Understanding the ecological role of root infecting fungi through phenomenological and trait based approaches

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Foreword

This Dissertation is a cumulative work of the following published or submitted manuscripts:

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List of Tables

List of Figures

Table of Contents

Chapter One

General Introduction

Interactions of roots with soil filamentous fungi

The soil represents one of the major habitats for filamentous fungi (Blackwell 2011). Within this habitat, filamentous fungi must procure carbon substrates, nutrients and water to keep active growth, or produce long term resting structures until resources are available. An important pool for those resources is organic matter. Certainly, soil fungi are major players in the decomposition of soil organic matter (Osono 2011).

Nonetheless, decomposing organic matter represents a costly enzymatic investment for fungi given its typically recalcitrant nature (Lynd et al. 2002). In contrast, actively growing roots represent a much more accessible resource pool, not only of carbon but also for other nutrients such as nitrogen and phosphorus. Thus, it is not surprising that most microbial activity in soil occurs around the root zone (in the so called rhizosphere) (Berendsen et al. 2012) or to detect a huge diversity of fungi growing on and within the root tissue (Rodriguez et al. 2009).

Given the importance of root systems for soil fungi, it is expected that most soil fungi have evolved traits that facilitate the interaction with plants. Likewise, this constant interaction of roots with roots-colonizers will result in the selection for root traits that would minimize the negative effect of the interaction (Poisot et al. 2011). As a result, it can be hypothesized that most soil fungi show some level of association with roots. The fact that both fungi and plants seem to interact since early in the evolution of angiosperms (Klymiuk et al. 2013), indicate that Root Infecting Fungi (RIF) are subject to a co-evolutionary dynamics with plants, resulting usually in a symbiosis. Symbiosis in this sense is understood as an intimate association between the host and the fungus, without implying any particular fitness effects of the interaction on the partners (Newton et al. 2010).

Among all soil fungal interactants, mycorrhizal fungi are the best characterized symbiotic relationship (van der Heijden et al. 2015). In this mutualisitic symbiosis the fungus helps the plant to acquire nutrients, while the plant provides carbon to the fungus. At its extreme, the fungi in the phylum Glomeromycota have lost all traits for a successful free-living lifestyle, while the mycorrhizal

dependent plants have suboptimal traits for a successful acquisition of nutrients on their own (Smith and Read 2010).

On the other hand, the symbiosis of soil fungi in the phyla Ascomycota and Basidiomycota, which represent the largest diversity of RIF in soil (O'Brien et al. 2005), is less understood. A small fraction has been classified as mycorrhizal given the clear presence of host-fungal nutrient exchange interfaces (e.g. Hartig net in ectomycorrhiza), particularly with shrubs and trees (Smith and Read 2010). However, most of them lack such structures, though exhibit extensive root colonization (Porras-Alfaro and Bayman 2011).

Furthermore, the same RIF genera which symptomlessly colonize root tissue in natural systems constitute the causal agents of most known soil-borne fungal diseases in agriculture, which in most cases derive in the death of plant tissue or the entire host (necrotrophic and wilting pathogens) (Agrios 1997). Hence, unlike mycorrhizal fungi, symbiosis within these fungal groups is extremely dynamic, shifting drastically along a parasitic-mutualistic continuum depending on the specific context where the interaction has evolved (Stukenbrock and McDonald 2008).

Given this scenario, it can be expected that community assembly processes of non-mycorrhizal RIF and plants feedback to one and another. Community assembly refers to a processes where the local abiotic environment and existing biota determine the numbers of species present at the local community (richness), the identity of the species (composition) and their relative abundance (structure) (HilleRisLambers et al. 2012). In the case of biotic interactions, this is because the presence of antagonistic species (competitors, herbivores or parasites), or the absence of key mutualists can greatly influence the establishment and fitness (vegetative growth, number of offspring) of plant species at certain location(s).

Yet there is an absence of empirical evidence to evaluate the role of community dynamics of non-mycorrhizal RIF on plant community assembly. Specifically, when surveying the literature two major gaps were identified in empirical research on this topic: (1) the lack of phenomenological experiments measuring the effects of the interactions between plants and non-mycorrhizal RIF on plant community structure and (2) a lack of mechanistic attempts to understand the effects of such interactions. The present thesis aims to address these gaps.

First gap: Lack of phenomenological evidence

The best phenomenological evidence to test the effects of RIF-plant interactions (or any species interaction) on community assembly, is by experimentally manipulating the presence (additions) or absence (removals) of species (Siepielski and McPeek 2010). Surprisingly, manipulations of soil fungal guilds have been mostly restricted to mycorrhizal interactions and are virtually absent with non-mycorrhizal RIF (van der Heijden et al. 1998; but see Rillig et al. 2014). Though compelling indirect evidence comes from studies under the Plant Soil Feedback (PSF) or "microbial wash" approach. In the former, the "manipulation" is indirect, by collecting soil from plant individuals after at least one growing period and measuring the effect of such "trained" soil on vegetative growth of con- and heterospecifics (Bever et al. 2010). Evidence points out that these effects can be species specific, potentially driving plant community dynamics. However, most PSF studies do not characterize the community structure in the soil biota, not providing conclusive evidence on relative the importance of non-mycorrhizal RIF in driving the observed effects. The "microbial wash approach" segregate AMF from non-AMF propagules based on spore size classes, however they pooled both RIF with less symbiotic (saprotrophic) fungi (e.g. Schnitzer et al. 2010), a distinction treated in detailed below. Chapter one of this thesis provides the result of an experimental manipulation directly aimed at measuring the effect of RIF on plant community structure.

Second gap: lack of mechanistic understanding of community dynamics and its consequences

Outcomes from phenomenological approaches are limited in that they merely report that the presence of a species exerts some effects on the fitness of another species. Determining, for example, whether or not the outcome of an interaction can be due to competition for resources or through indirect effects through shared enemies, requires understanding the similarity of the traits for resource acquisition and defense against enemies (Adler et al. 2013). Therefore, a mechanistic understanding of the consequences of species interactions requires understanding of the traits that makes them possible.

The same argument holds for understanding the interaction of species with the abiotic environment. A mechanistic understanding of species fitness along environmental gradients (resources, stress), requires knowledge of the traits necessary to withstand such gradients (e.g. traits for tolerance of high or low temperatures) (McGill et al. 2006). Since species do not only

passively respond to the abiotic environment, but also can modify it, trait information can also be used to understand the effects of the species on their ecosystem (Petchey and Gaston 2006). The importance of trait based approaches have long been recognized among plant and animal ecologists.

However, there are several limitations when using trait based fungal understanding for phenomenological experiments like the one of the first chapter. One limitation is a matter of perception: mycologists are largely unfamiliar with the general framework of trait-based ecology. Chapter two of this thesis develops a theoretical framework of how trait based approaches can be used to understand ecology of RIF. This framework is based on the dual lifestyle of most non-mycorrhizal RIF: progress on trait based RIF ecology can be achieved by identifying the traits reflecting their symbiosis with plants as well as the traits reflecting their free living status in the soil.

The second is a limitation of application: fungal trait data are limited, there is scarcity of established methods for measurement of *de novo* traits and there are few attempts to use trait based tests to understand fungal ecology. Chapter three of the present thesis builds a framework for the application of trait based approaches in fungal ecology in general. It also present a case study using trait fungal data applied to a trait based test originally developed to understand the role of abiotic parameters on plant community assembly.

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Chapter Two

Soil texture and root infecting ascomycete fungi interactively affect plant community structure

Background and aims Understanding the role soil fungal interactions in determining plant community structure is a major research interest in ecology. From all fungal interacts, non-mycorrhizal associates have received little attention despite constituting the most diverse group of root infecting fungi (RIF).

Methods A greenhouse experiment was conducted using experimental microcosm plant communities to test these fungi on plant community structure. Soil texture (high and low sand content) and fungal composition in soil (single inoculations with three distinct isolates and a mixture of all three) were manipulated in fully factorial fashion.

Results Each plant species present in the microcosms responded differently to infection, resulting in distinct patterns of plant community structure. Each fungus provided benefits to some host species while negatively affecting others. The host responses to infection were strongly dependent on soil texture: positive responses conferred to a host at one texture level were absent in the other level. Further, host responses to the higher fungal diversity treatment (mixture inoculation of 3 fungi) were also dependent on soil texture.

Conclusions Non-mycorrhizal RIF can exert significant effects on plant community structure as well as greatly modify the way soil abiotic factors shape plant community dynamics.

Keywords: Plant community structure, root infecting fungi, endophytic *Fusarium*.

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Introduction

Understanding the drivers behind changes in community structure represents a major goal in plant community ecology. Theory predicts that community structure depends on species specific differential responses to strong abiotic environmental gradients (Chesson 2000; Sarr et al. 2005). At local scales, where climatic conditions are considered to be homogenous, species specific responses to gradients in soil abiotic parameters are considered the most important factor in determining the identity, relative abundance and fitness of interacting plants (Wijesinghe et al. 2005).

However, plants also show differential responses to the matrix of fungal interactions in which they are embedded. For example, plants show strong interspecific variability in responses to mycorrhizal fungal composition and diversity (e.g. van der Heijden et al. 1998a; van der Heijden et al. 1998b) which varies at a local scale (Mummey and Rillig 2008; Horn et al. 2014). Furthermore, evidence from soil feedback studies reveals that non-mycorrhizal fungi could have similar effects on community structure (van der Heijden et al. 2008; Bever et al. 2010).

Among such non-mycorrhizal fungi, endophytic root-infecting fungi (RIF) in the Ascomycota are an important but understudied group that may drastically affect plant community structure (Rodriguez et al. 2009; Aguilar-Trigueros et al. 2014). This group of fungi has received little attention in plant community ecology, perhaps due to the cryptic nature of the interaction, i.e. symptomless colonization and lack of complex plant fungal interfaces like in mycorrhizal symbioses (the hallmarks of endophytic infection). Nevertheless, recent community surveys using DNA sequencing methods reveal that this group represents the largest fraction of fungal root interactants, in some cases being five times larger in terms of species richness compared to arbuscular mycorrhizal fungi (Wehner et al. 2014). Furthermore these RIF possess attributes that potentially make them strong drivers of community structure. First, they have broad host ranges (Hersh et al. 2011; Malcolm et al. 2013), meaning that interacting (neighboring) plants can be infected by the same fungal species, even by the same fungal genotype. Second, even a single isolate can exhibit negative, neutral or beneficial effects on the hosts (Mandyam et al. 2012; Mayerhofer et al. 2013), giving rise to indirect plant-plant interactions (e.g. apparent competition; Holah and Alexander 1999). Third, at least in the case of fungal foliar endophytes in the Ascomycota, observed plant responses to abiotic factors depend strongly on the presence of particular endophytes. Together, these attributes highlight the need to gather empirical evidence to assess the importance of host responses to this set of root fungi in contributing to changes in plant community structure.

In order to evaluate the differential host responses to this group of fungi, we conducted a greenhouse experiment where we manipulated the presence and composition of RIF in microcosms under two soil-treatment levels in a fully factorial fashion. This design allowed us to test the following specific questions: Can differential plant responses to endophytic RIF cause changes in plant community structure? Do plant responses to endophytic RIF change depending on soil type? Are responses different when increasing the diversity of endophytic RIF interactants? And if so, under which conditions?

Materials and Methods

The study system

The experimental set up was designed to recreate conditions found in a natural grassland located near the town of Mallnow, Lebus (Brandenburg, Germany, 52°127.77' N, 14° 129.349' E). This grassland belongs to the protection area "Oderhänge Mallnow" which is part of a large (60 km long and up to 20 km wide) post glacial region with dry grassland habitats occurring along the Oder River in north eastern Germany (Wehner et al. 2014). The grassland is managed by low intensity sheep grazing.

On October 2010, we sampled plant roots for subsequent isolation of root-infecting fungi (see description below) from a 15 x 15m plot located to encompass a strong gradient in soil texture from loamy to sandy soil along a hillside. This variation in soil texture also corresponded with a steep change in soil parameters such as C/N ration, available P and pH (Horn et al. 2014). Within this plot, the tussock grass, *Festuca brevipila* R.Tracey, was found to represent up to 70% of vegetation cover, which included 47 other herbaceous species (Horn et al. 2015). Common associated plant species were *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl*., Armeria elongata* Hoff*., and Rumex acetosella* L. which have been frequently reported for dry grassland in this region (Hensen 1997).

Fungal isolation and characterization

Roots of 27 individuals of *Festuca brevipila* were sampled within the plot. Each root system was sectioned into 1 cm long pieces, surface sterilized in a series of washes with 0.525% sodium hypochlorite for 3 minutes and 70% ethanol for one minute (Crous et al. 2009). Then, the root fragments were plated on Malt Extract Agar (MEA 2%) with Rose Bengal. This medium is frequently used for isolation of RIF. The fungal isolates were initially grouped into morphotypes according to colony characteristics (colony size, shape and color on Malt Extract Agar). From a clean culture of

each morphotype we took a piece of mycelium to extract DNA using the Power-Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the procedures in the manufacturer´s manual. Then we amplified the ITS region using the primers ITS1F and ITS4. PCR products were digested for 2 hours at 37^{o}C using the restriction enzymes *BsuRI, Hin61, HinfI* and *MboI.* These enzymes have been used previously for screening of soil borne fungi (Viaud et al. 2000). The digestion products were electrophoresed in 2% agarose gels, and RFLP profiles were then used to re-group morphotypes. The PCR products of each RFLP type were purified to remove non-incorporated ITS primers using a PCR clean-up kit (Macherey-Nagel, Düren, Germany) and sent to LGC Genomics (Berlin, Germany) for Sanger sequencing. Sequences were then used in a BLAST search to assign putative taxonomic affiliation.

For the present study, we used fungi which the closest BLAST matches identified as *Fusarium redolens* (accession number GU934525.1, 100% identity match)*, Gibberella sp.* (accession number JF773634.1, 100% identity match) and *Microdochium sp.* (accession number GQ923958.1, 99.25% identity match). The spore characteristics of the isolated fungi matched the genus descriptions. The three fungi produced very contrasting morphology on Malt Extract agar (Fig. AS1), even the two *Fusarium* species. We chose *Fusarium* and Gibberella (which is the teleomoph genus name for *Fusarium*) species because they are ubiquitous ascomycetous RIF with broad host ranges, and exhibit a wide array of host effects (from extremely pathogenic in agricultural systems to common endophytes in natural systems) (Gordon and Martyn 1997; Bacon and Yates 2006). *Microdochium* has similar characteristics, with some isolates being reported as pathogens of turf-grasses and cereals (Ren et al. 2014) while others are commonly found as endophytes in temperate grasslands (Mandyam et al. 2010). Moreover, in agricultural systems *Fusarium* and *Microdochium* species are commonly associated with cereal roots (Ren et al. 2014). For simplicity we use the genus names throughout. The fungi were characterized in terms of colony morphology, conidium type and intra-radical growth by *in vitro* inoculation on *Festuca brevipila* seedlings. The *in vitro* tests showed that none of the isolates were virulent necrotrophic pathogens, but instead had neutral to marginal positive effects on the growth of *Festuca brevipila*, typical of many endophytic fungi (Fig. AS2).

Inoculum production and soil inoculation

A single fungal colony growing on potato dextrose agar (PDA) petri dishes corresponding to the three chosen genera were used as the source for the production of inoculum for the experiment. The fungi were grown on sterilized oat kernels for inoculum production and used for soil inoculation

in the microcosms (Singleton et al. 1992). This method generates fungal propagules that are attached to organic material in the soil, reflecting the saprotrophic capabilities of this set of fungi. Briefly, the oat kernels were soaked in water overnight, autoclaved twice and inoculated with agar plugs (PDA) from 2 week old colonies. The fungi were grown in the kernels for one month in 500 ml Erlenmeyer flasks sealed with aluminum foil and parafilm. Gas exchange was allowed by frequently opening the flasks under a sterile hood to avoid contamination. The soil was mixed with the kernels at 1% of the final microcosm volume. Some kernels were plated on PDA prior soil infestation to verify the lack of contamination in the treatments. The soil used in the experiment was obtained near the 15 x 15 plot where the plants were sampled.

