Rules of acquisition: small-scale field studies on arbuscular mycorrhizal fungal and plant communities

Inaugural-Dissertation to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)

submitted to the
Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

by Sebastian Horn

Berlin, March 2015

This work was carried out between 2011 and 2015

under the supervision of

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Date of defense:

11.05.2015

Foreword

This dissertation is a cumulative work of three manuscripts, either published or in preparation for submission.

Therefore, this thesis is based on following papers, which are referred by their Roman numerals.

I) **Horn S**, Hempel S, Ristow M, Rillig MC, Kowarik I, Caruso T (2015). Plant community assembly at small scales: Spatial vs. environmental factors in a European grassland. *Acta Oecol* **63:** 56-62. https://dx.doi.org/10.1016/j.actao.2015.01.004

II) **Horn S**, Caruso T, Verbruggen E, Rillig MC, Hempel S (2014). Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. *ISME J* **8:** 2231-2242. https://dx.doi.org/10.1038/ismej.2014.72

III) **Horn S**, Caruso T, Verbruggen E, Rillig MC, Hempel S (2015). Does neighborhood plant community structure affect the AMF community of focal plants at small spatial scales? *submitted*.

Acknowledgements

Boy, what a ride. When I first started studying Biology, I was not really thinking I could ever complete a Ph.D. Now that I finished this incredible challenge, I feel different, but nevertheless, I am happy and proud, and incredibly thankful for all the people who have supported and believed in me until now.

First, I'd like to thank my supervisor Prof. Matthias Rillig for the opportunity to conduct research in an amazingly inspiring and supportive atmosphere, which greatly helped me to evolve into the scientist I am today.

I am also very grateful for all the colleagues that supplied me with support, scientific and non-scientific discussions, statistical insights and significant contributions to my sanity along the path, namely Dr. Stefan Hempel, Dr. Tancredi Caruso, Dr. Erik Verbruggen, Prof. Ingo Kowarik, my office girls Dr. Anika Lehmann, Dr. Tessa Camenzind and Weishuang Zheng, and all the other people from the Plant Ecology Lab.

I would also like to thank my family for their love and support. My deepest gratitude belongs to Dr. Nicola Pitsch, for without her I would not be where I am today. Her everlasting patience, understanding and undoubted belief in me has contributed so much more than she could imagine.

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Chapter I: General Introduction

Community ecology & the concepts of community assembly

The study of organism communities in the field using mathematical means and statistics is almost a century old. The principal view that species coexistence is based on the avoidance of competition for resources via trade-offs in life-traits forms the foundation of classical community ecology (Hall et al., 2003, Tilman and May 1982, Tilman and Downing 1994). Mathematical models describing the relationships between organisms went from being purely deterministic to the widely established niche-framework, with the introduction of demographic stochasticity to describe species diversity limits (MacArthur 1970, Tilman 2004). This framework was challenged by Hubbell with the proposition that community structures can be described solely by dispersal limitation and demographic stochasticity (Chave 2004, Gotelli and McGill 2006, Hubbell 2001). Although contradictory in their principal assumptions, niche and neutral processes were found to be convergent in terms of their abilities to predict species abundance distributions (Chave et al., 2002). Depending on the processes dominant in an ecosystem, community dynamics may change from patterns that are best modeled by purely neutral dynamics to niche-partitioning based competition systems (Cottenie 2005). Additionally to niche and neutral dynamics, other paradigms have been shown to be able to predict the assembly of communities, like priority effects (Alford and Wilbur 1985, Plückers et al., 2013), multitrophic networks (Cardinale et al., 2006, Van Der Putten 2009), or metacommunity frameworks (Leibold et al., 2004, Pillar and Duarte 2010), plus subsequent combinations of these approaches.

Soil ecosystems have several properties that make them ideal for the investigation of the relative roles of processes shaping ecosystems. The high species diversity within a distances of few cm to several m allows for the incorporation of processes operating at different scales in a single sampling, while sampling density can be sufficiently high to capture the majority of interactions. Additionally, many soil-borne species have limited dispersal capability (Mummey and Rillig 2008, Wolfe et al., 2006). The lack of studies addressing this topic with rigorous methodology proposed by theoretical biologists (Cottenie 2005, Gotelli 2000) as well as the absence of studies on multiple taxa and multiple scales

calls for further investigation of this subject. This work focuses on one of the key species groups in soil microbial communities, arbuscular mycorrhizal fungi, since they are closely connected to plants and their community ecology is only scarcely explored. Studies on AMF have established some interesting insights into the patterns underlying their community assembly (Dumbrell et al., 2010a, Dumbrell et al., 2010b, Lekberg et al., 2007), however, they lack spatially explicit design and therefore have difficulties disentangling the relative roles of niche and neutral processes in relation to the scale at which they did their analysis. This work aims at solving these obstacles.

The spatial scale at which field studies are conducted can directly link to the gained results and hence heavily influence conclusions and actions taken from these findings. The question at which scale competitive dynamics occur and how it relates to dispersal limitation needs to be taken into account. Plant species composition has been related to AMF diversity (Hiiesalu et al., 2014) and AMF community changes have been found to be associated with neighboring plants (Vandenkoornhuyse et al., 2003). The contrasting findings may be related to the different scales involved. For example, it has been shown that AMF can be highly structured at the sub-meter scale (Mummey and Rillig 2008), where dispersal and niche processes are prominent, missing out on the scales at which neutral processes take place. While hyphal networks have been shown to encompass large spatial areas, the sphere of interaction of a single AMF individual with the soil may be significantly smaller in comparison. It is therefore important to acknowledge this in designing field studies to assess community dynamics properly. The current work therefore uses a spatially explicit design that is capable of detecting both neutral and niche processes at the same time.

Oderhänge Mallnow - a study system for fungi and plants

In order to study the aforementioned processes in a sufficient manner, a study system is needed that fits the requirements of spatially explicit design and the capture of both niche and neutral dynamics. A high plant diversity is a necessity as well as a significant change of environmental characteristics over a short distance. Since this work addresses soil biota, environmental changes are primarily related to soil parameters like pH, phosphorus and carbon content. Dry, nutrient-poor grasslands are interesting ecosystems for the study of AMF, since they can harbor a lot of mycorrhizal plant species and the

plant communities in grasslands can be quite diverse. They often bear drastic changes in soil conditions, however, mostly these changes occur over the course of a few hundred meters to several kilometers. The Oderhänge Mallnow, located in Brandenburg, Germany, offer steep environmental gradients over the course of only a few meters. This region is a biodiversity hotspot which contains over 200 plant species and combines features of both steppes and oceanic habitats (Ristow et al., 2011). Being a historical battleground during World War II, the landscape is shaped by small hills and valleys, making it overall quite diverse. Some woody patches exist, but a significant amount of the 1200 square kilometer region consists of dry grasslands, dominated by grass species like *Arrhenatherum elatius*, *Festuca brevipila*, or *Stipa capillata*. It is a natural reserve, harboring several endangered plant species like *Adonis vernalis*, *Aster linosyris*, *Campanula sibirica* and *Hieracium echioides*. In this work slopes of the hillsides have been studied (Chapter 2, Fig. 1), since they offer the properties sought after: high diversity both in plants and root-associated fungi, and steep gradients in soil properties over relatively small scales below 15 meters (Wehner et al., 2014).

A brief overview of AMF research

The kingdom of fungi features a vast amount of ecologically and economically important species, including large amounts of saprotrophs, pathogens and mutualists in ecosystems worldwide. Mycorrhizal fungi are of particular importance since they associate with the majority of land plants (Smith and Read 2008) and bear a major role in acquiring nutrients for their plant host, therefore being of tremendous value both for plant diversity and agroecology. The group of mycorrhizal fungi can be divided into several subgroups that are historically classified by the phenotypical appearance of the association and the host plant, but have also received classification by phylogenetics in the recent two decades. Briefly, the main groups are ectomycorrhizal, ericoid mycorrhizal and arbuscular mycorrhizal fungi. Ectomycorrhizal fungi primarily associate with trees, infect root hairs and form a Hartig's net on the outside of the root. Ericoid mycorrhiza species primarily associate with plants of the Ericaceae and forms fungal coils in the root hairs. Arbuscular mycorrhizal fungi (AMF) associate with the majority of all land plants, forming tree-like structures within the root parenchym cells (Smith and Read 2008).

Due to the association with almost all important crop plants, AMF have received a significant amount of attention in research.

The classification of AMF, mainly cryptic, asexual fungi, has been historically based on spore morphology, but modern sequencing techniques have allowed for a more sophisticated approach and hence improved the understanding of diversity in this fungal group. According to fossils, the history of AMF symbiosis might date back up to 460 million years into the Early Devonian, indicating that their nutrient-transfer mutualism might have co-evolved with plants invading the land (Redecker et al., 2000). In the first descriptions of a genus named 'Glomus', they were only known for clusters of spores in the upper layers of soil, while at the beginning of the 19th century, their role as root-colonizing fungi had been discovered (Butler 1939). Later, evidence was found that a Glomus species was the source of mycorrhizal colonization in strawberries (Mosse 1953). After being assigned their own order *(Benjamin 1979),* a cladistic analysis based on morphology assigned the then-called Glomerales was established (Morton and Benny 1990). While they had originally been classified as imperfect Zygomycota, they now form an independent clade of the Glomeromycota. The phylogeny of the AMF remained highly debated until modern techniques have led to a phylum-level classification that is in use until today (Schüßler et al., 2001, Schüßler and Walker 2010, Stockinger et al., 2010). Together with modern classifications, studies arose that found a diversity among the AM fungi that was considerably higher than previously established with morphological means (Douds and Millner 1999, Lekberg et al., 2007, Lekberg et al., 2013, Torrecillas et al., 2011).

Arbuscular mycorrhizal fungi & their role in ecosystems

AMF form complex hyphal networks, allowing for plants to exchange of nutrients and allelochemicals over a significant distance far beyond the reach of the root system of a single plant (Achatz and Rillig 2014). They therefore extend the plant‘s ability to acquire phosphorous, but also nitrogen (Veresoglou et al., 2012), in exchange for carbon (Smith and Read 2008). The important process of soil aggregation is influenced by AMF (Leifheit et al., 2015), interactions between soil-borne pathogens and soil microarthropods have also been found (Hishi and Takeda 2008, Whipps 2004). A significant amount of soils on earth are nutrient-limited, and particularly nitrogen and phosphorous limitations are majorly

important for plant growth. AMF have an important role in the acquisition of phosphorous in soil for plants, therefore they provide a significant contribution to the plant diversity. They influence the possibilities of plant establishment in soils and extend the realized niche of several plant families (Bever et al., 2010, Bever et al., 2012). It has been shown that fungi and plant diversity can influence each other on several levels (Hiiesalu et al., 2014), which points out the importance of including fungal biodiversity in the judgment of conservation plans and restoration efforts. They may aid or prevent the establishment of invasive species (Williams et al., 2011), influence the biological activity of soil (Alguacil et al., 2014) and provide a possibility for controlling pathogens (Veresoglou and Rillig 2012).

While AMF have been demonstrated to carry significant ecosystem functions, the way how and why AMF species interact to form the diverse communities is still an active and developing topic in both ecology and molecular biology. AMF possess several traits that make them hard to study in a lab, including the obligate biotrophy (Smith and Read 2008), a supposed lack of a sexual lifecycle (Croll et al., 2009, Croll and Sanders 2009), heteromorphic spores (Walker and Vestberg 1998) or multiple karyotypes per nucleus (Hijri and Sanders 2004), while the assessment of diversity in the field has only been promoted to the species level identification since the development of next-generation sequencing tools.

One approach to AMF community assembly rules assumes that the fungi associate primarily via interactions with the environment, namely through niche-partitioning based species-sorting. Additionally, spatial effects like dispersal limitation have been shown to shape AMF community diversity on several spatial scales (Dumbrell et al., 2010a, Dumbrell et al., 2011, Lekberg et al., 2007, Lekberg et al., 2011). Another way to deal with AMF community composition is to look at the symbiotic partner, the plants, and their way how AMF and plants influence each other. The Passenger and Driver framework aims to account for the roles of plants shaping AMF and vice versa, while leaving the interactions with the environment as a null hypothesis (Zobel and Öpik 2014). This work is aimed at searching for evidence for both systemic and biotic processes in shaping of AM fungal communities.

DNA-based assessment of AMF communities

This work utilizes extensive DNA sequencing for studying AM fungal community composition; therefore a brief introduction of techniques and tools shall be given. The development of sequencing technology has made great improvements over the last ten years. While classical Sanger-sequencing only yields one sequence per run, current state-of-the-art sequencing tools enable the generation of billions of fragments in only one experimental procedure. While initially developed to deliver a cheap and fast technology for genome sequencing (chiefly the Human Genome Project), community ecologists quickly became aware of the benefits and possibilities of this new method. Traditional Sanger sequencing works by replicating target fragments one nucleotide after another and randomly introducing tagged nucleotides fused with a distinct fluorophore unique to each type of nucleotide that results in the termination of the replication process. The fragments then are separated from the donor strand and ordered according to their length. Detecting the fluorophore at the end of each fragment, from the smallest to the largest one, results in the sequence of a given molecule. Next-generation pyrosequencing sticks with the single-nucleotide detection system, but extends it to a massive parallel scale that creates millions of sequences in one run (Margulies et al., 2005).

The DNA strands which are to be amplified are tagged with an adapter and a barcode for later amplification and separated into single stands. In a first step, they are amplified in an emulsion-based PCR, where they are mixed with 28µm metal beads in a ratio that binds one molecule of DNA on one single bead. On the surface of these metal beads the amplification takes place, creating millions of copies of the target sequence bound to the metal. The beads loaded with DNA strands of one type are then distributed on a plate with picoliter wells, one bead per well, and the wells are filled with smaller beads carrying immobilized enzymes. The plate is subsequently flushed with solutions containing one type of nucleotide each in a cycle. Nucleotide incorporation is detected by the associated release of inorganic pyrophosphate, which is converted to a light emission via a set of enzymes (Ronaghi et al., 1998) and detected with a CCD camera. The information of flow nucleotide content in combination with the CCD sensor data allows for reconstruction of the sequence in each well with a bead. Bioinformatics tools help with distinguishing background and artifacts from real sequences (Hao et al., 2011, Quince et al., 2009, Quince et al., 2011).

Thesis outline

This work will provide evidence for processes leading to the assembly of plant and AM fungal communities. In Chapter II, the assembly rules that the plants in our ecosystem follow are analyzed, determining the influence of spatial and environmental patterns on the plant community composition. In Chapter III, the focus lies on AMF species and their community assembly patterns, linking 454-pyrosequencing data with environmental, spatial and phylogenetic patterns in order to answer how individual communities of AM fungi are assembled the way they are. In Chapter IV, the two organism groups are being combined in the analysis, and the sampling extent is increased. Therefore, a more detailed picture of the influence of AMF on plants or vice-versa can be drawn, comparing the relative roles of systemic and biotic patterns on community assembly. The results of the three chapters will be subjected to a general discussion in Chapter V.

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Chapter II: Plant community assembly at small scales: spatial vs. environmental factors in a European grassland

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Abstract

Dispersal limitation and environmental conditions are crucial drivers of plant species distribution and establishment. As these factors operate at different spatial scales, we asked: Do the environmental factors known to determine community assembly at broad scales operate at fine scales (few meters)? How much do these factors account for community variation at fine scales? In which way do biotic and abiotic interactions drive changes in species composition?

We surveyed the plant community within a dry grassland along a very steep gradient of soil characteristics like pH and nutrients. We used a spatially explicit sampling design, based on three replicated macroplots of 15x15, 12x12 and 12x12 meters in extent. Soil samples were taken to quantify several soil properties (carbon, nitrogen, plant available phosphorus, pH, water content and dehydrogenase activity as a proxy for overall microbial activity). We performed variance partitioning to assess the effect of these variables on plant composition and statistically controlled for spatial autocorrelation via eigenvector mapping. We also applied null model analysis to test for non-random patterns in species co-occurrence using randomization schemes that account for patterns expected under species interactions.

At a fine spatial scale, environmental factors explained 18% of variation when controlling for spatial autocorrelation in the distribution of plant species, whereas purely spatial processes accounted for 14% variation. Null model analysis showed that species spatially segregated in a non-random way and these spatial patterns could be due to a combination of environmental filtering and biotic interactions. Our grassland study suggests that environmental factors found to be directly relevant in broad scale studies are present also at small scales, but are supplemented by spatial processes and more direct interactions like competition.

Keywords: Assembly pattern; Dispersal limitation; *Festuca brevipila*; Niche partitioning; Null model; Plant community ecology; Variance partitioning

Introduction

Plant community assembly is significantly driven by processes on several scales, like competition (Aarssen, 1989), dispersal limitation (Ai et al., 2012) and environmental conditions (Latimer and Jacobs, 2012). Understanding the processes involved in the assembly of communities is considered one of the most important challenges in ecology today (HilleRisLambers et al., 2012; O'Neill, 1989; Turner and O'Neill, 1995). While the understanding of community assembly has advanced significantly within the last 50 years, ecologists still lack precise insight on how the interplay of organisms and their environment determines the structure of natural communities (Götzenberger et al., 2012; Naaf and Wulf, 2012).

One common idea in ecology about the assembly of a diverse community involves filtering by the environment and interactions of organisms that establish local populations. This led to the niche-partitioning concept (Leibold, 1995; Silva and Batalha, 2011), where assemblages of species are viewed as having different tolerances to the abiotic environment and differing abilities to exploit resources. With the rise of neutral theory (Hubbell, 2001; Rosindell et al., 2012), the debate on the processes influencing biodiversity was reinvigorated and the search for a unified theory has dominated the field (Adler et al., 2007). It has been suggested that the combination of investigating both local and short-term mechanisms as well as regional processes occurring over longer timescales may be crucial for a complete understanding of ecosystem assembly and function (HilleRisLambers et al., 2012).

Grasslands cover one fourth of the Earth's land surface and harbour the majority of annual plant diversity (Shantz, 1954). A significant amount of studies on grassland ecosystems are focused on the influence of soil characteristics on plant community composition (Wellstein et al., 2007), which, together with water, wind and sunlight, represents the bulk of abiotic influences on a plant community (Callaway, 1997; Parfitt et al., 2010). Soil characteristics can be strong predictors of plant community composition (Gough et al., 2000; Tilman and Olff, 1991), although the scale of the studies influences the predictive power of soil parameters like pH, carbon, nitrogen or phosphorus content (Sebastiá, 2004). But not only abiotic factors are influenced by the scale of a study; positive and negative

interspecies associations can occur at small scales and disappear with increasing scale (Wiegand et al., 2012).

In this study we aimed at increasing the understanding of scale-dependence in community patterns by analysing the plant community composition of a semi-natural grassland (Leibold et al., 2004). While a lot of studies on grasslands are trying to approach community composition mechanisms by inferring local interactions via the observation of larger-scale composition (Eckhardt et al., 1996; Thomas and Palmer, 2007; Toogood et al., 2008), we were aiming at understanding these processes by looking for patterns of species composition that could be either deterministically or stochastically structured while choosing the smallest local community possible: a single focal plant and its direct rhizosphere interaction partners, making the community unit as small and replicable as possible. Other small-scale studies have dealt with similar grain sizes like ours (Chu et al., 2007; Reitalu et al., 2009; Turtureanu et al., 2014), however, they do not approach single plants with their rhizosphere environment or combine small grain and extent. We consider the single plant rhizosphere environment a community, implicitly embodying the idea of interactions of plants with the environment and each other.

Our study area offers unique possibilities of studying steep environmental gradients within only a few meters in very species-rich grassland which also harbours one highly abundant plant species, enabling us to observe potential environmental filtering as well as spatial processes and biotic interactions in a spatially well-defined small-scale area. We selected this plant species, namely *Festuca brevipila* R. TRACEY (Aiken and Darbyshire, 1990; Klotz et al., 2002), as our focal plant to be able to target the whole gradient of environmental conditions which our study area offers, and still be able to standardize the community perspective on one species. We used patterns of co-variation among plant species, environmental and spatial variables derived from a neighbour matrix to answer the following questions: i) Do the environmental factors, specifically soil properties, that are known to determine community assembly at broad scales also operate at fine scales (1-15 meters) and how much do these factors account for community variation at fine scales? ii) In which way do biotic and abiotic factors drive changes in species composition? Our questions involve the disentanglement of patterns at various small scales, which calls for tools able to quantify the contributions of environmental and spatial patterns plus their shared effect. We therefore applied state-of-the-art multivariate analysis

(Borcard et al., 1992; Dray et al., 2006) to test our hypotheses and contrast patterns due to environmental variables with spatial patterns potentially due to biotic interactions (e.g. segregation caused by competition) and/or dispersal dynamics. Large-scale environmental effects that determine plant community structure in a range from a few to several hundred kilometres, include climatic gradients (Ludewig et al., 2014), altitudinal changes (Krömer et al., 2013) or differences in soil biogeochemistry (Khan et al., 2013). At the small scale of our study we focused on soil since this is the only variable forming gradients at such scales. Although our sampling design captured strong gradients in soil variables, we expected a relatively smaller influence of environmental variables on our plant communities in comparison to larger scaled studies, because biotic interactions or neutral-like effects could outweigh environmental drivers at the small scale of our study.

Materials and Methods

Data collection

The grassland studied is situated in a natural reserve (Mallnow Lebus, Brandenburg, Germany, 52°27.778' N, 14°29.349' E). The region is influenced by sub-continental climate with a mean annual precipitation of below 500 mm (Ristow et al., 2011) and the area is managed by sheep grazing twice a year. The sampling strategy was based on a hierarchical nesting of macroplots and plots, and was done at the end of June 2011 to minimize influences by spring ephemerals. Three macroplots of 15 x 15, 12 x 12 and 12 x 12 meters, respectively (Fig. 1), were located on the slopes of hills in an area of about five hectares. We found only minor traces of sheep trails which indicate a low grazing pressure on our macroplots, likely due to the strong slope. We ensured that all macroplots were part of two closely related grassland communities found in Mallnow, namely *Sileno otitae-Festucetum-brevipilae* Libbert 1933 corr. Kartzert & Dengler 1999 and *Festuco psammophilae-Koelerietum glaucae* Klika 1931. Our macroplots were comparable concerning vegetation and soil related factors like distance from trees, stone content or depth of A-horizon, as well as slope and sun exposure, and therefore can be considered a replicated design. The uphill-downhill axes of the macroplots are characterized by a steep textural gradient from highly sandy (downhill macroplot) to sandy-loamy (uphill macroplot) soils. Preliminary analyses revealed that this gradient causes gradients in many other soil parameters, namely pH, carbon, nitrogen and plant available phosphorus. Each macroplot was divided into 3 x 3 m plots (Fig. 1). From each macroplot the vegetation of the four corner plots (top left, top right, bottom left, bottom right) was sampled: For the measurement of soil properties one soil core per plant was taken atop of five randomly chosen *F. brevipila* plants per plot, creating 60 samples in total. In a radius of 15 cm around the chosen *F. brevipila* plant, the local plant community was assessed visually as presence or absence of plant species. This sampling unit represents our main community unit and below we refer to it as "sample". With regard to the smallest sampling unit ("sample"), the 15 cm radius ensures that interactions within the rhizosphere of *F. brevipila* plants were captured. We preferred this method to a totally random location of the sampling units (i.e. not having a focal species) for the following mutually reinforcing reasons: a random location would have been strongly

biased towards *F. brevipila* in a non-controlled way because *F. brevipila* is by far the most abundant species in the area (in some case the species can cover up to 70 % of one plot); by controlling for this critical source of certain bias, we could minimise possible very small scale environmental heterogeneity that could confound the interpretation of co-occurrence analysis based on null models (see methods below) and the comparison between null models and multivariate analyses based on RDA; the plant assemblage can be objectively defined at a biologically meaningful small scale (i.e. rhizosphere) as the neighbourhood community of the dominant species. This makes the community unit highly replicable: the average composition of this particular but representative assemblage can be assessed throughout plots and macroplots as a function of changes in the environment and the effects of the environments on how species interact within this assemblage. By having a focal species and defining the assemblage as a function of it, we lost some degree of generality but it is also true that our focal species and the genus to which it belongs (*Festuca*) is one of the most abundant if not the most abundant and widespread taxon in dry grasslands. Thus, we could compare total plant species richness of each plot with the species richness found in the proximity of each of the five randomly sampled *F. brevipila* plant per plot. The corner plots were chosen to use the maximum of the environmental gradient along one direction (the downhill-uphill axis) and a minimum of it in the direction orthogonal to the environmental gradient while keeping the spatial distances between plots equal. Thus, the three macroplots represent three spatial replicates while the environmental gradient is replicated twice within each macroplot.

Each soil core was thoroughly homogenized and representatively subsampled for the different analyses. Soil water content was measured as the weight difference between fresh and oven-dried soil cores. Soil carbon and nitrogen analysis was performed on a EuroEA 3000 Elemental Analyser (EuroVector, Milano, Spain) with a TDC detector using 25 mg of pulverized soil per core. Soil pH was measured in 10 mM $CaCl_2$ solution (van Lierop and Mackenzie, 1977) using 3 grams of soil per core. Plant available phosphorus was characterized following the CAL-method (Sparks, 1996) using 1 gram of soil per core. Dehydrogenase assays were conducted according to Rossel (1997), using 1 gram of soil per core.

Statistical analysis

Normality was checked with the Kolmogorov–Smirnov test and variables were transformed to meet the requirements of parametric analysis when necessary. The subsequent analysis of patterns in community structure was conducted in R 2.15.2 (R Core Team, 2013), with functions from the vegan (Oksanen et al., 2012) and the SPACEMAKER (Dray, 2011) package. Source code from the analysis in R is provided in the supporting information.

