Parallels between experimental and field arbuscular mycorrhizal fungal communities in response to habitat structure

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry, Pharmacy

of Freie Universität Berlin

by

LEONIE GRÜNFELD

Berlin, July 2022

This work was funded by the Deutsche Forschungsgemeinschaft as part of the Project Metacorrhiza (VE 736/2-1) granted to Dr. Stavros Veresoglou. The work was carried out between 2017 and 2022 under the supervision of Prof. Dr. Matthias C. Rillig at the Institute of Biology at Freie Universität Berlin. This dissertation was co-supervised by Dr. Stavros Veresoglou and by Prof. Dr. Monika Wulf in the framework of the Dahlem Research School´s graduate program „Biodiversity, Ecology and Evolution“.

First reviewer: Prof. Dr. Matthias C. Rillig

Second reviewer: Prof. Dr. Monika Wulf

Date of defense: 05.12.2022

I hereby certify that I have written my dissertation independently and that I have not used any sources and aids other than those indicated. I also declare that I have not submitted the dissertation in this or any other form to any other institution as a dissertation.

Acknowledgements

In the first place, I would like to thank Matthias for his supervision, support, and very constructive feedback at many stages of my PhD. Being part of his group was a very enriching experience both scientifically and personally. The great diversity of people with broad expertise and the extremely tolerant, friendly, and collaborative atmosphere gave me many opportunities to learn from a wide variety of perspectives. I will always remember this time and hope that many contacts, many of which became friends, will last.

I am very grateful to Stavros, who was not only the principal investigator of my PhD project, but also guided me through the entire PhD process from the first day. With his great enthusiasm and optimism, he always motivated me even during difficult periods. Without his great commitment and support in statistics, bioinformatics and writing, this thesis would not have been possible.

Many thanks to Monika for co-reviewing this work and for the great collaboration that made possible the observational studies that led to two chapters of this thesis. Monika was also a very supportive and encouraging member of my supervisory team, and I always enjoyed our stimulating conversations.

I would like to thank Annette and Maxi, who worked on this project as student assistants and moved it forward with their very proactive work style. I would also like to thank Florine, Magkdi, and Simon for helping collect the tons of soil needed. Magkdi has also been very supportive in the final stages of my molecular work. Our undergraduate students Max F., Svea and George helped with the analysis of the immense amount of root and soil samples. Many lab members supported me personally or scientifically, and I would like to thank especially Polina, Daniel, Stefan, India, Julien, Eva, Joana, Juan, Ludo, Max B., Tessa and Steffi for always having an open ear, or a useful advice. India, thank you also for the constructive proofreading of this thesis. For answering many questions and helping with various lab techniques, I thank our lab assistants Sabine, Gabi and Anja. My thanks also go to the gardeners Helga and Bernd, who supported the extensive work in the greenhouse.

Ich bin sehr dankbar, großartige Eltern zu haben, die mich immer uneingeschränkt unterstützt haben, insbesondere durch viele Stunden Babysitten während der Fertigstellung dieser Arbeit. Diego, danke für die gemeinsame Zeit, die mir viel Kraft für diese Doktorarbeit gegeben haben. Luna, danke für deine ansteckende Fröhlichkeit, die mich im letzten Jahr dieser Arbeit so bereichert hat.

Finally, I would like to thank everyone who has supported my doctoral process in any way, including many friends, family members, and colleagues whom I could not name here, you all are great!

Table of content

Foreword

This is a cumulative dissertation that includes author-formatted versions of the below listed four peer-reviewed and published articles. All references in the articles, as well as references in the general introduction and discussion (Chapters 1 and 5), have been merged and provided at the end of this dissertation.

I. **Grünfeld, L.**, Wulf, M., Rillig, M. C., Manntschke, A., & Veresoglou, S. D. (2020). Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. *New Phytologist*, 227, 10-13.
https://doi.org/10.1111/nph.16377 **Chapter 2**

II. **Grünfeld, L.**, Mola, M., Wulf, M., Hempel, S., & Veresoglou, S. D. (2021). Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest. *Mycorrhiza*, 31(5), 589-598.
https://doi.org/10.1007/s00572-021-01041-6 **Chapter 3**

III. **Grünfeld, L.**, Rillig, M. C., Skias, G., & Veresoglou, S. D. (2022). Arbuscular mycorrhizal root colonization depends on the spatial structure of the host plants. *Mycorrhiza*, 1-9.
https://doi.org/10.1007/s00572-022-01087-0 **Chapter 4a**

IV. Veresoglou, S. D., **Grünfeld, L.**, & Mola, M. (2021). Micro-Landscape dependent changes in arbuscular mycorrhizal fungal community structure. *Applied Sciences*, 11(11), 5297.
https://doi.org/10.3390/app11115297 **Chapter 4b**

Summary

Arbuscular mycorrhizal (AM) fungi have gained immense research interest due to their symbiotic relation with roots of most terrestrial land plants. Diverse positive effects on plant health and fitness, as well as a high ecological relevance for many other below-and aboveground processes have been recognized. Despite growing research ambitions, sufficient knowledge on drivers and their relative impacts on AM fungal communities at varying scales and ecosystems is lacking. Very little attention has been paid to the interrelation of the spatial structure of AM host plants (here meaning the small-scale distribution of host plant individuals or populations) and AM fungal community dynamics, although deeper insights could move forward the development of theoretical community frameworks applicable to these important endosymbionts. The work presented in this thesis aimed to study the effects of host plant spatial structure for the establishment of mycorrhizal symbiosis as well as for AM fungal diversity and community composition. To do so, controlled experiments mimicking micro-landscapes of AM fungal habitats were conducted and compared with observational data from temperate forests in Germany. In the experiments, we used meta-community designs to test the responses of root colonization, AM fungal diversity and community composition to levels of habitat connectance or habitat heterogeneity. We assayed rates of root colonization across forests sites differing in AM-associating plant cover. For assessing the importance of further spatio-temporal parameters and host-specificity we surveyed AM fungal communities within one of the forest sites over two years. As expected, we observed a consistent pattern of higher rates of root colonization at a higher proximity of host plants, which in the case of the field study was a higher cover of AM-associating woody plants and in the case of the experiment treatments a higher connectance of host plant patches. There were generally no clear effects of habitat structure on AM fungal diversity and community composition in both the field and the experimental study. In the field, the main finding was that spatial parameters, followed by host identity, more strongly affected AM fungal assemblages than temporal parameters. In the experiments, we observed within-experimental-unit differences of beta-diversity between pairs of long- and short-distance patches as well as between pairs of different habitat quality, which rendered those communities less predictable. In conclusion, we present empirical evidence for a co-variation of AM plant host structure and AM fungal abundance. These findings are of importance for the restoration or management of natural forest habitats, as well as for sustainable agriculture. Furthermore, this work supports previous findings of high stochasticity in AM fungal communities, highlighting the need for further research into spatial scale-specific drivers of this important organism group.

Zusammenfassung

Arbuskuläre Mykorrhizapilze (AM) haben aufgrund ihrer symbiotischen Beziehung mit den Wurzeln der meisten Landpflanzen breites Forschungsinteresse geweckt. Es sind vielfältige positive Auswirkungen auf die Gesundheit und Fitness von Pflanzen, sowie eine hohe Relevanz für viele weitere unter- und oberirdische ökologische Prozesse bekannt. Trotz wachsender Forschungsanstrengungen fehlt es jedoch an ausreichend Kenntnissen über die Einflussfaktoren auf AM-Pilzgemeinschaften auf verschiedenen räumlichen Ebenen unterschiedlichster Ökosysteme. Die Wechselbeziehung zwischen der räumlichen Struktur von AM-Wirtspflanzen (hier gemeint die kleinräumige Verteilung von Wirtspflanzenindividuen oder -populationen) und AM-Pilzgemeinschaften wurde bisher nur wenig untersucht, obwohl tiefere Einblicke die Entwicklung theoretischer Konzepte für diese wichtigen Endosymbionten voranbringen könnten. Die in dieser Dissertation vorgestellten Arbeiten zielen darauf ab, die Auswirkungen der räumlichen Struktur der Wirtspflanzen auf die Wurzelkolonisierung, sowie auf die Diversität und die Zusammensetzung der AM-Pilzgemeinschaft zu untersuchen. Zu diesem Zweck wurden Gewächshausexperimente durchgeführt, in denen Mikro-Landschaften bestehend aus Wirtspflanzen-Habitaten nachgeahmt wurden, und diese mit Beobachtungen aus Waldstandorten verglichen. In den Experimenten verwendeten wir meta-community Designs, um die Reaktionen von Wurzelkolonisierung, AM-Pilzdiversität und Artenzusammensetzung in Abhängigkeit von Habitat-Konnektivität und Habitat-Heterogenität zu testen. In den Wäldern untersuchten wir die Wurzelkolonisierungsrate in Standorten, die sich im Bedeckungsgrad von AM-assoziierten Pflanzen unterschieden. Um die Bedeutung weiterer räumlicher und zeitlicher Parameter und die der Wirtsspezifität zu beurteilen, untersuchten wir die AM-Pilzgemeinschaften an einem der Waldstandorte über zwei Jahre hinweg. Wie erwartet beobachteten wir ein konsistentes Muster höherer Wurzelkolonisierungssraten bei kleinerem Abstand zwischen den Wirtspflanzen, was in der Feldstudie eine höhere Deckung von AM-assoziierten holzigen Pflanzen, und in den Experimenten eine höhere Konnektivität von Wirtspflanzenfeldern bedeutete. Sowohl in der Feldstudie als auch in der experimentellen Studie gab es im Allgemeinen keine eindeutigen Auswirkungen der Habitatstruktur auf die Diversität der AM-Pilze, sowie die Artenzusammensetzung. Aus den Walddaten zeigte sich vor allem, dass räumliche Parameter, gefolgt von der Identität der Wirtspflanze, die Artenzusammensetzung der AM-Pilze stärker beeinflussten als zeitliche Parameter. In den Experimenten beobachteten wir innerhalb einzelner Mikro-Landschaften Unterschiede in der Beta-Diversität zwischen entfernten und nahen Habitat-Feldern, sowie zwischen Habitatfeldern mit unterschiedlicher Bodenqualität, was die Artenzusammensetzung der AM-Pilze weniger vorhersehbar machte. Zusammenfassend zeigen die Studien eine Kovariation von Wirtspflanzenstruktur und der Abundanz von AM-Pilzen. Diese

Erkenntnis ist bedeutend für die Wiederherstellung und Bewirtschaftung natürlicher Wälder, sowie für eine nachhaltige Landwirtschaft. Darüber hinaus bestätigt diese Arbeit frühere Erkenntnisse über eine hohe Stochastizität in AM-Pilzgemeinschaften und unterstreicht die Notwendigkeit weiterer Forschung an standortspezifischen Faktoren, die für die Arten-Zusammensetzung dieser wichtigen Organismengruppe von Bedeutung sind.

"In the end, we will conserve only what we love; we will love only what we understand, and we will understand only what we are taught." – **Baba Doum**

Chapter 1: General introduction

A primary goal of community ecology is to understand the factors responsible for the distribution and composition of organisms in a target environment. Due to alarming environmental and climate changes around the world, this branch of basic research has become increasingly important even outside the scientific community, not least to protect biodiversity-related ecosystem functions.

Our knowledge of species distribution varies drastically among groups of organisms. The study of microbial communities remains a methodological challenge because, for example, in situ cultivation in the laboratory can be difficult and suitable analytical methods are sometimes lacking. However, with the development of molecular techniques, particularly high-throughput sequencing, since the early 1990s, large strides have bene made in addressing knowledge gaps in microbial ecology.

The group of arbuscular mycorrhizal (AM) fungi is of particular interest due to their high ecological relevance. However, their symbiotic associations with host plants poses a challenge for unravelling the mechanisms responsible for dispersal and community composition. Among numerous abiotic and biotic factors structuring AM fungal communities, the role of local host plant distribution is indispensable, yet only poorly understood. Therefore, a major objective of this work is to explicitly investigate the influence of host plant spatial structure and thereby improve our understanding of AM fungal community dynamics.

Arbuscular mycorrhizal fungi and their ecological relevance

Arbuscular mycorrhiza represents a symbiosis between the roots of most terrestrial plants and a group of soil fungi belonging to the phylum Glomeromycota. Based on fossil evidence, it is estimated that the coevolution of AM fungi and plants dates back to about 500 million years and that prehistoric AM fungi may have played a critical role in plant land colonization (Redecker *et al.*, 2000).

AM fungi are obligate biotrophs that can – according to present knowledge – only complete their life cycle in the presence of a host plant. As endosymbionts they obtain photosynthetic carbohydrates from their host through absorptive structures called arbuscules, which are formed within the cortical cells of the host plant roots. In return, AM fungi provide phosphorous, nitrogen and micronutrients to the plant host. This often results in an enhanced nutrient acquisition from the soil by the plant and increased net primary production (Smith & Read, 2008). In addition to these direct nutritional effects, other benefits, such as increased resistance to drought (Augé, 2001; Ruiz-Lozano *et al.*, 2012; Bahadur *et al.*, 2019), heavy metals (Riaz *et al.*, 2021) and salinity (Evelin *et al.*, 2009) by the plants have been described in the presence of arbuscular mycorrhizae. Furthermore, AM hyphal networks play a role in connecting plant individuals by nutrient transport. In addition to their importance as symbionts, AM fungi interact with numerous other soil organisms, including microbes (bacteria and other fungi), earth worms or nematodes (e.g., Toljander et al., 2006; Veresoglou & Rillig,

2012; Paudel et al., 2016; Emmett et al., 2021). AM fungi even affect soil structure as their extraradical hyphae contribute to the stability of water stable soil aggregates (Rillig & Mummey, 2006; Leifheit *et al.*, 2014). Thus, AM fungi are important players in many below- and aboveground processes that affect the functioning of ecosystems.

The characteristics described above and the fact that many aspects of AM fungi are not yet well understood make them a highly relevant study object. Research is still needed to better understand mechanisms on the organism level up to their role in natural ecosystems. There is also a growing interest and debate on their potential for ecosystem restoration and ecological sustainable agricultural management, such as crop yield optimization (Hart & Trevors, 2005; van der Heijden *et al.*, 2008; Hart *et al.*, 2018; Rillig *et al.*, 2019)

Dispersal and distribution of AM fungi

AM fungi utilize various dispersal strategies including different propagule types and dispersal agents. Propagules exist in the form of spores, sporocarps, extraradical hyphae or fragments of colonized plant roots (Smith & Read, 2008). AM fungi actively disperse through growth of their extraradical mycelium. Furthermore, wind (e.g., Chaudhary et al., 2020) and water (e.g., LeBrun et al., 2018) have been identified as abiotic agents related to the long-distant dispersal of spores. There are several studies suggesting AM fungal spore dispersal through small mammals (Vašutová *et al.*, 2019). Other biotic agents, such as soil invertebrates, birds, and larger mammals (including human activities) play a role, but are relatively understudied (Paz et al., 2021).

Along with geohistorical factors, this variety of dispersal mechanisms is suspected to support widespread global distributions of the great majority of AM fungal taxa (Davison *et al.*, 2015). Despite a comparably low rate of endemism, several studies have observed significant variations in community composition at smaller scales (Wolfe *et al.*, 2007; Mummey & Rillig, 2008; Dumbrell *et al.*, 2010a; Hazard *et al.*, 2013; Avio *et al.*, 2020). At those more local or regional (sometimes termed as plot scale and landscape scale, respectively) several potential drivers have been proposed and investigated that shape AM fungal distributions.

Abiotic drivers

There is broad consensus that soil parameters affect AM fungi (Klichowska *et al.*, 2019) providing evidence for the "habitat hypothesis" postulating AM fungal community changes along differing abiotic conditions (Zobel & Öpik, 2014). Soil pH is one of the most studied environmental factors that often affects AM communities in the field (e.g., Dumbrell et al., 2010; Rasmussen et al., 2018; Davison et al., 2021). Along with pH, the concentrations of important chemical elements, such as carbon, nitrogen, phosphorous, as well as soil moisture can alter local AM fungal pools (Chaudhary *et al.*, 2008).

While the effects of soil chemistry on AM fungal community structure have been better explored, the influence of spatio-temporal factors is not well-resolved. Some contradictory results have been reported from the few studies that have investigated the relative effects of temporal and spatial factors. Dumbrell et al. (2011) for example found significantly distinct AM fungal diversity and communities in a British grassland site across the seasons within one year. In a different ecosystem, in the inner Mongolian steppe, Su et al. (2011) observed changes in AM fungal root colonization and rhizosphere spore diversity within a single growth season (between May and September). In contrast, Davison et al. (2012) found spatially distinct AM fungal communities in Estonian forest plots that did not vary over time. More data on seasonality and its indirect effects on AM fungi, probably driven through temperature and nutrient changes over the year, are highly required to further explore the observed discrepancies between different locations.

Biotic drivers

Because of their obligate association with plant roots, fungal distributions are likely to be interrelated with plant distributions (Davison *et al.*, 2015). Thus, most studies exploring AM fungal distribution patterns usually include vegetation type, or host plant identity (plant species) as one potential driving factor. Outcomes on the effects of host identity, however, vary as well across the published literature. While in some studies clear host identity effects were reported (Helgason *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002; Davison *et al.*, 2011; Su *et al.*, 2011) in others only weak or no effects were detected (Dumbrell *et al.*, 2010a; Rożek *et al.*, 2020), or appeared at the level of ecological groups rather than at the level of species (Öpik *et al.*, 2009)

Furthermore, synergistic, or competitive other microbes (in particular non-AM fungi and bacteria) affect AM fungal communities and vice versa (Smith & Read, 2008). AM-mycorrhizae do not present bipartite isolated fungal-plant associations but rather networks with bacterial communities colonizing the rhizosphere, extraradical hyphae or spores (Bonfante & Anca, 2009). So-called “mycorrhizae helper bacteria” have been described, referring to specific bacterial communities that promote either the establishment or the functioning of the mycorrhizal symbiosis (Garbaye, 1994; Frey-Klett *et al.*, 2007; Sangwan & Prasanna, 2021).

Challenges in AM fungal community ecology

Based on the current literature, complex interactions of abiotic and biotic parameters are likely to shape AM fungal communities. Unpredictable spatial and temporal processes additionally lead to usually high proportions of unexplained variations in natural AM fungal communities. This high complexity makes it difficult to understand and predict community dynamics. The fact that many parameters elicit idiosyncratic response patterns in communities of AM fungi may imply that they are relevant only under certain conditions or that they are masked (partially or completely) by other, more

critical factors, the latter being conceivable as a "law of the minimum" as suggested by Vályi et al. (2016).

AM habitat structure

There is evidence that AM fungal diversity underlies a species-area relationship, meaning that species-level richness decreases with habitat area in certain environments (see Boeraeve et al., 2019 for forests). Effects of habitat fragmentation on AM fungal diversity are likely related with a reduced connectance of suitable host plants. However, in contrast to host identity, the spatial structure – here meaning the explicit distribution patterns of host individuals – has rarely been addressed. The effect of host structure, however, might be highly relevant in areas with patchy AM-associating plant cover, such as in most temperate ecto-mycorrhiza dominated forests. Plant occurrence data from such a forest in Germany suggested facilitation between AM-associating over- and understory plants (Veresoglou *et al.*, 2017).

In the following Chapters we therefore mainly focussed on the underrepresented factor host plant structure and its effects on AM fungal communities. Building on the study of Veresoglou et al. (2017), in Chapter 2 we aimed to test whether the suspected AM fungal propagule limitation at ecto-mycorrhiza dominating overstory sites could be reflected by respective lower levels of root colonization in AM-associating understory plants. The studied forest sites have been described in earlier studies (Wulf, 1992; Naaf & Wulf, 2010) and thereafter we assumed relatively homogenous soil characteristics. This made the location suitable to investigate the relative important of spatial and temporal driving factors acting at the surveyed spatial scale (across one of the 50 m x 50 m forest sites). To do so, we monitored AM fungal diversity and community composition over two years in two herbaceous host plant species in Chapter 3. Since field observations always rely on regional conditions and typically do not produce generalizable results, in Chapters 4 we tested for the effect of habitat structure – *Plantago lanceolata* or *Medicago lupulina* host plant patches together with soil inoculum – alone in controlled greenhouse experiments. In Chapter 4a we focused on the responses of root colonization and in Chapter 4b on AM fungal diversity and community composition. Here, many of the known parameters could be well controlled, such as pH, soil moisture and temperature. Greenhouse experiments on AM community dynamics are lacking in the literature, probably because they require the implementation of realistic host plant distances, thereby easily exceeding the possible maximum size of the experimental units.

The framework of meta-community theory

Due to the spatial interplay of AM fungi and their hosts, many traditional community models do not apply to AM fungi. A promising approach might be to transfer ideas from meta-community theory to symbiotic microbes (Mihaljevic, 2012). Meta-community theory accounts for regional interactions

between local communities through dispersing individuals (Wilson, 1992; Leibold *et al.*, 2004). Veresoglou et al. (2012) pointed out chances and challenges of the application of meta-community theory to microbial symbionts, such as AM fungi. In our experimental designs in Chapter 4 we integrated the suggested considerations by the authors and used small populations of host plant patches to reflect local communities enabling migration between them. In one experiment, we used homogenous habitat patches – in accordance with the patch dynamic paradigm – and only manipulated the habitat connectance. The patch dynamic idea assumes that local communities establish based on two types of species interactions, competition for resources and colonization ability (Leibold *et al.*, 2004). The other two experiments were based on the source-and-sink paradigm using two soil types (fertilized and unfertilized) that varied in their relative proximity. Source-and-sink dynamics account for heterogeneous environments harboring high quality habitat patches with positive growth rates of species (source) and lower quality patches with negative growth rates (sink) (Winegardner *et al.*, 2012). Constant arrivals from migrating individuals from source habitats ensure the persistence of sink habitats. Through these specific experimental designs, we could not only investigate effects of the habitat structure, but also obtain insights about the applicability of meta-community theory to arbuscular mycorrhizal fungi.

Thesis outline

The overall aim of this thesis is to improve our understanding of AM fungal community dynamics. In two field studies and three large greenhouse experiments we focused from different angles on the effects of habitat structure, a widely overlooked factor (see Fig. 1 for a conceptual overview). Methodologically, classical biological techniques (here light microscopy - McGonigle et al., 1990) for the estimation of root colonization have been combined with modern molecular techniques (using Glomeromycota-specific primers and MiSeq Illumina sequencing) to assess taxonomical diversity serving subsequent community analyses. More specifically, we pursued the following goals in each chapter (see Fig.1 for Chapters 2-4):

In **Chapter 2** we examined whether the density of AM-associating overstory species affects AM fungal root colonization in herbaceous plants in temperate deciduous forest sites. In **Chapter 3** we investigated the relative importance of a set of spatio-temporal drivers for structuring AM fungal community composition in one of the previously studied forest sites (Chapter 2). In order to investigate isolated fungal-plant spatial interrelations three asynchronous controlled greenhouse experiments have been conducted using meta-community designs. In **Chapter 4a** we experimentally tested whether the spatial arrangements of host plant habitat (patch design), or the relative positions of habitat types (source-and-sink) within an artificial micro-landscape affected root colonization. In the same experiments we assessed in **Chapter 4b** effects on AM fungal diversity and community composition

across the spatial treatments. **Chapter 5** presents a summary of all results, as well as an overarching discussion to classify the findings according to the current state of knowledge.