Experimental design

The experimental community was composed of three perennial plant species that commonly co-occurred in the 15 x 15 m field plot, namely *Arrhenatherum elatius* (Poaceae*), Festuca brevipila* (Poaceae) and *Armeria elongata* (Plumbaginaceae). The experiment used a full factorial design with fungal identity and soil texture/ fertility as treatment factors. The fungal identity treatment had five levels: single species inoculations of each of the three fungal isolates (*Fusarium, Gibberella, Microdochium*), a mixture of the three (Mixture treatment), and a mock inoculated control (autoclaved kernels that were never inoculated with the fungi and which remained free of other fungal contaminants). For the Mixture treatment, the volume of inoculum was also 1% of soil volume, but it was divided into equal proportions of the fungal isolates. The soil texture/fertility treatment consisted of two levels: field collected soil, and a 1:1 mixture of field collected soil and sand (referred to as Low Sand and High Sand respectively). This treatment reflected the natural variability in soil texture of the 15 x 15 m field plot as reported in previous studies in the area (Horn et al. 2014) and corresponds to steep changes in soil fertility (C, N, P, pH). Prior to inoculation, the soil and sand were steam sterilized twice (4 hours, 100ºC) to eliminate other soil borne fungi. Each treatment combination was replicated 10 times (5 fungal treatments x 2 soil treatments x 10 replicates = 100 microcosms).

All the seeds used in the experiment came from the same area where the field plot was located. The seeds were surface sterilized with NaOCl (5%) and ethanol (70%) and germinated on sterile glass beads. Two week old seedlings were transferred to the 3 L gardening pots, which constituted our microcosms. In total, two individuals per species were used in each microcosm, giving a total density of 6 plant individuals per microcosm. The plants were arranged haphazardly in

the microcosm at least 3cm away from the border of the pot. All microcosm were maintained from January-April 2012 under glasshouse conditions (12 hours of light; 20^{o}C/18^{o}C temperature day/night; 45.5 % relative humidity). Plants were watered twice a day with 15 ml (first month), 30ml (second month) and 60ml of tap water until the end of the experiment.

Harvest

The first harvest was carried out three months after the start of the experiment. The shoots of the two grasses were cut 3 cm above the soil surface, but *Armeria elongata* was not cut given its rosette growth. This harvest was intended to reduce aboveground competition among the interacting species, especially from *Arrhenatherum elatius.* The second and final harvest was done one month after the first. All three species were clipped at ground level and placed in a drying oven at 50°C for one month and dry weight was measured. The whole root system of each microcosm was destructively harvested, and sub samples were taken by randomly clipping different portions. From 5 microcosm per fungal identity x soil combination (half of the replicates) thirty root segments of *A. elatius* and thirty of *F. brevipilla* were plated on PDA. The number of retrieved colonies per fungal isolates was recorded. Some of the colonies were transferred to individual plates to verify the identity of the fungus. Determinations of inoculated fungi and contaminants were easy to assess given characteristic features in colony morphology of the isolates when grown in pure culture in PDA at 25°C. *Fusarium redolens* produced fast growing colonies, with few aerial (flat) white mycelia and a margin with rhizoids. *Gibberella sp* produced very distinctive dome shaped mycelia with orange color from above and scarlet from below; within a week colonies became radially striate with a lobate edge. *Microdochium* sp produced very flat mycelium with an undulate margin; colonies initially were salmon colored from above but became olivaceous black as chlamydospores were produced after a week of growth (Fig. AS1).

From the same 5 microcosm, another subsample was taken and assessed for presence of fungal intra-radical structures by trypan blue staining and light microscopy (Phillips & Hayman 1970).

Data analysis

We used colonization and re-isolation data to assess which inoculated fungi were present at the end of the experiment. Data were analyzed as counts (number of retrieved colonies, number of observed fungal structures) using generalized linear models (GLMs), with quasi-poisson error structure to correct for overdispersion.

We performed a two way-ANOVA to detect the effect of RIF identity, soil type and their interactions on microcosm productivity. In the analysis, we used the combined biomass of the three species per microcosm for each harvest as the response variable. Next, we used a two-way MANOVA to test whether RIF identity, soil treatment and the interaction significantly modified community structure. In this case, the biomass of each species per microcosm was used as a proxy for abundance.

Next, we determined the effect of each fungus on the biomass of each plant species in each soil type. Within a soil type, we used one-way ANOVAs, where RIF identity was considered as a factor and the biomass of each plant species as a response variable. Then, we used model simplification by step-wise deletion to identify which and how each fungus influenced host plant biomass. This technique results in a simplified statistical model indicating only treatments that differ significantly from the control and one another (Crawley 2012). We further tested if the changes in relative biomass caused by the fungi altered competitive interactions by creating a correlation matrix of the biomass of the 3 interacting species for each soil x fungal treatment combination. Negative correlations indicate competitive interactions (Goldberg and Landa 1991). All the analyses were performed in R (R Development Core Team 2011).

Results

Re-isolations and microscopy

Re-isolation data showed that the control treatment remained contamination free while there was successful recovery of *Fusarium* and *Gibberella* (Fig. AS3) from treatments where they were used as inoculum. Although it was not possible to re-isolate *Microdochium,* fungal structures (hyphae, spores) were commonly observed in the roots of the two grasses in this treatment (*A. elongata* roots were difficult to localize and therefore were not assessed) (Fig. AS4). Furthermore, in this treatment typical *Microdochium* chlamydospores were frequently observed in the cortex and root hairs (such spores are also observed in pure *Microdochium* cultures on Malt extract agar (Fig. AS5). This type of chlamydospore has been frequently reported during colonization of endophytic *Microdochium* strains in temperate grasses (Mandyam et al. 2010).

Effects on aboveground productivity

Plant biomass data for the two grasses were pooled across the two harvests, as their biomass responses were similar at both (data not shown). We used non-transformed data of

aboveground productivity (total biomass of the three plant species) as there were no significant deviations from assumptions of normality of residuals and homoscedasticity of variances after removal of outliers (3 microcosms that were water-logged and one microcosm in the *Microdochium*/Low Sand treatment where the biomass was 2.1 times larger than the interquartile range above the third quartile of that group (Crawley 2012)).

Soil type, identity of inoculated RIF and their interaction significantly altered aboveground productivity in the microcosms (Table 2.1); while for belowground productivity the interaction term was not significant (Table 2.1). Total above- and belowground biomass was lower in the high sand treatment than in the low sand treatment (reduction of 20% and 28%, respectively, by comparing the two control treatments). Separate ANOVAs on each soil type showed that aboveground productivity was significantly affected by fungal treatment (High-Sand: F= 8.6, p<0.001; Low-Sand: F=6.82, p<0.001). In the High Sand treatment, the minimal adequate model indicated that aboveground productivity in the pooled treatments *Microdochium* and Mixture were significantly smaller than the pooled Control, *Fusarium* and *Gibberella* treatment (t=-5.5, P<0.001; pooled treatments do not differ significantly from one another) (Fig. 2.1). In contrast, belowground biomass in the High Sand was significantly larger in the *Fusasium* and *Gibberella* treatment with respect to pooled Control, *Microdochium* and Mixtures treatments (t=-4.39, P<0.001) (Fig.2.1).

In the Low Sand treatment, statistical significance was only detected in the comparison of the Mixture treatment to the control (t=5.17, P<0.001). Belowground productivity was not significantly different from the control in any of the treatment (Fig. 2.1).

Effects on community structure

In the case of the two grasses we used non-transformed data of the biomass as there were no significant deviations from normality of residuals or homoscedasticity of variances. In the case of *Armeria elongata*, Bartlett test detected heteroscedasticity and no transformation solved the issue. However, visual inspection of the error distribution indicated that the heteroscedasticity was due to three values in three different treatments while no correlation between the mean and error was observed. Therefore we also used non-transformed data of *Armeria elongata* in the analysis.

Plants of the three species had a greater biomass in the Low Sand treatment compared to High Sand, but responded differently to fungal treatment. MANOVA using the biomass of the three plant species as response variables indicated that soil type, identity of inoculated fungus and the

interaction had a significant effect on the biomass of these plant species (soil type: Pillai= 0.40, F=19.06, p<0.01; Fungal treatment: Pillai= 0.60, F=5.42, P<0.01; interaction: Pillai= 0.34; F=2.78; P< 0.01) (Table 2.2). Likewise, the biomass of each plant species was significantly affected by the experimental treatments: *A. elatius* was significantly affected by soil and fungal treatment as well as the interaction; in *F. brevipila* significance was detected only in the interaction term (soil x fungal treatment); while for *A. elongata,* fungal treatment and the interaction soil x fungal treatment were statistically significant (Table 2.1).

High Sand treatment

In the High Sand treatment individuals of *A. elatius* experienced mainly negative effects in all the fungal treatments, while for *F.brevipila* and *A. elongata* neutral to positive effects were predominant. Individuals of *A. elatius* growing in the *Fusarium* and *Gibberella* treatments were on average 3% larger compared to their respective controls while the ones in the *Microdochium* and Mixture treatments were 16% smaller compared to the control (Fig. 2.2). Statistical significance in the minimal adequate model was detected only in the treatment contrast between pooled *Microdochium* and Mixture treatments against the pooled Control, *Fusarium* and *Gibberella* treatments (t= -5.45, P<0.001) (treatments were pooled when differences were non-significant after performing model simplification by step-wise deletion of terms). In contrast there were large positive effects on the biomass of *F. brevipila* in single fungal inoculations and the Mixture (Fig 2.2). However, significance was only retained in the minimal adequate model in the contrast of the *Fusarium* treatment against the pooled Control, *Gibberella*, *Microdochium* and Mixture treatments (t=2.88, P=0.006). *A. elongata* showed similar patterns; in the *Fusarium*, *Microdochium* and Mixture treatments individuals were larger compared to the control; while the ones in the *Gibberella* treatment were smaller (Fig. 2.2). However in the minimal adequate model significance was detected only in the contrast between pooled *Gibberella* and Control against pooled *Fusarium, Microdochium* and Mixture treatments (t=4.75, P<0.001).

Thus, in this soil treatment, *F. brevipila* and *A. elongata* benefited from fungal treatment while *A. elatius* was unresponsive or negatively affected. Correlation coefficients among the interacting plants in this soil treatment (Table AS1), showed that there was a significant negative correlation between the biomass of the two grasses in the *Fusarium* and *Microdochium* treatments (*Fusarium,* r=0.78, p<0.05; *Microdochium,* r= -0.66, p<0.05) while there was no correlation observed

in the control suggesting that the presence of *Fusarium* and *Microdochium* increased competitive interactions between the grasses.

Low Sand treatments

In the Low Sand treatment, individuals of *A. elatius* growing in each fungal treatment were on average smaller relative to the control (Fig. 2.2). The minimal adequate model identified only the contrast between the *Microdochium* treatment with all other fungal treatments pooled into one group as significantly different (t= -4.23, $P<0.001$). For *F. brevipila*, individuals in each fungal treatment were also smaller on average than in the control. In the minimum adequate model the only significant effect was between the pooled *Gibberella* and Mixture treatment compared to the pooled Control, *Fusarium* and *Microdochium* treatment (t=-2.61; $p= 0.012$). Unlike the pattern observed in the High Sand treatment, individuals of *A. elongata* did not grow better in fungal treatments. Each single inoculation was on average smaller with respect to the control while the individuals in the Mixture treatment were larger (Fig. 2.2). The minimal adequate model identified as significant the contrast between the *Gibberella* treatment and the pooled Control, *Fusarium*, *Microdochium* and Mixture treatments. Overall, the *Gibberella* treatment caused reductions in the growth of *F. brevipila* and *A. elongata,* while *A. elatius* was unresponsive to them.

Correlation coefficients among the interacting plants in this soil treatment (Table AS1), showed significant negative correlation between the biomass of the two grasses only in the Mixture treatment ($r=-0.65$; $p<0.05$).

Table. 2.1. Two-way ANOVA results for total productivity (aboveground and belowground) and for each individual species using Soil texture and Fungal identity as factors. Analysis based on non-transformed data.

Source of variation	d.f.	*MS*	F-ratio
Total aboveground productivity			
Soil texture	1	125.45	48.70***
Fungal identity	4	28.86	11.20***
Soil texture x Fungal identity	4	10.94	4.25**
Residuals	86	2.58	
Total belowground productivity			
Soil texture	1	109.58	19.74***
Fungal identity	4	39.02	7.03***
Soil texture x Fungal identity	4	3.73	0.67
Residuals	86	5.55	
***A. elatius* biomass**			
Soil texture	1	132.12	47.89***
Fungal identity	4	25.68	9.31***
Soil texture x Fungal identity	4	8.71	3.16*
Residuals	86	2.76	
***F. brevipila* biomass**			
Soil texture	1	0.0005	0.0022
Fungal identity	4	0.52	2.21
Soil texture x Fungal identity	4	0.61	2.62*
Residuals	86	0.23	
***A. elongata* biomass**			
Soil texture	1	0.07	3.70
Fungal identity	4	0.11	5.70***
Soil texture x Fungal identity	4	0.05	2.57*
Residuals	86	0.01	

Table 2.2. Two-way MANOVA results for community structure (changes in biomass of the 3 plant species used in the experiment) having Soil textures and Fungal Identity as factors. Analysis based on non-transformed data.

Source of variation	*Df*	Pillai trace	*F*
Soil texture	1	0.40	19.06***
Fungal identity	4	0.60	5.42***
Soil texture x Fungal identity	4	0.34	2.78**
Residuals	86		

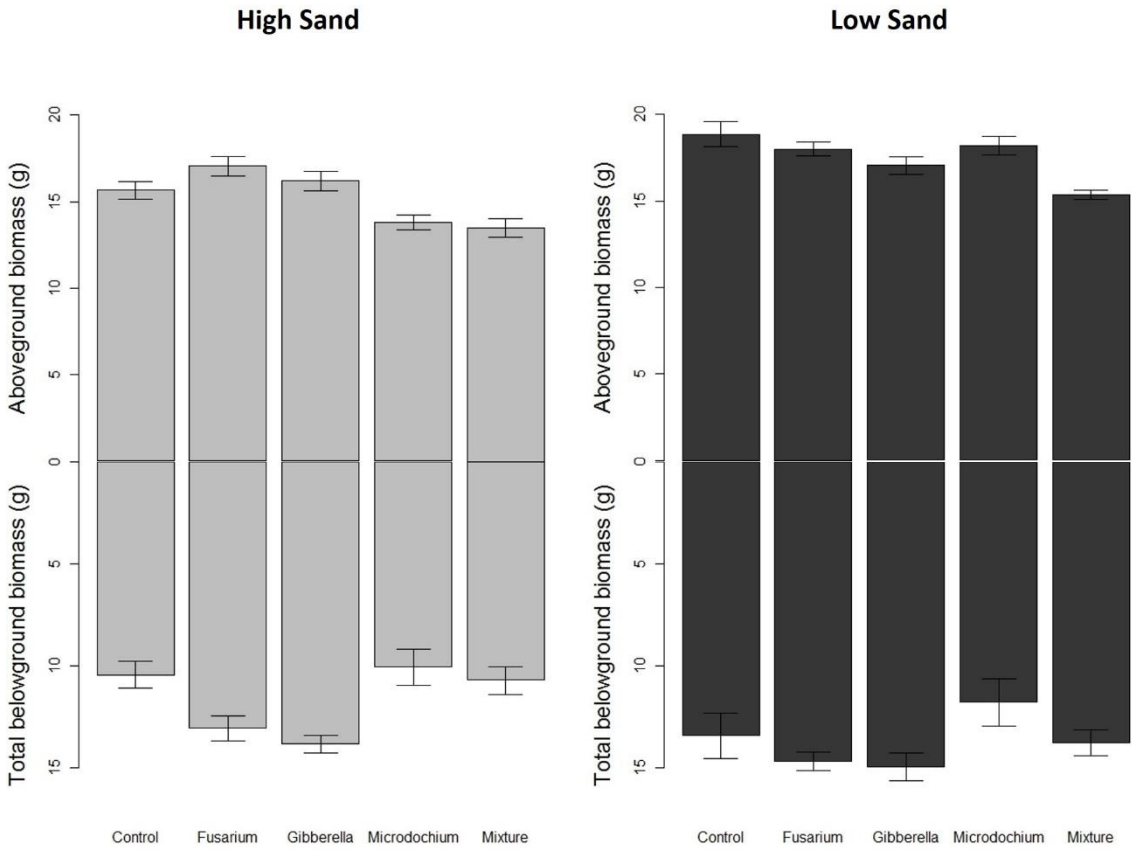

Fig. 2.1 Total productivity of microcosms in response to soil texture and fungal identity. Grey bars indicate High sand (low fertility) treatment (left); black bars indicate Low Sand (High fertility treatment (right). Bars indicate mean values and standard errors.

