Interactions between root-associated fungi and their host plants in a dry grassland

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Foreword

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

I **Wehner J**, Powell JR, Muller LAH, Caruso T, Veresoglou SD, Hempel S, Rillig MC. Determinants of root-associated Eumycota communities within Asteraceae in a semiarid grassland. (*resubmitted*)

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Contents

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General Introduction

Dry grassland habitats - Oderhänge Mallnow

In Central Europe, forest ecosystems would dominate the landscape without human influence. But forest clearance during the industrialization period has led to shifts in microclimatic conditions and, together with continous grazing pressure, to the establishment of grassland species adapted to high light intensity and higher soil temperatures especially in drier areas, with hot summers and cold winters. These so-called dry grassland habitats have similar microclimatic conditions to continental steppes or Mediterranean karst vegetation and are populated by relict species from the glacial time and thermophilic plants and animals immigrating from the border areas are well adapted to the extreme microclimatic conditions and low nutrient level (Ellenberg 1996). Plant communities in these grassland are often very diverse but also low competitive and are therefore an interesting study system. Without human management woody species would immigrate in these grasslands and outcompete the typical grassland species (Hensen 1995). Therefore, these systems are quite rare and do often have a certain nature protection status. They can still been found in Germany, for example in the Schwäbische Alp, on the southern slopes of the Kyffhäuser mountains (Sachsen-Anhalt), on the „Porphyrkuppen“ in the north of Halle (Sachsen-Anhalt) and in the Oder area in Brandenburg (Hensen 1995).

Our study site, the „Oderhänge Mallnow“ in the Oder region, is a nature protection area located in north-eastern Germany, approximately 120 km east of Berlin (52.4636°N, 14.4574°E next to the small village of Mallnow). It is a Natura 2000 hotspot of biodiversity containing over 200 different plant species combining flora elements of both steppes and oceanic habitats. It is part of a large (60 km long and up to 20 km wide), formerly glacial, region with dry grassland habitats along the Oder river, called “Oderbruch”. This is the northernmost and second largest arid environment in Germany with an annual precipitation of approximately 500 mm and a mean annual temperature of 8.7°C (Deutscher Wetterdienst 2010). The landscape is quite variable, with hills and valleys remaining from the final battles of World War II, which have a strong influence on plant species composition. It is partially wooded and woody area are mostly restricted to slopes because of higher access to groundwater. The main tree species are *Robinia pseudoacacia*, *Pinus sylvestris*, *Betula* spec. and some fruit-bearing trees. The tree free areas are dominated by different grass species, with *Arrhenaterum elatius*, *Festuca brevipila*, *Corynephorus canescens*, *Stipa capillata* as most common representatives, and herbaceous plants depending on soil type and exposition. Some of the herbaceous species are very rare in Germany and protected e.g. *Adonis vernalis*, *Anemone sylvestris*, *Aster linosyris*, *Campanula sibirica*, *Achillea pannonica*, *Hiercium echioides.* To reduce woody species and preserve these special vegetation the grassland is grazed by sheep twice a year. The soil is a calciferous boulder clay and very sandy (Hensen 1995) with a fairly extreme gradient from sandy clay on top of the hills and almost pure sand in the lower parts. The gradient in soil type, which is reflected in the vegetation and the variability in landscape might have important consequences for root fungal dispersal and community composition, because of differences in soil texture and sand blowing around. We hypothesize that the high plant diversity can be linked to a high diversity in root associated fungi, which we will investigate in Chapter 2 of these thesis. Due to its high diversity it is also a good system to study factors influencing co-existence of the different plant species, we do this partly in Chapter 4 by looking at plant responses to local pathogens.

Root associated fungi

The main goal of this thesis is to better understand the interaction between host plants and soil fungi. Soil fungi can have direct or indirect effect on plant performance ranging from beneficial to pathogenic (Bever 2003). They may influence plant community structure, diversity (van der Heijden *et al.* 1998a, Gilbert 2002, Stein *et al.* 2009), productivity (van der Heijden *et al*. 1998b, Klironomos *et al.* 2000) and play a key-role in plant community dynamics and thus contribute to coexistence of plant species (Bever 2003).

The mycelia of these fungi can be located inside (intraradical) or outside the roots by growing in the soil (extraradical). Fungi growing mainly inside the different plant tissues (e.g. leaves, stems, flowers, roots) without causing an obvious harm to their host (Saikkonen *et al.* 1998,
Schulz and Boyle 2005) are called endophytes. Different to the mycorrhizal symbioses, endophytes do not form cellular interfaces with specialized structures (e.g. arbuscules in case of arbuscular mycorrhizal fungi), have not synchronized their development to the plant partner, and both plant and fungal partner may not benefit from their interaction (Brundett 2006). The influence of colonization by root endophytic fungi on host plants performance can vary, as some studies found negative plant responses (Wilcox & Wang 1987, Stoyke & Currah 1993, Tellenbach *et al.* 2011, Mayerhofen *et al.* 2013) while others found positive (Newsham 1999; Usuki and Narisawa 2007; Upson *et al.* 2009; Wu *et al.* 2010). Negative effects might be explained by a translocation of carbon from the host plant to the fungus, while positive effects on plant performance might be promoted from endophyts by nutrient mineralization (Jumpponen 2001, Mandyam and Jumpponen 2005, Upson *et al*. 2009, Newsham 2011) and release of phytohormons (Mucciarelli *et al.* 2002, Schulz and Boyle 2005).

However, apart from these endophytes, plants can also be colonized by symbiontic arbuscular mycorrhizal fungi (AM fungi). Plant roots have been associated with AM fungi for over 400 Myr and mycorrhizal fungi may have played an important role in maintaining the colonization of land by plants (Redecker *et al*. 2000). AM fungi colonize plant roots and form special interfaces (i.e. arbuscules) for nutrient exchange between the symbiotic partners. Besides this intra-radical structure, AM fungi also form extraradical mycelia to take up nutrients from the surrounding soil beyond the rhizosphere depletion zone. They are completely dependent on host's carbon. In return the host plant achieves additional nutrients (Marschner & Dell 1994), water (Auge 2001), and pathogen protection from AM fungi (Newsham *et al.* 1995, Azcón-Aguilar and Barea 1996, Filion *et al.* 1999, 2003, Borowicz 2001).

Determinants of root associated fungal communities

As stated in the first paragraph the „Oderhänge Mallnow“ are a Biodiversity hotspot in Germany with more than 200 different plant species. However, it is unknown whether this aboveground diversity is also reflected in a high diversity of root-associated fungi. In Chapter 2 we will have a closer look at fungal communities associated with roots of different plant species within the Asteraceae in the same grassland and determine potential drivers of fungal community composition. Most root fungi are not always associated with the same host species but with several different ones. Due to the fact, that related host species share particular traits and a similar evolutionary history (Cavender-Bares *et al.* 2009, Crisp & Cook 2012) we hypothesized that this is reflected in community composition of root associated fungi. Next to host plant phylogeny, which has been shown being important for structuring fungal communities in tree species (Tedersoo *et al.* 2013), we want to further test the influence of the gradient in soil type and of spatial distances among host plants on community composition of root-associated fungi. These predictors have been shown to influence community composition of particular groups of fungi (e.g. AM fungi). So far no study exists testing the influence of those predictors on community composition of root-associated fungi of relatively closely related plant species in a very speciose dry grassland system.

The role of arbuscular mycorrhizal (AM) fungi in protecting host plants against pathogens

The mechanism of how pathogen protection arises via AM fungi is not well understood yet. There are several potential ways by which negative effects of pathogens might be negated by AM fungi. They can improve the nutrient status of the host plant, compete with other fungi for infections sites on the roots, induce changes in the root architecture, change the microbial community composition in the rhizosphere or activate the plants defense mechanism (Azcón-Aguilar and Barea 1996). Most of the studies on AM fungi-mediated pathogen protection focused on the interaction between a single pair of species (one beneficial and one pathogenic), and did not consider the vast microbial diversity associated with plant roots in a natural system. However, evidence exists that considering AM fungal diversity (see Chapter 3) in terms of pathogen protection is important and so far no studies exist testing this in a more natural system, like a dry grassland. With our study in Chapter 4 of this thesis, we wanted to answer the following questions:

- Does an interaction between local AM fungal and local parasitic/pathogenic organisms from a dry grassland system occur in a greenhouse experiment?
- If, so do plants benefit from the symbioses with AM fungi in terms of pathogen protection?

Thesis outline

In chapter 2 we focus on how root associated fungal communities relate to phylogenetic topology of host plants. This is done by 454 pyrosequencing of the fungal specific ITS region of DNA isolated from plant roots of 25 species within the Asteraceae growing in the "Oderhänge Mallnow". We will determine the influence of four different predictors of fungal community composition: the spatial component, soil type, host plant phylogeny and seasonality (of sampling).

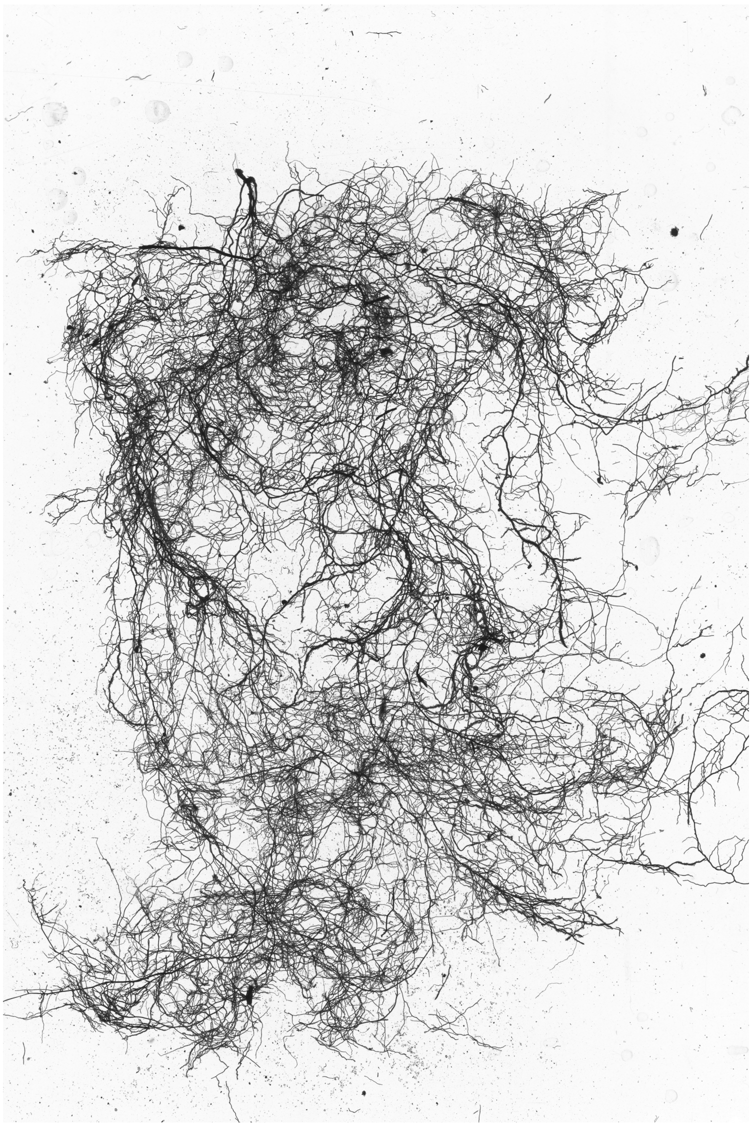
In chapter 3 we will shortly review the current literature on mechanism via which AM fungi could provide protection against pathogens to their host plants. In particular, we extend the focus beyond single AM fungal strains towards entire assemblages, asking whether a host plant may benefit more from a diverse AM fungal community in terms of pathogen protection or if this is likely.

In chapter 4 we analyze the interaction between local AM fungal assemblages and local saprobic/pathogenic fungal assemblages on plants in soil from the „Oderhänge Mallnow“. This is done by growing two plant species (*Hieracium umbellatum* L. and *Galium verum* L.), both very abundant in that grassland, in previously autoclaved local soil which is re-inoculated with an AM fungal spore suspension and a fraction of saprobic/pathogenic organisms. We will compare differences in above and belowground responses in biomass as well as certain root parameters between plants separately inoculated with AM fungi or saprobic/pathogenic organism and with both organism groups together.

In chapter 5 we will synthesize the results in a General Discussion.

2

Determinants of root-associated Eumycota communities within Asteraceae in a semiarid grassland



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Abstract

While plant-fungal interactions are important determinants of plant community assembly and ecosystem functioning, the processes underlying fungal community composition are poorly understood.

Here, we studied for the first time the root-associated eumycotan communities in a set of co-occurring plant species of varying relatedness in a species-rich semi-arid grassland in Germany. The study system provides an opportunity to evaluate the importance of host plants and gradients in soil type and landscape structure as drivers of fungal community structure on a relevant spatial scale. We used 454 pyrosequencing of the fungal ITS-region to analyze root-associated eumycotan communities of 25 species within the Asteraceae, which were sampled at different locations within a soil type gradient. We partitioned the variance accounted for by three predictors (host plant phylogeny, spatial distribution and soil type) to quantify their relative roles in determining fungal community composition and used null model analysis to determine whether community composition was influenced by biotic interactions among the fungi.

We found a high fungal diversity (156,816 sequences clustered in 1100 operational taxonomic units (OTUs)). Most OTUs belonged to the phylum Ascomycota (35.8 %); the most abundant phylotype best matched *Phialophora mustea*. Basidiomycota were represented by 18.3 %, with *Sebacina* as most abundant genus. The three predictors explained 30 % of variation in the community structure of root-associated fungi, with host plant phylogeny being the most important variance component. Null model analysis suggested that many fungal taxa co-occurred less often than expected by chance, which demonstrates spatial segregation and indicates that negative interactions may prevail in the assembly of fungal communities.

Our results show that the phylogenetic relationship of host plants is a major correlate of root-associated fungal community assembly, indicating that fungal colonization of host plants might be facilitated by certain plant traits that may be shared among closely related plant species.

Introduction

Plant roots interact with a range of soil fungi, which can influence plant growth and fitness (Lu & Koide 1994; Smith & Read 2008; Raaijmakers *et al.* 2008; Porras-Alfaro *et al.* 2011), plant community composition (van der Heijden *et al.* 1998), as well as ecosystem functioning (Bradley *et al.* 2008; Fisher *et al.* 2012). Depending on the identities of the host plant and fungus, these interactions can be of a mutualistic, neutral or parasitic nature. In grasslands, arbuscular mycorrhizal (AM) fungi are dominant symbiotic fungal partners and are known to increase nutrient status (Marschner & Dell 1994), improve water relations (Auge 2001) and protect host plants against pathogens (e.g. Borowicz 2001; Wehner *et al.* 2010; Veresoglou & Rillig 2012). In addition to AM fungi, which extend into the rhizosphere, plant roots are often colonized by fungal endophytes that reside completely within plant tissues (Rodriguez *et al.* 2009). One example of these endophytes in grasslands are the 'dark septate endophytes', which have been shown to provide benefits to the host plant but whose ecological function remains unclear (Porras-Alfaro *et al.* 2011; Jumpponen 2011). However, due to their microscopic nature and the difficulty of isolating many root-associated fungi, a large fraction of species remains unknown (Peay *et al.* 2008) and molecular methods are often essential to describe these fungal communities.

In general, community dynamics can be driven by local environmental gradients as well as by large-scale processes that determine the colonization and extinction of species within a region (Chase & Leibold 2003; Ricklefs 2006). For microorganisms, some have suggested that assembly processes are driven by a mixture of environmental filtering and random sorting from a regional species pool (Martiny *et al.* 2006). By contrast, if interspecific interactions among microbial taxa are important, assemblages will display nonrandom coexistence patterns (aggregation or segregation of species; e.g., Pan and May 2009).

Some of the local environmental factors that have been shown to affect fungal community composition include nitrogen availability, soil moisture and pH (Mulder & de Zwart 2003; Cox *et* *al.* 2010; Fujimura & Egger 2012). Root-associated fungal community composition also seems to depend on dispersal limitation (Lekberg *et al.* 2006) and on host-species identity; many fungal pathogens of plants are host-specific, whereas contradictory observations exist regarding host specificity of mycorrhizal fungi (Zhou & Hyde 2001). Evidence for host specificity has been found for AM fungi (Vandenkoornhuyse *et al.* 2003), for ectomycorrhizal (ECM) fungi (Morris *et al.* 2008) and also for endophytes in general (Wearn *et al.* 2012). Host range may also be linked to plant traits that facilitate infection and carbon/nutrient exchange; these traits may be constrained within phylogenetic lineages, resulting in covariance between host phylogenetic relationships and fungal species/communities (Ishida *et al.* 2007; Hibbett *et al.* 2000; Refrégier *et al.* 2008; Espiau *et al.* 1997; Gilbert & Webb 2007). Once the effects of these various environmental and host filters have exerted their influence on the fungal assemblages, interactions among fungal species may further influence assembly processes (Pan & May 2009). Such interactions among fungi can be negative (competition) or positive (facilitation) or random (predominant environmental filtering). For example, a potential negative interaction among fungi has been observed in that the presence of AM fungi in roots was negatively correlated with endophyte colonization (Wearn *et al.* 2012). By contrast, Pan & May (2009) found mainly positive interactions by examining endophytic fungi only; host infections with one fungus increased the vulnerability to infection by other fungi.

In the present study, which is the first investigating root-associated fungal (Eumycota) communities of co-occurring, phylogenetically relatively closely-related plant species in grasslands, we performed 454 pyrosequencing of internal transcribed spacer 1 (ITS1) amplicon libraries to characterize fungal diversity in roots. As a next generation parallel sequencing technology, 454 pyrosequencing increases the likelihood of detecting rare phylotypes due to the higher sampling depth, which would be difficult to reach with the classical cloning-Sanger sequencing methodology (Öpik *et al.* 2009). Specifically, we tested the hypothesis that host phylogeny, spatial structure and soil simultaneously affect the community composition of root-associated fungi of 25 different Asteraceae species in a semi-arid grassland. Since many ecological drivers exhibit spatial autocorrelation, we accounted for the potentially confounding effects of unmeasured drivers (dispersal limitation, other environmental variables) by explicitly estimating spatial structure in fungal communities, which allowed us to estimate the independent effects of host plant phylogeny and soil type. We also used null model analysis, testing whether species co-occurrence patterns follow a random distribution, to determine whether negative (segregation) and positive (aggregation) interactions among root-associated fungi may have observable effects on assembly processes.

Material and methods

Description of the study site

All plants were collected from a site in the nature protection area "Oderhänge Mallnow", located in north-eastern Germany approximately 120 km east of Berlin (52.4636°N, 14.4574°E). The site is a dry grassland habitat with over 200 different plant species combining elements of both steppes and more humid habitats, an annual precipitation of approximately 500 mm and a mean annual temperature of 8.7°C (Deutscher Wetterdienst 2010).It can be characterized as Adonido-Brachypodietum or rather Potentillo-Stipetum with *Adonis vernalis* and *Stipa capillata* as character species (Hensen 1997). The area is part of a large (60 km long and up to 20 km wide), formerly glacial region with dry grassland habitats occurring along the Oder river (called "Oderbruch"). The investigated grassland is grazed by sheep twice a year. The plant species were collected within an area of approximately 2 km² characterized by a gradient in soil type from sandy to loamy (see Figure S1). The pH ranged from 5.5 in sandy areas to 8.3 in more loamy areas. The sandy area had a lower water content but a higher C/N ratio in comparison to the loamy soil and within soil-type variance in these parameters is far lower than between soil-type variance. The soil factor exemplified a relatively steep gradient within a small spatial scale. Despite our soil assaying strategy having been conducted at a crude scale, it allowed us to estimate the importance of edaphic properties, in general, in driving fungal community assembly at the relevant spatial scales. The landscape is also variable, with hills and valleys remaining from the final battles of World War II, which may have important consequences for fungal dispersal.