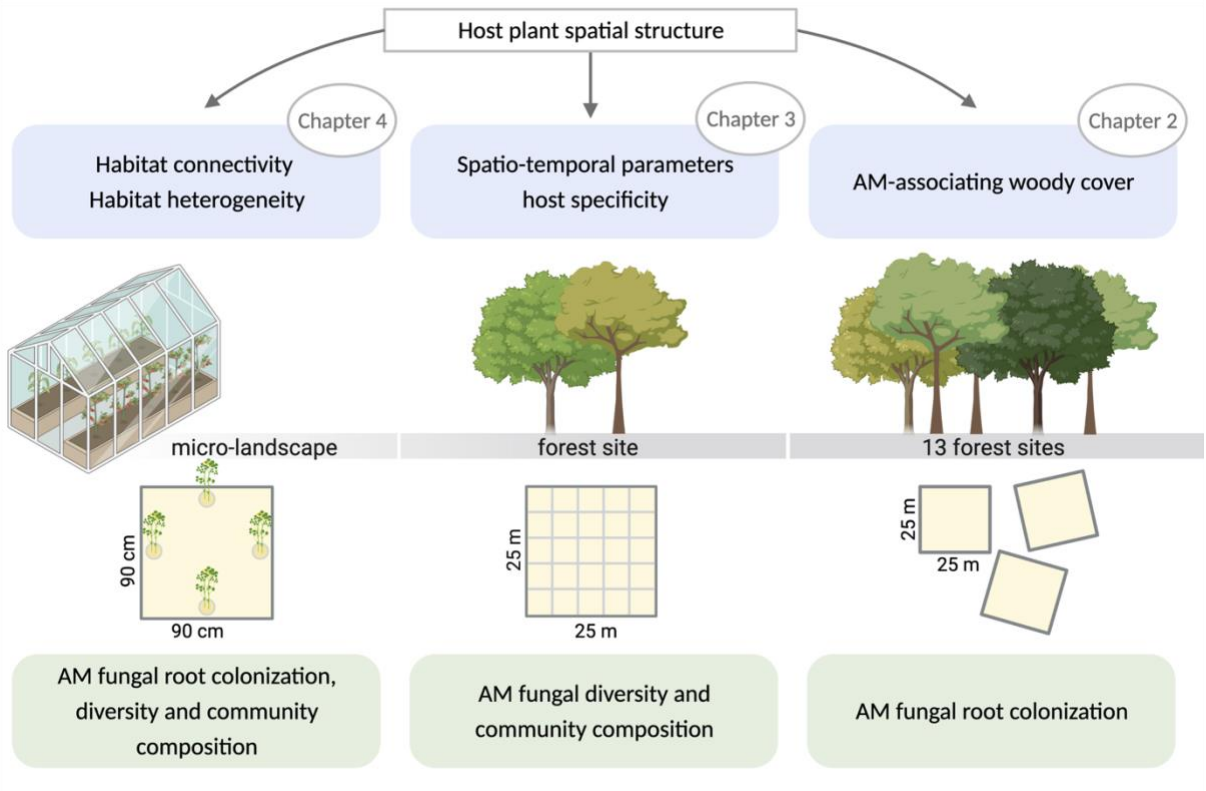


Figure 1 Conceptual overview on Chapters 2-4 showing how host plant spatial structure, the main driver of interest, was addressed across the studies. We worked at different spatial scales, from artificial micro-landscapes (< 1m^2) to a temperate forest site (525 m^2) to a set of 13 of forest sites. In three asynchronous greenhouse experiments we explored responses of AM root colonization, diversity and community composition across treatments representing either different levels of habitat connectance or heterogeneity (Chapters 4a and 4b). Across a temperate forest site, we investigated AM fungal diversity and community composition in response to spatial (including AM host plant cover) and temporal (year and season) parameters (Chapter 3). Across 13 forest sites varying in AM host plant cover we investigated the rate of root colonization in eight herbaceous AM host plants (Chapter 2). Figure created in Biorender.com.

Chapter 2

Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands

Grünfeld, L., Wulf, M., Rillig, M. C., Manntschke, A., & Veresoglou, S. D. (2020). Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. *New Phytologist*, *227*, 10-13. https://doi.org/10.1111/nph.16377

Arbuscular mycorrhiza represents a ubiquitous nutritional symbiosis between the roots of most terrestrial plant species and fungi of the subphylum Glomeromycotina (Spatafora *et al.*, 2016). Terrestrial habitats are unlikely to be limited in propagules of arbuscular mycorrhizal fungi (AMF), because AMF propagule densities build up fast in vegetated soil (e.g., Gould *et al.*, 1996). We start to appreciate, however, that shortages in AMF propagules are common in some habitats, such as agricultural fields subject to intensive farming (Schnoor *et al.*, 2011; Manoharan *et al.*, 2017). Forest habitats in the temperate region might also be occasionally AMF propagule limited (Veresoglou *et al.*, 2017), but to the best of our understanding this has not been shown with empirical data.

A particular feature of forest habitats is that two different strata of plants co-occur: the canopy consisting of woody plants and the understory mainly consisting of herbaceous plants. The two strata interact in various ways even though the underlying mechanisms remain mostly unknown (Sutherland *et al.*, 2013). Woody plants through intercepting light and via exploitative competition could alter germination and growth of herbaceous plants (Barbier *et al.*, 2008). However, it is unlikely that herbaceous plants can alter significantly the fitness of mature woody plants, rendering their interaction asymmetric. An underexplored factor in the way the two strata influence each other is mycorrhiza: the minority (i.e., coverage) of woody plants in Central European forests associating with arbuscular mycorrhizal (AM) might facilitate the herbaceous understory consisting mainly of AM plants.

In a recent study, Veresoglou *et al.* (2017) suggested that AM woody species in a temperate forest facilitate the establishment of AM herbaceous species of the understorey. AM woody species might be seen as islands of AM propagules in a large archipelago of non-AMF-associating trees which can support the AMF-associating ones (Van Der Heijden, 2004). The differences between stands dominated with AMF-associating trees and those dominated with woody plants that do not form AMF associations in Veresoglou *et al.* (2017) were inferred from plant community data and there have been

no comparable studies assaying *in situ* AMF availability. We here addressed this knowledge gap by testing if the abundance of AMF is higher in stands with a higher cover of AMF-associating woody species. For this purpose, we sampled roots of herbaceous plants that were widespread in these stands and assayed the proportion of roots colonized by AMF (term after McGonigle *et al*., 1990).

The stands were located in unmanaged, continuous, temperate European forests in northwest Germany (53.30°-53.66° latitude and 9.03°-9.49° longitude, Table S1). These are described in detail in Wulf (1992) and Naaf & Wulf (2010) and were used in the study of Veresoglou *et al.* (2017). We used vegetation records from (Wulf, 1992) and identified 13 of the 25 m x 25 m plots (we here described them as stands; Notes S1) so that the canopy of six of them was predominantly ectomycorrhizal-(ECM)-associating (*low* – below 7% relative cover of AMF-associating woody plants) and seven stands with a mostly AMF-associating canopy (*high* – above 49% relative cover of AMF-associating woody plants) (Table S2). Based on the vegetation records we *a priori* identified eight AMF-associating herbaceous plants that were present in the stands (Notes S1): *Ajuga reptans, Allium ursinum, Brachypodium sylvaticum, Circaea lutetiana, Geum urbanum, Pulmonaria obscura, Ranunculus auricomus,* and *Sanicula europaea*. Between 28 and 31 May 2017, we assayed roots of representatives of these plant species in as many stands as possible. We excavated the whole root system of the plants to a maximum depth of approximately 15 cm. In total we obtained 48 root samples from 13 stands to analyse. Six species were found in at least three different stands; however, *B. sylvaticum* and *S. europaea* were found exclusively in stands with a *high* AMF woody cover. We immediately stored plant material in 70% ethanol and kept it at 4°C until further examination. Roots were stained in Trypan blue (Gange *et al.*, 1999) and assessed with the magnified intersection method (McGonigle *et al.*, 1990) (Notes S1).

To address whether arbuscular colonization (i.e., proportion of root length containing arbuscules; McGonigle *et al.*, 1990) was higher in herbaceous plants in the stands with a *high* cover of AMF-associating woody plants (vs. low stands) we fitted a linear mixed effects model (Notes S1). We repeated the analysis using percent hyphal colonization (i.e., proportion of root length containing hyphae) of the roots as response variable (i.e., test whether hyphal colonization was higher in the *high* stands) and we report on this in Notes S2; here we use hyphal colonization to describe exclusively AMF hyphae.

Arbuscular colonization was higher at *high* stands (> 49% AMF-associating woody-cover) compared to *low* (<7% AMF-associating woody-cover) stands ($F_{1,27}$=6.75, P=0.015; Fig. 2a-b for a map; Notes S2). Conclusions did not change when we used hyphal colonization as a response variable, instead (Figs S3, S4). Arbuscular colonization can be seen as a proxy of symbiotic exchange, whereas hyphal colonization as a measure of mycorrhizal fungal biomass (Johnson *et al.*, 2010; Lekberg *et al.*, 2015), comprising two complementary parameters of a mycorrhizal association. Arbuscular and hyphal colonization varied from 0% to 72% and from 0% to 87.5%, respectively (Figs S1, S2; Table S3).

Exceptionally in roots of *G. urbanum* we found small differences in arbuscular colonization across the two stand types (23% at *low* and 25% at *high* stands; respective means for hyphal colonization were 28% and 32%; Fig. 2a). The fitted random effect values for stands, presenting proxies of overall AMF abundance per stand, also correlated positively with the relative availability (i.e., cover) of AM woody plants (r=0.67, P=0.01; Fig. 2c).

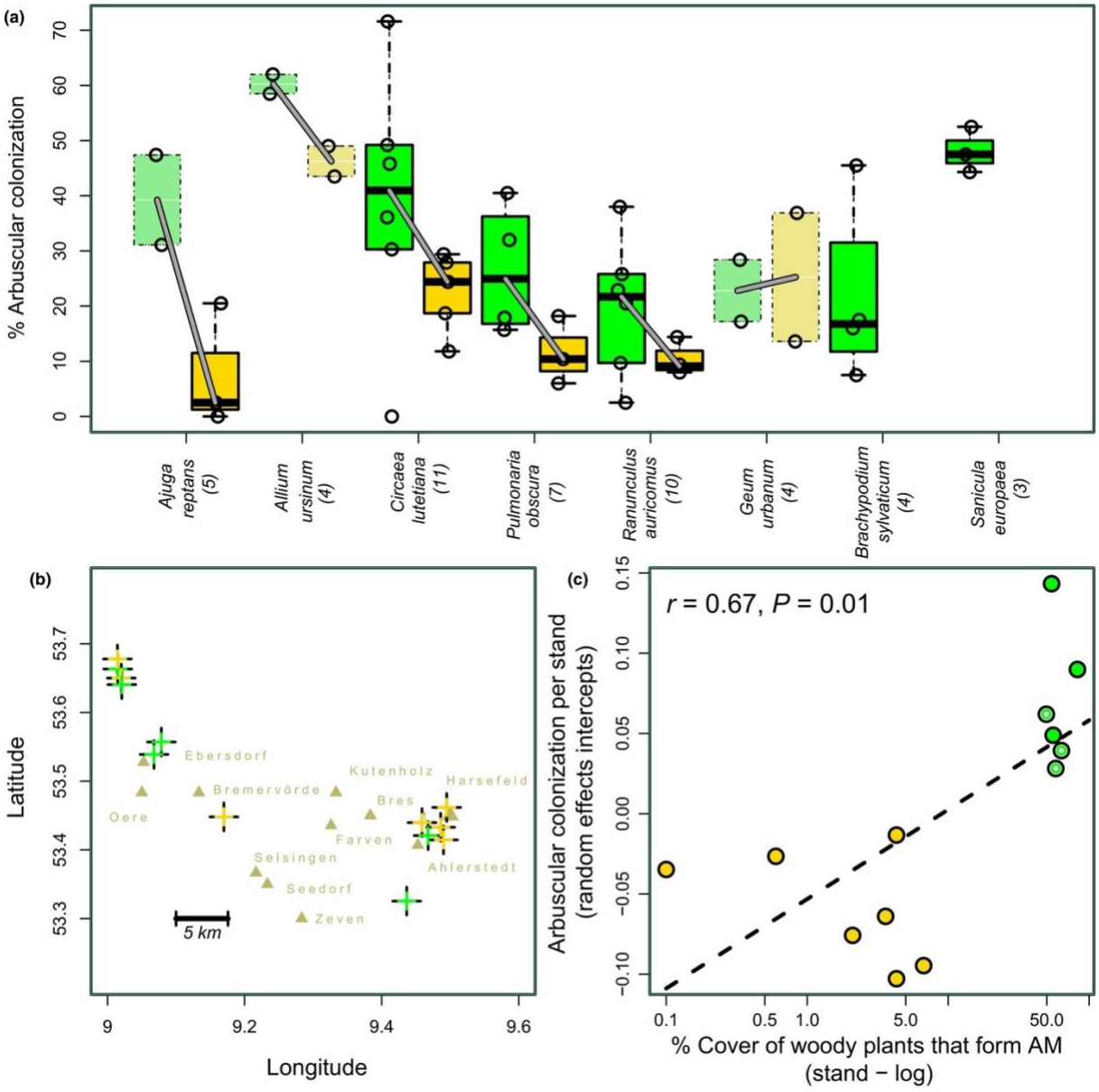


Figure 2 (a) Arbuscular colonization in the eight target herbaceous plant species. Jittered points present the statistics for the sampling stands with *high* (green) or *low* (yellow) coverage of AMF-associating woody plant and shrub species. Light coloured boxes originate from only two observations and thus contain higher uncertainties. Model statistics can be found in Notes S2. (b) Map highlighting the geographical position of the stands (green and yellow plus signs between 9.0 - 9.5 longitude and 53.3 - 53.7 latitude) in relation to villages (triangles) in the sampling area. (c) Arbuscular colonization per stand in the form of the respective fitted coefficients (y-axis) plotted against the percent cover of AMF-associating woody plants and shrubs that were observed in each stand (green – *high* and yellow – *low* dots).

We here present empirical evidence that AM fungal root colonization in herbaceous plants relates to AM woody plant coverage in ECM-dominated woody habitats. Our findings complement the analysis of (Veresoglou *et al.*, 2017) which was carried out in the same study region showing that there were fewer AMF-associating herbaceous plants, and a lower richness of such plant species, in stands with little cover of AMF-associating woody plants. Thereby we support an existing hypothesis that despite their ubiquity, AMF can face dispersal limitation and be present in some habitats at low densities (Zobel & Öpik, 2014).

We presume that the differences in arbuscular colonization have been due to the exclusion of certain AMF taxa from *low* in AMF-associating woody cover stands. We did not assay AMF community structure to test this assumption. Because all plant roots had detectable arbuscular colonization in all stands, it is unlikely that the absence of AMF propagules or nutrient availability-induced changes in host physiology alone explained differences in arbuscular colonization. *Geum urbanum*, for example, responded only weakly to stand type. This might be due to the fact that *G. urbanum* represents the only host plant that is not a forest specialist (Schmidt *et al.*, 2011) and therefore might associate with generalist AMF species, likely with good dispersal properties. Mutualistic networks between AMF and plants are known to be nested and asymmetric (Chagnon *et al.*, 2014). Because of a wider ecological niche, generalist plant species should have the ability to associate with many specialist AMF that could be found in ECM-dominated stands (i.e., habitat generalists come across a wider array of potentially compatible AMF partners) and this might explain the relatively constant colonization in *G. urbanum.* The six other forest specialist species might have a narrower range of AMF associates.

Even though it is woody plants that photosynthesize most in temperate forests, herbaceous plants show the highest species diversity and via contributing unique functional traits to the system, such as palatable leaves high in nitrogen (N) content and fine roots, could be of high ecological importance (Reiss *et al.*, 2009). Herbaceous plants might also interact with the woody canopy through various direct or indirect ways (Gilliam, 2007). As an example, the AM understory could cascade effects to numerous ecosystem processes in soil such as N-mineralization, nitrification and decomposition. We think that the higher litter quality of the AM-understory compared to the other woody plants could be priming these ecosystem processes (Van der Krift & Berendse, 2001; Veresoglou *et al.*, 2011; Luo *et al.*, 2016). The link through AMF between strata presented here implies that islands of AMF-associating trees or shrubs indirectly promote herbaceous plant growth, health and diversity (Azcón-Aguilar & Barea, 1996; Van Der Heijden *et al.*, 1998) by increasing AMF propagule availability. However, forest management practices are almost exclusively targeting woody species often associating with ECM. In conclusion, mixed stands containing AMF-associating woody plants may effectively promote multi-functionality and multi-diversity in temperate forests.

While root mycorrhizal fungal colonization is also controlled by factors other than propagule availability (including host factors, such as light, or soil factors such as nutrient availability), one interpretation of our results is that herbaceous plants in temperate forests do experience AMF propagule limitation. If this is true, which should be tested in additional work, our study highlights the need to better understand the efficiency of dispersal in Glomeromycotina.

Acknowledgements

The authors acknowledge funding from the Deutsche Forschungsgemeinschaft Project Metacorrhiza (VE 736/2-1) awarded to SDV.

Chapter 3

Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest

Grünfeld, L., Mola, M., Wulf, M., Hempel, S., & Veresoglou, S. D. (2021). Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest. *Mycorrhiza*, *31*(5), 589-598. https://doi.org/10.1007/s00572-021-01041-6

Abstract

Many woody and herbaceous plants in temperate forests cannot establish and survive in the absence of mycorrhizal associations. Most temperate forests are dominated by ectomycorrhizal woody plant species, which implies that the carrying capacity of the habitat for arbuscular mycorrhizal fungi (AMF) is relatively low and AMF could in some cases experience a limitation of propagules. Here we address how the AMF community composition varied in a small temperate forest site in Germany in relation to time, space, two plant host species, and also with regard to the degree to which plots were covered with AMF-associating woody species. The AMF communities in our study were non-random. We observed that space had a greater impact on fungal community composition than either time, mycorrhizal state of the close-by woody species, or the identity of the host plant. The identity of the host plant was the only parameter that modified AMF richness in the roots. The set of parameters which we addressed has rarely been studied together, and the resulting ranking could ease prioritizing some of them to be included in future surveys. AMF are crucial for the establishment of understory plants in temperate forests, making it desirable to further explore how they vary in time and space.

Introduction

Arbuscular mycorrhizal fungi (AMF; Phylum Glomeromycota) are globally distributed symbiotic fungi, which at large spatial scales show non-random distribution patterns, explained mainly by abiotic predictors such as pH (Dumbrell *et al.*, 2010a; Davison *et al.*, 2021), soil properties (Klichowska *et al.*, 2019) and climatic conditions (Dumbrell et al. 2011) but also host specificity (Vandenkoornhuyse *et al.*, 2002). Most studies addressing AMF, however, can only explain a small fraction of AMF community variance, suggesting that AMF communities are subject to a high degree of stochasticity (i.e., the fraction of community variance not explained by deterministic processes; Notes S3; (Dumbrell *et al.*,

2010b; Lekberg *et al.*, 2012; Goldmann *et al.*, 2020). Assaying stochastic drivers in large-scale mycorrhizal studies is challenging because they can inflate the sequencing effort required. We here aimed at ranking the relative importance, in terms of shaping arbuscular mycorrhizal fungal communities, of a set of stochastic (space, time) and biotic (host plant species and woody coverage of AMF-associating species) drivers rarely assayed together, in an effort, to smooth the way towards integrating stochastic predictors into mainstream studies of mycorrhizal fungus communities.

We present a spatio-temporal study at a forest site where we address the relative importance of (i) physical distance, (ii) sampling time (year and season), (iii) host specificity (here describing the impact of host plant identity on the AMF community composition in the roots of focal plant species) and (iv) relative coverage of AMF-associating woody species (Veresoglou *et al.*, 2017; Grünfeld *et al.*, 2019) in shaping the AMF community composition of the understory. To the best of our understanding, no other mycorrhizal study to date has simultaneously studied this specific set of parameters, even though studying subsets of them have generated highly-valued expectations: (Davison *et al.*, 2012), for example, studied the effects of seasonality and spatial structure in an Estonian temperate forest and observed considerable spatial heterogeneity in AMF species distributions, but minimal changes over the duration of a growth season. (Dumbrell *et al.*, 2011), by contrast, observed pronounced temporal changes in the composition of AMF grassland root communities over a single growth season. (Su *et al.*, 2011) addressed the relative strength of host specificity and seasonality to show that in the studied steppe of Inner Mongolia, seasonality masked any host preferences across five hosts. Therefore, our first expectation (*Hypothesis One*) was that physical distance would be relatively more important than temporal variance in shaping AMF communities in a woody habitat ((Davison *et al.*, 2012). It is likely that woody plants in such studies had strong effects on the understory because they had acted as islands of AMF propagules (Grünfeld *et al.*, 2019). If it is the presence of AMF-associating woody species which mainly shapes the regional pool of AMF species, then compared to grasslands, we might expect a lower relative importance of host specificity across the understory plants because tree root systems are comparably much larger than those of understorey plants. AMF propagule selectivity among hosts (i.e., which might lead to the evolution of host dependency on specific fungus species) in the understory hence is determined to a large degree by the identity of the neighbouring woody AMF-associates. We therefore additionally hypothesized (*Hypothesis Two*) that relative coverage of AMF-associating woody species would alter AMF community composition more than host specificity does. We addressed these two hypotheses in a forest site in the Elbe-Weser region in North-West Germany which we monitored over two years, totalling four harvests of root material.

Materials and Methods

Study site

The study site is a floristically well described (Wulf, 1992; Naaf & Wulf, 2010) temperate European deciduous forest in northwest Germany (53.44°N; 9.49°E). The soil is a humid to waterlogged pseudogley (Roeschmann, 1971). Biophysical characteristics were assumed to be relatively consistent over the homogeneous 625 m^2 site, and therefore we did not explicitly measure or include them in this study. Nevertheless, we report regarding previously assessed soil parameters at the same site by Wulf (1992) (see Table S5). Based on previous observations in the broader area, understory plants associating with AMF occurred at higher frequency (Veresoglou *et al.*, 2017) and were colonized more extensively by AMF (Grünfeld *et al.*, 2019) when there was a high relative coverage of woody plants forming arbuscular mycorrhizae. It is likely that the occurrence and density of arbuscular mycorrhizal (AM) woody plants facilitate the dispersal of AMF propagules and thus their availability to less dominant (with respect to biomass) understorey AM plants. To assess AMF community variability related to AM woody coverage we divided the forest site into 25 5 x 5 m rectangular plots and estimated in situ coverage by AMF-associating woody plants per plot. AM woody coverage ranged from 0% to 60% and we subsequently classified the plots into AM *high* and AM *low* classes (≥ 15% and < 15% AMF-associating woody coverage, respectively; Fig. S5, Table S4; we rationalize the choice of the threshold in Fig. S6).