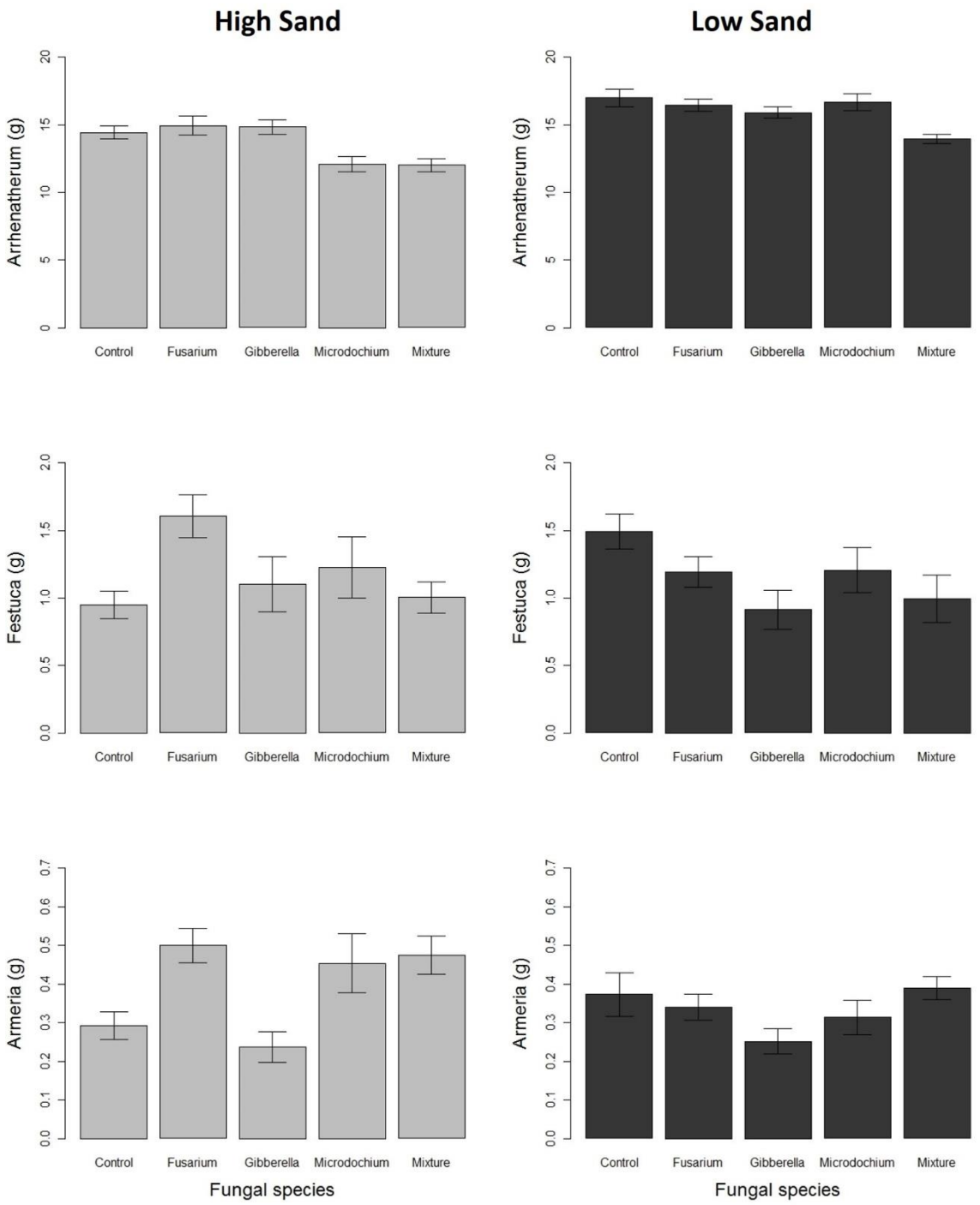

Fig. 2.2 Biomass responses to soil texture and fungal identity of the three plant species grown in microcosms. Top, *Arrhenatherum elatius;* middle, *Festuca brevipila;* bottom *Armeria elongata.* Grey bars indicate High sand (low fertility) treatment (left); black bars indicate Low Sand (High fertility treatment (right). Bars indicate mean values and standard errors.

Discussion

We provide evidence that common endophytic RIF caused differential host growth responses among interacting plants. Furthermore, such effects greatly modified the way plants respond to the strong change in soil texture imposed in this study. In the dry grassland to which our experiment relates, strong gradients in soil texture correspond to strong differences in soil parameters that change host growth (pH, and nutrient content), which are well known abiotic parameters to drive changes in plant community structure. Our data challenge the common view that fungal "endophytic" interactions are weak (Porras-Alfaro and Bayman 2011) and therefore play a minor role in plant community dynamics. This view stems from a lack of clearly visible disease symptoms during infection, or a lack of clear morphological evidence for plant-fungal interfaces (such as arbuscules or Hartig nets) for nutrient exchange like the ones observed in mycorrhizas (Rodriguez et al. 2009) rather than from actual experimental studies.

Many possible mechanisms could explain the plant growth responses in this experiment. Negative effects in response to root endophytic colonization can be seen as weak parasitism (Mandyam et al. 2013), or as induction of host resistance resulting in the allocation of carbon to the production of expensive defense compounds rather than to vegetative growth (Heil and Baldwin 2002; Aimé et al. 2013). Positive effects could be due to production of plant growth hormones (Schulz and Boyle 2005), or transport of nutrients to the host as a result of mineralization of organic matter (given the saprotrophic capabilities of these fungi) (Newsham 2011). Clearly, information about the symbiotic and saprotrophic traits of these fungi when present in a particular host would be necessary to disentangle all these possible mechanisms (Aguilar-Trigueros et al. 2014).

These differential growth effects by co-occurring hosts can result in interesting cases of indirect ecological interactions. For example, when some plant species suppress the growth of competing neighbors by hosting fungal species detrimental to others, this has been referred to as "apparent competition" (Hatcher et al. 2006; Beckstead et al. 2010). Indeed, previous studies have reported soil-borne fungi as drivers of this sort of indirect interactions (Putten and Peters 1997; Holah and Alexander 1999). In this experiment, apparent competition could explain the negative relationship between the yields of the two grasses *A. elatius* and *F. brevipila* in the presence of *Fusarium* and *Microdochium* in the High Sand treatment, and in the Mixture and Low Sand treatment combination.

Furthermore, it is evident that the exact nature of the host response, the mechanism driving it and its outcome on host communities greatly depends on soil conditions. For example, the large positive responses of *F. brevipila* and *A. elongata* to *Fusarium* were only observed in the High Sand treatment, while in the Low Sand treatment differences with respect to the control were small and non-significant. This effect may be due to fungal mineralization (saprotrophic) capabilities which is known to depend on availability of inorganic vs organic nutrient sources (Mayerhofer et al. 2013). At the High Sand treatment level of the present study, the organic material added to soil in the inoculum (oat kernels) could have constituted a pool of nutrients that was only available when fungi were present and not in the control. These positive effects of the fungi observed in the High Sand treatment resulted in the improved growth of the smaller species (*F. brevipila* and *A. elongata*). Thus, perhaps the presence of the fungi was able to reduce host fitness differences of the interacting plant species (they behave as "equalizing" factors *sensu* Chesson (2000)) and so also promote diversity maintenance.

Our results also provide some of the first empirical evidence for the importance of diversity of endophytic RIF on host community structure. Previous studies on diversity of root fungal symbionts have focused on mycorrhizae or soil borne pathogens (Wagg et al. 2011; Engelmoer et al. 2014) but see (Rillig et al. 2014). Here we show that increasing endophyte richness from 1 to 3 (single inoculations vs. Mixture) results in variable outcomes. Sometimes effects of all three fungi together are the same as those of a single isolate perhaps indicating that a specific fungus is not affected by other fungal interactions; while in other cases, the increased diversity reduces the effect of single inoculations (likely indicating antagonist interactions among the isolates). Although not the focus of our experiment, these results indicate that the process of endophytic community assembly within rhizospheres is dynamic, and has consequences for host-host interactions. Our inoculation scheme created a situation where a single strain could occupy most of the rhizosphere of three hosts without any other fungal competitors. This is an unlikely scenario in natural systems, but it would be an appropriate design to test competitive ability of fungal endophytes to invade rhizosphere patches in the presence of competitors and to resist invasion from other putative competitors. To our knowledge, these basic questions of community ecology are unexplored with plant associated fungal communities.

Because the experiment was carried out in microcosms designed to mimic an actual community module (Hatcher et al. 2006) it is likely that the responses observed in this experiment

play an important role in nature. Thus, this experiment must therefore be seen as an initial step to evaluate the extent to which endophytic RIF in the Ascomycota can drive plant community structure. Further studies should aim at evaluating long term effects of manipulations of RIF composition, structure and diversity on plant community dynamics.

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Chapter Three

Ecological understanding of root-infecting fungi using trait-based approaches

Abstract

Classification schemes have been popular to tame the diversity of root infecting fungi. However, the usefulness of these schemes is limited to descriptive purposes. We propose that a shift to a multidimensional trait-based approach to disentangle the saprotrophic-symbiotic continuum will provide a better framework to understand fungal evolutionary ecology. Trait information reflecting the separation of root infecting fungi from free living soil relatives will help to understand the evolutionary process of symbiosis, the role that species interactions play in maintaining their large diversity in soil and *in planta,* and their contributions at the ecosystem level. Methodological advances in several areas such as microscopy, plant immunology and metatranscriptomics represent emerging opportunities to populate trait data bases.

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Limitations of categorical approaches to study plant–soil fungal interactions

Understanding the effects of plant–soil fungal interactions in natural communities has become a major research area in plant science [1]. Interest in these interactions stem from increasing awareness that soil biota play an important role in plant performance, plant community assembly and ecosystem functioning [2].

However the complexity of soil fungal communities challenges our ability to understand the effects of such interactions on plant performance and on ecosystems processes. Recent surveys show that roots interact with phylogenetically diverse groups of fungi [3]. Moreover, the effects of particular plant-fungal combinations depend on environmental conditions and on the host and fungal genotypes [4]. In diverse communities and variable environments, net responses may be due to complex indirect interactions among co-occurring fungi and plants [5].

Given these complex associations, researchers regularly classify fungal taxa into broad categories according to particular criteria. These criteria may be based on nutritional mode (e.g., “biotroph”, “necrotroph” or “saprotroph” [6] (see Glossary)), presence of hyphal melanization and formation of septa (e.g."dark septate endophytes" [7]), or on a mix of taxonomic, morphological and physiological characteristics (e.g. “arbuscular mycorrhizal”; “ectomycorrhizal” [8]). These classificatory approaches have been important for distilling broad generalities from the rich brew of fungal–plant interactions such as the recognition of contrasting plant defense mechanisms against infecting fungi with different nutritional modes [9].

However, assignment of root infecting fungi into fixed categories is problematic for species labeled as “endophytes”. For example, some of the criteria used in delineating endophyte classes [10] are quantitative (number of potential hosts; number of co-infections within a host or degree of tissue colonization) but their delineation is imprecise (narrow vs. broad host range; low vs. high *in planta* diversity; extensive vs. limited *in planta* colonization). Similarly, a suggestion that “endophytic functional groups” should be based on their effects on host fitness resulted in the rather unsatisfying conclusion that "some endophytes may be latent pathogens, some may be derived from pathogens, and others may be latent saprotrophs, but many are neither"[11]. Such classification schemes can provide a useful initial framework to understand poorly studied plant–fungal interactions, but the resulting generalizations often include the

listing of so many exceptions to the proposed scheme that the framework is not useful operationally.

We argue that a shift in focus from classification schemes to a multidimensional trait-based approach reflecting the biology of the fungi is necessary for a better understanding of the ecology and evolution of root infecting fungi. These approaches consider species as a conglomerate of unique combinations of multiple traits, which could be depicted as species being points defined by multiple traits represented as dimensions (Fig 3.1a). This view directly link particular ecological and evolutionary questions with trait information. The proposed multidimensional trait-based program presented here is focused on the traits that allocate "endophytic" or "pathogenic" root fungi to a symbiotic lifestyle and separate them from free-living saprotrophic relatives. We explain how trait information can be used to address three essential questions: What are the mechanisms behind the evolution of root endophytic or pathogenic lifestyles from free-living fungi and *vice versa*? What is the importance of trait similarity in explaining the co-occurrence patterns of fungal genotypes or species *in planta* and in the soil? And, which traits might be used to understand the functional diversity of soil fungi? This is illustrated conceptually in Figure 3.1.

A fungal-trait approach: understanding the saprotrophic-symbiotic continuum.

Trait-based approaches rely on measurements of phenotypic characters or traits to guide inferences about particular ecological or evolutionary processes (Box 1). For example, plant scientists have successfully used such approaches to understand how fire has influenced the evolution within the Pinaceae by combining trait information with phylogenetic reconstructions [12], to measure the relative importance of abiotic factors and biotic interactions in shaping community assembly of tropical trees by measuring trait overdispersion in local communities [13], or to understand how plant diversity influences the variability of decomposition rates within climate regions by combining decomposition data with leaf traits from databases [14].

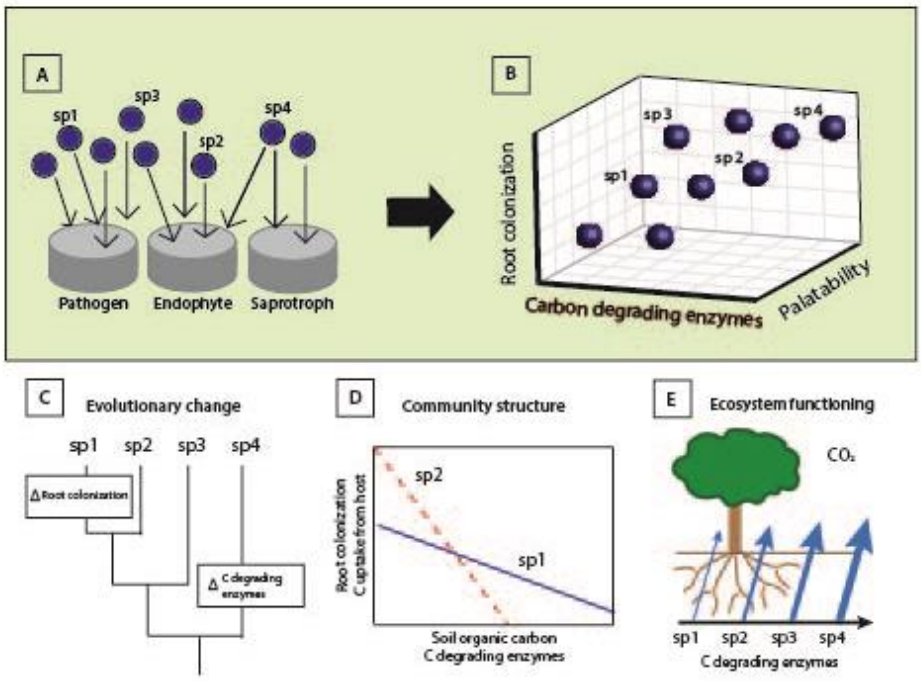

Figure 3.1. From categorization to trait analysis for root-infecting fungi. Schematic representation of a trait-based approach to understanding the ecology and evolution of root infecting fungi. Instead of placing species into fixed categories such as pathogen endophyte or saprotroph trait-based multivariate approaches represent species as particular combinations of traits in various dimensions. Such information can be coupled with: (A) phylogenic data () to understand evolutionary change; (B) their effect on species performance under different ecological factors () to understand mechanisms of community assembly and species co-existence and; (C) with their ecosystem function to explore their role on ecosystem processes. Note: The graphic in (B) represent the lower resource boundaries above which species 1 and 2 can still grow (zero net growth isoclines as in [54]). In this figure, the position of lines depends on the traits the species posses to exploit two resources: carbon from the host or from decaying matter.