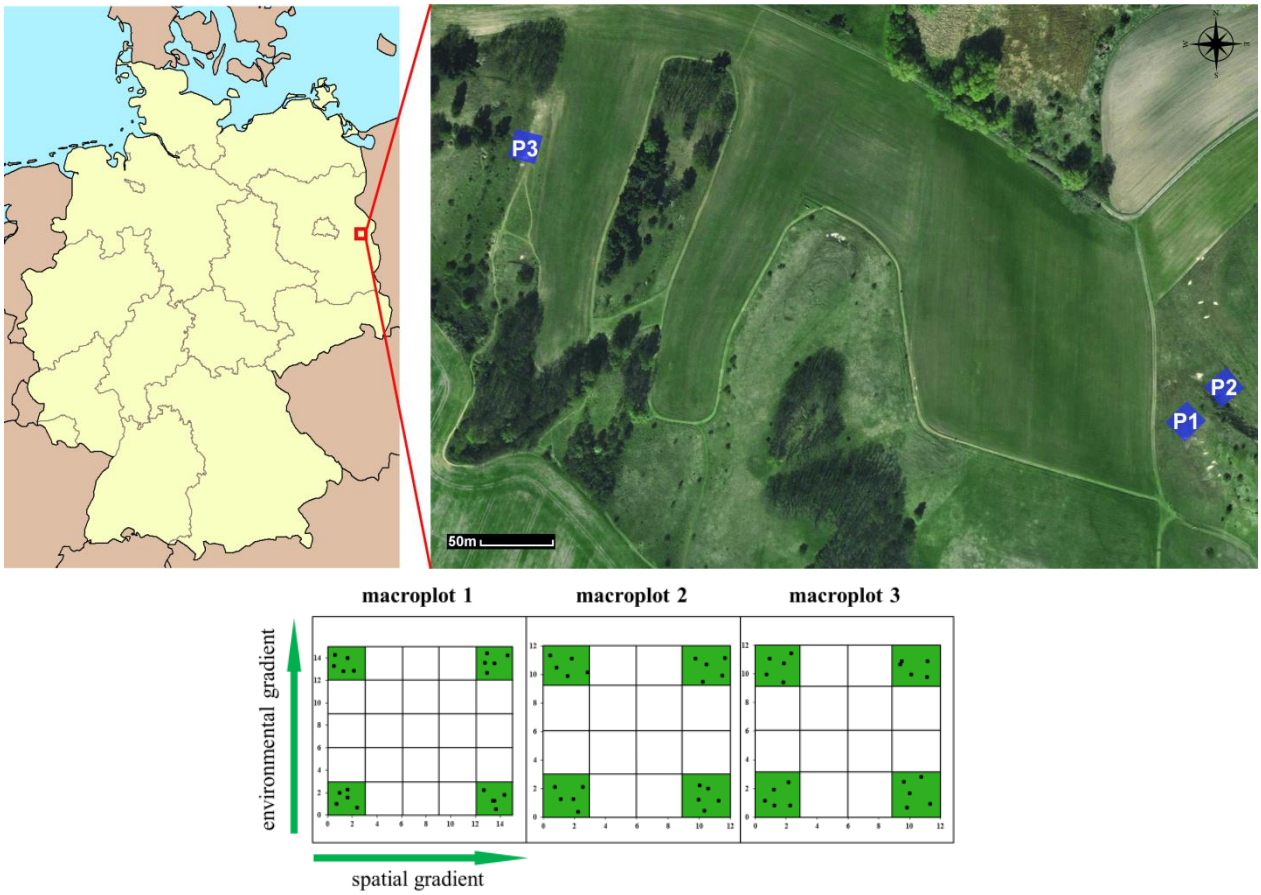

Figure 1: Sampling location and sampling design: all three macroplots were located on a hill slope in a German natural reserve close to the Polish border, offering a high environmental gradient within a few meters. A: A general map of Germany with the sampling area as red rectangle (left) and a satellite picture of the sampling area (right) (Google, 2013). Purple rectangles labelled as P1, P2 and P3 depict the location and orientation of the three macroplots. B: Diagrams of the three macroplots. The spatial gradient is oriented orthogonally to the environmental gradient. From the four corner plots (green), five *F. brevipila* plants were sampled randomly as described in the materials and methods section (black dots). Numbers on the diagrams represent the size of the respective macroplots in meters.

We created a presence / absence matrix for the plant species recorded in each sample, containing 60 samples and 68 identified plant species. Environmental factors for each sample were summarized in a matrix containing seven columns for the factors and 59 rows for the samples. Eventually, one row had to be omitted from all matrices since one soil core was lost prior to analysis. All the species matrices including the subsets used were stripped from zero-occurrences spots and species, respectively, prior to the subsequent analysis. For completeness reasons, the whole species matrix is included in the supplementary information. Multivariate analysis was done on a per-sample basis, while the null model analysis was conducted on various subsets of the whole data matrix (see below).

Species data were Hellinger transformed and subjected to a multivariate analysis to disentangle spatial and environmental factors influencing community variation (Legendre and Legendre, 1998). At first a canonical correspondence analysis (CCA) was conducted with the coordinates of the samples as constraints in order to remove linear spatial patterns. The remaining spatial patterns of the detrended community data were summarized, together with the geographical distance matrix of the samples, in the Moran eigenvector mapping matrix (MEM) that best accounted for autocorrelation (Dray et al., 2006). The final spatial matrix used for analysis then contained both the MEMs and the linear trends. Spatial autocorrelation represents the predictability of a locally observed response value by response variables observed in the surrounding area (Legendre, 1993). The MEM is calculated by multiplying a connectivity binary matrix with a weighting matrix. The connectivity matrix represents a graph in which samples are connected as networks while the weighting matrix is used to quantify the sample dissimilarity by weighting each link of the network (Caruso et al., 2012). In order to test multiple spatial patterns, the connectivity and/or weighting algorithms were modified and the best model was selected following the Akaike Information Criterion AIC (Akaike, 1973). Thus, the best linear combination of eigenvectors was chosen so the correlation with the data would be maximal and the AIC values would be minimal (Dray et al., 2006). An extracted eigenvector summarizes spatial patterns at a given scale; therefore the cumulative matrix of eigenvectors can describe several spatial scales. This matrix then can be used in multivariate regression approaches to predict spatial patterns (Dray et al., 2006). The eigenvector method we utilised is able to detect patterns down to a scale of 1m, which equals roughly twice the average distance between our samples. We used redundancy

analysis (RDA) and variance partitioning to resolve the contribution of environmental and spatial factors to the total variance (Legendre and Legendre, 1998). The community matrix was used as response matrix and measured environmental factors like carbon, nitrogen or pH, plus the MEM vectors representing spatial autocorrelation were used as explanatory factors for the response matrix. Since plants tend to respond more strongly to a change in nutrient availability when the nutrient is scarce than when it is abundant, we followed suggestions by Jones et al. (2008) and tried to transform the environmental data by taking their natural logarithm prior to variance partitioning. However, this did not change the results compared to untransformed data (see Table S4), we therefore only report results from the latter dataset. Variance partitioning is a tool to quantify the unique contribution of these two components plus the spatial patterning shared by the environmental data (Borcard et al., 1992). Multivariate variances were visualized using principal coordinate analysis (Anderson and Willis, 2003). Each of the variance partitions was subjected to a constrained redundancy analysis and subsequent statistical test at $P < 0.05$, based on permutation (Oksanen et al., 2012). We applied automatic stepwise model building for constrained ordination methods using the ordistep function (Blanchet et al., 2008) with forward and backwards selection to include important environmental variables only and calculate their respective P-values.

Since mosses and lichens can affect seedling establishment of higher plants (Soudzilovskaia et al., 2011), their cover was considered as an additional environmental factor; however, this did not increase the variance explained by the environment (data not shown). Lichens and mosses were thus excluded from further analysis albeit their inclusion slightly increased the explained variation of the spatial component.

Since variance partitioning quantifies variation in our community data but does not indicate a positive or negative trend in terms of species coexistence, which is necessary to infer biotic interactions, we applied a null model analysis in PAIRS (Ulrich, 2008). In our null model analysis the C-score index was used to compute values of co-occurrence for the given set of presence/absence data. Since the C-score does not require perfect checkerboard distributions and has a low susceptibility to type II errors, it seemed best suited for our purpose (Gotelli, 2000). The input matrix was randomized following the suggestions of Gotelli (2000) to minimize type I errors and test for patterns of co-occurrence expected

under non-random assembly processes and interacting species. The algorithm used fixed sums of rows and sums of columns and applied the Random Knight's Tour approach for shuffling the matrix. Retaining the row and column totals preserves differences in species richness among sites and differences in occurrence frequencies among species, therefore representing a conservative approach when testing for patterns in species composition. We applied a nestedness analysis using the matrix temperature method (Atmar and Patterson, 1993). Since the results indicated a strongly nested community, the data set was split up according to geographic orientation, and in addition to the whole community matrix, the subsets of the top, bottom, left and right plots were each subjected to a null model analysis. The top and bottom subsets represent the spatial distance since the gradient in each subset is minimized. The left and right subsets represent the whole gradient together with the spatial component (see Fig. 1) In addition, we included a subset of the diagonal patterns (that is, the top left plus the bottom right plots and the top right plus the bottom left plots) in order to account for potential tilting of the gradient orientation (compare Fig. 1B).

The null hypothesis was considered rejected when the observed C-Score was significantly different from the average simulated C-Scores ($P < 0.05$). A C-score lower than the simulated average represents an aggregated community, while a higher score represents a segregating community. Standardized effect sizes were used to compare results meaningfully and calculate probability values. The effect size is calculated as (observed C-score - simulated C-score) / (standard deviation of simulated C-score). Negative standardized effect sizes indicate that the observed index was less than the mean of the simulated indices while positive values indicate that the observed index was greater than the mean of the simulated indices (Gotelli and Entsminger, 2012).

Results

Sampling

We detected a total of 68 herb and grass species plus five different species of mosses and lichens in the survey of the entire plots, outlining the high species richness found in our sample region. Out of these herb and grass species, 47 species were found inside the 15 cm radius environment of sampled *Festuca brevipila* plants (see Table S1). Species not found in the 15 cm radius around samples were excluded from the species matrix prior to analysis so no zero-occurrences were present in the matrices subsequently used. The majority of plant cover was attributed to the grasses *Festuca brevipila* and *F. psammophila*, accounting together for up to 70% of the plant canopy in a plot. Other abundant plants were *Arrhenatherum elatius*, *Carex humilis* and *Rumex acetosella*, which are all common representatives of sunny-dry nutrient poor grassland habitats (Hensen, 1997).

All plots showed steep gradients in pH, carbon and nitrogen content (Fig. 1, Table S2), with macroplot 3 being generally richer in nutrients than macroplots 1 and 2. Plant available phosphorus content was poor in all three macroplots, ranging from 8.7 mg/kg soil to 42.2 mg/kg. Soil C to N ratios ranged between 11:1 and 23:1. Measured pH ranged from 3.7 to 7.6, encompassing four orders of magnitude in pH change. Macroplot 1 represented almost the whole pH range, while macroplot 2 was more acidic and macroplot 3 more alkaline than macroplot 1. Distances between samples in the plots ranged from 0.32 meters to 2.6 meters, with an average of 1.56 metres.

Variance partitioning

From the different models tested for the MEMs, the "Nearest Neighbour" approach for calculating the connectivity matrix with a concave-down weighting function yielded the lowest AIC and was subsequently used for calculating the eigenvector maps. The spatial component of the variation could be described by five low-rank MEMs and one high-ranking MEM, pointing out that in our community spatial influences are predominantly small-scaled, that is to say there is more significant spatial structure within plots and macroplots than between macroplots. The variance partition attributed 17.9% of the community variation to spatially independent environmental variables, from which

carbon, nitrogen and pH were significant at $P < 0.05$ (Table 1). The spatial component represented by the MEMs accounted for 14.5% of the community variation and was highly significant (Table 1), while the spatially structured environmental component (i.e. shared variation between spatial and environmental variables) accounted for 4.7% of the variation. Roughly 63% of variance remained unexplained (Table 1).

When we tested for the effect of environmental variables ignoring spatial autocorrelation (Table 1, second column), all tested environmental factors except water content and microbial activity were significant, indicating that spatial structure in the environment could drive some of the spatial changes observed in the plant community (Table 1, compare first two columns). The linear effect of the linear spatial coordinates alone accounted for 3.6% of total variation.

Table 1: P-Values of the RDA (redundancy analysis) based permutation tests and decomposition of the total variation in the community matrix into unique environmental (soil properties) and spatial (geographic position) components. Significant values are bold. Important variables were selected by applying automatic stepwise model building for constrained ordination methods including forward and backward selection. Values in italic were dismissed in the step-wise selection process from the model. The last line ("explained variation") shows the percentage of explained variation of each component. The amount of unexplained variation was 62.9%. P-values for the environmental variables in the column "env" are based on partial-RDA, which accounts for spatial autocorrelation. P-values for the same variables but in the column "space + env." are based on the RDA that does not correct for spatial autocorrelation, which can therefore include spatially structured environmental effects. Missing values marked with a "-" are non-testable.

component	env.	space + env.	space
Carbon	*0.48*	**0.01**	-
Nitrogen	0.06	**0.05**	-
C/N ratio	*0.85*	*0.21*	-
Phosphorus	0.07	**0.01**	-
pH	**0.01**	**0.01**	-
microbial activity	**0.04**	**0.02**	-
water content	*0.51*	*0.64*	-
cumulative	**<0.01**	-	**<0.01**
explained variation	17.9%	4.7%	14.5%

Null model analysis

The null model analysis revealed that the C-score was consistently higher in the sampled communities than in the random ones, making the matrix overall segregated. This was also true for every subset of the metacommunity we tested. This clearly shows that species associate non-randomly (Table 2). PAIRS reported a list of significant plant pair interactions, which we used to examine types of interactions between plants. When we tested the subsets of the community matrix, we noticed that the difference in effect size was higher for the left and right subset (i.e. along the environmental gradient) than for the top and bottom ones (i.e. orthogonal to the environmental gradient, Table 2). The effect size represents a measure of segregation strength, with larger effects sizes indicating more strongly segregating communities. The results indicate that the spatially structured environment is a bigger segregating factor than the environmental gradient alone, which is consistent with our variance partition results. In order to check for biases in the pooling of the subsets, we also compared the effect sizes of the three macroplots plus the possible two-macroplot-combinations (1 and 2; 2 and 3; 1 and 3), however, the effect sizes were comparable in all three subsets (Table 2). Since some of the individual gradients were not perfectly aligned with the sides of the macroplots, we also examined effect sizes of cross-plot subsets (that is, all plots in the southwest – northeast axis and all plots in the southeast – northwest axis). We noticed that the gradient axis oriented towards the pH causes a less segregating community than the axis oriented towards carbon and nitrogen (Table S2).

Mosses and lichens were not included in the null model analysis; however, including them did not change the result (data not shown).

Table 2: Null model analysis of community variation, using the C-Score index and the algorithm MOD9 in PAIRS as described by Ulrich & Gotelli (Gotelli and Entsminger, 2012; 2007). The effect sizes and P-values of different subdivisions of the plant community matrix are shown. Positive effect sizes imply a segregating community (species repel each other), negative values indicate an aggregating one (species attract each other). P-Values are depicted as stars: * = P<0.05; ** = P<0.01; *** = P<0.001; NS = not significant. The left table represents heterogeneous subsets used for inferences on community composition, while the right table represents homogenous subsets used to check the validity of our heterogeneous subset assumptions.

community matrix	effect size	P
all plots	7.25	***
top plots	5.48	***
bottom plots	3.24	**
left plots	2.24	*
right plots	8.04	***
macroplot 1	6.40	***
macroplot 2	2.75	*
macroplot 3	6.18	***
diagonal with gradient	6.49	***
diagonal w/o gradient	3.31	**

community matrix	effect size	P
macroplot 1 left	3.53	**
macroplot 2 left	1.85	NS
macroplot 3 left	4.41	**
macroplot 1 right	5.54	***
macroplot 2 right	1.55	NS
macroplot 3 right	-1.03	NS
plot 1	-1.19	*
plot 2	0.16	NS
plot 3	0.71	NS
plot 4	0.77	NS
plot 5	3.51	**
plot 6	1.15	NS
plot 7	1.14	NS
plot 8	0.66	NS
plot 9	-0.37	NS
plot 10	0.00	NS
plot 11	-0.15	NS
plot 12	-0.58	NS

Discussion

Do the environmental factors that are known to determine community assembly at broad scales operate at fine scales?

Given the steep gradients and the high species richness we found in our study area, we could initially expect that environmental filtering accounted for significantly more of community variance than the spatial component. We found that the environment is on par with spatial processes similar to the results found in other ecosystems (Li et al., 2011). The fact that environmental filtering plays a significant part in shaping plant communities is a common idea in community ecology (Medinski et al., 2010; Olff and Ritchie, 1998; Payne et al., 2011; Silva and Batalha, 2010; Tilman and Olff, 1991; Wellstein et al., 2007). We included the soil environmental factors that are generally considered important drivers of plant growth and distribution and that should cover the majority of abiotic influences (Bardgett, 2005). Even so, we lack a complete analysis of the micronutrients like Mg, Fe or Zn, and in general any aboveground environmental data like temperature, rainfall distribution or wind strength (even though these macroscopic factors definitely operate at scales much broader than those of our study). This might obscure some patterns currently not attributed to the environmental factors. Nonetheless, given the influence of key parameters like pH or phosphorus and the conservative analysis approach, it is unlikely that measuring more environmental variables would significantly increase the amount of variation accounted for by the environment. In fact, variables such as micronutrients generally correlate well with the general parameters (e.g., C and pH) we have measured. Since every environmental variable was spatially structured in our study area, it is possible that a significant influence from an unmeasured variable would be reflected in the spatial eigenvectors and could therefore be accounted for indirectly. Also, given the variables we measured, it is unlikely that we missed out major environmental predictors of plant communities. Next to the environmental part of the variation, a smaller fraction of variation was accounted for by the spatially structured environment component, which suggests that the environment might exert its effect in a spatially structured fashion (see below). The processes behind patterns found when analyzing communities

oriented along the different environmental gradients via null models may account for a significant part of the variation that remained unexplained after multivariate analysis.

Variance partitioning revealed that roughly half of the explained variance in species composition is due to the spatial position of the plant species in our sampled macroplots, regardless of environmental variation (Table 1). The permutation tests pointed out that the spatial structure of the environment can be a major determinant, given the significance of the environmental terms with and without spatial autocorrelation corrected (Table 1). This suggests that the prevalent environmental filtering could determine changes in species composition via its own spatial structure and/or by interacting with other processes, especially biotic interactions which are expressed by stabilizing niche differences (Hall et al., 2003). The scale of our study is so small that we can consider dispersal limitation a very minor source of spatial variation. Thus, we have a spatially structured effect of the environment, which could also be due to biotic interactions mediated by the environment, plus much spatial variation that neither the environment nor dispersal limitation can account for.

The remaining proportion of unexplained variation in community composition is likely to represent either random variation or variation related to unmeasured variables that are not spatially auto-correlated at the scales considered by our sampling design and MEM method (Table 1). It might be possible that processes in the sub-meter range may be responsible for parts of the unexplained variation; however, our analysis was designed to capture processes taking place between our community sampling units on a scale of more than one meter.

In which way do biotic and abiotic factors drive changes in species composition?

Null model analysis confirmed that changes in species between sampling spots are not random. We found that the segregation of species is higher in our studied area than expected by chance (Table 2).

The effect sizes of different subsets we analyzed were all positive, indicating that the segregating effects are ubiquitous and do not necessarily correlate with spatial changes in the environment, a result consistent with the multivariate results discussed two paragraphs above. Given that it is a fair assumption that dispersal limitation does not play a significant role in our study system, we can thus

assume that negative biotic interactions (consistent with segregation) can act as a structuring factor alongside the environmental filtering processes in our system. We noticed that the effect sizes differed noticeably in certain subsets of our data. These differences can be linked to some characteristics of the gradient in our plots, thereby suggesting a potential effect of environmental gradients exerted via biotic interactions. For example, we see that the plants in the top plots are segregating more strongly, thus we can infer that biotic processes like competitive exclusion should be more prevalent there. In fact, the upper part of the hills was less sandy and more densely populated with generally larger plants, which implies more competition for space or light. It has also been suggested that positive relationships between species are related to stressful conditions and negative relationships to productive environments (Callaway et al., 2002; Walker and Chapin, 1987), which is in consent with our observations given that the upper hill part of our sampling areas is indeed more productive due to higher resource availability (like water, nutrients and sunlight).

Complex interactions among conflicting processes such as competition for space, optimization of space utilization or demand for similar resources can facilitate exclusion (Sebastiá, 2004). We found a large difference in effect size and hence segregation when comparing the left and right subsets of the macroplots, which cannot be attributed just to environmental gradients, but also to patchy processes which remain to be investigated. In part, patterns of variation in the effect size of segregation seem to correlate with some environmental heterogeneity observed within macroplots (Table 2).

We never detected aggregation in any heterogeneous subset of the community matrix, which suggests that environmental filtering can take place mostly via niche partitioning, although care must be taken when inferring these processes from co-occurrence patterns. Given the small scale of our sampling design, we are not likely to find local coexistence, therefore any niche partitioning will be observed as segregation of species. The scale of observation may influence, how positive and negative interactions are related to biodiversity (Gotelli et al., 2010).

Conclusion

Overall, our data supports the hypothesis that at small scales steep environmental gradients share equal importance in structuring the plant assemblage dominated by *Festuca brevipila* with either spatially structured environmental effects or species spatial segregation due to negative interactions or a combination of these two factors. Small scale and high resolution sampling design will in the future allow teasing apart these two factors and scaling up their effects.

Acknowledgements

We would like to thank the Deutsche Forschungsgemeinschaft (DFG) for generous financial support.

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Chapter III: Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales

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Running title: Phylogeny and AMF community assembly

Keywords: arbuscular mycorrhizal fungi / community assembly / phylogenetic clustering / small-scale / spatially explicit / 454-pyrosequencing

Subject Category: Microbial population and community ecology

Abstract

Next-generation sequencing technologies with markers covering the full Glomeromycota phylum were used to uncover phylogenetic community structure of arbuscular mycorrhizal fungi (AMF) associated with *Festuca brevipila.* The study system was a semi-arid grassland with high plant diversity and a steep environmental gradient in pH, C, N, P and soil water content. The AMF community in roots and rhizosphere soil were analyzed separately and consisted of 74 distinct operational taxonomic units (OTUs) in total. Community-level variance partitioning showed that the role of environmental factors in determining AM species composition was marginal when controlling for spatial autocorrelation at multiple scales. Instead, phylogenetic distance and spatial distance were major correlates of AMF communities: OTUs that were more closely related (and which therefore may have similar traits) were more likely to co-occur. This pattern was insensitive to phylogenetic sampling breadth. Given the minor effects of the environment, we propose that at small scales closely related AMF positively associate through biotic factors such as plant-AMF filtering and interactions within the soil biota.

Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority of land plants, facilitating the uptake of nutrients from soil in exchange for plant-assimilated carbon (Smith and Read, 2008). They play important roles in ecosystem functioning through their influence on biogeochemical processes (van der Heijden *et al.*, 2008; Veresoglou *et al.*, 2012) and on the structure and productivity of plant communities (van der Heijden *et al.*, 1998; Jansa *et al.*, 2008). The question which effects predominate in structuring AMF communities remains only partially answered and detailed information on mechanisms is sparse in spite of recent advances (e.g. Öpik *et al.*, 2009; Öpik *et al.*, 2010; Caruso *et al.*, 2012; Lekberg *et al.*, 2013).

Grasslands cover one fourth of the Earth's land surface and harbor most of herbaceous plant diversity (Shantz, 1954). AM fungi are the dominant symbiotic fungi in these systems. Even though several recent studies deal with AMF in grasslands (Karanika *et al.*, 2008; Verbruggen *et al.*, 2010; Stover *et al.*, 2012), most of these studies simply catalogued species or use molecular techniques that preclude in-depth characterization of AMF communities. The scale of most studies generally exceeds the likely extent of an individual AM fungus, and this hampers inferences about species interactions at a local scale (Gai *et al.*, 2012; González-Cortés *et al.*, 2012; Verbruggen *et al.*, 2012; Zubek *et al.*, 2012). Moreover, AMF niche space is likely to be complex due to small scale heterogeneity of soil (Veresoglou *et al.*, 2013), and thus large scale studies may overlook important drivers of local AMF community assembly.

Recent research has shown that niche-based processes and environmental filtering are the dominating factors in structuring AMF communities, while neutral dynamics play a minor role (Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010a; Dumbrell *et al.*, 2010b). Yet, while AMF diversity in natural systems is typically measured by using taxon-based approaches, considering AMF phylogeny may provide additional information on processes impacting AMF community structure and functioning (Lekberg *et al.*, 2013). Indeed, a greenhouse study showed that the phylogenetic breadth of an AMF community can positively affect co-existence, and thus the resulting AMF species richness and plant performance (Maherali and Klironomos, 2007). It has also been shown that phylogenetic relatedness among AMF is

positively associated with coexistence (Roger *et al.*, 2013). However, the study of the predictive power of phylogeny relative to spatial and environmental determinants of fungal community structure is in its infancy, although mechanisms such as facilitation or the bidirectional interaction between plant and AMF in forming the symbiosis may be uniquely signaled by phylogenetic patterns.