Sampling Design

Over two years we collected root samples from the two most abundant perennial woody understorey plant species *Hedera helix* L. (Araliaceae, from now on *Hedera*) and *Euonymus europaeus* L. (Celastraceae, from now on *Euonymus*) in the forest site. In the beginning (May) and end (September) of the growing seasons of 2017 and 2018, respectively, we collected *Hedera* roots (78 samples) from pairs (i.e., two neighbouring plots of high and low relative coverage of AMF-associating woody species) of *high* and *low* AM plots. In September 2018, we additionally collected roots of *Euonymus* (19 samples; *Euonymus* could only be collected at the last harvest because there were only a few individuals of *Euonymus* in the forest site and their destructive harvest could modify meta-community dynamics of AMF species.). The two hosts were sampled independently of each other, meaning that *Euonymus* and *Hedera* separated by less than 50 cm potentially could have been sampled. Because both hosts were woody species, we expected them to phenologically vary less in time than herbaceous plants. Rarely, two individuals of a species were not available in a plot. Thus, there were a total of 97 root samples from the two host plants from the four sampling campaigns (see Table 1 and Fig. S5 for the specific sampling scheme). Roots were excavated to a maximum depth of about 10 cm from two plant individuals of each focal species per plot, which were processed independently. Assaying a depth of 0

– 10 cm maximized compatibility of our findings with the bulk of the literature and did not cause excessive disturbance to the forest site. The minimal distance between the two individuals of the same plant species was 50 cm to minimize the likelihood that the two root samples shared AMF individuals (Klironomos and Moutoglis 1999). The root samples were cleaned with water and transferred into falcon tubes with 95% ethanol.

Table 1: Sampling scheme showing the number of samples (n) and plots per sampling campaign and plant species.

	May 2017	**Sep 2017**	**May 2018**	**Sep 2018**
Hedera helix	n = 12 plots = 6	n = 20 plots = 10	n = 20 plots = 10	n = 26 plots = 14
Eunonymus europaea	not sampled	not sampled	not sampled	n = 19 plots = 10

Molecular analyses and bioinformatics

Roots were transported to the lab ln ethanol at 4°C and stored at -20°C. Root samples were freeze-dried and homogenized with a Retsch Mixer Mill MM 400 using metal balls of 1 mm diameter. DNA was extracted from 30 mg ground root material per sample with the DNeasy® PowerPlant® Pro Kit (Qiagen, Venlo, the Netherlands) and amplified with the AMF-specific-18S-rRNA gene targeting primer pair NS31-AML2 (Liu *et al.*, 2011) extended with the adaptors p5 (NS31) and p7 (AML2; (Kircher *et al.*, 2012). The amplification conditions were as follows: each of the 25 µl PCR reactions contained 1 µl DNA template, 2.5 µl (0.3 µM of each primer) primer mix, 0.25 µl KAPA HiFi DNA polymerase (1 U/µl), 0.5 µl KAPA dNTP mix (10 µM), 5 µl 5X KAPA HiFi Fidelity Buffer and 15.75 µl nuclease-free water. The PCR reactions were performed with a Biometra-Ton thermal cycler (Analytik Jena, Jena, Germany) under the following conditions: Initial denaturation at 95 °C for 2 min, 35 cycles with a denaturation phase of 98 °C for 45 s, an annealing phase of 65 °C for 45 s and an extension phase of 72 °C for 45 s and final elongation at 72 °C for 10 min. Samples that did not perform well on this initial PCR (~40% of the samples) were amplified instead with a GC-rich buffer from the kit. Four out of 97 samples did not show bands during gel electrophoresis and were excluded from further analysis. The NS31-AML2 amplicons were purified with the NucleoSpin® gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany). For indexing purposes, we used Miseq specific adaptors (NuGen) which we ligated to our products with an additional PCR. The PCR master mix for indexing consisted of 1 µl of the purified PCR template, 2.4 µl of the primer mix, 0.25 µl Phusion® high-fidelity DNA polymerase (BioLabs), 0.5 µl dNTPs (10 µM), 5 µl 5X Phusion® HF buffer and 15.85 µl nuclease-free water per 25 µl reaction. After indexing PCR -– thermocycling settings: 95 °C for 3 min, 15 cycles of 98 °C for 30 s, 55 °C for 30 s and

72 °C for 30 s, and 72 °C for 5 min – the DNA fragments were separated by gel electrophoresis to check the signal strengths. We used MiSeq Illumina chemistry (v3, 600 cycles) to sequence the amplicons. We processed the libraries with the uPARSE pipeline (Edgar, 2013) with uSearch v 10.0.240. In brief, forward and backward reads were merged with the fastq_mergepairs command, primers were stripped and sequence pairs with a length shorter than 400 bp or more than 1 expected error were removed. We used the cluster_otus command to construct the OTU table. Representative OTU sequences were blasted against MaarjAM (Öpik *et al.*, 2010) and non-specific to AMF OTUs (i.e., < 97.5% similarity or < 99% coverage) were excluded from further analyses. Representative sequences for each OTU were submitted to GenBank (submission MW017500-MW017533). We then rarefied to 2350 reads per sample, which excluded 2 samples from further analysis (i.e., analysis was carried out to the remaining 91 samples).

Statistical analyses

Null model analysis – to what degree were AMF distributions random?

To address the degree to which AMF communities were random, we conducted a null model analysis with the R package EcoSimR (Gotelli *et al.*, 2015). We compared C score occurrences in our dataset to distributions of 1000 random matrices that were generated with the *sim*4 algorithm. C score occurrences of checkerboards describe the cumulative number of occurrences across a pair of sites (= rows) and species (= columns) in the presence-absence community matrix where Species A has only been present at Site A and Species B has only been present at Site B. We z-score standardized effect sizes (SES) in relation to the set of simulated community matrices. We used presence-absence data and kept the total number of row sums in the community table fixed, describing how often species occurred. The row sums were proportional to those observed in the column sums, reflecting differences across samples. The *sim*4 algorithm effectively controls for Type I and II statistical errors and has been proposed for scenarios in which some rare species occasionally have been scored as absent even though present (i.e., incomplete lists; (Gotelli, 2000). Negative standardized effect sizes below -1.96 reflect aggregation of species within samples (= fungal species co-occur more often than expected by chance), positive values above 1.96 reflect segregation of species within samples (= fungal species co-occur less often than expected by chance), whereas values between -1.96 and 1.96 reflect a random species distribution among plots. Additionally, values differing by more than 1.96 standardized units were significantly different (analogous to a confidence interval). Because inadvertent pooling of heterogeneous samples (due to combining in the same analysis root samples differing in time, space and also plant host) might bias results towards appearing less random (Ulrich *et al.*, 2012), we additionally assessed null model statistics for several subsets of the combined community matrix.

Hypothesis One: physical distance is more important than temporal variance in structuring AMF

Because our study design was complex and difficult to be fully captured with statistical techniques, we tried whenever possible (such as in Fig. 4) to present effect sizes which assumed no specific statistical model. To address this hypothesis, we (i) visualized the raw data via unconstrained ordination; (ii) calculated effect sizes in the form of Bray-Curtis community distances for the major drivers of AMF community composition; and (iii) presented as a key result a summary for some characteristic groups of samples of community composition information at the AMF family level. First, we carried out a Principal Components Analysis (PCA) on Hellinger-transformed AMF OTU occurrence data (i.e., AMF community table with each OTU treated as an independent response variable) to visualize clustering patterns across the samples. Second, we presented how effect sizes differed among our variables of interest. We wanted to avoid statistical shortcomings of combining a redundancy analysis (i.e., a form of constrained ordination) with variance partitioning (Notes S4). Even though there are several techniques to address spatial autocorrelation in ordination analyses, to the best of our knowledge the only multivariate technique that works for temporal constraints is that of Palmer *et al.* (2008) which was specifically proposed for split-plot designs. To minimize the assumptions of our analyses we plotted the data with a PCA (i.e., meaning that we do not propose for this specific analysis any underlying model; Fig. 4a) and then calculated the distributions of pairwise Bray-Curtis distances. We visualized relative effect sizes by means of Bray-Curtis distances and only additionally fitted a predictive model in the form of a redundancy analysis (RDA) in which we addressed temporal constraints by restricting permutations (and thus calculation of resulting *P* values) to be only within plots. We further decomposed distance (i.e., spatial) information into three principal coordinate neighbouring matrices (PCNM; Borcard and Legendre 2002) which we then fitted into the RDA model. This approach may be an improvement compared to assuming full independence, but it still falls short of describing our spatio-temporal sampling design. For this reason, we cautiously interpreted the resulting variance partitioning exercise. To compare effect sizes, we randomly paired samples sharing specific attributes 9999 times and quantified Bray-Curtis distances. Third, we summarized how AMF community composition differed with each of the predictors by generating bar plots with relative abundance information on each AMF family. We finally created a heatmap (i.e., a two-dimension graphical representation of community data) presenting the frequencies with which individual AMF taxa were observed in habitats with specific attributes.

Hypothesis Two: Relative coverage of AMF-associating woody species would alter AMF community composition more than host specificity does

We first carried out a repeated-measures ANOVA to compare diversity metrics (i.e., richness, Shannon diversity and Pielou evenness; in the results section we only report on richness but the results were

comparable across all those diversity indices) between AM *high* and *low* plots. The response variable was the diversity metric; host species and *low* vs. *high* type of habitat were the predictors and time was the repeated measures parameter. In our repeated-measures ANOVA we corrected for spatial dependencies in the form of specifying the unit of the ANOVA analysis at the "plot" level. To address whether the communities in *high* and *low* plots differed in relation to how aggregated/segregated they were, we further compared the respective SES which we obtained from our null model analysis. We created a venn diagram depicting how host specificity and relative coverage of AMF-associating woody species influences AMF community composition to visualize compositional differences. To further address whether host plants or the two habitat types selected for specific OTUs, we finally carried out an indicator species analysis (we used the package indicspecies in R; (de Cáceres & Legendre, 2009) in relation to the following classes: the two host plants, the two habitat types (i.e., *high* vs *low*) and their meaningful combinations.

Results

Overall Statistics

Out of 853,811 quality-controlled reads, 696,451 described 32 AMF-specific OTUs (Table S6). Eighteen of them belonged to Glomeraceae, six to Claroideoglomeraceae, five to Archaeosporaceae, two to Diversisporaceae and one each to Gigasporaceae and Acaulosporaceae. We rarefied sequencing depth to 2350 reads per sample which resulted in the exclusion of two samples. AMF richness ranged from 4 to 17 OTUs per sample (median: 10 OTUs with the quartiles being 8 and 12; Fig. S7). Richness only differed with host plant (n = 93, t = -4.44, $P < 0.0001$; when we narrowed observations to those from the fourth harvest the respective statistics were n = 43, t = -3.14, P = 0.003; Fig. S7): *Euonymus* plants contained on average 8.2 AMF taxa, whereas *Hedera* plants contained 10.54.

The indicator species analysis classified 5 of the 55 species as indicators. OTU2 (Glomeraceae; P=0.045) was an indicator of *Euonymus* communities and OTU70 (Glomeraceae, P<0.001) an indicator of *Euonymus* community at *low* plots. OTU8 (Claroideoglomaceae; P=0.001) and OTU13 (Acaulosporaceae, P<0.01) were indicators of *Hedera* communities whereas OTU19 (Diversisporaceae; P=0.038) specifically associated with *Hedera* at *high* plots.

Null model analysis - to what degree were AMF distributions random?

In all our tests we observed significant species aggregation (Fig. 3). The standardized effect sizes (SES) ranged from -10.90 (combined community matrix) to -2.4 (*Hedera* roots in May 2017). AMF communities in *Hedera* roots from *low* plots (SES = -8.19) were more aggregated than those from *high* plots (SES = -4.81; any differences in the statistics exceeding 1.96 are significant). AMF communities in *Hedera* were more aggregated in autumn than in spring (the mean SES statistic for spring was -2.98

whereas for autumn it was -5.25). The results in SES statistics could not be explained based on sampling intensity (i.e., number of individuals assayed; there was no correlation between the two values).

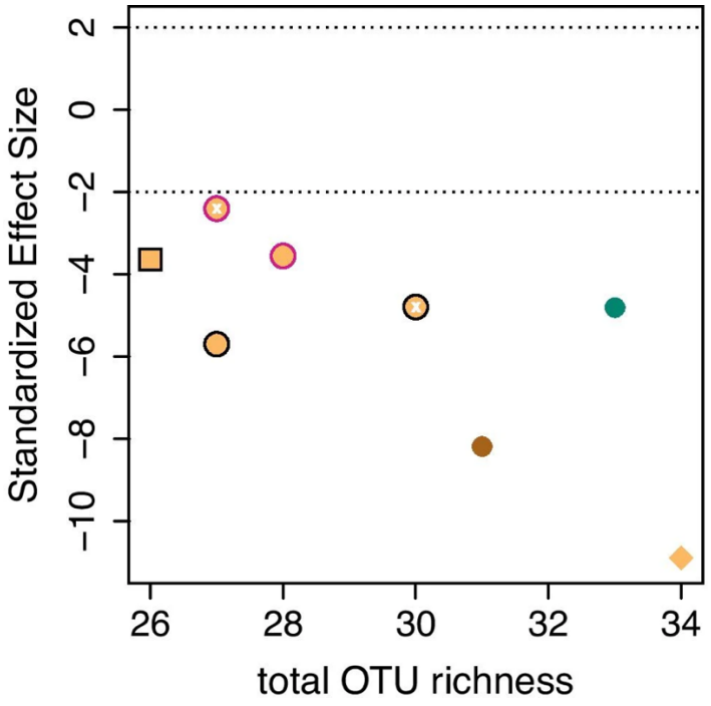


Figure 3 Standardized effect sizes of observed checkerboard scores which were compared against a null model generated with the sim4 algorithm (*y*-axis). We plotted these values against total OTU richness of the respective subsets of the dataset to capture a factor that may influence them. The two discontinuous lines highlight confidence intervals within which the community matrix can be considered random. The green point represents samples from AM *high* plots (> 15% woody AM-associating plant coverage), the brown from AM *low* plots (< 15% woody AM-associating plant coverage) and the orange points from combinations of the two. A pink border was used for spring and black for autumn; we used no border where we pooled samples from spring and autumn. We used white "x" symbols to highlight the location in the panel of samples taken over the first year. The square represents samples on *Euonymus* whereas circles those on *Hedera*. The diamond shows the complete data set. Differences in standardized effect sizes above 1.96 and below -1.96 are significant at a 0.05% confidence level.

Hypothesis One: physical distance is more important than temporal variance in structuring AMF

Our Principal Components Analysis on Hellinger-transformed occurrence data showed that any differences in AMF community composition across the samples were so subtle as to be little apparent (Fig. 4a). We plotted axes two and three because after excluding an outlier sample these two axes explained most rescaled variance. The take home message from the panel is that there were no apparent clustering patterns in our dataset against any parameter and any AMF community shifts in time or space thus were relatively small. The Bray-Curtis distributions overlapped considerably but spatial structure induced stronger effect sizes than temporal variability (Fig. 4b). In addition, community changes within a growth season were subtle (Fig. 4b). We also observed that the two host

plant species (Fig. 4b) shared more similar communities than expected by chance and that it was low plots that had the most divergent AMF communities. (Fig. 4c). *Euonymus*-associated AMF communities were dominated by Glomeraceae (94.8% on average compared to a maximum of 86% in *Hedera*; Fig. 4c). High occurrence of Glomeraceae was also observed at low AM plots (averaging 85.5%). Relative abundance differences of families were considerably more pronounced across years than across seasons (Fig. 4c). In the redundancy analysis with the drivers as predictors, we found that year, host plant and spatial autocorrelation axes explained AMF community shifts whereas season had no effect. AM-plant cover shared considerable variance with other predictors and significance depended on the ranking with which it was included among the predictors (Notes S5).

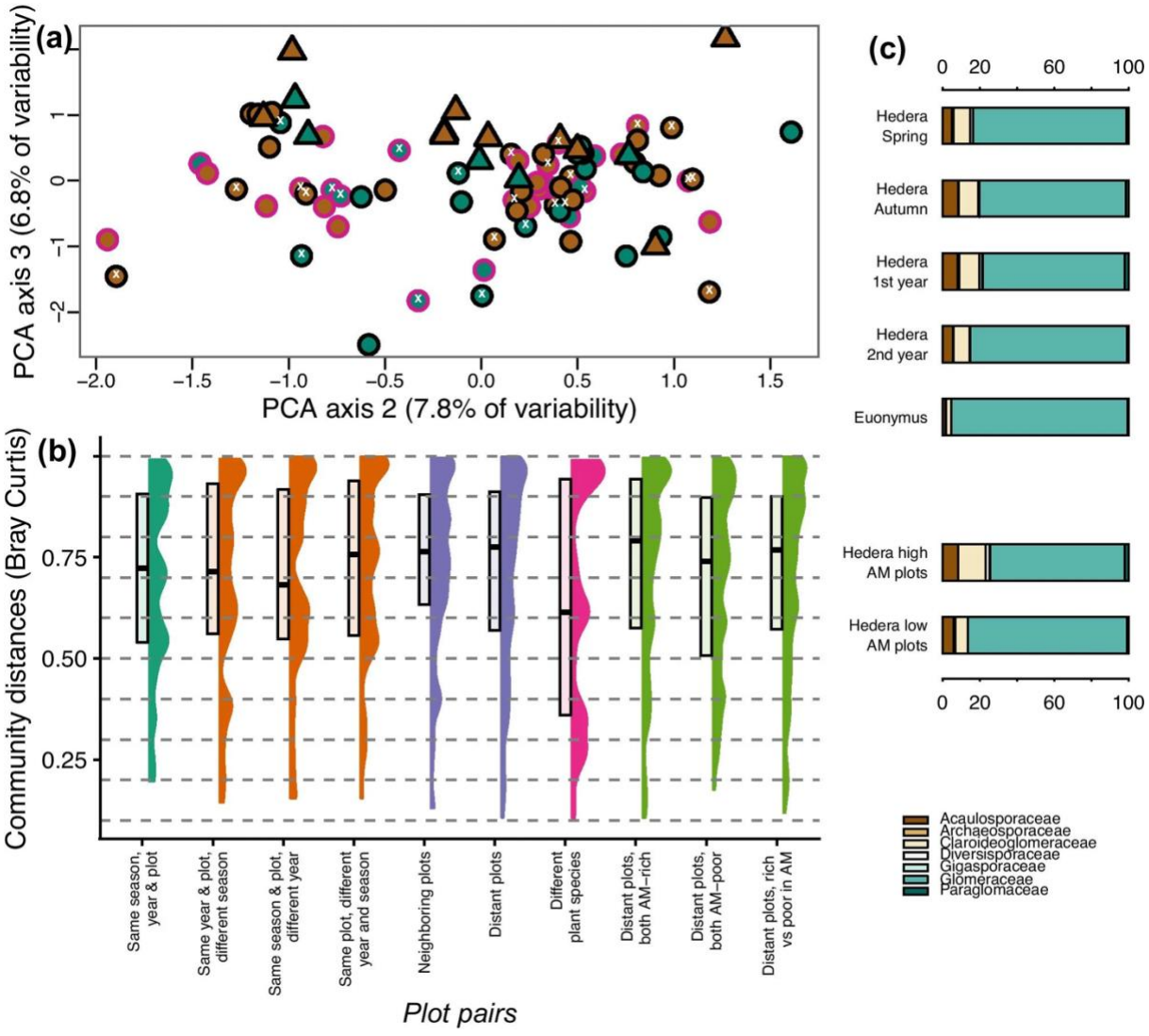


Figure 4 (a) Principal component analysis of Hellinger-transformed AMF community data (we plotted respective diagrams with axis one, explaining 13.5% of variability in Figs S8, S9). Green symbols represent samples from AM *high* (> 15% woody AM-associating plant coverage) whereas brown represent AM *low* plots (< 15% woody AM-associating plant coverage). A pink border was used for spring and a black for autumn. We used white "x" symbols to highlight the location in the panel of samples taken over the first year. Triangles describe samples on *Euonymus* whereas circles those on *Hedera*. (b) Distributions of pairwise community distances (Bray-Curtis distances) for a

range of pairwise combinations (dark green: within plots sampled at the same time; orange: same plot differing in sampling time; purple: same harvest but different plot; pink: same plot in the 4th harvest but different host plant; light green: same harvest but different plot grouped based on the relative coverage of AMF-associating woody plants). Larger values signify more dissimilar samples, meaning that the responsible factor induced a stronger AMF community shift than in the case of smaller values. As an example, the pairs belonging on the same plot which are presented in the four first histograms (in dark green and orange) consistently showed smaller values than those across different plots (two purples histograms) suggesting that space played a role in shaping AMF communities. Note that Bray-Curtis community distances between *Hedera* and *Euonymus* (in pink; same plot) were smaller than respective distances between individuals of *Hedera* (dark green). (c) Mean relative abundances of the seven AMF families (Acaulosporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, Glomeraceae, Paraglomaceae) grouped based on (top) the time of sampling, plant host and (bottom) our classification into high and low plots.

Hypothesis Two: Relative coverage of AMF-associating woody species would alter AMF community composition more than host specificity does

We observed no diversity differences in relation to *high* or *low* relative coverage of AMF-associating woody species (Fig. 3; $F_{1,81} = 0.048$, $P = 0.83$; The only significant effect was that of host plant; $F_{1,81} = 11.8$, $P < 0.001$). Roots from *low* plots contained consistently more aggregated AMF communities than the representatives from *high* plots (Fig. 3). There were minor compositional distances between *high* and *low* plots with 7 OTUs being specific to *high* plots and 2 to low plots (Fig. 5a). We observed, by contrast, twelve OTUs to be specific to *Hedera* samples (Fig. 5a), which might have been because of the most extensive sampling of *Hedera* individuals. Observation frequency, for most taxa, was higher at *high* plots than at *low* plots (Fig. 5b).