We explain how application of such trait-based approaches may be valuable at a conceptual level in understanding the saprotrophic-symbiotic continuum in root infecting fungi. This continuum is pertinent to this set of fungi as most root endophytes constitute a gray area along the saprotrophic–symbiotic spectrum [10] and facultative saprotrophy is a characteristic of most studied root pathogens [15]. Indeed, this issue has been debated among plant pathologists ever since the seminal work of Garrett [16]. More recently it has been a major focus of study among researchers on ectomycorrhizal fungi [17, 18].

Understanding the saprotrophic-symbiotic continuum is of particular relevance to plant–fungal associations that have been labeled "endophytic" or "root pathogenic". Despite being considered major components of natural communities [10, 19], "endophytes" are not well defined in terms of their nutritional mode, there is little empirical understanding of their life history strategies, which in turns makes predicting their community and ecosystem impacts difficult.

Specifically we aim at explaining how characterizing this continuum using a trait-based approach will make inroads on first, explaining the evolutionary trajectories of symbiotic fungi from soil-inhabiting relatives (and *vice versa*), second, on understanding and predicting the functional role of fungi along this continuum in nutrient and carbon dynamics within ecosystems, and third, providing a mechanistic understanding of fungal community assembly and maintenance of diversity both in soil and *in planta*. In the following section, we lay out the basis for such an approach, defining classes of traits relevant to this continuum, and suggest examples of relevant and measureable traits.

Symbiotic and saprotrophic traits: a starting point

In Table 3.1 we provide some examples of traits that we consider to be critical in assessing the ecology and evolution of “endophytic/pathogenic” root infecting fungi. As will be evident from the brief discussion of the rationale for each of them, these broad traits will themselves consist of many component traits that are operationally measurable. We expand on some of these points below.

Symbiotic traits are often related to the ability of fungi to avoid or overcome resistance responses driven by the plant immune system as host resistance is the main filter preventing the use of the root niche [20]. Other traits may be related to the metabolic interactions that occur in such symbioses; for example, systems for translocation and bidirectional transport of nutrient and carbon resources [21]. Identification of relevant component traits for “root endophytic” associations depends on understanding the molecular and physiological activities in fungal colonization structures [22]. Saprotrophic traits are related to the enzymatic machinery required to degrade the diversity of recalcitrant carbon sources [23], production of antimicrobial compounds during competition with other fungi [24] and with bacteria [25], and preventing or responding to fungivory by soil animals [26].

The traits in this continuum may help us to understand the evolutionary process behind the transitions from saprotrophism to symbiosis and *vice versa* [27]. This could be achieved using comparative phylogenetic approaches quantifying the variation in saprotrophic and symbiotic traits and identifying potential life history trade-offs, instead of using categories such as endophyte, saprotroph or pathogen as if they were characters [28]. It should also be possible to compare the evolutionary processes underlying the colonization and exploitation of roots vs. leaves by fungal endophytes or pathogens. The contrast between these two organs regarding their structure and their local environments [29] may help explain differences in colonization patterns between root and leaf “pathogens" or "endophytes” [4].

Trait-based approaches could also be used to unravel compositional differences in fungal communities inhabiting roots, the rhizosphere, and bulk soils. As roots are living organs, fungal species with “symbiotic" traits should dominate in the root compartment while species with “saprotrophic” ones should be more abundant in the rhizosphere and bulk soil. Trait information, if mapped onto fungal communities found in various soil compartments could be used to understand the ecological basis and predictability of these assemblages. For instance, canonical examples in agricultural research have led to the common view that root necrotrophic pathogens, despite having saprotrophic abilities, are outcompeted by “strict” soil saprotrophs [30]. Trait information could be used to test whether such putative trade-offs apply to root “endophytes” or “pathogens” in natural systems.

Trait information also allows the formulation of novel hypotheses. For example, does high trait diversity at the community level increase resistance to invasion by other fungi? Successful invasion depends on the number of open niches [31], and established fungal communities exhibiting high trait diversity may reduce resources (space, nutrients, organic matter) or limit access to resources (by priming host defenses or producing antibiotics). For fungi at the symbiotic end of the spectrum, is growth in the soil environment limited by the diversity of resident saprotrophic traits in the rhizosphere? Relationships between trait diversity and invasion resistance may help predict the likelihood of disease outbreaks by highly virulent root-infecting fungi in agricultural systems.

Assessing trait similarity provides a better means to assess the effect of competition among co-infecting fungi in the evolution, maintenance and frequency of mutualistic or pathogenic interactions [32]. Another application might be in coupling trait information with the

comparisons of root infecting fungal communities among invasive plants in their native and introduced ranges: such information may help explain the alleged reduction of pathogen attack of invasive plants in the introduced ranges if infecting fungi do not possess the traits to effectively exploit the new host [33].

A trait based approach for the saprotrophic-symbiotic continuum may better capture the importance of root infecting fungi in carbon and nutrient cycling at the ecosystem level, in a manner completely analogous to the use of "functional trait analysis" in plants [34, 35]. Little is currently known about the effects of fungal trait diversity on ecosystem processes although the recent frameworks proposed for mycorrhizal associations [36, 37] suggest that other root-inhabiting fungi may be important contributors to ecosystem function. Trait-based approaches would allow the recognition of factors that correlate with the main ecosystem processes, to identify the taxa driving such effects, and lead to a better understanding of ecosystem drivers.

Table 3.1. Examples of traits that are potentially important for characterizing fungi that fall along the symbiotic and saprotrophic continuum.

Fungal traits	**Description and rationale**	**Refs**
Production of cell-wall degrading enzymes	They broaden the spectrum of carbon sources for saprotrophic growth, but at the same time they trigger host defenses as a result of disruption of cell walls in living cells.	[60]
Lignolytic ability	The ability to decompose lignin (e.g. by white rot saprotrophs) expands availability of carbon substrates in woody systems. This trait seems rare in ectomycorrhizal fungi.	[61]
Antibiotic production	Key traits to cope with interference competition by fungi and bacteria are the production of antibiotics as well as the enzymes that degrade them.	[62]
Palatability	Soil fungi may produce toxic metabolites, melanin or crystalline cell inclusions to avoid fungivory by soil animals.	[26]
Resting structures	Resting structures permit survival under adverse conditions: a lack of appropriate hosts or inadequate carbon sources. Whether this is more or less likely in endophytic fungi is not known, and may depend on root or plant longevity.	[63]
Dispersal/Transmission mode	Root symbiotic fungi have both vertical and horizontal transmission, but how these transmission modes differ from corresponding dispersal modes of more saprotrophic fungi is not understood.	[10]
Degradation of antimicrobial root exudates	Root exudates (either inducible or constitutive) constrain growth patterns of fungal species and are a component of host resistance. Whether and how they are degraded, is likely to be important in colonization.	[64]
Penetration structures	Many symbiotic fungi have specialized structures for initial penetration of host tissue. These structures also produce physical, enzymatic and chemical signals that enable the early infection process.	[65]
Ratio of inter- to intracellular colonization	Extensive intercellular growth (apolastic growth) may reflect the ability of fungi to avoid the disruption of host cell walls, and so avoid the activation of resistance responses such as by reactive oxygen species, phenols, pathogenesis-related proteins, and phytoalexins.	[9]
Perifungal membrane	Some symbionts have structures that include the invagination of plant membranes (e.g. haustoria and arbuscules), but such invaginations may also occur in fungi with saprotrophic abilities (e.g. *Piriformospora indica*).	[66, 67]

Induction of host cell wall reinforcements	Cell wall thickening is one of the first general responses against pathogen invasion. The ability to avoid or prevent such responses during hyphal penetration may be essential for a symbiotic lifestyle.	[68]
Phytotoxin production and detoxification enzymes	Some fungi depend on the production of phytotoxins and the ability to degrade plant derived antimicrobial compounds for successful entry.	[60, 69]
Fungal invertases	Symbiotic fungi can obtain sugars from the host by either secreting fungal invertases or by being reliant on plant invertases. Fungal invertases are seemingly absent in ectomycorrhizal symbionts.	[70]
Transporter traits and their expression	In some fungi, establishment of symbiosis requires effective exchange of resources with the host plant. Gene expression is regulated in fungi based on their location in the host cells, the compounds being transported, and the direction of transport. While such processes are well established in mycorrhizal associations, there is little evidence whether other root symbionts exhibit similar mechanisms of resource exchange.	[21, 50]
Extra-radical mycelia and "foraging" pattern.	Root fungal symbionts often maintain dual growth in the root and the soil. Allocation of biomass to the extra-radical mycelium and the spatial distribution of hyphae in soil affect the transfer of nutrients. Several morphological “foraging types” have been identified in ectomycorrhizae and are likely in other root symbionts.	[55, 71, 72]

Trait measurement and storage of trait information in high quality databases

The full application of any trait-based approach depends on the accessibility of databases that are well curated, well funded, and linked to genomic and phylogenetic information. Phylogenetic databases have already been created for taxonomic purposes (e.g. UNITE [38]; http://www.deemy.de for ectomycorrhizal fungi). Thus, there is an excellent window of opportunity to integrate trait information generated from morphological and physiological characterization of newly isolated fungi with such platforms. Traits are measured on particular individuals, and meaningful extrapolations to species (or higher ranks) also depend on having estimates of trait variability among individuals and populations, as well as on the adequacy of the species concept for fungi [36].

Additionally, to be meaningful, trait information should be presented together with data on the conditions and circumstances under which the traits were measured ("metadata"). Trait information collected from culturable fungi under controlled conditions is useful to quantify a particular trait (e.g. phytotoxin production). Analogously, plant ecologists have populated trait-databases with measurements from controlled experimental conditions with standardized methods [39]. This level of control enables the use of meta-analytical tools to filter out the effect of variable conditions to better address broad scale functional diversity-ecosystem functioning questions [14].

Coupling this approach with *in situ* trait measurements under natural conditions (in mesocosms or the field) and with species abundance data will allow estimates of the contribution of a species in the context of environmental variability. Furthermore, *in situ* measurement of intraspecific trait variability could be used to refine models of community assembly [40]. However, caution must be taken when such trait information is intended to be used outside the particular system from which the traits were measured. It has been shown that traits with high levels of plasticity and measured from extreme habitats do not match well average values from databases [41].

In situ measurements are the only option to incorporate trait information for non-culturable fungi and are analogous to measurements for long-lived plant species (e.g., wood traits for tree species in the TRY database [42]). Hyphal length in soil or nutrient concentration in the hyphae can be measured following extraction of hyphae directly from the environment or from substrate-filled compartments [43, 44]. Enzyme activity assays performed on

ectomycorrhizal root tips [45] or fungal carbon substrate usage by fungi [46] are usually measured on excised material, but they may not accurately reflect process rates due to the effects of the manipulations. Meta-transcriptomic and other gene expression profiling approaches are promising and have the potential to lead to major insights at the community level [47, 48] and would be especially valuable if these could be targeted at fungal gene expression in particular. More sophisticated approaches at the individual level using "omics" tools (e.g., single cell genomics [49]; laser microdissection [50]) that can simultaneously examine fungal characters and identify the species being examined, hold exciting promise for future studies of soil fungal communities.

Concluding remarks

Rigorous comparative studies linked with phylogenetic information that is focused on traits, rather than qualitative categories, are necessary to determine what constitutes an adaptation to a particular life-style. In turn, traits can be used to make predictions about the impact and causes of community structure, and traits that strongly influence ecosystem function can be highlighted and measured for predicting outcomes. In this paper, as illustrated in Figure 3.1, we advocate a more objective trait-based approach to characterizing the processes involved in the interactions of root and soil inhabiting fungi, and hence provide a way to assess their importance at the evolutionary, community, and ecosystem levels. Others have strongly supported the use of trait-based approaches to move forward research on plant–fungal interactions [51, 52] and on microbial ecology [53]. In particular, there has been a recent call to explicitly use conceptual frameworks from functional ecology in ectomycorrhiza research, which promote integration of this important fungal group into research on diversity-ecosystem functioning relations .

Although broad categories have their place in the initial development of a discipline, they provide a rather abstract view of multi component processes at such a level of simplicity that they may actually inhibit understanding. In studies of root-inhabiting fungi, there has been a tendency to use qualitative categories to describe the saprotrophic-symbiotic continuum, rather than search for generalizations that come from more quantitative studies. Here we advocate a trait-based approach to understand the evolutionary ecology of root-inhabiting fungi and illustrate how this approach promises a clear way forward in this complex and technically difficult field.

Glossary box

Biotroph: nutritional mode in which a fungal symbiont relies exclusively on living host cells as a source of nutrients.

Functional trait: Species traits directly linked with a particular ecosystem process. Different species sharing similar functional traits are pooled into functional groups.

Life history trait: Traits reflecting allocation of resources of an individual into different fitness components.

Necrotroph: nutritional mode in which a fungal symbiont causes host cell death in order to acquire nutrients.

Saprotroph: nutritional mode in which a free-living fungus obtains nutrients from decaying organic matter, without inducing the death of the tissue.

Symbiosis: a physiological or structurally intimate interaction between phylogenetically unrelated organisms, without implying a specific effect on either organisms' fitness.

Trait: any morphological, physiological, or phenological character of an organism.

Box 1. Rationales for trait-based approaches

The rationale for collecting data for particular traits may differ. Traits may be chosen because they are likely to contribute to answer particular questions: a "top-down" approach. Alternatively, traits may be chosen because they can be integrated with the ever-increasing body of genomic and gene expression data allowing linkage of particular genes with tangible phenotypes, or traits, of ecological importance. This would be a "bottom-up" approach. Additionally, there is tremendous value in including traits simply because they are "easy to measure": databases are as valuable in generating hypotheses as they are in testing them, and unusual and unpredicted trait associations are a stimulus for deeper investigation.

Traits can be used to test hypotheses of evolutionary processes in conjunction with independently derived, usually sequence-based, phylogenetic information. Thus, they provide objective ways to identify and test potential evolutionary trade-offs in life history traits and relate them to environmental conditions using phylogenetic comparative methods. For example, this

approach has been used to evaluate allocation of carbon by arbuscular mycorrhiza fungal species to hyphal structures in roots and soil where percentage of root colonization and length of extra-radical mycelia were used as traits [55].

Trait-based approaches can also be used to understand community assembly. First, they allow the identification of potentially important physiological and ecological mechanisms by correlating traits with species performance along environmental gradients [56]. Second, trait similarity among species can be used to infer niche similarity and determine its effects on species sorting [57]. For example, the existence of trait trade-offs in important ecological factors or niche axes is fundamental for a mechanistic understanding of species co-existence [54].

At the ecosystem level, species sharing traits that influence particular ecosystem properties or species' responses to environmental conditions can be pooled into particular functional groups [58]. For example, Tilman [34] showed that functional trait diversity is a better predictor of community productivity than taxonomic diversity. This functional ecology perspective has focused on determining which traits allow species to face environmental changes –response traits- as well as which traits determine how species influence the environment –effect traits; and establishing the link between these set of traits to make better trait-based models of community assembly and ecosystem functioning [59]. Recently there has been a call to use this conceptual framework for ectomycorrhizal fungi [37].