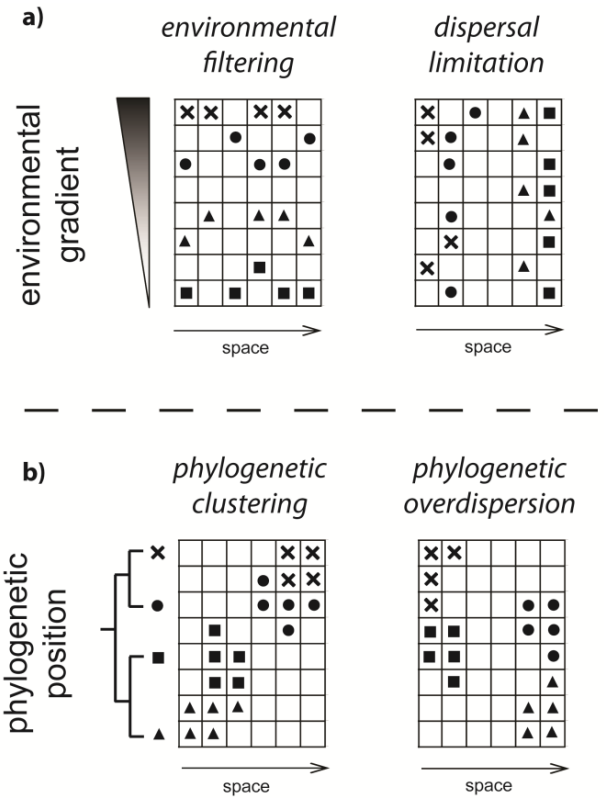

Figure 1: Proposed relationship between AM fungal community structure and the environmental, spatial and phylogenetic components. The symbols represent AMF communities of varying species composition. We expect the spatially structured environment (a) to be influencing AMF community composition either by environmental filtering or spatial processes. Environmental filtering will lead to fungal species aggregating along the environmental gradient. AMF communities in similar environments will be more similar to each other, no matter the spatial position (left diagram). Spatial processes like dispersal limitation will cause AMF communities to be more similar that are closer to each other, independent of the environmental properties in each sample (right diagram). The phylogenetic component (b) is expected to either cause segregation (overdispersion) or aggregation (clustering) of the AMF species co-occurrence in a sampled community. Phylogenetic clustering is expected when particular phylogenetically conserved trait values are selected in one sample over the other, and will show closely related species occurring more frequently (left diagram). Overdispersion is expected when AM fungi with increasingly different traits are increasingly more likely to co-occur, e.g. through limiting trait similarity and/or niche partitioning, and will show less closely related species occurring more frequently (right diagram). We expect the actual data to be composed of a mixture of all the depicted effects, which will be disentangled by variance partitioning.

In fact, phylogenetic distance can reflect trait convergence or displacement if traits are phylogenetically conserved, which implies that nonrandom phylogenetic patterns in species distribution can reflect underlying processes such as competition, interactions with the soil biota or habitat filtering (Kembel and Hubbel, 2006; Kembel *et al.*, 2010; HilleRisLambers *et al.*, 2012). For

example, phylogenetic dispersion (i.e. segregation) is expected to occur under competitive processes while trait selection processes may lead to phylogenetic clustering (i.e. aggregation).

AMF species identification has historically been based on spore morphology, which suffers from some significant shortcomings (Hempel *et al.*, 2007; Taylor *et al.*, 2013). Classical cloning and Sanger sequencing is costly, often preventing in-depth sequencing of environmental samples. The development of next generation sequencing techniques (Margulies *et al.*, 2005) facilitates the assessment of AM fungal communities at the species level in environmental samples (Öpik *et al.*, 2009; Öpik *et al.*, 2010), overcoming limitations inherent to morphological or genetic fingerprinting-based identification. The development of a new AMF-specific primer-set (Krüger *et al.*, 2009; Krüger *et al.*, 2012) allows access to an unprecedented amount of AMF diversity data in the field, as this primer set is both highly specific to AMF and amplifies all taxa within this group (Krüger *et al.*, 2009).

Here, we assessed the role of different factors that shape the AMF community in a semi-natural grassland. We had three main questions: 1) *Do environmental factors structure the AMF community*? 2) *How much influence do distance-based effects and stochastic events have on AMF community structure?* 3) *Is the AMF community phylogenetically structured?*

Our hypotheses regarding the community effects of each of the three components under investigation are further described in Figure 1. In order to disentangle the explanatory power of each of these three known factors shaping community composition, we extensively sampled the dominant plant species and used a variance partitioning approach to estimate variance explained by these factors while controlling for potential co-variation.

Materials and Methods

Study area and sample collection

The grassland we studied is situated in a natural reserve at Mallnow, Lebus (Brandenburg, Germany, 52°27.778' N, 14°29.349' E). The reserve is known for its different types of species rich dry grassland and has been managed by low-intensity sheep grazing for at least 500 years (Ristow *et al.*, 2011). At the beginning of October 2010, we sampled a larger plot of 15x15m (henceforth called "macroplot"), located on the slope of a hillside. The uphill-downhill axis of the hillside where the macroplot is located is characterized by a steep soil textural gradient from highly sandy (downhill) to sandy-loamy (uphill) soils. Geochemical analysis revealed that other soil parameters highly relevant for AMF communities, namely pH, carbon, nitrogen and plant available phosphorous (Kivlin *et al.*, 2011) strongly varied along the soil texture gradient (Table S1). Specifically, pH, which is known to have important effects on AMF (Dumbrell *et al.*, 2010b), varied from 5.5 to 8.3.

We assessed the local AM fungal community in the roots and surrounding soil of *Festuca brevipila* R. Tracey. *F. brevipila* is by far the most abundant species in our macroplot (coverage > 60 % and in some case > 80 %) as well as throughout the grassland of the study area. This approach standardized the observed AM fungal community on an organism of wide prevalence, allowing a precise, yet general definition of the community unit: the AM fungi associated with the rhizosphere of the dominant grass. We used nine plots of 3x3m, equally distributed across the macroplot in order to reduce the amount of sampling necessary for capturing the whole extent of the gradient. Despite this sub-partitioning of the macroplot, we analyzed the samples across sampling locations (i.e. the roots of an individual grass and its soil form the community unit), rather than based on plots. The sampling was replicated by taking soil cores (5 cm radius, 15 cm deep) centered on six randomly chosen *F. brevipila* individuals per plot, resulting in 54 (6 plants x 9 plots) sampling locations (henceforth called "samples") in total. This sampling allowed us to detect spatial patterns within and between plots with intervals ranging from about 1 to nearly 15 m. Each soil core was thoroughly homogenized prior to subsampling for the different analyses. Roots were washed in Millipore water before subsequent analysis. Soil variables were measured according to the protocol provided in the supplementary

information. To assess AMF colonization, a subsample of the roots was stained with Trypan Blue, assessed at 200X using the magnified gridline intersect method (McGonigle *et al.*, 1990).

DNA extraction, 454-pyrosequencing and OTU delineation

We used 250 mg of each soil and washed root material per core to extract DNA using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA) and the procedures in the manufacturer's manual. Then we created 454-pyrosequencing amplicon pools using AMF specific primers (Krüger *et al.*, 2009) following the protocol in the supplementary information, which involved three PCRs of 30 cycles each. We used a primer mixture, which increased competition for target molecules, delays exponential growth of products and therefore justifies an increased cycle number, but should theoretically also lead to a reduced PCR bias towards more abundant species. Since our results are consistent with the findings of other diversity studies on AMF concerning the representation of genera (Öpik *et al.*, 2010; Maherali and Klironomos, 2012), we assume no bias despite the high number of PCR cycles. Sequencing of the samples was done using the primer set LR3 and LR0R (Hofstetter *et al.*, 2002). These two primers span an area of about 900-950bp, including the variable D1-D2-D3 region of the LSU and are conserved among eukaryotes (Vilgalys and Hester, 1990), therefore preserving the diversity obtained by the AMF specific primers. LR3 was tagged with Adapter B, LR0R was fused with the Adapter A and error-correcting barcode sequence (Hamady *et al.*, 2008) for the 454 run, so we sequenced from the 3'-end of our target sequence towards the start of the LSU. 454-Pyrosequencing was done at Duke University Sequencing core facility (Durham, USA) using the Titanium chemistry from Roche (Basel, CH).

Resulting sequence sets were subjected to a denoising and clustering pipeline. Sequences were denoised using the PyroNoise approach implemented in Mothur (Schloss *et al.*, 2009). This denoising approach removes bad quality sequences, creates sequence clusters and removes chimera sequences, while being based on clustering flowgrams rather than sequences alone, which allows 454 errors to be modeled and removed naturally (Quince *et al.*, 2009; Quince *et al.*, 2011). We used the standard parameters for Titanium sequencing as suggested by Quince (2009), with a minimum flow amount of 360 and a maximum of 720. After denoising, the sequences of roots and soil were clustered using

CROP. The program uses a Bayesian clustering algorithm, which addresses species delineation uncertainty better than hierarchical clustering methods due to its flexible cut-off and therefore creates significantly fewer artificial OTUs than other fixed cut-off clustering approaches (Hao *et al.,* 2011). Runtime parameters and source code from the analysis in R described below are provided in the supplementary information.

Due to the nature of pyrosequencing, there were differences in read numbers for every sampling location, so we resampled the read numbers to equal amounts of 700 reads per sampling location, 350 each for root and soil subsample, using a bootstrap approach. Sampling locations with considerably lower read numbers than the resampling value (<260 per root or soil sample) were discarded (9 soil and no root samples). Based on read numbers, rarefaction curves were calculated for each root or soil sample per location. Since all species accumulation curves were leveling off, no sample was excluded. Singletons were removed from all samples. The resulting OTUs where represented by an abundance matrix.

Phylogenetic tree calculation

Tree calculation was done with RAxML (Stamatakis, 2006) and BEAST (Drummond and Rambaut, 2007). We added representative sequences of an SSU-ITS-LSU AMF reference alignment (Krüger *et al.*, 2012) to our dataset to determine the phylogenetic position of our OTUs. Using the position of an OTU in a phylogenetic tree relative to reference sequences creates more reliable species estimation than just using database queries (Ross *et al.*, 2008). In order to compare the quality of our tree and to add more description to the OTUs, we annotated them using the BLAST hit with the lowest E-value. The reference alignment was first trimmed to the region present in our sequences and then used as a template in Mothur to align our OTU sequences. The two alignments were combined and further refined in MUSCLE (Edgar, 2004). We used phylogenetic trees to further refine our OTU set and remove sequences which clustered outside the Glomeromycota and are therefore likely to be erroneous or non-AMF sequences. We used two different tree calculation approaches to validate the accuracy of the obtained phylogeny. Using RAxML, we calculated 1.000 rapid bootstrap trees and subsequently applied a thorough Maximum Likelihood analysis. BEAST was run with the Extended Bayesian

Skyline Plot as a tree model (Minin *et al.*, 2008) with a chain length of 10 million generations, from which the best tree was chosen for evaluation. Trees were then inspected and edited using FigTree (Rambaut, 2012).

Phylogenetic community structure

We addressed community structure by analyzing phylogenetic diversity (PD) between the AM fungal communities. Using the picante package (Kembel *et al.*, 2010) in R 3.0.2 (R Core Team, 2013), we obtained two estimates of PD: (1) the standardized effect size of mean pair wise distance (SES-MPD) which measures alpha-diversity, and (2) inter-community mean pair wise distance (IC-MPD) which measures beta-diversity. Phylogenetic distances between OTUs serving as input for the estimates of PDs were calculated using the Needleman-Wunsch implementation of Esprit (Sun *et al.*, 2009). The SES-MPD was calculated using the phylogenetic distance matrix of the OTUs plus the abundance matrix of the OTUs per sample and applied a null model randomization. The result was an NRI value for each sample, which is defined as $[-(MPD - MPD_{null})/_{SD}(MPD_{null})]$, where MPD is the mean pair-wise phylogenetic distance among species in the sample (Kembel and Hubbel, 2006). The mean values of the NRIs of all samples of roots and soil, respectively, were then used as the alpha-diversity measure to judge the clustering or segregation of the overall AMF community. Positive NRI values are correlated with clustering, negative values with overdispersion. The null model algorithm we used was "independentswap" with 999 randomized null communities. "Independentswap" retains column and row totals for null model analysis of species co-occurrence (Gotelli, 2000). This approach is particularly suited to problems that concern differences in species composition, because it accounts for variations in other community attributes such as diversity and richness. Significance of the calculated NRIs was tested using t-test.

IC-MPD calculates pair-wise phylogenetic distances of the samples, based on pair-wise genetic distances between OTUs and yields a sample x sample distance matrix as a measure of beta diversity. In order to include the IC-MPD information in a subsequent variance partitioning analysis (Legendre and Legendre, 1998), the distance matrix was subjected to a principal coordinate analysis (PCoA), a commonly used tool to reduce dimensionality which provides a measure of the amount of variance

explained in the a few independent principal axes (Legendre and Legendre, 1998). The first two PCoA axes which represented a major of amount of total phylogenetic variation were extracted and used as the phylogenetic explanatory variables.

Variance partitioning

The analysis of patterns in community structure was conducted in R, using the vegan (Oksanen *et al.*, 2012) and the SPACEMAKER (Dray, 2011) package and the abundance matrix obtained from processing the sequences as response matrix. Spatial information (pair-wise distance between samples), log-transformed environmental data (sample pH and C, N, P, and water content, and C/N ratio) and the estimates of phylogenetic beta diversity were used as explanatory variables.

The OTU abundance matrix was Hellinger transformed and subjected to a multivariate analysis to test for effects of spatial, environmental and phylogenetic variables influencing community variation. In variance partitioning "space" stands indeed for spatial autocorrelation: moran eigenvector mapping (MEM) was used to factor in spatial autocorrelation at multiple scales in community variance partitioning (Dray *et al.*, 2006). This method represents a general, more powerful version of the widely used PCNM (Borcard *et al.*, 2004), which allows testing for several types of spatial structure. Several competing spatial models are possible and the most parsimonious model is selected using a multivariate extension of the Akaike Information Criterion AIC (Akaike, 1973). This model provides the best linear combination of eigenvectors accounting for spatial autocorrelation at multiple spatial scales and each eigenvector represents a certain scale (Dray *et al.*, 2006). We used redundancy analysis (RDA) and variance partitioning to resolve the contribution of each of the factors to the total variance (Legendre and Legendre, 1998). As this was an observational study, we applied a conservative logic: unequivocal evidences of the effect of a certain factor are obtained only when controlling for the effect of other factors. For example, shared variation between spatial and environmental factors might depend on the fact that we sampled along an environmental gradient. However, this correlation does not imply causation since linear changes in community composition can also be observed along directions where there is little environmental variation or the gradient might not structure the community directly (Legendre and Legendre, 1998). Thus, a non-spatially

structured effect of the environment would suggest that communities are similar if their environments are similar, regardless of their spatial position. This is more robust evidence of independent effects of the environment in the framework of observational studies. This is also the reason why spatial autocorrelation at multiple scales needs to be accounted for in order to perform a robust test of factors that are spatially structured (Legendre and Legendre, 1998; Borcard *et al.*, 2004). In this sense, it is not the spatial variation in itself that is under investigation since this variation can be due to several possible and collinear factors that neither have been measured nor can be disentangled from measured factors. Given this logic, variance partitioning is the appropriate tool to quantify the unique contribution of the three factors investigated in the present study (Borcard *et al.*, 1992). Factors were tested using a partial-RDA based permutation approach, which tests for the focal factors by controlling for the other factors (Oksanen *et al.*, 2012).

Results

Study area and sample collection

The study area was characterized by steep gradients in all measured environmental components, following roughly the uphill-downhill direction (Figure S1). Plant available phosphorus concentration was low in most of the plots, with a range from 10.9 mg/kg soil to 85.0 mg/kg (median 30.1 mg/kg). Soil C to N ratios varied between 13:1 and 43:1 (median 19:1), pH showed a range from 5.5 to 8.3 (median 7.4), and root colonization by AMF ranged from 5% to 99% (median 77.5%). The colonization was significantly correlated with an increase in loam content of the soil, which linearly correlated with water content and organic content (Figure S2; for all environmental data see Table S1). We did not find a correlation between root colonization and phylogenetic distance. Correlation analysis shows that most of the environmental variables were correlated with each other, confirming our prediction of a single linear environmental gradient, with the exception of soil phosphorus (Table S3).

454-pyrosequencing and OTU delineation

After resampling and removal of singletons and non-AMF sequences, the root data set consisted of 54 OTUs and the soil dataset of 73 OTUs, with a total of 74 OTUs. Almost half of the detected OTUs (32 of 74) belonged to the genus *Glomus sensu* Schüßler and Walker (2010), and in most samples this was the most abundant taxon. The others were spread across all major AM fungal groups, spanning ten different AMF genera (Figure 2), suggesting that our methods are capable of detecting all major lineages within AMF. The dominance of *Glomus* was also found when comparing the read numbers of each of the AMF genera instead of OTU numbers (Figure 2). The highest abundance of sequence reads to any of the OTUs was attributed to a member of the *Rhizophagus* genus, which based on BLAST annotations is likely the cosmopolitan *Rhizophagus irregularis* found in high abundance in several studies (Öpik *et al.*, 2006; Lekberg *et al.*, 2013).

After denoising of a total of 67558 (roots) and 50594 (soil) sequences with PyroNoise and the Bayesian clustering step in CROP, 301 OTUs were obtained. Further removal of OTUs was based on

the elimination of singletons (164), the exclusion of OTUs which did not yield any BLAST result (33), resampling (13) and removal of non-AMF sequences identified from the trees. Our primers proved to be highly AMF-specific, with only a few non-target OTUs from the Chytridiomycota phylum and other fungi (17).

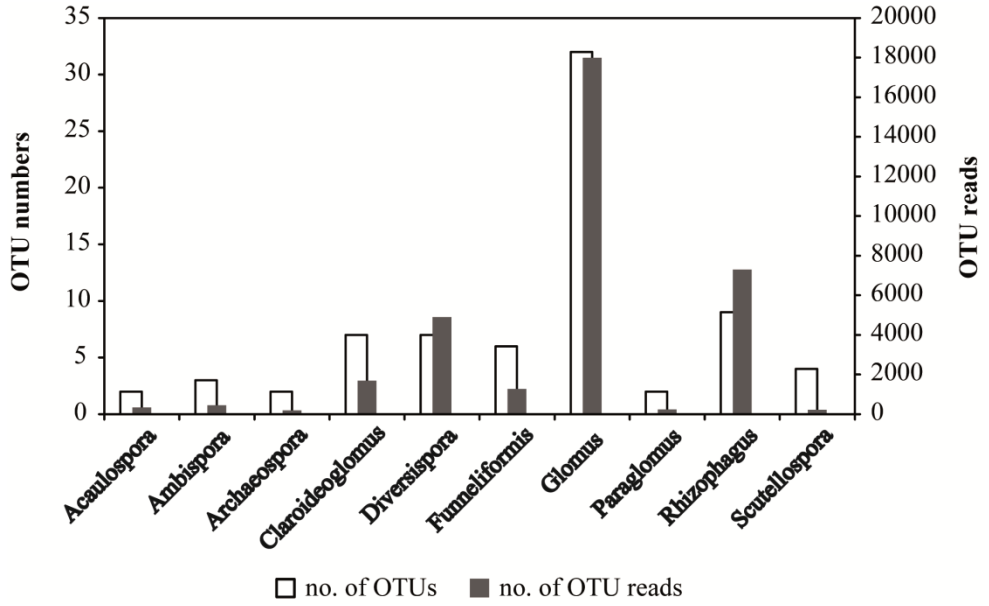

Figure 2: Number of OTUs and number of sequence reads per AMF genus. OTU numbers are represented by white bars (left y-axis). OTU sequence reads are represented by dark-gray bars (right y-axis).

If sampled sufficiently, the root community should ideally represent a subset of the soil community. We found only one OTU in the root dataset, which was not part of the soil dataset and which was very likely a sampling effect on the very rare OTU. The rarefaction curves (Figure S3) showed that all the communities were leveling off or were very close to saturation. The sequences clustered well with Glomeromycota reference data (Figure 3) as published in Krüger et al. (2012). In general phylogenetic position in the tree could assign many OTUs to genera that were only poorly annotated in the NCBI database (e.g. “uncultured Glomeromycetes”, Figure 3).

Phylogenetic community structure

The SES-MPD null model analysis showed significant differences from random distribution, when the abundance weighed data was used (Table 1). Mean net relatedness indices (NRIs) for both root and

soil datasets were positive with comparable sizes (0.27 and 0.26), which means that AMF communities contain taxa that are phylogenetically more related than expected by chance (i.e. significantly clustered). In the non-abundance weighted SES-MPD indices, the trend towards clustering is still present, albeit not significant. Since the number and relative abundance of OTUs was strongly biased towards the *Glomus* group (Figure 2), we split up the data into *Glomus* and non-*Glomus* OTUs and performed a separate analysis on each group. For both data sets the significant phylogenetic clustering persisted, suggesting the pattern is valid independent of whether closely or distantly related taxa are compared. In the *Glomus* dataset, significance was independent of abundance, with effect sizes being comparable in root and soil. In the non-*Glomus* OTU set, results were similar to the complete OTU set. The magnitude of the NRI was comparable in root and soil.

Variance partitioning and community clustering

The variance of the whole OTU set was significantly explained by spatial and phylogenetic patterns plus their combined effects (Figure 4, Table S2). The phylogenetically structured environmental effect was very small (<0.0001) in all of the treatments, so this partition was omitted. The influence of spatial position was more important in soil than in roots when abundance data were used, while with presence-absence data phylogenetic composition was more important in soil than in roots. Effects of spatially structured environment as well as environment alone remained comparable among root and soil, as well as between abundance and presence-absence data, but in general abundance data increased the amount of variation explained.

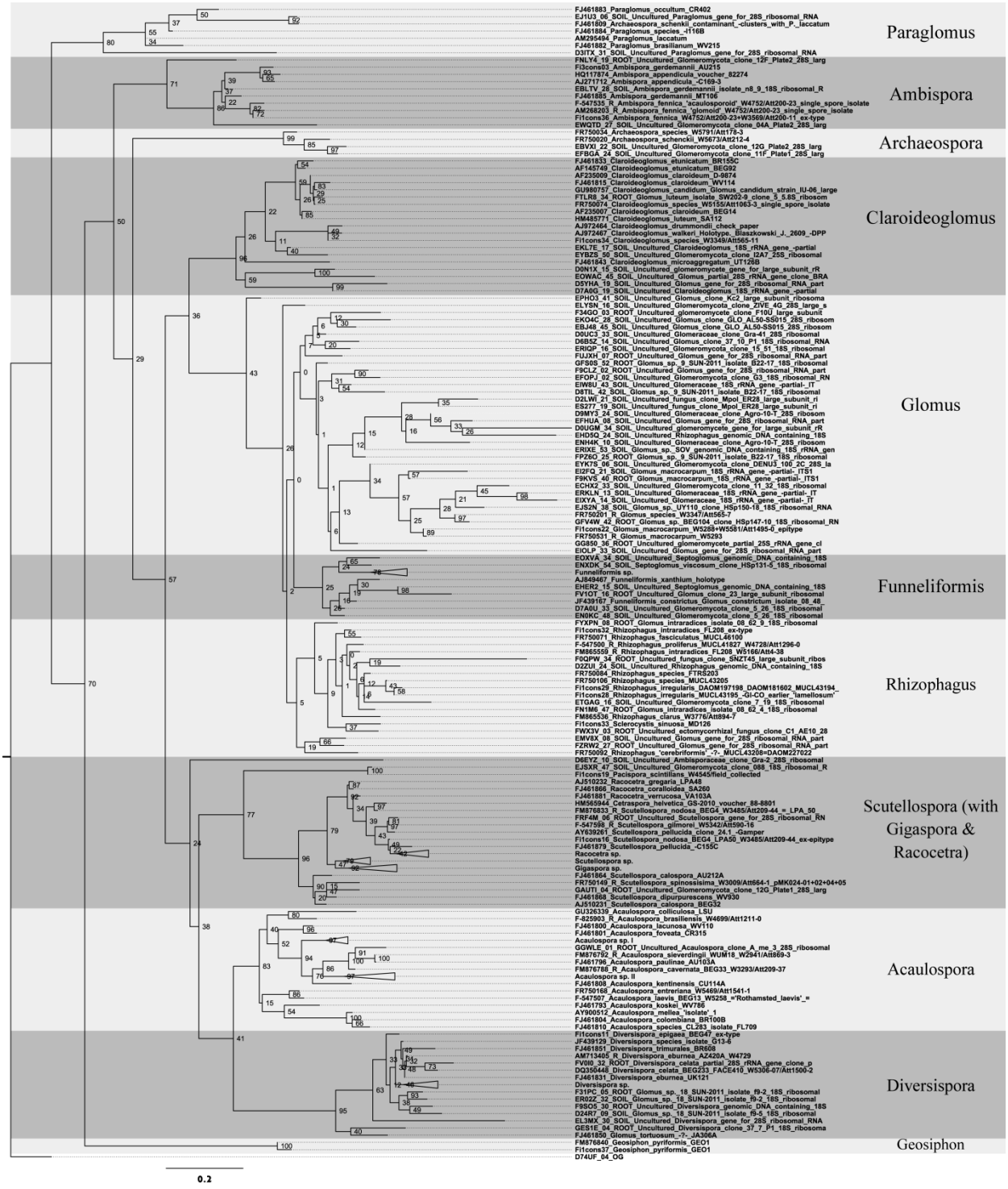

Figure 3: Maximum likelihood tree of 74 OTUs from the root and soil dataset, complemented with 114 sequences from the Krüger et al. (2012) SSU-ITS-LSU alignment and one non-AMF outgroup ("D74UF_OG", an unidentified member of the Chytridiomycota). Tree calculation was done in RAxML. Nodes ending in triangles represent collapsed species divisions which did not contain any of our OTUs, in order to increase visibility. Node numbers represent Bootstrap values. The node descriptions containing a "ROOT" or "SOIL" tag represent the OTUs defined in our study, while the other nodes represent the sequences from Krüger et al.

For the *Glomus* OTU variation, the major explanatory components were again phylogeny and the spatial signal (Figure 4, Table S2). Differences between root and soil indicated that environmental filtering is more selective in soil.