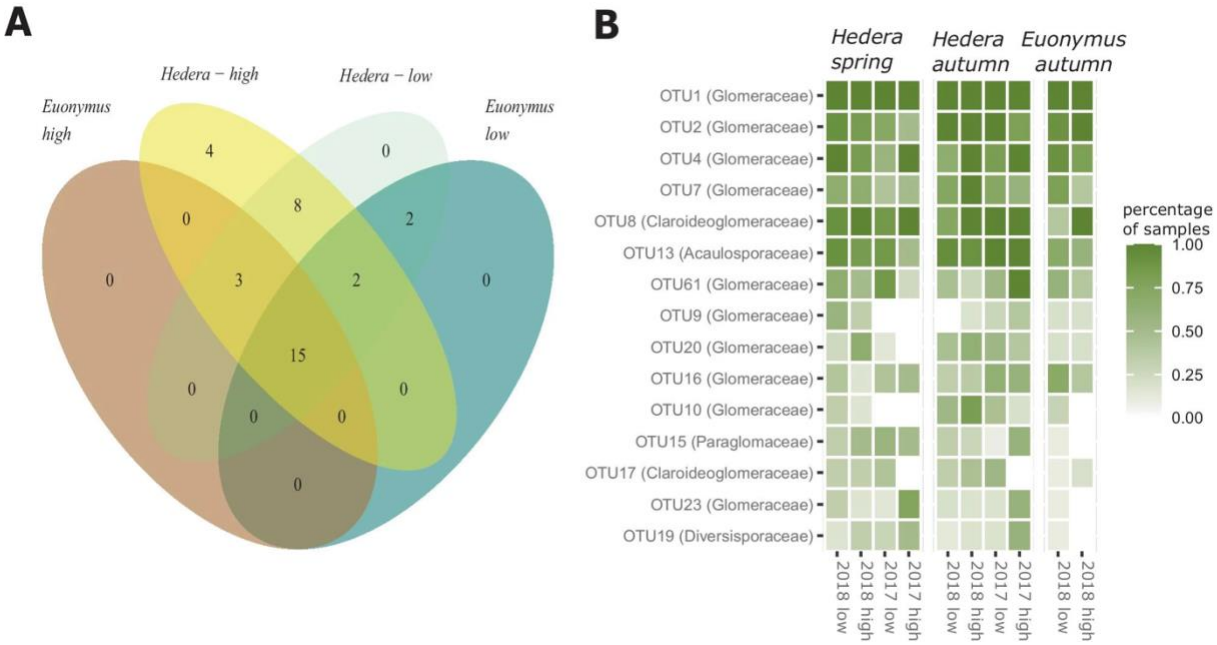


Figure 5 (A) Venn diagram depicting the distribution of OTUs across (i) AM *high* (> 15% woody AM-associating plant coverage) and AM *low* plots (< 15% woody AM-associating plant coverage) and (ii) the two plant hosts. Fifteen out of the thirty-two OTUs were observed in all four types of habitats. (B) Frequency of occurrence of the fifteen most abundant OTUs across ten groups of samples describing plant host, plot quality in relation to AMF abundance and season of sampling.

Ranking of spatio-temporal parameters and host specificity

Based on the variance partitioning exercise (Fig. 6), spatial parameters (4.54%) explained most variance followed by host specificity (2.32%). This was despite that the representation of hosts was unbalanced, meaning that the variance fraction allocated to host specificity actually should have been considerably larger. Temporal parameters explained 1.76% of the variance but this fraction was exclusively due to different years and not due to different seasons (Fig. 6, insert). The relative coverage of AMF-associating woody species (i.e., AMF cover in Fig. 6) explained no variance. These observations match well the results from Fig. 6.

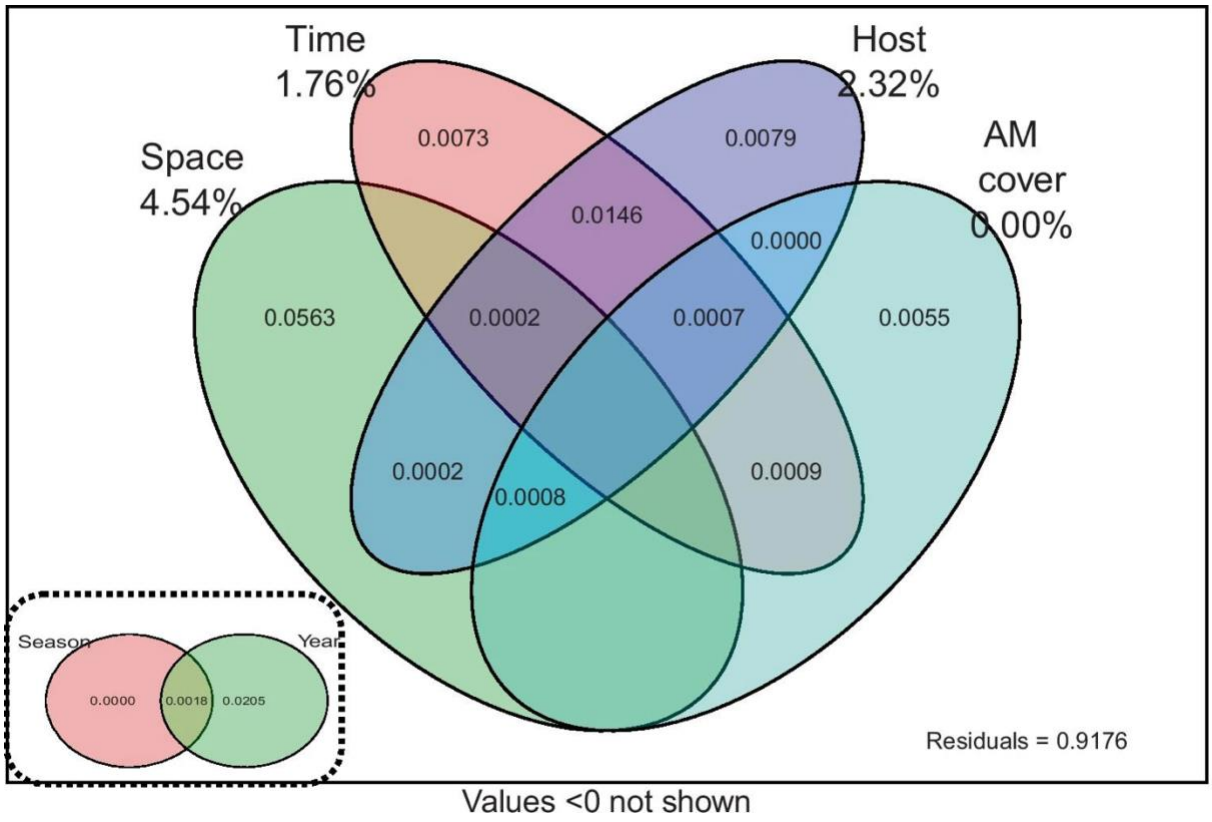


Figure 6 Partitioning of variance explained by spatial, temporal, host specific and AM plant cover related parameters across AMF communities in our forest site. Spatial parameters comprised three PCNM axes, and temporal parameters comprised the effects of season (i.e., explaining zero variance – insert at the bottom left) and year. The estimates are biased and are presented only for comparative purposes: for example, the impact of host effects on AMF community structure should have been considerably higher than shown, but because we harvested *Euonymus* only once the parameter explained a relatively small part of the total variance. The variance

partitioning additionally unrealistically assumes a completely balanced design with an equal representation of samples on all plots and invariable sampling effort across the four harvests. By including parameters that explained no variance such as season (insert at the bottom left), we further biased estimates. Finally, the analysis also does not capture that some plots have been assayed more than once and thus are not independent samples.

Discussion

A take home message of our study is that, in agreement with *Hypothesis One*, physical distance in the studied temperate forest exerts a stronger influence on AMF communities than either sampling time or host specificity. We also show that temporal variability is slightly higher across years than across seasons. Hence, our data agree with (Davison *et al.*, 2012) that there is low seasonality in forests in relation to AMF communities. The order of establishment of plant hosts, known as priority effects, could thus play an important role in structuring AMF communities (Hausmann & Hawkes, 2009). In natural systems this most likely occurs at the beginning of the growing season. Even though this idea remains underexplored, it could potentially explain why the effect sizes for different years were larger than for different seasons.

Through our null model analysis, we deduced that the plant root AMF colonization patterns in our study had been non-random (even though AMF community differences with time, space and hosts were weak - Fig. 4a) and showed extensive aggregation of species, meaning that the OTUs co-occurred more often than expected by chance. That our null model analysis supported that AMF root community composition was not random, was not surprising (e.g., Hu *et al.*, 2019). The outcome of co-occurrence analyses, however, depends strongly on how heterogenous the compared communities are (but also on sampling intensity): relatively homogenous communities such as those in our study are more likely to show aggregation whereas heterogeneous pools of samples such as those analysed with a comparable approach in Hu *et al.* (2019) are more likely to show segregation. It was important in our study to first show that the community matrix at the employed spatial scale is non-random (and thus our study had enough resolution to address community variance patterns in AMF communities), before addressing how spatio-temporal parameters and host specificity explained the community variance. Additionally, through our null model analyses we could observe some overarching patterns such as that *low* plots hosted more aggregated AMF communities than *high* plots. Species aggregation patterns often suggest shared habitat requirements across species compared to mechanisms such as competition and dispersal limitation which induce segregation (e.g., Cordero & Jackson, 2019). Thus, we might expect aggregating AMF taxa colonizing *Hedera* roots in *low* plots to have higher dispersal, but fewer competitive characteristics compared to communities on *high* plots.

In our RDAs, we observed pronounced plant host effects on AMF richness (Fig. 3), AMF community aggregation (Fig. 3) and community composition (Fig. 4c). The present study obviously did not fully address the role of host specificity: we only assayed two host plants and because of the low

abundance of *Euonymus*, we only assayed individuals at the last harvest. This mainly served the purpose of showing the degree to which our observations with *Hedera* corresponded to those with *Euonymus*. It nevertheless is likely that we could still get a reasonable (and hopefully representative) picture of how host specificity influences AMF communities. We present evidence, for example, that host specificity has a strong influence on AMF richness (i.e., plant host was the only parameter in our analyses that had an effect on AMF richness). We found of special interest, however, that pairwise differences between species (*Hedera*– vs. *Euonymus*–associating AMF communities; Fig 3.2b) were smaller than respective pairwise differences of conspecific individuals (randomly paired in RDA models). There is evidence that phylogenetically divergent co-occurring plant species share more similar AMF communities than closely related species (Veresoglou & Rillig, 2014) and our analysis hints towards that. Remarkably, most studies that have been carried out at a regional or global scale have found no evidence for host specificity (e.g., Davison *et al.*, 2015). This could mean that abiotic conditions mask host specificity at scales larger than that of the present study. Alternatively, inconspicuous factors at a smaller scale (i.e., such as that in the present study) driven by the environment such as priority effects or the availability of AMF propagules could modify how plant species select for AMF communities.

Contrary to our expectations that *low* and *high* plots would host distinct AMF communities (*Hypothesis Two*), we only observed small associated differences in diversity, and the factor AM plant cover in the RDA was only conditionally significant (Notes S5). This was despite that AMF communities across *low* plots appeared more divergent than across *high* plots (Fig. 4b) and that we observed differences in relation to the aggregation patterns (Fig. 3). (Grünfeld *et al.*, 2019) we had observed pronounced differences in root colonization between *high* and *low* plots across forests in the same general area, but we had worked at a relatively larger spatial scale. AMF can grow vegetatively to distances of about 50 cm (Klironomos & Moutoglis, 1999) but they could also potentially disperse by other means such as air and animal vectors (Egan *et al.*, 2014). We may have thus missed the relevant spatial scale, or differences in relation to the mycorrhizal state of the canopy affect percentage colonization to a greater degree than they affect AMF community composition.

We compare and rank relative effect sizes of drivers of AMF community composition operating at a small spatial scale (as compared to soil properties and climatic variables that operate at larger scales) that have rarely been addressed simultaneously. Several authors such as (Dumbrell *et al.*, 2010b) have highlighted the need to better understand stochastic processes in AMF and our study presents a ranking exercise which contributes towards satisfying that need.

Acknowledgements

The authors acknowledge funding from the Deutsche Forschungsgemeinschaft Project Metacorrhiza (VE 736/2-1) awarded to SDV. We thank Matthias Rillig for comments on an early version of the manuscript. We also thank Pierre-Luc Chagnon and David Janos for constructive feedback.

Chapter 4a

Arbuscular mycorrhizal root colonization depends on the spatial distribution of the host plants

Grünfeld, L., Rillig, M. C., Skias, G., & Veresoglou, S. D. (2022). Arbuscular mycorrhizal root colonization depends on the spatial structure of the host plants. *Mycorrhiza*, 1-9 https://doi.org/10.1007/s00572-022-01087-0

Chapter 4b

Micro-Landscape dependent changes in arbuscular mycorrhizal fungal community structure

Veresoglou, S. D., Grünfeld, L., & Mola, M. (2021). Micro-Landscape Dependent Changes in Arbuscular Mycorrhizal Fungal Community Structure. *Applied Sciences*, 11(11), 5297. https://doi.org/10.3390/app11115297

Abstract

The roots of most plants host diverse assemblages of arbuscular mycorrhizal fungi (AMF), which benefit the plant hosts in diverse ways. Even though we understand that such AMF assemblages are non-random, we do not fully appreciate whether and how environmental settings can make them more or less predictable in time and space. Here we present results from three controlled experiments, where we manipulated two environmental parameters, habitat connectance and habitat quality, to address the degree to which plant roots in archipelagos of high connectance and invariable habitats are colonized with (i) less diverse and (ii) easier to predict AMF assemblages. We observed no differences in diversity across our manipulations. We show, however, that mixing habitats and varying connectance render AMF assemblages less predictable, which we could only detect within and not between our experimental units. We also demonstrate that none of our manipulations favoured any specific AMF taxa. We present here evidence that the community structure of AMF is less responsive to spatio-temporal manipulations than root colonization rates which is a facet of the symbiosis which we currently poorly understand.

Introduction

Arbuscular mycorrhizal (AM) associations form direct nutritional symbioses between the roots of most terrestrial plants and a monophyletic group of soil-borne fungi belonging to the phylum Glomeromycota (Brundrett & Tedersoo, 2018). AM associations have attracted a lot of attention because they can promote net primary productivity (NPP) and agricultural production (Hoeksema *et al.*, 2010; Zhang *et al.*, 2019). NPP gains can partially determine how AM fungal communities in plant roots are structured (Van Der Heijden *et al.*, 1998; Maherali & Klironomos, 2007; Kiers *et al.*, 2011). As a result, a lot of the literature addresses practices that likely select for more beneficial communities of Glomeromycota in plant roots (e.g., Rillig et al., 2016; Pánková et al., 2018) and environmental parameters and practices that determine AM fungal community structure (e.g., Egerton-Warburton et

al., 2007; Veresoglou et al., 2013; Rillig et al., 2016). An alternative way to ask this question is via questioning how AM fungal diversity varies in space and time (i.e., which entails addressing the fraction of variance which is often classified in models as "unexplained"; Dumbrell et al., 2011; Davison et al., 2012).

Our general understanding so far is that AM fungal assemblages in the roots are non- random. This has been shown both in relation to null-model analyses (Davison *et al.*, 2011; Horn *et al.*, 2017), which assess the degree to which chance exclusively could have generated the observed community table (i.e., the occurrences of AM fungal species across root samples) of the study, and models exploring species-abundance distributions (Dumbrell *et al.*, 2010b; Unterseher *et al.*, 2011), which essentially test whether particular groups of species have been more abundant than expected by chance. Many studies observing preferential establishment of AMF taxa in specific habitats also hint towards this direction (e.g., Egerton-Warburton et al., 2007; Chen et al., 2017). Specific biotic and abiotic parameters of the habitat (besides exerting selectivity to specific AM fungal taxa), however, might also alter our ability to predict (i.e., modify the predictability of) mycorrhizal community structure in nature, but this point remains underexplored. Two syntheses which addressed this question found that anthropogenic disturbances, environmental heterogeneity and a plant host identity (i.e., being a monocotyledon) render AM fungal communities less predictable (i.e., more divergent) than they would have been expected to be by chance alone (Caruso *et al.*, 2012; Powell & Bennett, 2016). More recently, Deveautour et al. (2021) assayed AM fungal communities in the field to determine the degree to which AM fungal communities diverge with spatial distance but also when sampling from the root systems of the same or from a different plant-host individual. Deveautour et al. (2021) observed small differences in AMF community turnover between adjacent neighbouring plants (as compared to sampling from the same individual) but also that AMF community turnover increased for plant individuals further away from each other.

A particular feature of AM fungi is that they are obligate symbionts, meaning that they cannot fulfil their life cycle in the absence of a suitable host. This limits their ability to colonize soil in some environments because their vegetative growth ceases at distances of about 50 cm from the closest colonized root (Klironomos & Moutoglis, 1999). There is a large body of literature address- ing how dispersal constraints modify the community structure of organisms addressing variable types of landscape which can also occur at a micro level such as in soil in which case we can refer to them as micro-landscapes or meta-communities. There is a consensus that meta-communities simultaneously reduce local (α-) diversity and increase global (γ-) diversity because they make local community structure less predictable (e.g., Hubbell, 2001) which potentially allows persistence of less competitive species (Cadotte, 2007). This point remains underexplored in relation to AM-associations (Veresoglou *et al.*, 2012). Here we present a synthesis from three controlled studies with an overall aim to address

how spatial structure in plant mesocosms alters predictability in AM fungal communities. Based on the points we made (e.g., Dumbrell et al., 2010; Davison et al., 2011), we expected that in all experiments AM fungal communities were non-random (*Hypothesis One*) and that we would observe the highest γ-diversity in those cases in which the connectance of the patches in the archipelago is lowest (*Hypothesis Two*). Finally, we expected that lowering the connectance of plant and fungal mycorrhizal communities would increase segregation (i.e., the community table becomes more evenly dispersed via weakening pairwise interactions in agreement with the results from Hein et al. (2013) showing that strong pairwise interactions promote species aggregation) in Glomeromycota (*Hypothesis Three*). To the best of our understanding, the point that segregation in AM fungal communities could depend on the structure of the micro-landscape has never been addressed in the past for any fungal group and showcases the high potential (because they have an obligate symbiotic lifestyle and are ubiquitous in nature) of using Glomeromycota as model systems in fungal ecology.

Materials and Methods

Rationale of the Experiments

We worked with large mesocosms (i.e., 90 × 90 × 20 cm) as experimental units to which, for consistency with the meta-community literature, we refer to as archipelagos (Fig. 10). Within the mesocosms we established patches (i.e., patches in the form of 8 cm diameter × 20 cm height cylindrical inserts containing 30 µm mesh-covered windows to block root growth but allow growth of fungal hyphae) of vegetated habitat and manipulated the connectance of the patches either by means of distances across patches of the "meta- community" (Experiment One and Experiment Two) or the fertility of the patches within each mesocosm (Experiment Three). At the same time via manipulating the distances of the patches we altered the spatial availability of nutrients in the mesocosms and likely also that (i.e., the spatial distribution) of AMF propagules which were contained in those inserts (and were thus influenced by their spatial arrangement). We anticipated that the lack of prospective hosts between inserts (i.e., patches), over distances of up to 70 cm, hindered dispersal of AMF and would induce meta-community dynamics in our experimental units. The idea of using meta-community theory to model symbiotic systems has been developed and explained in larger detail by Mihaljevic (2012) (but see Veresoglou et al. (2012), for some AMF likely limitations of the approach in the particular case of AMF communities).

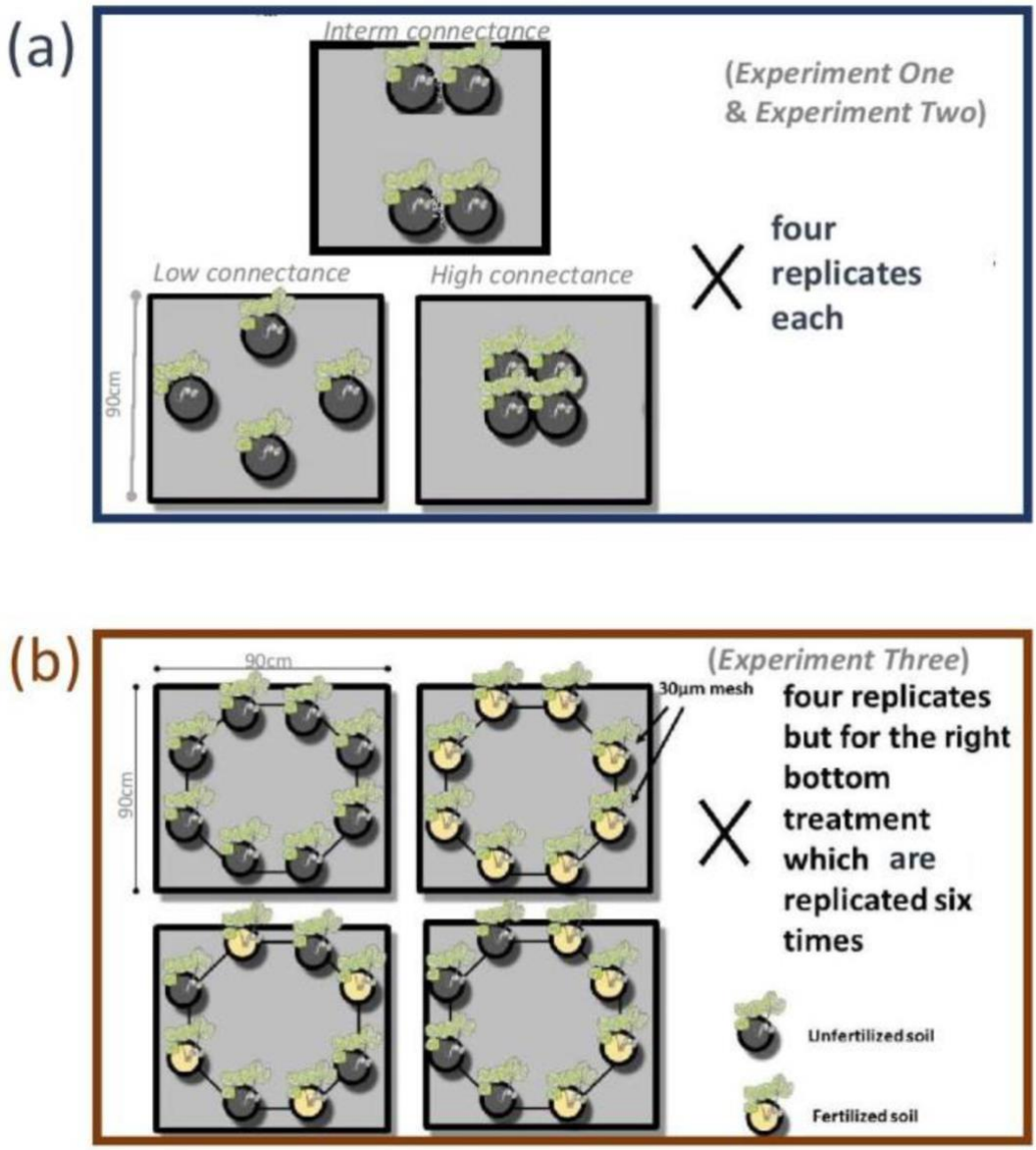


Figure 10 Schematic representation of the experimental design of the three experiments. (a) In Experiment One and Experiment Two we manipulated the connectance (low; intermediate and high) of four vegetated inserts (dark grey) over an unvegetated soil (sterilized and diluted with sand) matrix (in light grey). In Experiment One we used *Plantago lanceolata* as a host whereas in Experiment Two *Medicago lupulina*. (b) In Experiment Three we manipulated the diversity (i.e., only one habitat type; either fertilized or unfertilized or both habitat types) and spatial structure (overdispersed vs aggregated in the bottom two subpanels) of the vegetated inserts which we describe earlier (Top and bottom left archipelagos/treatments: 4 replicates/were each replicated four times; bottom right archipelagos/treatment: six replicates/ was replicated six times). We used *Medicago lupulina* as a host and the matrix soil was (like in the other experiments) sterilized, mixed with sand and was kept unvegetated (light grey).