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Chapter Four

Branching out: towards a trait-based understanding of fungal ecology

Abstract

Fungal ecology lags behind in the use of traits (i.e. phenotypic characteristics) to understand ecological phenomena. We argue this is a missed opportunity and that the selection and systematic collection of trait data throughout the fungal kingdom will reap major benefits in ecological and evolutionary understanding of fungi. To develop our argument, we first employ plant trait examples to show the power of trait-based approaches in understanding ecological phenomena such as identifying species allocation resources patterns, inferring community assembly and understanding diversity-ecosystem functioning relationships. Second, we discuss ecologically relevant traits in fungi that could be used to answer such ecological phenomena and can be measured on a large proportion of the fungal kingdom. Third, we identify major challenges and opportunities for widespread, coordinated collection and sharing of fungal trait data. The view that we propose has the potential to allow mycologists to contribute considerably more influential studies in the area of fungal ecology and evolution, as has been demonstrated by comparable earlier efforts by plant ecologists. This represent a change of paradigm, from community profiling efforts through massive sequencing tools, to a more mechanistic understanding of fungal ecology.

Keywords: Traits; resource allocation; community assembly; ecosystem processes

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Introduction

We live in a fungal world (de Boer et al., 2005); fungi profoundly impact population, community and ecosystem dynamics from local to global scales (Averill et al., 2014; Fisher et al., 2012). Yet fungal ecologists struggle to comprehensively understand fungal community assembly and its contribution to ecosystem functioning. Such understanding requires knowledge of the traits (i.e. phenotypic characteristics) of species that determine both their responses to environmental factors and their effect on ecosystem processes (Mcgill 2006; Petchey and Gaston 2006). So far, fungal traits have been used mainly for identification and classification (Kumar et al., 2011) but rarely for understanding fungal ecology. We argue that the selection and systematic collection of trait data throughout the fungal kingdom will reap major benefits in ecological and evolutionary understanding of fungi.

In this paper, we highlight how a core set of fungal traits can be used to address ecological phenomena. To do this, we employ plant trait examples, where the trait approach has been used successfully (e.g. Katabuchi et al., 2012). Second, we exemplify ecologically relevant traits in fungi, focusing on traits that can be measured for a large proportion of the fungal kingdom. Third, we identify major challenges and opportunities for widespread, coordinated collection and sharing of fungal trait data.

Using trait data in ecological research: examples from plant ecology

Trait data have been used in ecology for different purposes, but here we concentrate on three influential examples of the use of a core set of plant traits as a means of (i) identifying species trade-offs in resource use, (ii) detecting the relative importance of habitat filtering versus niche partitioning in community assembly, and (iii) understanding how biodiversity affects ecosystem processes by quantifying functional diversity. We focus on plant ecology because this field presents the most thorough development of a trait-based ecology (Adler et al., 2013) and provides examples analogous to many aspects of fungal biology.

(I) Identifying species trade-offs in resource use.

Trait data can be used to identify patterns of resource allocation to fitness components and physiological functions (Westoby et al., 2002). In a landmark study, Wright

et al. (2004) used six leaf traits to show that plant species can be placed along a major axis in the revenue obtained per leaf construction unit, which they termed the "leaf economic spectrum": at one extreme, there are species that invest few resources in leaf construction (e.g. thinner leaf, blade, shorter leaf lifespan) with short-term gains in photosynthates, while other species exhibit the opposite trait combinations (e.g. thicker leaf blades, longer leaf lifespan). This spectrum is consistent across a wide range of habitats, latitudes, and ecosystem types.

(II) Detecting the relative importance of habitat- filtering versus niche-partitioning in community assembly.

These approaches are based on measurements of trait means, variances and ranges at the community level. For example, habitat filtering (i.e., the extent to which abiotic factors like temperature, pH or nutrient levels prevent some species from establishing in local communities (HilleRisLambers et al., 2012)) is indicated by reductions in trait ranges at local scales. The rationale is that some species (and their traits) will be excluded in local communities with particular environmental conditions, and thus the trait range at local scales will be smaller than expected by chance as most species will have similar trait values (Cornwell et al., 2006). For example, in low resource patches (light, mineral nutrients) small-seeded plant species cannot establish given the lower amount of reserves they possess in comparison to large-seeded plant species. Thus, as only the large-seeded subset of the species pool can establish, the smaller the range of seed sizes (the difference between the species with largest and smallest seed) observed in the patch (Adler et al., 2013). At the other extreme, niche partitioning (i.e. the extent to which interacting species differ in their niches to stably co-exist) is inferred from increasing dissimilarities in trait values among co-occurring species, especially of traits related to the way they obtain resources and deal with stress and enemy attack. Thus, trait values among co-occurring species would be expected to be more different than expected by chance (Paine et al., 2011). For example, it has been shown that when plant species interact, they have dissimilar rooting depth values, reflecting partitioning of soil resources (Nobel, 1997).

(III) Understanding how biodiversity affects ecosystem processes by quantifying functional diversity.

Functional diversity refers to the number of functionally different species present in a community. The particular "function" a species performs is reflected by the sum of all the traits it possess that determine its contribution to an ecosystem process of interest (Petchey and Gaston, 2006). In plants, resource acquisition traits are commonly used (e.g., plant height reflects the ability to intercept light; leaf nitrogen concentration reflects the ability to acquire nitrogen). Further, multivariate statistical metrics have been developed to capture differences between species occurring in a given community using multiple traits (Petchey and Gaston, 2002). Functional diversity defined in this way has been shown to be a better predictor of, for example, aboveground productivity (an ecosystem process) than other measures of diversity such as species richness (e.g. Flynn et al., 2011).

Defining ecologically relevant fungal traits

In this section we identify the types of fungal traits that are good candidates for trait-based approaches mentioned in the previous section based on three criteria: (1) *ecological versatility of traits*, i.e. the traits should be representative for inferring fungal use of resources, community assembly mechanisms and multiple ecosystem processes, (2) a *wide scope throughout the fungal kingdom, i.e.* the traits should be relevant for a large pool of fungal species, and (3) *measurability*, i.e. methods should exist (or can be conceived) for their standardized measurement. In this way, data can be obtained from a large pool of species in a relatively short time using standardized protocols.

Ecological versatility of traits

Traits meeting this criterion (Table 4.1) are grouped into life-history, morphological or physiological traits. Life-history traits reflect resource investment during the life span of a species into different fitness components: survival, growth and reproduction (Flatt and Heyland, 2011). For example, life span of hyphae/fungal structures, number of spores/propagules, and allocation of biomass of either vegetative mycelia or reproductive structures represent fungal life history traits.

The morphological and physiological traits should correlate with fitness components, have predictive value in explaining species responses to environmental factors, or be relevant for ecosystem processes. Unlike plant trait data for which empirical support has been established

(Westoby et al., 2002), the ecological relevance for many fungal traits is based on expert opinion and has yet to be empirically tested.

We summarize the potential relevance of some of the traits in community assembly and ecosystem functioning in Table 4.2. For the investigation of community assembly, any trait that can be related to a major ecological axis such as resource acquisition, enemy avoidance (predation/fungivory), or stress tolerance (Chase and Leibold, 2003) may be useful. As fungi are involved in many ecological processes, an exhaustive list of fungal functional traits impacting ecosystem processes is beyond the scope of the paper. Instead we illustrate three key ecosystem processes for which we expect fungi to play an important role in terrestrial ecosystems: soil aggregation, plant productivity (host growth) and organic matter decomposition (Boddy, 2001; Mitchell, 2003; Rillig et al., 2014). Some of the traits, such as those related to mycelial architecture, may be linked to several ecosystem processes (Table 4.2).

Scope of the traits within the fungal kingdom

The traits in Table 4.1 are mostly applicable to terrestrial, filamentous fungi. We consider this group as a good starting point in the development of a trait-oriented approach because they include the largest known diversity of the fungal kingdom, exhibit a wide variety of lifestyles, and have a cosmopolitan distribution (Blackwell, 2011). However, traits relevant for aquatic and non-filamentous basal fungi require further consideration (Stajich et al., 2009).

Measurability of the traits

Traits are measured on individuals, but the modular growth of filamentous fungi challenges definitions of what an individual is (Pringle and Taylor, 2002). Here we propose trait measurements of fungal structures (e.g. hyphae, spores) important in colonizing a resource patch. A resource patch can be operationalized as a unit of host plant tissue, decaying material, or a Petri dish with a known medium under a narrow set of environmental conditions. This approach is aligned with models of fungal resource allocation (to mycelial growth vs. spore production), and focuses on the number or size of fungal structures within the resource patch (Gilchrist et al., 2006). Furthermore, measuring fungal traits under controlled conditions allows the standardization of trait measurements and the integration of existing data from the literature and databases on fungal growth rates on different substrates/media (discussed below). In fungi, data obtained under such controlled environmental

conditions have great potential for understanding ecological phenomena, as exemplified by the use of plant relative growth rate (measured in hydroponic conditions) to predict productivity in the field (Vile et al., 2006).

Table 4.1. Life-history and morphological/physiological traits hypothesized to be informative for fungal ecology.

Trait	*Measurable traits (per resource-patch defined individual/populations)*	*Example reference(s)*
Life history		
Life span	Persistence of vegetative and resting structures Persistence of fruiting structures (correlated with abundance patterns) Persistence of entire genotype in the environment Duration of metabolically active period Time to reproduction (sexual and asexual)	(Gange et al., 2011)
Reproductive output/dispersal	Spore diameter Spore production • Number of spores per unit of mycelium (mass, area, length) during active growth. Specialized hyphal modifications • Propagule dryness • Propagule motility • Propagule sliminess • Size of fruiting body • Frequency of fruiting (phenology) Dispersal vector Sexual reproduction: asexual, sexual, mating types Anastomosis groups (somatic compatibility)	(Hussein et al., 2013)
Propagule survival	Propagule type (spores, vegetative mycelia) Spore-wall thickness (diameter) Spore-wall thickness (Number of walls) Hyphal-wall thickness, composition Dormancy (half-life [time]) Number of resting structures per unit of mycelia (mass, area) Propagule germination rates	(Nara, 2009; Peay et al., 2009)
Morphological		
Mycelial architecture	Branching frequency per unit length hypha Branching angle (mean angle) Branching order Lateral dichotomies Rhizomorph/cord length and width Runner hyphae length and width Hyphal exploration type Fractal dimension	(Agerer, 2001; Heaton et al., 2012; Ritz and Crawford, 1990)

Colony/population size (or growth per unit of time)	Colony size • Mycelial mass (weight) • Hyphal length • Phospholipid-derived fatty acids • Colony forming units (CFU). • Maximum hyphal growth rate • Extent of mycelial colony growth Population size through molecular markers • Amplified fragment length polymorphism • Microsatellite	(Rayner et al., 1999)
Physiological		
Resource uptake	Enzyme spectrum (presence/absence and expression level, see databases: http://www.cazy.org/; http://pcwde.riceblast.snu.ac.kr) • Cellulases • Lignases • Oxidases • Phosphatases • Chitinases • Proteases Ion transporters and aquaporins (presence/absence and expression level) Specialized secreted molecules for ion uptake (presence/absence and concentration) • chelators • siderophores	(Eichlerová et al., 2015)
Mycelial construction investments (mycelial economics)	Mycelial nutrient concentrations Mycelial stoichiometry (C:N:P) Lipid content (mass per unit) Storage structures (number per unit) Production of non-enzymatic substances (presence/absence and concentrations) • Hormones • Antibiotics • Hydrophobins • Crystals • Melanin (concentration) Wall thickness Hyphal diameter	(Hammer et al., 2011)
Stress tolerance	Minimal and maximal growth temperatures Reaction norms to environmental gradients	(Crowther and Bradford 2013)

Table 4.2. Linking some classes of fungal traits to fungal ecology. Ecological relevant traits are indicative of how species interact with resources, enemies and stress. The same traits can be used to determine role how fungal species in key ecosystem processes. The traits are assessed during metabolically active growth periods, regardless of guild (e.g., symbiont, saprotroph) or habitat (e.g., terrestrial, marine).

	Allocation of resources/Community assembly			**Ecosystem processes**		
Trait type	Resource Acquisition	Enemy avoidance	Abiotic/ Host stress tolerance**	Soil Aggregation	Host growth	Decomposition
Mycelial Architecture	X	X	X	X	X	X
Colony/population size				X	X	?
Non-enzymatic exudates		X	X	X	X	X
Enzymatic capabilities	X	X			X	X
Mycelial construction investments	X	X	X	X	X	X
Life span	X	X	X	X	X	X

** For free-living fungi we consider stress driven by abiotic factors, while for symbiotic fungi, stress is also caused by plant immune responses.

Overcoming challenges to facilitate the widespread use of trait approaches in fungal ecology

Trait data collection

Currently, fungal trait measurements are made in a non-systematic fashion with a variety of protocols, often focusing on qualitative, rather than quantitative, differences and with taxonomic purposes. For instance, recent metabolic surveys of fungi measured enzyme activity using a variety of methods (as e.g. in Mandyam et al. (2010); or in Promputtha et al. (2010)). No "handbooks" exist for the measurement of ecologically relevant fungal traits as do for plants (e.g. Pérez-Harguindeguy et al., 2013). Such handbooks would provide an important resource for mycologists and additionally serve as a teaching tool. Undergraduate courses in mycology represent an excellent opportunity to obtain trait data from cultured isolates and environmental samples.

Use of intraspecific trait diversity

Most trait-based ecological studies for plants consider the species as the unit of interest. This results in the practice of using average trait values per species, often ignoring intraspecific trait variability (e.g. Kraft et al., 2011). However, incorporating this source of variability could lead to improved predictability (Bolnick et al., 2011; Violle et al., 2012). In fungi, intraspecific trait variability is expected to be high (Behm and Kiers, 2014), given inherent intraspecific variability, trait plasticity in different environments/hosts or complex saprotrophic-symbiotic cycles (Rodriguez et al., 2009). Methods have been proposed to incorporate intraspecific variability when measuring functional diversity (de Bello et al., 2011) and community ecology studies incorporate intraspecific variability to better understand community assembly (e.g. Jung et al., 2010).

Storage and availability of trait data

Currently, there is a wealth of valuable fungal trait data in culture collections, taxonomic keys and compendia. These data are often stored in a variety of formats and accessibility. These include mycological journals with species descriptions, compendia for identification of fungi (e.g. Domsch et al., 2007), and laboratory records of individual mycologists. Collating and making such data available should be a primary task. In addition, specialized databases are scattered over different locations, using different formats. Examples are the AFTOL structural and biochemical

fungal trait databases (https://aftol.umn.edu/), the CBS fungal growth on media/substrate database (http://www.fung-growth.org/), and the fungal plant cell-wall degrading enzyme database (http://pcwde.riceblast.snu.ac.kr). A global trait database for fungal ecology is a long-term goal and the immensity of this task should not intimidate researchers. Initially, plant trait data were similarly disparate and it took several years before they were successfully aggregated into comprehensive databases (Kattge et al. (2011).

Linkage to genomic data

Mycologists are inventorying fungal species using genomic methods at a massive scale in a multitude of ecosystems. The wealth of fungal genomic data obtained by this high-throughput sequencing is underused in terms of asking general ecological questions (Poisot et al., 2013), nor is it being linked to ecological relevant fungal traits. However, these DNA-based species have no corresponding morphotype; and thus there is little knowledge of what changes in species compositions means in terms of functional, or trait properties of communities (Prosser et al., 2007). If this wealth of information could be linked to a functional trait database, data generated in high-throughput sequencing could be used to better understand fungal community assembly and its relationship with ecosystem processes. A trait database could be linked to genetic barcodes (the choice of which has recently been agreed upon for fungi (Kõljalg et al., 2013; Schoch et al., 2012), and integrated with taxonomic databases such as UNITE and DEEMY for ectomycorrhizal fungi (Abarenkov et al., 2010; Agerer and Rambold, 2004). Clearly, concerted and co-ordinated characterization of fungi with regard to genomics, phylogenetics and traits is a major opportunity.

