkinson and Wittenberg, 2011); SWR can also be found at high pH or low pH (León et al., 2013; Terashima et al., 2004); and not only on sandy soil but also in clayey soil (DeBano, 2000). Soil scientists increasingly realize that SWR is caused by various mechanisms, with environmental conditions influencing the processes leading to SWR differently (Diehl, 2013).

AS and SWR connected by soil organic matter

Since both aggregate stability (AS) and SWR could regulate soil physical structure and soil chemical distribution, thus modifying soil biota habitats, it is necessary to study them together. Soil organic matter (SOM) plays a critical role in both processes. When soil aggregates are formed in a hierarchical way (Six et al., 2004; Tisdall and Oades, 1982), SOM serves as a binding agent (Sullivan and Koppi, 1987; Tisdall and Oades, 1982). This notion is supported by a positive correlation between AS and SOM under various environmental conditions in different soils (Chrenková et al., 2014; Miralles et al., 2009; Six et al., 1999).

To our understanding, SWR is mostly derived from hydrophobic SOM coating different sized soil components from soil particles to aggregates; however, SOM is not always correlated to SWR. This is because the quantity of SOM is not necessarily the most important factor, but sometimes the quality and even the distribution of SOM in the different size class of soil aggregates (Badía-Villas et al., 2014; Lugato et al., 2010), or whether SOM is located on the surface or contained inside an aggregate, can make a difference (Urbanek et al., 2007). In other words, SWR is correlated with the hydrophobic organic matter components on the surface of aggregates.

Soil water condition

Besides SOM, SWR and AS are connected by soil water conditions. Primarily, researchers consider SWR to play a negative role in soil and ecosystem functioning, because it stops water penetration after a seasonal drought or a fire, resulting in prolonged extreme dryness and consequently increasing erosion by preferential flow and surface flow, thus lowering the efficiency of agricultural irrigation (DeBano, 2000). However, SWR may also prevent soil aggregates from breaking down because of slower water infiltration (Bisdom et al., 1993; Vogelmann et al., 2013b). In the process of soil aggregate breakdown, fast wetting is more effective than slow wetting (Sullivan, 1990). This is because when water enters into the aggregate, there is a sudden increase of force (Kemper and Rosenau, 1986). The delayed time course of rewetting after extreme dryness is a way to protect fragile aggregates from the strong force of sudden wetting. Wet and dry cycles can rearrange the amphiphilic molecules coating soil aggregates that lead to SWR (Diehl, 2013; McHale et al., 2007); potentially, SWR could protect dehydrated aggregates as an ecological function. The wet and dry cycles could also change the microbial community (Schimel et al., 1999), since microbes have differential preferences for water content (Uhlírová et al., 2005). This could be a reason why fungi contribute to SWR, since fungi can remain operational at low water potentials.

SWR measurement methods

There are four basic ways to measure SWR: contact angle, intrinsic sorptivity or repellency index (R index), molarity of an ethanol droplet (MED) and water drop penetration time (WDPT). MED is also called alcohol percentage test in some literature (Dekker and Ritsema, 1994; King, 1981). Contact angle and R index are physically meaningful but hard to conduct, while MED and WDPT are easily carried out (Hallett, 2007). Many researchers tested how comparable those methods are (Chau et al., 2010; Doerr, 1998; Leelamanie et al., 2008; Roy and McGill, 1998). We are also interested in the question of whether SWR measuring methods introduce a bias in the study of SWR.

Focus of our study

In agriculture, prolonged irrigation caused by SWR is a big concern. In a review summarizing remedies for SWR, Müller et al. (2011) suggested that a better understanding of the fundamental mechanisms of how and why SWR develops was needed. We here conducted a meta-analysis based on published studies testing the relationship between SWR and AS. We specifically wanted to answer these questions: (1) is SWR positively correlated with AS? (2) is soil organic matter related to SWR? (3) do edaphic conditions affect SWR? (4) does experimental design interfere with the results?

Materials and methods

Data collection

A literature search for studies measuring AS and SWR was initiated with the following search string, [soil and aggregat* and (water repellen* or hydrophobic*)]. The search was conducted on 22 May 2014, using the Web of Science Citation Index Expanded database and including all articles published since 1956. The initial search returned 371 publications, however we only found 27 studies that reported control and treatment data for SWR, along with sample sizes (N) and either standard deviation (SD) or standard error (SE). The 27 studies are listed in the supplementary materials (S3). With the abovementioned search terms we found many studies that only mentioned SWR as a factor which might affect soil aggregation in the discussion but did not actually measure SWR. When necessary, the information was retrieved from digitized graphs with PlotDigitizer v2.6.6 (http://plotdigitizer.sourceforge.net). When SE was reported, we calculated SD as: SD = SE×sqrt (N). We assumed that unidentified error bars represented SE.

When one publication reported effects in more than one system, we treated each as a separate trial. In order to be considered as a separate system, experiments had to differ in at least one of the following factors: study site, plant type, type of organic matter added or temperature of fire set to induce SWR. When studies reported results for multiple time points, only the time point with the highest value of SWR was considered. Observations without replication in multiple sites whose soil parent material or texture were the same were recalculated to acquire means and SEs of SWR. In this case, non-repellent adjacent sites were used as control. The dataset of 27 studies yielded a total of 119 separate trials.

The effect size and factors

The effect size of SWR calculated for all statistical analyses was the log response ratio (rrSWR). It was calculated as

$$ln(\text{RR}) = ln\left(\frac{\bar{X}_1}{\bar{X}_2}\right), \quad (1)$$

where $\bar{X}_1$ is the mean of the treatment group and $\bar{X}_2$ is the mean of the control group. Variance was calculated as

$$V = \sqrt{\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}}\left(\frac{1}{n_1X_1^2}+\frac{1}{n_2X_2^2}\right), \quad (2)$$

where n_1 and n_2 are the sample sizes in the two groups, and SD_1 and SD_2 are the SDs in the two groups (Borenstein et al., 2009).

As shown in equation 2, if the SDs of both control and treatment groups are zero the variance will be zero, which causes an error (non-positive matrix was generated) and those studies will be excluded in the meta-analysis with our chosen method, which is residual maximum likelihood (REML). Even though all experiments included reported SDs, when SWR was measured by water drop penetration time (WDPT), the SDs of both groups could be zero. So, to avoid the error, the smallest meaningful number (which is 1 in WDPT) was assigned to the SD of the treatment group in the trials where both SDs were originally zero.

To answer the questions of how aggregate stability affected soil water repellency, we extracted the AS measurements of control and treatment groups of each trial. We calculated the log response ratio of AS following equation 1 (hereafter called rrAS) to standardize the differences of initial AS and treatments among different trials. Instead of the contents of soil organic carbon (SOC), the contents of soil organic matter were reported in some studies but less frequently. Thus we obtained the SOC content by following the equation that is soil organic carbon (%) = soil organic matter (%) ÷ 1.72 (Soil Quality, 2016). Similar calculations were done to obtain log response ratio of SOC (here after called rrSOC) from the content of SOC for exploring the SOC effects on SWR. Both rrAS and rrSOC were tested as moderators in a single variable meta-regression.

In addition, other factors that might affect SWR were extracted from the studies. They either influenced the results in terms of setting, such as the experiments taking place in the field or in the laboratory, or related to mechanism, such as soil pH and soil sand content. They were used

either as continuous or categorical explanatory variables. We minimized the number of levels of categorical moderators to maximize the statistical power of our tests. We either merged information on closely related categories or excluded poorly represented trials.

The remaining moderators tested in the meta-analysis were:

1) **Setting**: The location of the experiment had two levels: field and laboratory.
2) **Experiment duration**: Although experiment duration was reported as a continuous variable in studies, it varied from hours to more than 20 years. Thus, we grouped it as a categorical variable with two levels: short (less than 5 months) and long (more than 5 months). If there was more than one time point in a study, the time point of the highest SWR was taken.
3) **Treatment**: The causes of SWR were included as a categorical variable. It had three levels: organic matter addition, fire and microbe. **Burning temperature** had two levels: 'low' (0-200 °C) and 'high' (>200 °C).
4) **Sampling depth**: There was a large variability of soil sampling depth in the studies. In order to detect differences we included the following classes of sampling depth: 0-10 cm, 10-70 cm and 0-70 cm.
5) **Drying temperature**: There were two groups for the soil drying temperature, which were 'heated' and 'room temperature'.
6) **Plant type**: This variable had three groups: 'crops', 'tree' and 'non-tree'. Other plant groups were poorly represented.
7) **Soil pH**: This was included as a continuous variable. The solvents (H_2O, $CaCl_2$ or KCl) used to measure soil pH were not reported in most studies, therefore we could not differentiate between them.
8) **Soil sand content**: Values for sand content in the experimental soil were either directly reported or deduced from the information given on texture using the 'texture triangle' according to the classification of the United States Department of Agriculture. This was a continuous variable.
9) **Methods for soil water repellency measurement**: Four types of SWR data were reported,

including water drop penetration time (WDPT), molarity of ethanol droplet (MED), repellency index (R index) and contact angle.

Analysis and statistics

All analyses were conducted in R 3.2.2 (R Core Team, 2014), with the 'metafor' package (Viechtbauer, 2007). We performed a meta-analysis using a mixed-effects model where the overall analysis was performed with a random-effects model using the method of REML. The correct *p*-values were obtained from permutation tests (3999 times; Knapp and Hartung, 2003).

Two independent subgroups, studies that included burning and others, were tested separately. We set trials treated by either wildfire or experimental fire, as group *fire*, and trials in which SWR stemmed from other sources, i.e. microbes and organic matter, as group *non-fire*.

We conducted bootstrapping with the 'boot' package (Canty and Ripley, 2015) to double check if CIs were correctly estimated. The independency of categorical and continuous moderators was tested by Chi-squared contingency table analysis and Spearman correlation, respectively. Here, all graphs were drawn in R with the package 'ggplot2' (Wickham, 2009).

Publication biases were analyzed by plotting the effect size rrSWR against the sample size (replicates) and within-study variance (Egger et al., 1997). The robustness of the summary effect size estimates had to be verified for any disproportional impact of single studies. Therefore, a sensitivity analysis (Copas and Shi, 2000) was performed to identify studies with an exceptionally high or low effect.

Results

Data validation

We did not detect publication bias (Fig. A-1). Although three studies were identified as potential outliers in the stepwise sensitivity tests on moderator rrAS using the entire dataset (Fig. A-2 to A-5), no data were excluded, because we considered the potential outliers as representing different response patterns of SWR on aggregation stability.

The relationships among moderators

Chi-squared contingency table analysis showed that most combinations of moderators were dependent (Table 3-1), which means that effects of one moderator cannot be completely separated from effects of other moderators. Spearman correlation tests showed that sand content and soil pH were independent from other moderators except rrAS and rrSOC, which were highly correlated with each other (statistics not shown). Meta-regression of rrAS and rrSOC is shown in Fig. 3-1.

Table 3-1: Results of chi-squared contingency analysis for independence of moderators. Number of levels of each moderator are shown in bold on the diagonal. Chi-squared statistics are shown above the diagonal. *P-values* are shown below the diagonal.

	Setting	Treatment	Plant type	Duration	Depth	Drying	SWRM
Setting	2	20.04	12.85	55.54	10.01	7.63	21.39
Treatment	<0.001	3	36.2	17.33	6.75	26	51.39
Plant type	0.002	<0.001	3	11.51	9.34	6.76	22.24
Duration	<0.001	<0.001	0.003	2	16.23	6.48	27.44
Depth	0.007	0.15	0.053	<0.001	3	15.3	54.54
Drying	0.006	<0.001	0.034	0.011	<0.001	2	15.07
SWRM	<0.001	<0.001	0.001	<0.001	<0.001	0.002	4

Setting (experiment set in laboratory or field); Treatment (experiment treated by fire, organic matter and microbe); Plant type (tree, non-tree and crops); Duration (short-term and long-term); Depth (sampled from 0-10 cm, 10-70 cm and 0-70cm); Drying (samples dried at room temperature and in oven); SWRM (methods for SWR measurement)

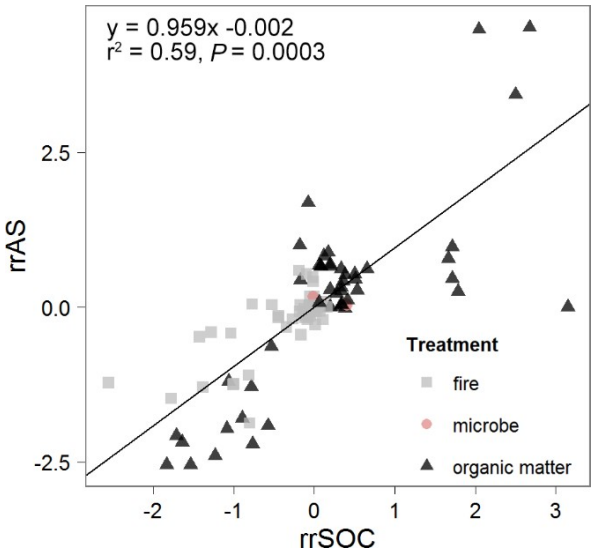


Fig. 3-1: Meta-regression for log response ratio of AS and log response ratio of SOC. The reported *p-value* was obtained from the permutation test.

The effects of moderators

Among the categorical moderators, experimental duration significantly affected the rrSWR, while the other tested moderators did not (Table 3-2 and Fig. 3-2). Experiments of short duration had significantly more positive responses (Table 3-2). All continuous moderators: rrAS, rrSOC, soil pH and soil sand content influenced rrSWR (Table 3-3 and Fig. 3-3).

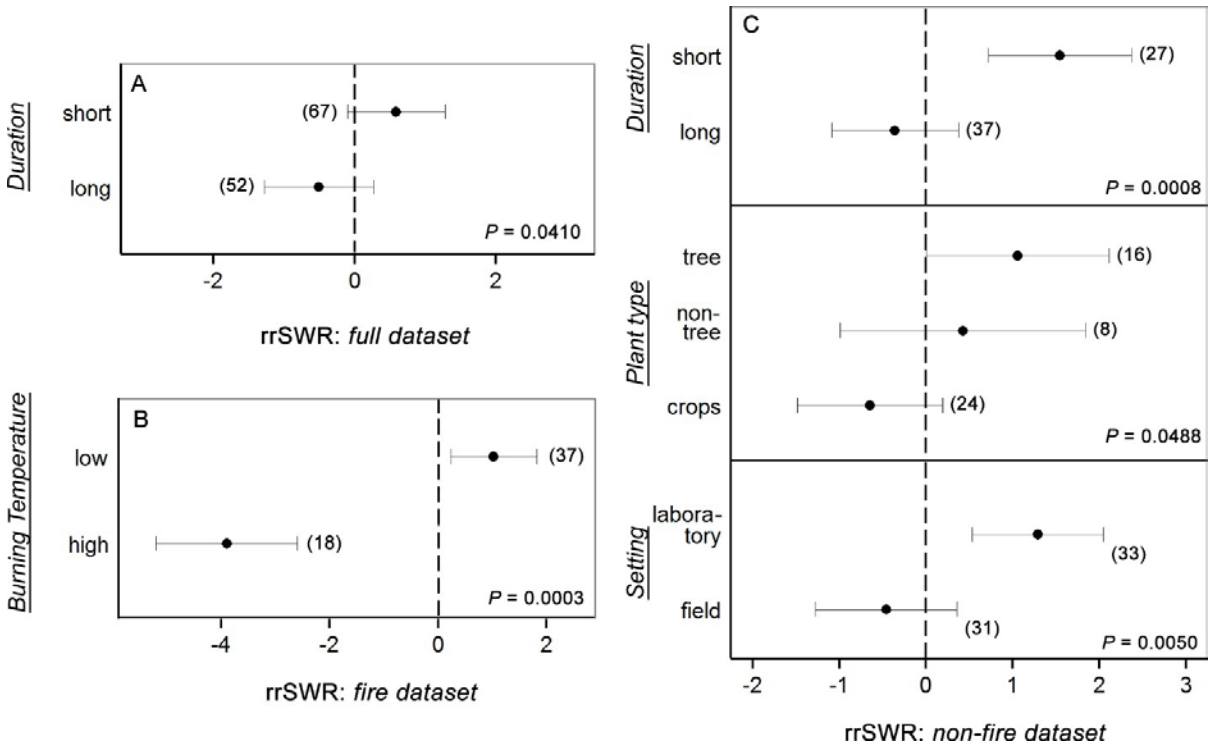


Fig. 3-2: Means and CIs of rrSWR for the significant moderators for the full dataset, fire dataset and non-fire dataset. Numbers in parentheses refer to the number of trials at the respective level. *P-values* were obtained from permutation tests (3999 times).

Table 3-2: *P-value*s of moderators effects tested on the full data set (3999 times iteration and $\alpha = 0.05$; significant moderator effects please refer to Fig. 3-1). Moderator levels are followed by the number of trials present in the level. The significant *p-values* are in bold.

Moderators	Level (No. of trials)	*p-values*
Setting	Laboratory (61) Field (58)	0.3840
Treatment	Fire (55) Microbe (16) Organic matter (48)	0.1600
Plant type	Tree (49) Non-tree (15) Crops (25)	0.1540
Experiment duration	Long (52) Short (67)	**0.0410**
Sampling depth	0-10 cm (75) 10 – 70 cm (17) 0-70 cm (10)	0.9790
Sample drying method	Room temperature (88) Oven dry (31)	0.1750
Measurement of SWR	Contact angle (12) MED (15) R index (16) WDPT (76)	0.7860

Table 3-3: Results of meta-regression on the full dataset, *fire* dataset and *non-fire* dataset. The *p-values* were obtained from permutation tests.