Sampling and DNA preparation

To ensure correct taxonomic identification, we sampled the 25 plant species of the Asteraceae and one species of the Campanulaceae (to serve as a reference taxon outside the Asteraceae; Tab. 1) during their flowering periods in either May, July or September 2010. We have chosen the Asteraceae because they are a large and diverse family of plants, often exhibiting high local diversity, making this an ideal group to target the questions that we address here. The family is at least 38 Million years (Myr) old, but the fossil record suggests that it may be much older (43–53 Myr) (Bremer & Gustafsson 1997).The distributions of the different plant species are very patchy and three specimens of each species, randomly selected in an area of approximately 10 m^2 and containing a single soil type, were sampled at a given time. Our intention was to address whether fungal community similarity was linked to the degree that host plants shared a common evolutionary history, which required us to establish a gradient of phylogenetic distances by sampling a large number of species. The important level of replication here was in the number of species, not the number of individuals within a species; replication within a species was performed as a means to determine reproducibility of host-associated communities.

Table 1: List of sampled plant species

Plant species	Family/Subfamily
Achillea pannonica Scheele	Asteraceae/ Asteroideae
Anthemis tinctoria L.	Asteraceae/ Asteroideae
Artemisia campestris L.	Asteraceae/ Asteroideae
Aster linosyris (L.) Bernh.	Asteraceae/ Asteroideae
Bellis perennis L.	Asteraceae/ Asteroideae
Conyza Canadensis (L.) Cronquist	Asteraceae/ Asteroideae
Erigeron muralis Lapeyr.	Asteraceae/ Asteroideae
Helianthus tuberosus L.	Asteraceae/ Asteroideae
Helichrysum arenarium (L.) Moench	Asteraceae/ Asteroideae
Leucanthemum vulgare Lam.	Asteraceae/ Asteroideae
Senecio jacobea L.	Asteraceae/ Asteroideae
Senecio vernalis Waldst. & Kit.	Asteraceae/ Asteroideae
Solidago virgaurea L.	Asteraceae/ Asteroideae
Carlina vulgaris L.s.str.	Asteraceae/ Carduoideae
Centaurea jacea L.s.l.	Asteraceae/ Carduoideae
Centaurea scabiosa L.s.l.	Asteraceae/ Carduoideae
Centaurea stoebe L.s.l.	Asteraceae/ Carduoideae
Chondrilla juncea L.	Asteraceae/ Cichorioideae
Cichorium intybus L.	Asteraceae/ Cichorioideae
Hieracium pilosella L.	Asteraceae/ Cichorioideae
Hieracium umbellatum L.	Asteraceae/ Cichorioideae
Hypochaeris radicata L.	Asteraceae/ Cichorioideae
Leontodon hispidus L.	Asteraceae/ Cichorioideae
Picris hieracioides L.	Asteraceae/ Cichorioideae
Taraxacum sp. F.H. Wigg.	Asteraceae/ Cichorioideae
Campanula rotundifolia L.	Campanulaceae

Whole plants were excavated, taking care that the root system was kept as intact as possible, and for each individual plant, the geographical location and the local soil type were recorded. After excavation, the roots were immediately cooled in the field and afterwards stored at -20 °C until further processing. Only plant species for which a permit was obtained were sampled. Roots were gently washed with deionized water to remove adhering soil particles. We did not surface-sterilize the root systems since we were concerned that interspecific variation in root anatomy could result in bias in the efficacy of the procedure. We acknowledge that rhizosphere-associated fungi (in contrast to those directly interacting with the roots) may have been sequenced but argue that these taxa are much less abundant relative to those directly associated with the roots and unlikely to be represented in the resampled communities included in analyses (see below). Roots were cut into 0.5 cm pieces, randomly chosen and directly filled in the 2 ml bead tubes (approx. 50-100 mg) provided as part of the PowerSoil DNA kit (Mobio, Carlsbad, California, USA) without any further processing. The DNA was extracted from these root tissues using this kit following the manufacturer's instructions except for an initial incubation at 65 °C for 10 min followed by a 10 min vortexing step. A soil DNA isolation kit was preferred to a plant tissue DNA isolation kit as it provides DNA of higher purity although it yields a lower DNA concentration (data not shown).

For pyrosequencing, triplicate PCR amplifications of the fungal internal transcribed spacer (ITS) region covering ITS1, 5.8s rDNA and ITS2 were performed for each sampled individual in 50 µL reactions, each containing 5 ng template DNA, 50 µM of each desoxynucleotide triphosphate (dNTP), 200 nM of each of the forward (ITS1F; Gardes & Bruns 1993) and the reverse primer (ITS4; White *et al.,* 1990) and 0.5 U *Taq* DNA polymerase (1000 U; Fermentas, St. Leon-Rot, Germany) in 1x PCR buffer (GenTherm, Rapidozym, Berlin, Germany). The PCR temperature profile consisted of an initial denaturation at 94°C for 2 min 30 s, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, and a final extension at 72°C for 10 min. PCR products were examined by agarose gel electrophoresis and quantified using a Nano Photometer (Implen, München, Germany). The PCR products were diluted to the same concentration and pooled per individual. The pooled PCR products were purified to remove the non-incorporated ITS primers of the first PCR which would compete with the longer primers used for the tagging step, using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany).

Amplicon libraries for pyrosequencing were prepared in a subsequent PCR amplification using the purified PCR products as templates. These PCRs were performed under the same conditions as described above, except the ITS1F primer was extended with a Roche 454 A pyrosequencing adapter and an error-correcting barcode sequence (Hamady et al. 2008), and the ITS4 primer was extended with a Roche 454 B sequencing adapter (see Table S1). In total we had 78 samples, split across three partitioned sections (each section representing 1/8th of the plate) in one pyrosequencing run. One sample from each species was included in each of the three sections, requiring the use of 26 different barcodes.

The PCR temperature profile consisted of an initial denaturation at 94°C for 2 min for 30 s, followed by 10 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, and a final extension at 72°C for 10 min. PCR products were examined by agarose gel electrophoresis and quantified using a Nano Photometer and purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). Equimolar pools of the pooled PCR products stemming from each of the three replicates of each plant species were made and sequencing was performed by the Genome Sequencing & Analysis Core Resource at Duke University (Durham, NC, USA) on a Roche FLX 454 pyrosequencing instrument.

Data analysis

Generating the phylogenetic tree for the host plants

A two-loci phylogeny was reconstructed for the host plant species and two outgroup species (*Campanula rotundifolia* and *Calycera* spec.) using DNA sequences of the plastid maturase K (*matK*) spacer region and the nuclear *ITS* region retrieved from GenBank (see Table S2 for accession numbers). Sequences not available in GenBank were replaced with available sequences of congeneric species (see e.g. Cadotte *et al.* 2008). The sequences were aligned with MAFFT v. 6 (Katoh *et al.* 2002) and species trees were estimated with BEAST v.1.6.1 (Drummond & Rambaut 2007) assuming a GTR+I+G model of nucleotide substitution. Trees were sampled every 1000 generations for a total of 10 million generations and the maximum clade-credibility tree with node heights based on sample averages, generated from the final 9001 sampled trees, was calculated. Due to missing intrageneric sequence information, branch lengths within the genera *Senecio* and *Centaurea* were standardized to branch lengths within *Hieracium* where intrageneric information was available. In order to assess the effects of this adjustment, subsequent analyses were performed with a set of 100 trees containing randomized intrageneric branch lengths for the three *Centaurea* species (see Figure S2 for the phylogenetic tree).

Fungal diversity

To reduce sequencing errors, e.g. due to the presence of ambiguous bases or homopolymers, sequences were denoised using the PyroNoise algorithm as implemented in Mothur v. 1.25.1 (Schloss *et al.* 2009). Subsequently, reads without a valid barcode or primer sequence were excluded and the ITS1 region was extracted from the remaining raw sequence reads using the FungalITSextractor Perl script (Nilsson *et al.* 2009), which only considers reads with complete ITS1 regions.

Operational taxonomic units (OTUs) were generated using Bayesian clustering as implemented in CROP v. 1.33, which generates OTUs without using a hard cut-off regarding sequence similarity for species discrimination thus reducing common overestimation of the number of OTUs (Hao *et al.* 2011). Briefly, the method splits all sequences into blocks and a center sequence is identified that characterizes the cluster. Pairwise distances among sequences are then calculated; the probability that a sequence belongs to a cluster is a function of its distance to the center sequence. The process is repeated after pooling/splitting clusters into separate blocks in a Markov Chain Monte Carlo chain (here, 10,000 iterations). More detail is provided in the original publication. We also clustered DNA sequences using CD-HIT (Huang *et al.* 2010) to validate the results of the algorithm implemented in CROP. CD-HIT uses a greedy heuristic clustering algorithm that is fast (ideal for this purpose). The number of clusters predicted using CD-HIT and the CROP algorithm were very different (details; Table S5); therefore, we used the CROP output in subsequent analyses. The number of reads that were obtained per sample varied widely. Because measures of diversity are highly dependent on sample size (e.g. Smith & van Belle 1984), we performed a procedure to assess sample-based richness and abundance by standardizing each sample to 1000 reads using the bootstrapping method (Efron 1979), which provides a conservative approach to control for inconsistencies in the number of reads. In brief, 1000 subsamples of 1000 reads were obtained with replacement from each plant sample and fungal community OTU richness was assessed for each subsample. The mean across all 1000 subsamples was then assessed (the distribution of richness estimates did not deviate from normality) and was rounded to the closest integer. Subsequently, OTU information on relative number of sequence reads for the most abundant taxa (aggregating to the richness estimate obtained from the bootstrapping procedure; when two sequences were equally represented then ranking was based on a randomization procedure) were extrapolated to sum up to 1000 reads. Singletons were filtered out during this re-sampling procedure. All further analyses were done on the re-sampled dataset.

Taxonomic identities of the OTUs were obtained by comparing their representative sequences with all fungal ITS1 sequences in the GenBank nucleotide sequence database (as of January 2013) using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1997). Sequences belonging to fungal species in GenBank are often not confirmed by morphological characteristics and many identifications may be erroneous; however, this imperfect approach is currently the best means of assigning tentative identities to DNA sequences (Porter & Golding 2011) and curated databases of ITS are only available for specific groups of fungi (i.e. UNITE (http://unite.ut.ee/index.php) for ectomycorrhizal fungi). Subsequent analyses of the BLAST results to assign OTUs at phylum or order level were performed using the software package MEGAN v. 4 (Huson *et al.* 2007). OTUs not of fungal origin were excluded.

All subsequent analyses were done in R v. 2.14.1 (R Development Core Team 2011). First, we calculated rarefaction curves (function *rarefaction* for package “vegan”, Oksanen *et al.* 2009) for the different individuals of each plant species to gauge adequacy of sampling depth. To examine the distribution of fungal OTUs among the three replicates of one plant species, we generated a dendrogram after performing cluster analysis based on Canberra distances.

Variance partitioning and null model analysis

The spatial positions of the plant individuals were used to run Principal Coordinate Analysis of the

Neighborhood Matrix (PCNM; Borcard *et al.* 2002, 2004). The resulting eigenvectors account for the multivariate spatial autocorrelation of our plant individuals at all spatial scales that could be resolved by the sampling strategy (Borcard & Legendre 2002, Desdevises *et al.* 2003). This was done to separate the plant individuals spatially and test for the influence of space on fungal community composition. Space is a proxy for all the factors that are spatially structured and that determine the spatial pattern in species distributions but have not been measured explicitly.

To determine to effect of host plant phylogeny, we first generated a phylogenetic distance matrix of the phylogenetic tree of the Asteraceae. This distance matrix was used to calculate a Constrained Analysis of Principal Coordinates to estimate phylogenetic inertia (Desdevises *et al.* 2003). The principal coordinates represent the phylogenetic relationship among the terminal taxa at all levels. Coordinate axes that were determined to be significant predictors of fungal community composition using the forward.sel function in R (package “packfor”) were extracted and included in the variance partitioning as one variance component (Desdevises *et al.* 2003). Variance partitioning was done using redundancy analysis (RDA), with the re-sampled fungal community matrix as response variable and three explanatory matrices: the PCNM eigenvectors (‘spatial effect’), the significant coordinate axis of plant phylogeny (‘host phylogeny’), and the two-level categorical factor (sand/loam) of the soil (‘soil’). We also tested for the effect of the three different sampling times (that we needed because of different flowering time) and sampling time was included as fourth factor in the variance partitioning. The variance partitioning accounted for the variance explained by each of our predictors separately but also for their combined effects.

Constrained RDA followed by a pseudo-*F* test was used to assess the significance of the explanatory variables. Calculations concerning the variance partitioning were also done in package “vegan”.

To specifically address the importance of biotic interactions, which are not adequately assessed through variance partitioning (e.g. Smith & Lundholm 2010), we conducted a null model analysis

(Gotelli 2000; Gotelli & Ulrich 2010) to test for species co-occurrence patterns. We used the C-scores (a checkerboard index assessing the extent of species segregation/ aggregation, compared to random species distribution, Stone & Roberts 1990) to measure the extent to which fungal species co-occur. The higher the index the more species are negatively associated with each other whereas a low index indicates positive interaction. To create null distributions of the C-score we used an algorithm equivalent to SIM 9 in Gotelli (2000), which is a randomization algorithm where both row and column sums are fixed. This specific algorithm is considered to be ideal for testing for patterns of co-occurrence arising from species interactions. The analysis was implemented in R using the package "bipartite" (Dormann *et al.* 2009) and the function *oecosimu* in the package "vegan" and 5,000 random fungal community matrices for comparing the central tendency of null distribution to the observed C-scores.

Results

Sequence recovery

A total of 158,721 sequence reads met our quality criteria across all host plants and from these, 156,816 ITS1 sequences, with a length varying between 101 base pairs (bp) and 530 bp (median of 170 bp), were used for further analysis. Two plant individuals, one from *Carlina vulgaris* and one from *Hypochaeris radicata,* resulted in only a single sequence and were therefore excluded from our analyses. Excluding these individuals, the number of ITS1 sequences varied from 7,060 and 52 (median of 1,615) per individual plant.

The Bayesian OTU clustering resulted in a total of 1,793 OTUs, of which 811 OTUs were singletons. After resampling, 1,100 non-singleton OTUs remained; note that these numbers do not add up since some of the singletons from the original dataset might be picked up twice during resampling, with replacement, and are then no longer singletons.

Of these 1,100 OTUs, 966 OTUs were confirmed to be of fungal origin (identification is based on

MEGAN v. 4) and used in subsequent analyses. Each plant species on average hosted 65 OTUs in their roots, but variation was high (standard deviation: 19.08). Per individual, the highest number of OTUs was found in the roots of one *Hieracium pilosella* (114 OTUs) and one *Taraxacum* sp. (107 OTUs) whereas the lowest number of OTUs was recorded from one *Helianthus tuberosus* (18 OTUs) (Table S3).

Rarefaction curves approached saturation for most individuals (Figures S4-S5).

Identification of the main groups of fungi

The taxonomic identification with MEGAN v. 4 found 87.8 % of the OTUs to be of fungal origin. The remaining 12.2 % of all OTUs (134 OTUs) (Fig. 1) could not be assigned to a known organism group. Most of the fungal OTUs belonged to the phylum Ascomycota (394 OTU, 35.8 % of all OTUs) with the most abundant sequence matching *Phialophora mustea* (Table S6) and the Basidiomycota (201 OTUs, 18.3 % of all OTUs) with *Sebacina* as most abundant genus (Table S6). We also found 7.2 % of the OTUs (79 OTUs) belonging to Glomeromycota (Fig. 1), with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* as one of the 50 most abundant OTUs.

Chytridiomycota (15 OTUs) accounted for 1.4 % of OTUs (Fig. 1). About 25.2% of the OTUs (277 OTUs) could be assigned to fungi but not to a certain phylum and are classified in Figure 1 as “unassigned fungal OTUs”.

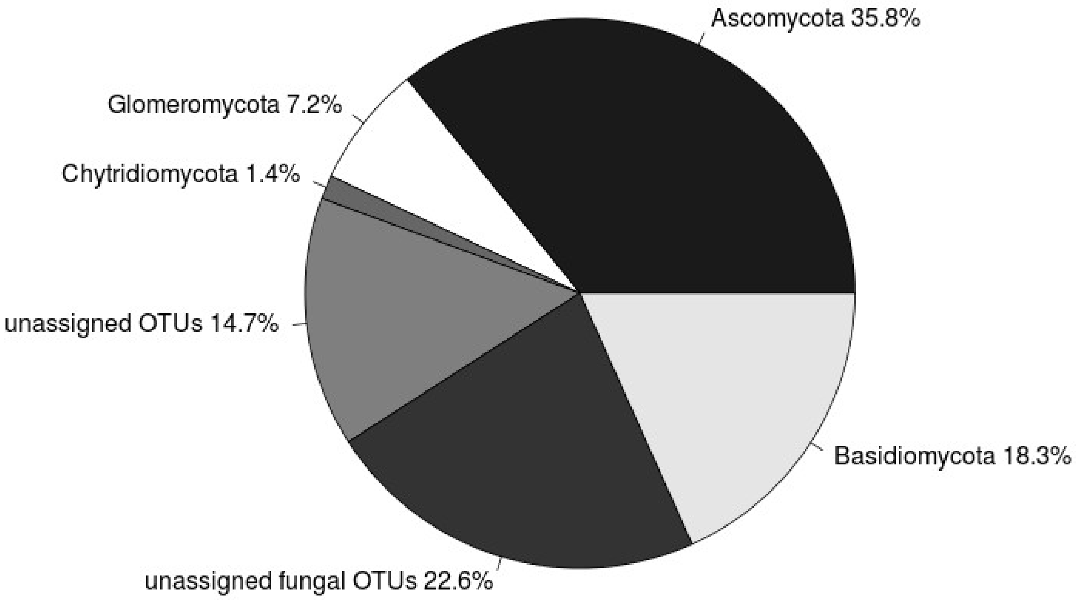

Fig. 1: Relative abundance of fungal phyla in the OTU taxa definitions that were delineated with CROP

Effects of species identity, spatial structure, host plant phylogeny and soil on fungal communities and biotic interactions

The cluster analysis gives a broad overview of variation in fungal community composition among the different plant species (Fig. 2). Variation in fungal community composition is generally associated with plant species identity; for some plant species all three individuals appeared in a single cluster (e.g. *Hieracium pilosella*, *Leucanthemum vulgare*, *Anthemis tinctoria*), while only a few plant species displayed large intra-specific variation and did not cluster (e.g. *Achillea pannonica*, *Bellis perennis*). In most cases at least two individuals appeared in a single cluster (e.g. *Solidago virgaurea*, *Centaurea jacea*).