Table 1: t-Test results of the NRI of mean pair wise distance (SES-MPD) of the root and soil community matrices, including a division of the data set into *Glomus* only and non-*Glomus* OTUs. Either abundance data (+abu) or presence / absence data (-abu) was used when calculating the effect sizes and P-values.

data set			t	df	p-value
all OTUs	root	+abu	2.644	53	**0.011**
		-abu	0.929	53	0.357
	soil	+abu	2.031	47	**0.048**
		-abu	1.156	47	0.254
Glomus OTUs only	root	+abu	2.889	46	**0.006**
		-abu	2.588	46	**0.013**
	soil	+abu	2.750	44	**0.009**
		-abu	3.227	44	**0.002**
all OTUs except *Glomus*	root	+abu	2.994	42	**0.005**
		-abu	1.479	42	0.147
	soil	+abu	3.347	43	**0.002**
		-abu	1.477	43	0.147

In the data set of all OTUs except *Glomus*, spatial and phylogenetic components were again the major variables contributing to explained variation (Figure 4, Table S2) and major differences were found between root and soil. Phylogeny was a major explanatory variable, but it decreased significantly from root to soil. In the roots, the decrease in phylogenetic signal was also found in the joint effect of spatial structure and phylogeny. Finding comparable results when removing the most abundant taxon group shows that the patterns are not exclusively shaped by *Glomus* alone.

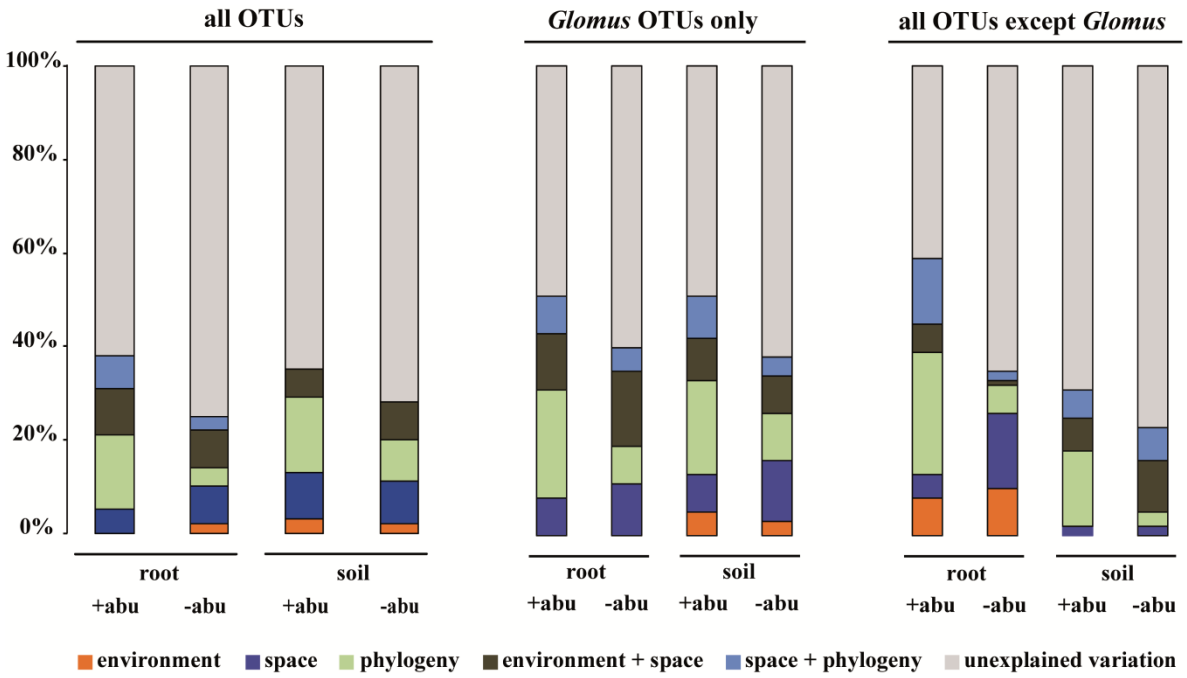

Figure 4: Percentage of variation explained by permutation tests based on RDA (redundancy analysis) and decomposition of the total variation in the community matrix into unique environmental (soil properties), spatial (geographic position) and phylogenetic (genetic distance) components. Bars of combined effects represent the shared variation between these two components. Either abundance data (+abu) or presence-absence data (-abu) was used when calculating the phylogenetic component for the variance partitioning. Values are based either on the whole OTU set or on the *Glomus* OTUs and the non-*Glomus* groups, respectively.

Discussion

In the present study we have been able to quantify the relative predictive power of different factors in explaining small-scale AMF community composition in a semi-natural grassland. The three main community factors under investigation were environmental drivers, spatial structure and phylogenetic distance and below we discuss each of them with regard to our three main questions.

Do environmental factors structure AMF communities?

Previous studies addressing AMF community structure and applying variance partitioning have shown the dependence of AM fungi on the environment (e.g. Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010b). In our study, the non-spatially structured environmental component explained only little of the variation in community structure. Despite our expectations that a gradient like the one we found in our field site should significantly shape a soil community, the environment was only found to be significant in the "*Glomus* only" and "non-*Glomus*" subsets. Given that these two groups might respond slightly differently to environmental properties, this could then lead to diminished significance in the overall dataset. However, environmental effects can definitely exert their effect along a gradient in a spatially structured manner, as indicated by the variance fraction accounting for spatially structured effects of the environment. Nevertheless, even if one sums that amount of variation accounted for by spatially structured and not spatially structured environmental effect, the total contribution of the environment remains small relative to the other investigated factors.

Instead, our results suggest that the AMF communities in our study area are predicted mainly by the spatial distance between samples and phylogenetic distance between OTUs, when the effect of the environment has been taken into account. Since our environmental gradient was quite steep and concentrated in a small area, we have reduced confounding factors such as historical events and/or dispersal limitation, which are present in broad scale studies. Moreover, confounding effects due to plant identity are also absent given that the observed AM fungal community is standardized on an organism of wide prevalence that belong to a genus (*Festuca*) that very often dominates dry grasslands world-wide. Certainly, at broader spatial scales the relative role of the various drivers of community

composition may change and we stress that the local community we are here investigating must represent a local subset of the regional AMF pool. Ultimately, our local community is therefore also the result of broad scale dispersal processes and environmental filtering processes that we cannot resolve in our study. For this same reason, we believe that, given the state-of-the-art, our approach offers a fair compromise between the ecologists' quest for general conclusions derived from large scale fully randomized design (e.g. no focal plant) and the need for the collection of robust patterns from field studies performed at local spatial, temporal and taxonomic scales. In other words, the locality of our study is showing fairly dominant non random phylogenetic and spatial patterns in AMF communities: these patterns could have been neglected in the past given the multitude of factors that structure AMF assemblages from very local to regional scales. Indeed, in other studies stronger environmental effects have been found: Dumbrell et al. (2010b) studied an extremely pronounced pH gradient (<4-8), the study of Lekberg et al. (2007) focuses on agricultural fields at larger scales, and thus different community-structuring mechanisms may operate under different ecological settings. It is also possible that significant effects of the environment on AM fungi might be confounded with environmental effects on the host plants (Sharma *et al.*, 2009).

The results therefore indicate that spatial and phylogenetic distance are the major representatives of the underlying processes shaping the community at small spatial scales, with soil results being similar to the roots, but more clearly separated into spatial and phylogenetic components (Figure 4, Table S2). An explanation for the higher amount of variation explained in soil is that root communities may be strongly shaped by heavily root colonizing (i.e. abundant taxa). The communities may also be more (temporally) dynamic, and thus more prone to sampling effects, that is, which plant species and when during their life cycle is sampled.

How much influence do distance-based effects and stochastic events have on AMF community structure?

We observed a large fraction of AMF community variance explained by spatial patterns after controlling for environmental factors and phylogenetic distance. Dispersal or unmeasured environmental factors as well as biotic interactions not leaving a phylogenetic signal are all possible

factors behind these spatial patterns (Chang *et al.*, 2013). Given the variables we measured, it is unlikely that we missed out major environmental predictors of AMF communities. In addition to that, every measured environmental variable was spatially structured in our study area along the sampled gradient and it is therefore reasonable to assume that effects of unmeasured environmental variables are included in the variation shared by spatial eigenvectors and the measured environmental variables. On the small scale of our study, dispersal limitation is less likely but AM communities can be exceptionally patterned already at a sub-meter scale (Mummey and Rillig, 2008), so that dispersal constraints can indeed play a role at a 15 meter scale. Stochastic population dynamics due to irregular, unpredictable environmental or demographic fluctuation might also contribute to these patterns. Spatial structure that is independent of environmental factors indicates that chance-events play a role in community composition although biotic interactions such as competition may also contribute to spatial patterning. Dumbrell et al. (2010a) suggested that chance-events could lead to a positive feedback mechanism on any taxon in the community, which could be random and self-reinforcing. This hypothesis could explain a diminished environmental signal and a strong spatial patterning. Regardless of the contribution of stochastic effects, the significant phylogenetic structure of the assemblages shows that AMF communities are also significantly shaped by deterministic processes.

Is the AMF community phylogenetically structured?

We find that phylogenetic distance can account for a relative large and statistically significant fraction of AMF community: AMF communities consist of taxa that are more related than expected by chance. This can be an effect of at least three processes: convergence via habitat filtering because taxa that are similar in traits respond in a similar way to environmental factors; or plant-AMF interactions are such that the focal plant selects phylogenetically clustered AMF assemblages. Third, fungal interactions with the soil biotic community (e.g. arthropods) could create interactions that support assemblages of conserved traits: the selected AMF are those that share traits that allow them to coexist. Whichever the cause, the effect propagates to the soil AMF assemblage and seems even stronger in some cases in the soil than in the roots. Given that the soil abiotic environment has little effect on AMF, especially when controlling for spatial autocorrelation, our results suggest that biotic interactions are more likely to be

responsible for the AMF phylogenetic community pattern, although we cannot completely rule out environmental filtering as one of the source of the observed phylogenetic signal.

In AMF, phylogenetic community patterns can inform on assembly processes (HilleRisLambers *et al.*, 2012) because AMF traits are phylogenetically conserved (Powell *et al.*, 2009). The fact that phylogenetic clustering was more intense when abundance was taken into account suggests that taxa within the most abundant group, *Glomus*, share traits that allow them to coexist. This coexistence can take place due to similar, positive interactions with the host: if the host plant selects for a particular set of conserved AMF traits from a pool that varies from one place to the other, this will result in higher clustering than expected by chance. Besides this process, the neighboring plant community of our focal species could also play a role in determining phylogenetic patterns in the AMF community: analyzing the neighboring plant community of the *F. brevipila* plants showed that significant plant-plant interactions contribute to plant community composition in close proximity of *F. brevipila* (Horn *et al.*, *unpublished*), and this could in turn also influence the AMF communities of the focal plant (Hausmann and Hawkes, 2009), but it is not straightforward what the effect would be in terms of expected phylogenetic pattern (clustering vs. dispersion). Our results are similar to those of Roger *et al.* (2013), who found closely related AMF to be more likely to coexist, presumably due to lack of competitive exclusion. This counterintuitive agreement between studies appears to indicate a general pattern and warrants future study. It might indicate that closely related AMF are similar in traits that are favored by plants (due to spatial-temporal dynamics), and that this is not offset by competition for root or soil space because competition should reduce phylogenetic clustering if traits involved in the competition processes are phylogenetically conserved.

Other members of the plant microbiome have been shown to exhibit similar community patterns (i.e. phylogenetic clustering) as we find here for AMF, for instance rhizobia (Sachs *et al.*, 2009) and endophytic bacteria (Sessitsch *et al.*, 2004). Facilitative interactions between fungi have been shown in ectomycorrhiza (Shaw *et al.*, 1995; Koide *et al.*, 2005), ericoid mycorrhiza species (Gorzelak *et al.*, 2012) and have been recently indicated for AMF as well (Thonar *et al.*, 2014). Facilitation between closely related AMF as well as antagonism between distantly related taxa would ultimately result in a phylogenetically clustered AMF community. Only more mechanistic, experimental studies will in the

future tell which of the proposed mechanisms contribute to community phylogenetic clustering in AMF.

Conclusions

Here we report that in AMF communities spatial and phylogenetic patterns independent of environmental factors appear to be a major source of community variation even at the small scale of the present study, which suggests that environmentally independent and even stochastic events can deeply affect AMF assemblages already at fairly small (1-10 m) scales. AMF communities are strongly structured in terms of phylogenetic relationships between fungi as evidenced from their phylogenetic clustering. Given the weak effects of the environment, we propose that this pattern is explained by direct or indirect positive interactions among fungi and their biotic environment. Phylogenetic clustering was observed both in the roots and the soil and in some cases phylogeny explained more variation in soil. In order to elucidate the mechanisms behind these patterns, the study of fungal traits offers a promising research avenue in microbial ecology.

Acknowledgements

We would like to thank Janis Antonovics for his insightful and inspiring comments on the manuscript, as well as the Deutsche Forschungsgemeinschaft (DFG) for their generous financial support.

Conflict of interest statement

The authors declare no conflict of interest.

Supplementary Material

Supplementary information is available at ISMEJ's website.

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Chapter IV: Does neighborhood plant community structure affect the arbuscular mycorrhizal fungal community of focal plants at small spatial scales?

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Running title: Plant and AMF community assembly

Keywords: arbuscular mycorrhizal fungi / community assembly / phylogenetic clustering / plant ecology / small-scale / spatially explicit / 454-pyrosequencing

Subject Category: Microbial population and community ecology

Abstract

Arbuscular mycorrhizal fungi (AMF) associated with *Festuca brevipila* in a semi-arid grassland with steep environmental gradients were analyzed using next-generation sequencing and markers covering the full Glomeromycota phylum. Additionally, plant diversity in the rhizosphere environment was assessed in order to address interactions between these two groups in the context of the Passenger and Driver framework, which provides hypotheses for the environment shaping AMF and plant communities (independent), plant communities driving AMF (passenger) or AMF communities driving the plants (driver). The AMF community consisted of 73 OTUs, the plant community of 47 different species. Community-level variance partitioning showed that environmental factors played a very minor role in determining AMF community composition. Instead, phylogeny and spatial structure were main determinants of the AMF community. Analysis of the plant neighborhood of focal plant AMF communities via plant phylogeny and presence / absence as a predictor for AMF composition and vice-versa showed significant, but small interaction between these two organism groups. This points out that, in context of the Passenger-Driver framework, at small scales these groups appear mostly "independent" and to a small extent passengers. This suggests that plant and AMF community assembly rules differ on small scales, leading to a decoupling of the two species groups from each other. We therefore propose that plant and AMF communities are mainly dependent on spatio-environmental factors, interactions within their species groups and their phylogenetic properties, while interactions between the two organism groups lack major contributions to the community assembly in the field.

Introduction

Arbuscular mycorrhizal fungi (AMF) are one of the most important symbiont groups for plants, forming relationships with the majority of land plants and playing a significant role in the acquisition of phosphorus (Smith and Read 2008). They facilitate the uptake of nutrients from soil and are considered to have been important for the transition from aquatic to terrestrial habitats by plants. Yet, the community ecology of these organisms remains only coarsely understood, mainly due to the cryptic nature of the group and the complexity of the three-way interaction between plants, AMF and the environment. Additionally, indirect interactions further complicate the picture.

The study of AMF in grasslands is of particular importance since grassland ecosystems cover a significant proportion of earth's surface and harbor the majority of herbaceous plant diversity (Shantz 1954). Understanding the interaction of plant diversity and fungal community ecology is crucial for understanding ecosystem functions (Wardle et al., 2011). Studies on plant biodiversity in grassland ecosystems on small scales have revealed connections between species richness of AMF and plants (Hiiesalu et al., 2014) and clear host plant effects on AMF community composition (Vályi et al., 2015). Despite the fact that AMF can form hyphal networks, the influences of the environment happen on a fairly small scale (Mummey and Rillig 2008). To date, only a few studies have dealt with this fact and applied a sufficiently fine sampling scheme to provide a solid statistical basis for the analysis of these local biotic interactions in the field (Horn et al., 2014).

The AMF-plant symbiosis represents a very close relationship. However, plants and AMF interact as two sets of communities associated with each other due to their independent dispersal, with the plant set potentially driving the structure of the fungal set and vice versa (Fig. 1). The Passenger and Driver hypothesis (Hart et al., 2001, Zobel and Öpik 2014) creates a theoretical framework which states that AMF are considered the Driver if their communities shapes the plants, while they may be considered the Passenger if plants shape AMF communities. If neither is influenced by the other, they behave independently. According to the Driver hypothesis, an AMF community shapes the composition of a local plant community through processes such as AMF taxon-specific impacts on plant performance.

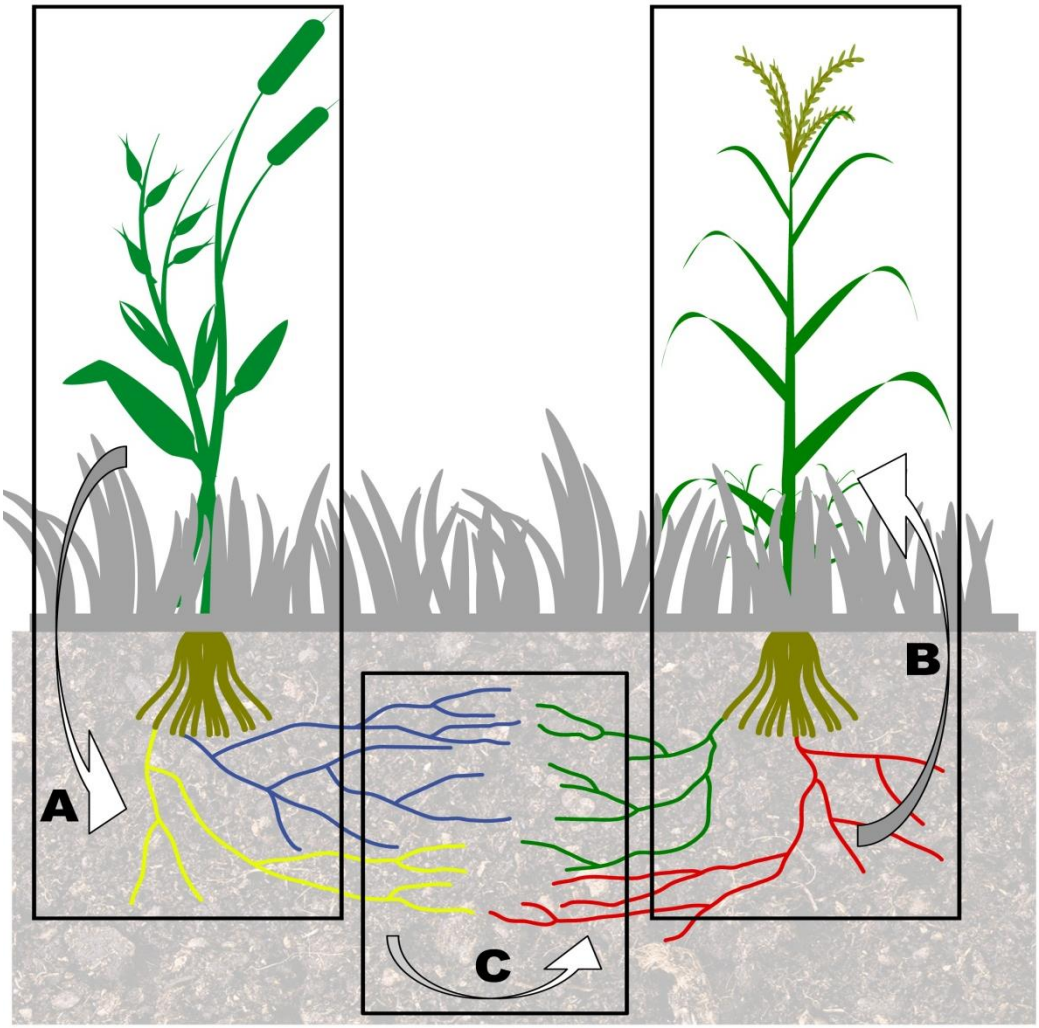

Figure 1: Potential interactions of AMF and plants in their respective community assembly. A) Plant community composition may influence AMF community composition. Zobel & Öpik (2014) called this the Passenger hypothesis. Different plant communities will lead to different AMF communities (compare B). B) AMF community composition may shape the plant community. This was called the Driver hypothesis. Different AMF communities lead to different plant communities (compare A). C) AMF may interact with each other, spatial and environmental processes in shaping their communities, independently of plants. This represents the Null hypothesis.

By contrast, the Passenger hypothesis postulates that an existing community of host plants shapes the composition of a local AMF community, e.g. through differential rewarding of AMF taxa with carbohydrates (Zobel and Öpik 2014). The Independence hypothesis from Zobel and Öpik (2014) represents a null hypothesis for the above working hypotheses and states that plant and AMF communities do not influence each other, while they can be related through e.g. shared environmental associations. Field studies on this particular subject are rare, given the difficulties in distinguishing

cause and effect in correlations between the two organism groups and hence making judgment difficult who is the passenger and who is the driver. Therefore, in order to overcome this issue, in-depth sampling efforts need to be combined with a small-scale approach to account for the specific traits of AMF, combined with a conservative analysis devoted to find correlations after accounting for a set of other potential factors influencing community composition. Therefore one would need a dedicated analysis of AMF and plant community plus their environment at a given location to paint a more complete picture of who influences whom, or if there is influence at all. General contributions to each other's community composition could be addressed by variance partitioning or linear modeling. Our study aims at generating hypotheses about possible mechanisms in the small-scale interaction sphere by using a spatially explicit sampling design, a standardized focal organism of high abundance and an exhaustive sampling approach at sufficiently small scales, taking into account AMF, plants, and their environment.

AMF and plant communities are thought to be influenced by niche-based processes and environmental filtering (Dumbrell et al., 2010a, Dumbrell et al., 2010b, Lekberg et al., 2007, Peng et al., 2009, Silva and Batalha 2011), including dispersal, abiotic filtering and biotic interactions (Götzenberger et al., 2012). Negative interactions among AMF species in competition for the same root space would result in the superior competitor persisting on the root (Hart et al., 2001, Thonar et al., 2014). Greenhouse studies as well as field observational work has shown that phylogenetic patterns are connected to co-occurrence (Horn et al., 2014, Maherali and Klironomos 2007). However, understanding the predictive power of phylogeny in relation to the environmental determinants of fungal communities is still in its infancy. Studies suggest that AMF traits are phylogenetically conserved (Powell et al., 2009), therefore phylogenetic distance can reflect displacement or trait dissimilarity. Mechanisms like facilitation or feedbacks between plants and AMF may be uniquely signaled by phylogenetic patterns, since closely related species would receive similar facilitation due to similar traits (Anacker et al., 2014). That implies that phylogenetic patterns in communities may reflect underlying processes such as competition, interactions with soil and plant biota or habitat filtering (HilleRisLambers et al., 2012, Kembel and Hubbel 2006, Kembel et al., 2010).

The identification of AMF species has been facilitated by the development of modern parallel sequencing techniques (Margulies et al., 2005), overcoming issues connected to spore morphology or classical Sanger sequencing, which often prevented identification of AMF on a species level (Öpik et al., 2009). New primers which combine high specificity to AMF and broad coverage within the Glomeromycota have allowed unprecedented access to the AMF diversity in the field (Krüger et al., 2009).

In this study we aimed at elucidating patterns shaping AMF communities colonizing a single plant species (*F. brevipila*) in a semi-natural grassland in comparison with their surrounding plant community on a small scale. We set our results in the context of the Passenger and Driver framework and tested for patterns that are consistent with this framework. If the plant community composition variation surrounding our focal plant is related to the AM fungal communities in soil or the roots of the focal plant, this would provide evidence for the Driver framework. If plants were able to act as predictor for AM fungal community composition, this would point to the Passenger hypothesis. If neither is prevalent, interactions among the AMF, based on phylogeny, space or environment, could be determinants for their community composition, indicating an independent behavior. Therefore our main questions were: Are AMF communities influenced by present plant identity and vice-versa? Are AMF communities assembled through interspecific interactions? How important is the role of the variation in environmental parameters?

We conducted an intense sampling of the AMF species on three macroplots and added the plant biodiversity to our analysis. By partitioning variance caused by the respective species group individually for AMF on plants and plants on AMF, and by using generalized linear models to look for contributions on each other after removing all other effects, we aimed at qualifying and quantifying individual influences of plants on AMF and vice-versa to generate a statement on the potential Passenger or Driver effects of our analyzed community. We also tried to estimate processes explained by the environment, spatial factors, AMF and plant phylogeny, while controlling for potential co-variation. Sampling was conducted in a nature protection area located in north-eastern Germany, a Natura 2000 biodiversity hotspot which contains over 200 different plant species and combines floral elements of steppes and oceanic habitats. Given the high diversity of plants and (Ristow et al., 2011)

and a previously found high diversity in AMF (Horn et al., 2014), this sampling location is very suitable for the questions we pose in this study.

Methods

Study area and sample collection

The grassland of this study is located in a natural reserve at Mallnow, Lebus (Brandenburg, Germany, 52°27.778' N, 14°29.349' E). The reserve consists of different types of species rich dry grassland and has been managed by sheep herding for several hundred years (Ristow et al., 2011). The mean annual precipitation in this sub-continental climate is below 500 mm. Our sampling strategy was based on a hierarchical nesting of plots, and was done at the beginning of spring in April. Twelve plots of 3x3m were sampled. They represented the four corners of three larger plots of 15x15m (henceforth called "macroplots"), which were located on the slopes of a hillside. The uphill-downhill axes of the macroplots were characterized by a steep textural gradient from sandy-loamy (uphill) to highly sandy (downhill) soils. Geochemical analysis showed that soil parameters like pH, C, N and P varied along the texture gradient (Horn et al., 2015).