Dataset	Moderator (N)	Equation	*p-value*
full			
	rrAS (N = 118)	y = 1.83x + 0.12	0.0003
	rrSOC (N = 101)	y = 1.84x - 0.07	0.0003
	Soil pH (N = 115)	y = 0.54x - 3.03	0.0175
	Soil sand content (N = 114)	y = 0.03x - 1.17	0.0113
fire			
	rrAS (N = 55)	y = 4.08x + 0.45	0.0003
	rrSOC (N = 49)	y = 4.18x + 0.98	0.0003
	Soil pH (N = 55)	y = 0.58x - 3.48	ns
	Soil sand content (N = 55)	y = -0.01x +0.07	ns
non-fire			
	rrAS (N = 47)	y = 1.01x + 0.34	0.0003
	rrSOC (N =44)	y = 1.26x - 0.08	0.0003
	Soil pH (N = 43)	y = -0.02x + 0.04	ns
	Soil sand content (N = 43)	y = 0.04x -1.32	0.0003

ns: not significant

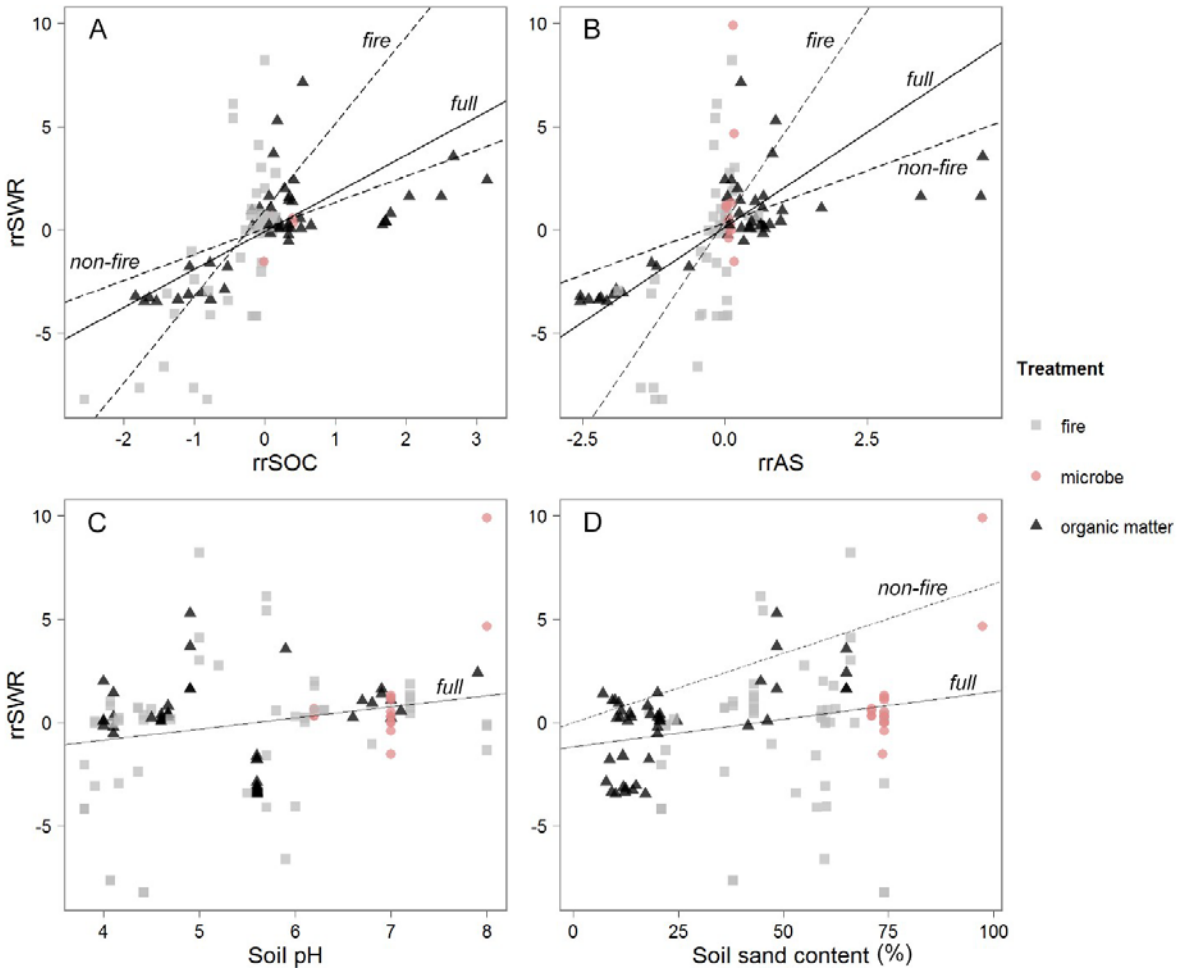


Fig. 3-3: Meta-regression between rrSWR and continuous moderators (rrAS, rrSOC, soil pH and soil sand content). The regression equations and *p-values* are shown in Table 3. Solid lines are on the full dataset and dashed lines are on data subset fire or non-fire.

The effects of moderators on data subset

In the *fire* dataset the burning temperature had a significant effect on rrSWR (Fig. 3-2B). Burning at high temperatures (> 300°C, N = 18) led to a sharp decrease of SWR, while low temperatures induced SWR (< 300°C, N = 37). In Fig. 3-2C (*non-fire* dataset), short-term and/or laboratory studies had a positive impact on rrSWR, but not long-term and/or field studies. Studies with trees as experimental plants had positive SWR responses (N = 16).

Discussion

Meta-regression among SWR, AS and SOC

As expected, we found positive relationships between the log response ratios of SWR and SOC (Fig. 3-3A) using our meta-analytical approach. This result is in line with the results of individual studies; for example, a study on 864 samples which found positive relationships between soil organic matter and SWR (Täumer et al., 2005) and another one on calcareous topsoils from fire-affected pine forest (Mataix-Solera and Doerr, 2004). Blanco-Canqui and Lal (2009) found that SOC explained more variation in AS than in SWR using soil with different crop residues, which is similar to our results. For AS, the importance of SOC is usually discussed in the context of quantity, whereas for SWR, the quality of soil organic matter is more important (Badía-Villas et al., 2014; Lugato et al., 2010; Urbanek et al., 2007). To function as a binding agent gluing particles together, organic matter does not have to be hydrophobic, but only hydrophobic organic matter can induce SWR. If the functionally important compounds have both effects, we can observe a correlation among AS, SOC and SWR (e.g. Giovannini et al., 1983). If SOC compounds are amphiphilic, relationships become more complicated (Diehl, 2013). They can turn hydrophobic under certain conditions, e.g. increased soil moisture (Kajiura et al., 2012), and hypothetically bind together aggregates with different surface polarities (Shein and Milanovskii, 2003). Hydrophobins produced by filamentous fungi are a group of amphiphilic compounds that potentially contribute to the connection between SWR and AS (Rillig, 2005).

We propose that the correlation between rrSWR and rrAS is not just a covariance caused by shared organic matter compounds, but reflects a mechanistic link. For example, swelling, slaking or mechanical forces like raindrops on the soil surface and traffic affecting the soil body are involved in aggregate breakdown; SWR could play a positive role in protecting soil aggregates against these consequences of swelling and slaking, which are water-related disturbance processes (Zaher et al., 2005; Vogelmann et al., 2013b).

In a review, Goebel et al. (2011) proposed that SWR was an important soil organic matter stabilization mechanism that could become more important because of the increase in extreme events with global change. This hypothesis can be true under two terms (1) the extreme conditions, i.e. abiotic factors, can induce increased production of hydrophobic compounds and/ or these conditions could be more suitable for turning amphiphilic compounds into hydrophobic ones; (2) the hydrophobicity of soil itself could contribute to soil stability. For example, SWR slows down the wetting process and therefore stabilizes aggregates (Sullivan, 1990). Since Goebel et al. (2011) used more frequent and intensive droughts and heat waves as the examples of extreme conditions, the causality appears reasonable.

However, if the extreme condition considered is flooding, the effects of SWR on soil stability may be the opposite. SWR plays a negative role in disturbances like raindrops. Increasing SWR was associated with higher splash erosion (Ahn et al., 2013; Fox et al., 2007) and increased amounts of surface runoff (Pierson et al., 2011). Erosion is another aspect of soil stability which is different from aggregate stability, so the overall effect of SWR on soil stability should be broken down into different aspects associated with partially opposite mechanisms.

The causal relationship between SWR and AS is not unidirectional. McHale et al. (2005) showed in a model that the roughness of a surface could also change its hydrophobicity, because uneven surfaces create gaps filled with air that enhanced hydrophobicity; and in the simplified model a rougher surface was equivalent to a surface with larger air gaps. The pore size of a soil changes as macro-aggregates form, leading to more, larger air gaps on the surface, hence connecting AS and SWR. González-Peñaloza et al., (2013) showed that larger aggregates were more hydrophobic. However, Goebel et al., (2004) found that water-repellency was higher in homogeneous soil samples, perhaps because grinding could change both surface roughness and organic matter distribution. Clearly, the relationship between AS and SWR is complex.

Fire and its burning temperature affect SWR

For the first time, we show that burning alters the relationships between SWR, SOC, and AS from those seen when organic matter and microbes were used as treatments (see Fig. 3-3A and 3B), despite the fact that the treatments (fire, organic matter and microbe) did not influence the overall SWR response (Table 3-2). When soils were burnt (wild fire, setting fire in the field or setting fire in the laboratory) the burning severity (temperature and time; Thomaz et al., 2014) determined the response of SWR. In our analysis, at temperatures higher than 300 °C, burning decreased rrSWR, whereas lower temperatures increased SWR (Fig. 3-2B). This agrees with the idea that high heat eliminated most organic matter compounds which cause SWR, while moderate heating modified organic matter compounds which can increase SWR (Thomaz et al., 2014; Zavala et al., 2010). However, not all experimental findings agree with this pattern. Llovet et al. (2008) reported that SWR increased at a high burning intensity while it decreased at lower intensities. They found that burning changed neither organic matter content in quantity nor aggregate stability (measured with artificial raindrops). Fox et al. (2007) found that with decreased organic matter, soil had increased MWD and WDPT. This may suggest that hydrophobic features of organic compounds are associated with heat-resistance. However, during burning, fire may have already broken down aggregates at low temperatures (Urbanek, 2012), and in the later rewetting phase SWR could protect already damaged aggregates from breaking down completely.

Microbes and SWR

Research on contributions of microbes to soil stability is biased towards certain organism groups. Ideas about mechanisms of soil microbe action on AS are well developed (Rillig and Mummey, 2006; Six et al., 2004), while for SWR they are still mostly unclear (DeBano, 2000; Dekker et al., 2005; Rillig, 2005). Most studies about microbes were focused on observations of microbes promoting SWR but not on the underlying mechanism (Bond and Harris, 1964; Savage et al., 1969; Zheng et al., 2014). In this study, we found only 16 trials that targeted

microbes as causal agents or treatments, far less than burning (N= 55) and organic matter (N= 48) treatments. In some studies soil microbes were indirectly involved through measuring respiration when organic matter was added. The connection between microbial hydrophobic exudates and SWR has been made (DeBano, 2000), but adding microbes producing hydrophobic compounds to an experimental system did not always show hydrophobicity (Bond and Harris, 1964; Zheng et al., 2014). We summarize four potential scenarios corresponding to suppression of SWR when hydrophobic microbes are present in the environment: (1) microbial growth conditions are not sufficient to produce hydrophobic compounds (Wessels, 2000); (2) abiotic conditions, such as soil pH and moisture, are not conducive to turning amphiphilic organic matter hydrophobic by outward orientating hydrophobic organic moieties (Diehl, 2013; Linder et al., 2005); (3) hydrophobic compounds are not stable, being consumed by soil organisms and only increasing SWR for a limited period, thus perhaps not even being recorded; (4) microbes are not able to explore the vast soil surface area efficiently, and thus cannot spread hydrophobic compounds to coat enough particles to produce macroscopically detectable SWR. We do not consider any of these scenarios more likely than another, but their complex interaction could explain the high heterogeneity of SWR in soil. More research is required to define the conditions under which each of these scenarios operates.

Effects of soil pH and sand content on SWR

As we expected, rrSWR increased with soil sand content. Based on the regression equation we obtained, using soil with higher than 40% sand content is likely to produce a positive response in SWR. SWR has a higher chance of occurring in sandy soil, but this is not exclusively so. Soil texture can influence both AS and SWR through changing particle distribution, as discussed in the first section in discussion.

Soil pH had a weak positive effect on rrSWR in acid soil. The range of pH reported in the published studies was from 4 to 8, with 17 trials reporting non-acid soil pH. Thus, the positive relationship cannot be extrapolated outside of this range. A neutral pH could be optimal for maximizing SWR, because when the density of charged sites is minimal, either increasing or

decreasing pH along with the development of (negative or positive) surface charges could rearrange micelles to have outward-oriented hydrophilic groups (Diehl, 2013).

Experimental setting influenced SWR

We found that when adding organic matter into the study system, short-term laboratory trials (N = 27; all short-term trials were done in the laboratory) had positive rrSWR. In this type of experiment, freshly added organic matter caused increased microbial growth and even though the associated organic matter was not stable in the long-term, positive results were still recorded. However, in the *full* dataset, there was no effect of experimental setting and duration indicating that the overrepresentation of short-term laboratory results likely did not bias our results.

Measurements for SWR

In line with previous studies finding a high correlation between different measurement methods of SWR (Cosentino et al., 2010; Keizer et al., 2005a; Wessel, 1988; Zavala et al., 2010), we did not find differences among the different methods. One aspect to note is that the thresholds for soil to be categorized as hydrophobic or hydrophilic based on the different measurements are not comparable. Each method has its own hydrophobicity threshold, for contact angle it is 90°, for R index 1.95, for MED 3% (v/v) and for WDPT it is 5s or 10s (Doerr, 1998; Moody and Schlossberg, 2010; Wessel, 1988). For example, a sample whose WDPT is greater than 10s does not necessarily have a contact angle greater than 90°. We suggest the use of response ratios to compare results obtained from different measurements.

Other potential factors and SWR

We did not find evidence of plant type and soil sampling depth affecting rrSWR, which perhaps arises from coarsely reported data for these moderators. In addition, there are more factors to consider, including particle size distribution, water potential or water content of the soil sample. But there were insufficient data on these moderators to include them in the meta-analysis. Many studies on SWR have many potentially confounding factors; they were either not specific

enough in terms of work on certain mechanisms for our purposes (Shakesby et al., 2000) or did not report key factors like water content. Water status, measured commonly as soil water content and water potential, has been shown to influence SWR measurements (Goebel et al., 2004). One study showed that SWR could be altered by different sample preparation methods at different temperatures (air, oven or freeze drying; Spohn and Rillig, 2012). Karunarathna et al. (2010) showed that at different water contents soil has the highest value of SWR and for some soils air dried samples were not water-repellent. And Kawamoto et al. (2007) found similar results in volcanic ash soil. Actually, when measuring SWR as WDPT, Blanco-Canqui and Lal (2009) suggested that soil be at a standard water potential of -166 MPa (equal to air-dry). Even if we do not use a standard water status for SWR measurements, if the chosen conditions are not reported, comparisons among studies are challenging.

Conclusions

We found 27 studies that quantitatively addressed the question of the relationships among SWR, AS and SOC; the results showed a positive relationship among these variables, which supports the hypothesis that SWR and AS are affected by SOC and interact with each other. More studies are needed to reveal the mechanisms behind these interactions. In addition, we found fire as an extreme treatment altered interactions between SWR and AS. The role of microbes in SWR needs more attention, since they contribute to the production and distribution of SOC. We found that experimental setting (laboratory or field), the commonly used methods for assessing SWR and sample drying temperature did not affect SWR in our dataset.

The current studies on this topic covered few aspects of numerous antagonistically and synergistically interacting environmental factors of SWR. We found SWR increased with increasing soil sand content and soil pH (ranging from 4 to 8). However, the data quality of the moderators plant type and sampling depth was not optimal. Furthermore, soil water content was rarely reported in studies. In the future, more constrained experimental designs and more detailed reporting of the conditions potentially affecting SWR would allow for a greater understanding of the mechanisms controlling SWR.

Chapter 4

Enzyme activities, growth features and water related traits of saprotrophic fungi: studying saprotrophic fungi affecting soil formation and stability using a trait-based approach

Abstract

Using a trait-based approach to address how saprotrophic fungi affect soil formation and stability, we tested fungal traits and their phylogenetic signals on growth features (colony extension rate (K_r) and biomass density), enzyme activities (acid phosphatase, cellobiohydrolase, leucine aminopeptidase and laccase) and water-related features (mycelial water content and hydrophobicity of fungal surface (HFS)) of 31 saprotrophic fungi from Basidiomycota, Ascomycota and Mucoromycotina isolated from a grassland. The factors which could influence traits distribution were also tested: the growing surface of agar and cellophane overlaid agar and the growth phase of the colony. We found that the cellophane membrane affected some of the strains morphologically and physiologically. The colonies had higher enzyme activities at a younger age. But the shifts of enzyme activities did not lead to changes in phylogenetic signals. We also found that HFS, the activity of leucine aminopeptidase and biomass density had phylogenetic signals. The fungal lifestyles showed by the combination of chosen traits were grouped highly similarly to the phylogenetic groups of the strains. Our results suggest that HFS and mycelial water content are two crucial fungal traits, and that plasticity of fungal traits should be concerned in building fungal trait databases.