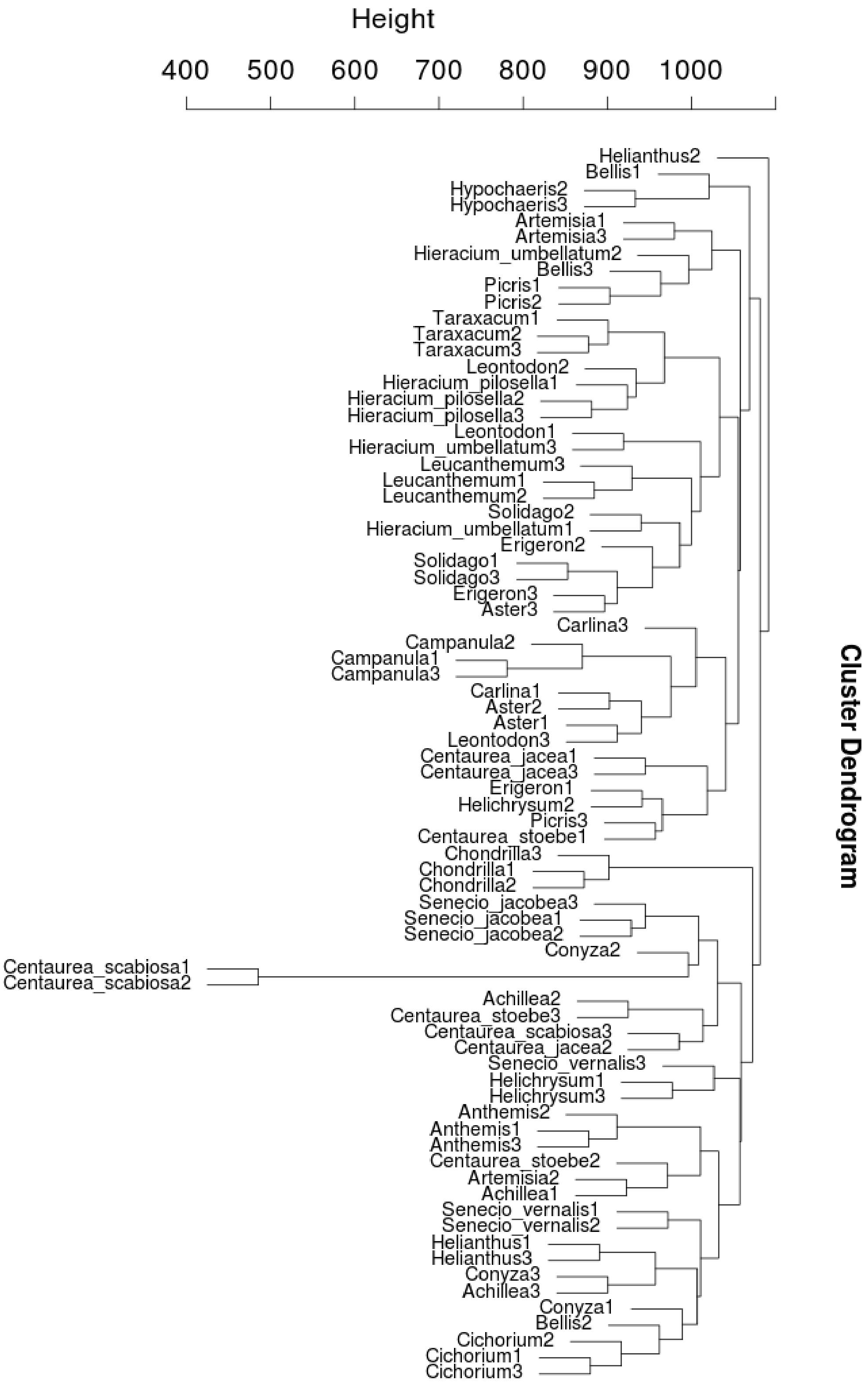

Fig. 2: Canberra distance based cluster dendrogram of ITS fungal communities as these were sampled from the replicated (three replicates) host plants.

To separate the direct contribution of these host plant effects from other drivers of fungal community composition, we partitioned variance in fungal communities to 'spatial effect', ‘host phylogeny', and ‘soil type’ components. Each class of variables accounted for a significant proportion of the multivariate variance in OTU distributions (constrained RDA followed by pseudo-*F* test; $p < 0.005$). The three main predictors together explained 30 % of the variance in fungal community composition (Table 2), with ‘host phylogeny' (20 %) explaining most, followed by space with 9 % and soil type with 1 %.

Table 2: Results of the variance partitioning showing the explained variance of 'spatial effect', 'host phylogeny','soil type' and 'season'. The analysis was done on all fungal OTUs and subgroups (79 OTUs of Glomeromycota, 390 OTUs of Ascomycota, 201 OTUs of Basidiomycota) defined by MEGAN v. 4, transformed by Hellinger transformation.

	All fungi	**Ascomycota** (adjusted R^2 of the individual fractions)	**Basidiomycota** (adjusted R^2 of the individual fractions)	**Glomeromycota** (adjusted R^2 of the individual fractions)
Spatial effect	0.09	0.07	0.08	0.06
Host phylogeny	0.20	0.15	0.07	0.03
Soil type	0.01	0.002	0.01	0.00
Seasonality	0.01	0.002	0.01	0.00

Sampling time, which exhibited some co-linearity with host plant phylogeny, accounted for 1 % of the explained variance, suggesting that compositional differences were driven by host plant phylogeny (which still explained 20 % of the variance) and that these effects were not strongly confounded by sampling time. We plotted fungal community composition on the phylogeny of the Asteraceae to visualize the main pattern: closely-related plant species share similar fungal communities (Fig. 3).

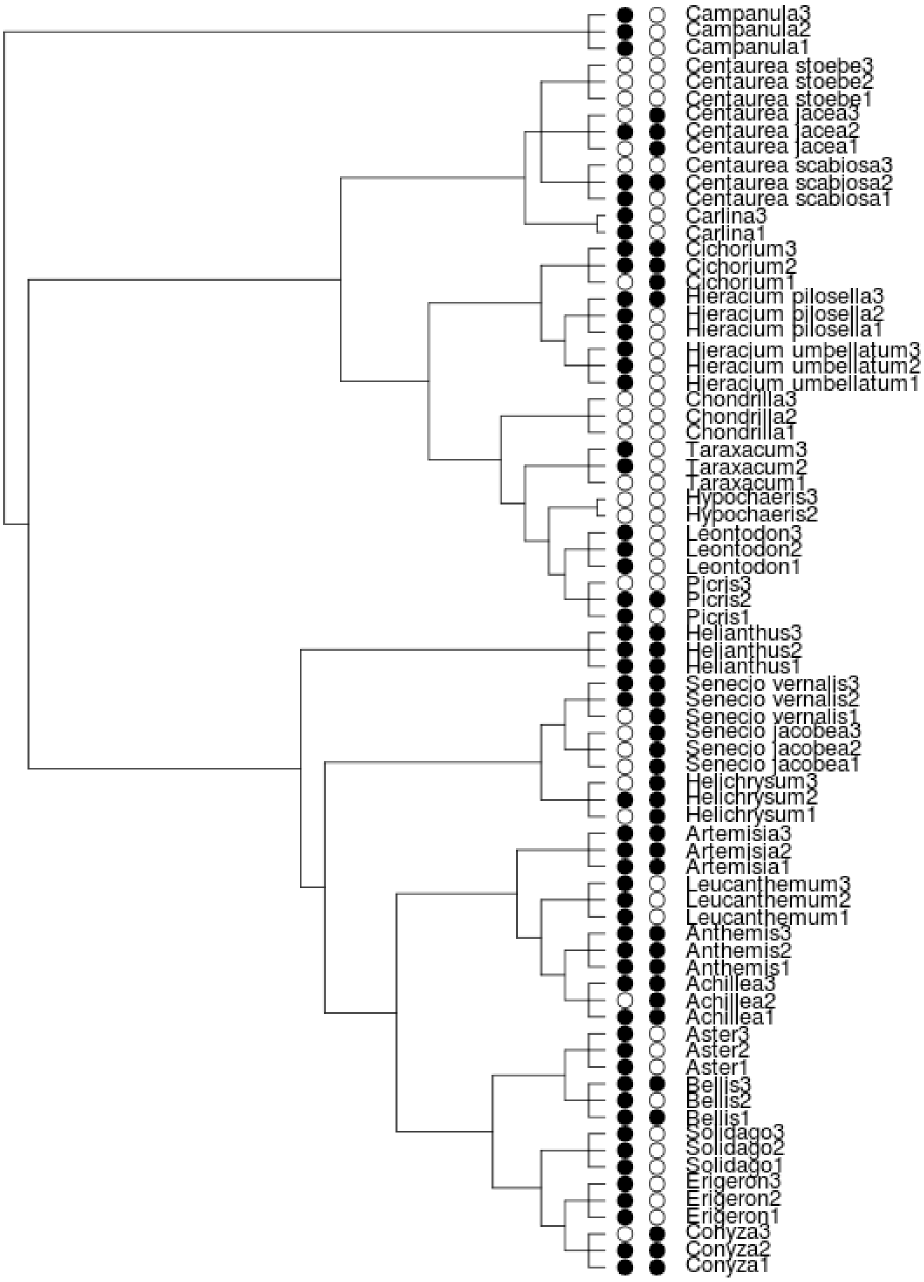

Fig. 3: Phylogenetic comparative analysis of fungal community composition metrics of the plant hosts. The phylogenetic tree is a reconstruction of the evolutionary history of the Asteraceae based on the ITS and matk region. The two metrics (white and black bullets) of the fungal community are the axes loadings of the first and second nonmetric multidimensional scaling (NMDS) axes, respectively, following ordination analysis that was applied to the entire fungal community. For NMDS1, the black and white dots represent NMDS loadings between -0.51 and 0.13 and between 0.13 and 0.75, respectively. For NMDS2, the black and white dots represent NMDS loadings between -0.51 and -0.02 and between -0.02 and 0.45, respectively.

The null model analysis yielded a significantly lower C-score than expected by chance (positive effect size; Table 3): this implies species segregation (Gotelli 2000), i.e. most pairs of species co-occur less often than expected by chance.

Table 3: Results of the null model analysis on the presence/absence matrix of total fungal community composition and the Glomero-, Asco- and Basidiomycota subset per plant individual. The analysis was done on the complete re-sampled dataset (76 individuals and 1100 fungal OTUs). The P-value indicates more observed checkerboards than expected suggesting negative species co-occurrences.

	Observed C-score	**Effect size**	**Mean of the expected C-score**	**P-value**
Fungi vs. plant individual	9.85	3.50	9.78	**0.00099**
Glomeromycota vs. plant individual	12.171	3.93	11.641	**0.00099**
Ascomycota vs. plant individual	14.11	5.876	13.893	**0.00099**
Basidiomycota vs. plant individual	7.217	0.28471	7.2050	0.788

Looking only at the Ascomycota subset we found a similar pattern: factors explaining the most were host plant phylogeny (15 %) and space (7 %). The other two predictors explained less than 1 % of the variance (Table 2). For the Basidiomycota and Glomeromycota subset we could not find the same pattern as for all fungi together (Table 2). In the null model analysis of the subsets, for Ascomycota and Glomeromycota OTUs demonstrated segregation, but a neutral pattern was observed for the Basidiomycota.

Discussion

Alpha diversity

It is common for 454 sequencing studies to report considerably higher diversity than cloning and sequencing studies (e.g. Öpik *et al.* 2009; Becklin *et al.* 2012). Our 156,816 sequences resulted in a large number of OTUs, considering that we focused on root-associated fungi of plants in a single plant family (the Asteraceae) in a small area. We attribute this to the high plant diversity in the investigated area, which is a biodiversity hotspot in Germany with about 300 different plant species. The grassland is quite heterogeneous consisting of small hills with steep slopes and interspersed woodland stands, which may provide a range of different micro-habitats for fungal colonization.

The number of sequence reads varied strongly between the different individuals. This variability could be caused by errors during the amplification or during sequencing. For example, a fungal community on a certain plant individual, which has an unequal distribution of short and long ITS fragments could introduce amplification errors during PCR reactions, since shorter DNA fragments are preferentially amplified (Bellemain *et al.* 2010). Another potential factor causing this variation could be the barcodes used (Hamady *et al.* 2008). However, we used a re-sampling procedure to correct for these differences in our analysis.

The plant species with the highest number of OTUs was *Hieracium pilosella,* while the species with the lowest number was *Helianthus tuberosus*. The low number of OTUs in *Helianthus* might be due to its root system, a tuber-forming root with a relatively coarse architecture, compared to the highly branched root system of *Hieracium*, which might therefore be more susceptible to fungal infection (Newsham *et al.* 1995).

Taxonomic composition of the fungal communities

Most of the OTUs (35.8 %) belonged to the Ascomycota, which are commonly found to be the dominant root-colonizing fungal group in semiarid grasslands (Porras-Alfaro *et al.* 2011). The most abundant OTU in our sampling area was identified as closely related to *Phialophora mustea*, the function of which is not well understood. *Phialophora* species are known to form a complex group of fungi with endophytes, saprobes and plant pathogens (Yan *et al.* 1995, Ko *et al.* 2011). The second most abundant fungus was identified as related to *Paraphoma chrysanthemicola,* section *Phoma* (de Gruyter *et al.* 2010), a common dark septate endophyte in grasslands (Porras-Alfaro *et al.* 2011). The roles of these endophytes in ecosystems still remain unclear but under controlled condition they can enhance plant performance (Newsham *et al.* 2011).

Surprisingly, we also found a relatively large number of Basidiomycota (18.3 %), which are typically more frequent in forest soils (Buée *et al.* 2009). The relative abundance we report is almost double the relative abundance that Porras-Alfaro *et al.* (2011) retrieved in their study, conducted in a semiarid grassland. The most abundant genus of Basidiomycota and the third most abundant overall was *Sebacina* (Order Sebacinales). Sebacinalean fungi are common endophytes in many plant roots (Selosse *et al.* 2009, Weiss *et al.* 2011) and may enhance plant growth and pathogen protection (Schäfer *et al.* 2007). The AM fungus *Rhizophagus irregularis* (Phylum Glomeromycota) was relatively abundant, and among the 50 most abundant sequences. In total, Glomeromycota accounted for 7.2 % of all OTUs. Glomeromycota are widespread and dominant in grasslands, and Asteraceae generally form symbioses with them (Hempel *et al*. 2013).

We also found that 22.6 % of OTUs were from fungi of uncertain identity, which likely reflects the fact that only a low percentage of fungi have been formally described (Mueller & Schmit 2007).

Predictors explaining community composition

The multivariate distribution of fungal species, which is an aspect of beta-diversity (Legendre *et al.* 2005), could be explained to a certain extent by space, phylogeny, soil type and sampling time; all four predictors had a significant influence on the fungal communities.

However, the most important factor explaining total fungal community composition was 'host phylogeny', accounting for 20 % of the variance and suggesting that closely related species shared more similar fungal communities than expected by chance (i.e., root-associated fungal communities exhibited conservatism across the host phylogeny). The cluster analysis showed that fungal community composition tended to be more similar among individuals of the same plant species. Such a relationship between host identity/ phylogeny and associated fungi has been observed for plant pathogenic fungi, like the anther smut fungi and their caryophyllaceous hosts (Refrégier *et al.* 2008) and necrotrophic leaf fungi (Gilbert *et al.* 2007). Similar relationships have also been observed for mycorrhizal fungal associations in *Orchis* (Jacquemyn *et al.* 2011) and ectomycorrhizal fungi (Tedersoo *et al.* 2013).

Fungal colonization of host plants is likely to be limited or facilitated by certain plant traits, which may be shared among closely related plant species. For example, root traits linked to the architecture of the root system (e.g. root length, root diameter, ratio of fine and coarse roots) and foraging behavior, which exhibit phylogenetic constraints (Kembel & Cahill 2005), are likely to influence fungal colonization due to variation in surface area-to-volume ratios and the frequency of infection points (Newsham *et al.* 1995). For example, very dense root systems with many fine roots might be more susceptible to fungal infections compared to less branched, thick roots (Newsham *et al.* 1995).

On the other hand, this phylogenetic signal in fungal community composition could be simply due to patterns of co-occurrence, with fungi and their hosts limited by the same or co-varying environmental conditions. However, our results suggest this is not the case since 20 % of the variation accounted for phylogeny was independent of the other predictors. Furthermore, in a recent study Tedersoo *et al.* (2013) found similar effects of host plant phylogeny on fungal community composition within the Salicaceae, which indicates that our pattern might be independent of the investigated system.

The second most important factor for structuring the fungal communities was space, which could be due to a strong fragmentation of the landscape, dispersal dynamics, or an interaction between these dynamics and environmental drivers not accounted for by our soil factor. The alternation of hills and valleys might limit dispersal and gene flow between the communities on the roots of the different host plants. For example, for AM fungi, habitat filtering and dispersal limitation are both drivers of assembly processes (Kivlin *et al.* 2011; Caruso *et al.* 2012), but the total amount of variation in species turnover explained by environmental parameters (edaphic variables, host species, etc.) may be small due to broad tolerances in some taxa (Powell *et al. 2*011).

Despite the statistical significance of differences in soil type we only detected comparably small effects of soil type. This may imply generally weak niche differentiation with respect to this factor or be due to the coarse categorical factor used to represent soil type (i.e., unmeasured edaphic variables may account for more variation). Edaphic variables are likely to be spatially structured and we contend that this variation, if important, would be accounted for by the spatial component. Our results are in line with those of others who have observed that soil type and other soil chemical properties have a small but significant influence on fungal community composition (e.g., Verbruggen *et al.* 2012 for AM fungi).

By looking only at fungal subsets we found Ascomycota showing the same strong phylogenetic pattern as total fungi, whereas for Glomeromycota and Basidiomycota the spatial effect is more important than host plant phylogeny. Thus, our subgroups contributed differently to our variance partitioning pattern: Ascomycota mostly determined the large variance accounted for by host plant phylogeny while Basidiomycota determined the fraction of variance accounted for soil type. All subgroups contributed more or less equally to the variance explained by space. The results of the subgroups have to be carefully interpreted because of potential differences in sampling depth among the subgroups. Glomeromycota for example represent only 7.2 % of all OTUs which may not be a representative sampling amount. The same might be true for Basidiomycota. Another aspect could be a real difference in host specificity, which is especially known for Glomeromycota (Smith & Read 2008). Studies on host specificity of Basidiomycota mostly addressed ectomycorrhizal fungi, which we may not have here.

Spatial position and soil type may be confounded by species identity because species usually were sampled in the same soil type and area. Because we were limited to sampling plants in the areas where they could be found and as their distributions were patchy, there was a degree of non-independence among the factors we tested. That said, this had little impact on our analysis as the variation was generally attributed to individual components and not to the dimensions in which multiple components overlapped. Our results are therefore conservative as they accounted for possible sources of non-independence among the tested factors. Indeed, this is a strength of our study since we are studying these patterns under the natural conditions in which they occur.

It was not the aim of our study to test for effects of species identity on fungal community composition. However, we tested for this effect and results suggested that host species identity is a relatively poor predictor of fungal community relative to host plant phylogeny as it explains a similar proportion of variation but with substantially more costs associated with degrees of freedom (data not shown).