We assessed the local AM fungal community in the roots and surrounding soil of *Festuca brevipila* plants plus the neighboring plant species around these *Festuca* plants. *Festuca brevipila* is one of the most abundant species in our macroplots (Ristow et al., 2011, Horn et al., 2015). Soil cores (5 cm radius, 15 cm deep) were taken from five randomly chosen *F. brevipila* plants per plot, resulting in 60 (5 plants x 12 plots) sampling locations in total., Plant presence / absence was assessed in the surrounding area in a radius of 15cm around each soil core to target local interactions present in the rhizosphere of our focal plant. We chose presence / absence instead of abundance since above- and belowground abundances are not linearly correlated, representing a more conservative approach (Hiiesalu et al., 2012, Hiiesalu et al., 2014).

Each soil core was thoroughly homogenized and representatively subsampled for soil geochemical analyses of water content, pH, carbon, nitrogen, phosphorus and dehydrogenase activity. Roots were washed in Millipore water before analysis. Soil properties were analyzed following the procedure utilized in a recent study (Horn et al., 2014).

DNA extraction, 454-pyrosequencing and OTU delineation

We used 250 mg of each soil and washed root material per core to extract DNA using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc.) following the procedure described in the manufacturer's manual., Then we created 454-pyrosequencing amplicon pools for the AMF using specific primers (Hofstetter et al., 2002, Krüger et al., 2009, Vilgalys and Hester 1990), following the protocol presented in our previous study (Horn et al., 2014).

Sequences were denoised using the PyroNoise approach (Quince et al., 2009) implemented in Mothur (Schloss et al., 2009). The denoising approach removes bad quality sequences, creates sequence clusters and removes chimera sequences. After denoising and preclustering, sequences of roots and soil were clustered into operational taxonomic units (OTUs) using CROP, which utilizes a Bayesian clustering algorithm. This approach addresses species delineation uncertainty better than hierarchical clustering methods due to its flexible cut-off and therefore creates significantly less artifact OTUs than fixed cut-off clustering approaches (Hao et al., 2011).

Due to the nature of pyrosequencing, we found differences in read numbers for every sampling location, so we resampled the read numbers to equal amounts of 500 reads per sample using a bootstrap approach with 10.000 iterations per sample (Wehner et al., 2014). Samples with considerably lower read numbers than the estimated resampling threshold (<70%) were discarded. Additionally, singletons were removed. All subsequent statistical analysis was done in R 3.1 (Team 2013), the source code from the analysis is provided in the supporting information.

Phylogenetic tree calculation

We calculated a phylogenetic tree for the AMF OTUs using RAxML (Stamatakis 2006) in order to further refine the OTU definitions following our approach from a previous study (Horn et al., 2014). About 110 representative sequences of an SSU-ITS-LSU AMF reference alignment (Krüger et al., 2012) plus an outgroup sequence from the Chytridiomycota were added to our own sequences to determine the phylogenetic position of our OTUs. With the help of the phylogenetic tree we removed sequences which clustered outside the Glomeromycota and are therefore likely to be erroneous or non-

AMF sequences. OTUs were annotated according to the results of a BLAST search prior to phylogenetic tree calculation.

Phylogenetic community structure

When testing for Driver and Passenger hypothesis, we wanted to include not only abundance data but also the phylogenetic sorting of the respective communities. In order to account for the different roles of plant and AMF communities influencing each other, we started with analyzing phylogenetic diversity (PD) within the AM fungal and plant communities. We chose the Daphne plant tree for our plant phylogenetic analysis (Durka and Michalski 2012), a tree consisting of most major Central European plant species, since it provides a complete set of phylogenetic distances for our plant dataset. Using the picante package (Kembel et al., 2010), we obtained two estimates of PD: the standardized effect size of mean pair wise distance (SES-MPD), which calculates the net relatedness index (NRI from beta-diversity with a null model, and inter-community mean pair wise distance (IC-MPD), which calculates beta-diversity as phylogenetic distance between communities. Phylogenetic distances between AMF OTUs were calculated using the Needleman-Wunsch implementation of Esprit (Quince et al., 2009, Sun et al., 2009). The distances between plant species were calculated as pairwise distances from the trimmed Daphne phylogenetic tree using the cophenetic.phylo function of the ape package (Paradis et al., 2004). The SES-MPD represents a net related index (NRI) value for each sample, which is defined as $[-(MPD - MPD_{null})/_{SD}(MPD_{null})]$, where MPD is the mean pair-wise phylogenetic distance among species in the sample (Kembel and Hubbel 2006, Li et al., 2014). The mean values of the NRIs of all samples of AMF were then used as the alpha-diversity measure to judge the clustering or segregation of the overall AMF community. Positive NRI values are related to clustering, negative values to overdispersion. The same approach was applied to the plant community. The null model algorithm we used was “independentswap” with 999 randomized null communities. “Independentswap” retains column and row totals for null model analysis of species co-occurrence (Caruso et al., 2012a, Gotelli 2000). This approach is particularly suited to problems that concern differences in species composition, because it accounts for variations in other community attributes such as diversity and richness. Significance of the calculated NRIs was tested using t-test.

IC-MPD were calculated as pair-wise phylogenetic distances of the samples, based on pair-wise genetic distances between OTUs and plant species, respectively, and yielded a sample x sample distance matrix as a measure of beta diversity. In order to include the IC-MPD information in a subsequent variance partitioning analysis (Caruso et al., 2012b, Legendre and Legendre 1998), the distance matrices of plants and AMF were subjected to a principal coordinate analysis (PCoA), a commonly used tool to reduce dimensionality which provides a measure of the amount of variance explained in the few independent principal axes (Legendre and Legendre 1998). The PCoA axes were extracted and used as the phylogenetic explanatory variables in order to quantify the individual contributions of plant phylogeny on AMF community composition and vice-versa in the variance partitioning analysis. The use of phylogeny allows for dealing with beta-diversity correlations, while abundance data will be utilized as means for alpha-diversity in the subsequent variance analysis.

Null model analysis

In order to account for non-random species associations that are linked to biotic influences of AMF and plant on each other, we performed null models on plant and AMF species, respectively. Null model analysis was done in EcoSim (Gotelli and Entsminger 2012). In our null model analysis the C-score index was used to compute values of co-occurrence for the given set of presence/absence data. Since the C-score does not require perfect checkerboard distributions and has a low susceptibility to type II errors, it seemed best suited for our purpose (Gotelli 2000). The input matrix was randomized following the suggestions of Gotelli (2000) to minimize type I errors and test for patterns of co-occurrence expected under non-random assembly processes and interacting species. The algorithm used fixed sums of rows and sums of columns and applied the Random Knight's Tour approach for shuffling the matrix. Retaining the row and column totals preserves differences in species richness among sites and differences in occurrence frequencies among species, therefore representing a conservative approach when testing for patterns in species composition. The null hypothesis was considered rejected when the observed C-Score was significantly different from the simulated C-Scores ($P < 0.05$). A C-score lower than the simulated average represents an aggregated community, while a higher score represents a segregating community. Segregating communities indicate species

sorting processes by niche-partitioning or biotic interactions, which would, in combination with results from variance partitioning, show potential influences of AMF on plants and vice-versa.

Models of correlations between plants and AMF

In order to judge whether AM fungi are the Driver or the Passenger in the plant-AMF interaction, we quantified the variation in phylogenetic distance and abundance of AMF that explained the respective plant community, plus the vice-versa analysis using plant phylogenetics and plant presence / absence as a predictor for AM fungal community composition. We combined the extracted PCA axes of the IC-MPD (see above) with a forward selection model to choose the proper number of axes to include in the analysis. Additionally, we checked the generalized linear response of the relative abundance of AM fungal taxa on plant presence / absence and vice-versa using the manyglm function from the mvabund package (Wang et al., 2012, Warton et al., 2012) with a negative binominal error distribution. We removed contributions from environment and space by conducting an RDA on the predictor's abundance or presence / absence table, respectively, and used the residuals with the generalized linear model function.

The analysis of patterns in community structure was conducted so that the Null hypothesis of the Driver and Passenger framework as well as potential influences of plants on AMF and vice-versa could be quantified. Calculation was done in R, using the vegan (Oksanen et al., 2012) and the spacemakeR (Dray 2011) package. We analyzed the relative AMF abundance obtained from processing the sequences as response variable. Spatial information (pair-wise distance between samples), log-transformed environmental data (sample pH, carbon, nitrogen, phosphorus, water content, and dehydrogenase activity as a proxy for microbial activity) and the plant presence / absence matrix plus the respective phylogenetic distance matrices were used as additional explanatory variables. Furthermore, plant presence / absence and phylogeny were used as response variable while using environment, space and the AMF as explanatory variables.

In variance partitioning “space” represents spatial autocorrelation: Moran eigenvector mapping (MEM) was used to account for spatial autocorrelation at multiple scales in community variance partitioning (Dray et al., 2006, Legendre et al., 2009). This method represents a general, more

powerful version of the widely used PCNM (Borcard et al., 2004), which allows testing for several types of spatial structure. Several competing spatial models are possible and the most parsimonious model is selected using a multivariate extension of the Akaike Information Criterion AIC (Akaike 1973). This model provides the best linear combination of eigenvectors accounting for spatial autocorrelation at multiple spatial scales and each eigenvector represents a certain scale (Dray et al., 2006, Horn et al., 2014).

Mixed partitions in variance partitioning represent the difference between the sums of the modeled variance for the individual partitions and the modeled variance of all partitions, therefore they do not have a testable null-hypothesis and thus no attributable P-value in an ANOVA.

Results

454-pyrosequencing and OTU delineation

The clustered and denoised data set consisted of 325 OTUs. During the resampling, we removed 7 root and 1 soil sample based on minimal read numbers of 500 reads. After resampling and removal of singletons, we found the majority of the remaining 130 OTUs to cluster well within our tree with the reference sequences (Fig. 2), but 57 OTUs were removed prior to matrix calculation since they clustered outside the reference sequences and thus membership to Glomeromycota could not be ascertained. The OTUs found in our tree span across most of the known AMF families, indicating that the primers we utilized are fairly exhaustive in covering the AMF phylum (Fig. 2). From the families included in the reference sequences, we only lacked OTUs of the Geosiphon clade, while all other families were represented by at least one OTU.

Table 1: Percentage of explained AMF community variation by decomposition of total variance in the AMF community matrix into unique predictors: environment (soil properties), space (geographic position) and phylogeny (phylogenetic position). Depicted are percentage values of total variance in all macroplots and the individual macroplots each in root and soil. MP = macroplot. Significance: *** = P<0.001; ** = P<0.01; * = P<0.05; NS = not significant, NT = not testable.

	environment	space	phylogeny	env + space	space + phylo	env + space + phylo	not explained
all MPs root	1 ^{NS}	16 ***	11 ***	1 ^{NT}	14 ^{NT}	6 ^{NT}	51
MP1 root	13 ***	20 ***	18 ***	0 ^{NT}	19 ^{NT}	0 ^{NT}	30
MP2 root	0 ^{NS}	31 ***	28 ***	0 ^{NT}	0 ^{NT}	24 ^{NT}	17
MP3 root	0 ^{NS}	4 **	30 ***	0 ^{NT}	9 ^{NT}	12 ^{NT}	45
all MPs soil	4 ^{NS}	24 ***	15 ***	0 ^{NT}	10 ^{NT}	6 ^{NT}	41
MP1 soil	3 ^{NS}	5 **	14 ***	9 ^{NT}	13 ^{NT}	0 ^{NT}	56
MP2 soil	9 ***	17 ***	15 ***	8 ^{NT}	42 ^{NT}	0 ^{NT}	9
MP3 soil	0 ^{NS}	21 ***	25 ***	0 ^{NT}	7 ^{NT}	6 ^{NT}	41

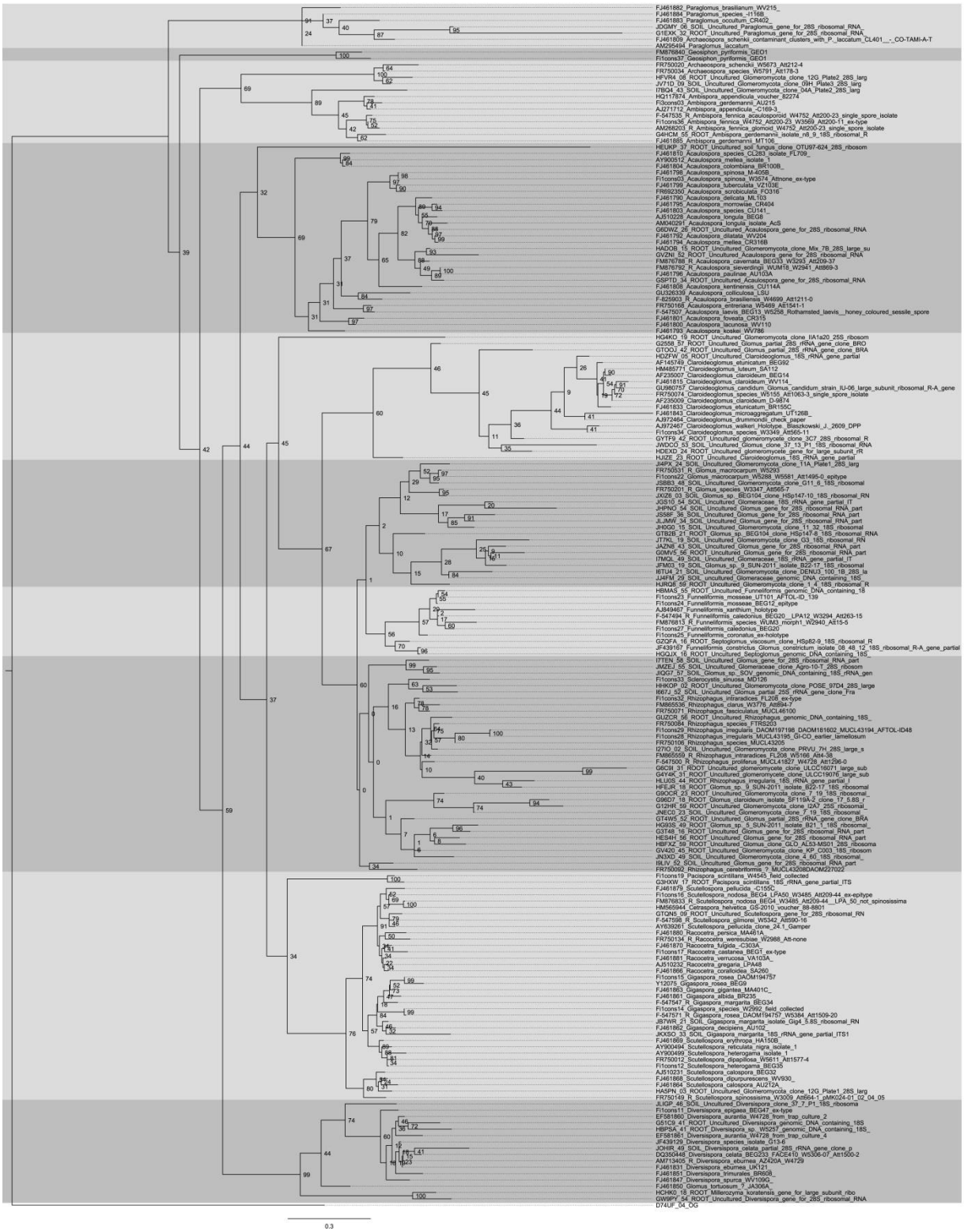

Figure 2: Maximum likelihood tree of 73 OTUs from the root and soil dataset, complemented with 110 sequences from the Krüger et al. (2012) SSU-ITS-LSU alignment and one non-AMF outgroup ("D74UF_OG", an unidentified member of the Chytridiomycota). Tree calculation was done in RAxML. Node numbers represent Bootstrap values. The node descriptions containing a "ROOT" or "SOIL" tag represent the OTUs defined in our study, while the other nodes represent the sequences from Krüger et al.

In the end, the root data set consisted of 68 OTUs and the soil dataset of 59 OTUs, with a total of 73 OTUs. Overall OTU richness per macroplot was comparable, ranging from 31 to 44 in roots and from 28 to 41 in soil (Table 2). The dominant fungal groups in our soils and roots were *Glomus* ssp. and *Rhizophagus* ssp.

Table 2: AMF phylogeny and null model results from community abundance data. The OTU numbers for all macroplots and each individual macroplot in root and soil are depicted in the "OTUs" column. The mean pair wise distance between the communities of all macroplots and each individual macroplot in root and soil are depicted in the "MPD" column. The effect size and respective P-value for the null model analysis of the communities in all macroplots and the individual macroplots in root and soil are depicted in the columns headed with "null model". Positive effect sizes / mean pair wise distances indicate a segregated community (species repel each other), while negative values represent an aggregated community (species attract each other). MP = macroplot.

	phylogeny		null model	
	OTUs	MPD	effect size	P
all MPs root	68	0.01	11.75	<0.001
MP1 root	42	-0.02	4.08	0.002
MP2 root	31	-0.07	1.13	0.137
MP3 root	44	0.00	-0.73	0.250
all MPs soil	59	0.01	19.42	<0.001
MP1 soil	41	0.08	10.96	<0.001
MP2 soil	28	-0.14	10.66	<0.001
MP3 soil	39	0.08	1.61	0.068

Null model interactions

The AMF community was found to be significantly segregated overall. In roots the effect was only significant for the first macroplot and the whole dataset (Table 2). In soil the first, second, and overall datasets were significantly segregated. Effect sizes were considerably higher in soil than in root data sets (Table 2).

Phylogenetic diversity

Despite the fact that we found significant portions of variation in AMF community composition explained by AMF phylogenetic distance between samples (see below), we could not find a significant NRI difference overall. While neither the root nor the soil sets of the phylogenetic data showed significantly segregated or aggregated communities on a per-macroplot or per-data-set basis (Table 2), individual distances of the spots themselves were showing a broad range of phylogenetically segregated and aggregated communities.

Variance partitioning and AMF-plant correlations

The overall effect of the environment on AMF alone was very low. With an exception of the root data set of macroplot 1, environmental data explained less than 10%. Pure space was a major predictor of the overall data set and even within the macroplots showed significant and large proportions (up to 31%) of explained variation (Table 1). About 5-7 eigenvectors were responsible for this effect, and these were predominantly from the very low (>100m) or very high (<10m) rank class. Phylogeny was the second largest explanatory component in the variance partitioning of the AMF without plants. Up to 30% of variation could be explained by the phylogenetic distance of the AMF in our data set (Table 1). Additionally, we found the spatial-phylogenetic partition (spatial phylogenetic turnover) explained large parts of the AMF variance.

The generalized linear models (GLM) we applied showed a significant role of plant species presence / absence in prediction of AMF species abundance in roots ($P<0.001$) and soil ($P<0.001$). The vice-versa approach did not show any significance. In the variance partitioning analysis, the plant presence / absence accounted for a significant amount of explained variation in the root AMF communities, confirming the above results from the GLM approach (Table 3). However, the phylogeny was no significant predictor for fungal community pattern. We found a weak signal from the partition of spatial variation plus plant community in the AMF data set, which was stronger for presence / absence than for phylogeny (Table 3). When using the AMF as a predictor for the plant community, the explained variation by the fungi was very low, yielding a maximum of 3% variation of fungal phylogeny explained in the root data set (albeit not significant), and otherwise negligible amounts. The

spatially structured AMF community was a slightly better predictor and explained up to 5% of the plant community variation (Table 4).

Table 3: AMF / plant interaction by decomposition of total variance in the AMF community matrix into unique predictors: Plant phylogeny (left) or plant presence / absence (right) + environment (soil properties) + space (geographic position). Depicted are percentage values of total variance. Significance: *** = P<0.001; ** = P<0.01; NS = not significant, NT = not testable.

	AMF vs. plant phylo		AMF vs. plant p/a	
	root	soil	root	soil
environment	0 ^{NS}	0 ^{NS}	3 ***	0 ^{NS}
space	30 ***	29 ***	19 ***	24 ***
plants	0 ^{NS}	0 ^{NS}	4 **	0 ^{NS}
env + space	4 ^{NT}	3 ^{NT}	11 ^{NT}	5 ^{NT}
space + plants	0 ^{NT}	6 ^{NT}	11 ^{NT}	10 ^{NT}
env + plants	0 ^{NT}	0 ^{NT}	0 ^{NT}	0 ^{NT}
env + space + plants	3 ^{NT}	3 ^{NT}	0 ^{NT}	2 ^{NT}
unexplained	63	59	52	54

Table 4: AMF / plant interaction by decomposition of total variance in the plant community matrix into unique predictors: AMF phylogeny (left) or AMF abundance (right) + environment (soil properties) + space (geographic position). Depicted are percentage values of total variance. Significance: *** = P<0.001; ** = P<0.01; NS = not significant, NT = not testable.

	plants vs. AMF phylo		plants vs. AMF abu	
	root	soil	root	soil
environment	8 ***	7 ***	20 ***	19 ***
space	12 ***	8 ***	5 **	6 ***
AMF	3 ^{NS}	0 ^{NS}	0 ^{NS}	0 ^{NS}
env + space	1 ^{NT}	1 ^{NT}	0 ^{NT}	0 ^{NT}
space + AMF	0 ^{NT}	3 ^{NT}	5 ^{NT}	4 ^{NT}
env + AMF	11 ^{NT}	12 ^{NT}	0 ^{NT}	0 ^{NT}
env + space + AMF	0 ^{NT}	0 ^{NT}	0 ^{NT}	0 ^{NT}
unexplained	65	69	70	71

Discussion

Are AMF communities influenced by plant community composition or vice-versa?

The Passenger and Driver framework is working with the assumption that AMF and plants influence each other's community dynamics. Our analysis showed that AMF community variance is mostly influenced by their spatial position and their phylogenetic composition (Table 1, Table 3), while the plants are strongly influenced by the environment (Table 4), as well as spatial position. Plants were found to be significantly segregated in a previous study (Horn et al., 2015). The generalized linear models we applied, however, showed a significant role of plant presence / absence in prediction of AMF abundance, while the vice-versa analysis was not significant, pointing to the Passenger role of AM fungi. This was confirmed in part by the variance analysis. It has been shown that plants may reward the best fungal partners with more carbohydrates (Bever et al., 2009) or that particular plant communities may cause the development of specific AMF communities (Hausmann and Hawkes 2009). While our AMF communities are fairly diverse, the plant diversity in our sampling area is dominated by a set of a few indicator species, which may explain the diminished effect of plants found in the variance analysis of AMF compared with the generalized linear models. The variance analysis is using RDA-based matrix regressions while the generalized linear model we apply here is fitted separately to each species of the response variable, therefore allowing higher contributions of individual species / OTUs to surface.

When it comes to support for the Driver hypothesis, our data can only provide a negligible amount of support. In the root data set, AMF phylogeny showed to explain a small amount of plant community variation (Table 4). However, given that we sampled from a single plant species, the root colonizing fungi might have limited ability to influence the surrounding plant communities, and one would rather expect such an effect to be more dominant in the soil. Since the effect was not present in the surrounding soil, the phylogenetic effect of AMF on plants is not very convincing, and moreover, cannot be causally attributed to either AMF or plants unambiguously. The dominance of *Glomus* ssp., *Rhizophagus irregularis* and other generalist taxa in the AMF community might, in combination with the phylogenetic pattern, be another explanation for the lack of community interactions on a small

scale between plants and AMF. Grassland plant communities harbor a lot of C3 grasses which are not very dependent of mycorrhiza. There is evidence that these plants prefer generalist AMF taxa (Helgason et al., 2007, Öpik et al., 2009, Vályi et al., 2015), and therefore might not apply strong selection processes on specialist AMF species.

Are AMF communities assembled through interspecific interactions?

Our analyzed AMF communities were found to be structured non-randomly. Null model analysis showed strongly segregating communities, while community beta-diversity indices indicating phylogenetic aggregation or segregation patterns were not significant. In a previous analysis of AMF communities in this sampling area, we found phylogenetic aggregation (Horn et al., 2014), however, the overall segregation in a non-phylogenetic null model analysis was found in both analyzes. Despite that, we found that the phylogenetic signal explained a significant amount of variance of the AMF community. These segregation results may be caused by our sampling being at the beginning of the vegetative period in our sampling area, which in turn leads to lessened interactions and coexistence mainly due to spatial and temporal cohabitation of a certain spot in soil. While the vegetation in our grassland decays over winter and rebuilds in the spring, AMF communities are of a more perennial nature and the mycorrhizal networks have been shown to survive winter periods (McGonigle and Miller 1999). It might be that in the beginning of the growth season, plant-mycorrhiza interactions were still at the beginning and therefore selection pressure differed, forcing a changed balance between competitors and persistors.

Nevertheless, AMF community structure is determined by their phylogeny and spatial position. Given the amount of variation explained by these two parameters, compared to the plant data, it is reasonable to conclude that the AMF in our sampling area assemble mostly independently from the plants. We see in our variance partitioning that especially on the single-macroplot level, environment, space and phylogeny are not clearly separated from each other in their influence on AM fungal communities, however, dispersal limitation found to exist both in the AMF and in the plant community may be an explanation for this effect. The presence of low-ranking MEM eigenvectors (indicating the influence of large-scale spatial segregation) emphasized the findings of the non-phylogenetic null model that the

spatial range of AMF was limited at the time of sampling and that dispersal limitation could be a main contributor to the spatial signal we found. We do find that the AMF exhibit a stronger spatial signal than plants, however, it is difficult to disentangle random effects from actual dispersal limitation when trying to quantify this effect. Given our small scale, the assumptions of Zobel and Öpik (2014) might not apply here.

The replication of macroplots confirmed findings from a previous sampling regarding the phylogenetic sorting of the AMF community (Horn et al., 2014), which may be caused by interspecific interactions, meaning that the majority of AMF on a local scale is interacting rather with other AMF species in establishing communities than with plants. Trait convergence (Powell et al., 2009) could be an effect that causes this kind of selection process.

How important is the variation in environmental parameters?