Introduction

Trait-based approach

The trait-based approach is frequently used in evolutionary biology and community, ecosystem and microbial ecology to unravel mechanisms and functioning at the system level by exploring features on the individual level (Ackerly and Cornwell, 2007; Garnier and Navas, 2012; Powell et al., 2013; Rillig et al., 2014). This method is well established for understanding functions in plant ecology, shown by the elaborate plant trait databases, such as TRY: an integrated database that includes 5 million plant trait records (www.try-db.org/). In microbial ecology, despite the attention received from soil and fungal scientists on the potential of the trait-based approach, its development is held back by the absence of microbial traits databases (Aguilar-Trigueros et al., 2014; de Vries et al., 2012; Powell et al., 2013). Therefore, we have initiated a project about how fungal traits are related to soil structure formation and stabilization. We focus on soil and soil fungi, because soil aggregation as an ecosystem function has so far only been cursorily addressed in the context of trait diversity (Pérès et al., 2013) and not at all in its relation with soil fungi. Soil fungi are a group of the most abundant and active microbes in the soil ecosystem whose contribution to soil aggregation has long been noted and studied (Rillig and Mummey, 2006; Ritz and Young, 2004; Six et al., 2004).

A fungal trait describes a certain morphological, physiological or phenological feature measured on an individual, in the case of fungi for example a colony (Aguilar-Trigueros et al., 2015; Violle et al., 2007). In this study we collected quantitative data of chosen traits to build a trait database as a first step and to lay the foundation of the project. One concern in regards to trait databases is how interspecies and intraspecies traits data from a broad gradient of environmental or experimental conditions can vary as a result of the plasticity of trait expression (Powell et al., 2013; Reich et al., 1999). Despite this potential source of variation, different plant traits still correlated with each other and formed certain life strategies across diverse taxa (Reich et al., 1999; Westoby et al., 2002). Here, we also looked into whether the

same convergence of traits are also shown in fungal species based on the selected three groups of traits: growth, enzyme and water-related.

Fungal growth traits in linear growing phase

Traits of living organisms vary along their lifespans. For filamentous fungi, generally four distinct phases in their life spans could be distinguished, namely, the lag phase, the log phase, the linear growing phase and the stationary phase (see Fig. 4.7 from Moore et al., 2011 modified from Trinci, 1969). The last three phases correspond to the exponential growth zone, productive zone, fruiting zone and aging zone of a fungal colony growing on solid nutrient medium (see Fig. 4.10 from Moore et al., 2011). When fungi grow on solid medium, colony extension rate (K_r), a constant in the linear growth phase, is used to describe the colony while exploring nutrients horizontally; and biomass density (dry biomass per area) represents how fungi accumulate biomass, also including the thickness of the colony. These growth related traits are relatively well quantified since the measurement is easy. Thus, we chose K_r and biomass density to represent growth traits in this study. It is well documented that fungal growth rates can range from a few micrometers to millimeters per hour (Moore et al., 2011), therefore, if we standardize the total cultivation period in an *in vitro* petri dish system, at the sampling point, different fungi could be in different growth phases, thus leading to problems in the comparison of traits. Additionally, hyphae in different phases could have different properties. Thus, we standardized growth phases of fungi by measuring all traits in the linear growth phase of each fungus (the specific period was determined based on a preliminary experiment).

As decomposers in the ecosystem, the functions of fungi are realized by producing a large variety of enzymes (Burns et al., 2013; Caldwell, 2005), for instance during litter decomposition (Allison, 2012). Fungi also obtain access to food resources by regulating enzyme exudation (Moore et al., 2011). There are three large groups of enzymes working on decomposing carbon, nitrogen and phosphorus resources; fungi from different taxa could cover very limited or broad types of enzymes depending on their life strategy (Caldwell, 2005). To

better understand strategies of fungi in enzyme exudation, we chose acid phosphatase and aminopeptidase that decompose phosphorus and nitrogen, respectively; in addition, laccase and cellobiohydrolase were used to study carbon decomposition. Fungi vary in producing enzyme acting on easily decomposing substrates (e.g. glucose, starch) or such that are harder to attack (e.g. lignin and cellulose).

Fungal enzyme traits and cellophane effects on traits collection

Enzyme traits of fungi are well known to be growth phase dependent; different combinations of enzymes can be secreted in the early or later phase depending on their function (Dorn and Rivera, 1966). To disentangle the growth phase influences on enzyme activities we sampled from the actively growing outer zone (equivalent to exponential growth zone and productive zone) and the more stationary inner zone (equivalent to aging zone, sometimes fruiting zone) (Fig. 4-1).

Cellophane membranes have long been widely used to separate fungal materials from solid medium while allowing the diffusion of water and nutrients (Cassago et al., 2002; Dusseau, 1938; Katz et al., 1972; Liu et al., 2010; Reeslev and Kjoller, 1995). However, the effect of cellophane membranes on fungal traits have not been carefully studied. Thus, we used cellophane membranes not only to collect fungal mycelia for enzyme measurements, but also to test for cellophane effects on fungal traits by dividing the medium surface into agar surface and cellophane-overlaid surface (Fig. 4-2). Besides enzyme traits, all other tested traits were measured on both sides as well.

Mycelial water content and hydrophobicity of fungal surface

Water is crucial for all organisms. It is not only the solvent providing the medium for all reactions (Hudson, 1992), but also the major constituent of fluids in organisms including fungi. In fungal biology, water is usually studied in terms of how water activity (water availability) affects fungi. For example, soil moisture influenced fungal enzyme activity and community composition (Brockett et al., 2012; Herron et al., 2009; Uhlírová et al., 2005). *Fusarium* species

and *Aspergillus niger* had different preferred water activity for producing mycotoxins (Mogensen et al., 2009) and moisture was important for fungal growth (Ayerst, 1969). The moisture condition is simply thought as an environmental factor acting on fungi. Then, does water play a role as a constituent of fungi? Back in 1927, Richards found that fresh sporocarps held different amount of water after growing under similar conditions. This water content consisted of intracellular water (water contained in the tissues and cells) and extracellular water; both are intrinsic fungal features related to water. Thus, we include mycelial water content as a fungal trait rather than an environmental factor.

Besides mycelial water content, hydrophobicity of the fungal surface (HFS) is another potential water related trait. Hydrophobicity is a physicochemical property of cell surfaces; when a fungal surface is hydrophobic, water droplets form on the colony mat or along a hypha (Chau et al., 2009; Unestam and Sun, 1995). Studies on HFS mostly focused on morphological functions indicating that HFS was related to cell adhesion and cell elasticity of fungi (Dague et al., 2007; Unestam and Sun, 1995) and morphology of monomorphic and dimorphic fungi (Hazen et al., 1988). By contrast, physiological functions of HFS are less studied. There are hypotheses based on observations of ectomycorrhizae; Unestam and Sun (1995) proposed hydrophobicity is related to nutrient and water translocation: in both hydrophobic and hydrophilic fungi, large molecules related to nutrient uptake are exuded at fungal tips through cell wall pores, but pores along hydrophobic hypha are closed by hydrophobic compounds, forming vessels similar to water-tight pipes. Agerer (2001) found hydrophilic fungal species (e.g. *Laccaria* species) that can produce phenoloxidases to decompose lignin can obtain sufficient amounts of nutrients from their immediate vicinity without the need to explore large areas. On the other hand, hydrophobic fungal species (e.g. Boletales) are prone to explore large areas while lacking phenoloxidases since they have more choices of nutrients. Although the hypotheses are preliminary, they point out that HFS is a crucial fungal trait and there may be trade-offs among fungal growth traits, enzyme traits and water-related traits.

The ecological functions of HFS are rarely addressed. Intuitively, HFS is thought to correlate with soil water repellency, a surface property of soil which reduces or prevents water

infiltration into the soil, since hydrophobic compounds could affect both soil and fungal surface properties (Bond and Harris, 1964; DeBano, 2000). The hypothesis is supported by a solid body of evidence from field observation (Savage et al., 1969; Young et al., 2012) to *in vitro* studies (Chau et al., 2012; Rillig et al., 2010; Zheng et al., 2014) although the underlying mechanisms still remain uncertain. Both soil fungi and soil water repellency are close to another important soil process: soil aggregation. On the one hand, ideas for mechanisms of soil fungi in enhancing soil aggregation have been intensively studied and are comparatively well established (Rillig and Mummey, 2006; Ritz and Young, 2004; Six et al., 2004). On the other hand, soil water repellency could affect soil aggregation by changing soil water regime (Dal Ferro et al., 2012; Hallett and Young, 1999; Vogelmann et al., 2013a). Thus, there is a need to include HFS in measurements of traits related to soil aggregation, as proposed by Rillig et al. (2014).

Fungal traits and phylogeny

The trait-based approach is developed with phylogenetic comparative methods (Blomberg et al., 2003; Felsenstein, 1985; Harvey et al., 1995). Out of necessity data should be phylogenetically "corrected" to prevent phylogenetic pseudoreplication, which in extreme cases can override the true results (de Bello et al., 2015; Felsenstein, 1985). Thus, we incorporate methods to calculated phylogenetically independent contrasts (Felsenstein, 1985) and phylogenetic signal (Blomberg et al., 2003) to address four scientific questions in the study: (1) are fungal traits influenced by cellophane membranes? (2) do enzymatic traits and mycelial hydrophobicity change with sampling position related to different growth phases? (3) what are the variations of growth traits, enzyme traits and water related traits and do they have phylogenetic signal? (4) are there trade-off related to different lifestyles?

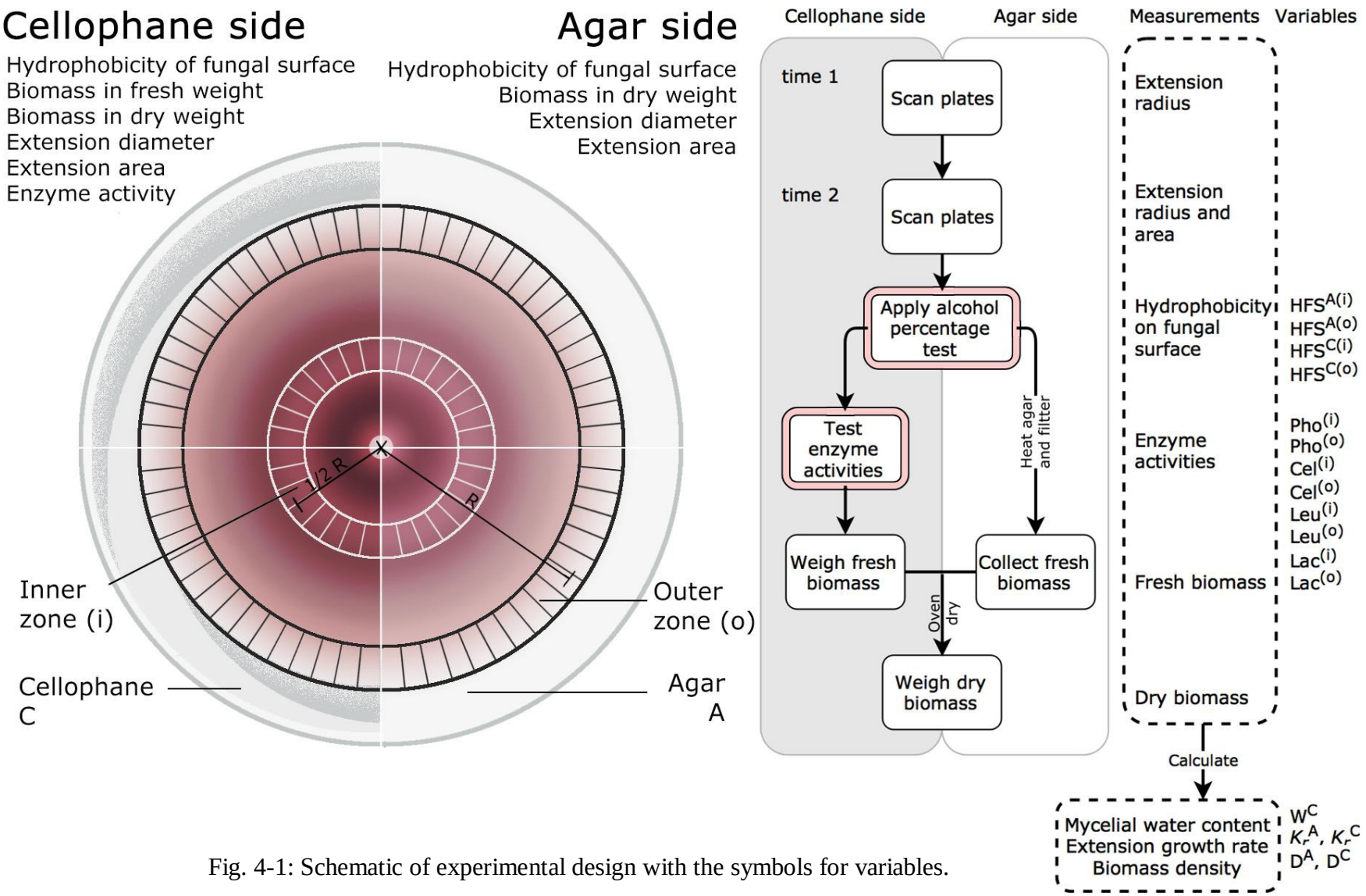


Fig. 4-1: Schematic of experimental design with the symbols for variables.

Materials and methods

Fungal material and cultivation

The 31 saprotrophic fungal strains were isolated from soil and root tissue samples collected in Oderhänge Mallnow, Germany, which is a 300 ha large nature conservation area. The isolates were identified (details see Table B-1; phylogenetic tree see Fig. 4-1) and screened for the basic growth traits of colony extension rate (K_r): the cultivation period for fungi to obtain maximal growth and the corresponding final diameter at the same experimental conditions for this study (Fig. B-1).

In this study, the experimental unit was a petri dish (9 cm in diameter) with 25 mL potato dextrose agar (PDA). To prevent hydrophilic mycelia growing into agar, a membrane of cellophane membrane was placed along the central line covering half circle of the agar surface. In this way, agar-free mycelia could be easily collected for measuring enzyme activities (Liu et al., 2010). Cellophane membranes (Cellophane Membrane Backing #165-0963) were sterilized by autoclaving at 121 ˚C for 5 min while soaking in deionized (DI) water (Cassago et al., 2002). Afterwards, a plug (5 mm in diameter) of fungal inoculum was placed in the center of the plate. Plates were sealed by parafilm and cultivated at room temperature (25 ˚C) until harvest. Each strain had a different cultivation time from 6 days to 36 days, listed in Table B-1, to insure all strains were in the linear growth phase when harvested. The inocula were cultivated at the same condition for 2 weeks in normal PDA before being used. Hereafter the side overlaid with cellophane is called cellophane side and the agar-only side is called agar side. To distinguish which side the measurements were obtained from, a superscript ‘A’ and ‘C’ in variables demonstrated that the origin was agar side and cellophane side, respectively. Six replicates were prepared for each strain. In total, there are 31 x 6 = 186 petri dishes.

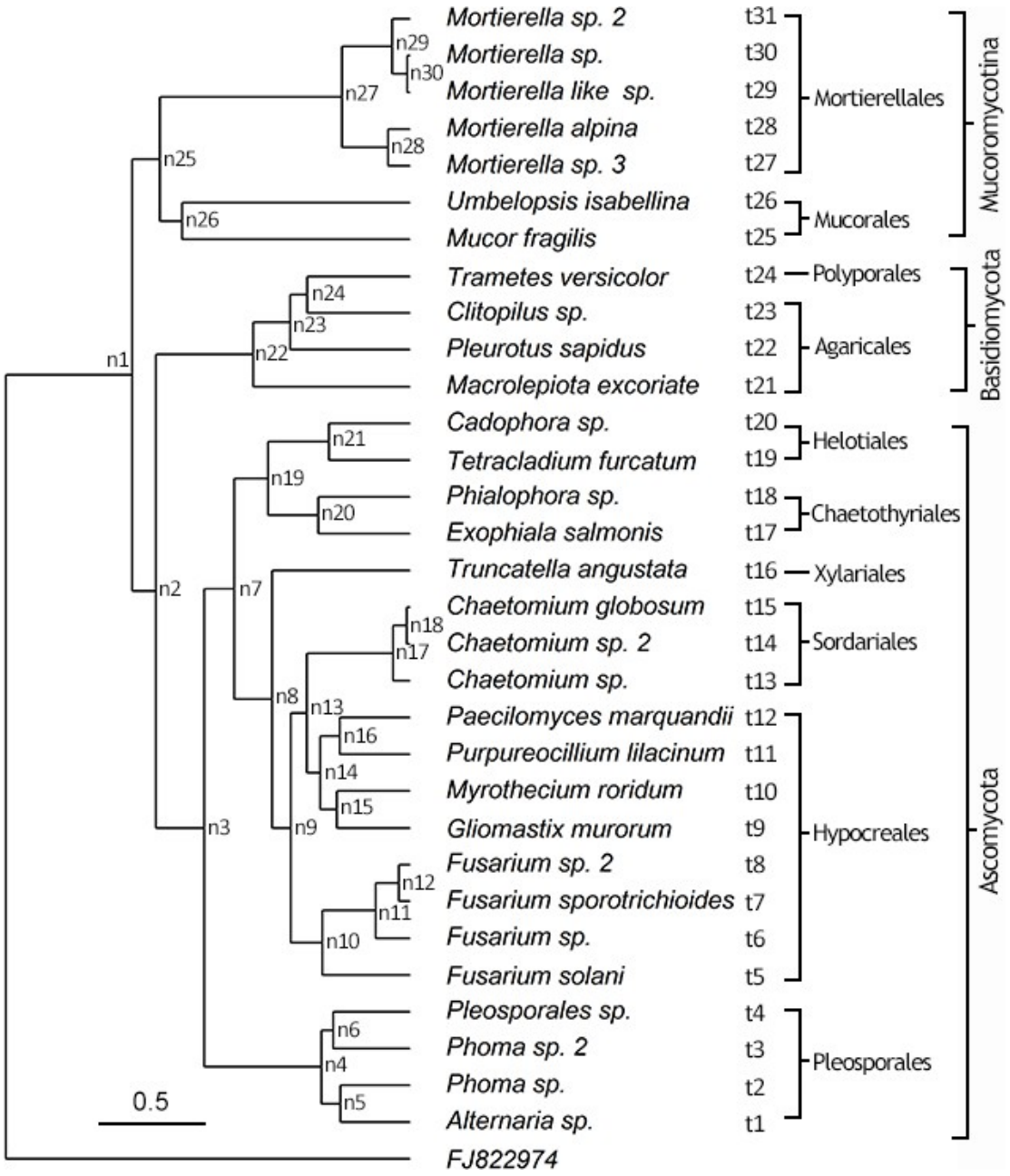


Fig. 4-2: Maximum clade-credibility tree with node heights based on sample averages (generated from the final 39991 sampled trees) for the chosen 31 saprotrophic fungal isolates with the numbering of tips (t1 to t31) and nodes (n1 to n30).