All in all, we could explain 30 % of the variance with the three predictor variables (when correcting for co-linearity in sampling time), which indicates that assembly processes in root-associated fungal communities are predictable to a certain extent, at least in our system. Additional sources of variation could include, for example, neighbor effects or stochastic colonization followed by priority effects (Hausmann & Hawkes 2009; Mummey *et al.* 2009; Dickie *et al.* 2012) or biotic interactions among the fungal species (Pan & May 2009). It was clear from the null model analysis of species co-occurrence that the root fungal communities (especially Ascomycota and Glomeromycota) were not random assemblages but demonstrated strong spatial segregation. This latter result suggests that negative interactions among fungi led to limiting similarity in their composition (e.g. Gotelli 2000), and indicate competition among fungi. Next to environmental filtering, which allows only certain fungi to persist (as shown in variance partitioning), these negative interaction could be an additional explanation for the pattern of community composition found in our study. These processes likely combine to contribute to the coexistence of similar species (Shigesada *et al.* 1979), and may thus help explain the observed level of fungal diversity in our system. Analysis of the Basidiomycota, however, suggested that interactions among these species had negligible effects on community assembly. Basidiomycota communities may have, therefore, been driven by environmental factors, but variance explained by our environmental factors was low. Further investigations might be necessary to explain the factors driving

Basidiomycota community composition.

Conclusion

Our sequence dataset enabled analysis of the impact of host phylogeny, space and soil on root-associated fungal community composition. The variance explained by the predictors is quite high and significant. Therefore, we can conclude that the fungal community assembly in this system is predictable to a certain extent. However, other factors, e.g. community-level interactions among plant species, interactions between root-associated fungi and other soil microbes, fine-scale soil heterogeneity, and stochastic colonization and extinction events, may be also important. We found patterns of species co-occurrence consistent with the hypothesis that negative interactions might prevail in the assembly of root-inhabiting fungal assemblages. This limiting similarity, in combination with environmental filtering, is likely to be an important reason for the high level of diversity observed in this semi-arid grassland.

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Data accessibility

Raw sequence data is submitted to the European Nucleotide Archive (ENA) XXXX.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 List of primers with error-correcting barcode sequences for the respective samples

Table S2 Accession numbers of sequences of the different taxa used for generating the tree of the host plant species

Table S3 Number of sequence reads and OTUs among the different individuals

Table S4 Sample characterization

Table S5 Results of the OTU clustering with CD-HIT

Table S6 Best BLAST hits for the representative sequences of the 50 most abundant OTUs

Figure S1 Map of the investigation area

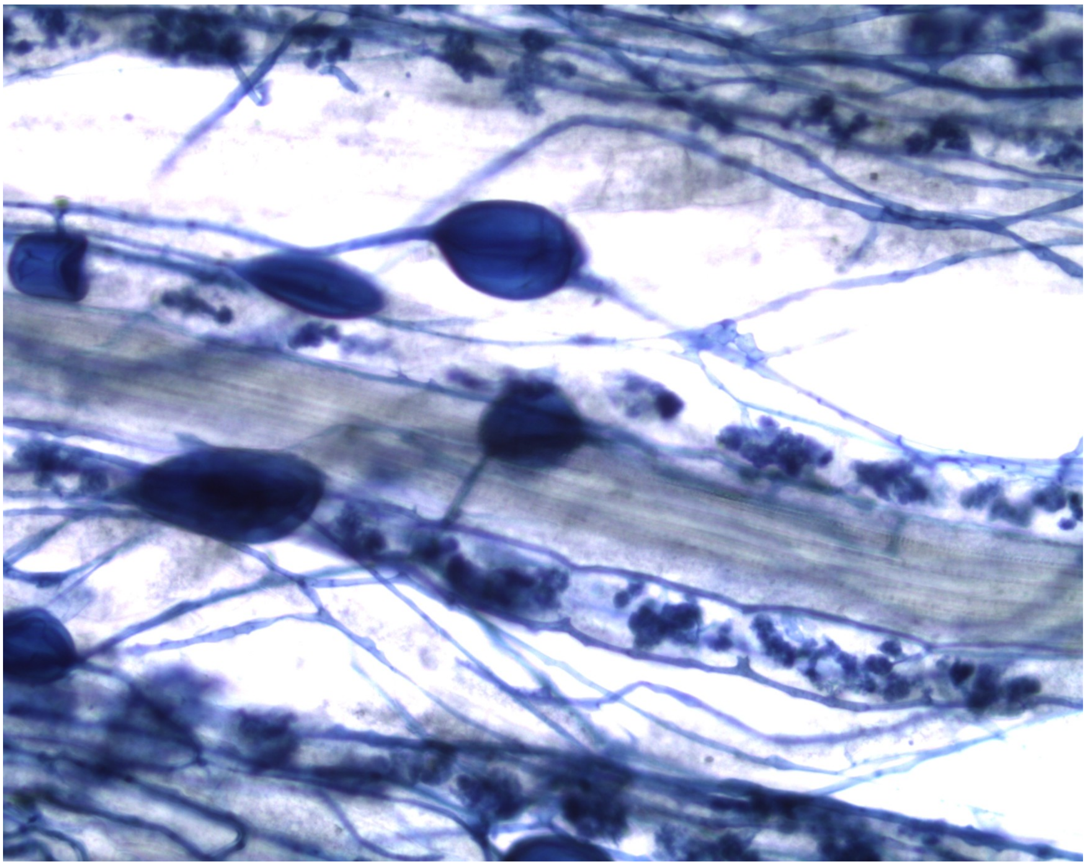
Figure S2 Phylogenetic tree of the Asteraceae

Figure S3 Principal component analysis with plotted confidence intervals for OTU numbers of each individual of the re-sampled dataset

Figure S4-S5 Rarefaction curves of the different plant species

3

Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity?



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Abstract

Arbuscular mycorrhizal (AM) fungi can confer protection to host plants against some root pathogens, and several mechanisms for these phenomena have been proposed. If AM fungal taxa vary in the ways that they limit the negative effects of pathogens on host plants, additive and/or synergistic interactions among members of diverse AM fungal assemblages and communities may result in a greater pathogen protection than is currently predicted. However, in a review of the literature on interactions between AM and pathogenic fungi, we found few examples that compared the effectiveness of single- and multi-species AM fungal assemblages. Here, we briefly recount the generally recognized mechanisms of pathogen protection by AM fungi and present evidence, where appropriate, for functional diversity among AM fungal taxa with regard to these mechanisms. We propose that functional complementarity of AM fungal taxa in interactions with pathogens could mimic, or even be the cause of, previously observed relationships between AM fungal diversity and plant productivity.

Why consider diversity?

The role of below-ground soil organisms interacting with plant roots has gained increased attention in recent years (e.g. Callaway *et al.* 2004, Reynolds *et al.* 2003, van der Putten *et al.* 2003), and the interactions between beneficial and pathogenic organisms have been identified as being particularly relevant due to their important implications for plant fitness (e.g. Bever 2003, Fitter and Garbaye 1994, Schippers *et al.* 1987). Most studies, however, focus on interactions between a single pair of species (i.e. one beneficial and one pathogen) and do not take into consideration the vast microbial diversity within these functional groups that coexist in, on, and near plant roots. This means that the existing body of knowledge is rather unrealistic and not concordant with the emerging view that many communities include guilds of interacting species (Stanton, 2003). This is particularly evident with the arbuscular mycorrhizal (AM) fungi, which are obligate symbionts of a majority of all plant species and common across most terrestrial biomes (Smith and Read 2008). Although not necessarily the primary function of the symbiosis, AM plants have been observed to receive protection from pathogens relative to their nonmycorrhizal counterparts in experimental studies (e.g. Borowicz 2001, Newsham *et al.* 1995a, Filion *et al.* 1999, 2003). Several mechanisms have been proposed to explain how this protection arises (Azcón-Aguilar and Barea 1996, Whipps 2004, Dalpé 2005). Studies suggest that AM fungal taxa vary both in the expression of traits associated with some of these mechanisms (Hart and Reader 2002, Pozo & Azcón-Aguilar 2007) and in their ability to protect host plants against pathogens (Newsham *et al.* 1995b, Klironomos 2000, Maherali and Klironomos 2007, Sikes *et al.* 2009). Therefore, it is plausible that assemblages of AM fungi derived from multiple species may exhibit greater potential to protect host plants against pathogens than a single AM fungal species; this concept could be analogous to (or even contribute to) the positive relationships observed between the richness of AM fungal assemblages and the growth of individual plants or the diversity and productivity of plant communities associating with these assemblages (van der Heijden *et al.* 1998, Maherali & Klironomos 2007).

Most research on AM fungal-mediated pathogen protection, especially studies addressing the mechanisms of this interaction, fails to consider the richness of AM fungal communities in natural systems (Azcón-Aguilar and Barea 1996, Borowicz 2001, Whipps 2004, Dalpé 2005). We propose that it is timely to revisit the mechanisms known to mediate pathogen protection and consider ways through which AM fungal diversity may influence the outcomes of these interactions.

Improved nutrient status of the host plant

It is well known that AM fungi can improve the nutrient status of their host plants (Smith & Read 2008). Several mineral nutrients, especially P, are allocated through the symbiosis to the plant in exchange for carbon (e.g. Pearson & Jakobsen 1993). There is evidence that plants that took up larger amounts of nutrients through their AM fungal symbiont *(*e.g. *Glomus intraradices* and *Glomus mosseae)* have an increased tolerance for pathogenic infections (e.g. Bodker *et al.* 1998, Karagiannidis *et al.* 2002). However, it is not clear whether this increased tolerance was a direct consequence of improved nutrition. In other studies not involving the AM symbiosis, increased nutrient uptake did not result in improved pathogen tolerance (Shaul *et al.* 1999, Fritz *et al.* 2006). Maherali and Klironomos (2007) found taxonomic variation within the AM fungal phylum Glomeromycota, both in terms of nutrient responses and pathogen protection. While members of the Gigasporaceae enhanced nutrient uptake to a larger extent than those of the Glomeraceae, the latter reduced pathogen abundance in roots to a greater extent. This suggests that an enhancement of P by the AM symbiosis may increase plant development, but not decrease pathogen infections (Trotta *et al.* 1996). Accordingly, Newsham *et al.* (1995a) found no effects of AM fungi on the P level of host plants, only pathogen protection against *Fusarium oxysporum*.

Studies unrelated to pathogen protection have shown wide variation in terms of AM fungal mediated nutrient uptake among AM fungal species (Jakobsen *et al.* 1992, van der Heijden *et al.*

1998, Smith *et al.* 2000, van Aarle *et al.* 2002, van der Heijden *et al.* 2003). This can be explained to a certain extent through the variation of traits such as mycelium development both in soil and roots and P uptake efficiency (Jansa *et al.* 2005). Consequently, one can anticipate that increased AM fungal richness may result in improved nutrient supply of their host plants, due to trait variation and complementarity.

If multiple AM fungal species are better able to enhance the nutrient status of a host plant (also for different nutrients, such as N, Ca, and P), compared to a single fungus, host defenses of AM plants may be enhanced under limiting nutrient conditions when plants are weakened and, consequently, more susceptible to pathogen attack.

Competitive interactions with pathogenic fungi

Direct (via interference competition, including chemical interactions) and indirect (via exploitation competition) interactions have been suggested as mechanisms by which AM fungi can reduce the abundance of pathogenic fungi in roots. These have generally been proposed in response to observations of negative correlations in the abundance of AM fungal structures and pathogenic microorganisms in roots and soil (St-Arnaud *et al.* 1994, Bodker *et al.* 2002, Filion *et al.* 2003) or on growth medium (St-Arnaud *et al.* 1995). Presumably, pathogenic and AM fungi exploit common resources within the root, including infection sites, space, and photosynthate within the root (recently reviewed in Whipps 2004). Interference competition may also arise if carbon availability within intercellular spaces and the rhizosphere (Graham 2001) or the number of infection loci within the root system (Vigo *et al.* 2000) is reduced as a result of AM fungal colonization.

Increasing the richness of AM fungal taxa colonizing the root system may result in more intense competition with a pathogenic fungus. AM fungi in multi-species assemblages vary in competitive ability, but the total level of colonization generally does not exceed that of the most abundant fungus when grown in isolation (Abbott and Robson 1981, White 1984, Jansa *et al.*

2008). *Glomus fasciculatus* 92 and *Glomus constrictus* 122, when inoculated in combination, did not increase the frequency of AM fungal root colonization or reduce symptoms of *Phytophthora parasitica* on citrus relative to when each were inoculated in isolation (Davis and Menge 1981). In another study, inoculation with a multi-species AM fungal assemblage from a field soil increased the intensity (and, to a lesser extent, the frequency) of AM fungal colonization of date palm roots, when compared with *Glomus monosporus*, *Glomus clarum*, or *Glomus deserticola* in isolation, but this did not result in enhanced amelioration of the negative effects of *Fusarium oxysporum* f. sp. *albedinis* on plant growth (Jaiti *et al.* 2007). Recent research suggests that if competition between AM and pathogenic fungi is occurring, it is for resources other than just the occupation of space within the root system. The majority of variation in root colonization by AM fungi is explained by the divergence of the two most species-rich fungal clades: the extensively colonizing Glomerales and the poorly colonizing Diversisporales (Hart and Reader 2002, Powell *et al.* 2009). Surveys of the ability of various AM fungal taxa to reduce the abundance of *Fusarium oxysporum* and/or a *Pythium sp*. in host root systems also suggest that variation in this trait is largely constrained to this divergence, with low levels of pathogen abundance in root systems inoculated with various *Glomus* species relative to those inoculated with various *Gigaspora*, *Scutellospora*, and *Acaulospora* species (Powell *et al.* 2009, Sikes *et al.* 2009). Using comparative methods that account for the statistical nonindependence of taxa within these clades, these studies found no evidence that higher frequency of AM fungal colonization within the host root system resulted in a linear reduction in colonization by the pathogenic fungi. This suggests that variation in traits other than the extent of root colonization should be a focus of future surveys. For example, variation exists among AM fungal species in the frequency of infection points within root systems (Liu and Luo 1994), but this has not been subject to widespread, systematic survey. Predicting the contributions of AM fungal assemblages to reduction of pathogen loads will require the identification and characterization of traits that contribute to AM fungal competitive abilities.

Anatomical or architectural changes in the root system

AM fungal colonization influences root architecture of the host plant in most studies by causing a more profusely branched root system (Price 1989, Yano 1996, Paszkowski 2002, Olah *et al.* 2005, Gutjahr *et al.* 2009).

Interactions between changes in the root system and protection of plant roots from pathogen attack have been demonstrated for several AM species. Matsubara *et al.* (1995) have shown that eggplants colonized by *Glomus etunicatum* or *Gigaspora margarita* contained higher lignin concentrations in first order and second order roots compared to non-mycorrhizal plants, when *Verticillium dahliae* was present. In addition, those AM fungi caused the plant to produce thicker third order roots. In contrast to the induced higher branching of the root system, *Glomus mosseae* decreased the root branching of tomato under high P conditions if a plant was attacked by *Phytophthora nicotianae* (Trotta *et al.* 1996). Alternatively, branching of the tomato root system was not affected by inoculation with *Glomus mosseae*, but decreased the number of infection loci of *Phytophthora parasitica* (Vigo *et al.* 2000).

Newsham *et al.* (1995b) proposed that an abundance of lateral root tips and developing meristems make highly branched root systems more susceptible for pathogen attack, resulting in an increasing demand for AM fungi to protect them. Norman *et al.* (1996) could support this hypothesis as they compared plants with inherently highly branched root systems and found that mycorrhizal plants had fewer necroses compared to nonmycorrhizal ones. If mycorrhizal fungi frequently cause increased branching of the roots, but increased branching in itself leads to higher susceptibility to root pathogen attack, AM fungi must confer protection through additional mechanisms.

We found no studies which consider AM fungal diversity for this mechanism, per se. However, variation exists among studies with respect to how AM fungi influence root architecture, suggesting that this may be a promising avenue for further research.

Microbial community changes in the rhizosphere

Different factors (e.g. altered exudation patterns, putative direct AM fungal effects, different root size and architecture, altered physiology) may contribute to quantitative and qualitative microbial community changes in the mycorrhizosphere caused by AM fungi (Andrade 1998, Hodge 2000, Artursson 2006, Toljander 2007, Finlay 2008). Changes due to the influence of single AM fungi on mycorrhizosphere microbial communities may in turn lead directly to the reduction of fungal pathogen populations (Meyer & Linderman 1986, Citernesi *et al.* 1996, Filion *et al.* 1999, Larsen *et al.* 2003). The mechanisms for such direct effects remain for the most part elusive. In a recent study Siasou *et al.* (2009) provide evidence that rhizosphere fluorescent *Pseudomonas* strains, which produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) that confers plant protection against *Gaeumannomyces graminis* var. *tritici*, produced significantly larger amounts of DAPG in the presence of soluble carbon from soil containing *Glomus intraradices*. Even though the majority of studies provide support for this mechanism of pathogen protection (see Whipps 2004), that is not universal. Li *et al.* (2007) found that bacterial strains putatively associated with AM fungi were equally effective at providing pathogen protection as their counterparts isolated from non-mycorrhizal soils.

Studies considering AM fungal diversity in relation to this mechanism are rare (Whipps 2004). Secilia and Bagyaraj (1987) analysed microbial communities associated with different AM fungi and found that actinomycetes antagonistic to the fungal pathogen *Fusarium solani* were more prevalent with certain AM fungal species.

A diverse AM fungal assemblage may promote more diverse assemblages or greater populations of benefical microbes and therefore enhance the tolerance of a host plant to pathogenic infections. However, we found no studies that directly tested this idea.

Activation of plant defense mechanisms

The recognition dialogue established between the host plant and AM fungi even before physical contact, results in specific shifts in the host's gene regulation (Genre *et al.* 2009, Oldroyd *et al.* 2009) with the consequent production of specific multifunctional compounds (i.e. both involved in transduction pathways and capable to confer disease resistance) (Pozo & Azcón-Aguilar 2007, Liu *et al.* 2007, van Wees *et al.* 2008). It is possible that the processes of AM fungal establishment rather than resulting in the constitutive expression of defense, which is costly for the plant, enhance the plant's ability to activate defense mechanisms more efficiently when under attack (Pozo *et al.* 2009). Induced pathogen protection through this mechanism may either be systemic within the plant (e.g. Guillon *et al.* 2002) and/or through root exudation (Lioussanne 2008). Consistent with most other mechanisms of AM fungal mediated plant pathogen protection little is known about the role of AM fungal diversity. Pozo *et al.* (2002) compared *Glomus mosseae* and *Glomus intraradices* in their capacity to induce local and systemic resistance to *Phytophthora parasitica* and found that only the former was effective at reducing disease symptoms through the induction of different hydrolytic enzymes. Liu *et al.* (2007) compared the transcriptional response of *Medicago truncatula* to different AM fungi, including *Gigaspora gigantea*. A core set of genes, including some associated with defense mechanisms, were induced in response to the different AM fungi, suggesting that gene induction specific to a single AM fungus might be rare. One gene predicted to encode a cysteine-rich protein (also named 'defensin' due to anti-fungal activity) was only up-regulated in roots colonized by *G. intraradices*. Pozo *et al.* (2009) compared the response of non-mycorrhizal plants or plants colonized by either *G. mosseae* or *G. intraradices* to the application of different defense-related stimuli in the shoots and found stronger induction of defense-associated genes, particularly in *G. mosseae* colonized plants.

We found no studies which consider AM fungal assemblages of varying richness. The studies discussed above show differences between AM fungi in activating plant defense mechanisms. A diverse AM fungal assemblage might be able to induce more defense-related genes than one fungus alone.