Patterns within our macroplots differed despite the relative similarity in plant diversity and environmental features. In the soil data set, the environment showed a small signal mainly attributed to pH variance, which was found more strongly in macroplot 1 and 2. Macroplot 3 showed a generally different behavior. We believe this is due to the more sandy soil structure of this macroplot, which in turn selects for a set of more drought persistent individuals, both among plant and AMF species. Therefore species selection processes may be different here. In roots only macroplot 1 showed a significant environmental signal, which indicates a AMF population that is more dependent on the environmental gradient there. Spatially structured phylogeny was also among the important partitions, especially in the individual macroplots, suggesting that the phylogenetic species sorting process is of a perennial nature and is still able to structure the community at the beginning of the growth season, albeit being influenced by a lower amount of living plant roots and therefore creating phylogenetic islands which are mainly spatially structured.

When we compare the results found here with the ones found in a previous study (Horn et al., 2014), we see several similarities, be it the level of AMF diversity or the general structure along space and phylogeny. This provides evidence that our utilized response variables could be not highly sensitive to seasonal effects. Since the spatial extent of spore distribution in AMF is quite low, it comes to no

surprise that community patterns in the soil in a previous year are also found in the subsequent growing season. The cover of live plant biomass in German grasslands vanishes to a great extent during winter every year, which leads to the necessity to reestablish a certain amount of mycorrhizal colonization in the beginning of every growth period (Kabir et al., 1997). This provides some indications for a certain seasonal effect and a possible explanation that the interactions of the AMF species are stronger in our previous study which was conducted at the end of the growth period. However, given we only have two sampling points in time, the implications of seasonality we can conclusively assess are limited.

Conclusion

The tenet of the Passenger hypothesis, meaning direct influence of plant communities on AMF communities, could be shown, but their overall influence on AMF composition was low. Data indicate that the AMF could be following primarily a species-sorting process led by phylogenetic dynamics and dispersal limitation, pointing to a mainly independent behavior. The shape of these processes may be linked to the seasonal state of the biotic interaction in our research area, pointing out the different spatio-temporal dynamics of plants and AMF.

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Chapter V: General discussion

In this study the community assembly principles of plants and AMF in a highly diverse system were analyzed on a small scale, enabling the discovery of processes linked to direct influences on these organisms. Community ecology dealing with plants and / or AMF in the field tends to address fairly large areas covering a wide range of organisms to paint a full picture of processes taking place in an ecosystem, however, the actual selection processes always happen at a single spot at a given time, therefore the inference of patterns is increasingly more difficult the more broad the applied scale is. The success of neutral theory in describing systems with a high species density like tropical rainforests (Hubbell 2001) demonstrates that the amount of local interactions and selection processes constructed in multiple layers illustrates that stochastic processes or multitudes of interactions may be so dominant they overlay the common assumptions of classical niche theory. For my thesis it was important to not only focus on organisms whose interactions may happen at a more confined spatial scale but also adjust the size of the analyzed ecosystem to an area suitable to capture the majority of the significant interactions taking place and therefore being able to model a more inclusive approach. The study design was spatially explicit and utilized a fine-scale approach, linking high sampling density and small spatial extent in a way not done before. The hypotheses in the chapters tried to approach everything from a conservative stance, since field observational studies lack manipulative testing for mechanisms. Hence, the analysis of patterns focused on major factors known for their importance, the AMF OTU assignment involved a conservative approach to both clustering and sequence removal, and the calculation of spatial portions of variance followed the strict selection among a diversity of models using Akaike Information Criterion (AIC) (Akaike 1973).

The idea behind the chapters was to initially analyze the individual organism groups to determine how likely a strong interaction between the two species groups was. Given that plant assemblages are commonly thought to be shaped by their environment and interactions via niche selection for almost half a century and that only in recent decades have been linked to belowground microbial diversity, it seemed fair to assess them alone at first, so that a general baseline existed when comparing them with the AMF. Since AMF are highly important plant symbionts (Smith and Read 2008), they were the first

choice for elucidating plant-microbe interactions. So subsequently in Chapter II the plant community assembly was assessed at a fairly small scale. This assembly is driven by processes taking place on several scales like competition (Aarssen 1989), dispersal limitation (Ai et al 2012) and environmental conditions (Latimer and Jacobs 2012). The results of Chapter II indicate that plants behave according to niche sorting processes and dispersal limitations, which is likely expressed through competitive exclusion. The plant communities were significantly overdispersed in the Null model analysis, indicating that species sorting processes influence the establishment of a plant species at a given spot (Gotelli 2000). Since the variance partitioning illustrated the importance of spatial processes and environmental filtering in plant community composition, this overdispersion was likely caused by species establishment according to niche differences (Chave et al 2002), which prevented other species from establishing at a given spot. Examples for this behavior were different *Festuca* species, with *Festuca brevipila* being more dominant in the upper regions of the plots where soil contained more clay and hence more moisture, while *Festuca psammophila* was almost exclusively found in the lower regions of the hill slopes, where the high sand content caused the soil pH to be lower and the soil overall to be dryer. The strong spatial signal could be an indicator of dispersal limitation, however, given the short distances between the different macroplots, it was unlikely that dispersal limitation was a dominating process there. Nevertheless, examples of plants were found that were spatially segregated, for example *Bromus sterilis*, which was found only in the most distant macroplot 3, or *Stipa capillata*, which was only found in macroplot 1. Neutral behavior or random influences were the more favorable explanation for the spatial signal. The findings confirm the baseline assumption that plants are strongly connected to aboveground interactions, even at a very small scale, indicating that current assumptions for plant community assembly are also valid in the chosen study system. It also reassures the common concepts of community composition rules function at very small scales, which might also be an outlook for neutral behavior in that sense that decreasing scale of analysis might actually capture a more fine-scaled subset of interactions and therefore remove the neutral properties of a community.

After acquiring a baseline idea of plant community assembly, the same approach was taken for the AMF. Arbuscular mycorrhizal fungi have a yet to be conclusively described diversity, therefore

limiting the amounts of generalizable findings from greenhouse experiments. Field studies using exhaustive parallel sequencing are currently the only feasible approach of dealing with such unknown diversity in a community context. The design of the study of Chapter III allowed to find general AMF community assembly rules and to differentiate the role different abundance can play. General trait manipulation is exceptionally difficult for field studies on AMF, and only a few proper studies exist on this subject (van der Heijden and Scheublin 2007). Therefore approaching the community assembly the way it was done in Chapter III provides one means to overcome or at least diminish this restriction. This includes that the different assembly rules of dominant and rare AMF are likely to be connected to the individual traits of these groups and can act as a proxy to understand their role in the ecosystem.

When looking at the AMF in Chapter III, where sampling took place roughly 6 months before the sampling used for Chapter II and IV, the analysis pipeline revealed a surprising amount of AMF OTUs for the small sampling area (15 x 15m), namely 74. Most of them belonged to the *Rhizophagus* and *Glomus* genus, but altogether 10 different families of AMF could be found. In contrast to the plants, the environment played only an insignificant role in AMF community composition, while spatial processes and phylogenetic sorting were dominant. Previous studies had found a significant influence of the environment (Dumbrell et al 2010, Lekberg et al 2011, Macek et al 2011), however, these studies did not share the same small-scale approach that was applied here. In contrast to the plants, AMF do seem to be affected significantly by the scale that is applied to analyzing them, which comes to no great surprise given that AMF are thought to be more spatially restricted than plants (Wolfe et al 2006). The famous and now clearly outdated microbial concept of "everything is everywhere but the environment selects" (Baas Becking 1934) is therefore not valid, given that AMF are known to be inefficient dispersers (Smith and Read 2008). While there are cosmopolits among the AMF (Lekberg et al 2013, Öpik et al 2006), the actual community composition analysis indicated other selection processes than the environment. The AMF in our study area behaved phylogenetically aggregated, which lead to the assumption that selection processes beneficial for the establishment of one AMF species would also be beneficial for genetically closely related species (Powell et al 2009). Given that several studies speculate on the selective role of plants on AM fungal species (Bever et al 2009, Bever et al 2010), this seems to be a valid assumption, since the phylogenetic signal was found not only in

this but also the subsequent sampling in Chapter IV. The seasonal effects and spatial influence of plant-microbial interactions has been shown (Davey et al 2012, Dumbrell et al 2011, Lekberg et al 2011), so the aggregated behavior might be an effect depending on seasonal situations or the spatial position of the sampling, emphasized by the fact that the particular observation of phylogenetic aggregation could not be reproduced in the subsequent sampling that took place in spring. The strong spatial signal is an indicator of the described spatial limitations of AMF species in terms of dispersal and the general extent of a single AMF individual. Although the definition of AMF species had to rely on a mathematical background, the modern approach that was chosen closed the gap between the actual species and the definition of an OTU to a certain extent (Powell 2012). It is therefore a valid assumption that spatial limitations observed in Chapter III were indeed related to the actual behavior of AMF species. Even though a strongly skewed abundance distribution was observed, with a significant dominance of *Glomus* and *Rhizophagus* species, the effect of spatial limitation and phylogenetic sorting could also be found in the rare families, further emphasizing that the observed effect is indeed real. That proved interesting since the common idea of the rare species in a species-rich ecosystem describes them as a sort of functional redundancy system, which is used as an explanation why species-poor ecosystems are more prone to losing functional traits and therefore are less stable than the species-rich systems. The conclusion from this would be that the rarity of species is more dependent on the selection principles that apply, and therefore their community composition should be more dependent on spatial position and niche dynamics rather than their phylogenetic relationship amongst each other. The opposite was true in Chapter III, which pointed to the idea that either the influence of plants on the AMF might be a significant selector or that abundance and rareness are mere traits of an AM fungal species that are shaped by the distribution capabilities of that species. They would be expressed by the amounts of spores or the amount of hyphal growth rather than biotic selection procedures like in plant communities.

Given the noticeably different processes of species establishment in AMF and plants already apparent in Chapter II and III, it became clear that the analysis of the influence of the two organism groups on each other was a necessity to understand more about the processes in the study system and was subsequently done in Chapter IV. It was possible to reaffirm previous findings that point to a niche-

based sorting process among the plants and a spatially-phylogenetic sorting paradigm among the AMF. But the more important question dealt with the interactions between the two groups. The variance partitioning indicated that AMF and plants are independent of each other, not only in light of abundance or presence-absence, but also when looking at the phylogenetic systematics of the two organism groups that were sampled. Some support was found for the Passenger hypothesis which states that AMF communities are influenced by the plant communities surrounding them, and evidence for the Driver hypothesis was negligible. Findings that point to influences both of AMF on plants and vice-versa only could be found when applying generalized linear models. A problem with such an analysis is that correlation is not necessarily causality, which makes it exceptionally difficult to disentangle Driver and Passenger effects from each other in a field study. Despite the fact that Zobel and Öpik stated that in reality, AMF and plant community interaction might represent a mixture of Passenger, Driver and independent behavior, the AMF communities in the applied sampling regime in Chapter IV behaved primarily independently. This might be influenced by dispersal limitations of either plants (Turnbull et al 2000) or AMF (Mummey and Rillig 2008, Smith and Read 2008), especially when looking at the strong spatial signal observed in both communities. When looking at the results from Chapters II and III, the mixture of different selection processes comes to no real surprise, given the strong dependence on environmental (for the plants) or intraspecific (for the AMF) processes. This is also the context in which the linear model result should be seen: If plants select for certain genetic traits in AM fungi, this will eventually lead to species sorting along a phylogenetic gradient, while the actual interaction can appear to be dependent on plant community composition. Since field observational studies generally lack the possibility to conclude the exact mechanisms causing the observed patterns, it is difficult to tell if plants actively "lock out" undesired AM fungal species or if genetically closely related AMF species are able to "break in", once a favorable species has found its way into the plant root. Given that there is evidence for both, it might as well be a mixture of the two processes.

The interaction of plants and AMF in assembling their respective communities has been shown to be limited in the small scale analysis conducted here. Instead, evidence suggests that both groups have their own sets of assembly rules, and that they are characterized by the individual traits of the

respective group. It has been shown that AMF do influence plant communities by enhancing growth of rare species, for example by supplying additional resources along the mycorrhizal network to plants with higher mycorrhizal dependency, therefore promoting species diversity (van der Heijden et al 1998a, van der Heijden et al 1998b). However, this view is based on studies dealing with artificial communities, field samplings with outdated sampling methods for AMF and neglect of the different spatial extents of the two organism groups. In the present work, it was possible to quantify the influence of the whole AMF community on whole plant communities in relation to their individual assembly patterns. It was therefore possible to put into perspective the contributions of individual AMF species on isolated plant species that are commonly cited as evidence for the AMF influence on plants. They without doubt do provide important ecosystem services, but the actual contribution to the assembly of a plant community remained not fully understood. While the potential influence of AMF on plants has been conclusively shown, the relative influence in comparison with other factors has only been addressed in a few studies (Klironomos et al 2011). The present work provided insight into the fact that AMF and plants, despite their close mutualistic relationship, exist in their own individual domains and their interaction might be based on a system of individual benefits for the plant and AMF species. I would suggest in the context of this work, that AMF and plants perceive each other merely as an extended environmental factor providing nutrients for each other.

Naturally, the field studies presented here could analyze only a snapshot of a highly dynamic ecosystem. The role of seasonal effects and the differences in the spatio-temporal properties of AMF and plant species could not be addressed to a satisfying extent, which warrants further sampling taken at a more dense system of points in time. Additionally, the analysis presented here represents only a look at one ecosystem, however, the community composition processes of the AMF were verified twice here and a third time in a Eucalyptus woodland in Sydney, Australia (Horn et al., unpublished). In that study, AMF community differences could be related to a certain extent to the age of their surrounding host trees, but were again mainly spatially and phylogenetically structured, despite the fact that the ecosystem analyzed was of a different nature, in a different climate zone and in the Southern hemisphere half-way around the world. Therefore, the insights gained here may very well be of a common validity and should help gain a balanced perspective towards the role of AM fungi in

plant assemblages. In the future, this view should be more refined by adding more ecosystems to the analysis, and by adding a more extended examination of plant and AMF interaction via the analysis of more individual plants over a wider range of species and their respective AM fungal communities. Given that sequencing technology is advancing even as this work is written, the feasibility of doing so will be given even more in the future. Further perspectives may shift the paradigm of the ubiquitous dependence of plant communities on AMF more towards an egalitarian perspective or provide evidence for an inherent difference of AM fungal behavior in different ecological contexts.

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Chapter VI: Summary

The study of assembly of plants and microbial communities is an important subject of ecological research as it aids our understanding of species diversity and hence ecosystem functions. Mycorrhizal fungi are of particular importance due to their role as prime symbionts for most land plants. The community composition of plants and mycorrhizal fungi are governed by environmental, spatial and biotic patterns, whose individual contributions are an active field of research and ongoing debate. Since the processes are tightly interwoven, greenhouse experiments offer limited access to the actual processes which take place in nature.

In the present dissertation, the community assembly rules of plants, AMF and the interplay between the two organism groups were studied in a high diversity ecosystem in the "Oderhänge Mallnow", offering a large diversity of plants and steep gradients in soil environmental properties on a small scale. Two field samplings were conducted, one studying a single macroplot of 15x15m and analyzing the AM fungi community composition of the focal plant on the study site, *Festuca brevipila* roots and surrounding soil. The second sampling was conducted 6 months later, using three macroplots of 15x15m, 12x12m and 12x12m, and focused on AMF communities in root and soil of *Festuca brevipila* plus the composition of the surrounding plant communities.

In Chapter II, the plant community assembly patterns were studied. It was found that important roles of environmental factors that prevail at larger scales also are present at smaller scales. Additionally, biotic interactions causing species segregation and effects of the spatially structured environment share a significant influence in plant community composition.

In Chapter III, the AM fungal communities from the first sampling and their respective assembly patterns were analyzed. Results indicate that environmental influences are negligible for AMF, but rather spatial and phylogenetic patterns dominate the assembly of communities. Phylogenetic clustering was observed not only in the dominant but also in the rare species, indicating that trait conservatism and resulting selection principles are a main route for AMF community assembly. Apart from this, dispersal limitation and stochastic position events contribute to the composition of an AMF

community at a given spot, as evident due to the low mobility of AM fungi in the soil. These patterns may be related to direct or indirect positive interactions among fungi and their biotic environment.

In Chapter IV, the interactions of AMF and plant communities from the second sampling were analyzed in context of the Passenger and Driver framework. AMF follow their strong spatial structure and phylogenetic sorting patterns, albeit strong phylogenetic clustering could not be observed in this particular sampling, indicating potential seasonal influences. They only have insignificant influence on either their surrounding plant community composition or their phylogenetic distribution, hence rejecting the Driver hypothesis. Environment only has minor influences on AMF community composition, confirming findings from Chapter III. The plants follow more or less the same patterns as in Chapter II, even when adding AMF communities as explanatory variables to the equation. They do however have a significant influence on AM fungi community composition in context of generalized linear models, indicating evidence for the Passenger hypothesis.

In summary, plant and AMF may share a close mututalistic relationship, however, the rules governing diversity and composition of their respective communities seem to be independent of each other. While plants follow classical niche-partitioning based systematics, AMF possess a stronger focus on biotic patterns, be it intra-specific as shown by phylogenetic sorting, or inter-specific as in their influence by plants. Their low mobility and limited dispersal capabilities add a layer of random spatial position, leading to the conclusion that there is no single rule of acquisition regarding AM fungi community and diversity in an ecosystem.

Zusammenfassung

Die Ökologie der Artengemeinschaften von Pflanzen und Mikroorganismen ist ein wichtiges Thema ökologischer Forschung, da es ein primärer Faktor bei der Gestaltung von Artenvielfalt und daraus folgend Ökosystem-Funktionen ist. Mykorrhizapilze sind von besonderer Bedeutung aufgrund ihrer Rolle als Hauptsymbionten für die meisten Landpflanzen. Die Zusammensetzung der Gemeinschaften von Pflanzen und Mykorrhizapilzen werden von Umweltfaktoren, räumlichen und biologischen Mustern bestimmt, deren individuelle Einflüsse ein aktives Forschungsfeld und Thema laufender Debatten sind. Da diese Prozesse eng mit einander verwoben sind, bieten Gewächshausexperimente nur begrenzten Zugang zu den tatsächlich in der Natur stattfindenden Prozessen.

In der vorliegenden Dissertation wurden die Regeln der Zusammensetzung von Pflanzen- und Pilzgemeinschaften sowie das Zusammenspiel der beiden Organismengruppen in einem hochdiversen Ökosystem in den "Oderhängen Mallnow" untersucht, welches eine große Diversität von Pflanzen sowie steile Gradienten in Umweltfaktoren des Bodens in geringer räumlicher Ausbreitung bietet. Zwei Probennahmen im Feld wurden durchgeführt, wobei die erste einen einzelnen Macroplot von 15x15 Metern untersuchte und dabei die Zusammensetzung der AM-Pilzgemeinschaften in den Wurzeln und dem umgebenden Boden der Fokalart des beprobten Feldes *Festuca brevipila* analysierte. Die zweite Probenahme wurde 6 Monate später auf drei Macroplots der Maße 15x15m, 12x12m und 12x12m durchgeführt und konzentrierte sich auf die AM-Pilzgemeinschaften in Wurzeln und umgebendem Boden von *Festuca brevipila* und zusätzlich die Zusammensetzung der umgebenden Pflanzengemeinschaften.

In Kapitel II wurden die Zusammensetzungsmuster der Pflanzengemeinschaften studiert. Im Ergebnis war die wichtige Rolle der Umweltfaktoren, die auf größeren Skalen vorherrscht, auch hier zu finden. Zusätzlich stellten biotische Interaktionen, die zur Abspaltung von Arten führten, sowie räumlich strukturierte Umweltfaktoren einen wichtigen Einfluss auf die Zusammensetzung der Pflanzengemeinschaften dar.

In Kapitel III wurden die AM-Pilzgemeinschaften der ersten Probennahme und ihre jeweiligen Zusammensetzungsmuster untersucht. Die Resultate weisen darauf hin dass Einflüsse von Umweltfaktoren vernachlässigbar waren, stattdessen dominieren räumliche und phylogenetische Muster die Zusammensetzung der Gemeinschaften. Phylogenetische Aggregation wurde nicht nur bei den dominierenden, sondern ebenfalls in den seltenen Arten beobachtet, was darauf hinweist dass die Konservierung von Eigenschaften und die daraus resultierenden Selektionsprinzipien einer der Hauptwege für die Zusammensetzung von AM-Pilzgemeinschaften darstellen. Daneben wirken die Begrenzung der Ausbreitung und zufällige Positionierungsereignisse an der Zusammensetzung einer AM-Pilzgemeinschaft an einer bestimmten Stelle mit, wie sich durch die geringen Mobilität der AM-Pilze im Boden zeigt. Diese Muster könnten durch direkte oder indirekte positive Interaktionen der Pilze mit ihrer lebenden Umgebung verursacht werden.

In Kapitel IV wurden die Interaktionen der AM-Pilz- und Pflanzengemeinschaften der zweiten Probennahme im Rahmen des Fahrer-und-Beifahrer Systems analysiert. AM-Pilze folgen ihren Muster der ausgeprägten räumlichen Struktur und phylogenetischer Sortierung, gleichwohl konnte die phylogenetische Aggregation in dieser Probennahme nicht beobachtet werden, was auf einen möglichen saisonalen Einfluss hindeutet. Sie haben nur einen insignifikanten Einfluss auf die Zusammensetzung der sie jeweils umgebenden Pflanzengemeinschaften, was zur Ablehnung der Fahrerhypothese führt. Die Umweltfaktoren haben nur geringen Einfluss auf die Zusammensetzung der AM-Pilzgemeinschaften, was die Ergebnisse aus Kapitel III bestätigt. Die Pflanzen folgen mehr oder weniger denselben Mustern wie in Kapitel II, auch wenn man die AM-Pilzgemeinschaften als erklärenden Variablen der Gleichung hinzufügt. Sie haben jedoch einen signifikanten Einfluss auf die Zusammensetzung der Pilzgemeinschaften im Kontext des generalisierten Linearmodells, was auf einen Beweis für die Beifahrer-Hypothese hindeutet.

Zusammenfassend ist zu bemerken, dass Pflanzen und AM-Pilze eine enge mutualistische Beziehung teilen, die Regeln, nach denen ihre Diversität und die Zusammensetzung ihrer jeweiligen Gemeinschaften erfolgt, jedoch unabhängig voneinander zu sein scheinen. Während Pflanzen der klassischen Systematik der Nischenaufteilung folgen, liegt der Fokus bei AM-Pilzen stärker auf biotischen Mustern, sei es innerhalb ihrer Artengruppe durch phylogenetische Sortierung, oder

außerhalb beispielsweise durch den Einfluss der Pflanzen. Ihre geringe Mobilität und die begrenzte Ausbreitungsfähigkeit fügt eine zusätzliche Schicht zufälliger Positionsereignisse hinzu, was zu der Schlussfolgerung führt, dass es keine einzelne Erwerbsregel bezüglich der AM-Pilzgemeinschaften und ihrer Diversität in einem Ökosystem gibt.

Contributions to the publications

I) **Horn S**, Hempel S, Ristow M, Kowarik I, Rillig MC, Caruso T (2015). Plant community assembly at small scales: spatial vs. environmental factors in a European grassland. *Acta Oecologica*

Own contributions: I conducted the field sampling, analyzed the data, performed laboratory work including soil parameter analysis, carried out the interpretation and graphical realization of results and wrote the manuscript.

II) **Horn S**, Caruso T, Verbruggen E, Rillig MC, Hempel S (2014). Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. *ISME J*.

Own contributions: I conducted the field sampling, performed laboratory work including 454-amplicon generation, AMF colonization and soil parameter analysis, analyzed the data, carried out the interpretation and graphical realization of results and wrote the manuscript.

III) **Horn S**, Caruso T, Verbruggen E, Rillig MC, Hempel S (2015). Does neighborhood plant community structure affect the AMF community of focal plants at small spatial scales? *submitted*.

Own contributions: I conducted the field sampling, performed laboratory work including 454-amplicon generation and soil parameter analysis, analyzed the data, carried out the interpretation and graphical realization of results and wrote the manuscript.

Appendix A: Supplementary Material for Chapter II

Table S1: Plant species presence / absence list, part 1.