Harvest and measurements

The experimental design and variables are illustrated in Fig. 4-2. All the plates were scanned from the back side twice during the experiment. The first time point was approximately the half time of cultivation of each strain and the second time point was by the end of cultivation. The pictures were analyzed by Image-J (Schneider et al., 2012) for colony radius (for both time points) and colony extension area (only at the second time point). The radius was measured in triplicates in each picture. These data were used to calculate the colony radial growth rate (K_r) following the function: $K_r = (\text{diameter}_{t_2} - \text{diameter}_{t_1})/(t_2 - t_1)$. Later on, the colony extension area was used to calculated biomass density.

As mentioned above, mycelium in the outer zone of the colony is younger than in the inner zone. Thus, we sampled at inner and outer zones of colonies in order to test for potential changes of HFS and enzyme activities in different growth phases. Here, the inner zone was an area around the middle point along the radius (aging zone) and the outer zone was an area close to the edge (exponential growth zone and productive zone) (see Fig.4-2); the variable abbreviation with a superscript (i) or (o) refers to the measurement of the inner or outer zone, respectively.

The hydrophobicity on fungal surface (HFS) was measured by the alcohol percentage test on cellophane side and agar side. This is a rapid and simple way widely used in quantifying soil water repellency and HFS (Dekker and Ritsema, 1994; King, 1981) and produces as reliable results as other methods, e.g. contact angle (Chau et al., 2010; Leelamanie et al., 2008; Roy and McGill, 1998). The test was conducted as described in our previous study (Zheng et al., 2014): a series of aqueous ethanol solutions from 0 to 100% ethanol in 5% increments was made. Four-microliter droplets of ethanol solutions were applied on the surface of fungal colonies, and the time interval used for infiltration of the solution droplets was > 5-s. Triplicate droplets on the inner and outer zones were examined, of which the mean was taken as data. If mycelia on the agar side went into the media, hyphae were hydrophilic and the results of alcohol percentage test were zero.

In order to profile fungal enzyme activities in different growth phases, acid phosphatase (Pho), cellobiohydrolase (Cel), leucine aminopeptidase (Leu) and laccase (Lac) were tested by a microplate photometric method. The method was adapted from a procedure used for testing enzyme activities of ectomycorrhizal root tips (Courty et al., 2006). Because our samples were mycelia rather than soil or dead woods, a few adjustments to incubation time and substrate concentration were made. A small piece of mycelium (3-5 mm^2 in area) was cut out from cellophane side either in the inner or the outer zone to measure one type of enzyme activity immediately. All enzyme assays were done within 24 hours after the plates were opened and samples were stored at 4°C. The superscript 'A' or 'C' was not used in enzyme variables, because the enzyme activities were only tested on the cellophane side.

The acid phosphatase activity test was based on the hydrolysis of pNPP (para-NitroPhenyl Phosphate, a synthetic substrate) into pNP (para-NitroPhenol) + P. The reaction mixture contained 100 µl of acetate buffer (50 mM, pH 5.5) with 100 µl substrate (5 mM). After incubation at 37 ˚C for 15 min, the absorbance at 410 nm (ε_{410} = 18.3 mM^{-1} cm^{-1}) was measured. One unit of acid phosphatase activity was defined as the amount of enzyme releasing 1 µmol of pNP min^{-1}. The results of enzymatic tests were expressed against the dry weight of mycelia. The leucine aminopeptidase activity and cellobiohydrolase activity were measured in the same way with variations in incubation time, the incubation temperature, the buffer pH and the substrate (Table 4-1).

The laccase activity was determined as follows: twelve pieces of mycelium (4 replicates x 3 subsamples) were placed in wells of a 96-well microplate with 100 µl of acetate buffer (pH 5) and 100 µl of 2,2'- azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) solution (2 mM). After incubating at 25 ˚C for 25 min, the mycelia were removed from the plate and the oxidization of ABTS was monitored by determining the increase at 405 (ε_{405} = 36.8 mM^{-1} cm^{-1}) with a plate reader (Bio-rad). One unit (U) of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS min^{-1}.

Table 4-1: Conditions of the enzyme activity tests including concentration of substrates, incubation time, incubation temperature, buffer systems and pH.

Tested enzyme (EC number)	Substrate (consentration)/ mM	Time/ min	Temperature / °C	Buffer	pH
Acid phosphatase (3.1.3.2)	pNPP (5)	15	37	Acetate buffer	5.5
Cellobiohydrolase (3.2.1.91)	pNP-cellobioside (2)	120-240	37	Acetate buffer	5.5
Leucine aminopeptidase (3.4.11.1)	Leucine-p-Nitroanilide (5)	30	50	Tris buffer	8.0
Laccase (1.10.3.2)	ABTS (2)	15	25	Acetate buffer	5.0

Finally, a quarter of the colony from the cellophane side was lifted and weighed to measure the fresh biomass. Another quarter of the colony from the agar side was collected by heating the medium in a microwave for 30 sec or longer until it melted, and then the mycelia were carefully washed out. The fresh mycelia were dried in an oven at 45 °C over-night to obtain the dry biomass for both sides. In our study we did not develop a more precise concept to describe the physiological water content of the fungus. The water content contains intercellular and extracellular water in fungal tissue, which we called “mycelial water content”. The mycelial water content was calculated as (fresh biomass – dry biomass)/fresh biomass. The biomass density of a colony was calculated as the dry weight in micrograms divided by the area of the colony in square millimeters (Reeslev and Kjoller, 1995).

Statistical analysis

Assumptions of normality and homogeneity of variables were tested by Shapiro-Wilk and Bartlett’s test, respectively. Data were then transformed by Box-Cox transformation to meet corresponding assumptions of parametric statistics if needed; however, non-transformed values are reported in figures and tables if transformation is not specified. First an ANOVA was used to test if the traits were different among the strains (n = 186). Then a Student t-test or a Wilcoxon test was used to test whether changing growing surfaces or sampling positions affected the traits over all the strains (n = 186). After that, Wilcoxon test was used to test the

difference of traits for each strain pairs. The relationships among all fungal traits were tested by a pairwise Spearman correlation (n = 31). The phylogenetic signal of all the traits (n = 31) were tested based on Blomberg' K statistics (Blomberg et al., 2003) using an r package "picante" (Kembel et al., 2010).

We then tested the traits interactions. Here the growth phase effect was not included, so samples from different zones are regarded as subsamples. The traits (K_r, biomass density, the mycelial water content, HFS, Pho activity, Cel activity, Leu activity and Lac activity) of the 31 strains went through the following tests (n = 31). A principal component analysis (PCA) was carried out to visualize the strains grouping on traits data. The phylogenetic signals of the first three principal components drawn from the PCA results were checked. To reveal the relationship among traits, generalized linear regression were fitted on phylogenetic independent contracts (PIC) of variables done by the functions pic( ) and compar.gee( ) from an r package "ape" (Paradis et al., 2004). The correct *p-values* of generalized linear regression were calculated on 1000 times permutation allowing for removal of the effects of extreme data points. The effect of extreme data points were checked by fitting models without them. All analyses were performed with R software v. 312 (R Core Team, 2014). The significance level of the statistical analysis was 0.05.

Results

Fungal traits ant their phylogenetic signals

All the traits (biomass density, K_r, hydrophobicity on fungal surface, mycelial water content, Pho activity, Cel activity, Leu activity and Lac activity) showed variation from strain to strain with all *p*-values < 0.0001 (details of statistics are not shown). The strains grew comparable to previous experiments shown in Table B-1 and Fig. B-1 indicating colonies were in linear growth phase when sampled. On agar, the K_r ranged from 23.6 to 215.3 µm h^{-1}. The top three fastest growing strains were all from the Mucomycotina while the species from the clade of Helotiales and Chaetathyriales grew more slowly with K_r less than 30 µm h^{-1}. An opposite trend was shown in biomass density across the strains since K_r and biomass density were negatively correlated (Table 4-4). The colony surface of *Phoma* sp., *Te.furcatum*, *Mortierella* sp., *Fusarium* sp., *Ma.excoriate*, *U.isabellina*, *E.salmonis* and *G.murorum* were hydrophilic, the others were hydrophobic growing on PDA surface (Fig. 4-3). The mycelial water content of colonies collected from cellophane surface varied from 67.9% to 92.6%. Fig. 4-4 shows relative distributions of enzyme activity. Basidiomycota had much higher Lac activity than Ascomycota and Mucoromycotina which were lacking laccase. The order Mortierellales did not exude Cel while other strains had comparable levels of it. In contrast, Mucoromycotina had higher Leu activity compared to others. All the strains generally exuded acid phosphatase.

To our surprise K_r did not have phylogenetic signal, but biomass density did. We also found HFS and Leu activity which was the only enzyme having phylogenetic signal (Table 4-3). HFS measured at four locations on the colony always had phylogenetic signals indicating the phylogenetic signals did not depend on the sampling positions and growth surfaces in our experimental setting.

Table 4-2: The growth traits (K_r and biomass density) on agar and cellophane membrane and the mycelial water content on the cellophane side. The values were mean with standard deviation in brackets (n = 6). The solid lines divide the table into Mucoromycotina, Basidiomycota and Ascomycota parts downwards, the dashed lines separate orders.

Strains	K_r^A/ µm h^{-1}	K_r^C/ µm h^{-1}	D^A/ µg mm^{-2}	D^C/µg mm^{-2}	W^C/ %
*Mo.*species 2	116.0 (20.0)	142.6 (20.4)	25.5 (9.8)	43.3 (12.9)	89.0 (1.5)
*Mo.*species	213.8 (11.1)	250.2 (17.1)	32.6 (3.2)	57.3 (7.5)	81.4 (1.3)
*Mo.*like species	135.7 (19.3)	95.1 (50.3)	25.1 (6.1)	52.2 (12.9)	80.9 (3.0)
Mo.alpina	210.5 (10.8)	236.2 (17.5)	42.5 (11.7)	45.9 (6.4)	82.2 (3.0)
*Mo.*species 3	162.6 (24.2)	168.6 (21.3)	29.6 (5.6)	33.8 (7.9)	85.7 (1.5)
U.isabellina	82.8 (14.7)	81.4 (12.8)	53.1 (12.1)	71.6 (10.8)	87.3 (2.2)
Mu.fragilis	215.3 (39.6)	200.1 (57.5)	21.5 (4.3)	19.8 (8.9)	92.6 (2.4)
Tra.versicolor	145.4 (27.0)	163.6 (23.2)	22.7 (5.3)	44.5 (5.5)	88.8 (0.3)
Cl.species	70.3 (9.4)	67.7 (5.2)	71.2 (7.8)	81.2 (5.5)	78.2 (1.3)
Ple.sapidus	95.5 (9.5)	100.2 (11.3)	51.3 (14.1)	18.9 (3.6)	77.5 (2.0)
Ma.excoriate	33.5 (6.0)	n.a.	37.8 (5.6)	36.2 (3.5)	82.4 (1.9)
*Ca.*species	12.0 (2.1)	9.7 (5.2)	70.4 (22.8)	122.5 (26.2)	81.3 (1.2)
Te.furcatum	25.5 (2.3)	33.3 (1.9)	108.5 (18.2)	116.7 (8.5)	82.3 (0.9)
*Phi.*species	22.7 (1.3)	22.3 (2.3)	89.0 (8.2)	160.4 (25.2)	85.6 (0.4)
Ex.salmonis	23.6 (1.2)	24.6 (0.6)	78.1 (19.1)	102.4 (11.6)	86.4 (1.1)
Tru.angustata	136.0 (12.6)	67.9 (20.4)	29.2 (4.8)	57.6 (16.2)	77.3 (7.3)
Ch.globosum	107.3 (7.4)	138.3 (10.8)	24.9 (4.8)	45.6 (6.5)	83.9 (1.1)
*Ch.*species 2	167.4 (25.2)	198.1 (22.6)	34.8 (15.2)	42.1 (11.1)	75.9 (2.9)
*Ch.*species	23.1 (6.7)	27.3 (3.5)	81.8 (21.4)	107.3 (16.4)	83.1 (2.9)
Pa.marquandii	60.6 (8.3)	60.0 (11.7)	92.7 (41.4)	73.8 (8.9)	88.8 (3.2)
Pu.lilacinum	61.1 (11.7)	56.7 (7.4)	72.8 (12.2)	67.0 (12.7)	91.1 (1.3)
My.roridum	50.5 (4.8)	50.2 (3.8)	59.3 (5.8)	80.0 (3.4)	86.4 (0.7)
G.murorum	54.3 (6.4)	53.7 (5.9)	46.5 (20.0)	87.7 (18.7)	87.2 (1.3)
*F.*species 2	94.5 (21.1)	132.8 (13.6)	56.8 (17.1)	61.6 (11.1)	89.4 (3.4)
F.sporotrichioides	109.5 (6.1)	99.8 (12.0)	30.7 (8.2)	63.3 (13.5)	87.4 (2.3)
F.species	150.3 (8.4)	147.1 (7.8)	13.0 (2.8)	24.5 (5.3)	90.6 (0.8)
F.solani	120.1 (27.0)	122.9 (36.3)	30.8 (18.1)	64.1 (17.1)	90.0 (3.7)
*Ple.*species	19.7 (5.5)	18.4 (3.7)	100.4 (15.6)	144.0 (17.3)	88.9 (2.9)
*Pho.*species 2	34.9 (7.2)	9.2 (7.2)	59.1 (8.4)	126.8 (52.3)	90.4 (2.6)
*Pho.*species	143.4 (7.8)	138.0 (7.7)	29.3 (2.7)	67.6 (7.9)	87.6 (2.0)
*A.*species	137.6 (41.3)	115.2 (48.8)	64.4 (17.2)	87.9 (7.0)	67.9 (3.0)

Notes: colony extension rate (K_r), biomass density (D), mycelial water content (W), ^{A} means colonies grown on agar, ^{C} means colonies grown on cellophane membrane and n.a. (data not available)

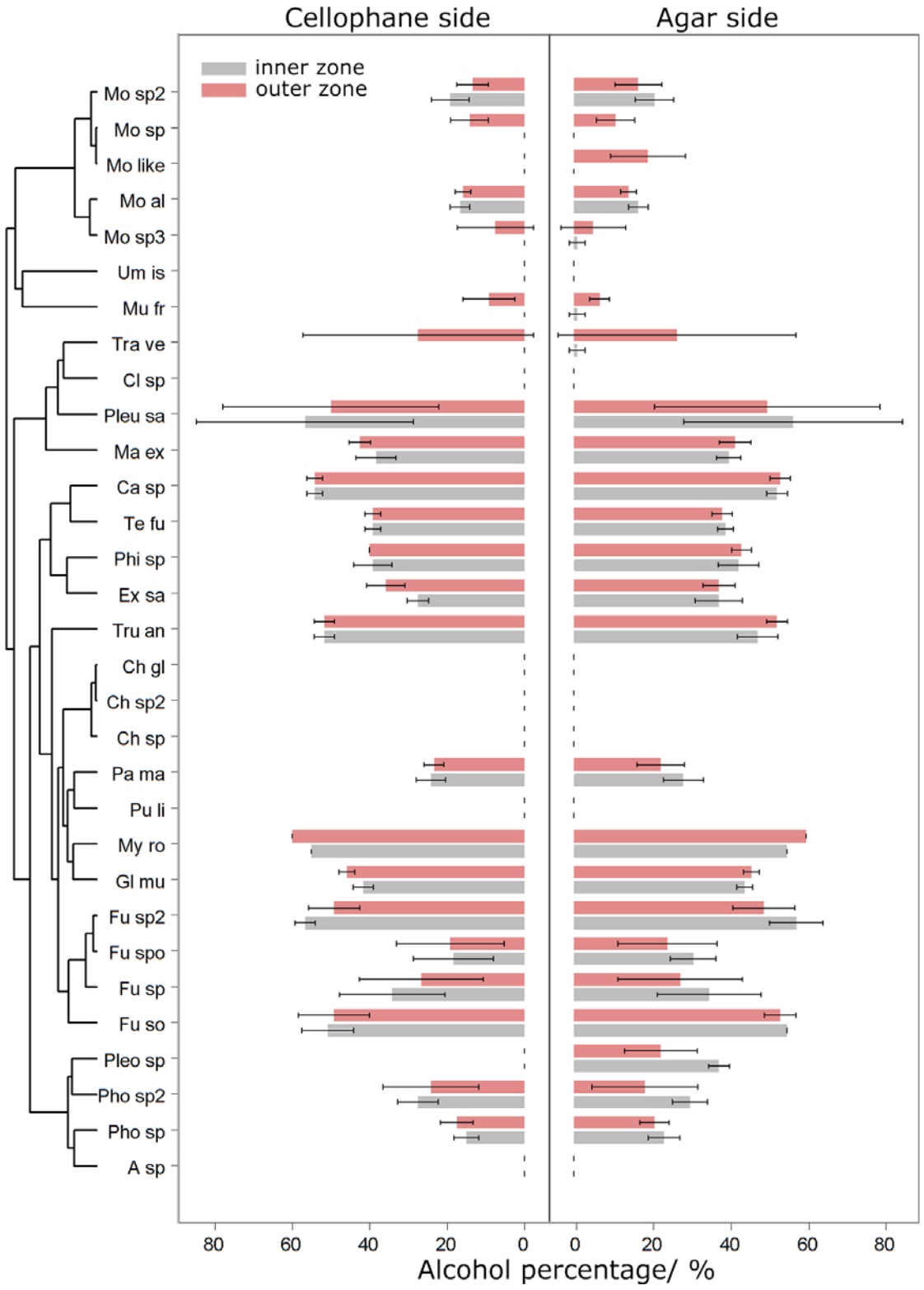


Fig. 4-3: Phylogenetic distribution of hydrophobicity of fungal surface measured by alcohol percent test in inner and outer zones of colonies growing on agar or cellophane surfaces. Error bars are standard deviation, n=6.