Conclusions

Our discussion above suggests that different AM fungal species may confer protection of host plants from root pathogenic fungi via different mechanisms, although there is sparse evidence to either support or disprove this. There is presently only one report (Maherali and Klironomos, 2007) that compared AM fungal effects on reduced pathogen abundance using a taxonomically diverse set of AM fungal species in the same experimental setting. Even though important taxonomic patterns were uncovered in this study, there was no test for actual richness effects of AM fungi on pathogen root colonization. Testing for these effects and uncovering their mechanistic basis represents the next logical step in this line of research. There may be functional complementarity among AM species in regard to processes leading to pathogen tolerance or resistance, both among and within groups of mechanisms. We would therefore expect that pathogen protection increases with AM fungal diversity if single AM fungal species within AM fungal assemblages provide different benefits (i.e. nutrient uptake, competition with pathogen, lignification of roots etc.). Because plants, AM fungi and pathogens in natural and managed systems likely interact within a diverse community of potential partners and competitors (Kniskern *et al.* 2007; Barrett *et al.* 2007). The results of these interactions may be underestimated by studies considering only single AM fungal species. Species rich AM fungal assemblages have been observed to enhance the diversity and productivity of host plants and communities (van der Heijden *et al.* 1998; Maherali and Klironomos, 2007); we propose that the existence of functionally complementary mechanisms of pathogen protection within these assemblages may be a significant driver of these phenomena.

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4

Indigenous arbuscular mycorrhizal fungal assemblages protect grassland host plants from pathogens

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Abstract

Plant roots can establish associations with neutral, beneficial and pathogenic groups of soil organisms. Although it has been recognized from the study of individual isolates that these associations are individually important for plant growth, little is known about interactions of whole assemblages of beneficial and pathogenic microorganisms associating with plants. We investigated the influence of an interaction between local arbuscular mycorrhizal (AM) fungal and pathogenic/saprobic microbial assemblages on the growth of two different plant species from semi-arid grasslands in NE Germany (Mallnow near Berlin). In a greenhouse experiment each plant species was grown for six months in either sterile soil or in sterile soil with one of three different treatments: 1) an AM fungal spore fraction isolated from field soil from Mallnow; 2) a soil pathogen/saprobe fraction consisting of a microbial community prepared with field soil from Mallnow and; 3) the combined AM fungal and pathogen/ saprobe fractions. While both plant species grew significantly larger in the presence of AM fungi, they responded negatively to the pathogen/saprobe treatment. For both plant species, we found evidence of pathogen protection effects provided by the AM fungal assemblages. These results indicate that interactions between assemblages of beneficial and pathogenic microorganisms can influence the growth of host plants, but that the magnitude of these effects is plant species-specific.

Introduction

Under natural conditions plant roots interact with different soil organisms, which can be beneficial, neutral or pathogenic. The role of these interactions has been increasingly recognized (e.g., Reynolds *et al.* 2003, van der Putten 2003, Callaway *et al.* 2004). Beneficial organisms like arbuscular mycorrhizal (AM) fungi may influence plant community structure, diversity (van der Heijden et al. 1998a, Stein et al. 2009) and productivity (van der Heijden *et al.* 1998b, Klironomos *et al.* 2000) in a natural ecosystem. Pathogenic and parasitic soil organisms, such as viruses, bacteria, insects or fungi, have been also observed to promote plant diversity and significantly determine plant community composition (Gilbert 2002).

AM fungi are the most common symbiotic fungi, associated with many different plant species (Smith & Read 2008) and completely dependent on their host's carbon. In return the plant receives additional nutrients (Marschner & Dell 1994), improved water relations (Auge 2001) and pathogen protection (e.g., Newsham *et al.* 1995a, Filion *et al.* 1999, 2003, Borowicz 2001) from AM fungi. Several mechanisms whereby AM fungi could cause pathogen protection are known, including changes in root architecture, improved nutrient status, activation of plant defense mechanisms, or competition for infection sites (Azcón-Aguilar & Barea 1996). However, most studies on AM fungal mediated pathogen protection were carried out with selected, usually single, isolates of both pathogenic and AM fungi. For instance, Newsham et al. 1995a used one *Glomus* sp. as AM fungal treatment and *Fusarium oxysporum* as pathogen treatment. While the AM fungus provided protection against *Fusarium*, it did not increase the nutrient status of the host plant. In natural ecosystems a mycorrhizal plant species is associated not only with one AM fungus but with an entire AM fungal assemblage (Clapp *et al.* 1995) and, as a consequence, community-level emergent properties in determining the outcomes of AM fungal-pathogen interactions are poorly understood (Wehner *et al.* 2010). For example, AM fungi may display functional complementarity in terms of protecting the host plant from a pathogen (Barrett *et al.* 2009): while one species might provide nutrients to their host, another one might increase the tolerance or resistance against pathogens, both leading to protection from the pathogen. Therefore, it might be important to consider AM fungal assemblages in the context of pathogen protection. However, not only AM diversity can influence pathogen protection, but also the assemblage of pathogens may need to be considered. It is known that host plants are often exposed to several pathogens simultaneously (Barrett *et al.* 2009), but there are only relatively few studies with multiple pathogens approaches (e.g., Klironomos 2002, Bradley *et al.* 2008, Vandemark *et al.* 2010).

Here we investigated the interaction of local AM fungal and soil pathogen/ saprobe assemblages for two different plant species. Building on work on single isolates, we hypothesized that a natural AM fungal assemblage would provide protection against soil pathogen/ saprobe assemblages.

Material and Methods

Ethics statement

All necessary permits were obtained for the described field studies at the nature protection area "Oderhänge Mallnow" from the Landesumweltamt Brandenburg, Referat Arten- und Biotopschutz, Potsdam, Germany.

Field site

The study area from where seeds of both plant species and soil were collected is located in north-eastern Germany, approximately 120 km east of Berlin in the nature protection area “Oderhänge Mallnow” (52.4636°N, 14.4574°E, next to the small village of Mallnow), a Natura 2000 hotspot of biodiversity containing over 200 different plant species combining floral elements of both steppes and oceanic habitats. The site is a dry grassland habitat with *Adonis vernalis* and *Stipa capillata* as character species and part of the Adonido-Brachypodietum or rather Potentillo-Stipetum (Hensen 1997). It is part of a large (60 km long and up to 20 km wide), formerly glacial, region with dry grassland habitats along the Oder river, called “Oderbruch”. The area is the most northerly-situated dry- and summer-warm region in Germany (Hensen 1997) and is characterized through its strongly continental climate with a mean annual precipitation around 500 mm and a mean annual temperature of 8.7°C (Deutscher Wetterdienst 2010).

Soil and inoculum preparation

In June 2008 we collected soil from the grassland site. All the soil was sieved (2 mm), air dried and stored in boxes under cover until the experiment was set to start. A portion of approximately 100 kg was autoclaved (60 min at 121°C) for use as growth substrate for the plants. The soil contained 10.2 mg P kg^{-1}, 30 mg K kg^{-1} and had a pH of 7.0.

For inoculum preparation soil cores (0- 20 cm) were randomly collected at numerous locations (> 20) spread across the entire grassland in January 2009. The AM fungal inoculum represented by a resident spore community was obtained from this field soil by wet sieving and sucrose gradient centrifugation using a method modified from Klironomos (2002): 1) soil cores were air dried and mixed to obtain a homogenous sample; 2) portions of 5 kg of soil (for a total of 40 kg) were suspended in 5 L water and passed through stacked 2 mm, 212 µm and 38 µm sieves; 3) AM fungal spores retained in the 38 µm sieve were surface sterilized for 1 min with 10 % bleach, washed with tap water and collected in a beaker; 4) all spore collections were finally cleaned by suspending in 60 % sucrose solution and centrifuging for 2 min at 960 x g.

A pathogen/ saprobe fraction (for simplicity we refer to this as ‘Pathogen’ treatment; but see discussion below) was obtained by passing a soil suspension, extracted from 2 kg of non-autoclaved soil, through a 20 µm sieve, which excludes AM fungal propagules. Although this fraction may contain non-pathogenic biota, comprised, for example, of saprobic or beneficial fungi or bacteria, this approach has been used as an effective method of isolating a community of pathogenic soil microorganisms able to negatively impact plant growth in an experimental system

(Klironomos 2002). We spiked the pathogen fraction with a fungus isolated from plant roots randomly collected from our soil using selective media (Conway 1985). The isolated fungus was determined to be highly similar to *Ulocladium tuberculatum* by sequencing the ITS-region and doing a BLAST search in GenBank [National Center for Biotechnology Information (www.ncbi.nlm.nih.gov)]. Fungi in this genus are known as plant pathogens (Andersen & Hollensted 2008). Controls were inoculated with an autoclaved AM spore suspension, pathogen community and cornmeal medium (20 min at 121°C).

Seed collection and pre-germination

Seeds of *Galium verum* (Rubiaceae) and *Hieracium umbellatum* (Asteraceae), which are both common at the site, were collected at the end of the growing season in 2008. We chose these species because in addition to being abundant, they have similar root architecture despite being members of different plant families. The root systems of both species consist of a few coarse roots with branched fine roots. Root architecture is one of the most prominent traits in relation to pathogen susceptibility (Newsham *et al.* 1995b). The seeds were surface sterilized with 70 % ethanol, germinated in small plastic boxes with sterile soil from the field site and left to grow until the primary leaves had developed. Seedlings were transplanted to experimental units, which consisted of 4 x 20.5 cm ‘conetainers’ (Stuewe and Sons Inc., Corvallis, OR, USA).

Experimental design

The experiment consisted of two crossed factors: presence/absence of AM fungi and pathogen, resulting in four treatment combinations per plant species. Containers were completely randomized on the greenhouse bench with 10 replicates per treatment for a total of 80 units. Plants were grown for three months with the AM fungal inoculum before adding the pathogen community. Since our goal was to test for AM fungal mediated pathogen protection, the gap between treatments was intended to let the AM fungal symbiosis establish first before challenging with the pathogen inoculum. We added 7 ml of a low P Hoagland's solution as fertilizer bi-weekly.

Harvesting and post-harvest measurements

The experiment was harvested after six months of growth, which is equivalent to one growth season for these plant species in Central Europe. Due to poor survival rates in the treatments without AM fungi, treatment replication was reduced to five. The number of replicates in the AM fungal treatment was reduced by randomly choosing five individuals from all surviving replicates. Roots were separated from shoots, cleaned, and both parts were oven dried at 40°C for one week before being weighed for calculation of total biomass. Dried roots of all individuals of *G. verum* and *H. umbellatum* were re-hydrated, stained with ink-vinegar (Vierheilig *et al.* 1998) and assessed for percentage of AM fungal colonisation (McGonigle *et al.* 1990). We counted the number of AM and non-AM structures for 100 root intersections under the microscope using 200 x magnification. For determining different colonization pattern of AM fungi we used the INVAM homepage (http://invam.caf.wvu.edu/). With this method we only captured fungal colonisation; we would have missed root colonisation by other microbiota like bacteria or viruses, which could be pathogenic as well. However, there is evidence that in grasslands, fungi appear to be often important root pathogens (Klironomos 2002) and may contribute to plant species coexistence (Westover & Bever 2001).

Furthermore we measured the length of fine (< 2mm) and coarse roots (>2 mm) and average root diameter with a flatbed scanner and the package WinRhizo (Régent Instruments Inc., 2007).

Statistical analysis

All analyses were performed with the R software (R Development Core Team 2008).

We started by calculating a MANOVA with the response variables total biomass, fine root length, coarse root length and root diameter. In fact, response variables showed different degrees of correlation, which requires a multivariate approach to protect posterior analysis on single variables. We then calculated single ANOVAs with two factors, "AM fungi" and "Pathogen" inoculation on total biomass, fine root length, coarse root length and root diameter for each species separately. Data were transformed as needed to meet ANOVA assumptions, however, untransformed values are reported in the figures. The Kruskal Wallis test was used to analyse percent root colonisation with AM and non-AM fungal structures within the treatments.

Results

Performance of the plant species

We generally found significant AM fungal and pathogen effects as well as a significant interaction in both *Hieracium umbellatum* and *Galium verum* (see MANOVAs; Tables 1 and 2).

Table 1: Multivariate analysis of variance for *Hieracium umbellatum* for the response variables total biomass, fine root length, coarse root length and root diameter

	Df	Pillai	Approx F	Num Df	Den Df	Pr(>F)
myco	1	0.93947	50.442	4	13	**<0.0001*****
patho	1	0.77373	11.113	4	13	**0.0003****
myco: patho	1	0.58083	4.503	4	13	**0.015***

(*= p < 0.05; ** p < 0.001; ***= p < 0.0001, myco= AM fungal treatment, patho= Pathogen treatment, myco:patho= Interaction of AM fungal and pathogen treatment)

Table 2: Results from the Multivariate analysis of variance for *Galium verum* for the response variables total biomass, fine root length, coarse root length and root diameter

	Df	Pillai	Approx F	Num Df	Den Df	Pr(>F)
myco	1	0.91414	34.602	4	13	**<0.0001*****
patho	1	0.73940	9.221	4	13	**0.0009****
myco: patho	1	0.82527	15.351	4	13	**<0.0001*****

(*= p < 0.05; ** p < 0.001; ***= p < 0.0001, myco= AM fungal treatment, patho= Pathogen treatment, myco: patho= Interaction of AM fungal and pathogen treatment)

More specifically, individuals of both species inoculated with the AM fungal fraction had a significantly higher total biomass than those without AM fungi (Fig. 1). In contrast, inoculation with the pathogen/saprobe fraction significantly reduced total biomass.

While *G. verum* responded negatively to pathogen inoculation only in the absence of AM fungi (Table 3), *H. umbellatum* showed this response independent of AM fungal inoculation (Table 4).

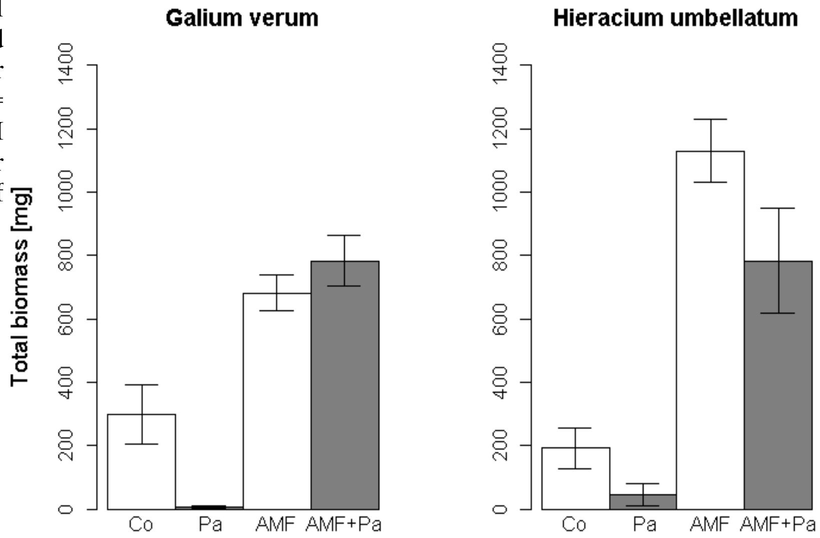

Fig. 1: Effect of indigenous soil microbial treatment on the total biomass of *Galium verum* and *Hieracium umbellatum* in the four treatments (Co= Control, Pa= Pathogen community, AMF= AM fungal community, AMF + Pa). Error bars represent the standard error of the mean.

Table 3: Results from analyses of variance on different response variables for *Galium verum.*

	Effect	Df	Sum Sq	F-value	p-value
Total biomass	myco	1	413946	49.4803	**<0.0001*****
	patho	1	3172	0.3792	0.5467
	myco: patho	1	85017	10.1624	**0.006***
	Residuals	16	130.4		
Fine root length (<2mm)	myco	1	9433182	42.7560	**<0.0001*****
	patho	1	507064	2.2983	0.1490
	myco: patho	1	5043393	22.8592	**0.0002****
	Residuals	16	3530055		
Coarse root length (>2mm)	myco	1	1296085	117.4398	**<0.0001*****
	patho	1	60160	5.4512	**0.03291***
	myco: patho	1	78746	7.1353	**0.0167***
	Residuals	16	176579		
Root diameter	myco	1	0.8	0.1074	0.7474
	patho	1	192.2	25.7987	**<0.0001*****
	myco: patho	1	352.8	47.3557	**<0.0001*****
	Residuals	16	119.2		

(*= $p < 0.05$; ** $p < 0.001$; ***= $p < 0.0001$, myco= AM fungal treatment, patho= Pathogen treatment, myco: patho= Interaction of AM fungal and pathogen treatment)

Root morphology

The root morphology followed similar patterns as total biomass. Fine root length and coarse root length had the highest values in the AM fungal treatments of both plant species (Fig. 2).

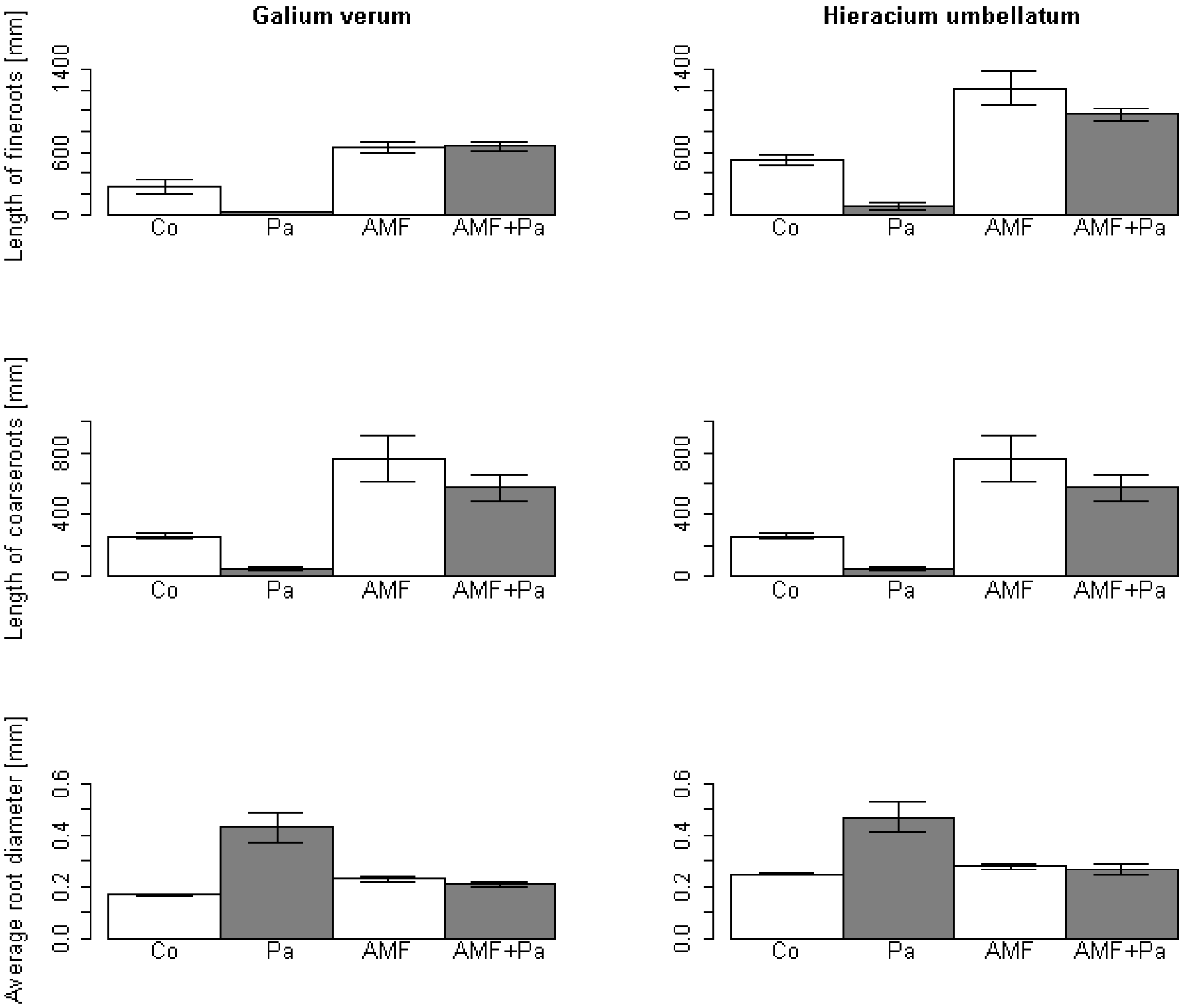

Fig. 2: Effect of indigenous soil microbial treatment on the length of fine roots, length of coarse roots and average root diameter of *Galium verum* and *Hieracium umbellatum* in the four treatments (Co = Control, Pa= Pathogen community, AMF = AM fungal community, AMF + Pa) Error bars represent the standard error of the mean.