Experimental Work

The experimental work on Experiment Two and Experiment Three has been described in detail in Grünfeld et al. (2019); the two experiments are described there as *Experiment One* and *Experiment Two*, respectively; Fig. 10). In brief, we carried out three controlled experiments with rectangular mesocosms sized 90 x 90 x 20 cm (width x length x height; Fig. 10). Experiment One and Experiment Two used identical experimental designs consisting of four inserts per mesocosm positioned at different distances (three different levels each replicated four times generating archipelagos of low, intermediate and high connectance) from each other but were carried out with different hosts (*Plantago lanceolata* and *Medicago lupulina*; Fig. 10a). In Experiment Three we experimented with two different habitats (unfertilized soil and soil fertilized with 1.8 g superphosphate per insert) and the spatial structure of mixtures of them (i.e., aggregated vs overdispersed spatial structure). In Experiment One some of the *P. lanceolata* roots penetrated the 30 µm mesh barriers and explored the unvegetated compartment. In Experiment Two and Experiment Three we observed differences in AMF colonization across the treatments which we presented in detail in Grünfeld et al. (2022).

The soil that was used for the three experiments was collected from a location in northwest Berlin (52.51◦ N, 13.14◦ E), had a pH of 6.7 and contained on average 1.75% organic C and 1.3 g kg−1 N. The freshly collected soil used for the experiments was stored at room temperature for less than two weeks before setting up the experiments. The soil used to fill the patches was unsterilized providing natural microbiota. The soil used to fill the main compartment of the experimental units was mixed 1:1 with sand and steam-sterilized (99 ◦C for 2 h) in order to destroy AMF propagules. To each of the inserts we added 200–250 seeds (B&T World Seeds, Aigues-Vives, France) of either *P. laneolata* (Experiment One) or *M. lupulina* (Experiments Two and Three) to approximate a plant density of one seedling per square cm (e.g., Scotton, 2019).

In the three experiments we used a fully randomized design. Because of the size of the mesocosms it was impossible to re-randomize the experimental units over the duration of the experiment. The temperature in the air-conditioned glasshouse was maintained close to 20 ◦C. In all three experiments, two weeks after germination of the seedlings, we set up an automatic irrigation system so that the plants were watered daily (over the first two weeks of the experiments watering was carried out manually). We further controlled growth conditions with five soil moisture sensors (ECH20 EC-5 soil moisture sensors and an Em50 data logger, METERs) positioned in three experimental units: in each experimental unit one of the sensors was in the unvegetated compartment and one in one of the inserts. Watering was adjusted so that soil moisture ranged between 60% and 75% of the water holding capacity. We inspected plant growth daily and removed any unwanted seedlings.

All three experiments were harvested 12 weeks after sowing, respectively, and cleaned root samples (50 mL core) were frozen at −20 ◦C before DNA extraction. Plant biomass was dried at 60 ◦C

for three days and weighted. Root material from each insert was used to assess root colonization (McGonigle *et al.*, 1990). Soil cores (five per experimental unit with more details in Grünfeld et al. (2022) were used to assay extraradical hyphae in soil. These results are described in Grünfeld et al. (2022).

Molecular Analyses and Bioinformatics

Roots from each individual insert per experiment were treated as one sample.Root samples were freeze-dried and homogenized with a Retsch Mixer Mill MM 400 and DNA was extracted from 30 mg ground root material per sample with the DNeasy® PowerPlant® Pro Kit (Qiagen). DNA was amplified with a proofreading polymerase (Kapa HiFi; Kapa Biosystems) and the primer pair NS31-AML2 targeting Glomeromycota (Lee et al., 2008). Thermocycling conditions were as follows: Initial denaturation at 95 ◦C for 2 min, 35 cycles with first 98 ◦C for 45 s, then 65 ◦C for 45 s and 72 ◦C for 45 s and final elongation at 72 ◦C for 10 min. The PCR master mix for indexing consisted of 1 µL of the purified polymerase chain reaction (PCR) template, 2.4 µL of the primer mix, 0.25 µL polymerase, 0.5 µL dNTPs (10 µM), 5 µL PCR buffer and 15.85 µL nuclease-free water per 25 µL reaction. Amplicons were purified with the NucleoSpin® gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) and indexed for MiSeq sequencing by means of an additional PCR with the same conditions as described earlier but with only 15 cycles. Amplicons were purified with magnetic beads (GC Biotech, Alphen van den Rijn, The Netherlands), and were pooled at equimolar quantities. Sequencing was carried out at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv, Berlin, Germany).

Raw sequences were processed with the UPARSE pipeline (Edgar, 2013) with USEARCH v 10.0.240 and default settings and were clustered into phylotypes (i.e., Operational Tax- onomic Units - OTUs) at a threshold of 97% sequence similarity. Representative OTU sequences were blasted against MaarjAM (Öpik *et al.*, 2010) and non-specific to Glomeromycota (i.e., > 97.5% similarity or > 99% coverage) OTUs were excluded from further analyses. We then rarefied these to 2200 reads which filtered out two samples from further analyses (i.e., analysis was carried out on the remaining 240 samples).

Statistical Analyses

To address Hypothesis One, stating that AM fungal local communities were non- random, we compared C score (i.e., checkerboards) occurrences in our presence-absence community tables with 1000 randomizations in which we maintained the total number of row sums fixed and the column sums proportional to those of the original community table.

This was carried out through the *sim4* algorithm (i.e., which is appropriate for assessing incomplete lists, Gotelli, 2000) which we implemented through the R package EcoSimR (Gotelli *et al.*, 2015). We presented the results in the form of *z*-score standardized effect sizes (SES) which can be

interpreted as (1) random community structure in the case of scores with absolute SES values below 1.96; (2) aggregation for negative SES values below −1.96; and (3) segregation for positive values above 1.96.

To address Hypothesis Two, stating that low connectance promoted a high γ-diversity in Glomeromycotan communities we used a fixed-effects linear models. We assayed how the experimental design (a categorical predictor with three levels: high connectance vs. intermediate vs. low connectance archipelagos; Fig. 10) modified γ-diversity (response variable) in the experimental units. To further gauge the impact of connectance on α- and γ- diversity we calculated those indices (i.e., local to the inserts and global for the entire mesocosm richness estimates, describing essentially the observed in the resulting community table number of OTUs at each of the two hierarchical levels) for individual inserts and modelled them after a repeated-measures analysis of variance (ANOVA) approach in which we used as response variables the diversity indices and the type of meta-community as predictors with additional error terms to model the nesting of inserts within experimental units. To further address the possibility that the treatments induced differences at a community level we implemented redundancy analyses (RDA) with the Hellinger transformed community tables as responses and the treatments as predictors. Additionally, we carried out an indicator species analysis to assess the degree to which phylotypes preferably established in some spatial designs.

To address Hypothesis Three, stating that low connectance of plant communities increases segregation, we used the Jaccard index (i.e., Jaccard similarity coefficient), defined for any pairwise combination of habitats as the ratio of common species over total number of species, as a metric of similarity across communities. We calculated Jaccard similarities for all pairwise combinations of inserts within individual experimental units. To avoid inflating the degrees of freedom we averaged the similarity coefficients describing the similarity of any given insert across habitats of any particular class (i.e., short distance/long distance/(un)fertilized soil patches). To model similarity coefficients we used a repeated- measures ANOVA approach with the Jaccard coefficients as response variable and a structure identical to the models we used to model α- and γ- diversity.

Results

Overall Statistics

Alpha diversity varied in the experiments between 6 and 44 phylotypes (i.e., 12–40 in Experiment One; 17 to 44 in Experiment Two and 6 to 41 in Experiment Three; Fig. 11). Gamma diversity varied between 30 and 53 phylotypes (i.e., 30–48 in Experiment One; 39 to 52 in Experiment Two; 35 to 53 in Experiment Three). In none of the three experiments could we explain alpha (F values varied between 0.38 and 2.1 with respective *p* values larger than 0.11) or gamma diversity (F values varied between 0.2 and 1.3 with respective *p* values larger than 0.3) based on the experimental treatments.

Community differences across the treatments were not significant in any of the three experiment specific RDAs (F values varied between 0.96 and 1.22 with adjective R2 values were in all cases below 0.005). Indicator species analysis yielded inconsistent results and a low occurrence frequency of indicators: there were no indicator OTU in Experiment One, there was a single indicator OTU in Experiment Two specific to low connectance archipelagos (with p = 0.03) and there were two OTUs specific to the unfertilized control and one to the fertilized control but not to any of the mixes of them in Experiment Three. Such low frequencies of indicators could have been explained, at least in the case of the two first experiments, by chance.

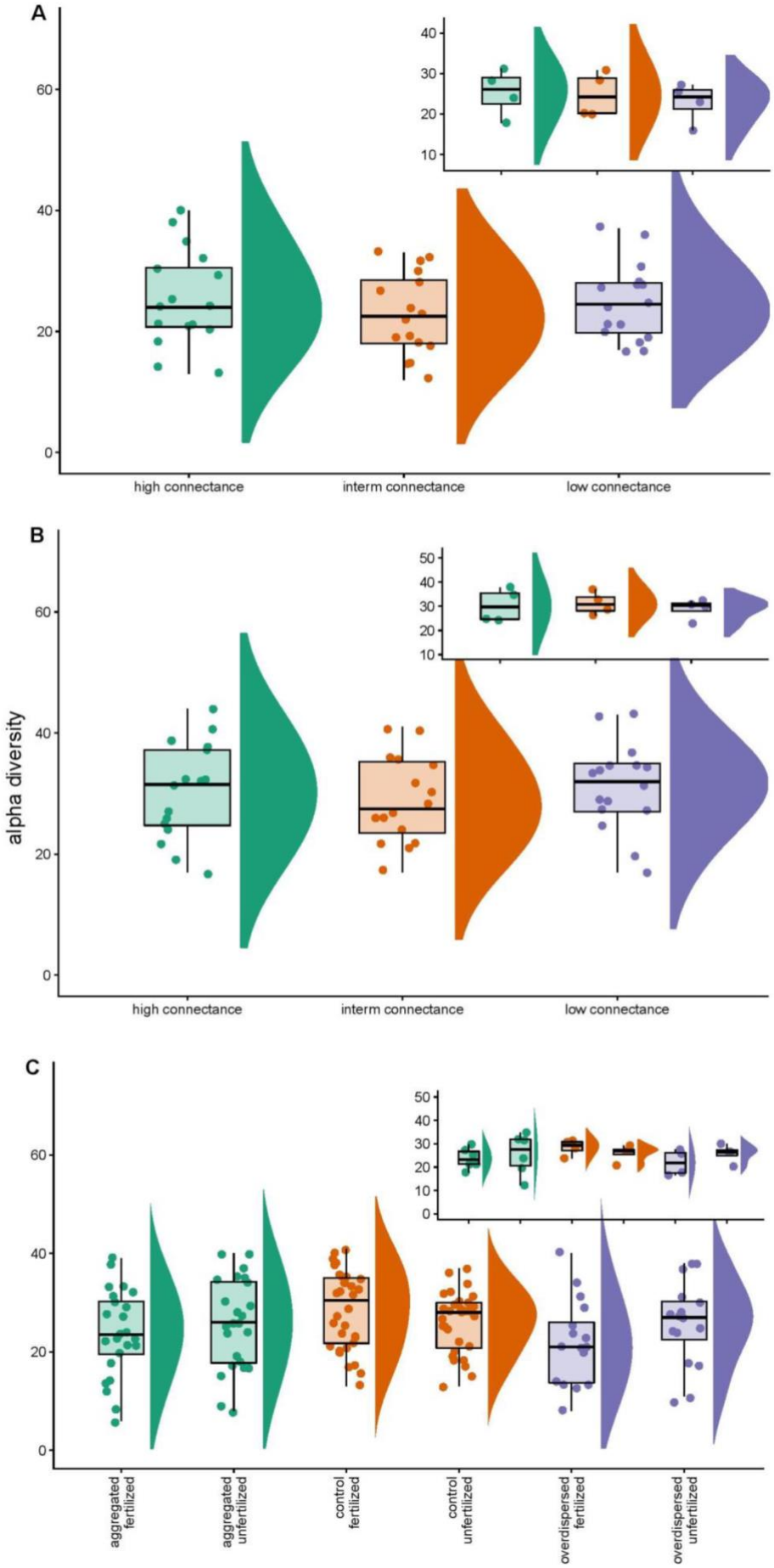

A
60
40
20
0
high connectance
interm connectance
low connectance
B
alpha diversity
60
40
20
0
high connectance
interm connectance
low connectance
C
60
40
20
0
aggregated fertilized
aggregated unfertilized
control fertilized
control unfertilized
overdispersed fertilized
overdispersed unfertilized

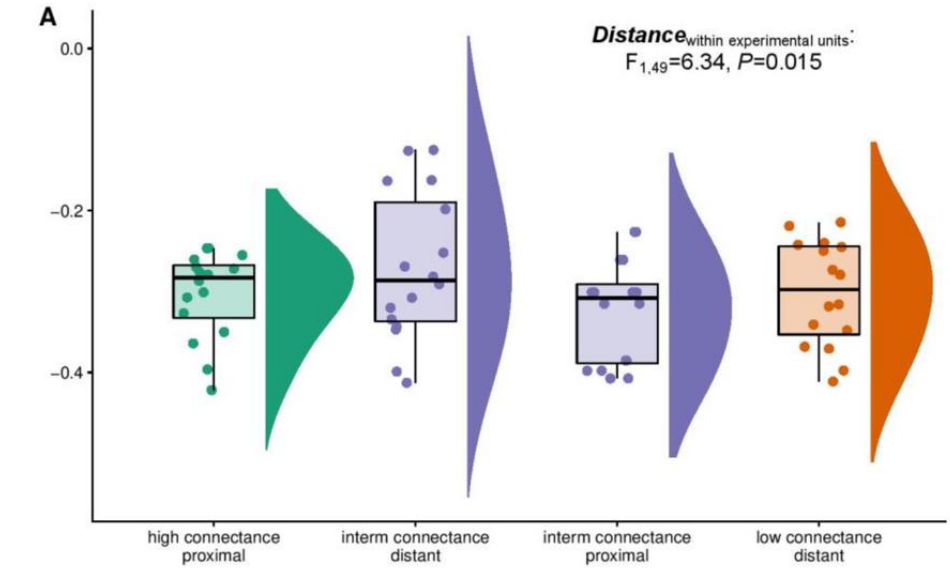
Figure 11 Alpha diversity statistics across treatments and habitats in (**A**) Experiment One; (**B**) Experiment Two; (**C**) Experiment Three. Each experimental unit contained several inserts and we assayed the mycorrhizal community independently for each insert. We observed no differences in alpha diversity in all three experiments. Main panels depict alpha diversity across individual samples whereas the panel inserts show the results after averaging the four (Experiment One and Experiment Two) or eight (Experiment Three) estimates of alpha diversity per experimental unit. Note the lack of differences in relation to alpha diversity. We observed comparable trends for gamma diversity.

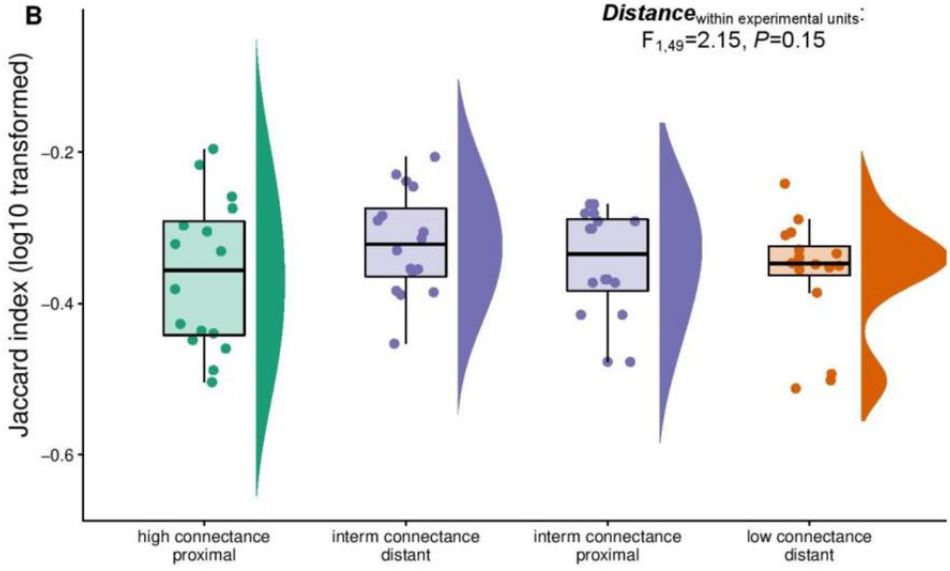
Null Model Analyses

All standardized effect size statistics differed from zero and ranged between −9.6 and −20.6 (Experiment One: −9.6; Experiment Two: −10.99 and Experiment Three: −20.66), suggesting community aggregation.

Comparative Analysis of Jaccard Similarities across Experiments

Jaccard similarities did not differ across treatments but within experimental units between short-distance and long-distance inserts in the intermediate connectance treatment of Experiment One ($F_{1, 49} = 6.3$, $p = 0.015$; Fig. 12a; Appendix IV, Test 5.1). There was a comparable trend with Jaccard similarities ($F_{1, 49} = 2.12$, $p = 0.15$) in Experiment Two (Fig. 12b; Appendix IV: Test 6.1). In Experiment Three, there were differences in Jaccard similarities only between observations within experimental units which differed in their habitat type (i.e., unfertilized vs. fertilized; unfertilized vs. unfertilized; fertilized vs. fertilized; $F_{2, 207} = 4.0$, $p = 0.02$; Fig. 12c; Appendix IV: Test 7.1). Jaccard similarities were on average larger in the overdispersed; Appendix IV: Test 7.2).


A
Distance within experimental units:
F1,49=6.34, P=0.015
0.0
−0.2
−0.4
high connectance proximal
interm connectance distant
interm connectance proximal
low connectance distant


B
Distance within experimental units:
F1,49=2.15, P=0.15
Jaccard index (log10 transformed)
−0.2
−0.4
−0.6
high connectance proximal
interm connectance distant
interm connectance proximal
low connectance distant

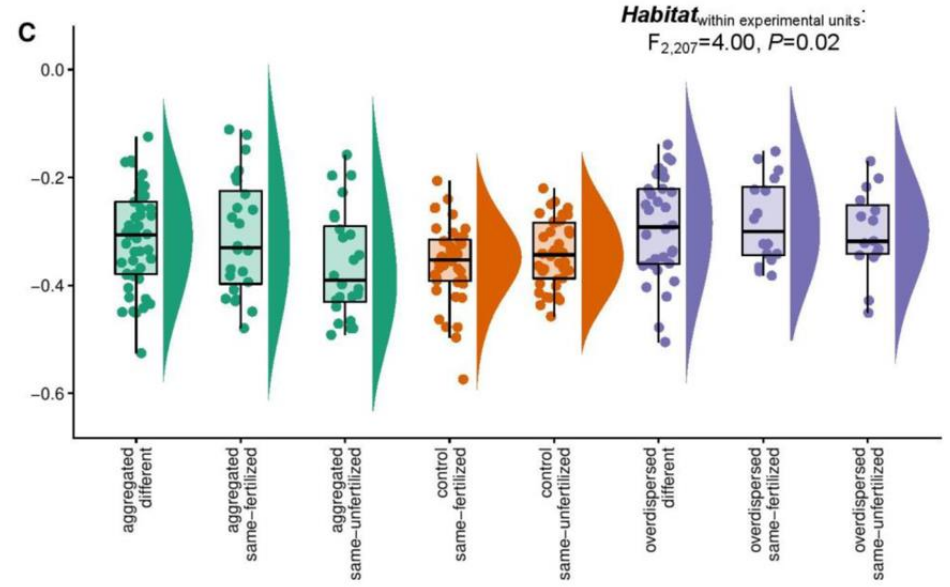

C
Habitat within experimental units:
F2,207=4.00, P=0.02
0.0
−0.2
−0.4
−0.6
aggregated different
aggregated same−fertilized
aggregated same−unfertilized
control same−fertilized
control same−unfertilized
overdispersed different
overdispersed same−fertilized
overdispersed same−unfertilized

Figure 12 Jaccard index statistics from pairwise comparisons of samples within experimental units in (**A**) Experiment One; (**B**) Experiment Two; (**C**) Experiment Three. Note in panels (**A**,**B**) that in intermediate connected spatial arrangements we observe higher Jaccard distances between long- distance compared to short-distance "patches" (in the case of Experiment Two a trend) within-subjects distance effect; $F_{1,49} = 6.34$, $p = 0.015$ in Experiment One; $F_{1,49} = 2.12$, $p = 0.15$ in Experiment Two) and in panel (**C**) that Jaccard distances differed ($F_{2, 207} = 4$, $p = 0.02$) within experimental units in the overdispersed and aggregated treatment.

We thus observed that within experimental units there were differences in AMF community turnover (assayed with the Jaccard index) which peaked for pairs of distantly placed patches (Experiment One and Experiment Two; as compared to closely placed patches) and pairs of patches containing different habitats (Experiment Three). However, we observed no comparable differences between treatments only containing distantly placed vs. only closely placed patches or high fertility vs. low fertility patches (and this is why the predictor treatment was not significant). A high community turnover, in the absence of diversity differences, is evidence of a lower predictability.

Discussion

We present evidence from three controlled experiments supporting that small scale micro-landscape heterogeneity (i.e., here describing either experimental units with a low patch-connectance or experimental units containing habitats of different quality) hardly alters diversity patterns in AMF communities. AMF community structure, however, remains non-random. At the same time, we observed that archipelagos combining habitats of both low- and high-connectance (which implies that AMF might have needed to combine traits of long-distance and short-distance dispersal), as well as overdispersed micro-landscapes displayed differences in community turnover (and thus predictability; Appendix IV, Test 7.2) across their patches, with pairs of highly connected patches and patches sharing comparable habitats being the most similar to each other. Some conceptual models predict that AMF communities become less random at small (local) spatial scales (i.e., manifested in the form of a low community turnover, Vályi et al., 2016). With this study we provide experimental evidence that even at such small spatial scales, micro-landscape variability continues to structure AMF communities and can alter their stochasticity (i.e., used here as an opposite to predict).