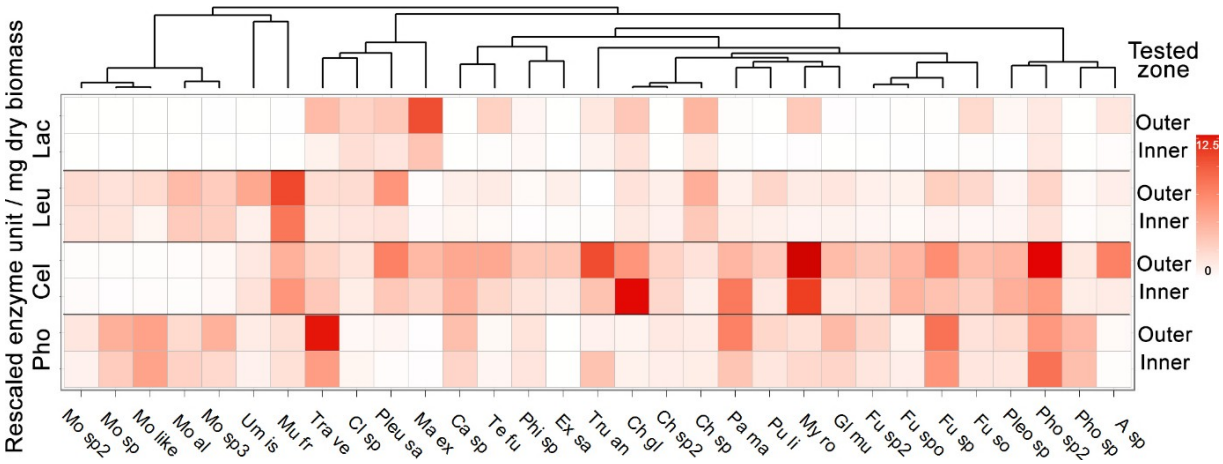


Fig. 4-4: Phylogenetic distribution of rescaled enzyme activities of the 31 strains tested in inner and outer zones of colonies growing on cellophane overlaid PDA. The rescaled data are 5 X acid phosphatase (Pho) activity, 100 X cellobiohydrolase (Cel) activity, 1.5 X leucine aminopeptidase (Leu) activity and 1 X laccase (Lac) activity. Notice that the data is rescaled for better visualization, grayness of different enzymes is not comparable across enzymes and for the original data see in Table B-2 and Table B-3.

Table 4-3: Variables and principal components show phylogenetic signal (K-statistics), n = 31. PC1 and PC2 are obtained from the principal components analysis (Fig. 4-7).

Variables	K	*p*
D^{A}	0.334	0.026
D^{C}	0.571	0.001
$Leu^{(i)}$	0.567	0.046
$Leu^{(o)}$	0.706	0.007
$HFS^{A(i)}$	0.496	0.005
$HFS^{A(o)}$	0.388	0.016
$HFS^{C(i)}$	0.293	0.043
$HFS^{C(o)}$	0.320	0.023
PC1	0.447	0.004
PC2	0.314	0.039

Note: biomass density (D), ^{A} means colonies grown on agar, ^{C} means colonies grown on cellophane membrane.

Fungal trait distributions influenced by cellophane membrane and growth phase

The different sampling positions and growth surfaces could shift trait distributions. The Student's t tests (t) and Wilcoxon's rank tests (V) showed cellophane membranes on the agar

changed dry biomass (t(185) = 6.49, $p < 0.001$), fresh biomass (t(185) = 2.46, $p = 0.014$), extension area (V(185) = 2724, *p-value* < 0.001) and biomass density (t(185) = 10.44, *p-value* < 0.001), HFS in the inner zone of the colony (t(185) = -4.89, *p-value* < 0.001) and HFS in the outer zone of the colony (t(185) = -2.30, *p-value* = 0.022) but not K_r (t(185) = -0.72, *p-value* = 0.472) for all strains. Biomass density was either kept neutral or increased with cellophane except for *Ple.sapdius* (Fig. 4-5). Although the overall effect of cellophane on K_r was not significant, *Tru.angustata* and *Phoma* sp. grew faster on agar while *Mo.alpina*, *Mortierlla* sp., *Mortierlla* sp. 2, *Fusarium* sp., *Te.furcatum* and *Ch.globosum* grew more slowly on the cellophane (Fig. 4-5). The cellophane membrane inhibited surface hydrophobicity in the strains *Mo.alpina* and *Phoma* sp. 2 (Fig. 4-5).

The mycelium sampling positions, i.e. growth phases, affected Cel activity (t(185) = 4.17, *p-value* < 0.001), Leu activity (t(185) = 8.38, *p-value* < 0.001) and Lac activity (t(185) = 6.60, *p-value* < 0.001) but not Pho activity (t(185) = 1.55, *p-value* = 0.123). Generally, the enzyme activities and Lac activity were higher in the outer zone (Fig. 4-4). On the agar side HFS was the same at both sampling positions (t(185) = -0.31, *p-value* = 0.757), while HFS on the cellophane side (t(185) = -2.37, *p-value* = 0.02) was marginally different.

However, the trends for each trait were stable. In Table 4-4, the correlation of the variables measured in pairs of the different sampling zones or different sides were all highly correlated with each other (correlation coefficients greater than 0.55). Therefore, in the subsequent PCA and regressions we used means averaged across inner and outer zone data.

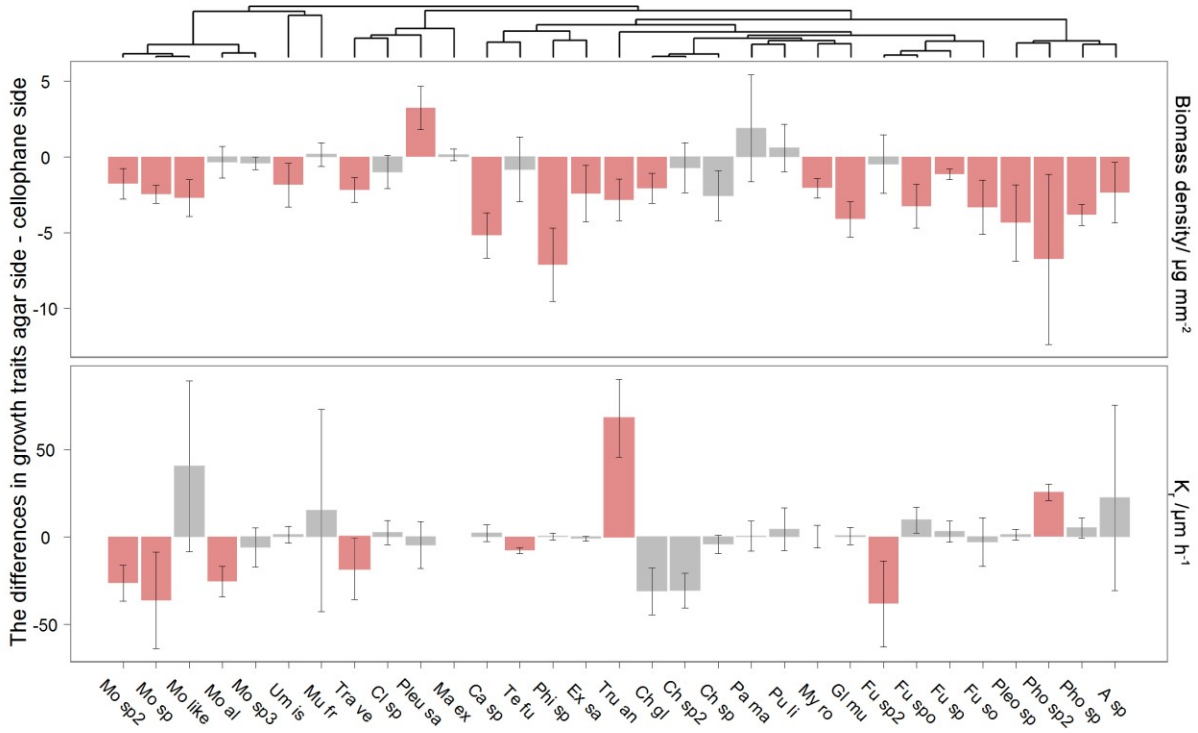


Fig. 4-5: Phylogenetic distribution of the differences in K_r and biomass density between measurements on agar and for cellophane for the 31 strains. The grey bars indicate the traits were the same on both sides (tested by Wilcoxon test on untransformed data, $p > 0.05$, $n = 6$). Error bars are standard deviation, $n = 6$. The K_r on cellophane side of *Ma. excorate* is missing.

On cellophane membranes, colonies had different sporulation time (Fig. 4-6 G to I). *Mu. fragilis* and *Tru. angustata* could grow on the cellophane surface although this was not optimal (Fig.4-6 C and D). In Fig 4-6F *Ma. excoriate* seemed to grow symmetrically on the cellophane and agar side, but actually on the cellophane side mycelium grew below the membrane. This is why K_r on the cellophane side was missing in Fig 4-5 and table 4-3. On the cellophane side *Phoma* sp. 2 stopped growing after extending a short distance while exuding a large amount of mucilage covering the whole cellophane membrane (Fig. 4-6 A). The mycelia of *Cadophora* sp. were very brittle and could not attach to cellophane membrane (see Fig.4-6 B).

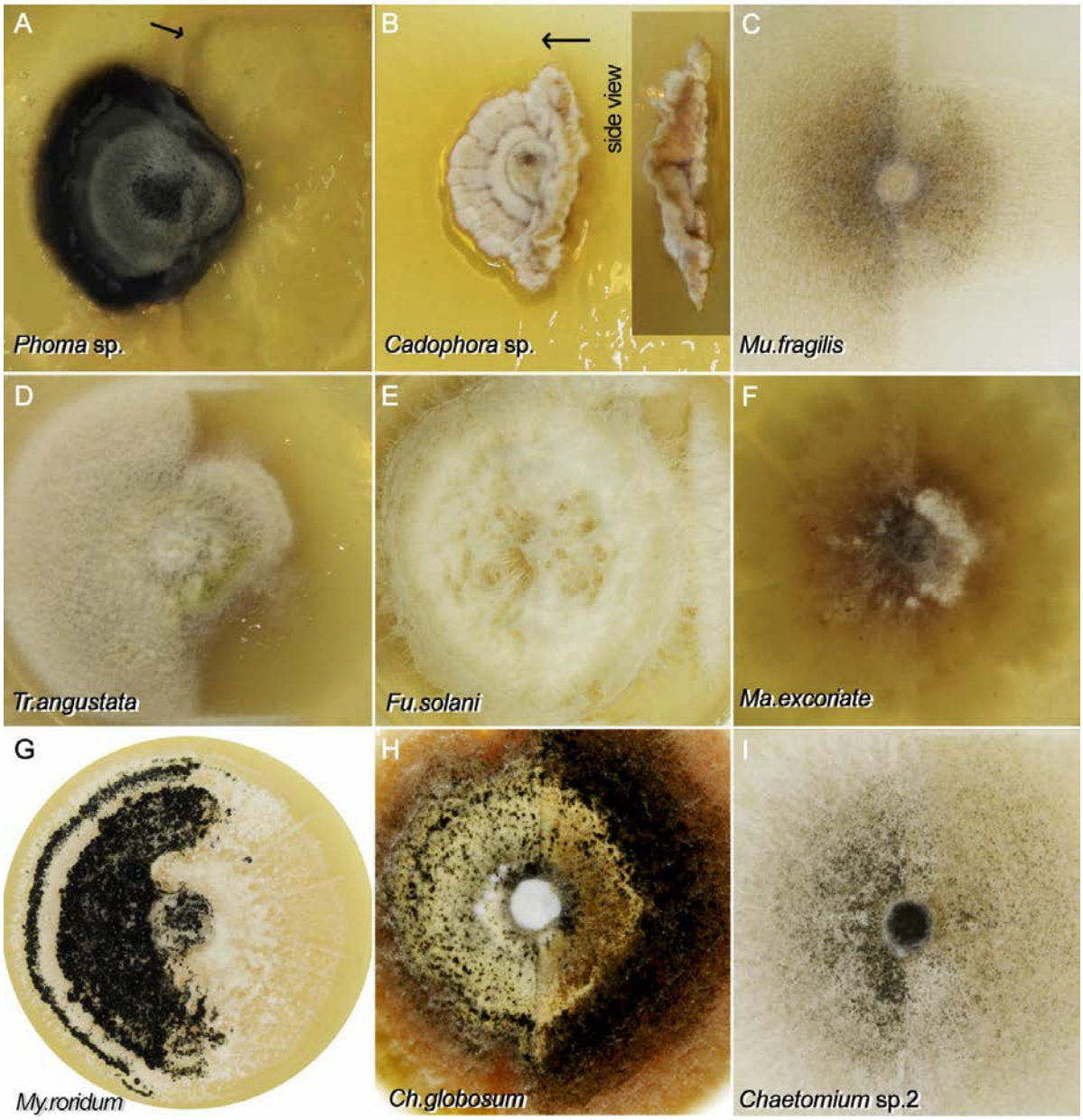


Fig. 4-6: Photos of colonies growing differently on the cellophane surface than on agar. In A, the arrow points at mucilage accumulating around the cellophane membrane. In B, flipped colony shown from the side.

Table 4-4: Spearman correlation matrix for 16 variables (n = 31). Correlation coefficients greater than 0.55 are in bold, m: $p < 0.1$, *: $p < 0.05$, †: $p < 0.01$, ‡: $p < 0.001$.

Vari-ables	K_r^A	K_r^C	D^A	D^C	W^C	$HFS^{A(i)}$	$HFS^{A(o)}$	$HFS^{C(i)}$	$HFS^{C(o)}$	$Pho^{(i)}$	$Pho^{(o)}$	$Cel^{(i)}$	$Cel^{(o)}$	$Leu^{(i)}$	$Leu^{(o)}$	$Lac^{(i)}$
K_r^C	**0.92**‡	1														
D^A	**-0.73**‡	**-0.68**‡	1													
D^C	**-0.69**‡	**-0.70**‡	**0.73**‡	1												
W^C	-0.03	-0.01	-0.14	-0.08	1											
$HFS^{A(i)}$	0.23	0.22	-0.01	-0.25	-0.26	1										
$HFS^{A(o)}$	0.21	0.19	0.02	-0.22	-0.18	**0.89**‡	1									
$HFS^{C(i)}$	0.11	0.08	0.03	-0.16	-0.24m	**0.94**‡	**0.86**‡	1								
$HFS^{C(o)}$	0.14	0.13	0.02	-0.19	-0.19	**0.85**‡	**0.91**‡	**0.90**‡	1							
$Pho^{(i)}$	0.19	0.08	-0.25m	-0.1	0.29	0.03	0.04	0	-0.01	1						
$Pho^{(o)}$	0.26	0.22	-0.23	-0.12	0.33*	0.06	0.1	0.03	0.05	**0.59**‡	1					
$Cel^{(i)}$	-0.18	-0.19	0	-0.02	0.23m	0.05	0.17	0.13	0.18	0.07	0	1				
$Cel^{(o)}$	-0.29	-0.34*	0.17	0.11	0.05	-0.09	0.01	-0.01	0.01	-0.1	-0.22	**0.56**‡	1			
$Leu^{(i)}$	0.27m	0.34*	-0.17	-0.36*	0.11	0.42†	0.40†	0.32*	0.28m	0.13	0.29	0.02	-0.21	1		
$Leu^{(o)}$	0.25	0.28m	-0.19	-0.33m	0.22	0.25	0.18	0.17	0.1	0.05	0.19	-0.05	-0.16	**0.65**‡	1	
$Lac^{(i)}$	-0.13	-0.16	0.05	-0.05	-0.23	0.14	0.17	0.14	0.14	-0.03	-0.2	0.35*	0.26†	0.12	-0.07	1
$Lac^{(o)}$	-0.33	-0.32	0.17	0.11	-0.16	0.13	0.15	0.19	0.17	-0.17	-0.29m	0.33*	0.42*	-0.03	-0.08	**0.62**‡

Notes: colony extension rate (Kr), biomass density (D), fungal water content (W), A means samples grown on agar, C means samples grown on cellophane, (o) means data were from the outer zone, (i) means from inner zone and all enzyme actives (Pho, Cel, Leu and Lac) were tested on the cellophane side only.