We also found a significant decrease of these variables in the pathogen treatment compared to the controls for *H. umbellatum* (Table 4).

Table 4: Results from analyses of variance on different response variables for *Hieracium umbellatum.*

	Effect	Df	Sum Sq	F-value	p-value
Total biomass	myco	1	500.0	109.4391	**<0.0001*****
	patho	1	88.2	19.3051	**0.0004****
	myco: patho	1	3.2	0.7004	0.4150
	Residuals	16	73.1		
Fine root length (<2mm)	myco	1	3162235	75.0230	**<0.0001*****
	patho	1	611228	14.5012	**0.0015***
	myco: patho	1	47981	1.1383	0.3018
	Residuals	16	674403		
Coarse root length (>2mm)	myco	1	969.36	67.4333	**<0.0001*****
	patho	1	200.21	13.9275	**0.0018***
	myco: patho	1	46.48	3.2335	0.0910.
	Residuals	16	230.0		
Root diameter	myco	1	16.2	0.9818	0.3365
	patho	1	180.0	10.9091	**0.0045***
	myco: patho	1	204.8	12.4121	**0.0028***
	Residuals	16	264.0		

(*= $p < 0.05$; ** $p < 0.001$; ***= $p < 0.0001$, myco= AM fungal treatment, patho= Pathogen treatment, myco: patho= Interaction of AM fungal and pathogen treatment)

For *G. verum* we only detected the pathogen effect for coarse root length, but a significant AM fungal x Pathogen interaction in all root length variables (Table 3). Furthermore, both plant species showed a significant increase in root diameter in the pathogen treatment by comparison with the AM fungal treatments and controls (Fig. 2).

Fungal colonisation of roots

We found AM fungal hyphae in AM fungal and AM fungi + Pathogen treatments in roots of both plants species, confirming that the AM fungal treatment was effective. AM fungal colonisation was similar in *G. verum* and *H. umbellatum* (Fig. 3).

We found the highest colonisation by AM fungal hyphae in the two AM fungal treatments, which were significantly different from the non-AM fungal treatments (for *G. verum* df= 3, P = 0.0012 and *H. umbellatum* df= 3, P = 0.0012). Furthermore, we observed morphologically distinct colonization patterns within the roots, supporting the notion that plants were colonized by an assemblage of AM fungi. Only a few AM fungal-like hyphae were observed as background in the non-AM fungal treatments.

The extent of colonisation by non-AM fungal structures was low; these structures were found in all treatments (Fig. 3). Both plant species accumulated significantly more non-AM fungal structures in the pathogen treatment than in the non-mycorrhizal controls and AM fungal treatments (for *G. verum* df=3, P = 0.014 and *H. umbellatum* df=3, P = 0.007). We observed several hyphal types and other structures (spores, microsclerotia) that support the assessment that an assemblage of pathogens infected the plants. Furthermore, we detected a trend towards the reduction of non-AM fungal structures in the AM fungal treatment.

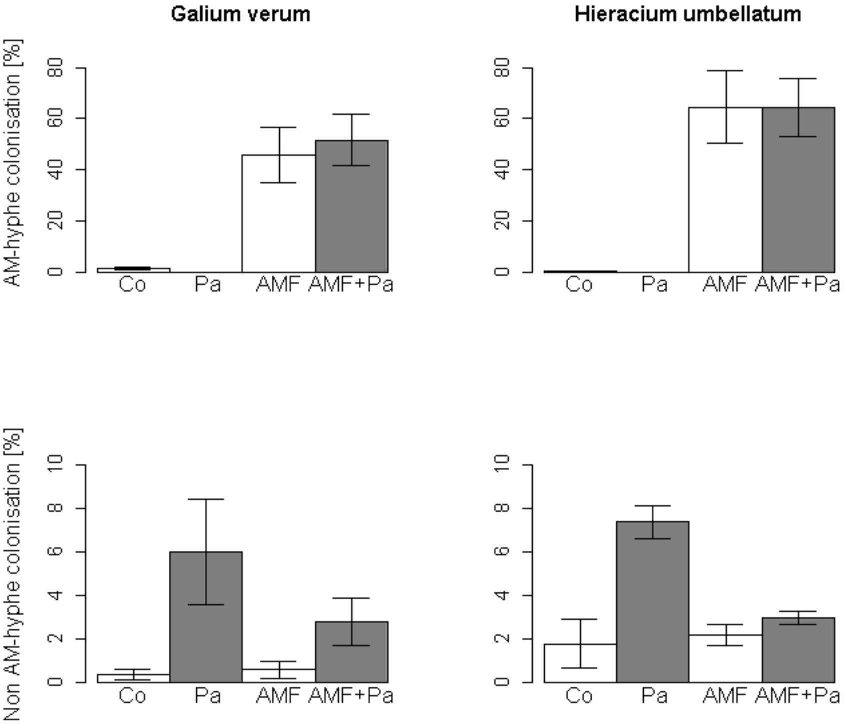

Fig. 3: Percent root colonisation by AM fungal hyphae and non AM fungal hyphae in the roots of *Galium verum* and *Hieracium umbellatum* in the four treatments (Co = Control, Pa= Pathogen community, AMF = AM fungal community, AMF + Pa). Error bars represent the standard error of the mean.

Discussion

In this study we examined the interactions of indigenous AM fungal and pathogen/ saprobe assemblages, both stemming from the same field site as the two plant species and the soil used. Overall, our results support the hypothesis that interactions between beneficial and pathogenic plant-soil microbial assemblages can influence the performance of certain plant species in a community.

AM fungi strongly increased the performance of both plant species compared to the non-AM fungal treatments. The effects of the pathogen/ saprobe fraction were species dependent. While we found a significant decrease in the total biomass in presence of the pathogen/ saprobe fraction compared to the controls and the sole AM fungal treatment in *H. umbellatum*, *G. verum* only negatively responded to the pathogen/ saprobe fraction in absence of AM fungi. This indicates that the pathogen/ saprobe fraction acted indeed as a pathogen treatment, as observed in previous experiments (Klironomos 2002).

When AM fungi were present the negative effects of the pathogen/ saprobe fraction were totally offset for *G. verum* whereas *H. umbellatum* also performed better than with the pathogen/ saprobe fraction alone. Given that we found no significant interaction, this effect may not be caused by tolerance to the pathogen but rather be due to better nutrient supply in the presence of AM fungi. However, taken together, our results provide evidence for AM fungal mediated protection against the local pathogen/ saprobe fraction.

Such pathogen protection is consistent with effects observed by Newsham *et al.* (1995a); however, unlike these authors, we found an additional growth promoting effect caused by AM fungi, which was likely due to a better nutrient supply provided by different AM fungi in the assemblage, In our study we may have added different functional groups in the Glomeromycota, for which pathogen protection is suspected to be a phylogenetically-conserved trait (Maherali & Klironomos 2007, Powell *et al*. 2009). Some species within these AM fungal assemblages may have provided pathogen protection and some may have increased the nutrient status, which supports the hypothesis that functional complementarity could arise between different AM fungal species. Belowground, the results obtained for root morphology were consistent with those observed aboveground. AM fungi increased the length of fine and coarse roots in both species. Although a highly branched root system should increase nutrient supply, it can also raise susceptibility against pathogens (Newsham *et al.* 1995b) and the plants benefit more from AM fungi via pathogen protection. However, we found a reduction in length of fine and coarse roots for both plant species in the pathogen treatment. This reduction might be due to direct damage to the root exodermis upon pathogen infection (van der Putten 2003) thereby counteracting the AM fungal effect on root branching.

We consistently found an increase in root diameter in the pathogen treatment, which might be caused by the loss of fine roots either through pathogen attack or handling mistakes during harvest. Young and thinner roots are more susceptible against pathogens (Schwarz & Grosch 2003) and might have been destroyed.

We found pathogenic structures in both Pathogen and AMF + Pathogen treatments, but the presence of AM fungi reduced the pathogenic structures in the root tissue compared to the sole Pathogen treatment. However, we found pathogenic structures (i.e., conidia) in cells with AM fungi, which suggest that AM fungi might cause tolerance against pathogenic fungi in native plants. Due to the pathogen/ saprobe fraction we used as pathogen treatment we cannot know if fungi alone caused the pathogenic effects or if other organisms played also an important role (Petermann *et al.* 2008).

This is the first study to provide evidence for local AM fungal mediated plant pathogen protection by a resident AM fungal assemblage. We conclude that pathogen effects in natural ecosystems may be overestimated by studying single species and not considering diversity. In the field most plant species are associated with AM fungi, which can protect their host plants against pathogen attack under given conditions. Since such an important interaction between assemblages of beneficial and pathogenic organisms can take place, future research to understand factors controlling plant communities should focus on both organism groups and their interactions.

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5

General Discussion

In our study system "Oderhänge Mallnow" we found an unexpected diversity of fungi associated with roots of plant species within the family of Asteraceae (see Chapter 2). Due to the fact that we only sampled in one plant family, and that plant phylogeny was the most important predictor for structuring community composition of root associated fungi, we would expect an even higher diversity if roots of more plant families had been considered. This high diversity of root associated fungi could be linked to the high plant diversity in our study area providing many different niches. At a small scale-level roots of different plant species can be seen as micro-habitats for fungi with conditions varying between different plant species (e.g. root architecture, root exudate, associated microorganisms). Some of these root associated fungi might be generalist and able to colonize roots of many different plant species independent of the prevailing conditions. However, our study provides evidence that the phylogenetic relationship among the plant species is important for fungal colonization, which indicates that regardless of their function many of these fungi might be selective or even specific for certain phylogenetic groups of plants within a family due. This specialization of fungi to different plant taxa might allow coexistence of certain fungal species, while in conjunction fungi were found to engage in negative pair-wise interactions, as indicated by Null model analysis (spatial segregation) in Chapter 2. However, it would be interesting to test in further studies if host plant phylogeny remains the most important predictor for explaining fungal community composition when considering other plant families or different phylogenetic level (dicotyledons, monocotyledons).

For studying the root associated fungal community composition in Chapter 2 we applied 454 pyrosequencing a next generation sequencing technology which allows considering organismal diversity in one sequencing step by obtaining a large number of parallel-sequence reads per sample, and it therefore eliminates the cloning step (Öpik *et al.* 2009). Moreover, the likelihood of detecting rare organisms increases with this technology due to the higher sampling depth. In contrast, several disadvantages make it difficult to investigate fungal community composition compared to bacteria (Lindahl *et al.* 2013), for example the absence of a general pipeline from sequence processing to the generation of operational taxonomic units (OTUs) and the absence of a good database to BLAST (Basic Local Alignment Search Tool) the sequences to identify genera or species (Lindahl *et al.* 2013). Moreover, the species concept in fungi is still unclear (Giraud *et al.* 2008) and the drawback of only using DNA sequences for identification of species exists. However, for studying beta-diversity our approach of defining "species" based on sequence similarities in OTUs by using a Bayeasian model for defining the cut off (Hao *et al.* 2011) circumvents most of these issues, but data have to be carefully interpreted when considering alpha-diversity because of a lack in the species concept in fungi. At the moment there is no good way to distinguish fungal species only by sequencing, because "species delineation thresholds" vary among the different groups of fungi (Nilsson *et al.* 2008). By using a general cut off of 97% sequence similarity for all groups of fungi, like other authors mostly do (Lindahl *et al.* 2013), one will underestimate later evolved groups like the Ascomycota and overestimate the earlier lineages like Glomeromycota (Schoch *et al.* 2012). Apart from all these disadvantages data are still meaningful because we mainly considered beta-diversity pattern and not the absolute OTU richness, which may be not sensitive to all these issues.

However, future work has to be done to look for a better species concept in fungi which allows considering alpha-diversity in fungal community studies. If this is done one has to generate a general pipeline to process fungal sequence data to OTUs.

As shown in Chapter 2, fungal coexistence is strongly driven by host plants identity and phylogeny and the interaction among the different fungal species. In Chapter 3 and 4 we analyzed a potential mechanism for plant coexistence: AM fungal mediated pathogen protection. In a literature study reviewing the mechanisms by which AM fungi may protect their host plants against pathogen attack, we found evidence that diversity of AM fungi and pathogens may modify the effect (Wehner *et al.* 2010). These findings triggered a greenhouse experiment with an AM fungal spore community and a community of saprobic/pathogenic fungi (see Chapter 4).

In Chapter 4 we found that an AM fungal community can be very effective in protecting their host plants against pathogen attack. In contrast to Newham *et al.* 1995, who inoculated with only one AM fungus and only found a pathogen protection effect, we found an additional growth promoting effect for host plants inoculated with the AM fungal community in the absence of pathogens. This may reflect the different functions of the organisms in our AM fungal community. Some AM fungal species might provide pathogen protection and others may deliver more nutrients to the host plant, which results in an increased plant growth. Evidence for this trait conservatism in AM fungi has been shown by Powell *et al.* 2009 who found *Glomus* species being more effective in terms of pathogen protection while *Gigaspora* are better nutrient suppliers. AM fungal mediated pathogen protection might be an important trade off for plant coexistence: while some plant species may benefit from an association with AM fungi in terms of pathogen protection others might not and may be negatively affected by pathogen attack. The negatively effected plants might be reduced in their abundance and become rarer in comparison to plants receiving this type of protection.

I conclude form the results of Chapter 3 and 4, that in manipulative experiments with either AM fungi or pathogens or other root-associated fungi it would be always important to consider diversity of these organism to generate more realism in the experimental setup, because of different interactions between the groups of organism.

From an evolutionary perspective one could imagine that AM fungi drive diversification of plant pathogens like host plants do, where phylogenetically closely related plant species are hosts for phylogenetically closely related pathogens (e.g., Refrégier *et al.* 2009). This hypothesis could be tested in a later study by comparing communities of root associated fungi of non AM fungal plants with AM fungal plants in a molecular based approach by performing pyrosequencing and sampling of many different plant species in different plant families to decrease effects of host specificity. The resulting data have to be corrected for similarities in fungal community composition based on relatedness of plant species; and tested for differences in fungal community composition in both plant groups (non AM and AM colonized) to find an indication that AM fungi may contribute to the diversification of pathogens or endophytes.

Conclusion

The motivation of this research was to investigate the interactions of host plants and fungal partners in a highly diverse plant community to find determinants of coexistence. With our studies we have been shown that aboveground diversity is likely reflected in a high diversity of root associated fungi. Root fungal community composition is influenced by host plant phylogeny, spatial pattern and soil type; also direct interactions between the root-associated organisms may exist. Our results shed a different light on the ecosystem functioning where belowground organism especially fungi play an important role for coexistence of plant species and may contribute to the high plant diversity in the “Oderhänge Mallnow”.

6

Appendix Chapter 2

Table S1 List of primers with error-correcting barcode sequences for the respective samples

	Adapter	Tag	ITS-Primer
1	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCAACCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
2	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCAAGGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
3	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCATCGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
4	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCATGCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
5	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCGCATCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
6	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCGCTACA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
7	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCGGAACA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
8	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCGGTTCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
9	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCTACGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
10	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCTAGCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
11	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCTTCCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
12	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACCTTGGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
13	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGAACGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
14	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGAAGCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
15	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGATCCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
16	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGATGGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
17	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGCCATCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
18	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGCCTACA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
19	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGCGAACA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
20	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGCGTTCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
21	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGGCAACA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
22	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGGCTTCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
23	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGTACCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
24	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGTAGGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
25	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGTTCGCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4
26	A: 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG	AACGTTGCCA	CTTGGTCATTTAGAGGAAGTAA-Its1f
	B: 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	TC	TCCTCCGCTTATTGATATGC-Its4

Table S2 Accession numbers of sequences of the different taxa used for generating the tree of the host plant species

Sampled species	Species/Genera and accession number used for matk tree	Species/Genera and accession number used for ITS tree
Taraxacum sp.	*Taraxacum* (HM989734.1)	*Taraxacum* (AM946534.1)
Aster linosyris	*Aster* (HM989804.1)	*Aster* (HQ154046.1)
Conyza canadensis	*	*Conyza* (AY875694.1)
Erigeron muralis	*Erigeron* (HM989796.1)	*Erigeron* (AF511587.2)
Solidago virgaurea	*S. virgaurea* (EU749423.1)	*S. virgaurea* (EU125358.1)
Helianthus tuberosus	*Ambrosia artemisifolia* (GQ248077.1)	*H. tuberosus* (AF047953.1)
Carlina vulgaris	*C. vulgaris* (AY013533.1)	*C. vulgaris* (AY826246.1)
Centaurea jacea	*Centaurea* (AY013496.1)	*Centaurea* (AM114332.1)
Centaurea stoebe	*Centaurea* (AY013496.1)	*Centaurea* (AM114332.1)
Centaurea scabiosa	*Centaurea* (AY013496.1)	*Centaurea* (AM114332.1)
Cichorium intybus	*C. intybus* (AJ633133.1)	*C. intybus* (AJ633451.1)
Hypochaeris radicata	*H. radicata* (AF528421.1)	*H. radicata* (GU011987.1)
Leontodon hispidus	*L. hispidus* (AF528431.1)	*L. hispidus* (DQ451769.1)
Picris hieracioides	*P. hieracioides* (AF528432.1)	*P. hieracioides* (EF107658.1)
Hieracium pilosella	*Hieracium* (AJ633177.1)	*H. pilosella* (HQ161945.1)
Hieracium umbellatum	*H. umbellatum* (AJ633195.1)	*H. umbellatum* (AJ633422.1)
Senecio jacobaea	*Jacobea vernalis* (FJ395360.1)	*Senecio* (AJ400806.1)
Senecio vernalis	*Jacobea vernalis* (FJ395360.1)	*Senecio* (AJ400806.1)
Helichrysum arenarium	*Helichrysum* (HM445637.1)	*H. arenarium* (AY445199.1)
Anthemis tinctoria	*Ursinia trifida* (AF456803.1)	*Anthemis* (EU179214.1)
Achillea pannonica	*Achillea* (HM989801.1)	*A. pannonica* (AY603184.1)
Artemisia campestris	*Artemisia* (HM989753.1)	*A. campestris* (AM398856.1)
Leucanthemum vulgare	*L. vulgare* (FJ395389.1)	*L. vulgare* (EF577315.1)
Chondrilla juncea	*C. juncea* (AJ633135.1)	*C. juncea* (AJ633348.1)
Bellis perennis	*	*Bellis* (AF494007.1)
Campanula rotundifolia	*C. rotundifolia* (EU713335.1)	*C. rotundifolia* (DQ304615.1)

* For these species no sequence information was available and we included sequences containing gaps instead of bases