Concluding remarks

Among mycologists, efforts are increasing to implement trait-based approaches both conceptually (Aguilar-Trigueros et al., 2014; Crowther et al., 2014; Chagnon et al., 2013; Falconer et al., 2011; Koide et al., 2014) and empirically (Pena and Polle, 2014; Philibert et al., 2011). While these efforts have been valuable, their scope has been limited to defined functional groups (e.g., root-associated fungi, forest pathogens) or for specific purposes (e.g., characterizing fungal niches). We propose to build on these approaches and present the versatility of the use of trait data in ecology. This process represents a change of paradigm, from community profiling efforts through sequencing tools and a focus on species composition to a more intimate, deeper understanding of fungi in ecosystems. This mechanistic understanding will allow key ecological questions to be

addressed including, for example: What are the consequences of fungal diversity loss in terms of ecosystem functioning? Can we predict fungal community change due to climate or land-use change? Can we manipulate fungal communities to better support ecosystem services?

While the development of a trait-based understanding for fungi may seem like a daunting task, its time has certainly come and is within our means. Critical understanding of the aforementioned questions can be gained from controlled experimental approaches. For such experiments, traits measured under controlled laboratory conditions would be of great value for understanding effects of manipulated functional fungal diversity and its role in ecosystem processes. Clearly, this development will require a dedicated effort and *de novo* collection of data for explicit ecological purposes. In the long run, the collection of trait data from as many context as possible would allow objective evaluation of trait plasticity and its use under more realistic conditions (outside experimental set ups), as plant ecologist have done in the past for plant traits (Kattge et al., 2011). The traits summarized here represent only a starting point. Our goal is to inspire and integrate the participation of the broader mycological community in this process. As such, this paper represents an invitation to the international community to contribute to the vision for this approach: we hope that mycologists, regardless of system, taxon or scale of study, will contribute to identifying and describing ecologically relevant traits, share the information with the community and use it to understand ecological phenomena. Mycological meetings and workshops would represent excellent opportunities to start this task. Eventually, such discussion would culminate in a consensus on the traits that could be used in ecology as well as on standardized protocols for their measurement; this in turn would eventually allow the integration of data from different systems.

Case study:

Trait based fungal ecology: evaluating spore size as a trait to infer habitat filtering during AMF community assembly.

Introduction

Habitat filtering refers to the process by which local abiotic conditions restrict the ability of a species to establish and reproduce depending on its traits (Kraft et al. 2014). This process relies on the existence of two phenomena: heterogeneity in the abiotic environment and species trade-offs in their ability to interact with such heterogeneity (Chase and Leibold 2003). The rationale is that some species possess traits that allow them to establish and reproduce under some set of abiotic conditions but do not allow them to establish in another set of conditions.

There has been substantial research in plant and animal ecology to identify the traits behind such species trade-offs. In plants (Westoby et al. 2002), propagule (seed) size has received a lot of attention in plant ecology. Results from this research indicate that: i) there are large differences in seed size among plant species, even several orders of magnitude within local communities (Leishman et al. 1995); ii) there is a negative relationship between seed size and seed number reflecting a life history trade-off (Westoby et al. 2002); iii) this trade-off can be linked to a tolerance-fecundity trade-off where large seeded species perform better under low resources (water, nutrients, light) while small seeded species have higher fecundity but poor recruitment in low resource patches (Muller-Landau 2010).

Studies of such species trait trade-offs in general, and propagule size in particular, are virtually absent in fungal ecology, even though speculative statements abound in the literature. For example, it has been hypothesized that large spore AMF species would perform better at low disturbance levels, while the opposite would apply to small spored species (Chagnon et al. 2013). Further, based on AMF community surveys, it has been argued that high nitrogen levels prevent reproduction of large spore sized AMF species (Egerton-Warburton and Allen 2000). However there are no explicit tests whether abiotic factors do differentiate AMF species according to spore size.

In this study, we tested whether habitat filtering acts on AMF communities depending on their spore size. We make use of the statistical framework developed by Cornwell et al. (2006). This

framework is based on the expectation that if particular abiotic factors restrict the community membership of species depending on a trait, metrics that measures trait dispersion at local communities (ranges, variances) should be smaller than expected by chance. To test this premise, we used the study of Oehl et al. (2010) reporting changes in AMF community structure along a land use intensity gradient and different soil types. These abiotic factors are known to drive AMF community structure and the magnitude of the heterogeneity included in that study was strong enough to cause significant changes in AMF community structure. As such, this study represent a case for a habitat filtering process that could resulted in restricted variation in spore sizes in local communities. Specifically we ask whether local communities differing in land use intensity and/or soil types show a greater reduction in trait dispersion metrics than expected by chance.

Material and methods

Data collection

Trait dispersion analysis relies on two data sets: a presence/absence species matrix along several local communities and trait values for each species. The study of Oehl et al. (2010) provided the presence/absence species matrix. Briefly, this study characterized AMF communities on 16 sites (each site considered here as a local community) based on spore morphology and species identity.

The sites were chosen to reflect three different soil types (Cambisols, Fluvisols and Leptosols) and two land use intensities (grassland and arable land). The sites also varied in other abiotic parameters (pH, organic carbon, phosphorus levels) that are known to modify AMF community structure (See Appendix 1). These sites are located throughout a total area of 4000 km^2 in central Europe.

Data on spore sizes (not provided in the study) were obtained from the original descriptions of the species available from the Arthur Schüssler lab (website: http://schuessler.userweb.mwn.de/amphylo/), from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM) and from species description of the Janusz Błaszkowski Lab (http://www.zor.zut.edu.pl/Glomeromycota/index.html). As a measure of spore size we used projected area of spore. This was calculated from spore descriptions which provide an average radius for spherical spores or two radii for ellipsoid ones. This trait was chosen over volume or weight because: a) all spore description include at much two radii, providing a uniform trait measure

through all glomeromycota. Calculating volume is not possible for ellipsoid spores given that descriptions do not include a third radii for "depth"; while weight is rarely measured); 2) mathematically projected area and volume are positively correlated and likely the two radii chosen for description are the ones that encompass most of the variation in size. Trait data was log transformed for analysis.

Null models

Trait analysis for habitat filtering relies on comparing observed measures of trait central tendency and/or dispersion metrics (means, variances and ranges) in local communities to a null expectation (HilleRisLambers et al. 2012). The null expectation is obtained by calculating the trait dispersion metrics (ranges and variances) on randomly reshuffled communities under the assumption that community membership is independent of local abiotic parameters.

We used the independent swap algorithm in the package picante in R (Kembel et al. 2010) to obtain randomized communities. This algorithm randomly reshuffles the entire presence/absence matrix and creates local communities with two restrictions: richness of local communities as well as the frequency of occurrence of species is kept constant through all communities. By doing so, we are specifically asking about the probability of detecting observed local communities out of a distribution of randomized communities of the exact same species richness and where species are limited to occur to a subset of sites. We repeated the algorithm to create 1000 randomized communities. For all randomizations we excluded species that were reported based on a single spore in each local community in the original data matrix, as these are more likely to be present because of recent dispersal events, rather than established species in the community.

Statistical significance

Observed trait dispersion metrics at each community were considered non-random if they fell in the 5% extremes of the null distribution. One tailed tests were performed on trait dispersion (ranges and variances) because habitat filtering results in trait dispersion metrics that are expected to be smaller than expected. We also computed community trait means to evaluate whether they were smaller or bigger than expected by chance; two tailed tests were performed for trait means.

Results

Spore size varied from 3 to 5 orders of magnitude over the 16 sites. The largest spore size was of *Gigaspora margarita* (100,098.2μ^2) while the smallest one was *Glomus microcarpum* (1,194.6 μ^2). However, seventy five percent of the species varied in spore size from 3 to 4 orders of magnitude (Fig. 4.1). Species richness varied from 14 to 31 species with a median of 23 species.

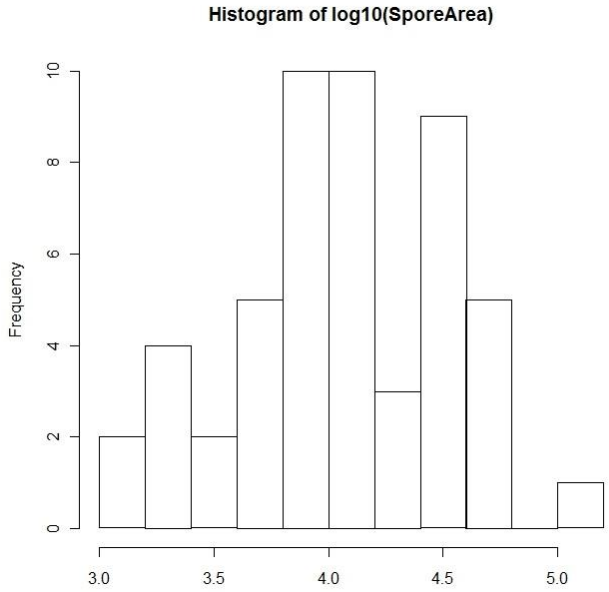

Fig. 4.1. Distribution of spore sizes in the study of Oehl et al. (2010). Histogram of spore size (projected area) in logarithmic scales.

Results indicate that there is not systematic segregation ("filtering") of species depending on spore size thoughouot the different soil types and land use intensities. Observed trait ranges or variances were only observed to be smaller than expected by chance in two different grasslands (one detected by reduced variance and the other by reduced range) (Table 4.1). The community trait mean on the grassland with significantly smaller trait range (Grassland "JLhg") was also smaller than expected by chance (Table 4.1; Fig. 4.2).

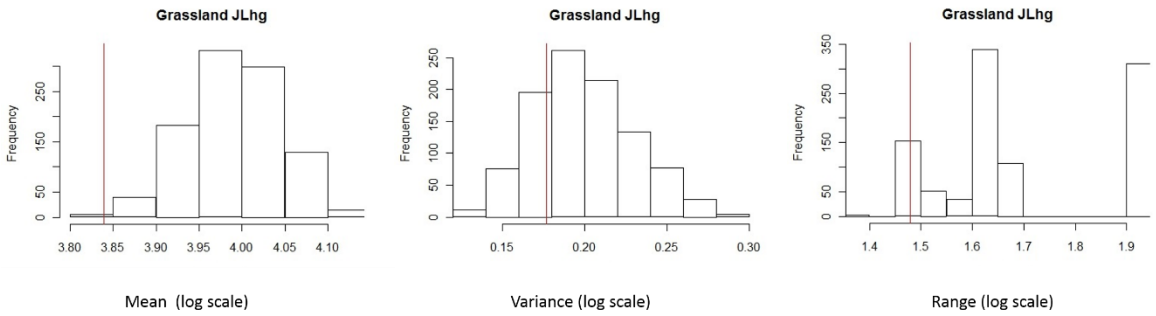

Fig. 4.2. Histograms of the null distributions for ranges (left), variances (middle) and mean (right) for spore sizes in the Calcareous grassland "JLhg" based on 1000 randomizations. Position of the observed values is marked with a red line. Both range and mean values are smaller than expected by chance (P<0.05).

Table 4.3 Community trait (spore size) metrics calculated from the presence/absence matrix presented in Oehl et al. (2010). Statistical significant deviations from null models (P<0.05) are in bold (two tailed tests for means; one tail test for variances and ranges). Values from log transformed data.

Site	Mean	Variance	Range
Grassland PS-hg	3.96	0.23	1.9
Grassland PS-g	3.99	**0.14**	1.62
Arable PS-a	3.92	0.19	1.49
Grassland TS-g	4.00	0.19	1.62
Arable TS-a	4.00	0.23	1.62
Grassland GR-hg	3.98	0.24	1.9
Grassland DG-g1	4.05	0.21	1.9
Arable DG-a1	4.01	0.22	1.66
Grassland DG-g2	3.97	0.18	1.51
Arable DG-a2	3.95	0.21	1.62
Grassland AG-g	4.02	0.25	1.92
Arable AG-a	4.02	0.18	1.5
Grassland JL-hg	**3.84**	0.18	**1.48**
Arable JL-ha	3.92	0.20	1.5
Grassland JL-g	3.97	0.17	1.6
Arable JL-a	3.90	0.21	1-46

Discussion

Overall, the results indicate that land use intensity or soil type represented in the Oehl et al. (2010) does not strongly differentiate AMF communities with respect to spores sizes. Indeed the

only case when habitat filtering was detected through reduced trait ranges was in a site characterized by "very low" land use intensity practices: a grassland (mown meadow) with no crops and with the lowest fertilization inputs out of all sites (50 kg/ha for N; 10 kg/ha for P). The site also had relatively high pH levels (7.6, the third highest out of the 16 sites) and the second highest level of soil organic carbon (47.6 mg/kg) as well as available P (23.6 mg/kg); this high level of P is probably explained by former high fertilization inputs. Given these extreme characteristics of grassland "JL-hg" relative to others it would be unlikely that the significant detection of both trait range and mean is the result of false positive given the number of statistical tests made.

We emphasize that this analysis does not contradict the results of the original study. The authors show significant changes in community structure according to land use intensity. Our analysis makes the point that those changes were not greatly determined by spore sizes, except for one case where the conditions seem to be extreme. In other words, spore size for most part is neutral in the community assembly process.

There are several potential reasons for this outcome. First, variation in spore sizes was lower compared to variation in seed sizes typically observed in plant communities. In this study, variation was around 2 orders of magnitude, while seed sizes can vary around 3 to 5 orders of magnitude in a single community (Leishman et al. 1995, although this study relies on seed mass not seed projected area). Under this setting, filtering would need to select strongly for spore sizes in order to detect habitat filtering by means of trait dispersion metrics.

Secondly, it could be that variation in spore sizes does not reflect strong life-history or ecological trade-offs compared to plants. Seeds represent the main units on which angiosperm recruitment and establishment is based. Therefore, any variation in seed traits, such as size, is likely to have fitness consequences. For AMF, recruitment and establishment do not depend solely on their spores. Both plant to plant mycelial contact as well as colonized root fragments serve as units to establish on new hosts (Smith and Read 2010). Thus, a clear relationship between variation in spore size and AMF species fitness would not be as clear as with seeds for plants.

There is a need for more research to determine the ecological meaning of variation in spore size for AMF (and other fungi). So far there is no empirical study showing the relationship between spore size and spore number, as in plants. Although, some studies use spore output as a measure of AMF fitness (Bever et al. 2009), large spore sized AMF species might be at an advantage because of a greater chance of establishment. So far, there are no empirical studies regarding this issue.

In conclusion, this study represents the first attempt to use AMF trait data to infer community assembly mechanisms. Information on more traits would be very desirable to better assess the role of abiotic factors in shaping AMF community structure. Certainly, spore size is not the only (or the most important) trait to test community assembly processes. This study is therefore also a call to collect more trait data, as has been proposed for AMF (van der Heijden and Scheublin 2007) and for other root-infecting fungi (Aguilar-Trigueros et al. 2014).

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Chapter Five

Discussion

The aim of the present thesis was to increase our understanding of the community assembly of Root Infecting Fungi (RIF) and its effect on plant community and ecosystem dynamics. As explained in Chapter 1, we used a phenomenological approach (experimental manipulations of RIF communities) as a starting point to show the relevance of RIF in plant community patterns. Then we move to a complementary trait based approach in order to provide mechanistic understanding of RIF ecology. The work presented here is relevant to most groups of soil inhabiting fungi, however, we were purposely biased towards non-mycorrhizal RIF as they are the most common and diverse group of RIF but have received less attention.

Outcomes from phenomenological approach and future work

In Chapter 2, we found that experimental manipulations of the three common non-mycorrhizal fungi (Ascomycota) alter community structure of microcosm plant communities. The outcome was driven by differential host growth responses to the presence of the different isolates. This is surprising given the asymptomatic nature of the colonization (Appendix of Chapter 2; Chapter 6). It also makes evident that many of the observed fitness differences (here measured as vegetative growth) among co-occurring plants depend strongly on the composition of the local soil fungal biota.