Fungal lifestyles and trade-offs

The PCA grouped the different strains, which revealed commonalities in lifestyles (Fig. 4-8). The first three principal components explained 30%, 18% and 18% of the total variance, respectively. PC1 was mostly loaded on K_r and biomass density, PC2 on HFS and Lac activity and PC3 on mycelial water content and Cel activity. The PCA grouping shared high similarity with phylogenetic groupings. This agrees with results showing that both PC1 and PC2 have phylogenetic signals (table 4-4).

The first group is the Mucoromycotina strains; they were fast-growing species with relatively high Leu activity and low biomass density. *Mu. fragilis* is a good example as it extended the fastest on the agar side (K_r, 215.3 μm h^{-1}) and had the highest mycelial water content (92.6%) and also the highest Leu activity (6.3 U mg^{-1} dry biomass). The second group is dominated by the Ascomycota clade, which was grouped by biomass density. *Alternaria* sp. had the lowest mycelial water content (67.9 %) while showing moderate growth rates (K_r, 137.6 μm h^{-1}), biomass accumulation (biomass density, 64.4 μg mm^{-2}), enzyme exudation (Fig. 4-5) and HFS (21%). The third group is the strains with high HFS and high Cel activity. At the opposite side, the last group are strains with higher mycelial water content and Lac activity which are mostly from order Hypocrealas.

The correlations of traits shown in table 4-4 arise from their common environmental attributes and/or phylogenetic similarity. After removing phylogenetic dependency the relationships between traits shifted. The significant fitting tested by 1000 permutations are shown in Fig. 4-7. The model fitted with PIC of K_r and biomass density was not significant anymore (Fig. 4-7A_2), as well as on mycelial water content and Pho activity (Fig. 4-7C_2). On the contrary, PIC of Cel activity and Lac activity became significant (Fig. 4-7B_2). However, three extreme points: n17, n18 (the clade of *Sordariales)* and n30 (the node connecting *Mortierella* like sp. and *Mortierella* sp.) strongly affected the model fitting results. Removing the extreme points either revealed (Fig. 4-7A_2) or eliminated the relationship (Fig. 4-7B_2)

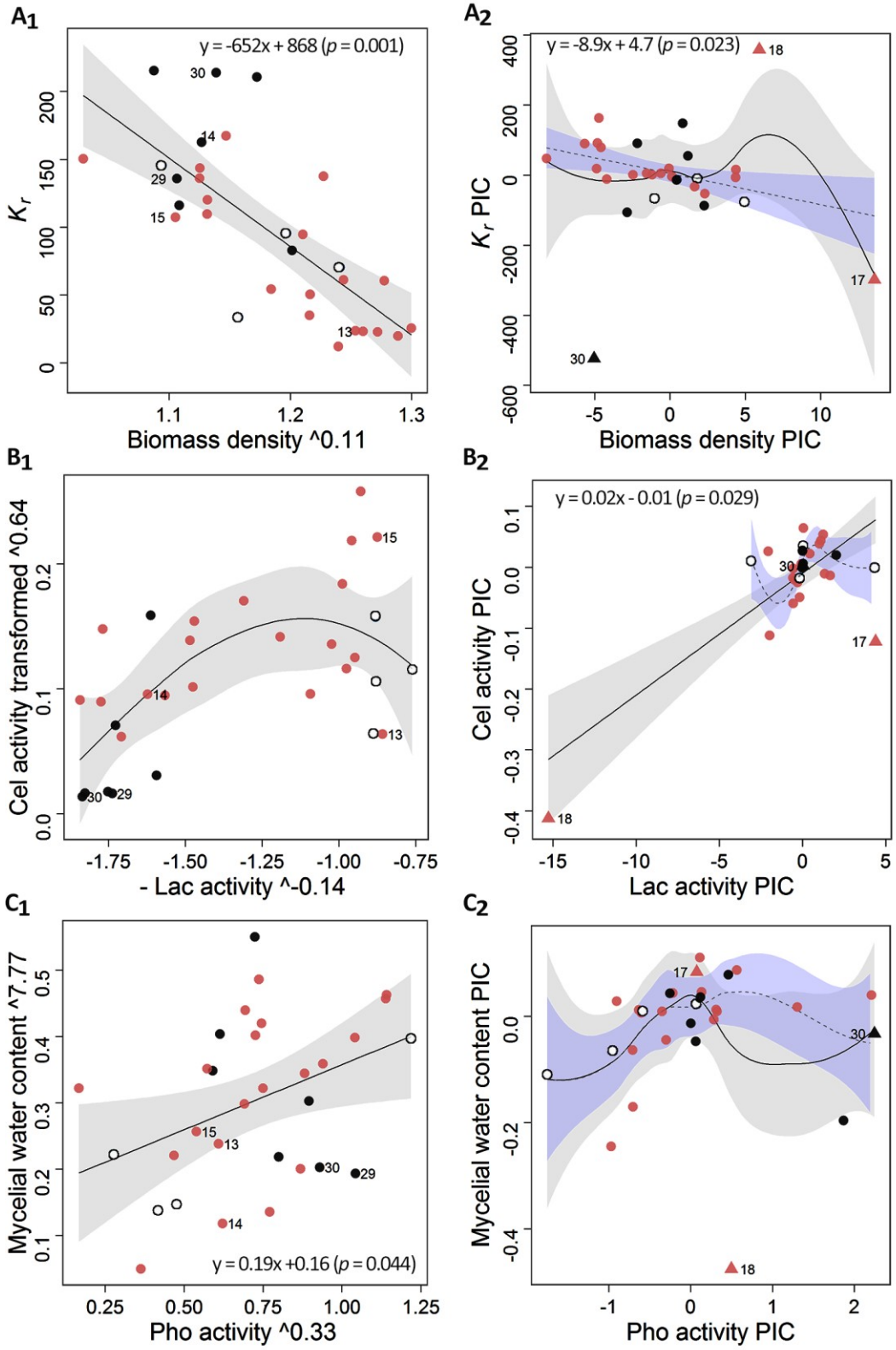
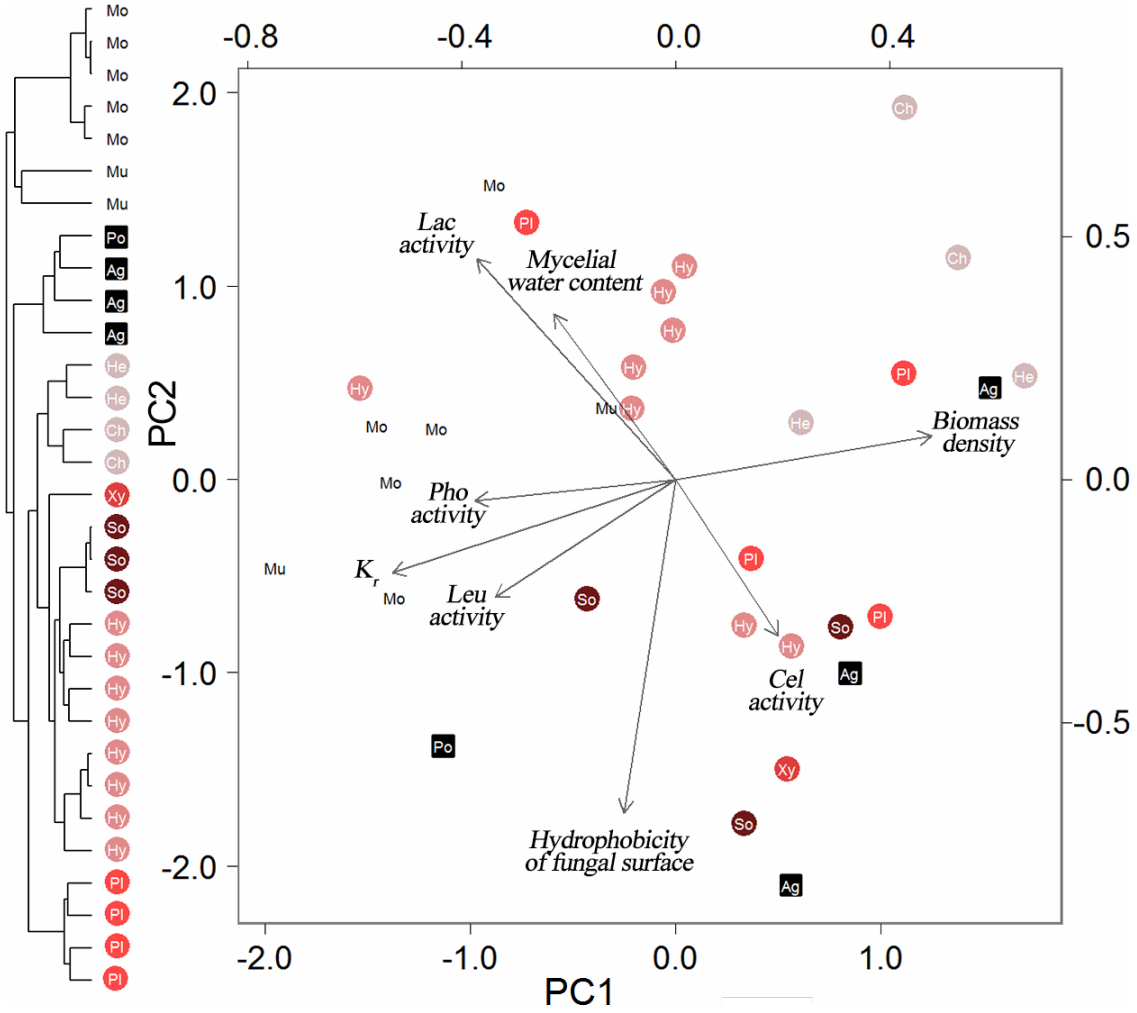


Fig. 4-7: Plots comparing regressions on transformed data (A1, B1 and C1) and their phylogenetically independent contrasts (A2, B2 and C2). The extreme node points (triangles) in PIC data and the corresponding tips are labeled (tip 13 and tip 14 to node 17; tip 14 and tip 15 to node 18; tip 29 and tip 30 to node 30). The regressions on data with removed node 17, 18 and 30, are shown as a dashed line with darker shadow. Red, hollow and black dots denotes Ascomycota, *Basidiomycota* and Mucoromycotina respectively. The significant relationships

(*p-values* < 0.05, calculated by 1000 permutations) fit a generalized linear model with the equation shown on the side and others a loess model.

Fig. 4-8: Principal component analysis (PCA) of K_r, biomass density, HFS, mycelial water content, Pho activity, Cel activity, Leu activity and Lac activities of the 31 strains. The abbreviations are the first two letters of order names, Mortierellales, Mucorales, Polyporales, Agaricales, Helotiales, Chaetathyriales, Xylariales, Sordariales, Hypocrealaes and Pleosporales shown in the phylogenetic tree on the side.



Discussion

Effects of cellophane on trait data

Our experiment confirms cellophane membrane could trigger species-specific morphological and physiological changes in fungi, which is in line with Kerr (1958). It also efficiently separated fungal mycelia from the medium as in previous studies (Cassago et al., 2002; Katz et al., 1972; Liu et al., 2010; Reeslev and Kjoller, 1995). Cellophane membrane changed biomass density more strongly than K_r (Fig. 4-3), which is consistent with a previous study (Reeslev and Kjoller, 1995). However, we did not expect cellophane to cause thicker colonies, since biomass density was thought to be positively related to nutrient availability under the colony (Moore et al., 2011; Trinci, 1969) and overlaying cellophane could not increase nutrient availability. Usually, fungal physiological changes are in response to spatial and temporal environmental changes (Cairney and Burke, 1996). For example, when stressed by addition copper and cadmium, fungal colonies became thicker (Gadd et al., 2001). Then, how does cellophane affect fungi as an environmental factor?

Essentially, a cellophane membrane is a water-proof cellulose membrane (Gillespie and Williams, 1966). As an abiotic factor, cellophane can potentially trigger or enhance fungal cellulase secretion. We have noticed that some cellophane membranes were softer than others at harvest time, which means that they would more easily break into pieces when lifted from colonies, as a sign of slight decomposition. Previous studies have also reported fungi caused mass loss of the cellulose film (Deacon, 1979). Cellophane attracting cellulose decomposing aquatic Chytridiales species growing on the edges of membranes also suggests an interaction between cellophane and fungi (Willoughby, 1998).

Filamentous fungi can grow on various surfaces (Harding et al., 2009), and the fundamental change due to cellophane is the growth surface. Willoughby (2001) described the morphology of an aquatic fungus, which has richly-branched rhizoids which only developed around cellophane membranes. If a stain does not have structures to attach to cellophane surface, it may grow like *Cadophora* sp. (Fig. 4-6B). We did not find another study showing a fungus producing a large amount of exudate on the cellophane as *Phoma* sp. did in our study (Fig. 4-6A). Since it would be a large investment of energy to produce the mass, we thought that the exudates contained enzymes, but the results of enzyme tests did not support this (data not shown).

Although cellophane showed influences on fungal traits, since using cellophane is still a practicable way to collect trait data, we suggest that in studies on the individual level, cellophane effects should be checked to avoid cellophane membrane changing observed traits -at least for cellulase activity and HFS-, and caution should be taken when using it to separate fungal mycelia from media when fungi are cellulose-decomposers.

Fungal traits and phylogenetic signals

In a trait-based approach one crucial part is identifying functional traits. In the approach species are treated as functional groups rather than phylogenic groups, because during evolution there are trait changes leading to divergent traits in close species (Aguilar-Trigueros et al., 2014; Violle et al., 2007). For the first mycelial water content and hydrophobicity of fungal surface were tested on 31 saprotrophic fungi showing significant variations. We found that the difference in mycelial water content among the strains could be as large as 24.7% (Table 4-2) in the same moisture saturated condition ; the humiditiy in a PDA plate is 100% equaling to a water activity of 1 (Mogensen et al., 2009). This result extends the findings of Richards (1927) who showed that sporocarps had different water content in the same humidity condition. If mycelial water content is only a response to environment humidity, the strains should have all reached the same mycelial water content that is why we suggest that mycelial water content is an intrinsic fungal trait. In addition, Richards (1927) showed that the water content of sporocarps of 8 species were positively correlated to the respiration rate across taxa. This phenomenon is an analogy to the relationship between initial seed water content and respiration (Vertucci and Leopold, 1984).

Enzyme activities are highly plastic traits (Burns et al., 2013). It is not surprising that we found that Cel, Leu and Lac activities varied in different growth phases, which is in line with results from Heinonsalo et al. (2012). Not only does the age of colony in *in vitro* experiments interfere with enzyme trait data, but also using the data collected from an artificial condition to represent in-site enzyme activities would be problematic. In plant ecology, Cordlandwehr et al. (2013) have indicated that using data retrieved from database of plastic traits (canopy height, leaf dry matter content and specific leaf area) was less accurate than using in-site measurements of these traits. Plasticity of traits shown in plants and fungi is a way of adaption to a heterogeneous environment (Cairney and Burke, 1996; Cordlandwehr et al., 2013; Cornwell and Ackerly, 2009). Thus, to build a fungal trait database for predicting ecological functions of fungi, traits like enzyme activity need to be recorded in a way that captures their plasticity.

The HFS was less variable in the study conditions. The growth phases marginally affected HFS and only two strains turned hydrophilic because of cellophane membranes. Nevertheless, Smits et al. (2003) reported *Laccaria bicolor* WSL 73.1 developed hydrophobicity with time and after 20 days had a second patchy layer with higher hydrophobicity. It could thus be possible that we did not capture the development of HFS at our measuring time points. Chau et al. (2009) showed HFS of colonies of *Cladosporium cladosporioides* and *Suillus tomentosus* decreased from central to peripheral. Additionally, in both studies they showed that some types of solidified nutrient media (PDA, Melin Norkrans Media and malt agar) could inhibit or promote HFS in different strains. Generally, HFS is prone to be a time-dependent trait and can change with the heterogeneous soil environment (Hazen et al., 1988), sporulation (Wösten and Willey, 2000), nutrient exploration, nutrient storage (Agerer, 2001) and drought resistance strategy. HFS could be an important dimension of niche space as a strategy for successful competition and adapting to periodical wet-dry cycles.

A trait having phylogenetic signal means that closely related species display similar trait values due to their common ancestry (Adams, 2014). For the first time, we identified empirically HFS as a phylogenetically conservative trait of fungi as Agerer (2001) proposed based on field observation of ectomycorrhizal fungi. Phylogenetic signals in traits are labile to different degrees (Blomberg et al., 2003) as traits expression varies in response to environment. But in our experimental condition, growth phases and growth surface feature did not change the phylogenetical signals (Table 4-3), which means that the variable which had phylogenetic signal would always have the signal, no matter where it was measured.

Fungal lifestyles and trade-offs

Different but phylogenetically constrained lifestyles in chemical microenvironments might enable fungi to explore different niches and optimally use nutrients therein. Our PCA result indicates the chosen traits of growth, enzymes production, mycelial water content and HFS tended to evolve convergently, i.e. the strains clustered in PCA are highly similar to phylogenetic clusters (Fig. 4-8) and PC1 and PC2 showed phylogenetic signals (Table 4-3).