#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Achillea millefolium agg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agrostis capillaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alyssum alyssoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthericum ramosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arenaria serpyllifolia	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Armeria elongata	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Arrhenatherum elatius	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	1
Artemisia campestris	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0
Bromus inermis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bromus sterilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carex humilis	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Centaurea stoebe	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerastium semidecandrum	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0
Chenopodium strictum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Corynephorus canescens	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactylis glomerata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dianthus carthusianorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Echium vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elytrigia repens	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erigeron canadensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erodium cicutarium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Euphorbia cyparissias	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca brevipila	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Festuca psammophila	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galium verum	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicotrichon pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
Hieracium pilosella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium umbellatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypochaeris radicata	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Koeleria macrantha	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0
Medicago minima	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myosotis stricta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petrorhagia prolifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peucedanum oreoselinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phleum phleoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potentilla incana	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Rumex acetosella	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1
Salvia pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sedum sexangulare	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0
Silene conica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Silene otitis	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stipa capillata	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Thymus serpyllum	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Trifolium arvense	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica arvensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica spicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica verna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Table S1: Plant species presence / absence list, part 2

#	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Achillea millefolium agg	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agrostis capillaris	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Alyssum alyssoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthericum ramosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Arenaria serpyllifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Armeria elongata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arrhenatherum elatius	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0
Artemisia campestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Bromus inermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Bromus sterilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
Carex humilis	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea stoebe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerastium semidecandrum	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Chenopodium strictum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynephorus canescens	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactylis glomerata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Dianthus carthusianorum	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Echium vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Elytrigia repens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erigeron canadensis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
Erodium cicutarium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euphorbia cyparissias	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Festuca brevipila	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
Festuca psammophila	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1
Galium verum	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicotrichon pratensis	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium pilosella	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium umbellatum	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypochaeris radicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Koeleria macrantha	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1
Medicago minima	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myosotis stricta	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petrorhagia prolifera	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peucedanum oreoselinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Phleum phleoides	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Potentilla incana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rumex acetosella	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salvia pratensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Sedum sexangulare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	0	1
Silene conica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silene otitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Stipa capillata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus serpyllum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trifolium arvense	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica arvensis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica spicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veronica verna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S2: Environmental parameters measured from the soil cores

#	MP	Plot	H_2O (%)	pH	C	N	C/N	P (mg/kg)	Dehyd.
1	1	1	0.42	4.97	0.76	0.06	13.16	28.12	1.46
2	1	1	3.06	4.33	1.13	0.09	12.11	35.22	1.15
3	1	1	4.59	4.52	0.54	0.05	11.92	27.55	1.12
4	1	1	2.48	4.62	0.68	0.06	12.28	36.27	1.70
5	1	1	3.59	4.51	0.64	0.05	13.33	35.69	3.11
6	1	2	3.15	5.09	0.45	0.03	13.17	24.12	2.12
7	1	2	4.23	5.24	0.36	0.03	13.55	21.92	1.29
8	1	2	2.93	5.06	0.61	0.04	14.10	23.34	1.50
9	1	2	5.00	5.17	0.69	0.05	13.09	23.63	1.70
10	1	2	2.36	4.98	0.68	0.05	12.81	20.53	2.22
11	1	3	4.61	5.48	0.69	0.06	12.37	12.32	7.03
12	1	3	4.19	5.35	1.25	0.10	12.25	11.08	2.53
13	1	3	4.61	5.31	1.75	0.15	11.94	9.72	2.52
14	1	3	6.35	5.47	1.73	0.15	11.70	8.74	10.67
15	1	3	6.11	5.51	0.96	0.08	12.16	9.37	7.69
16	1	4	3.95	7.57	1.05	0.06	23.20	20.22	5.91
17	1	4	3.64	7.61	1.14	0.07	16.55	28.20	5.41
18	1	4	4.82	7.61	0.99	0.06	18.19	19.35	8.28
19	1	4	4.29	7.27	1.58	0.12	12.83	30.27	14.25
20	1	4	6.78	6.73	1.11	0.08	14.61	22.55	13.33
21	2	5	3.62	3.95	0.63	0.05	12.78	24.29	0.67
22	2	5	4.51	6.55	1.32	0.10	12.72	25.44	8.26
23	2	5	3.38	4.74	1.01	0.08	12.50	23.24	1.92
24	2	5	4.87	6.87	1.17	0.09	13.60	19.03	11.84
25	2	5	4.83	4.17	1.18	0.09	12.82	16.53	0.67
26	2	6	3.90	3.97	0.94	0.07	12.90	14.78	0.95
27	2	6	3.38	4.27	0.76	0.06	13.15	18.33	0.43
28	2	6	7.04	4.01	0.53	0.04	13.79	17.07	0.53
29	2	6	2.68	3.97	0.61	0.05	12.14	13.05	0.77
30	2	6	0.01	4.00	0.50	0.04	13.68	10.40	0.57
31	2	7	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.
32	2	7	5.98	4.04	1.34	0.12	11.16	16.75	2.08
33	2	7	7.73	3.91	1.60	0.14	11.08	17.55	0.98
34	2	7	5.70	3.96	2.07	0.18	11.76	23.54	1.39
35	2	7	4.74	4.00	1.94	0.16	11.83	22.30	1.80
36	2	8	7.46	3.87	0.78	0.07	11.61	10.30	2.07
37	2	8	9.33	3.80	0.62	0.05	12.07	25.70	1.39
38	2	8	7.53	3.73	0.99	0.08	12.15	11.71	2.18
39	2	8	8.59	3.89	1.42	0.12	11.57	15.06	1.40
40	2	8	8.52	3.84	0.82	0.07	12.67	15.60	0.60
41	3	9	3.43	7.05	0.84	0.06	13.96	19.23	19.85
42	3	9	3.38	7.46	0.85	0.05	17.85	19.11	10.48
43	3	9	2.66	7.59	0.79	0.04	20.22	15.99	6.51
44	3	9	4.66	7.53	1.21	0.09	13.95	18.61	10.44
45	3	9	4.18	7.40	0.87	0.06	14.35	18.31	6.27
46	3	10	4.51	7.22	1.05	0.09	11.91	14.95	11.95
47	3	10	8.34	7.10	0.96	0.09	11.09	12.92	15.39
48	3	10	4.91	6.74	1.05	0.08	13.30	24.21	14.18
49	3	10	4.43	6.93	1.30	0.11	12.20	12.91	11.44
50	3	10	2.96	7.12	1.34	0.11	12.00	17.85	9.64
51	3	11	8.47	7.59	1.73	0.11	16.51	33.38	16.32
52	3	11	9.78	7.53	2.58	0.18	14.47	26.38	24.10
53	3	11	11.90	7.51	3.22	0.22	14.89	37.53	32.83
54	3	11	9.87	7.56	3.41	0.21	15.98	42.19	18.94
55	3	11	10.01	7.43	2.54	0.21	12.22	30.02	23.69
56	3	12	5.55	7.24	1.26	0.10	13.21	15.59	11.99
57	3	12	5.89	7.41	1.39	0.08	17.66	14.91	10.05
58	3	12	4.45	7.07	1.76	0.14	12.97	19.05	11.44
59	3	12	5.38	7.51	1.21	0.07	18.62	14.49	13.08
60	3	12	6.04	7.34	1.60	0.12	13.72	17.06	17.66

Table S3: P-values from the analysis of variance of the environmental data used in the null model subsets. MP1 = macroplot 1; MP2 = macroplot 2; MP3 = macroplot 3; top = top plots; bottom = bottom plots; left = left plots; right = right plots; diag w/G = diagonal plots along the gradient; diag wo/G = diagonal plots orthogonal to the gradient. P-values are depicted as stars: *** = P<0.001; ** = P<0.01; * = P<0.05; NS = P>0.05.
The function call was: `aov(environmental.factor~plot.subset)`

	MP1	MP2	MP3	top	bottom	left	right	diag w/G	diag wo/G
C	**	***	***	***	***	***	***	***	***
N	**	***	***	***	***	***	***	***	***
C/N	***	***	*	***	**	***	***	**	***
P	***	NS	***	***	***	***	**	***	***
pH	***	*	**	***	***	***	***	***	***
micro	***	NS	**	***	***	***	***	***	***

Table S4: Variance partitioning results before and after the natural log-transformation of the environmental data

	untransformed	log-transformed
environment	17.9 %	17.2 %
env + space	4.7 %	4.1 %
space	14.5 %	15.1 %
unexplained	62.9 %	63.6 %

Source code of the statistical analysis in R

```
# PCNM analysis and variance partitioning of mallnow plant and soil data
# read in the source data and create some variables from it
slist<-read.table("mps.txt", header=TRUE) # species list
env<-read.table("mallnow.env.txt", header=TRUE)   # environment data
coords<-data.frame(easting=slist$easting,northing=slist$northing)

species<-slist[,4:dim(slist)[2]] # stripping first 3 colums for species only
xyir<-as.matrix(coords) # make a matrix from coordinates

library(vegan)
species <- decostand(species,method="hellinger")
detach("package:vegan")

library(spdep)
library(tripack)

nbtri <- tri2nb(xyir)
nbgab <- graph2nb(gabrielneigh(xyir), sym = TRUE)
nbrel <- graph2nb(relativeneigh(xyir), sym = TRUE)
nbsoi <- graph2nb(soi.graph(nbtri, xyir), sym = TRUE)

library(vegan)
d<-dist(coords,method="euclidean") #euclidiean distance map
detach("package:vegan")
library(ade4)
nbmst<-neig2nb(mstree(d, ngmax = 1)) # create minimum spanning tree
detach("package:ade4")

par(mfrow = c(2, 2))
plot(nbtri, xyir, col = "red", pch = 20, cex = 0.2)
title(main = "Delaunay triangulation")
plot(nbgab, xyir, col = "red", pch = 20, cex = 0.2)
title(main = "Gabriel Graph")
plot(nbrel, xyir, col = "red", pch = 20, cex = 0.2)
title(main = "Relative Neighbor Graph")#
plot(nbmst, xyir, col = "red", pch = 20, cex = 0.2)
title(main = "Minimum Spanning tree")
par(mfrow = c(1, 1))

# First, we de-trend community structure with respect to N and E
library(vegan)
ccaTrend<-cca(species~easting+northing,data=coords)
resCom<-residuals(ccaTrend)
detach("package:vegan")

library(spacemakeR) # PCNM library
# test.W is a function to compute and test eigenvectors of spatial weighting matrices
## Binary ##
tri.top<-test.W(resCom, nbtri)
```

```
gab.top <- test.W(resCom, nbgab)
rel.top<-test.W(resCom, nbrel)
mst.top<-test.W(resCom,nbmst)
##
## Binary + weighting function 2 (concave down) ##
f2 <- function(x, dmax, y) {1 - (x^y)/(dmax)^y} # a weighting distance function
maxi <- max(unlist(nbdists(nbgab, xyir)))
# maximum of a flattened spatial distance list of the gabriel graph aka maximum spatial
distance
tri.f2 <- test.W(resCom, nbtri, f = f2, y = 1:10, dmax = maxi, xy = xyir)
gab.f2 <- test.W(resCom, nbgab, f = f2, y = 1:10, dmax = maxi, xy = xyir)
rel.f2 <- test.W(resCom, nbrel, f = f2, y = 1:10, dmax = maxi, xy = xyir)
mst.f2 <- test.W(resCom, nbmst, f = f2, y = 1:10, dmax = maxi, xy = xyir)
##
## Binary + weighting function 3 (concave up) ##
f3 <- function(x, b) {1/(x)^b}
tri.f3 <- test.W(resCom, nbtri, f = f3, b = 1:10,xy = xyir)
gab.f3 <- test.W(resCom, nbgab, f = f3, b = 1:10, xy = xyir)
rel.f3 <- test.W(resCom, nbrel, f = f3, b = 1:10, xy = xyir)
mst.f3 <- test.W(resCom, nbmst, f = f3, b = 1:10, xy = xyir)
##
## Neighborhood approach
dxy <- seq(give.thresh(dist(coords)), 15, le =100)
nbdnnlist <- lapply(dxy, dnearneigh, x = as.matrix(coords), d1 = 0)
dnn.bin <- lapply(nbdnnlist, test.W, Y = resCom)
minAIC <- sapply(dnn.bin, function(x) min(x$best$AICc,na.rm = T))
dnn.bin[[which.min(minAIC)]]$all[1]
##
## Neighborhood approach with weighting function 2 ##
dnn.f2 <- lapply(nbdnnlist, function(x) test.W(x, Y = resCom,f = f2, y = 2:10, dmax =
max(unlist(nbdists(x, as.matrix(coords)))),xy = as.matrix(coords)))
minAIC <- sapply(dnn.f2, function(x) min(x$best$AICc,na.rm = T))
min(dnn.f2[[which.min(minAIC)]]$all[3])
##
## Neighborhood approach with weighting function 3 ##
dnn.f3 <- lapply(nbdnnlist, function(x) test.W(x, Y = resCom,f = f3, b = 1:10,xy =
as.matrix(coords)))
minAIC <- sapply(dnn.f3, function(x) min(x$best$AICc,na.rm = T))
min(dnn.f3[[which.min(minAIC)]]$all[2])
##
## PCNM approach ##
pcnm_M<-pcnm(dist(coords),give.thresh(dist(coords)))
AIC.pcnm<-ortho.AIC(resCom, pcnm_M$vec, ord.var=T)
min(AIC.pcnm$AICc)
##
## Polynomial for classical trend-surface approach, remember to close ade4! ##
detach("package:spacemakeR")
detach("package:ade4")
library(vegan)
X2<-(slist$easting)^2
X3<-(slist$easting)^3
Y2<-(slist$northing)^2
```

```
Y3<-(slist$northing)^3
Y<-(slist$northing)
X<-(slist$easting)
rda_Poly<-rda(resCom~X2+X3+Y2+Y3+X+Y)
#extractAIC.cca(rda_Poly)[2]
extractAIC(rda_Poly)[2]

AIC_matrix<-
as.matrix(c(tri.top$all[1],min(tri.f2$all[3]),min(tri.f3$all[2]),gab.top$all[1],min(gab.f2$all
[3]),min(gab.f3$all[2]),rel.top$all[1],min(rel.f2$all[3]),min(rel.f3$all[2]),mst.top$all[1],mi
n(mst.f2$all[3]),min(mst.f3$all[2]),dnn.bin[[which.min(minAIC)]]$all[1],min(dnn.f2[[which.min(
minAIC)]]$all[3]),min(dnn.f3[[which.min(minAIC)]]$all[2]),min(AIC.pcnm$AICc),extractAIC(rda_Po
ly)[2]))
rownames(AIC_matrix)=c("bintri","tri.f2","tri.f3","bingab","gab.f2","gab.f3","binres","res.f2"
,"res.f3","binmst","mst.f2","mst.f3","dnn","dnn.f2","dnn.f3","pcnm","poly")
AIC_matrix

dnn.f2[which.min(minAIC)] # format for dnn approach
dnn.f2_model<-dnn.f2[which.min(minAIC)]

# 4, 6, 5, 3, 8 and 57 were the AIC improving MEMs
MEM_4<-dnn.f2_model[[1]]$best$vectors[,4]
MEM_6<-dnn.f2_model[[1]]$best$vectors[,6]
MEM_5<-dnn.f2_model[[1]]$best$vectors[,5]
MEM_3<-dnn.f2_model[[1]]$best$vectors[,3]
MEM_8<-dnn.f2_model[[1]]$best$vectors[,8]
MEM_57<-dnn.f2_model[[1]]$best$vectors[,57]
r.da.mems.v <- cbind(MEM_4,MEM_6,MEM_5,MEM_3,MEM_8,MEM_57)

library(ade4)
# First, we run a PCA of Env for visualising main pattern of correlation in Explanatory
Variables
Env.set<-
data.frame(C=env$Ko,N=env$Nit,Water=env$water,pH=env$pH,C_N=(env$Ko)/(env$N),P=env$Phos,micro=
env$Dehy)
# PCA #
PCA_ENV<-prcomp(log(Env.set+1),scale=TRUE,scores=TRUE)
summary(PCA_ENV)
PCA_ENV

#Variance Partitioning#
MEM<-data.frame(MEM_4=MEM_4,MEM_6=MEM_6,MEM_5=MEM_5,MEM_3=MEM_3,MEM_8=MEM_8,MEM_57=MEM_57)
Env.sel<-
data.frame(Ko=env$Ko,N=env$Nit,Water=env$water,pH=env$pH,C_N=(env$Ko)/(env$N),P=env$Phos,micro
=env$Dehy)
mod <- varpart(species,Env.sel,MEM)
mod
par(mfrow = c(1, 1))
showvarparts(2)
plot(mod)

# ANOVAs
```

```
rda.resultENV <- rda(species, Env.set, MEM)
anova(rda.resultENV, step=200, perm.max=200)

#Testing single variables#
attach(Env.sel)
rda.resultENV_term <- rda(species~Ko+N+C_N+P+pH+micro+Water+Condition(r.da.mems.v))
ordistep(rda.resultENV_term, scope = formula(rda.resultENV_term), perm.max = 9999)
detach(Env.sel)

#Testing single variables by ignoring spatial autocorrelation#
attach(Env.sel)
rda.resultENV_term_only_env <- rda(species~Ko+N+C_N+P+pH+micro+Water)
ordistep(rda.resultENV_term_only_env, scope = formula(rda.resultENV_term_only_env), perm.max =
9999)
detach(Env.sel)

# Testing spatial variables
rda.resultMEM <- rda(species, MEM, Env.sel)
anova(rda.resultMEM, step=200, perm.max=200)
```

Appendix B: Supplementary Material for Chapter III

Methods

Amplicon pool generation

We ran two nested PCRs on each sample using AMF specific primers which amplify a ~ 1300 bp fragment spanning parts of the SSU, the complete ITS region and a large part of the LSU (Krüger et al 2009), including the D1, D2 and D3 region (Liu et al 2012). The following PCR conditions were used for all PCRs: 95°C for 2 min, following 30 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 40 sec, following 72°C for 2 min. In order to reduce PCR-related sequence errors, we used the Kappa HiFi Enzyme (Kappa Biosystems, Woburn, MA) which possess a proof-reading function for all PCR steps. The first PCR was amplified with the SSUmAf and the LSUmAr set. 1µl of a 1:100 dilution of the first PCR was used as a template for the second one. The primer set for the second PCR was SSUmCf and LSUmBr (Krüger et al 2009). The resulting products were separated on a gel, bands excised, cleaned up with a NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany), and tagged with the appropriate 454-adaptors (Roche) and barcoded primers (Hamady et al 2008) in a third PCR using the general primer set LR3 and LR0R (Hofstetter et al 2002).

Tagged root and soil PCR products from every soil core were gel-separated, cleaned and their concentration determined with a NanoPhotometer® (Implen GmbH, München, Germany). The amplicons were pooled by mixing equimolar amounts of each sample. One pool each was created for the soil and the root DNA sequences.

Environmental variables measurement

Soil water content was measured as the weight difference between fresh and oven-dried soil-core subsamples. Samples were taken two days after a substantial raining event so that soil was at water holding capacity at the sampling point in time. Soil carbon and nitrogen analysis was performed on a EuroEA 3000 Elemental Analyzer (EuroVector, Milano, Italy) with a TDC detector using 25 mg of

pulverized soil per core. Soil pH was measured in 10 mM $CaCl_2$ solution (van Lierop and Mackenzie 1977) using 3 grams of soil per core. Plant available phosphorus was characterized following the CAL-method (Sparks 1996) using 1 gram of soil per core. Dehydrogenase assays were conducted according to Rossel (Rossel et al 1997), using 1 gram of soil per core.

Results

Phylogenetic community structure

Abundance-weighted IC-MPD principal coordinate analysis (PCoA) of the root data set attributed 8.3% and 5.9% variation to the first and second ordination axis, respectively. In the soil data set, the first axis accounted for 12.5% of total variation, the second axis for 4.5%. Without the abundance weighting, the amount of variation explained by each axis was considerably lower (4.5% and 3.1% for root, 4.2% and 3.5% for soil). Variation of IC-MPD PCoA ordination axes was comparable among *Glomus*, non-*Glomus* and all OTU data sets (data not shown).

Tables

Table S1: Environmental data used for variance partitioning. Water, carbon and nitrogen content are given in percent; phosphorus is given in mg / kg soil; pH values, C/N ratio and coordinates are absolute numbers.

code	Plot	x	y	water	pH	C	N	C/N	P
1	1	2.5	0.6	8.725	5.840	0.767	0.035	24.825	31.263
2	1	0.6	1.5	11.373	5.575	0.947	0.056	17.016	26.003
3	1	0.3	1.8	12.979	6.120	0.967	0.040	24.046	25.185
4	1	0.9	2.1	11.037	6.120	1.150	0.070	16.465	30.540
5	1	2.2	1.57	8.214	5.840	0.835	0.043	21.053	26.223
6	1	2.5	0.5	10.159	5.700	0.833	0.047	17.503	25.770
7	2	1.5	7.5	13.070	6.830	2.529	0.112	23.050	21.893
8	2	1.35	7.86	14.029	6.430	2.108	0.102	21.795	23.650
9	2	0.45	7.9	12.174	6.930	1.510	0.066	23.474	12.898
10	2	1.6	7.2	12.131	7.100	1.444	0.073	20.357	16.243
11	2	0.7	8.95	17.692	6.675	2.342	0.106	22.090	19.148
12	2	2.6	7.65	14.124	6.305	2.448	0.137	18.256	20.900
13	3	2.4	13.5	17.765	8.320	2.511	0.134	19.082	34.078
14	3	0.3	14.4	19.077	7.865	3.632	0.229	15.896	34.655
15	3	1.2	14.4	21.289	7.425	2.519	0.157	18.335	52.143
16	3	1.2	13.2	17.964	7.515	2.858	0.181	15.831	25.833
17	3	2.4	14	23.420	7.850	2.175	0.141	15.415	53.543
18	3	2.9	12.8	18.681	7.485	3.030	0.170	17.823	29.650
19	4	7.9	1.2	8.861	7.230	1.488	0.090	15.436	35.615
20	4	7.4	2.5	9.375	7.125	0.673	0.051	12.847	28.980
21	4	7.8	1.57	10.714	7.050	1.315	0.042	32.468	85.023
22	4	6.9	0.3	8.883	6.775	0.981	0.071	13.891	29.210
23	4	7.1	0.4	9.113	6.385	0.512	0.031	20.742	34.650
24	4	6.65	0.9	8.971	6.335	1.807	0.086	21.527	56.488
25	5	7.65	8.15	11.111	7.500	1.194	0.054	22.460	45.100
26	5	6.3	7.1	12.938	7.545	1.297	0.065	20.065	40.675
27	5	6.4	7.8	11.172	8.060	1.718	0.058	29.815	42.785
28	5	7.1	7.9	13.281	7.650	1.430	0.080	18.037	37.663
29	5	7.4	8.7	12.500	7.720	1.396	0.059	23.770	18.275
30	5	7.3	6.9	12.230	7.545	1.235	0.088	15.460	19.153
31	6	8.8	13	16.987	7.530	3.480	0.259	13.472	32.653
32	6	6.6	12.8	14.355	7.765	1.641	0.096	18.667	37.810
33	6	7.2	13.6	18.153	7.630	2.525	0.162	15.962	30.993
34	6	8	13.9	15.297	8.015	2.445	0.125	21.220	35.130
35	6	8.5	13.4	14.851	7.860	2.032	0.127	16.098	28.693
36	6	8.5	12.6	14.634	8.015	2.206	0.111	20.702	28.928
37	7	12.6	2.3	10.830	7.405	1.395	0.062	22.540	64.975
38	7	12.65	1.1	8.772	7.105	1.067	0.067	15.742	40.870
39	7	12.5	2.9	9.199	7.450	0.866	0.030	34.700	69.223
40	7	14	1.7	7.967	6.025	0.933	0.022	42.982	34.305
41	7	14.9	2.1	9.012	6.170	1.166	0.054	21.840	16.718
42	7	12.2	1.2	7.781	7.065	0.631	0.022	38.266	50.430
43	8	14.1	6.7	10.095	7.290	1.308	0.087	15.837	10.938
44	8	14.8	7.95	9.827	7.450	1.132	0.068	16.485	15.563
45	8	13.1	8.3	10.027	7.180	0.967	0.062	15.635	34.685
46	8	12.3	8.4	12.538	7.645	1.353	0.072	18.968	42.353
47	8	13.6	6.4	13.636	7.380	1.284	0.065	19.749	54.708
48	8	13.5	7.2	11.644	7.190	0.876	0.044	20.068	25.373
49	9	14.2	12.8	12.121	8.235	1.327	0.072	19.036	20.905
50	9	13.3	13.3	15.238	7.795	2.558	0.193	13.187	24.775
51	9	12.9	12.4	12.814	7.915	2.205	0.147	15.209	27.278
52	9	12.7	12.6	16.138	7.725	2.752	0.215	12.781	51.545
53	9	13.5	12.6	15.072	8.070	1.653	0.100	16.956	25.278
54	9	13	13.5	14.462	7.755	2.193	0.108	19.441	21.293

Table S2: Percentage values of variance partitioning used for figure 4 in the manuscript. Significant values (P<0.05) are bold, non-testable values are depicted in italics.

			environment	space	phylogeny	env + space	space + phylo	unexplained variation
all OTUs	Root	+abu	0	**5**	**16**	*10*	*7*	62
		-abu	2	**8**	**4**	*8*	*3*	75
	Soil	+abu	3	**10**	**16**	*6*	*0*	65
		-abu	2	**9**	**9**	*8*	*0*	72
Glomus OTUs only	Root	+abu	0	**8**	**23**	*12*	*8*	49
		-abu	0	**11**	**8**	*16*	*5*	60
	Soil	+abu	**5**	**8**	**20**	*9*	*9*	49
		-abu	**3**	**13**	**10**	*8*	*4*	62
all OTUs except *Glomus*	Root	+abu	**8**	**5**	**26**	*6*	*14*	41
		-abu	**10**	**16**	**6**	*1*	*2*	65
	Soil	+abu	0	**2**	**16**	*7*	*6*	69
		-abu	0	**2**	**3**	*11*	*7*	77

Table S3: P-values of correlations between the environmental variables. The P-values were calculated by computing a matrix of Pearson's r correlation coefficients for all possible pairs of columns of the environmental matrix, using the rcorr function from the Hmisc package in R. Significant values are bold.

pH	**<0.001**				
carbon	**<0.001**	**<0.001**			
nitrogen	**<0.001**	**<0.001**	**<0.001**		
C/N ratio	**0.007**	0.101	**0.015**	**<0.001**	
phosphorus	0.976	0.315	0.719	0.570	**0.010**
	water	pH	carbon	nitrogen	C/N ratio

Figures

Figure S1: Kriging maps of the sampled plots, visualizing the environmental gradient.