This type of phenomenological greenhouse studies provides the best means of studying the ecological consequences of RIF interactions given the power to detect causality. This type of experiments in grasslands has overwhelmingly been carried out with arbuscular mycorrhizal fungi (AMF) in terms of soil-inhabiting fungi (van der Heijden et al. 1998; but see Rillig et al. 2014). Field manipulations, although more realistic, provide more noisy results given the likelihood of uncontrolled colonization by other soil biota. Fungal cross-contamination as well as presence of other fungal interactants was very low. Further greenhouse microcosm studies could be used to determine the effect of increasing diversity of RIF or changes in relative abundance of fungal interactants on host phenotypes and plant-plant dynamics as has been done recently for AMF interactions (Wagg et al. 2011). To achieve external validity in these greenhouse experiments, they could be coupled with ecological modelling approaches, as it is done with Plant Soil Feedback studies (e.g. Mangan et al. 2010).

Development of a trait-based platform and its further use

We continued developing a framework for a trait based approach pertinent to RIF in Chapter 3 and 4. In doing so, we extended it to include most fungi that occur in soil, as it was evident that the distinction between soil and root fungi is artificial (Chapter 3).

We envision that the proposed framework can be further used for three main objectives. First, elucidate the mechanisms by which RIF influence plant community dynamics. Second, identification of fungal life history trade-offs for RIF and understanding their ecological consequences. Third, promoting the exchange of ideas from the different scientific fields related to the study of RIF-plant interactions.

Elucidating mechanisms by which RIF influence plant community dynamics

The main driver to develop a trait-based framework was the difficulty to attribute mechanism behind the outcomes of Chapter 2. Figure 5.1 represents the many pathways through which the isolates used could have driven the observed outcome. It is clear that two main types of mechanisms can operate. First, a symbiotic mechanism where differential host growth is due to a host specific parasitism, commensalism or mutualism. Second, a saprotrophic mechanism, where fungi are predominantly free-living soil organism with the ability to immobilize or release nutrients modifying the resource conditions where the hosts grow. Clearly knowledge of the saprotrophic and symbiotic capabilities of RIF would be needed to disentangle these mechanisms.

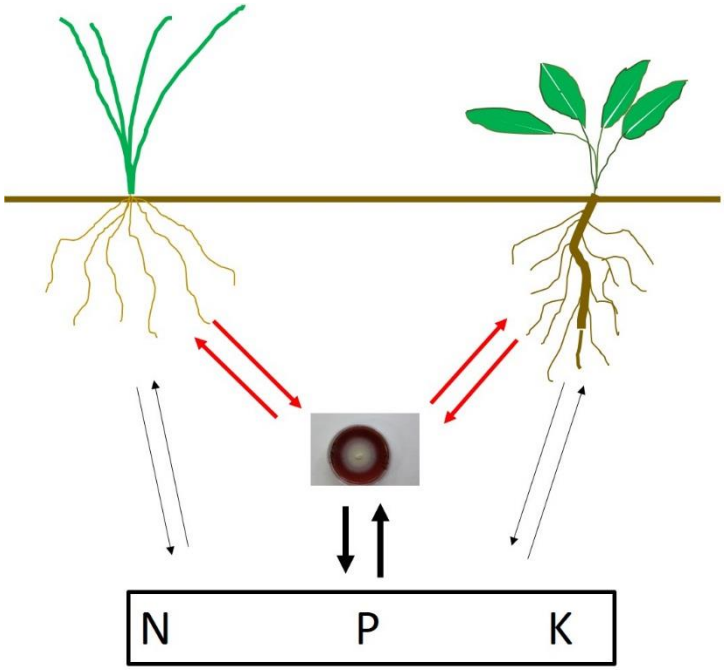

Fig. 5.1 Different mechanism by which RIF (non-mycorrhizal) could modify plant-plant dynamics. Arrows in red denote effects driven by a symbiosis (positive, neutral or negative effects on hosts). Black arrows indicate indirect effect driven by the use of mineral resource during free living stage (saprotrophy).

Identification of life-history trade-offs for RIF and understanding of their ecological consequences

Chapter 3 emphasized that little is known about how the transition from the soil to root interfaces constrained the evolution of soil fungi. Likely, such transition have resulted in life-history trade-offs (trade-offs in energy investments to different fitness component). Such trade-offs could be detected using the traits proposed in Table 3.1 and table 4.1. The study of trade-offs in mycology has been restricted to fungal pathogens and to some extent ectomycorrhizal fungi (Olson et al. 2012; Bässler et al. 2014).

This type of information can be used to understand, for example, the maintenance of the large diversity of fungi present in roots and soil. All diversity maintenance mechanism rely on the existence of species trade-offs in the presence of environmental heterogeneity (Chesson 2000). These ideas are untested in mycology. In this sense, the case study provided in Chapter 4 represents an example for the identification of a putative of life-history trade-off (spore size and spore output)

and the environmental conditions that favor either type of trait combination (small spores in locations with high P availability).

Promoting communication from different fields relevant to RIF ecology

The information presented in Chapter 3 and 4 was intended for the use of researchers historically working in different fields. First, it combines approaches from different fields within mycology and fungal ecology. In the soil many fungal groups converge and interact, but mycologist study each group separately. For example, most calls for trait based approaches were directed to mycorrhizal research audience (Chagnon et al. 2013; Koide et al. 2014) (similar calls to a broader mycological audience are just coming out (Crowther et al. 2014)). These proposals neglect or underestimate their role in the decomposing organic matter. On the other hand, mycologist interested in saprotrophy have restricted their attention to wood decaying fungi and neglect soil fungi (Woodward and Boddy 2008). Table 3.1 represents a combination of techniques developed in each field for a better understanding of soil fungi in general. The need for communication to a broader mycological audience better explained in chapter 4.

Second, and more importantly, the trait-based framework aimed to establish bridges between fungal ecologists and mainstream ecologists. On the one hand mainstream ecology (particularly plant community and ecosystem ecology) largely ignore fungi when developing theories of community assembly and ecosystem functioning. For example, Siepielski and McPeek (2010) in reviewing empirical studies on species co-existence, out of the 323 reported studies only 2 included fungi (of them a yeast). On the other hand, the information presented will help mycologist to test hypotheses of mainstream ecology and thus, propel fungi from the current state of underrepresentation in comparison to other organismal groups (Pautasso 2013).

Conclusion

The present thesis showed that non-mycorrhizal RIF are important (but largely ignored) players in determining the plant phenotypes and fitness differences among plants in natural communities. It further develops a framework to understand the mechanisms behind such effects and move forward fungal ecology into mainstream ecology and, to some extent, evolutionary biology. Ultimately, we hope that here we provided strong arguments to showcase that the study

of ecological interaction among root (and soil) inhabiting fungi and the interaction with their hosts represent an exciting, fruitful but largely unexplored research field.

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Summary

The aim of the present thesis was to increase our understanding of the community assembly of Root Infecting Fungi (RIF) and its effect on plant community and ecosystem dynamics. It specifically targets non-mycorrhizal RIF belonging to the phyla Asco- and Basdiomycota, which represent the largest diversity of soil fungal interactans, but have received less attention compared to mycorrhizal associates. Two approaches were used in addressing this aim.

First, a phenomenological approach was used by experimentally manipulating the presence of RIF and quantify its effect on plant community structure. This work was based on the isolation of RIF from a natural grassland in north eastern Germany which were further screened to determine the type of interaction. Three isolates related to *Fusarium* species (Ascomycota) were selected to conduct a greenhouse microcosm experiment (Chapter 2). This experiment had the objective to test whether plant species, which co-occur in the same natural grassland where the fungi were isolated, respond in species specific manner to each fungal isolate and whether the fungi alter the plant response to changes in soil abiotic characteristics. To achieve this objective, the identity of the fungal isolate together with soil texture were manipulated in a fully factorial fashion in experimental plant community microcosms. It was observed that each plant species responded differently to infection, resulting in distinct patterns of plant community structure depending on the fungus present. Each fungus provided benefits to some host species while negatively affecting others. The host responses to infection were strongly dependent on soil texture: positive responses conferred to a host at one texture level were absent in the other level. Further, host responses to the higher fungal diversity treatment (mixture inoculation of 3 fungi) were also dependent on soil texture. Based on this results, it can be concluded that non-mycorrhizal RIF can exert significant effects on plant community structure as well as greatly modify the way soil abiotic factors shape plant community dynamics.

Second a trait-based approach was developed to understand the mechanisms behind community assembly process and ecosystem functioning. It is proposed that a shift to a multidimensional trait-based approach to disentangle the saprotrophic-symbiotic continuum will provide a better framework to understand fungal evolutionary ecology (in contrast to current classification schemes). Trait information reflecting the separation of root infecting fungi from free living soil relatives will help to understand the evolutionary process of symbiosis, the role that species interactions play in maintaining their large diversity in soil and *in planta,* and their

contributions at the ecosystem level. Methodological advances in several areas such as microscopy, plant immunology and metatranscriptomics represent emerging opportunities to gather trait data pertinent to this continuum. In chapter four, it is further stressed the necessity of fungal trait frameworks by arguing that the current underrepresentation of trait based studies in mycology is a missed opportunity. Selection and systematic collection of trait data throughout the fungal kingdom will reap major benefits in ecological and evolutionary understanding of fungi. To develop the argument, plant trait examples were used to show the power of trait-based approaches in understanding ecological phenomena such as identifying species allocation resources patterns, inferring community assembly and understanding diversity-ecosystem functioning relationships. To expand on this point, a case study is presented to showcase how fungal spore trait data can be used to infer life history trade-offs and to test the role of habitat filtering during fungal community assembly. Second, ecologically relevant traits in fungi are presented which could be used to answer such ecological phenomena and can be measured on a large proportion of the fungal kingdom. Third, major challenges and opportunities are identified for widespread, coordinated collection and sharing of fungal trait data. The proposed view has the potential to allow mycologists to contribute considerably more influential studies in the area of fungal ecology and evolution, as has been demonstrated by comparable earlier efforts by plant ecologists. This represent a change of paradigm, from community profiling efforts through massive sequencing tools, to a more mechanistic understanding of fungal ecology.

Zusammenfassung

Das Ziel dieser Arbeit war es, unser Verständnis zur Zusammensetzung von Lebensgemeinschaften wurzel-infizierender Pilze (RIF (root infecting fungi)) und deren Auswirkung auf Planzenlebensgemeinschaften und Ökosystemdynamiken zu verbessern. Im Speziellen geht es um die Untersuchung von „Nicht-Mykorrhiza“ Pilzen, welche zu den Phyla Asco- und Basidiomyceten gehören und den größten Anteil an bodenpilzlichen Interaktionspartnern stellen, obwohl sie weit weniger Aufmerksamkeit erhalten als Mykorrhizapilze. Um dieses Ziel zu erreichen, wurden zwei Ansätze verwendet.

Zuerst wurde ein phänomenologischer Ansatz angewandt, bei dem die Präsenz von RIF experimentell modifiziert und deren Effekt auf die Struktur der Pflanzenlebensgemeinschaft quantifiziert wurde. Dieses Experiment basierte auf RIF-Isolaten aus einem natürlichen Grasland in Nordost Deutschland, welche weiterführend untersucht wurden um den Interaktionstyp zu bestimmen. Drei Isolate (nahe verwandt mit der Gattung *Fusarium* (Ascomycota)) wurden ausgewählt um ein Mikrokosmos-Experiment im Gewächshaus durchzuführen (Kapitel 2). Das Ziel dieses Experimentes war es herauszufinden ob Pflanzenarten, welche im gleichen Grasland auftreten in welchem die Pilze isoliert wurden, in artspezifischer Weise auf die jeweiligen Pilzisolate reagieren und ob diese Pilze die Reaktionen der Pflanzen auf abiotische Bodenparameter verändern. Um das zu erreichen wurde die Identität der Pilzisolate zusammen mit der Bodentextur voll-faktoriell in experimentellen Pflanzenlebensgemeinschafts-Mikrokosmen manipuliert. Es wurde beobachtet, dass jede Pflanzenart unterschiedlich auf die Pilzinfektion reagierte, was abhängig vom zugegebenen Pilzisolat zu individuellen Mustern in der Struktur der Pflanzenlebensgemeinschaft führte. Jedes Pilzisolat beeinflusste gleichzeitig bestimmte Wirtspflanzen positiv und andere Arten negativ. Die Reaktion der Wirtspflanzen auf Pilzinfektionen war stark abhängig von der Bodentextur: Positive Effekte auf eine Wirtspflanze bei einer bestimmten Bodentextur waren nicht ersichtlich bei einem anderen Texturlevel. Die Reaktion der Wirtspflanzen auf die Behandlung mit höherer Pilzdiversitat (Inokulation von 3 Pilzisolaten) war ebenfalls abhängig von der Bodentextur. Basierend auf diesen Ergebnissen kann geschlussfolgert werden, dass Nicht-Mykorrhiza RIF einen signifikanten Effekt auf die Pflanzenlebensgemeinschaft ausüben und gleichzeitig den Einfluss von abiotischen Faktoren auf die Dynamik der Lebensgemeinschaft beeinflussen.

Der zweite Ansatz, basierend auf Eigenschaften („traits“), wurde entwickelt um die Mechanismen, welche hinter den Prozessen zur Zusammensetzung von Lebensgemeinschaften sowie Ökosystemfunktionen stehen, zu verstehen. Eine Verschiebung hin zu multidimensionalen auf Eigenschaften basierenden Ansätzen, welche das Kontinuum Saprotroph-Symbiotisch auflösen sollen, wurde im Gegensatz zu jetzigen Klassifikationssystemen als geeigneterer Rahmen für das Verständnis der evolutionären Ökologie von Pilzen vorgeschlagen. Kenntnisse über Eigenschaften, welche den Unterschied von wurzel-infizierenden Pilzen zu ihren im Boden lebenden Verwandten widerspiegeln, werden zum Verständnis des Evolutionsprozesses der Symbiose, der Rolle von interspezifischen Interaktionen bei der Erhaltung der großen Diversität im Boden und *in planta* und deren Mitwirken auf dem Ökosystemlevel beitragen. Methodologische Fortschritte auf unterschiedlichen Gebieten wie Mikroskopie, Pflanzenimmunologie und Metatranscriptomics stellen neue Möglichkeiten dar um Daten zu Eigenschaften passend zu diesem Kontinuum aufzunehmen. In Kapitel vier wird die Notwendigkeit des Einbeziehens von Pilzeigenschaften aufgezeigt, mit dem Argument dass die momentane Unterrepräsentation von Eigenschaften-basierenden Studien in der Mykologie eine verpasste Möglichkeit darstellt. Die Auswahl und systematische Aufnahme von Daten zu Eigenschaften quer durch das Reich der Pilze wird großen Nutzen für das ökologische und evolutive Verständnis von Pilzen bringen. Zur Untermauerung dieser Argumentation wurden Beispiele von Pflanzeneigenschaften herangezogen, welche die Stärke von Eigenschaften-basierenden Ansätzen für das Verständnis ökologischer Phänomene aufzeigen. Dies sind beispielsweise die Identifikation von Mustern artspezifischer Ressourcenallokation, Rückschlüsse auf die Zusammensetzung von Lebensgemeinschaften oder das Verständnis von Zusammenhängen zwischen Diversität und Ökosystemfunktionen. Um diesen Punkt weiter auszuführen wurde als erstes eine Fallstudie präsentiert, welche aufzeigt wie Daten zu Pilzsporen genutzt werden können um Rückschlüsse zu „trade-offs“ im Lebenszyklus zu ziehen und die Rolle des Habitatfilters während der Ausbildung von Lebensgemeinschaften zu testen. Zweitens wurden ökologisch relevante Eigenschaften in Pilzen präsentiert, welche an großen Teilen des Pilzreiches gemessen werden und zur Untersuchung solch ökologischer Phänomene genutzt werden könnten. Als drittes wurden die Hauptherausforderungen und –möglichkeiten für eine ausgedehnte und koordinierte Erfassung und Nutzung von Daten zu Pilzeigenschaften identifiziert.