We did not find enough evidence to support whether there is a trade-off between K_r and biomass density independent of phylogeny. In the raw data, K_r and biomass density had a negative linear relationship (Fig. 4-7 A_1), but the potential trade-off only shows up when 3 extreme points were removed (Fig. 4-7A_2). The strong effect of extreme data points was also reported by Lipson et al., (2009). The existence of trade-offs between K_r and biomass density

and the divergence of traits in certain groups could be true at the same time, nevertheless both require further investigation.

For enzyme production, we showed in Fig. 4-4 that the strains cannot invest energy in all types of enzymes, a fact established previously (e.g., Eichlerová et al., 2015; Wang et al., 2014). But we did not observe trade-offs among any pairs of enzyme, except between Lac activity and Cel activity, but possibly caused by the suspicious extreme points (Fig. 4-7 B_2). The number of enzymes we tested in the study is far less than the number of enzymes that can be exuded by fungi in general. It would be worth exploring more enzymes to uncover potential trade-offs, and to include studies in more realistic conditions.

Chapter 5

Summary

The main goal of this dissertation was to explore the connection between soil aggregation and soil water repellency trough soil fungi under the umbrella of the trait-based approach. In the study two types of soil fungi, ectomycorrhizal (EcM) fungi and saprotrophic fungi, were included.

In **Chapter 2** we show that: (1) ectomycorrhiza establishment in *Pinus sylvestris L.* plants inoculated with different isolates of EcM fungi produced an increase in water-stable aggregates (WSA), compared to initial values – in contrast with non-mycorrhizal plants, which did not produce any change but increased the mean weight diameter (MWD) ; (2) soil water repellency (SWR), which measured as water drop penetration time (WDPT) was induced by three EcM fungal isolates which also exhibited mycelium hydrophobicity; (3) root development was only weakly positively correlated with both WSA and WDPT; (4) different EcM fungal isolates affected stability of soil aggregates and repellency of soil differently. This suggests that EcM fungi play a role in both soil aggregation and soil water repellency and using a trait-based approach for the observation of fungal effects on soil structure will be fruitful.

In **Chapter 3** we performed a quantitative data synthesis (meta-analysis), in which we tested whether AS and SWR are two interrelated soil processes, as well as whether the edaphic factors (SOC, soil pH, and soil sand content) and experimental factors (experimental setting, duration, sampling depth, sample drying temperature, measuring methods and burning temperature when treated with fire) affect SWR. We found (1) correlations among SWR, AS and SOC indicating SWR and AS were joint processes connected by SOC; (2) soil pH and sand content both positively related to SWR in the reported ranges; (3) the methods used to quantify SWR did not bias the results; (4) when soil was treated with fire, SWR increases more strongly with increasing AS; and at higher temperature SWR disappeared whereas at lower temperature SWR showed up. The included studied covered less frequently microbial treatments and did not report some crucial abiotic factors like soil water content. SWR can associate with SOC and AS in multiple ways and influenced by abiotic conditions. Our study emphasizes the

importance of integrating AS and SWR in the study of soil stability, and of controlling or recording specific conditions to reveal different aspects of mechanisms.

In **Chapter 4**, we experimentally tested fungal traits of 31 saprotrophic fungi; the traits examined are related to soil aggregation and stability. The chosen traits are growth features (colony extension rate (K_r) and biomass density), enzyme activities (acid phosphatase, cellobiohydrolase, leucine aminopeptidase and laccase) and water-related features (mycelial water content and hydrophobicity of fungal surface (HFS). We showed that fungal traits were affected by cellophane membrane morphologically and physiologically. The colonies had higher enzyme activities at younger age. But the shifts of enzyme activities were not lead to changes in phylogenetic signal. We showed that mycelial water content was an intrinsic trait that should be included in future studies. For the first time we found that HFS, the activity of leucine aminopeptidase and biomass density had phylogenetic signals.

We found the chosen traits were phylogenetically convergent, which means the grouping of the strains based on trait information were similar to their phylogenetic groups. In trade-offs among the traits, K_r and biomass density were potentially negatively related to each when three extreme data points were removed. At the same time the relationship between cellobiohydrolase and laccase activities was strongly influenced by the suspicious extreme points. Here, the extreme data points arise from divergent traits of the phylogenetically close strains, which suggests divergent evolution.

In all, our results suggest that HFS and mycelial water content are two crucial fungal traits, that using cellophane membrane to facilitate collecting fungal traits needs to be employed with care, and that plasticity of fungal traits is a concern when building trait databases.

Chapter 6

Zusammenfassung

Die Hauptzielsetzung dieser Dissertation war es, den Zusammenhang zwischen Aggregation (BA) und dem Wasserabweisungsvermögen des Bodens (BWV) mittels Boden-assoziierter Pilze unter Anwendung des Merkmal-basierten Ansatzes („trait-based approach“) zu ergründen. Im Rahmen dieser Studie wurden zwei Pilztypen in Experimente einbezogen – Ektomykorrhizapilze (EkM) und saprobische Pilze (SP).

In **Kapitel 2** zeigten wir, dass (1) die Etablierung von Ektomykorrhiza in *Pinus sylvestris L.* Pflanzen mittels verschiedener EkM-Isolate die Wasserstabilität von Bodenaggregaten (WSA) sowie die Größenklassenverteilung („mean weight diameter“, MWD) signifikant erhöht im Verhältnis zum Ausgangsboden und den unmykorrhizierten Kontrollpflanzen; (2) Das Wasserabweisungsvermögen des Bodens (BWV), welches als Wassertropfeneindringungszeit („water drop penetration time“, WDPT) gemessen wurde, wurde durch drei der getestete Isolate verstärkt, die ebenfalls nachweisbar ein hydrophobes Myzel aufwiesen; (3) Die Wurzelentwicklung (Wurzelbiomasse und –länge) korrelierte nur geringfügig positiv mit WSA und WDPT; (4) Der Einfluss der verschiedenen EkM-Isolate auf die Faktoren WSA und BWV variierte zwischen den einzelnen Pilzgenotypen. Diese Fakten legen nahe, dass EkM-Pilze eine entscheidende Rolle in der Bodenaggregation sowie dem Wasserabweisungsvermögen von Boden spielen. Diese sollte unter Anwendung des Merkmal-basierten Ansatzes weiter ergründet werden.

In **Kapitel 3** wurde eine quantitative Datensynthese (Meta-Analyse) durchgeführt, mit der wir die Fragen klären wollten, (1) ob BA und BWV zwei Bodenprozesse sind, die in gegenseitiger Wechselwirkung stehen und (2) ob edaphische Faktoren (organischer Bodenkohlenstoff, Boden-pH und Sandgehalt) und experimentelle Faktoren (experimentelle Umgebung, Experimentdauer, Beprobungstiefe, Probentrocknungstemperatur, Messmethode sowie Temperatur von Feuerbehandlungen) BWV beeinflussen. Wir fanden (1) Korrelationen zwischen BWV, BA und organischem Bodenkohlenstoff, was darauf schließen lässt, dass der organische Kohlenstoff im Boden ein verbindender Prozess zwischen BWV und BA ist; (2)

Boden-pH und Sandgehalt korrelieren positiv mit BWV; (3) Die Messmethode zur Bestimmung des BWV hatte keinen verzerrenden Einfluss auf die Analysen; (4) Wenn die Testböden mit Feuer behandelt wurden, konnte eine stärkere Korrelation zwischen BWV und BA festgestellt werden. Dies lag unter anderem daran, dass bei hohen Feuertemperaturen BWV nicht nachweisbar war im Gegensatz zu niedrigen Feuertemperaturen. Die Studien, die in diese Meta-Analyse einbezogen wurden, untersuchten und dokumentierten selten mikrobielle Behandlungsgruppen und Informationen über abiotische Faktoren wie z.B. Bodenwassergehalt. Diese Informationen sind jedoch wichtig, da BWV, BA und organsicher Bodenkohlenstoff auf vielfältige Weisen miteinander interagieren können. Unsere Studie erbringt einen wichtigen Beweis für die Bedeutsamkeit von BA und BWV für die Bodenstabilität.

In **Kapitel 4** untersuchten wir experimentell Charakteristika von 31 saprobischen Pilzen, die wir als bedeutsam für die Bodenaggregation und –stabilität einstuften. Die ausgewählten Merkmale waren Wachstumseigenschaften der Pilzkolonien (Kolonieausdehnungsrate (K_r) und Biomassendichte), Enzymaktivitäten der sauren Phosphatase, Cellobiohydrolase, Leucin-Aminopeptidase und Laccase sowie Wasser-bezogene Merkmale (Myzelwassergehalt und Hydrophobizität der Pilzoberfläche (HPO)). Die Experimente wurden auf Nährmedien mit und ohne Cellophan durchgeführt und wir konnten nachweisen, dass Cellophan die getesteten Pilze physiologisch und morphologisch beeinflussen kann. Des Weiteren war das Koloniealter ein entscheidender Faktor: Die Pilzkolonien hatten eine höhere Enzymaktivität im jüngeren Außenbereich als im älteren Zentrum. Diese Aktivitätsverschiebung war jedoch nicht phylogenetisch manifestiert. Ebenfalls konnten wir zeigen, dass der Myzelwassergehalt ein intrinsisches Merkmal war, welches in zukünftigen Studien Berücksichtigung finden muss. Mit unserer Studie konnte nun zum ersten Mal nachgewiesen werden, dass HPO, die Aktivität der Leucin-Aminopeptidase und die Biomassendichte phylogenetisch konserviert sind.

Darüber hinaus fanden wir, dass die untersuchten Pilzmerkmale phylogenetisch konserviert waren; dies bedeutet, dass die Gruppierung der Pilzisolate basierend auf ihrer Merkmalsinformation mit der phylogenetischen Gruppierung übereinstimmte. Korrelationen innerhalb dieser Merkmale zeigte, dass K_r und die Myzelbiomassendichte negative zusammenhängen, wenn drei Extremwerte aus der Analyse ausgeschlossen wurden. Die Beziehung zwischen Cellobiohydrolase- und Laccaseaktivität wurde stark durch eben diese drei Extremwerte beeinflusst. Diese Werte beruhten auf divergenten Merkmalen phylogenetisch sehr naher Pilzisolate; dies weist auf eine potentielle divergente Evolution hin.

Zusammengefasst zeigen unsere Ergebnisse auf, dass HPO und der Myzelwassergehalt zwei wesentliche Pilzcharacteristika sind, und dass der Einsatz von Cellophanmembranen in Experimenten zur Merkmalserhebung mit Vorsicht durchzuführen ist. Des Weiteren zeitg sich deutlich, dass die Plastizität der Merkmalsausprägungen von Pilzen bei der Etablierung von Datenbank berücksichtigt werden muss.

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Contribution to the publications

I. Zheng W, Morris EK, Rillig MC. (2014). Ectomycorrhizal fungi in association with *Pinus sylvestris* seedlings promote soil aggregation and soil water repellency. Soil Biol Biochem **78**:326–331.

Own contribution: WZ performed all the experiments and analyses and wrote the manuscript. EKM mentored the analysis. EKM and MCR revised the manuscript.

II. Zheng W, Morris EK, Lehmann A, Rillig MC. Does soil water repellency promote soil water-stable aggregation? A meta-analysis (in preparation).

Own contribution: WZ collected the entire dataset, performed analyses and wrote the manuscript. AL and EKM mentored the analysis. All authors reviewed the manuscript.

III. Zheng W, Lehmann A, Rillig MC. Enzyme activities, growth features and water related traits of saprotrophic fungi: studying saprotrophic fungi affecting soil formation and stability using a trait-based approach (in preparation)

Own contribution: WZ performed all the experiments and analyses and wrote the manuscript. All authors reviewed the manuscript.

Appendix A

Supplementary Material for Chapter 3

We tested for a publication bias by plotting the effect size rrSWR against the sample size (replicates) and within-study variance (Egger et al., 1997). Although the studies had negative rrSWR, they were included in the publication.

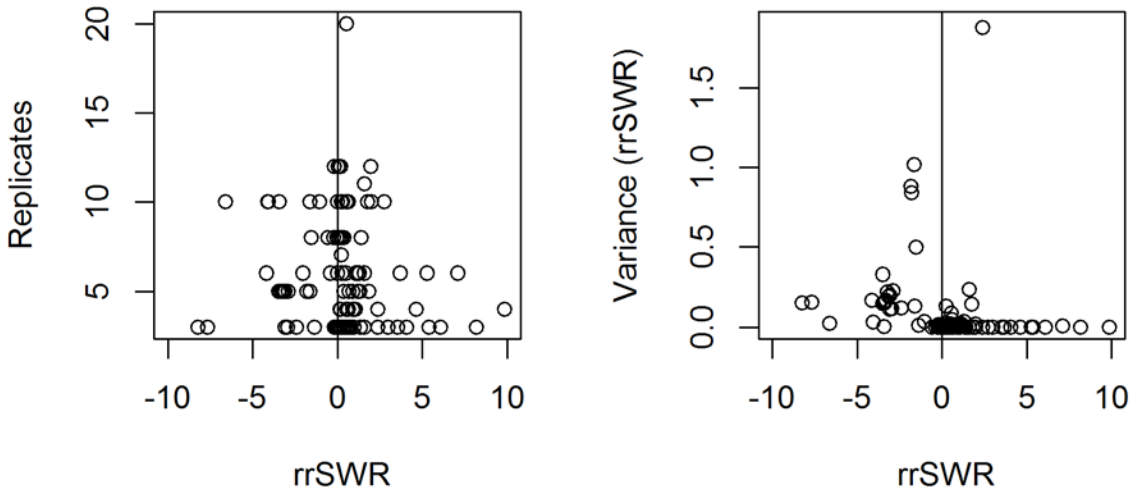

Fig. A-1: Scatterplots of effect size (rrSWR) against sample size and sample variance, respectively.

The robustness of the summary effect size estimates had to be verified for any disproportional impact of single studies. Therefore, a sensitivity analysis (Copas and Shi, 2000) was performed to identify studies with an exceptionally high or low effect (Fig. A-2 to A-5).

However, we only applied this procedure on independent variables significantly affecting rrSWR. After excluding a study, a new random effects meta-analysis was performed and the effect size estimate and the bias CIs were compared with those of the complete dataset. Effect size estimates and bias CIs for each level of the categorical independent variables were investigated. The points in Fig. A-2 to A-5 that are not in the range of the initial effect size had a disproportional impact. Consequently, the meta-analysis had to be re-run without this specific study.

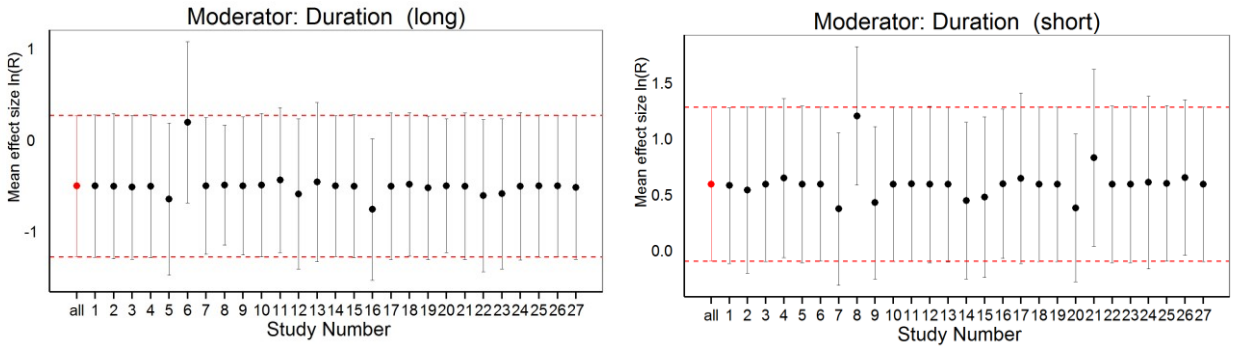

Fig. A-2: Sensitivity analysis of the moderator duration. The values on the x-axes represent study ID of the excluded study.

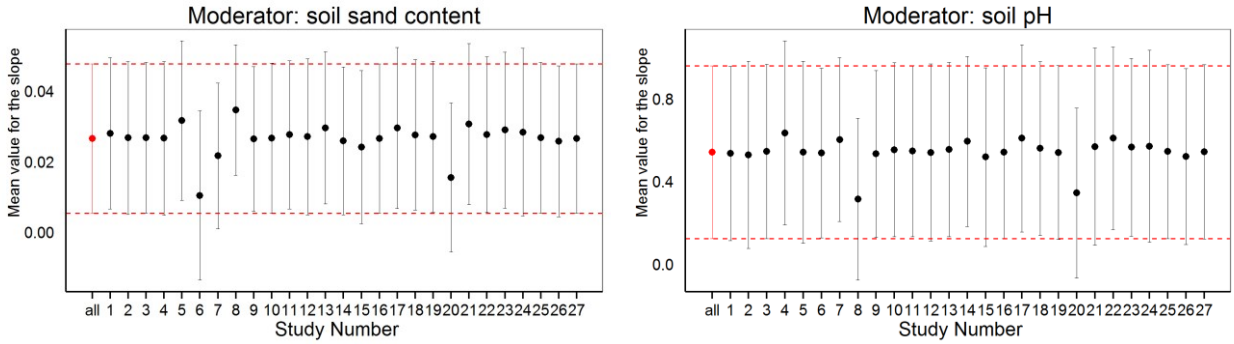

Fig. A-3: Sensitivity analysis of the moderator soil sand content and soil pH. The values on the x-axes represent study ID of the excluded study.