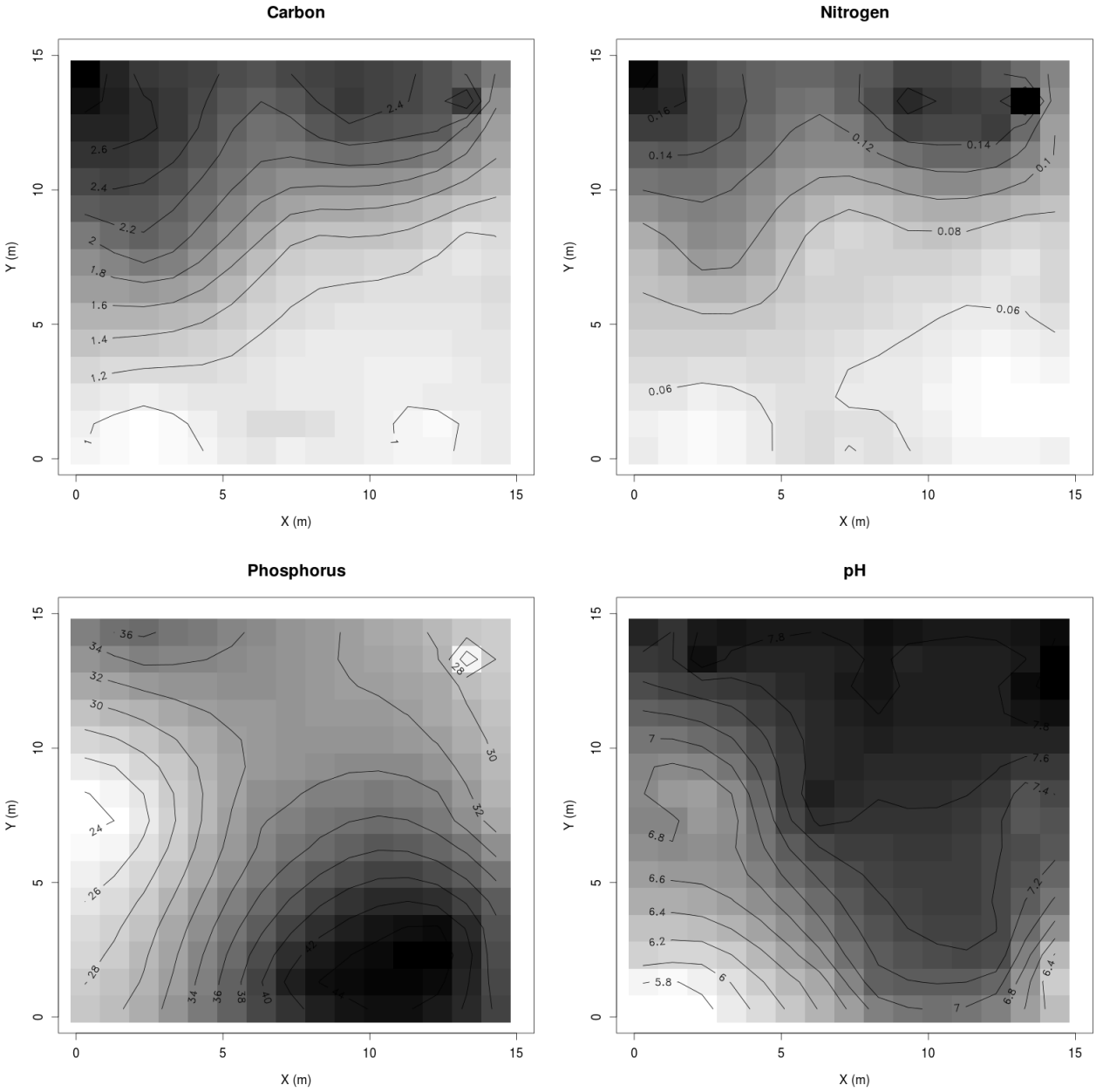


Figure S2: Correlation between root colonization (y axis, in percent) and soil texture in our macroplot.

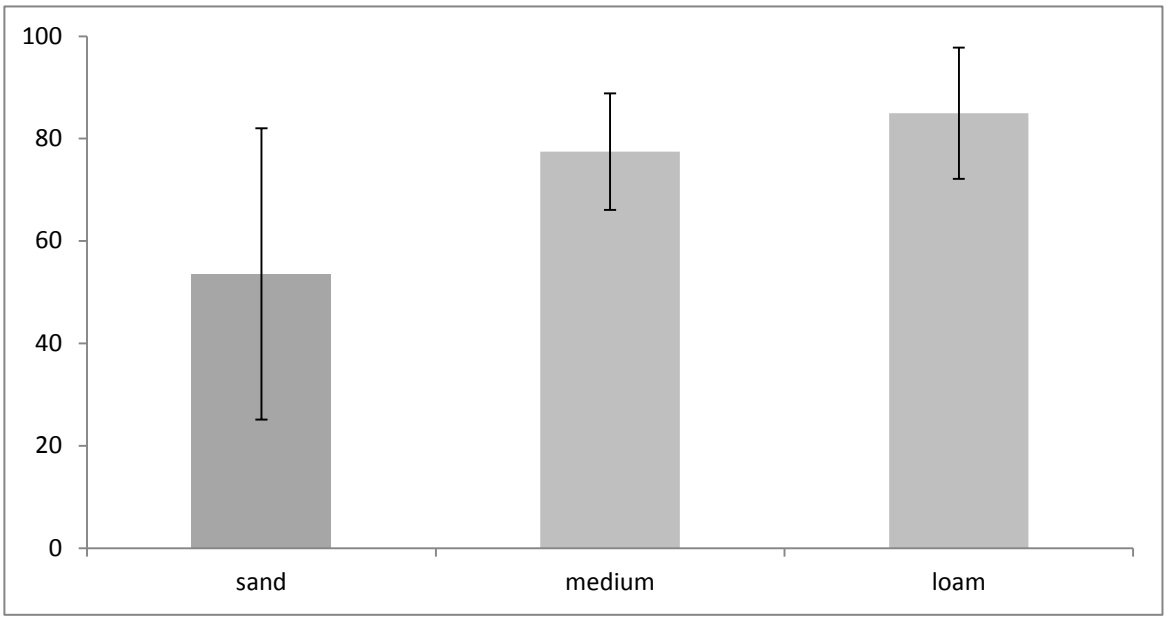
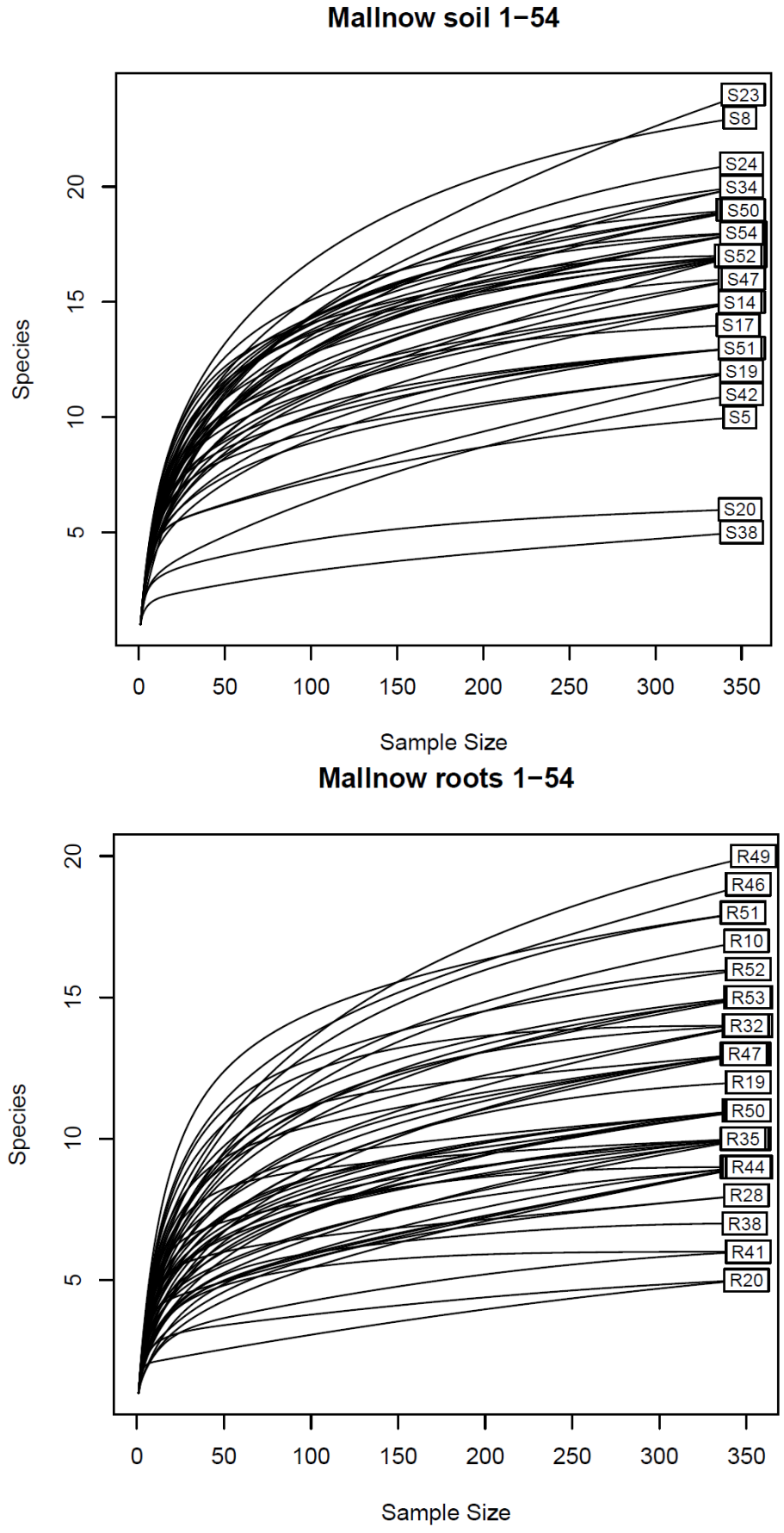


Figure S3: Rarefaction curves of soil (a) and root (b)



Pipeline for 454 data analysis

```
### mothur part ###

sffinfo(sff=m.10.root.sff, flow=T)
sffinfo(sff=m.10.soil.sff, flow=T)
trim.flows(flow=m.10.root.flow, oligos=mallnow.oligos, minflows=360,
maxflows=720, processors=4, pdiffs=4, bdiffs=0)
trim.flows(flow=m.10.soil.flow, oligos=mallnow.oligos, minflows=360,
maxflows=720, processors=4, pdiffs=4, bdiffs=1)
shhh.flows(file=m.10.root.flow.files, maxiter=5000, processors=4)
shhh.flows(file=m.10.soil.flow.files, maxiter=5000, processors=4)
trim.seqs(fasta=m.10.root.shhh.fasta, oligos=mallnow.oligos, maxhomop=8,
maxambig=0, processors=4, pdiffs=4, bdiffs=1)
trim.seqs(fasta=m.10.soil.shhh.fasta, oligos=mallnow.oligos, maxhomop=8,
maxambig=0, processors=4, pdiffs=4, bdiffs=1)

### run rename script and concatenate root & soil fastas ###

### CROP part ###

crop -i m.10.all.b0.b1.fasta -b 660 -z 400 -e 15000 -o m.10.all.b0.b1.crop
> crop.all.out &

### run matrix creation script & multivariate statistics ###
```

Appendix C: Supplementary Material for Chapter IV

Table S1: AMF OTU table used in the statistical analysis - root samples, part 1

no	name	R1	R2	R3	R5	R6	R7	R8	R9	R12	R14	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35
1	G0MV5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	G12HR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	G1EXK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
4	G2558	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	G3HXW	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	G3T48	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	G4HCM	0	0	0	0	0	0	0	0	0	0	13	0	1	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	G4Y4K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0
9	G51C9	0	0	41	6	0	0	0	4	6	0	61	123	14	35	48	0	0	4	56	0	0	0	0	0	0	0	7	0	5	0
10	G6C9I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	0	0	0	0
11	G6DWZ	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	137	286	2	0	0	0	0	0	0	0
12	G96D7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	G9OCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0
14	GSPTD	0	0	0	0	0	0	0	0	14	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	4	85	0
15	GT4W5	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	GTB2B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
17	GTOOJ	0	0	0	0	0	0	0	0	0	0	10	0	13	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	GTQN5	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	GUZCR	0	0	0	0	0	0	4	0	0	0	6	0	30	0	2	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
20	GV420	0	0	43	32	0	0	1	2	11	51	27	16	162	113	60	0	302	113	232	0	0	0	0	0	0	0	0	0	0	0
21	GW9PY	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	GYAJ1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	GYTF9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	GZQFA	0	0	0	0	0	0	0	0	0	0	25	45	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	HA5PN	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	HADOB	0	0	0	0	0	0	0	0	98	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	HBFXZ	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	HBMAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	HBPSA	0	0	0	0	0	0	0	0	1	0	96	164	11	231	76	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
30	HCHK0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	HDEXD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
32	HDZFW	3	0	0	5	0	0	0	63	79	4	3	20	21	18	28	0	12	115	5	0	0	0	0	0	0	1	0	0	0	0
33	HES4H	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	HEUKP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	HFEJR	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	HFVR4	0	0	0	0	0	65	124	0	5	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
37	HG4KO	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - root samples, part 2

no	name	R1	R2	R3	R5	R6	R7	R8	R9	R12	R14	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35
38	HG93S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	HGQJX	0	0	0	0	0	0	0	0	0	0	10	4	5	37	0	0	1	0	6	0	0	0	0	0	0	0	0	0	0	0
40	HHKOP	0	113	101	148	0	0	0	0	7	0	0	0	0	0	0	0	152	0	0	0	0	1	0	0	0	0	0	0	0	0
41	HJIZE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
42	HJRQ8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	HLU0S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	I27IO	77	21	4	36	60	152	361	197	7	10	87	3	188	2	197	0	32	76	164	0	0	0	0	0	0	0	15	97	0	0
45	I667J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	I6TU4	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
47	I7BQ4	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	I7MQL	0	0	0	0	0	0	0	0	0	0	5	0	35	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	I7TEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	I9LIV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	JAZN8	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	JB7WR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
53	JDGMY	3	39	1	3	85	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	1	0
54	JFM03	0	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	JGS10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	JH0G0	0	0	0	0	0	0	0	0	135	21	24	61	0	8	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
57	JI4PX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
58	JIQG7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	JJ4FM	316	317	270	268	353	280	0	0	132	248	0	61	2	4	1	397	2	176	3	496	286	208	493	244	498	333	473	290	194	236
60	JKXSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	JLIGP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	JLJMW	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	32	0	104	211	259
63	JMZEJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	JN3XD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	JNEC0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	JOHIR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	JS58F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
68	JSBB3	0	0	0	0	0	0	0	82	1	0	5	0	0	15	1	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0
69	JT7KL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	JV71D	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	JWDCO	102	7	0	1	0	0	0	148	2	0	118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	JXIZ6	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	94	0	1	0	0	77	0	5	252	0	0	0	0	3	0
73	JZIXS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - root samples, part 3

no	name	R36	R37	R38	R40	R41	R42	R43	R44	R45	R46	R47	R48	R49	R50	R51	R52	R53	R55	R56	R57	R58	R59
1	G0MV5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	G12HR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
3	G1EXK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	G2558	0	0	0	3	0	1	0	0	0	0	2	0	0	1	0	0	0	0	0	1	0	0
5	G3HXW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	G3T48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	G4HCM	0	0	0	0	17	2	0	6	0	31	54	11	8	4	0	0	6	10	52	22	13	45
8	G4Y4K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	G51C9	0	0	0	17	74	2	26	61	1	87	44	128	3	0	39	93	64	54	12	4	10	43
10	G6C9I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	G6DWZ	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	G96D7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	G9OCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	GSPTD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	GT4W5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
16	GTB2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	GTOOJ	0	0	0	6	7	84	0	9	1	9	4	20	0	71	120	0	2	30	3	103	55	8
18	GTQN5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	GUZCR	0	0	0	12	3	0	19	12	4	8	12	6	30	5	0	3	6	2	28	11	15	3
20	GV420	2	0	0	233	165	37	87	152	221	91	177	7	8	82	59	76	67	46	80	120	16	82
21	GW9PY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	GYAJ1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
23	GYTF9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
24	GZQFA	0	0	0	0	0	1	104	4	26	25	18	1	0	6	0	0	2	47	1	3	11	34
25	HA5PN	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	HADOB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
27	HBFXZ	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
28	HBMAS	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	66	0	0	0	0
29	HBPSA	0	0	0	86	109	195	17	41	4	84	33	113	199	169	253	191	91	185	18	95	120	51
30	HCHK0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	2	0	0	0	2	0	0
31	HDEXD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	HDZFW	0	0	0	0	54	10	0	1	0	35	10	86	0	1	9	14	16	9	2	4	0	16
33	HES4H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
34	HEUKP	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	HFEJR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
36	HFVR4	0	0	0	0	0	0	0	0	15	0	5	0	0	0	0	0	0	0	0	0	0	0
37	HG4KO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - root samples, part 4

no	name	R36	R37	R38	R40	R41	R42	R43	R44	R45	R46	R47	R48	R49	R50	R51	R52	R53	R55	R56	R57	R58	R59
38	HG93S	0	0	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	0	22	0
39	HGQJX	0	0	0	4	0	3	37	4	9	45	9	15	0	37	0	4	54	17	1	2	6	29
40	HHKOP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	HJIZE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	HJRQ8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
43	HLU0S	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	I27IO	0	0	0	133	21	9	185	102	61	65	112	78	202	80	5	51	95	20	181	61	218	50
45	I667J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
46	I6TU4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	I7BQ4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	15	1	0	0
48	I7MQL	0	0	0	1	29	81	18	28	22	0	11	9	1	31	0	8	2	0	81	19	5	8
49	I7TEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
50	I9LIV	0	0	0	0	0	0	0	0	121	0	3	0	0	7	0	28	3	0	6	0	1	13
51	JAZN8	0	0	0	0	3	2	0	0	2	0	0	0	0	0	0	0	0	0	4	1	1	0
52	JB7WR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	JDGMY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	JFM03	0	0	0	3	7	11	5	13	4	4	3	0	0	4	0	10	0	0	4	5	3	0
55	JGS10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0
56	JH0G0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	47	0	2	0	0	30
57	JI4PX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	JIQG7	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	4	12	3	1	0	0
59	JJ4FM	493	498	444	0	0	1	0	6	4	0	1	0	0	2	2	1	0	0	6	1	1	5
60	JKXSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	JLIGP	0	0	0	3	0	59	0	36	2	14	0	10	0	0	0	11	25	0	0	8	0	3
62	JLJMW	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	JMZEJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	JN3XD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	JNEC0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	JOHIR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	JS58F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	JSBB3	0	0	0	0	7	0	0	12	0	0	0	13	6	0	6	0	0	0	0	0	0	20
69	JT7KL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	JV71D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	JWDCO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	31	0	49
72	JXIZ6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
73	JZIXS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - soil samples, part 1

no	name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
1	G0MV5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	G12HR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	G1EXK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	G2558	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	G3HXW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	G3T48	0	0	0	0	10	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	G4HCM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	67	0	14	0	0	0	0	0	0	0	0	0	0	0
8	G4Y4K	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	G51C9	0	0	0	213	0	0	0	0	0	0	119	0	80	136	12	43	117	132	39	121	0	119	3	9	0	0	95	0	0	0
10	G6C9I	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	G6DWZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	382	245	142	0	0
12	G96D7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	G9OCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
14	GSPTD	0	0	0	0	0	0	0	0	0	0	2	0	0	2	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	GT4W5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
16	GTB2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	GTOOJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	16	0	0	0	0	0	0	0	0	0	0	0	0
18	GTQN5	6	0	0	0	2	0	0	0	192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	GUZCR	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0
20	GV420	0	0	0	0	12	0	0	0	0	0	1	0	0	2	6	24	2	31	95	25	0	286	320	422	0	0	0	1	0	0
21	GW9PY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	GYAJ1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	GYTF9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	GZQFA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	56	5	0	0	0	0	0	0	0	0	0	0	0
25	HA5PN	2	1	0	0	0	0	23	0	268	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	4	0
26	HADOB	0	0	0	0	0	0	0	0	0	0	21	0	0	79	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	HBFXZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	HBMAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
29	HBPSA	0	0	0	25	0	0	0	0	0	0	12	0	3	131	0	193	134	160	110	67	0	12	0	2	0	0	10	0	0	0
30	HCHK0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
31	HDEXD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	HDZFW	0	0	0	4	192	0	0	0	0	0	2	76	3	0	21	33	2	4	4	0	2	0	163	0	0	0	0	0	0	0
33	HES4H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	HEUKP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	HFEJR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	HFVR4	0	0	0	0	1	0	106	175	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	HG4KO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - soil samples, part 2

no	name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
38	HG93S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	HGQJX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	32	33	104	0	0	51	0	2	0	0	0	0	0	0
40	HHKOP	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0
41	HJIZE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	HJRQ8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	HLU0S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	I27IO	0	4	11	2	1	0	21	148	0	0	0	0	0	0	11	4	0	6	9	3	0	2	5	7	0	0	0	0	0	0
45	I667J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	I6TU4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
47	I7BQ4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	2	0	0	0	0	0	0	0	0	0	0	0
48	I7MQL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	64	47	57	24	0	0	0	0	0	0	0	0	0	0
49	I7TEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
50	I9LIV	0	0	0	0	0	0	0	0	0	0	76	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0
51	JAZN8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
52	JB7WR	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	3	1	0
53	JDGMY	124	200	125	18	39	177	0	175	0	17	0	2	142	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	JFM03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	20	7	23	3	0	0	0	0	0	0	0	0	0	0
55	JGS10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	JH0G0	0	0	0	0	0	0	0	0	0	0	124	346	251	149	294	0	23	0	0	39	0	0	0	0	0	0	0	0	0	0
57	JI4PX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
58	JIQG7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
59	JJ4FM	364	296	0	236	243	323	349	0	0	482	102	75	0	0	0	2	1	3	12	5	485	0	1	0	496	118	150	353	494	499
60	JKXSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	JLIGP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
62	JLJMW	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	JMZEJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	JN3XD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	JNEC0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
66	JOHIR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	JS58F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	JSBB3	0	0	0	0	0	0	0	0	0	0	22	0	21	0	0	0	0	0	13	200	0	0	0	48	0	0	0	0	0	0
69	JT7KL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
70	JV71D	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	JWDCO	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	JXIZ6	0	0	357	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	4	0	0	0	0	1
73	JZIXS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - soil samples, part 3

no	name	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
1	G0MV5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2	G12HR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	G1EXK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	G2558	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	G3HXW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	G3T48	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	2	0	0	0
7	G4HCM	0	0	0	0	0	0	0	0	0	5	1	13	31	8	2	0	3	1	0	0	0	0	19	2	45	40	46	117
8	G4Y4K	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	G51C9	0	0	0	0	0	0	0	0	0	12	192	18	57	35	244	7	154	12	78	59	58	57	82	89	2	20	17	42
10	G6C9I	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	G6DWZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44	0	0	0	0	0	0	0	0
12	G96D7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	G9OCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	GSPTD	0	0	54	0	0	0	0	0	121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	GT4W5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	GTB2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
17	GTOOJ	0	0	0	0	0	0	0	0	0	4	3	15	1	0	0	0	0	0	0	115	0	0	1	5	0	0	2	0
18	GTQN5	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	GUZCR	0	0	0	0	0	0	0	0	0	3	2	3	0	0	0	0	2	2	0	0	0	4	1	2	1	2	0	1
20	GV420	0	0	0	0	0	0	0	0	1	75	62	77	15	182	68	210	125	113	94	2	45	65	70	16	29	91	3	134
21	GW9PY	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0
22	GYAJ1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	GYTF9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	GZQFA	0	0	0	0	0	0	0	0	0	1	0	2	54	99	86	0	2	2	22	4	2	19	20	63	34	5	24	11
25	HA5PN	4	16	7	19	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	HADOB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	HBFXZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	HBMAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	3	12	0	0	0	0
29	HBPSA	0	0	0	0	0	0	0	0	0	281	42	176	149	48	0	38	89	106	169	252	168	67	71	215	204	183	315	66
30	HCHK0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0
31	HDEXD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	HDZFW	0	0	0	0	0	0	0	0	0	15	53	2	43	21	0	14	23	7	0	0	28	1	0	0	0	6	0	0
33	HES4H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
34	HEUKP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	HFEJR	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	HFVR4	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	HG4KO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1: AMF OTU table used in the statistical analysis - soil samples, part 4

no	name	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
38	HG93S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	41	0
39	HGQJX	0	0	0	0	0	0	0	0	0	5	11	122	44	20	36	51	4	13	50	2	30	91	28	35	37	1	0	23
40	HHKOP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	HJIZE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	HJRQ8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	HLU0S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	I27IO	0	0	0	0	0	0	0	0	0	7	36	28	17	4	3	0	11	25	6	9	3	11	16	34	18	70	5	24
45	I667J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0
46	I6TU4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	I7BQ4	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	1	0	0	0	0	0	0	0	10	3	1	7
48	I7MQL	0	0	0	0	0	0	0	0	0	60	67	5	47	23	36	89	8	23	24	0	19	0	0	0	86	30	6	0
49	I7TEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	7	7
50	I9LIV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	11	0	19	0	0	0	15	0	0	35
51	JAZN8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	2	0	1	0	0	0	1	1	0	0
52	JB7WR	5	5	3	16	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	JDGMY	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	JFM03	0	0	0	0	0	0	0	0	0	0	27	2	24	11	5	11	0	6	2	0	7	0	0	0	5	2	7	1
55	JGS10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	4	121	0	0	0	0	0
56	JH0G0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	1	78	36	0	7	0	0	0
57	JI4PX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	JIQG7	0	0	0	0	0	0	0	0	0	0	0	0	1	8	0	0	0	0	0	5	0	0	0	26	0	2	0	2
59	JJ4FM	482	478	366	120	191	465	332	485	368	3	2	0	1	0	3	4	0	3	2	0	0	0	0	0	3	2	0	1
60	JKXSO	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	JLIGP	0	0	0	0	0	0	0	0	0	3	0	36	5	35	2	65	60	0	0	0	0	102	3	0	0	44	0	1
62	JLJMW	0	0	57	342	219	34	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	JMZEJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
64	JN3XD	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
65	JNEC0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	JOHIR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	0	0	0	0	0	0	0	0	0	0
67	JS58F	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	JSBB3	0	0	0	0	0	0	0	0	0	0	0	1	1	3	0	6	18	45	0	0	1	0	0	0	0	0	0	25
69	JT7KL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	JV71D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	JWDCO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	25	0
72	JXIZ6	0	0	0	0	89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	29	0	0	0	0	0
73	JZIXS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0