Die vorgeschlagene Sichtweise hat das Potential Mykologen zu befähigen, deutlich mehr einflussreiche Studien im Feld der Pilzökologie und –evolution beizutragen, so wie es durch

frühere vergleichbare Bemühungen der Pflanzenökologen aufgezeigt wurde. Dies stellt einen Paradigmenwechsel dar von Bemühungen zur Darstellung der Lebensgemeinschaft über „massive sequencing“ Methoden hin zu einem stärker mechanistischen Verständnis von Pilzökologie.

Contribution to the publications

Aguilar-Trigueros CA, Rillig MC. 2015. Soil texture and root infecting ascomycete fungi interactively affect plant community structure. Plant and Soil *Under Revision.*

Own contribution

CA A-T and MC Rillig designed the experiment. CA-AT executed the experiment, gathered the data and analyzed it. CA-AT wrote the manuscript, all authors reviewed the manuscript.

Aguilar-Trigueros CA, Powell JR, Anderson IC, Antonovics J, Rillig MC (2014) Ecological understanding of root-infecting fungi using trait-based approaches. Trends Plant Sci. 19:432-438. DOI: 10.1016/j.tplants.2014.02.006

Own contribution

CA-AT developed the idea and gathered the information on fungal traits presented in the table. CA-AT and JA drew the figure. CA-AT wrote the manuscript. All authors reviewed the manuscript.

Aguilar-Trigueros CA, Hempel S, Powell JR , Anderson IC , Antonovics J, Bergmann J, Cavagnaro TR, Chen B, Hart MM, Klironomos J, Petermann JS, Verbruggen E, Veresoglou SD, Rillig MC.2015. Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biology Reviews* (Accepted) DOI: 10.1016/j.fbr.2015.03.001

Own contribution

CA-AT together with all authors developed the idea. CA-AT developed the examples of plant traits discussed. CA-AT developed the idea of the case study, gathered the data and performed the analysis with Hempel S. All authors contributed to the construction of the table. All authors reviewed the manuscript.

Appendix

Supplementary Information Chapter 2

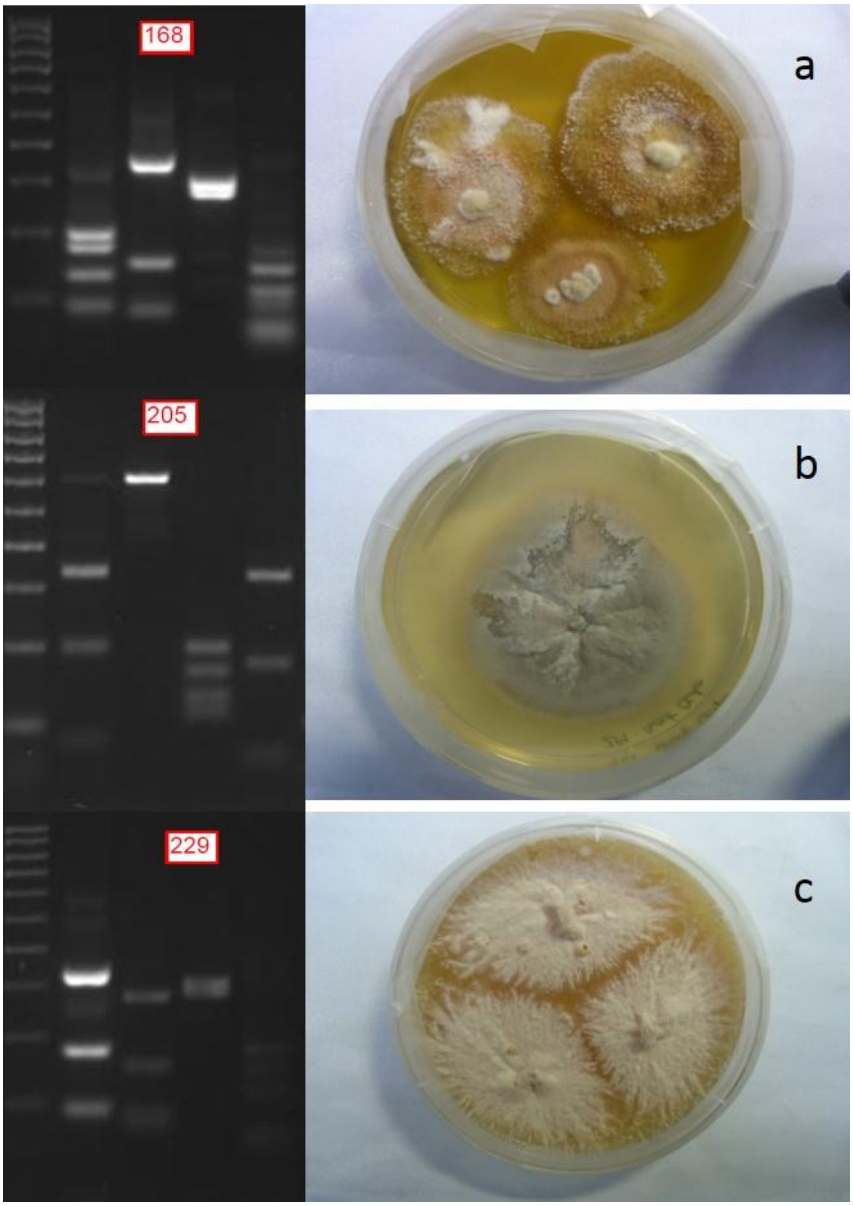

Fig. AS1. RFLP patterns and matching colony morphology types. a) Isolate 168 corresponding to *Gibberella sp;* b) Isolate 205, *Microdochium sp*; c) Isolate 229, *Fusarium sp*.

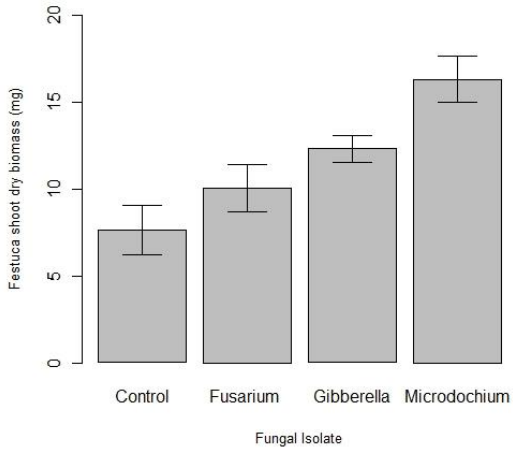

Fig. AS2. Responses of *Festuca brevipila* to *in vitro* inoculations with the three fungal isolates used in the main experiment. Responses obtained from two week old seedlings that were inoculated with each isolates and let them grow together for growing 6 weeks (Sample size: Control: 8 seedlings; Fusarium: 5 seedlings, Gibberella: 5 seedlings, Microdochium 6 seedlings) . Bars indicate means, whiskers standard errors.

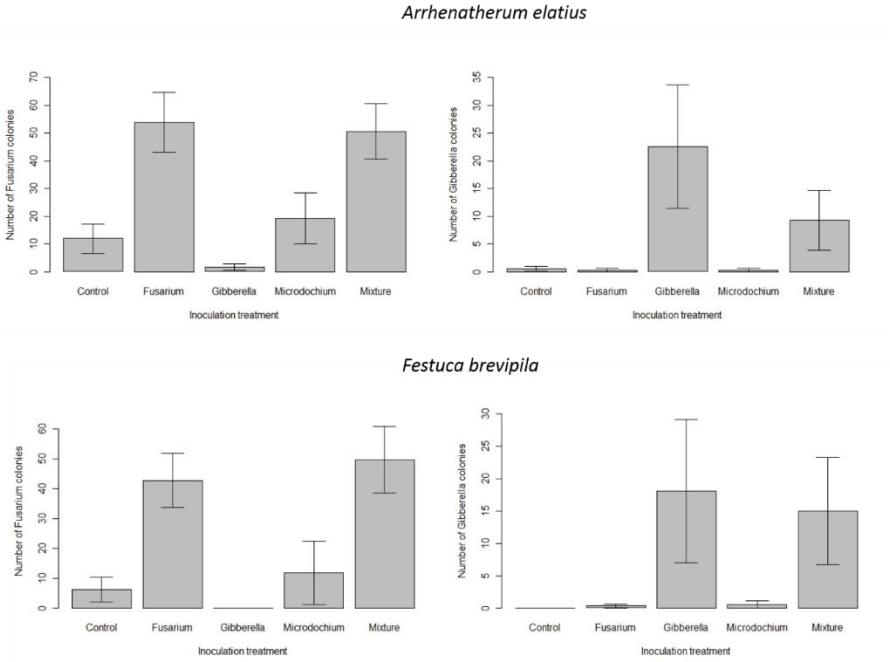

Fig. AS3. Fungal re-isolations from grasses growing in the microcosms. The *y*-axis corresponds to the total number (mean and standard error) of fungal colonies of *Fusarium* (left barplots) and *Gibberella* (right barplots) retrieved from 10 pots from eachfungal treatment (5 in Low Sand and 5 in High Sand).

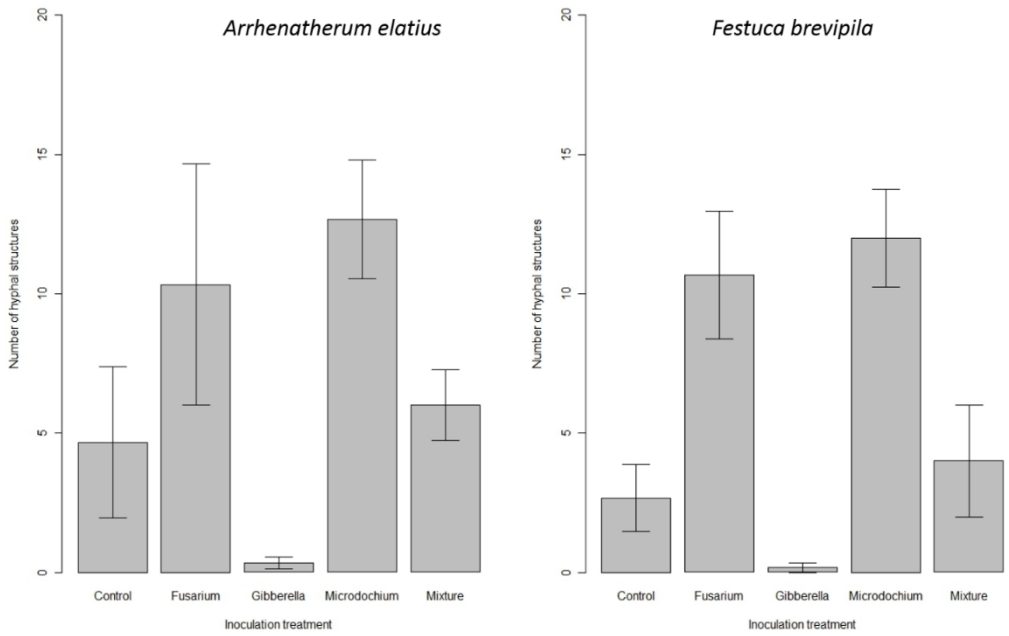

Fig. AS4 Number (mean and standard error) of fungal structures found in the roots of *Arrhenatherum elatius* and *Festuca brevipila* in each fungal treatment.

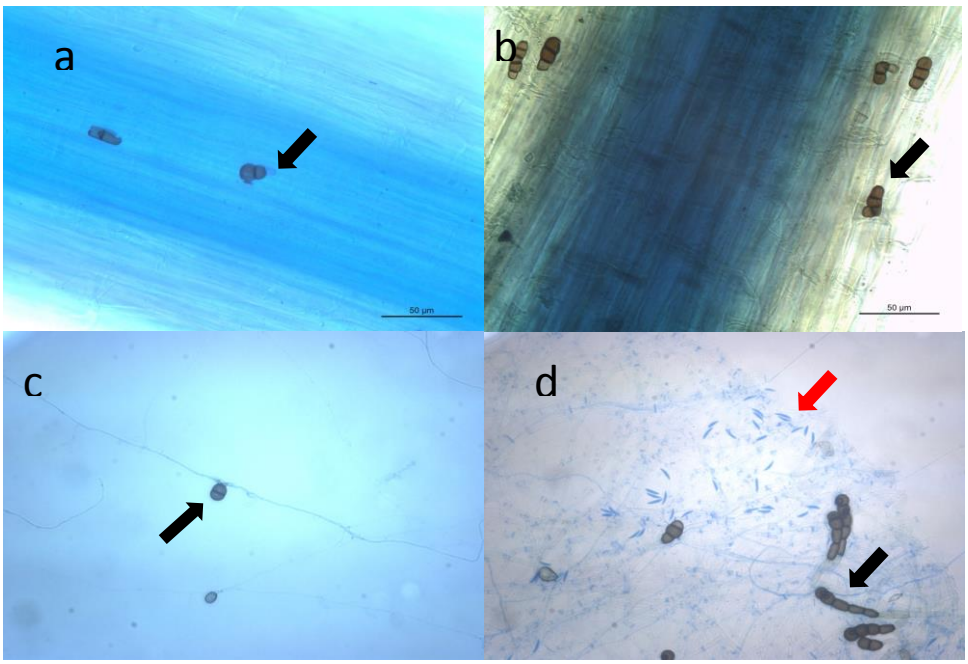

Fig. AS5.Comparison of chlamydospores of *Microdochium* isolates in pure culture (c and d) with observed ones in *Microdochium* treatments growing in the roots of *F. brevipilla* (a and b). In b, the chlamydospores are inside the root cortical cells (black arrow), where they were frequently observed. In d) the *Microdochium* isolates also produce microconidia (red arrow).

Table AS1. Correlation matrix of the biomass of each interacting species in each fungal treatment within soil types. Values indicate correlation coefficients (r), asterisks indicate statistical significance (p<0.05).

	High Sand soil type				Low Sand soil type		
CONTROL HS	**Arrhenatherum**	**Festuca**	**Armeria**	**CONTROL LS**	**Arrhenatherum**	**Festuca**	**Armeria**
Arrhenatherum	1	0.11	0.51	**Arrhenatherum**	1	0.03	0.2
Festuca		1	-0.08	**Festuca**		1	0.66*
Armeria			1	**Armeria**			1
FUSARIUM HS	**Arrhenatherum**	**Festuca**	**Armeria**	**FUSARIUM LS**	**Arrhenatherum**	**Festuca**	**Armeria**
Arrhenatherum	1	-0.78*	-0.56	**Arrhenatherum**	1	-0.55	-0.35
Festuca		1	0.22	**Festuca**		1	0.31
Armeria			1	**Armeria**			1
GIBBERELLA HS	**Arrhenatherum**	**Festuca**	**Armeria**	**GIBBERELLA LS**	**Arrhenatherum**	**Festuca**	**Armeria**
Arrhenatherum	1	-0.2	0.53	**Arrhenatherum**	1	0.26	0.85*
Festuca		1	-0.04	**Festuca**		1	0.59
Armeria			1	**Armeria**			1
MICRODOCHIUM HS	**Arrhenatherum**	**Festuca**	**Armeria**	**MICRODOCHIUM LS**	**Arrhenatherum**	**Festuca**	**Armeria**
Arrhenatherum	1	-0.66*	-0.48	**Arrhenatherum**	1	-0.45	-0.33
Festuca		1	0.37	**Festuca**		1	0.15
Armeria			1	**Armeria**			1
MIXTURE HS	**Arrhenatherum**	**Festuca**	**Armeria**	**MIXTURE LS**	**Arrhenatherum**	**Festuca**	**Armeria**
Arrhenatherum	1	0.18	0.15	**Arrhenatherum**	1	-0.65*	-0.55
Festuca		1	0.33	**Festuca**		1	0.56
Armeria			1	**Armeria**			1