Our Hypothesis Two stated that we would observe the highest γ-diversity in the cases in which they were most fragmented, but we observed that the differences across treatments in our experiments were unrelated to AMF diversity. Evidence suggests that AMF richness (either in the form of alpha, here defined as OTUs observed per insert, or gamma diversity, here describing the number of OTUs per mesocosm) stays relatively constant across a range of environmental gradients in AMF systems (e.g., Lekberg et al., 2012; Kivlin & Hawkes, 2016; Maitra et al., 2019) albeit this is not the case with nutrient availability gradients as has been for example shown in Camenzind et al. (2014). It has

actually been proposed that plants impose a strong filter on the number of partners they simultaneously associate with (Hammer *et al.*, 2011; Kiers *et al.*, 2011; Veresoglou & Halley, 2012), which could determine AMF richness in plant roots. In our experimental set ups, manipulations of the spatial design altered AMF root colonization (Grünfeld *et al.*, 2022). The exact reasons why in mixed micro-landscapes we observed a higher root colonization and variable AMF community turnover (which was masked when comparing across less diverse micro-landscapes) are not clear. We suspect that the underlying mechanism relates to alternative growth strategies across AMF taxa. AMF have been proposed to contain two types of hyphae, absorptive and explorative, which differ in their functions (e.g., Staddon et al., 2003). Mixed micro-landscapes might necessitate both types of hyphae to be present at high densities which likely weakens interspecific pairwise co-occurrence interactions across AMF species (i.e., pairs of species found together more frequently than expected by chance and pairs of species co-occurring less frequently than expected by chance; e.g., Bar-Massada, 2015). High densities of both types of hyphae should theoretically result in a higher diversity of pairwise interactions, including many combinations of short-distance dispersers and long-distance dispersers. Also, mixed micro-landscapes could render the benefits that plants acquire from the different AMF species more variable with long-distance dispersers being favoured in some parts of the micro-landscape whereas short-distance ones in others and thus generate conditions with unclear investment optima. In doing so, mixed micro-landscapes favour a more diverse set of AMF (Kiers *et al.*, 2011). An alternative explanation is that within experimental units we could better control for idiosyncratic parameters that can sometimes determine AMF community structure in the early stages, such as the quality and quantity of the AMF propagules and soil moisture settings throughout the experiment. We think that through controlling those idiosyncratic parameters in our within experimental units comparisons, we might had a higher statistical power to detect differences in community turnover (and thus predictability) than across experimental units.

We found support for Hypothesis One that AM fungal communities were non-random which, however, was not surprising. A large body of the mycorrhizal literature supports the idea as we reviewed in the introduction (e.g., Davison et al., 2011; Horn et al., 2017). What makes our study novel is that across three controlled experiments we found consistent results on a parameter that determines how random AM fungal communities might be micro-landscape structure. We observed differences in community turnover in mixed micro-landscapes (that were masked in their homogenized counterparts) which was higher for distant patches and patches differing in their habitat quality. This observation aligns well with expectations based on meta-community theory (Hubbell, 2001). There have only been a few studies so far quantitatively (i.e., assessing effect sizes on the degree of predictability, rather than simply obtaining a qualitative result such as whether the community is segregated) assessing how predictable synthetic microbial systems can be. A recent meta-analysis on

the topic examining 21 datasets showed that organic additions make microbial communities less predictable (i.e., more stochastic; Ning et al., 2019) which was later further supported by an additional study (Silva & Pernthaler, 2020). In another study, Fodelianakis et al., (2021) showed that evolutionary drift in synthetic bacterial communities rendered them less predictable than in their original cultures. We used here an important for the functioning of ecosystems, system, arbuscular mycorrhizae, to show that also spatial structure can induce less predictable microbial communities and that this happens when we mix different micro-landscape features.

Arbuscular mycorrhizal fungi are most likely to experience dispersal constraints in urban and agricultural landscapes as well as woody habitats (Grünfeld et al., 2019). In the case of agricultural landscapes, the growth settings most likely select for short-distance dispersal traits (i.e., there are uniform distances across crop individuals, which ease the proliferation of AMF species from close by patches of AMF diversity). In contrast, in woody habitats the growth settings most likely select for a combination of long- and short-distance dispersal (i.e., distances between AMF-associating plants most likely vary in time and space). Based on the results of our study, plant hosts in woody habitats could, therefore, experience a higher stochasticity in relation to harbouring AMF community structure than other hosts. This might actually benefit AMF-associating plants in forests, in the longer term. Woody plants, in particular, experience a high mortality at early life-stages. If plant fitness to a certain degree depends on the benefits they acquire from associating with AMF (as we suggest in Veresoglou et al. (2017) and Grünfeld et al. (2019), stochasticity in AMF community structure could render plant fitness more variable in both time and space and ensure that the surviving individuals are those that associate with strongly mutualistic AMF (e.g., Johnson et al., 2014). Further studying parameters that determine stochasticity in mycorrhizal fungal communities, could be key to explaining why and how plant-soil feedback varies in time and space (e.g., Kadowaki et al., 2018; Liang et al., 2020).

Conclusions

In conclusion we present evidence that mixing micro-habitat features, such as distances across hosts and fertility levels, makes AMF communities more stochastic (i.e., less predictable). This observation presents a range of opportunities to increase AMF diversity (via facilitating establishment of less competitive species) and hopefully productivity in silviculture and agriculture. Glomeromycota, clearly, present a special case of fungi because of their obligate symbiotic lifestyle, meaning that it is possible to control their spatial structure through manipulating the location of their plant hosts. A follow up question revolves around assessing the degree to which there are comparable patterns in other systems of fungi and the overall consequences for ecosystem functioning.

Acknowledgments

We want to thank Matthias Rillig and Maraike Probst for constructive comments. The publication of this article was funded by Freie Universität Berlin.

Chapter 5: General discussion

The primary goal of this dissertation is to improve the understanding of AM fungal community dynamics in relation to the factor host plant spatial structure. In two field studies and three asynchronous greenhouse experiments we therefore assessed different aspects of AM fungal diversity, such as root colonization as a measure of abundance, as well as species-level richness and community composition (Fig. 1).

In Chapter 2 we showed that the proximity of AM-associating woody plants increased AM fungal root colonization in eight herbaceous understorey plants across 13 temperate deciduous forest sites. We concluded that close-by AM plant species acted as sources of AM fungal inoculum for each other. Connectance through AM fungal hyphal networks could be an important mechanistic aspect of facilitation between plant species (Veresoglou *et al.*, 2017). In Chapter 3 we compared and ranked the effects of a set of spatio-temporal parameters on AM fungal diversity and community composition. Spatial parameters followed by host plant identity were relatively more important than temporal parameters. Overall, only a small fraction of the observed variation in community composition could be explained by the investigated factors suggesting high stochasticity in the formation of the regarded communities. In Chapter 4a we found that continuous and heterogenous artificial micro-landscapes consisting of *Planatgo lanceolata* or *Medicago lupulina* host patches induced higher rates of root colonization with higher proportions of functional structures than respective discontinuous or homogeneous micro-landscapes. In Chapter 4b we observed that AM fungal diversity was generally not affected by micro-landscape structure. However, we found higher community turnover rates within micro-landscapes containing different distances (Experiments 1 and 2) and different types (Experiment 3) of habitats rendering those communities less predictable.

AM fungal communities in temperate forests

It has been suggested that dispersal and consequent propagule limitation in AM fungi occur at the local scale in certain ecosystems, but data supporting this hypothesis are lacking (Davison *et al.*, 2011; Zobel & Öpik, 2014). In Chapter 2 we presented evidence that indeed AM fungal propagule availability in temperate forests varies and might be reduced at forest sites with relatively lower AM-associating woody plant cover. There is a slowly increasing number of studies exploring the effects of plant mycorrhizal type and plant cover on AM fungal communities in temperate forests. Our findings for example are in line with recent research conducted in experimental forests in Poland. Here, the authors found higher abundances of AM fungi at forest plots with increasing number of AM-associating tree species, respectively (Rożek *et al.*, 2020; Zubek *et al.*, 2021). A limitation to date is that none of the studies have examined the effects on host plant fitness at sites with lower AM host plant densities, which would be an interesting follow-up question. However, it can be concluded that the abundance

and mycorrhizal type of tree species is likely to be of great importance for the distribution of AM fungi in comparable ecto-mycorrhiza dominated temperate forests, which is relevant for the protection and restoration of these ecosystems.

Assessing AM fungal community dynamics over two years in response to spatial and temporal parameters, as well as host plant specificity in Chapter 3 revealed more complex results. We observed that spatial parameters and host identity (but not AM-associating plant cover) were more important for structuring AM fungal communities than temporal parameters (year and season), and overall, we could only explain little variation in fungal community structure. Thus, we encountered similar difficulties as many other field studies that could explain only a small fraction of the variation in natural AM fungal communities by deterministic processes (e.g., Dumbrell et al., 2010; Kohout et al., 2015; Rasmussen et al., 2018; Maciel Rabelo Pereira et al., 2020). In our case, we worked at temperate forest site (50 m x 50 m) with likely quite homogenous abiotic conditions, thus increasing the chance of observing potential effects of the spatial structure of AM host plants. However, in future studies in the same area, it may be beneficial to expand the study range (by comparing multiple sites) and to include environmental variables that typically influence AM fungal distribution, such as soil pH and nutrient concentrations.

In general, similar to the results of the experimental work, AM-associating woody plant cover did not affect AM fungal communities but did affect AM root colonization. Based on these results, it is therefore possible that AM fungi are not generally dispersal-limited in temperate forests as also recently suggested by Boeraeve et al. (2019). However, lower densities of AM-associating plants can probably cause propagule limitation and thus reducing the level of mycorrhization.

Differential responses of AM fungal root colonization and community composition to host plant spatial structure

We observed parallels in natural and experimental AM associations, namely an increased AM fungal root colonization at relatively higher host plant densities. These findings were derived from observations in eight herbaceous host plant species across 13 temperate forest sites (Chapter 2) and from greenhouse experiments where distance or heterogeneity of AM habitat patches were manipulated (Chapter 4a). Zubek et al. (2021) showed similar results in a recent study from a polish nature reserve in which AM root colonization was higher in herbaceous understory plants in riparian compared to beech forests, which was attributed to a lower number of AM host plants in the latter.

Interestingly, in our case, in contrast to colonization patterns, the diversity or composition of AM fungal taxa did not show comparable strong responses to host plant structure in the same experiments (Chapter 2), or sampling area (within one of the previously studied forest sites – Chapter 4b). This was unexpected as AM fungal richness often co-varies with root colonization rates (e.g., see

Verbruggen et al., 2012 for agricultural soils). Therefore, the question arises why host spatial structure induced changes in root colonization while fungal communities remained rather unaffected. In contrast to our findings, Rożek et al. (2020) observed that not only root colonization, but also species richness increased at forest sites with a higher AM plant cover. Similarly, Rożek et al. (2020) reported distinct AM fungal community compositions in relation to overstory tree type (deciduous vs. coniferous) within an experimental forest. In another, earlier study in a seminatural forest in Great Britain, shifts in AM fungal community composition in roots of an herbaceous plant were found between plots with AM-associating vs. ectomycorrhizal-associating dominant overstory tree species, while soil properties were not considered (Helgason *et al.*, 1999). In the referred studies, however, the soils between compared sites were highly divergent, thus effects of pH and macronutrients could not be delineated from those of the host plants.

Therefore, with respect to the current state of knowledge, the single factor host plant spatial structure might not be of great importance for the diversity or composition of AM fungi in temperate forests. Effects that do appear in some studies are probably strongly linked to site-specific (mostly soil) conditions, which in turn are linked to specific plant communities. These effects become more visible the more heterogeneous or spatially separated sampling sites are from each other. This may explain why we did not observe any effects of AM host plant cover in our spatio-temporal study, where we worked at a relatively small spatial scale with likely low soil heterogeneity. It would be highly interesting whether this also applies to other study systems, in particular to those where AM fungal dispersal may be more restricted. This could be the case in urban environments, or for example on sandy coasts with fragmented habitat islands (Koske *et al.*, 2004).

Experimental limitations

To assess the generality of the effects of habitat structure, the results of the experimental work could be valuable. A post-hoc expectation derived from the results of the above discussed observational data would be that AM fungal diversity or community composition would be altered in micro-landscapes with varying habitat quality, but not with varying habitat connectance. However, in contrast to these, as well as to the initial expectations, diversity and community composition was overall unaffected by the implemented treatments (except for some within-unit differences, see following paragraph).

One possible explanation for the different responses of root colonization and the diversity-measures is that our experimental design did not reflect well regional and local pools of AM fungal taxa. We expected that differences in dispersal and competition within our micro-landscapes would have altered AM fungal assemblages across habitat patches over time. Thus, the distances between habitat patches, the experimental duration, or both, might not have been sufficient to reveal differences in dispersal, or competitive ability across fungal species. Also, it is possible that species

filtering through the plant host or soil conditions acted within homogenized habitats in the beginning of the experiment producing rather similar and stable subsets of AM fungal communities across micro-landscapes.

Consequently, the observed changes in root colonization could also reflect rather intraspecific than interspecific dynamics within the experimental fungal communities. The proximity between habitat patches could have facilitated migration of individuals and thereby promoted an accelerated build-up of extraradical mycelium. Stronger hyphal networks connecting the neighbouring plant patches could have led to more efficient information and nutrient fluxes resulting in higher root colonization (Hart & Reader, 2005; Barto *et al.*, 2012). It is likely that at larger distances AM fungi require to invest more in growth of exploratory hyphae resulting in decreased colonization.

The fact that the observed communities were not random (Chapter 4b), but not related with the spatial treatments finally could mean that we missed any other relevant factor driving assemblage processes. With our data, we can however conclude, that it was not species richness that induced variations in root colonization. A relationship between species richness and colonization rate is often implicitly assumed but has, to our knowledge, not been explicitly studied for AM fungi. This result highlights the importance of capturing both, functionally relevant and diversity-related measures, in order to comprehensively assess AM fungal communities.

Conclusions for the applicability of meta-community theory

Meta-community theory represents a promising approach for a suitable description of community dynamics in host-dependent microbes (Mihaljevic, 2012). Therefore, we designed the greenhouse experiments on host plant structure in a way that they could detect such dynamics in AM fungi (Veresoglou et al., 2012). We used small patches of host plant populations and considered them as islands of “local” communities of AM fungi connected by an initially sterile matrix soil. Thereby we anticipated variations in AM fungal species richness and composition in response to levels of connectance and heterogeneity across micro-landscapes reflecting the “regional” community. Contrary to our expectations we could not detect variations in fungal diversity estimates across our spatial treatments. We however observed variations in β-diversity (species turnover) between patches within the experimental units with mixed connectance (Experiments 1 and 2) and with different habitat types (Experiment 3). In Chapter 4b we assumed that higher turnover rates in treatments with a heterogeneous landscape would be indicative of source-and-sink dynamics (Experiment 3). The explanation is, that if even at high connectance of neighbouring habitat patches species turnover is detectable, this will most likely be due to environmental filtering through a varying environmental factor (here P-fertilization). On the other hand, under source-sink dynamics γ-diversity should also be higher in the treatments with both habitat types, as co-existence of AM fungal taxa across the micro-

landscape will be promoted when different habitat types are present (niche differentiation), which we could not observe. Furthermore, habitat distance alone did not affect AM fungal communities, but the combination of short- and long-distance patches within micro-landscapes induced higher β-diversity. In Chapter 4b we explained this by the possible co-existence of AM fungal taxa with different dispersal abilities, however, the respective micro-landscapes did not promote an overall higher γ-diversity. Consequently, these micro-landscapes hosted less predictable communities but did not show a clear signal of meta-community dynamics as would be required to support the theory.

In conclusion, our data neither support the applicability of meta-community theory nor exclude it entirely. Studying mycorrhizal fungi in a meta-community context is challenging because experiments at this scale are difficult to implement and, in the field, the precise boundaries of local communities are undefined. Compared to AM fungi, ecto-mycorrhizal fungi may offer an advantage through their association with trees, as these large host plants can be considered as clearly delineated islands (Peay *et al.*, 2007, 2010). For AM systems, field experiments with some degree of control of the spatial host distribution and longer durations could be very informative.

Future methodological challenges

Comparisons to other AM fungal community studies are often difficult as methods, sampling scale and design differ. For example, for the assessment of overall abundance a range of techniques exists. Spore density or root colonization are often measured, but so are phospholipid and neutral lipid fatty acid (PLFA and NLFP) biomarkers (Olsson *et al.*, 1997), or even sequencing-derived surrogates such as sequencing depth (Veresoglou *et al.*, 2014).

Similarly, despite the general advances in molecular techniques, traditional methods for the assessment of AM fungal diversity such as spore identification are still used (e.g., Rożek et al., 2020; Zubek et al., 2021). This complicates the comparison of absolute species number estimates with estimates derived from molecular data, such as the widely applied terminal restriction fragment length polymorphism (t-RFLP) analysis (Dickie & Fitzjohn, 2007). The most common approach for species-level taxa estimation based on next-generation sequencing is determining operational taxonomic units (OTUs) via the online database MaarjAM (Öpik *et al.*, 2010). However, recently, the usage of amplicon sequence variants ASVs has been proposed to be beneficial for fungal sequences (Joos *et al.*, 2020). Comparing ASV-based denoising and OTU-based clustering methods revealed significantly different results in microbial diversity (Joos et al., 2020; Chiarello et al., 2022). Results and interpretations also strongly depend on the bioinformatic pipelines (Pauvert *et al.*, 2019; Joos *et al.*, 2020). To what degree outcomes are comparable is insufficiently studied and often the main difficulty is the delineation between rare taxa and measured noise. In terms of subsequent community analyses, Veresoglou et al.

(2014) suggested the implementation of diversity profiles (Leinster & Cobbold, 2012) into microbial studies, as the integration of different diversity metrics can better account for rare taxa.

In summary, the wide range of profiling techniques, often time-consuming and costly, combined with the extensive knowledge gaps in microbiology (definition of "individual" and "species") and limited taxonomical resolution (Bruns & Taylor, 2016) present challenges for future research. Further efforts to identify the most promising techniques and standardization among studies to the greatest extent possible are urgently needed.

General conclusion

The studies presented in this thesis provide empirical evidence for host plant structure-related changes in AM fungal abundance assessed by root colonization. We observed similar patterns in both natural and experimental communities, supporting the growing recognition that AM fungi are affected by habitat fragmentation and propagule limitation in certain environments with likely consequences for the symbiotic efficiency. Advances in molecular techniques such as next generation sequencing have allowed deeper insights into host-fungal community dynamics, although data are still lacking to draw more general conclusions. This would be of great importance to disentangle the relative influence of different abiotic and biotic drivers across various environments at distinct spatial scales. The high degree of stochasticity commonly found in natural AM fungal communities demonstrates knowledge gaps and challenges for future research. A major task will be to better understand how biotic drivers, in particular interactions with other soil microorganisms, contribute to the distribution of AM fungi.

Own contributions

Chapter 2: Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands

I carried out the sample collection together with MW and SDV, I stained the root samples together with AM, I estimated root colonization and I wrote the article with contributions from SDV.

Chapter 3: Disentangling the relative importance of spatio-temporal parameters and host selectivity in shaping AMF communities in temperate forests

I carried out the sample collections (partly together with MW and SDV), I extracted the DNA and amplified the samples (with help of MM at the final pooling) and I wrote the article with contributions from SDV.

Chapter 4a: Arbuscular mycorrhizal fungal abundance influenced by different host plant and inoculum spatial structure

I carried out the experiments and the harvests; I estimated hyphal density; I did the statistical analyses and wrote the paper with contributions from SDV and MCR.

Chapter 4b: Micro-landscape dependent changes in arbuscular mycorrhizal fungal community structure

I carried out the experiments and the harvests; I extracted the DNA and amplified the samples (with help of MM at the final pooling) and I contributed to write the paper (shared first authorship with SDV).

Appendix I: Chapter 2

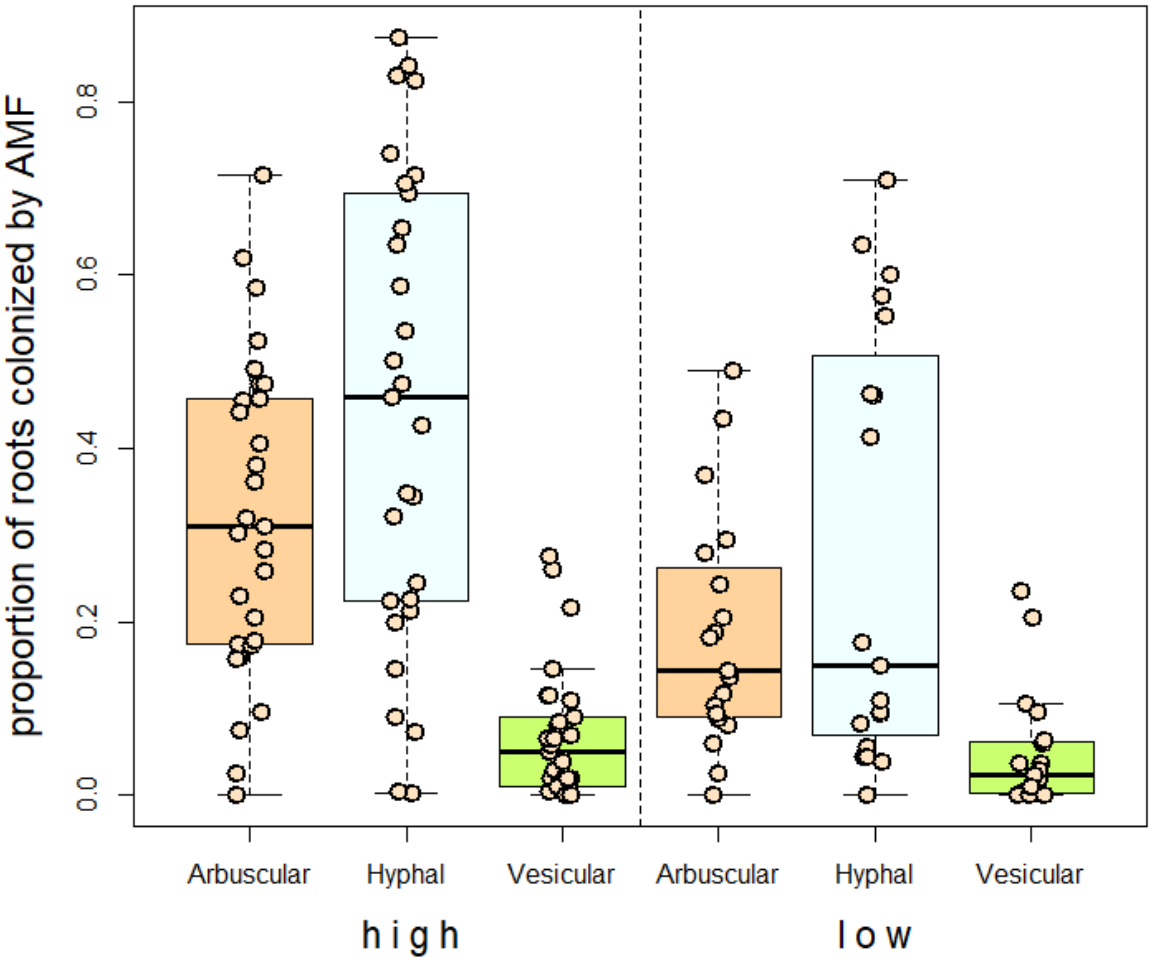


Figure S1 Variance in arbuscular, hyphal and vesicular colonization across plant species in high (left) and low (right) stands ("high" stands had a high percent cover of woody plants associating with AMF whereas "low" stands a low percent cover of woody plants associating with AMF). Arbuscular colonization was almost as variable as hyphal colonization, with better distribution properties (i.e., Gaussian like) but also showed distinctiveness between high and low stands. Among other reasons (see Notes S1: Estimation of root colonization) this is why arbuscular colonization represented the suitable response value for this study.