Table S3 Number of sequence reads and OTUs among the different individuals

Individual	No. of sequence reads before re-sampling	No. of OTUs before re-sampling	No. of OTUs of 1000 re-sampled sequences
Achillea pannonica1	3867	132	74
Achillea pannonica2	6328	168	64
Achillea pannonica3	3153	72	80
Anthemis tinctoria1	1719	127	95
Anthemis tinctoria2	4478	152	84
Anthemis tinctoria3	2153	123	83
Artemisia campestris1	706	59	53
Artemisia campestris2	4415	137	75
Artemisia campestris3	1667	71	52
Aster linosyris1	1362	112	89
Aster linosyris2	3049	92	53
Aster linosyris3	986	66	53
Bellis perennis1	139	31	31
Bellis perennis2	316	62	61
Bellis perennis3	336	39	38
Campanula rotundifolia1	3264	114	62
Campanula rotundifolia2	2249	106	68

Campanula rotundifolia3	1853	97	67
Carlina vulgaris1	2138	99	65
Carlina vulgaris3	1502	124	88
Centaurea jacea1	1164	115	89
Centaurea jacea2	541	82	75
Centaurea jacea3	2060	141	87
Centaurea scabiosa1	7060	131	65
Centaurea scabiosa2	2465	69	50
Centaurea scabiosa3	149	37	37
Centaurea stoebe1	1299	72	56
Centaurea stoebe2	2465	65	40
Centaurea stoebe3	2266	95	61
Chondrilla juncea1	1212	97	72
Chondrilla juncea2	554	60	56
Chondrilla juncea3	927	88	74
Cichorium intybus1	280	58	57
Cichorium intybus2	2410	114	80
Cichorium intybus3	4821	112	61
Conyza canadensis1	463	73	69
Conyza canadensis2	466	71	67
Conyza canadensis3	2432	143	92
Erigeron muralis1	693	47	42
Erigeron muralis2	1140	88	68
Erigeron muralis3	1461	78	57
Helianthus tuberosus1	2068	136	90
Helianthus tuberosus2	52	18	18
Helianthus tuberosus3	1672	136	97
Helichrysum arenarium1	757	32	28
Helichrysum arenarium2	998	72	58
Helichrysum arenarium3	5378	138	65
Hieracium pilosella1	5940	212	114
Hieracium pilosella2	4995	182	90
Hieracium pilosella3	5832	153	76
Hieracium umbellatum1	274	50	49
Hieracium umbellatum2	3100	119	70
Hieracium umbellatum3	369	48	46
Hypochaeris radicata2	364	39	38
Hypochaeris radicata3	1086	60	49
Leontodon hispidus1	1359	63	48
Leontodon hispidus2	4386	153	89
Leontodon hispidus3	1260	71	55

Leucanthemum vulgare1	394	66	63
Leucanthemum vulgare2	2441	137	84
Leucanthemum vulgare3	2063	56	41
Picris hieracioides1	1563	89	64
Picris hieracioides2	4523	100	49
Picris hieracioides3	6499	119	50
Senecio jacobaea1	1071	85	65
Senecio jacobaea2	363	73	71
Senecio jacobaea3	555	102	94
Senecio vernalis1	857	97	83
Senecio vernalis2	1138	58	48
Senecio vernalis3	523	50	47
Solidago virgaurea1	1941	109	76
Solidago virgaurea2	1222	65	50
Solidago virgaurea3	2779	106	58
Taraxacum sp1	2806	173	107
Taraxacum sp2	2400	101	62
Taraxacum sp3	1778	111	78

Table S4 Sample characterization

Plant individual	**X-coordinate (in decimals)**	**Y-coordinate (in decimals)**	**Soil type**
Achillea pannonica1	14.4914027	52.4644333333	loamy
Achillea pannonica2	14.4914027	52.4648333333	loamy
Achillea pannonica3	14.4914972	52.464675	loamy
Anthemis tinctoria1	14.476266	52.4659333333	sandy
Anthemis tinctoria2	14.4764305	52.4657638888	sandy
Anthemis tinctoria3	14.4762638	52.4655972222	sandy
Artemisia campestris1	14.4862083	52.4637722222	sandy
Artemisia campestris2	14.4862722	52.4635916666	sandy
Artemisia campestris3	14.4861444	52.4638888888	sandy
Aster linosyris1	14.4861388	52.4634638888	loamy
Aster linosyris2	14.4862805	52.4632555555	loamy
Aster linosyris3	14.4861222	52.4633888888	loamy
Bellis perennis1	14.4919444	52.4630555555	loamy
Bellis perennis2	14.4919444	52.4627777777	loamy
Bellis perennis3	14.4919444	52.4630555555	loamy
Campanula rotundifolia1	14.4858361	52.4640722222	loamy
Campanula rotundifolia2	14.4857111	52.4640111111	loamy
Campanula rotundifolia3	14.48555	52.4640722222	loamy
Carlina vulgaris1	14.4910027	52.4637305555	loamy

Carlina vulgaris3	14.4906027	52.4639722222	loamy
Centaurea jacea1	14.4826	52.4648055555	loamy
Centaurea jacea2	14.4825944	52.46485	loamy
Centaurea jacea3	14.4828	52.4648888888	loamy
Centaurea scabiosa1	14.4907944	52.4636694444	loamy
Centaurea scabiosa2	14.4909666	52.4638	loamy
Centaurea scabiosa3	14.4907638	52.4639111111	loamy
Centaurea stoebe1	14.4936111	52.4652777777	sandy
Centaurea stoebe2	14.4936111	52.465	sandy
Centaurea stoebe3	14.4938888	52.4652777777	sandy
Chondrilla juncea1	14.480844	52.4652166666	sandy
Chondrilla juncea2	14.480725	52.4652583333	Sandy
Chondrilla juncea3	14.480672	52.4652972222	sandy
Cichorium intybus1	14.4894444	52.4645305555	sandy
Cichorium intybus2	14.4892944	52.4644333333	sandy
Cichorium intybus3	14.4893333	52.4644527777	sandy
Conyza canadensis1	14.480872	52.4651027777	sandy
Conyza canadensis2	14.481188	52.4652666666	sandy
Conyza canadensis3	14.481044	52.465275	sandy
Erigeron muralis1	14.4864111	52.4627111111	loamy
Erigeron muralis2	14.4863722	52.4627833333	loamy
Erigeron muralis3	14.4866944	52.4626689444	loamy
Helianthus tuberosus1	14.476327	52.4652722222	sandy
Helianthus tuberosus2	14.476283	52.4653194444	sandy
Helianthus tuberosus3	14.476633	52.4655	sandy
Helichrysum arenarium1	14.4863722	52.4631305555	sandy
Helichrysum arenarium2	14.48645	52.4630722222	sandy
Helichrysum arenarium3	14.4862777	52.4630472222	sandy
Hieracium pilosella1	14.4925	52.4644444	loamy
Hieracium pilosella2	14.4922	52.4641666	loamy
Hieracium pilosella3	14.4925	52.3888888	loamy
Hieracium umbellatum1	14.4861944	52.4636472222	loamy
Hieracium umbellatum2	14.4861444	52.4635805555	loamy
Hieracium umbellatum3	14.4862138	52.4637694444	loamy
Hypochaeris radicata2	14.4902722	52.4649083333	sandy
Hypochaeris radicata3	14.490325	52.4648777777	sandy
Leontodon hispidus1	14.4855527	52.4641194444	loamy
Leontodon hispidus2	14.4856583	52.4640444444	loamy
Leontodon hispidus3	14.4856555	52.464075	loamy
Leucanthemum vulgare1	14.4748944	52.4649194444	loamy
Leucanthemum vulgare2	14.4749083	52.4648555555	loamy

Leucanthemum vulgare3	14.4747305	52.4648027777	loamy
Picris hieracioides1	14.4866833	52.4627527777	loamy
Picris hieracioides2	14.4867583	52.462675	loamy
Picris hieracioides3	14.4865472	52.4627111111	loamy
Senecio jacobaea1	14.4912861	52.4647694444	loamy
Senecio jacobaea2	14.4917277	52.4647694444	loamy
Senecio jacobaea3	14.4916111	52.464675	loamy
Senecio vernalis1	14.4845527	52.4651638888	sandy
Senecio vernalis2	14.4846194	52.465175	sandy
Senecio vernalis3	14.4842166	52.4651527777	sandy
Solidago virgaurea1	14.4865472	52.4628527777	loamy
Solidago virgaurea2	14.4866305	52.4627777777	loamy
Solidago virgaurea3	14.4865083	52.4627111111	loamy
Taraxacum spec.1	14.484325	52.4645888888	loamy
Taraxacum spec.2	14.4844361	52.4646388888	loamy
Taraxacum spec.3	14.4842416	52.464775	loamy

Table S5 Results of the OTU clustering with CD-HIT. We used the CD-HIT-EST method for clustering nucleotide sequences aL, aS, s were set to 0.9.

Cluster algorithm	**Number of OTUs**	**Number of singletons**
CD-HIT 97 % cut off	3151	1108
CD-HIT 95 % cut off	2458	1691

Table S6 NCBI BLAST results with the closest match to a species within the criteria of 97% sequence identity and 90% coverage of the 50 most abundant OTUs

No.	**Best hit in INSD with a known species/genus/family/order**	**Accession number INSD**	**Sequence identity (%)**	**Query coverage (%)**	**Phylum**	**Order**	**No. of reads (original/ resampled to 1000 sequence reads per individual)**	**Percentage of all reads (original/ resampled to 1000 sequence reads per individual)**
1	*Phialophora mustea*	JX145399.1	99% (207/208)	100%	Ascomycota	Magnaporthales	13235 / 5963	8.44 / 7.85
2	*Paraphoma chrysanthemicola*	JN123358.1	99% (167/168)	100%	Ascomycota	Pleosporales	12658 / 5522	8.07 / 7.26
3	*Tetracladium maxilliforme*	HM036615.1	99% (160/161)	100%	Ascomycota	Helotiales	8454 / 3996	5.39 / 5.26
4	*Helotiales sp.*	JN859278.1	100% (159/159)	100%	Ascomycota	Helotiales	5970 / 3052	3.81 / 4.01
5	*Leptosphaeria sp.*	HQ713770.1	99% (153/155)	100%	Ascomycota	Pleosporales	5566 / 2691	3.55 / 3.54
6	*uncultured Sebacina*	HQ154383.1	100% (180/180)	100%	Basidiomycota	Sebacinales	4151 / 2585	2.65 / 3.40
7	*Ceratobasidium sp.*	JF912482.1	99% (182/183)	100%	Basidiomycota	Cantharellales	4213 / 1956	2.69 / 2.57
8	*Fusarium redolens*	JN631749.1	100% (148/148)	100%	Ascomycota	Hypocreales	3150 / 1935	2.01 / 2.55
9	-	No match with criteria*	No match with criteria*	No match with criteria*	-	-	3384 / 1817	2.16 / 2.39
10	-	No match with criteria*	No match with criteria*	No match with criteria*	-	-	3655 / 1683	2.33 / 2.21
11	*Exophiala salmonis*	GU586858.1	100% (193/193)	100%	Ascomycota	Chaetothyriales	3146 / 1480	2.00 / 1.95
12	*Ilyonectria radicola*	JN129416.1	99% (136/137)	100%	Ascomycota	Hypocreales	2208 / 1055	1.41 / 1.39
13	*Cladosporium allii*	JN906977.1	99% (153/154)	100%	Ascomycota	Davidiellaceae	3869 / 1034	2.47 / 1.36
14	Uncultured fungus	FN397328.1	99% (196/198)	100%	-	-	2193 / 1001	1.40 / 1.32
15	Uncultured *Ceratobasidium*	JF912475.1	99% (178/181)	100%	Basidiomycota	Cantharellales	1985 / 948	1.26 / 1.25
16	Fungal endophyte	AF373058.1	98% (193/196)	100%	Ascomycota	-	1564 / 936	0.99 / 1.23
17	*Olpidium brassicae*	AB625456.1	100% (132/132)	99%	Incertae sedis	Incertae sedis	1076 / 924	0.69 / 1.22
18	*Cryptococcus victoriae*	JQ769319.1	99% (132/133)	100%	Basidiomycota	Tremellales	1843 / 922	1.17 / 1.21
19	uncultured Helotiales	JQ318662.1	100% (157/157)	99%	Ascomycota	Helotiales	1671 / 894	1.06 / 1.18
20	*Pilidium concavum*	GU126750.1	100% (106/106)	98%	Ascomycota	Helotiales	742 / 867	0.47 / 1.14
21	uncultured Helotiales	JF691173.1	97% (166/171)	100%	Ascomycota	Helotiales	758 / 830	0.48 / 1.09

22	*Fusarium equiseti*	KF274675.1	100% (148/148)	100%	Ascomycota	Hypocreales	944 / 723	0.60 / 0.95
23	*Phlebiella christiansenii*	EU118659.1	97% (176/182)	100%	Basidiomycota	Corticiales	3434 / 659	2.19 / 0.8
24	Uncultured Ascomycota	HM358998.1	99% (204/206)	100%	Ascomycota	-	1135 / 646	0.72 / 0.85
25	*Peyronellaea glomerata*	JX885584.1	100% (139/139)	100%	Ascomycota	Pleosporales	938 / 638	0.60 / 0.84
26	-	No match with criteria*	No match with criteria*	-	-	-	1403 / 637	0.89 / 0.84
27	-	No match with criteria*	No match with criteria*	-	-	-	1299 / 632	0.83 / 0.83
28	*Rhizophagus irregularis*	FR750084.1	100% (109/109)	100%	Glomeromycota	Glomerales	977 / 590	0.62 / 0.78
29	uncultured *Cryptococcus*	JF495242.1	99% (135/136)	100%	Basidiomycota	Tremellales	1147 / 569	0.73 / 0.75
30	*Alternaria alternata*	KC916665.1	99% (163/164)	100%	Ascomycota	Pleosporales	955 / 539	0.61 / 0.71
31	*Gastrosporium simplex*	GQ981504.1	98% (164/167)	100%	Basidiomycota	Phallales	1194 / 510	0.76 / 0.67
32	-	No match with criteria*	No match with criteria*	-	-	-	3038 / 505	1.94 / 0.66
33	Uncultured *Alternaria* clone	FJ708614.1	100% (150/150)	100%	Ascomycota	Pleosporales	61 / 504	0.04 / 0.66
34	uncultured fungus	HM136627.1	99% (203/204)	89%	-	-	772 / 442	0.49 / 0.58
35	Mycorrhizal fungal sp.	EU880591.1	100% (167/167)	100%	-	-	238 / 441	0.15 / 0.58
36	uncultured fungus	FJ777112.1	97% (189/195)	100%	-	-	756 / 417	0.48 / 0.55
37	Uncultured *Cladosporium*	JQ951582.1	100% (155/155)	100%	Ascomycota	Capnodiales	889 / 390	0.57 / 0.51
38	-	No match in Genbank#	No match in Genbank#	-	-	-	137 / 384	0.09 / 0.50
39	Uncultured *Cryptococcus*	FR838294.1	99% (164/165)	100%	Basidiomycota	Tremellales	690/ 378	0.44 / 0.50
40	*Cyathicula*	AM262410.1	99% (159/160)	100%	Ascomycota	-	500 / 375	0.32 / 0.49
41	*Plectosphaerella cucumerina*	JQ796755.1	100% (137/137)	99%	Ascomycota	Phyllachorales	321 / 368	0.20 / 0.48
42	Uncultured Pyronemataceae	GU327419.1	98% (106/108)	100%	Ascomycota	Pezizales	730/ 362	0.46 / 0.48
43	Uncultured *Chalara*	FR838454.1	99% (158/159)	100%	Ascomycota	Microascales	576 / 353	0.37 / 0.46
44	Uncultured Helotiales	GQ223461.1	99% (154/155)	100%	Ascomycota	Helotiales	683 / 349	0.43 / 0.46
45	*Hygrocybe conica*	EU784298.1	99% (212/214)	100%	Basidiomycota	Agaricales	482 / 339	0.31 / 0.45

46	*Periconia macrospinosa*	JN859365.1	99% (150/151)	99%	Ascomycota	Microascales	500 / 337	0.32 / 0.44
47	*Alternaria* sp.	GU934500.1	99% (160/161)	100%	Ascomycota	Pleosporales	277 / 326	0.18 / 0.43
48	-	No match with criteria*	No match with criteria*	-	-	-	974 / 320	0.62 / 0.42
49	*Peziza granularis*	JF908558.1	99% (202/205)	95%	Ascomycota	Pezizales	484 / 293	0.31 / 0.38
50	*Lewia infectoria*	JQ781841.1	100% (193/193)	100%	Ascomycota	Pleosporales	531/ 293	0.34 / 0.38

*No match with criteria- no BLAST result with at least 97% sequence identity and 90% coverage; #Not in the database- no similar sequence present in NCBI database

Figure S1 Map of the investigation area

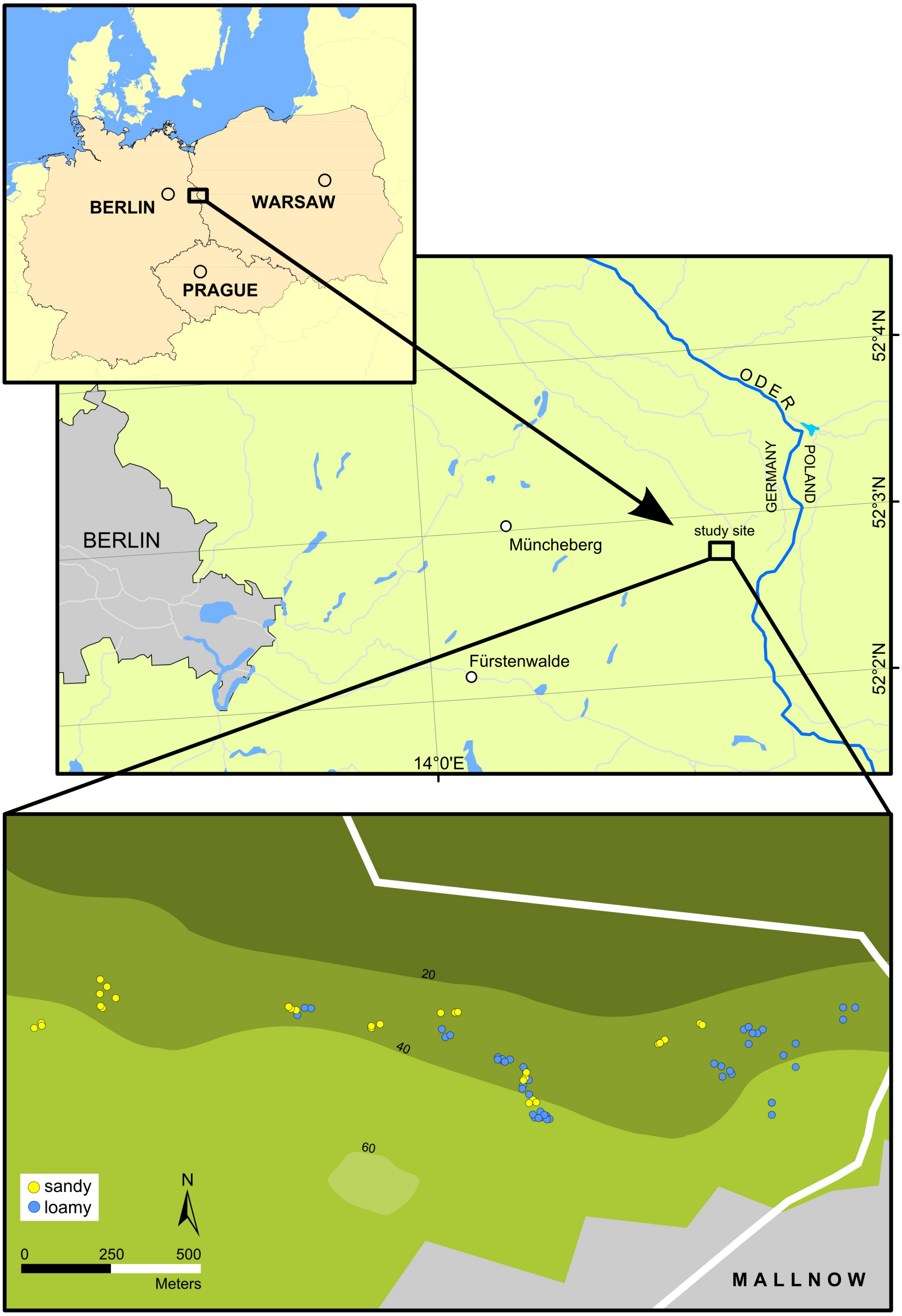


Figure S2 Phylogenetic tree of the Asteraceae with branch length used in the variance partitioning. Campanula rotundifolia was used as reference taxon outside of the Asteraceae, whereas Calycera was used as outgroup species for rooting the tree but removed for all subsequent analyses.