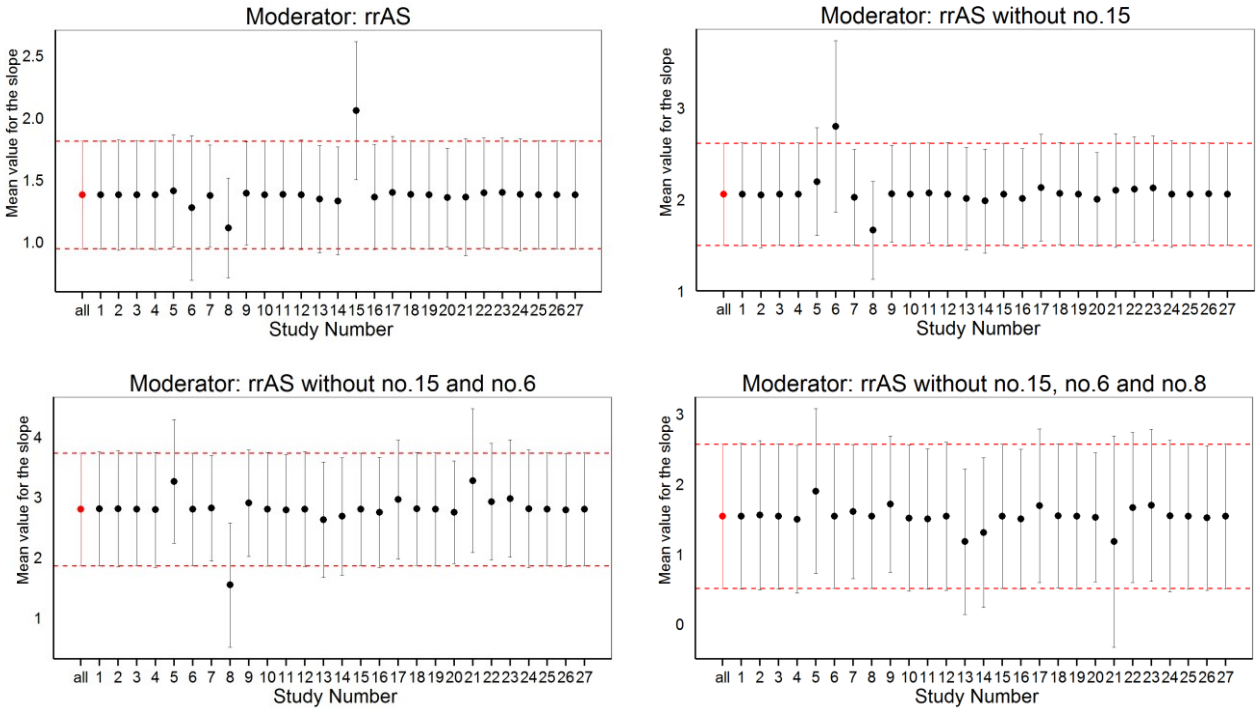

Fig. A-4: Sensitivity analysis of the moderator rrAS. The values on the x-axes represent study ID of the excluded study.

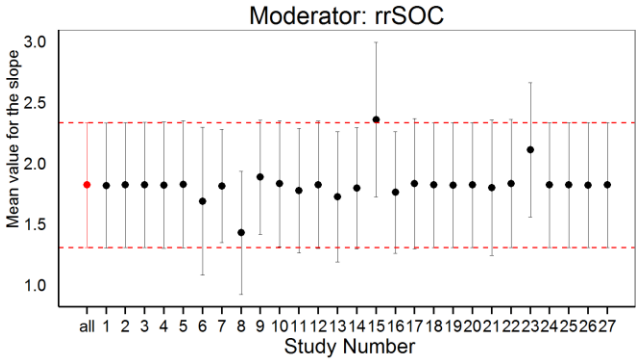

Fig. A-5: Sensitivity analysis of the moderator rrSOC. The values on the x-axes represent study ID of the excluded study.

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Appendix B

Supplementary Material for Chapter 4

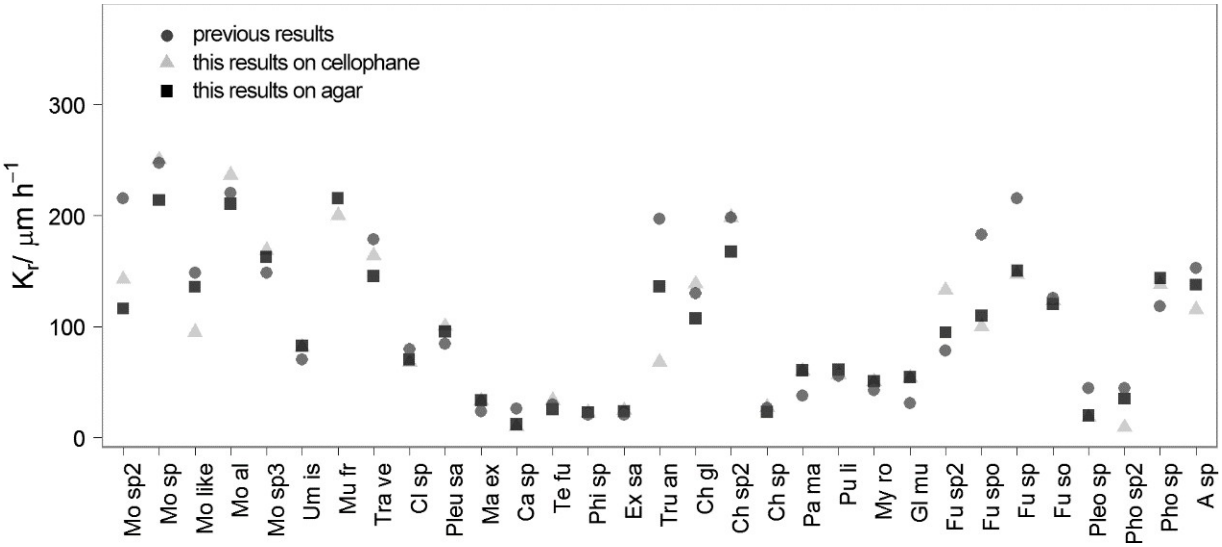
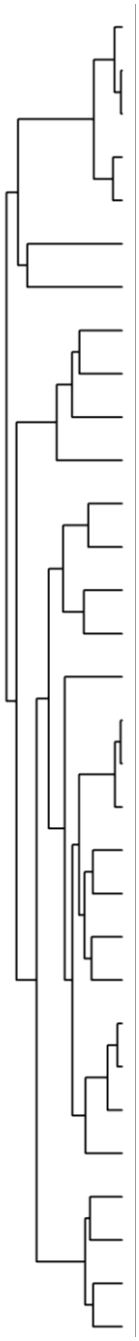


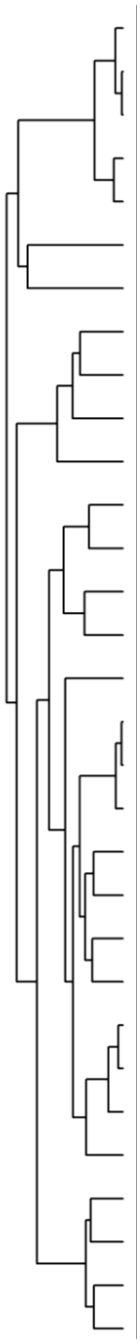
Fig. B-1: Comparing K_r obtained from this study and the preliminary study.

Table B-1: Cultivation period in the experiment according to data from two preliminary studies of the same strains growing in petri dishes containing PDA at 22 °C. When colonies stopped growing or reached the edge of the petri dish maximal diameter and cultivation period were recorded. The Kr is the colony extension rate from another study.



Strains	Cultivation period/ day	K_r/ µm h^{-1}	Maximal cultivation period/ day	Maximal diameter/ mm
*Mo.*species 2	10	215.1	16	62
*Mo.*species	6	247.3	11	85
*Mo.*like species	10	148.5	16	77
Mo.alpina	6	220.0	11	83
*Mo.*species 3	11	148.1	18	85
U.isabellina	13	70.4	35	40
Mu.fragilis	6	372.7	7	85
Tra.versicolor	11	178.6	7	85
*Cl.*species	14	79.5	16	74
Ple.sapidus	13	84.6	35	85
Ma.excoriate	33	23.2	35	70
*Ca.*species	36	25.7	35	51
Te.furcatum	26	30.0	35	42
*Phi.*species	36	20.2	35	36
Ex.salmonis	33	20.7	35	44
Tru.angustata	13	196.8	35	80
Ch.globosum	12	129.7	11	85
*Ch.*species 2	9	198.5	31	81
*Ch.*species	26	26.8	35	29
Pa.marquandii	20	37.6	9	85
Pu.lilacinum	13	55.2	35	42
My.roridum	26	42.6	35	55
G.murorum	22	31.1	35	78
*F.*species 2	13	78.1	35	78
F.sporotrichioides	14	183.0	16	62
*F.*species	11	215.2	16	82
F.solani	14	125.5	15	80
*Ple.*species	36	44.6	35	75
*Pho.*species 2	26	44.7	35	76
*Pho.*species	12	117.9	9	85
*A.*species	17	152.4	16	85

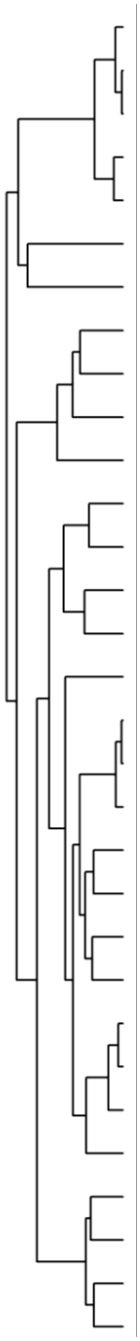
Table B-2: Acid phosphatase (Pho) and leucine aminopeptidase (Leu) activities in inner and outer zones of colonies growing on agar with cellophane (standard deviation in brackets, n=6). The activities were units per mg dry biomass. The solid lines divide the table into Mucoromycotina, Basidiomycota and Ascomycota parts downwards, the dashed lines separate orders.



Strains	Pho$^{(i)}$	Pho$^{(o)}$	Leu$^{(i)}$	Leu$^{(o)}$
*Mo.*species 2	0.16(0.11)	0.30(0.25)	1.22(0.66)	1.42(0.63)
*Mo.*species	0.63(0.20)	0.97(0.40)	1.09(0.45)	1.21(0.68)
*Mo.*like species	1.13(0.85)	1.15(0.72)	0.43(0.27)	1.49(0.86)
Mo.alpina	0.56(0.19)	0.46(0.23)	2.14(0.39)	2.82(1.12)
*Mo.*species 3	0.47(0.27)	0.96(0.52)	1.95(0.77)	2.09(0.92)
U.isabellina	0.17(0.12)	0.24(0.09)	0.60(0.40)	3.58(1.70)
Mu.fragilis	0.37(0.28)	0.38(0.19)	5.50(1.73)	7.09(2.57)
Tra.versicolor	1.02(1.33)	2.45(2.78)	0.96(0.67)	1.41(0.92)
*Cl.*species	0.13(0.08)	0.08(0.04)	1.06(0.47)	1.44(1.31)
Ple.sapidus	0.03(0.01)	0.11(0.07)	1.22(0.80)	4.28(3.07)
Ma.excoriate	0.02(0.03)	0.02(0.02)	0.19(0.24)	0.13(0.19)
*Ca.*species	0.52(0.42)	0.79(1.03)	0.43(0.11)	0.65(0.23)
Te.furcatum	0.12(0.08)	0.08(0.04)	0.23(0.14)	0.84(0.21)
*Phi.*species	0.32(0.20)	0.33(0.27)	0.06(0.06)	0.25(0.08)
Ex.salmonis	0.00(0.00)	0.00(0.00)	0.09(0.09)	0.65(0.29)
Tru.angustata	0.74(0.57)	0.17(0.11)	0.09(0.08)	0.00(0.14)
Ch.globosum	0.17(0.05)	0.14(0.07)	0.89(0.40)	1.15(0.91)
*Ch.*species 2	0.20(0.14)	0.27(0.31)	0.56(0.50)	0.64(0.40)
*Ch.*species	0.22(0.10)	0.23(0.07)	2.21(0.57)	3.30(1.32)
Pa.marquandii	0.72(0.49)	1.54(1.84)	0.72(0.23)	0.62(0.27)
Pu.lilacinum	0.30(0.15)	0.50(0.40)	0.61(0.41)	1.70(0.48)
My.roridum	0.47(0.47)	0.37(0.55)	0.44(0.16)	0.81(0.36)
G.murorum	0.52(0.39)	0.85(0.41)	0.49(0.10)	1.00(0.36)
*F.*species 2	0.32(0.27)	0.50(0.56)	0.40(0.24)	0.60(0.37)
F.sporotrichioides	0.20(0.14)	0.17(0.06)	0.23(0.08)	0.56(0.45)
*F.*species	1.29(0.48)	1.70(1.66)	0.44(0.35)	2.00(0.79)
F.solani	0.30(0.35)	0.36(0.39)	0.32(0.23)	1.59(1.74)
*Ple.*species	0.31(0.19)	0.45(0.35)	0.38(0.15)	0.45(0.20)
*Pho.*species 2	1.70(0.98)	1.26(1.54)	1.14(0.57)	1.73(0.69)
*Pho.*species	0.79(0.52)	0.87(1.39)	0.11(0.04)	0.23(0.09)
*A.*species	0.02(0.02)	0.07(0.05)	0.26(0.17)	0.68(0.76)

Table B-3: Cellobiohydrolase (Cel) and laccase (Lac) activities in inner and outer zones of colonies growing on agar with cellophane (standard deviation in brackets, n=6). The activities were units per mg dry biomass. The solid lines divide the table into Mucoromycotina, Basidiomycota and Ascomycota parts downwards, the dashed lines separate orders.

Strains	Cel$^{(i)}$	Cel$^{(o)}$	Lac$^{(i)}$	Lac$^{(o)}$



*Mo.*species 2	0.00(0.00)	0.00(0.00)	0.02(0.01)	0.02(0.01)
*Mo.*species	0.00(0.00)	0.00(0.00)	0.01(0.01)	0.01(0.01)
*Mo.*like species	0.00(0.00)	0.00(0.00)	0.02(0.01)	0.02(0.02)
Mo.alpina	0.00(0.00)	0.00(0.00)	0.01(0.01)	0.02(0.01)
*Mo.*species 3	0.00(0.00)	0.00(0.00)	0.02(0.03)	0.05(0.05)
U.isabellina	0.02(0.01)	0.01(0.01)	0.01(0.01)	0.03(0.02)
Mu.fragilis	0.06(0.05)	0.05(0.02)	0.02(0.02)	0.04(0.03)
Tra.versicolor	0.03(0.02)	0.03(0.01)	0.83(0.37)	4.19(7.65)
*Cl.*species	0.01(0.00)	0.02(0.01)	2.00(0.95)	2.68(0.97)
Ple.sapidus	0.03(0.02)	0.08(0.04)	1.63(1.20)	3.31(1.94)
Ma.excoriate	0.03(0.01)	0.04(0.02)	3.67(2.07)	10.38(5.29)
*Ca.*species	0.05(0.02)	0.05(0.02)	0.02(0.02)	0.01(0.02)
Te.furcatum	0.02(0.01)	0.05(0.01)	0.15(0.14)	2.79(1.17)
*Phi.*species	0.02(0.01)	0.03(0.01)	0.43(0.19)	0.63(0.29)
Ex.salmonis	0.01(0.01)	0.03(0.02)	0.01(0.01)	0.02(0.01)
Tru.angustata	0.04(0.04)	0.10(0.09)	0.72(0.65)	1.45(1.29)
Ch.globosum	0.12(0.07)	0.07(0.02)	1.72(1.14)	3.46(2.16)
*Ch.*species 2	0.02(0.01)	0.03(0.02)	0.03(0.01)	0.03(0.02)
*Ch.*species	0.01(0.00)	0.02(0.01)	1.45(1.07)	4.54(3.43)
Pa.marquandii	0.08(0.03)	0.04(0.02)	0.16(0.10)	0.13(0.20)
Pu.lilacinum	0.01(0.01)	0.03(0.02)	0.01(0.00)	0.02(0.01)
My.roridum	0.11(0.16)	0.13(0.25)	0.09(0.05)	3.29(2.58)
G.murorum	0.01(0.01)	0.04(0.02)	0.03(0.01)	0.09(0.04)
*F.*species 2	0.02(0.01)	0.03(0.01)	0.03(0.02)	0.05(0.04)
F.sporotrichioides	0.05(0.05)	0.04(0.04)	0.04(0.03)	0.08(0.05)
*F.*species	0.04(0.01)	0.07(0.03)	0.06(0.04)	0.07(0.06)
F.solani	0.03(0.02)	0.04(0.07)	0.11(0.17)	2.27(4.06)
*Ple.*species	0.05(0.02)	0.05(0.03)	0.05(0.02)	0.52(0.64)
*Pho.*species 2	0.06(0.02)	0.12(0.10)	1.35(0.62)	1.35(0.69)
*Pho.*species	0.01(0.00)	0.01(0.00)	0.02(0.01)	0.02(0.01)
*A.*species	0.01(0.01)	0.08(0.01)	0.18(0.19)	1.51(2.01)

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