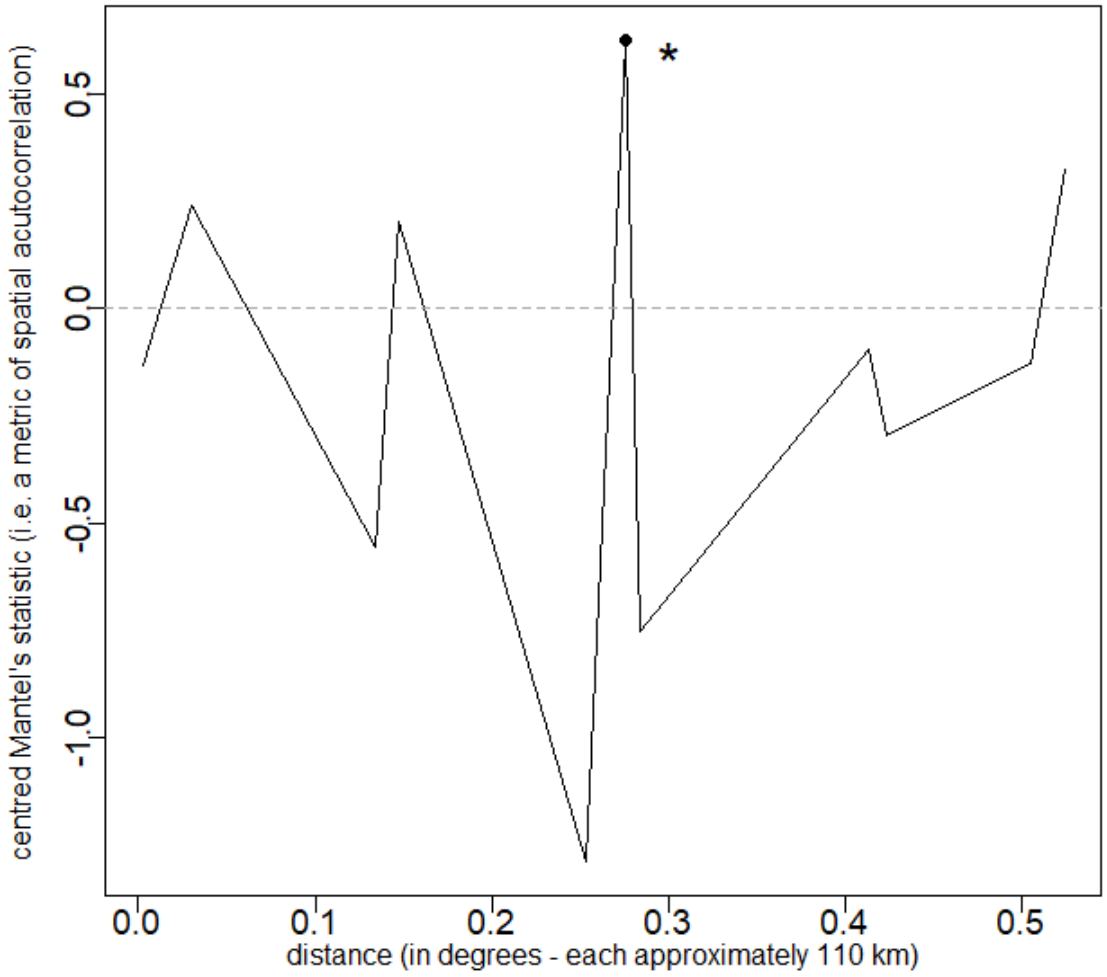


Figure S2 Correlogram of percent arbuscular colonization (y-axis) with distance (x-axis). We carried out the correlogram with the function correlog in the R package ncf (Bjornstad, 2018). The specific correlogram uses as a statistic a centred Mantel test which is carried out independently at each different distance. Whenever significant (i.e., here only at approximately 0.28 degrees) we highlight the result with a star. Because only one of the ten tests were significant and that the sign of the Mantel statistic varied with distance there was insufficient evidence that our percent arbuscular colonization showed any spatial constraints.

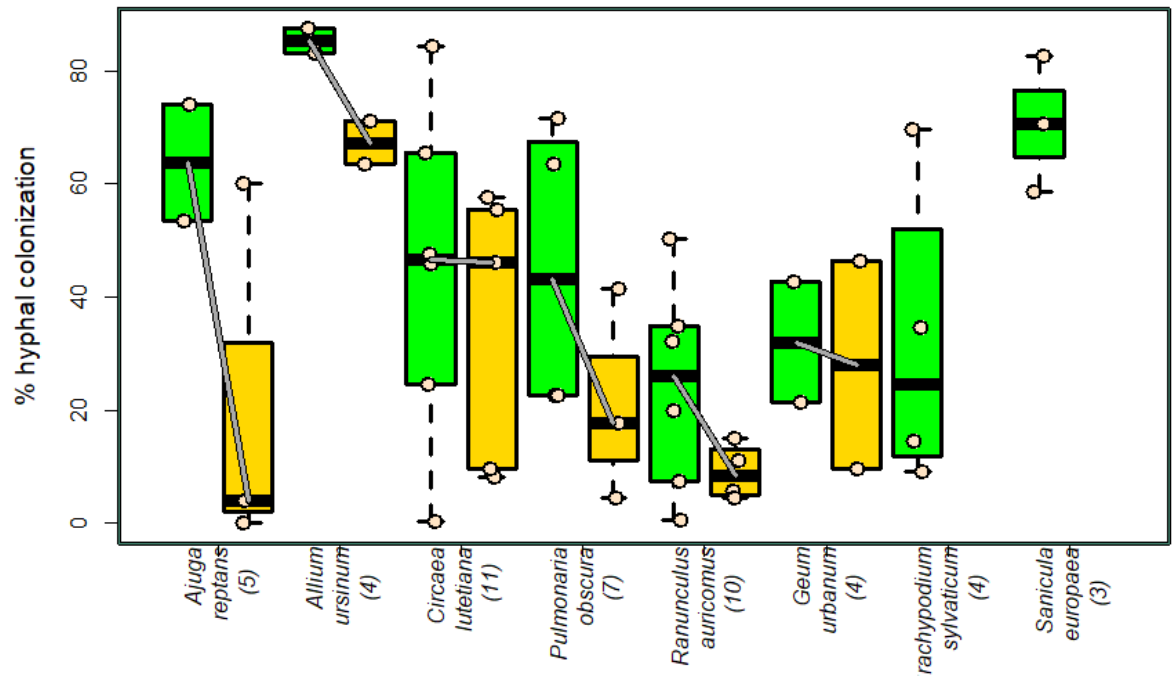


Figure S3 Hyphal colonization in the eight target herbaceous plant species. Jittered boxplots show the number of observations (pale yellow dots) on sampling stands with low (yellow) or high (green) coverage of AMF-associating woody plant and shrub species. Model statistics can be found in Notes S2.

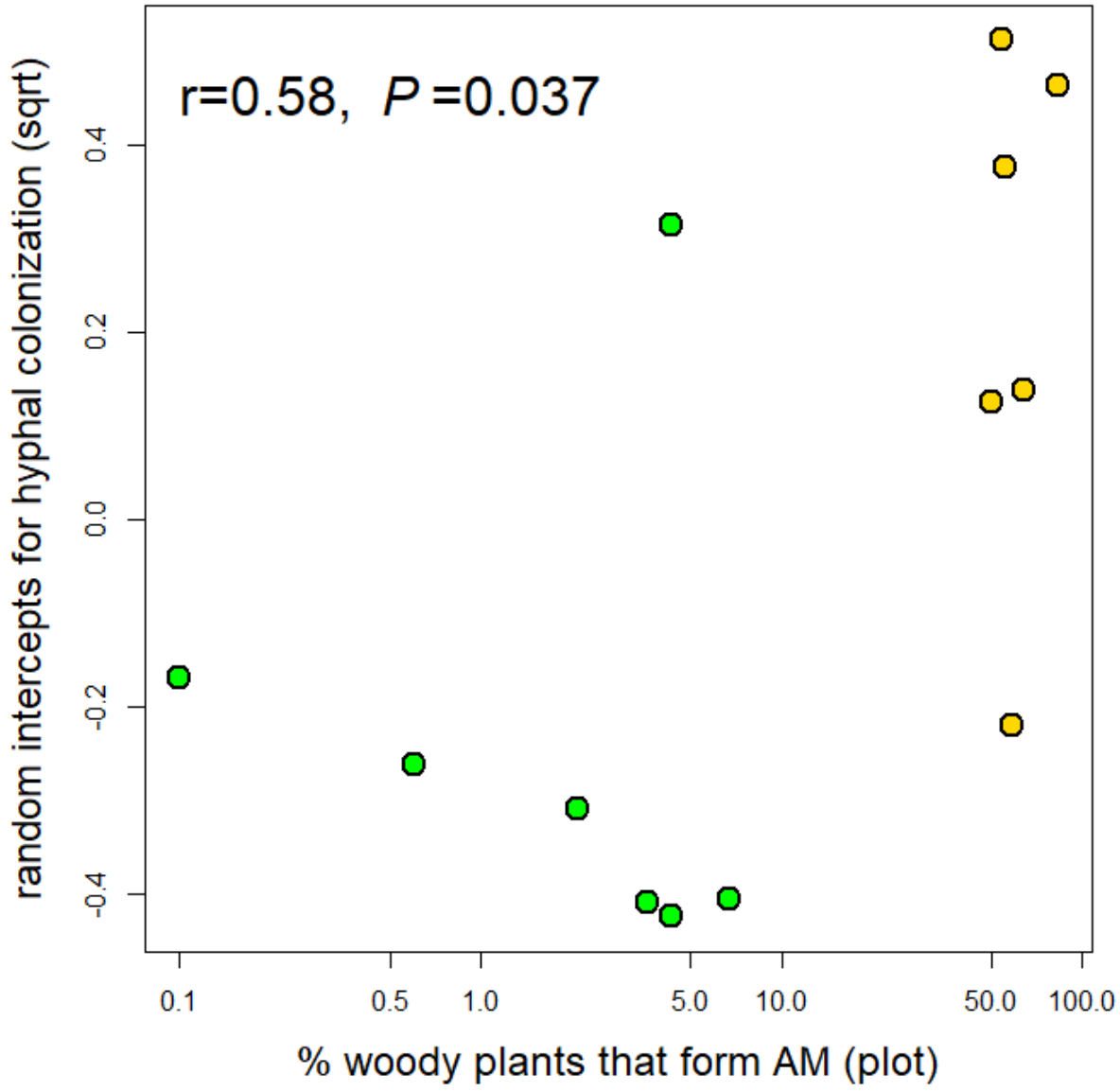


Figure S4 Fits for the random effects variable stand (y-axis) plotted against the proportion of AMF-associating woody plants and shrubs that were observed in each stand (green and yellow dots).

Table S1: Location information (i.e., latitude and longitude) as well as percent AMF and ECM cover for the 13 stands which we assayed for herbaceous plants. Based on the percent cover of woody plants that associate with AMF we classified that stands into the classes of “high” and “low” AMF woody cover.

standID	% woody coverage that associate with AMF*	% woody coverage that associate with ECM**	classification (percent of woody plants the associate with AMF)	latitude	longitude
60	63.6	92	high	53°, 25', 8.23	9°, 28', 42.62
80	55.6	162	high	53°, 39', 49.14	9°, 2', 0.67
86	0	326	low	53°, 26', 37.78	9°, 29', 8.84
103	4.3	331	low	53°, 39', 49.14	9°, 2', 0.67
105	4.3	247	low	53°, 39', 49.14	9°, 2', 0.67
112	6.7	330	high	53°, 25', 8.23	9°, 28', 42.62
113	0.6	297	low	53°, 25', 8.23	9°, 28', 42.62
115	3.6	367	low	53°, 25', 8.23	9°, 28', 42.62
233	82.6	163	high	53°, 18', 17.68	9°, 24', 33.65
286	54.2	118	high	53°, 39', 49.14	9°, 2', 0.67
296	49.7	137	high	53°, 31', 35.69	9°, 4', 7.25
297	58	100	high	53°, 31', 35.69	9°, 4', 7.25
358	2.1	256	low	53°, 26', 36.13	9°, 13', 12.82

*% coverage values were derived after converting extended Braun-Blanquet classes to % woody cover with the **coefficients proposed by van der Maarel (2007). As a result, they do not add up to (and in most cases exceed) 100%.

Table S2 List of the woody species we observed in the stands and their classification into the three mycorrhizal classes (i.e., here represented with scores 0 (not forming arbuscular mycorrhiza), 0.5 (occasionally forming arbuscular mycorrhiza) and 1 (always forming arbuscular mycorrhiza). For the classification we used data from Wang & Qiu (2006). To combine mycorrhizal information with community structure we multiplied abundance of species with their respective AM score. For more information on the approach kindly check Veresoglou et al. (2017).

plant species	**mycorrhizal status (Wang and Qui 2006)**	**AM score**
Acer pseudoplatanus	AM+ECM+NM	0.5
Alnus glutinosa	AM+ECM+EEM+NM	0.5
Betula pubescens	ECM+EEM	0
Carpinus betulus	ECM	0
Corylus avellana	ECM	0
Crataegus laevigata	AM+ECM	0.5
Euonymus europaea	AM	1
Fagus sylvatica	ECM	0
Fraxinus excelsior	AM+ECM	0.5
Hedera helix	AM	1
Ilex aquifolium	AM+ECM	0.5
Lonicera periclymenum	AM	1
Populus x canadensis	AM+ECM	0.5
Prunus padus	AM+ECM	0.5
Quercus robur	ECM	0
Rubus fruticosus	AM	1
Rubus idaeus	AM+NM	0.5
Sorbus aucuparia	AM+ECM+NM	0.5
Ulmus glabra	AM	1
Viburnum opulus	AM	1

Table S3 Percent arbuscular colonization in the target herbaceous host species across sampling stands.

host species	stand	% arbuscular colonization
Ajuga reptans	286	31.1
	297	47.4
	103	2.5
	105	20.5
	358	0
Allium ursinum	80	58.5
	286	62
	103	43.5
	105	49
*Brachypodium sylvaticum**	80	7.5
	233	45.5
	286	17.5
	296	16
Circaea lutetiana	60	49.2
	112	0
	233	36.1
	286	71.6
	296	45.8
	297	30.3
	86	27.9
	103	11.8
	105	29.4
	115	18.7
	358	24.4
Geum urbanum	60	17.2
	233	28.4
	86	13.6
	286	36.9
Pulmonaria obscura	60	17.9
	80	32
	112	15.7
	286	40.5
	103	10.4
	105	18.2
	115	6
Ranunculus auricomus	60	22.9
	112	9.7
	233	20.5
	286	38
	296	25.8
	297	2.5
	86	8
	113	8.8
	115	14.4
	358	9.4
*Sanicula europaea**	80	52.5
	233	44.3
	286	47.5

* species found exclusively in *high* stands

Notes S1: Extended Materials and Methods

Semi-permanent stands

We used semi-permanent forest stands which were surveyed in 1988 for the first and in 2008 for the second time. We reinvestigated a subset of the originally 415 forest stands in 2017. A comparison between the first two surveys revealed a slight trend towards species homogenization as stated by Naaf & Wulf (2010). Hence, we here selected solely undisturbed stands that held a required diversity in herbaceous plant species.

Several abiotic parameters can alter the proportion of the plant root colonized by AMF, such as temperature, moisture, light availability and nutrient availability. We here assayed understory plants for stands with a closed canopy from a relatively small geographic area. This way we partially controlled for cross-stand differences in temperature, moisture and light availability. Because of the conservation state of the forests it is additionally unlikely that there were large differences in herbivory; the herbaceous plants which we assayed were all intact. It is likely, however, that there were systemic differences in nutrient availability between AMF-dominated and ECM-dominated stands which we explore in the main text. Selection criteria for plants

The classification of the mycorrhizal status of occurring plant species was based on Wang & Qiu (2006). Hereafter, we selected eight herbaceous species that were classified as AM-associating and were abundant at our forest stands.

Estimating the proportion of root colonized by AMF

Studies in mycorrhizal ecology often use a confusing terminology when referring to mycorrhizal structures. Here, in agreement with McGonigle et al. (1990), we describe the total occurrence of mycorrhizal structures as proportion of root colonized by AMF and the occurrences of arbuscules, hyphae and vesicles in the roots as % arbuscular, hyphal and vesicular, respectively, colonization. Two replicate slides per sample included approximately 60 root fragments with a length between 1-2 cm. Root fragments were cut at different distances from the main root axis to represent a variety of root thicknesses. Per sample arbuscular, hyphal and vesicular colonization for a minimum of approximately 200 intersections was assessed using the magnified intersection method (McGonigle et al., 1990; Fig. S1) but focused our statistical analyses on % arbuscular and hyphal colonization.

Statistics

We analysed our data with two linear mixed effects model with arbuscular colonization (i.e., proportion of root length containing arbuscules) and hyphal colonization (i.e., proportion of root length containing hyphae) as response variables, woody AM plant species coverage (i.e., the relative cover of woody plants that associated with AMF in the woody stratum with two levels: high or low)

and host species fixed effects predictors and sampling stand as a random effects categorical factor. Because of the low number of replicates and the several different species which we included in the analysis we assume Gaussian-distributed errors instead of using a generalized linear model approach. To address issues with spatial autocorrelation we added in our model a spatial autocorrelation structure (i.e., modelling spatial autocorrelation in arbuscular colonization across neighbouring stands; (Pinheiro et al., 2014) but this did not improve the fit (i.e., AIC values of the model) and the results we present are without this structure. We further examined spatial autocorrelation in arbuscular colonization through a correlogram which we present in the form of Fig. S2. Correlograms visualize the output of iterative spatial autocorrelation tests (each at $P<0.05$) at different spatial distances, which can result from either positive or negative spatial autocorrelation. We observed no spatial autocorrelation at P=0.05 but for a distance of 0.3^{o} (latitude & longitude; approximately 33 km) which should have been because we carried out multiple tests (i.e., there was one test at $P=0.05$ per distance and the sign of the test varied with distance).

Sensitivity analysis

We repeated the analysis described in the main manuscript with % hyphal colonization as response variable. We had to transform the hyphal data (square root transformation; full model; Fig. S3) and the stand coefficients we used in our regression (Fig. S4) to meet the assumptions of the analysis of variance. In the full model we fitted the categorical parameter "stand" as a block factor. The conclusions did not change compared to our main analysis where we used arbuscular colonization as response variable (e.g., Notes S2; Fig. S4).

Notes S2: Detailed statistics

S2.1 *Model output – the two full models*

Key to variables

species: categorical variable with eight levels describing the species used in the experiment.

AMwoody: categorical variable with two levels: "high" and "low" describing classification of stands (we refer to this variable in the letter as "stand").

standID: a categorical variable having a unique ID for each stand

1. Type of model: mixed effects linear model fitted with the command *lme*

Response variable: % Arbuscular colonization

```
numDFdenDF   F-value p-value
(Intercept)     1    27 104.53286  <.0001
species         7    27   5.91597  0.0003
AMwoody         1    27   6.74568  0.0150
# standID was fitted as a random effects parameter
```

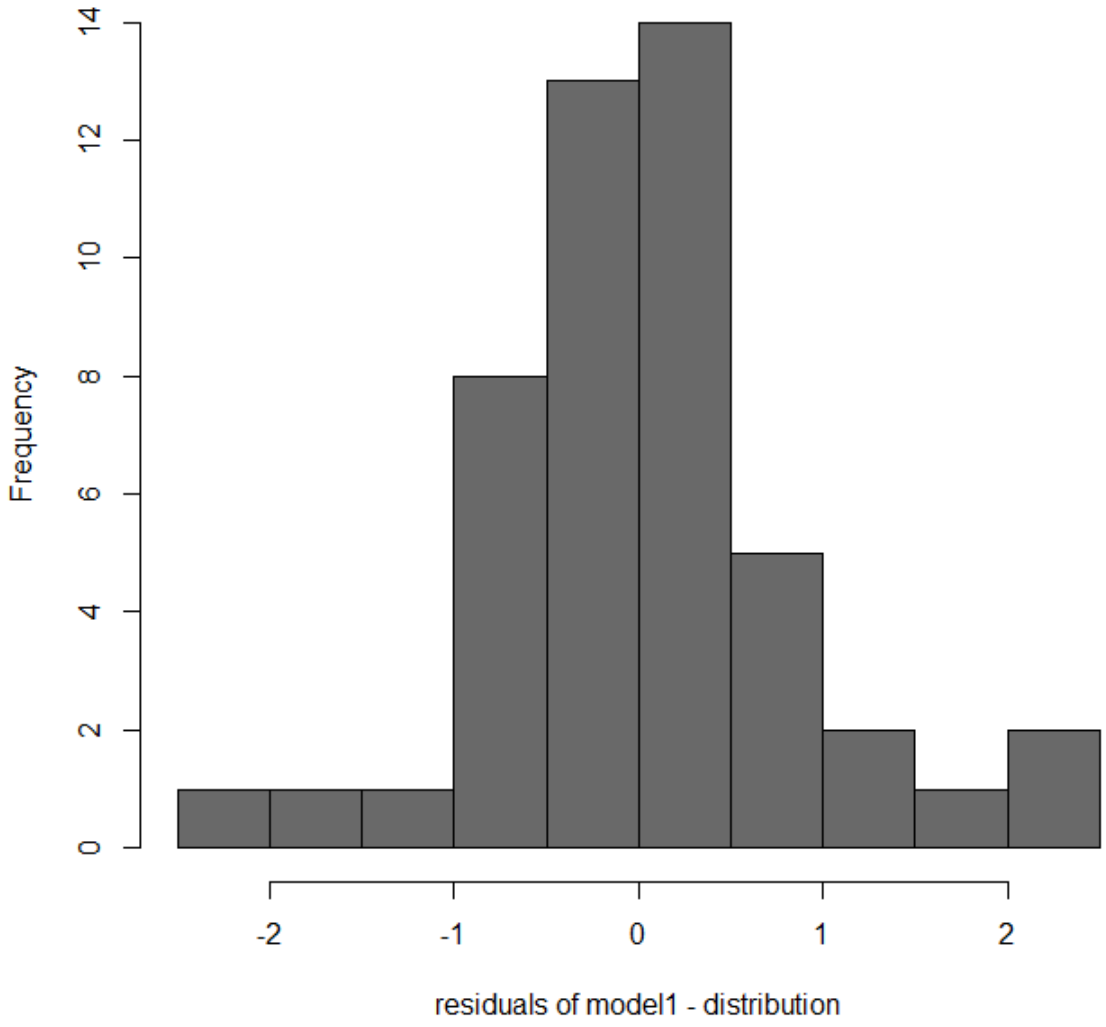
2. Type of model: general linear model

Response variable: % Hyphal colonization - sqrt transformed

```
Df  SumSq Mean Sq F value  Pr(>F)
species     7  96.301 13.7573  2.7094 0.02215 *
AMwoody     1  26.040 26.0397  5.1283 0.02933 *
standID     1   4.177  4.1773  0.8227 0.37011
Residuals 38 192.949  5.0776
```