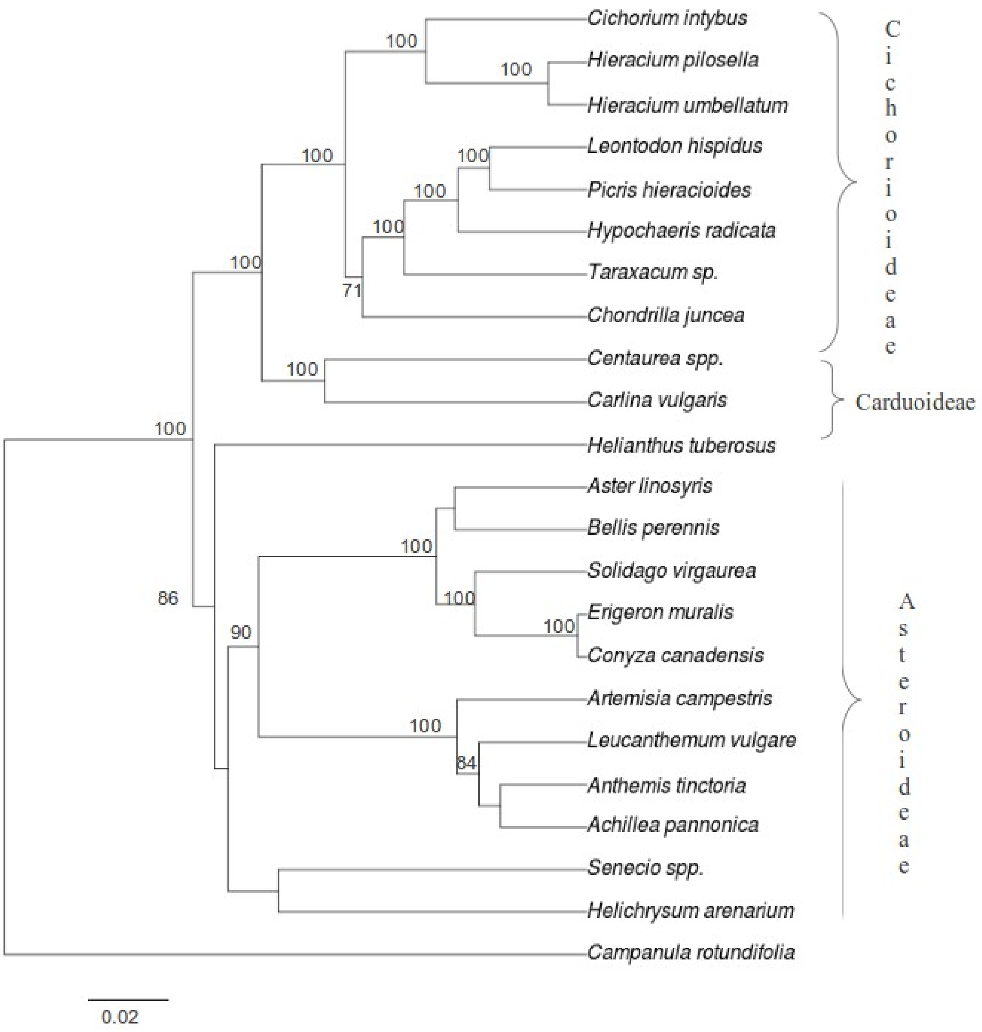


Figure S4 Rarefaction curves per plant species Mean species richness is based on the re-sampled number of OTUs; Individuals in subsample shows the number of fungal sequences per plant individual.

Rarefaction curves were performed using the function *rarefaction* in R downloaded from www.jennajacobs.org/R/rarefaction.html.

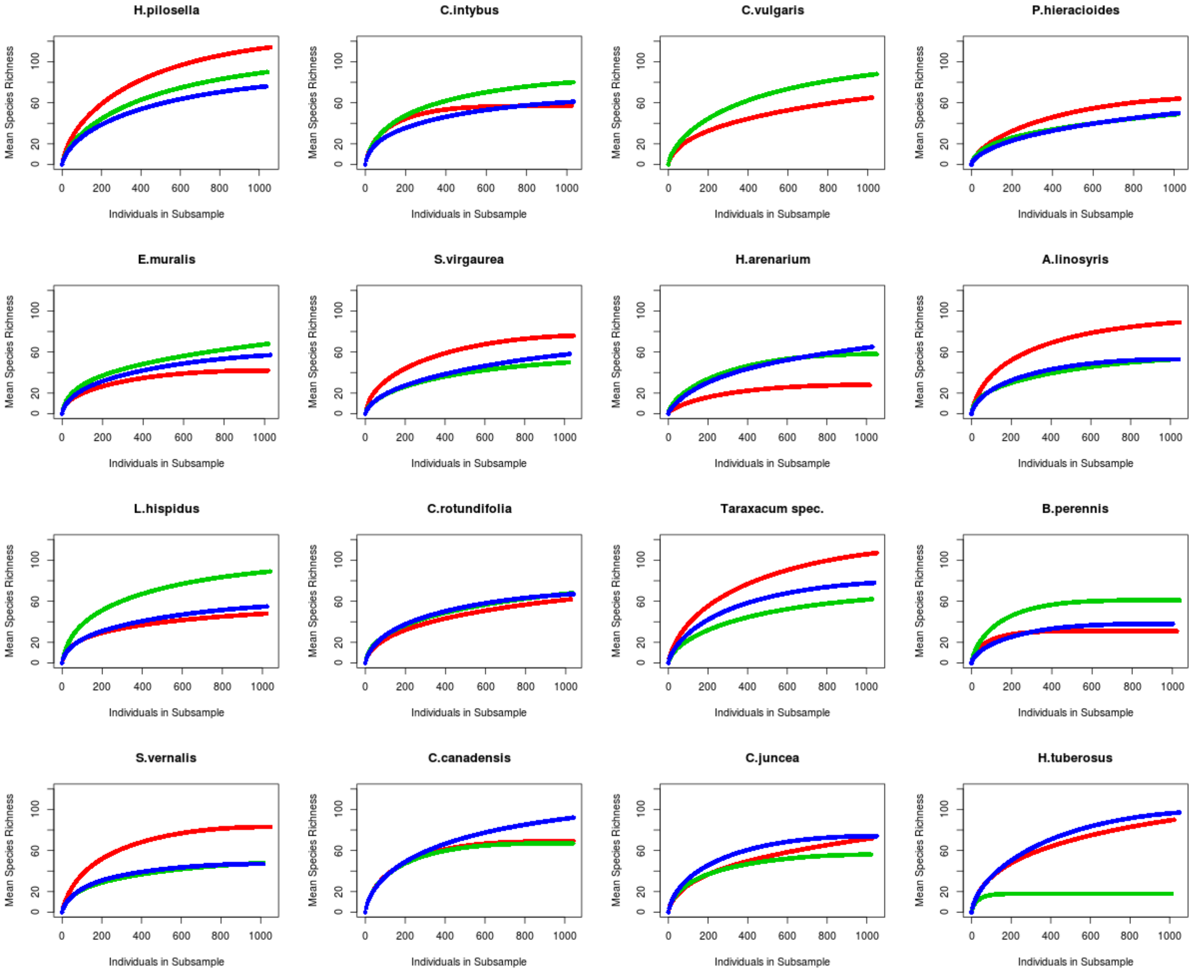
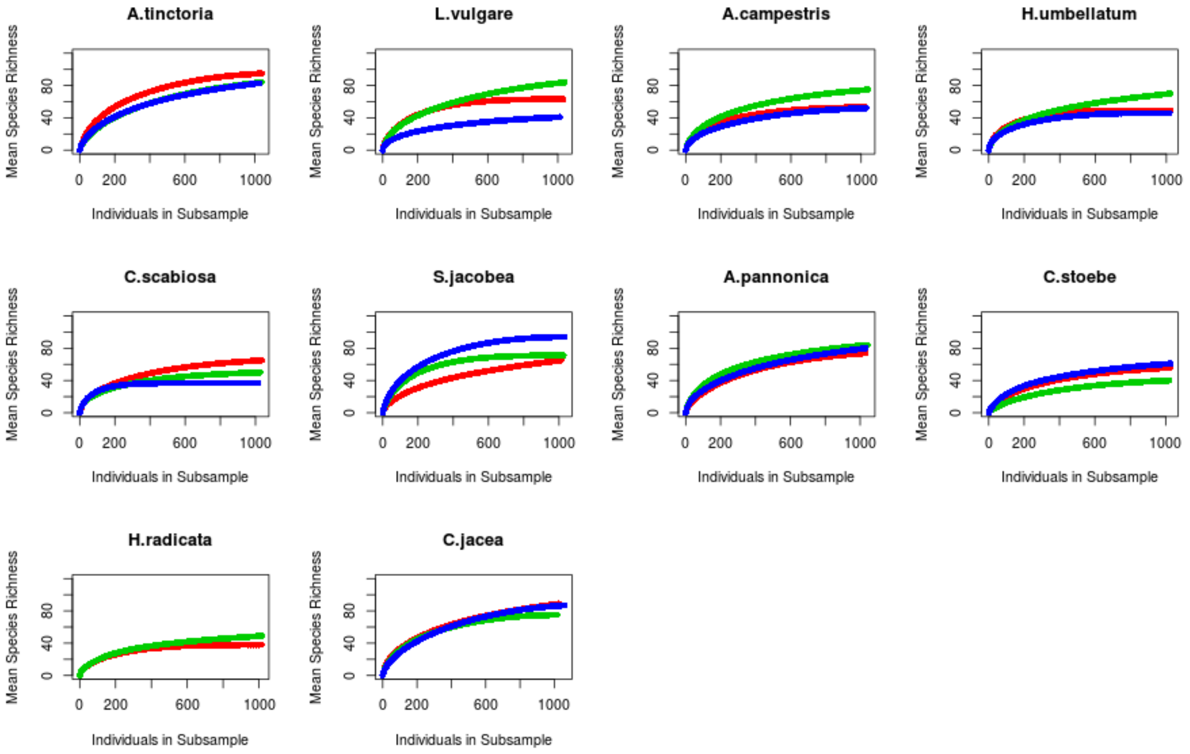


Figure S5 Rarefaction curves per plant spieces. Mean species richness is based on the re-sampled number of OTUs; Individuals in subsample shows the number of fungal sequences per plant individual

Rarefaction curves were performed using the 'rarefaction' function in R downloaded from www.jennajacobs.org/R/rarefaction.html.



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Summary

This doctoral thesis presents the study of potential determinants for coexistence of root associated fungi and mechanism for coexistence of plant species in a dry grassland habitat.

In Chapter 2 we used 454 pyrosequencing of the fungal specific ITS region to study root-associated fungi in 25 plant species within the family of Asteraceae and tested the influence of three main predictors; host plant phylogeny, spatial effects and a gradient in soil type on community composition of root associated fungi. Fungal diversity in the investigated roots was high with 156,816 sequences clustered in 1100 operational taxonomic units (OTUs). In variance partitioning we found all three predictors explaining fungal community composition to a certain percentage. While plant phylogeny was the most important predictor, explaining 20 % variance, followed by space with 9 % and soil type with only 1 % of explained variance. Null model analysis suggested that fungal taxa co-occurred less often than expected by chance, which demonstrates spatial segregation and indicates negative interactions within fungal communities. With this study, we demonstrated, for the first time in a natural setting, that biotic interactions among fungi and with their plant hosts can be more important than the present edaphic properties and that host plant phylogeny can constrain these interactions.

In Chapter 3 and 4 we analyzed the importance of AM fungal diversity for protecting host plants against pathogen attack by reviewing the different mechanism how pathogen protection could arise when considering a divers community of AM fungi. Furthermore, we directly tested the effects of an local AM fungal and a local community of saprobic/pathogenic fungi on two different host plants growing in sterile soil, all from the “Oderhänge Mallnow” in greenhouse experiment.

In a literature study we found evidence that considering AM diversity might be important in terms of the different mechanism how pathogen protection could arise.

In the greenhouse experiment in Chapter 4, the AM fungal community compensated the negative influence of the community of saprobic/pathogenic fungi on the growth of their host plant. We could further show, that root colonization of non-AM fungi was significantly reduced in the AM fungal treatment compared to the non-AM fungal treatment. These results indicate that interactions between assemblages of beneficial and pathogenic microorganisms can influence the growth of host plants, but that the magnitude of these effects might be plant species-specific.

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Zusammenfassung

Im Rahmen dieser Doktorarbeit wurden zum Einen verschiedene Faktoren untersucht, die die Zusammensetzung von Pilzgemeinschaften im Wurzelraum beeinflussen und somit auch deren Koexistenz ermöglichen. Zum Anderen wurde noch ein wichtiger pilzinduzierter Mechanismus untersucht, der zur Koexistenz verschiedener Pflanzen in einem Trockenrasen beitragen könnte.

In Kapitel 2 pyrosequenzierten wir die pilzspezifische ITS-Region von Pilzen aus dem Wurzelraum von 25 verschiedenen Arten aus der Familie der Asteraceae und testeten den Einfluss von drei Hauptfaktoren; Pflanzenphylogenie, räumlicher Effekt und einem Gradient im Bodentyp auf die Zusammensetzung der Pilzgemeinschaften. In den untersuchten Wurzel fanden wir eine sehr hohe Pilzdiversität, 156,816 Sequenzen konnten 1100 sogenannten *operational taxonomic units* (OTUs) zugeordnet werden. Durch Partitionierung der Varianz zwischen den verschiedenen Prädiktoren konnte gezeigt werden, dass alle drei einen wichtigen Beitrag zur Erklärung der Zusammensetzung der Pilzgemeinschaften in den verschiedenen Pflanzenarten leisten. Die Pflanzenphylogenie stellte dabei mit 20 % den wichtigsten erklärenden Faktor dar, gefolgt von räumlichen Effekten mit 9 % und Bodentyp mit 1 % erklärter Varianz. Die Ergebnisse einer durchgeführten Null model Analyse zeigten, dass die Pilze weniger oft miteinander assoziert waren als durch Zufall erwartet werden würde. Das gefundene Muster gibt Hinweise auf eine räumliche Trennung der verschiedenen Pilzarten und deutet somit auf negative Interaktionen innerhalb der Pilzgemeinschaften hin. Mit dieser Studie konnten wir zum ersten Mal in einer Freilandstudie zeigen, dass die biotischen Interaktionen zwischen Pilzen und ihren Wirtspflanzen wichtiger sein können als edaphische Eigenschaften des Untersuchungsgebietes. Vorallem die Phylogenie der

Wirtspflanzen spielt eine zentrale Rolle für die Zusammensetzung der Pilzgemeinschaften.

In Kapitel 3 und 4 wurde analysiert, wie wichtig die Diversität von arbuskulären Mykorrhizapilzen (im folgenden nur Mykorrhizapilze genannt) ist, um ihre Wirtspflanzen vor Pathogenen zu schützen. Dafür wurde zunächst eine Literaturstudie durchgeführt. In dieser wurde kritisch hinterfragt, was passieren würde, wenn man anstelle von einem Mykorrhizapilz, eine ganze Gemeinschaft hinsichtlich der unterschiedlichen Mechanismen des Mykorrhizapilz induzierten Pathogenschutzes betrachten würde. In einem Gewächshausexperiment testen wir den direkten Einfluss einer lokalen Mykorrhizapilzgemeinschaft und einer saprobisch/pathogenen Pilzgemeinschaft auf das Wachstum von zwei verschiedenen Wirtspflanzen aus dem selben Herkunftsgebiet, "Oderhänge Mallnow" in sterilem Boden.

Durch die Literaturstudie fanden wir Hinweise, dass die Diversität von Mykorrhizapilzen eine wichtige Rolle für die unterschiedlichen Mechanismen des Mykorrhizapilz induzierten Schutz vor Pathogenen spielt und das man sie daher zukünftig mit betrachten sollte.

In unserem Gewächshausexperiment konnten die negativen Effekte der saprobisch/pathogenen Pilzgemeinschaft auf das Wachstum der Wirtspflanzen durch die Anwesenheit von Mykorrhizapilzen kompensiert werden. Die Wurzelkolonisation mit nicht-Mykorrhizapilzen war in Anwesenheit von Mykorrhizapilzen signifikant reduziert im Vergleich zu der Behandlung ohne Mykorrhizapilzen. Die gefundenen Ergebnisse deuten daraufhin, dass Interaktionen zwischen Gemeinschaften aus nützlichen und pathogenen Mikroorganismen das Pflanzenwachstum beeinflussen können. Die Größe dieses Effekts wird aber stark von der jeweiligen Pflanzenart abhängen.

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Contributions to Chapters

Chapter 2: Wehner J, Powell JR, Muller LAH, Caruso T, Veresoglou SD, Hempel S, Rillig MC. Determinants of root-associated Eumycota communities within Asteraceae in a semiarid grassland. (*submitted* to Journal of Ecology)

Own contributions: This work was a DNA based field study, where the different Authors contributed to different parts during data processing, analysing and writing. I designed the experminentell set up together with Jeff Powell and Matthias Rillig, did the field sampling with help of Stefan Hempel and Ruth Lintermann, conducted the lab work with some help of Ruth Lintermann, processed the raw sequence data, did the statistical analysis in R with the help of Tancredi Caruso and Jeff Powell and wrote the manuscript.

Chapter 3: Wehner J, Antunes PM, Powell JR, Mazukatow M, Rillig MC (2010). Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? *Pedobiologia*, **53**: 197-201.

Own contributions: This work was a colletive literature study, where different Authors contributed too. I coordinated the writing of the manuscript and wrote the introduction section: *Why consider diversity?*, the section about *Improved nutrient status of the host plant*, the section about *Anatomical or architectural changes in the root system* and the Conclusion.

Chapter 4: Wehner J, Antunes PM, Powell JR, Caruso T, Rillig MC (2011). Indigenous arbuscular mycorrhizal fungal assemblages protect grassland host plants from pathogens. *PLoSone*, **6**: e27381.

Own contributions: This work was a greenhouse study where different Authors contributed to the experimentell design and statistical analysis. I designed the greenhouse experiment together with Pedro Antunes, who also helped by collecting soil from the study site. I run the greenhouse experiment and performed the lab work, did the statistical anaylsis (with the help of Tancredi Caruso) and wrote the manuscript.

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