S2.2 *Model diagnostics for the first model*

Assumption of normality:




```
        Shapiro-Wilk normality test
W = 0.95825, p-value = 0.08578
```

Assumption of homoscedasticity:

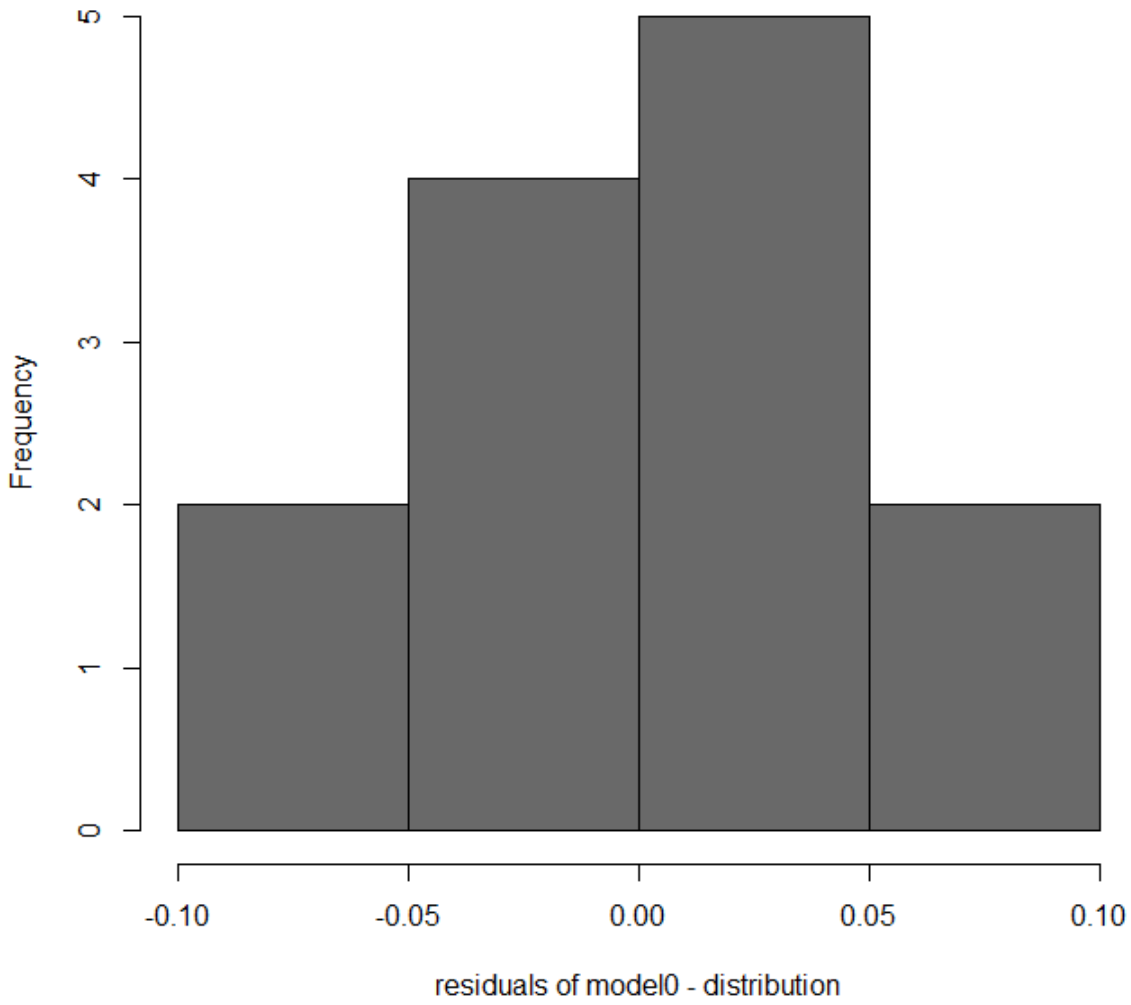
```
> levene.test(data$AC, data$species , kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  data$AC
Test Statistic = 5.0291, p-value = 0.6564

> levene.test(data$AC, data$stand, kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  data$AC
Test Statistic = 3.6412, p-value = 0.05637
```

S2.3 Model formulation and diagnostics when stands are used as experimental units

Model formulation:

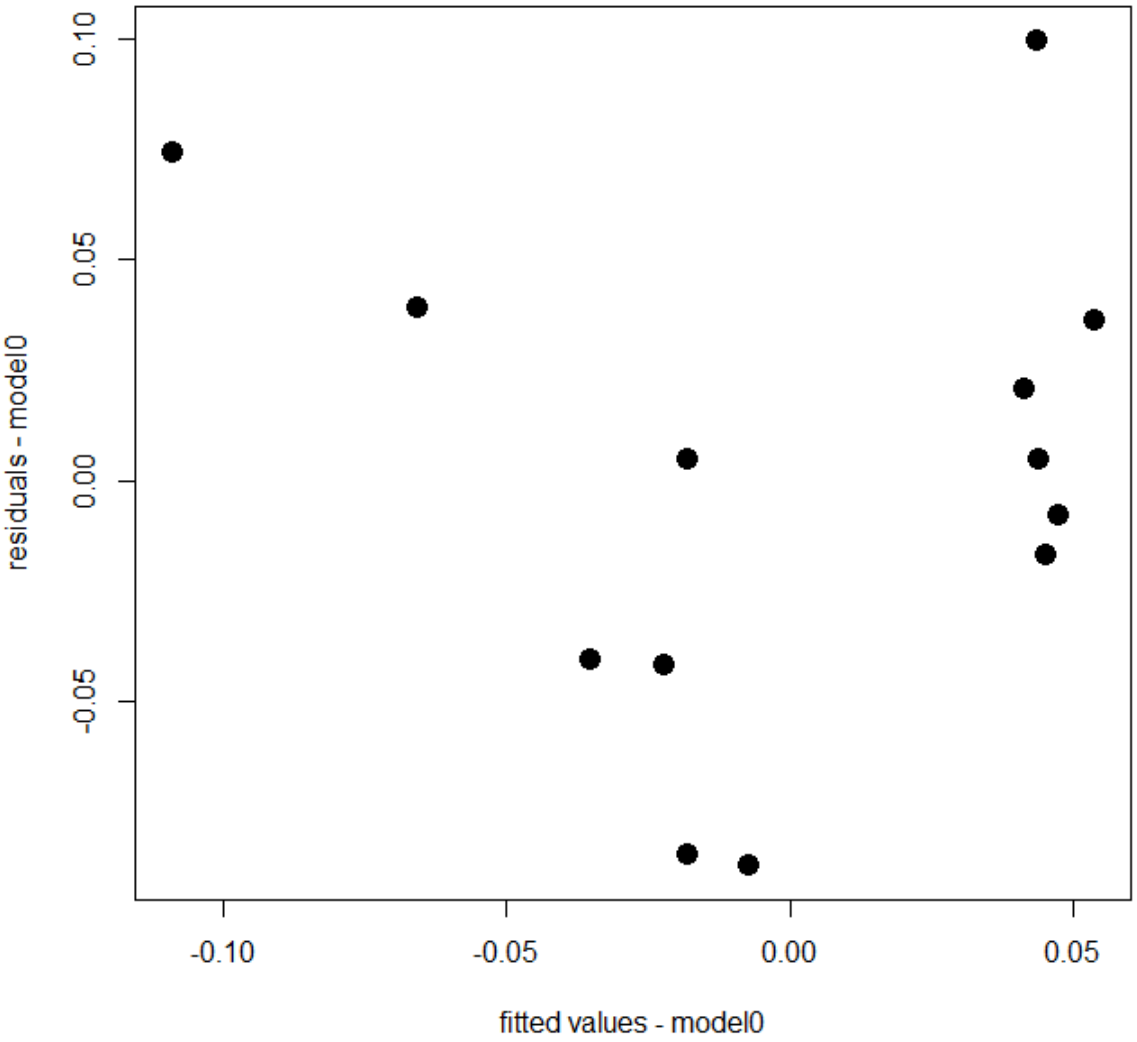
```
basemodel<-lme(AC~species, random=~1|standID,  data=data)
anova(basemodel)
coeff<-(summary(basemodel))$coefficients[[2]][[1]][,1]      # extract coefficients
                                                            # for random effects
                                                            # factor

model0<-lm(coeff~log(myc))
modela<-gls(coeff~log(myc), correlation=corLin(form=~long+lat), data=coords)
modelb<-gls(coeff~log(myc), correlation=corRatio(form=~long+lat), data=coords)
modelc<-gls(coeff~log(myc), correlation=corGaus(form=~long+lat), data=coords)
modeld<-gls(coeff~log(myc), correlation=corExp(form=~long+lat), data=coords)
modele<-gls(coeff~log(myc), correlation=corSpher(form=~long+lat), data=coords)
AIC(model0, modela, modelb, modelc, modeld, modele)         # assess which type
                                                            # of correction for
                                                            # spatial autocorrelation
                                                            # works best

> anova(model0)                                             # best model statistics
Analysis of Variance Table

Response: coeff
          Df   Sum Sq   Mean Sq F value  Pr(>F)
log(myc)   1 0.031247 0.0312471  9.1911 0.01141 *
Residuals 11 0.037397 0.0033997
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Assumption of normality:




```
> shapiro.test(resid(model0))                # assumption of normality
        Shapiro-Wilk normality test
data:  resid(model0)
W = 0.97291, p-value = 0.9264
```

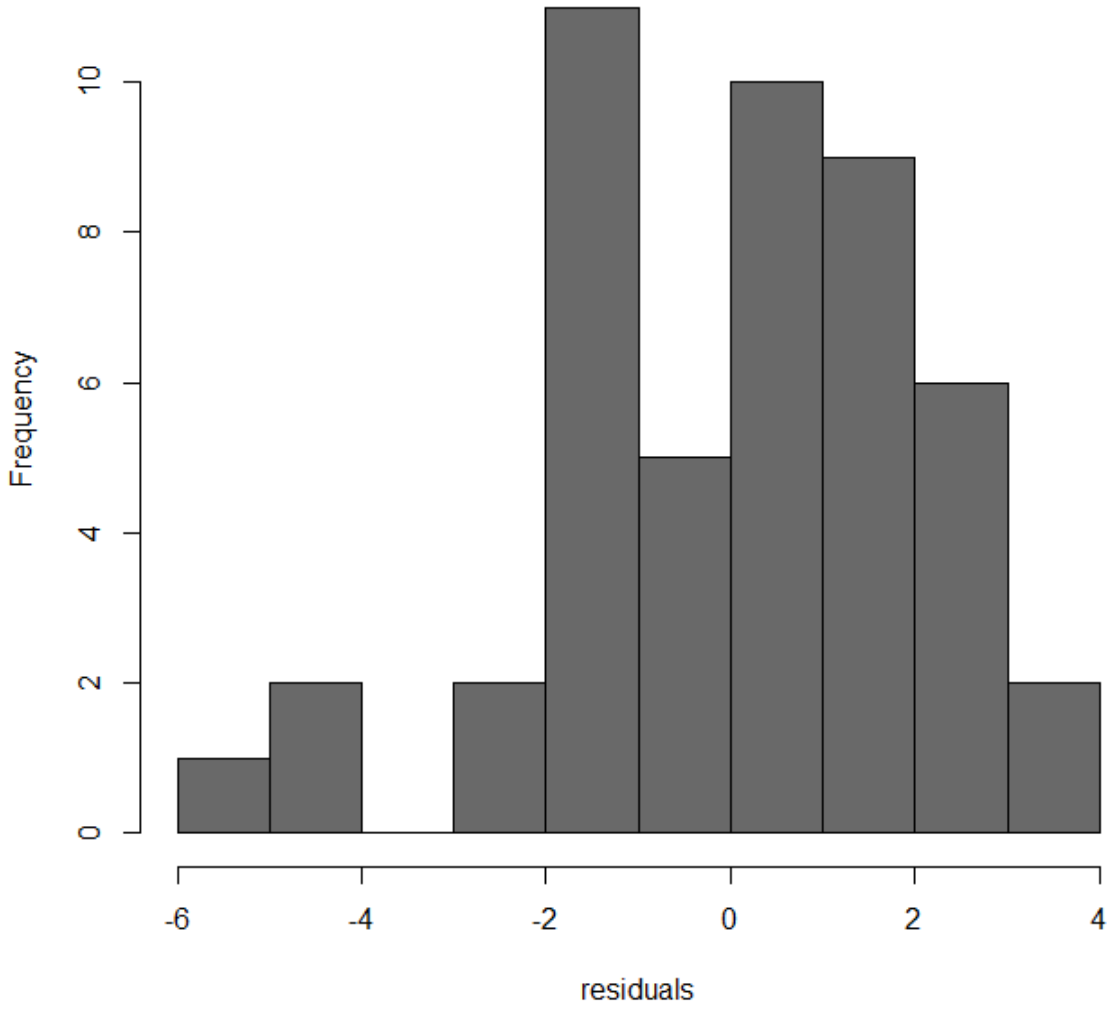
Assumption of homoscedasticity:




```
>levene.test(coeff, log(myc), kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  coeff
Test Statistic = 12, p-value = 0.3636
```

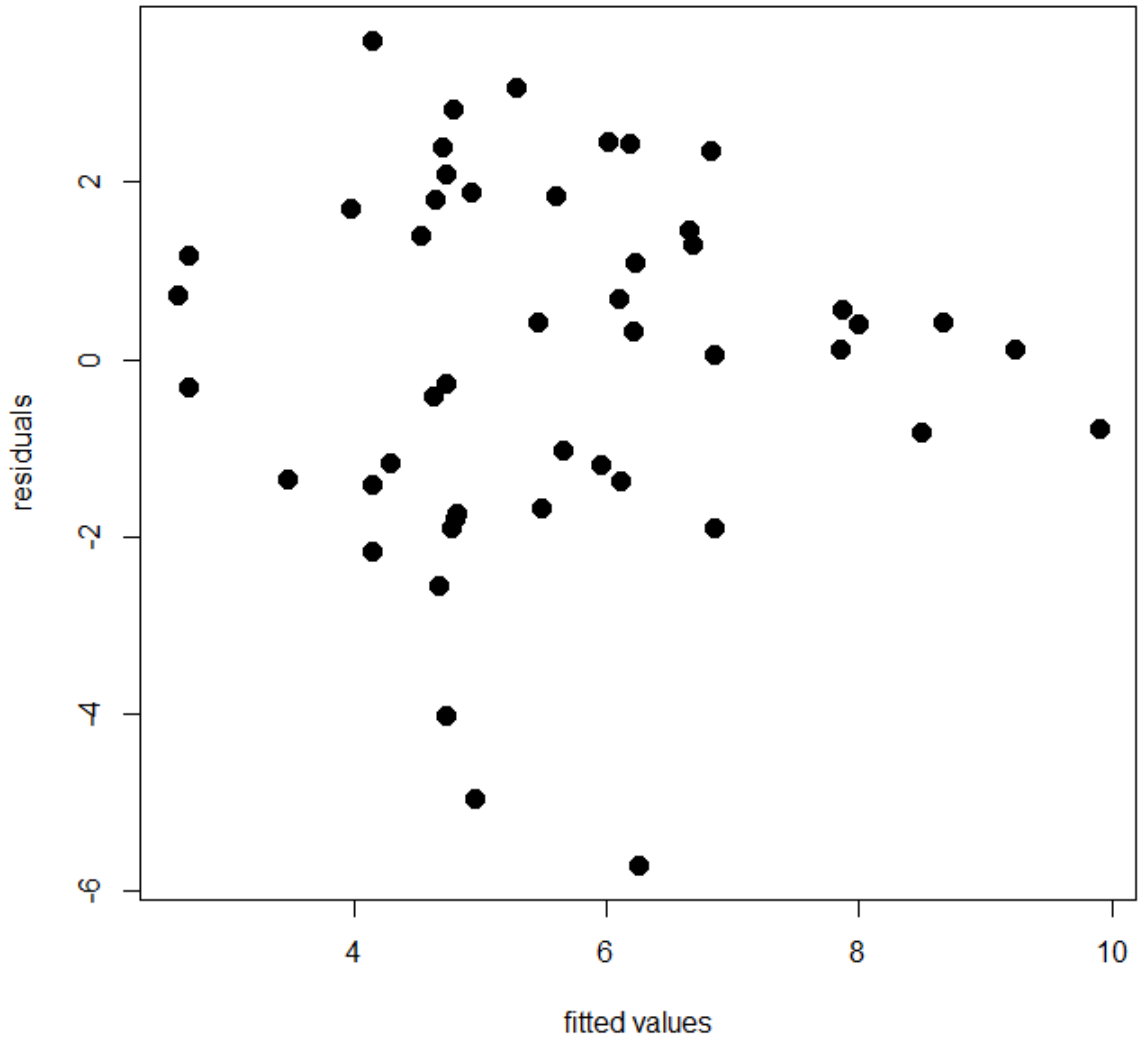
S2.4 *Model diagnostics for the second model*

Assumption of normality:




```
> shapiro.test(resid(model2))
        Shapiro-Wilk normality test
data:  resid(model2)
W = 0.96346, p-value = 0.1393
```

Assumption of homoscedasticity:




```
> levene.test(data$HC2, data$species, kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  data$HC2
Test Statistic = 6.2823, p-value = 0.5072

> levene.test(data$HC2, data$stand, kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  data$HC2
Test Statistic = 0.51385, p-value = 0.4735

> levene.test(data$HC2, data$standID, kruskal.test=T)
        rank-based (Kruskal-Wallis) Modified robust Brown-Forsythe Levene-type
        test based on the absolute deviations from the median
data:  data$HC2
Test Statistic = 5.711, p-value = 0.9299
```

Appendix II: Chapter 3

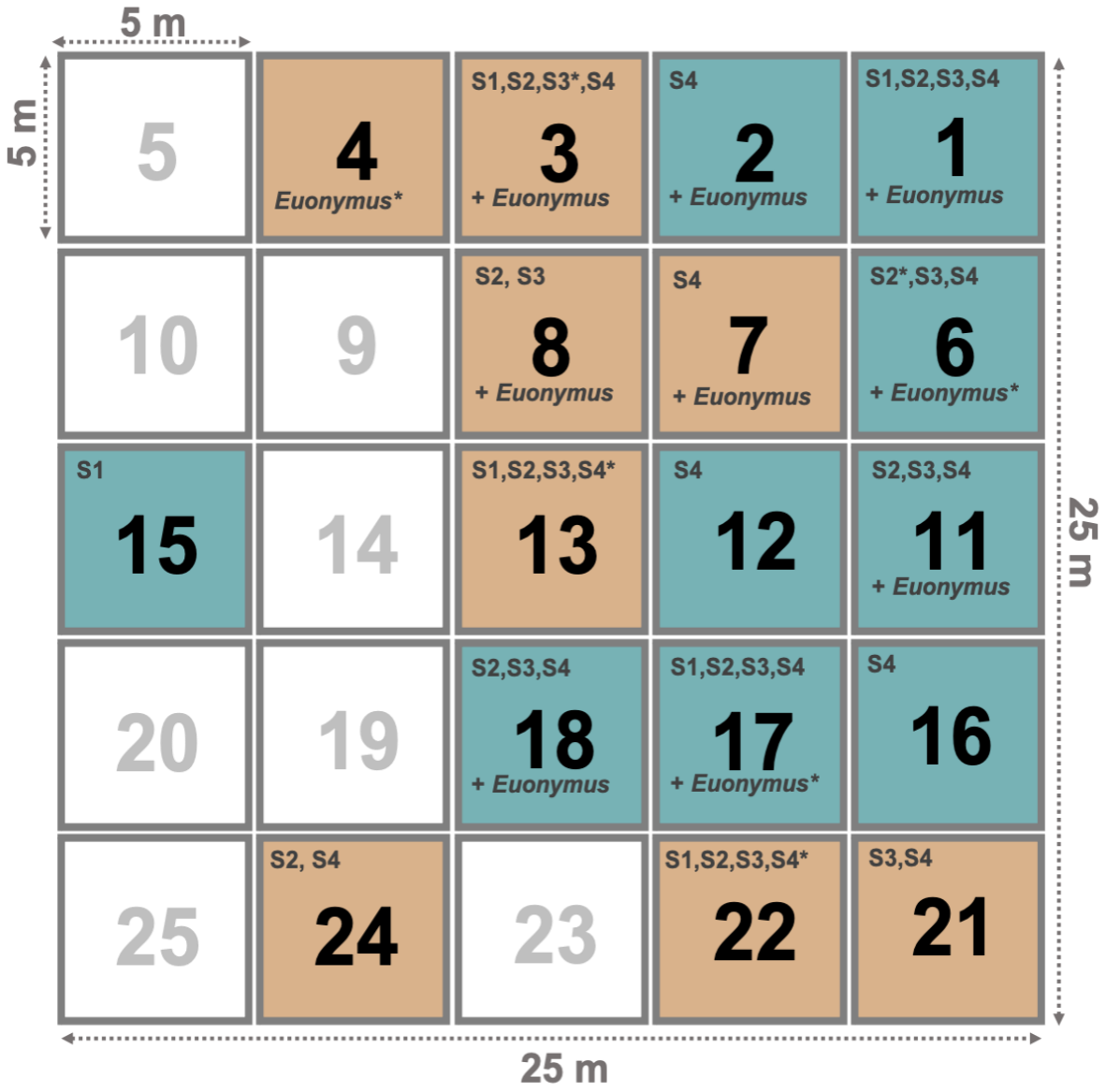


Figure S5 Schematic plan of the 25 5 m x 5 m plots labelled with study plot identity numbers (1-25) at the forest site. Green squares indicate plots with *high* (≥ 15% AM plant cover) and brown squares indicate plots with *low* (< 15% AM plant cover). White squares indicate plots where no root samples were collected. Root samples of *Hedera* are labelled S1 (May 2017), S2 (September 2017), S3 (May 2018), and S4 (September 2018) on respective plots; root samples of *Euonymus* collected in September 2018 only are noted. In general, two root samples from each of two individuals were collected with a minimum distance of 50 cm between them. Exceptions where only one individual was sampled, because no other plant was present, are indicated with an asterisk.

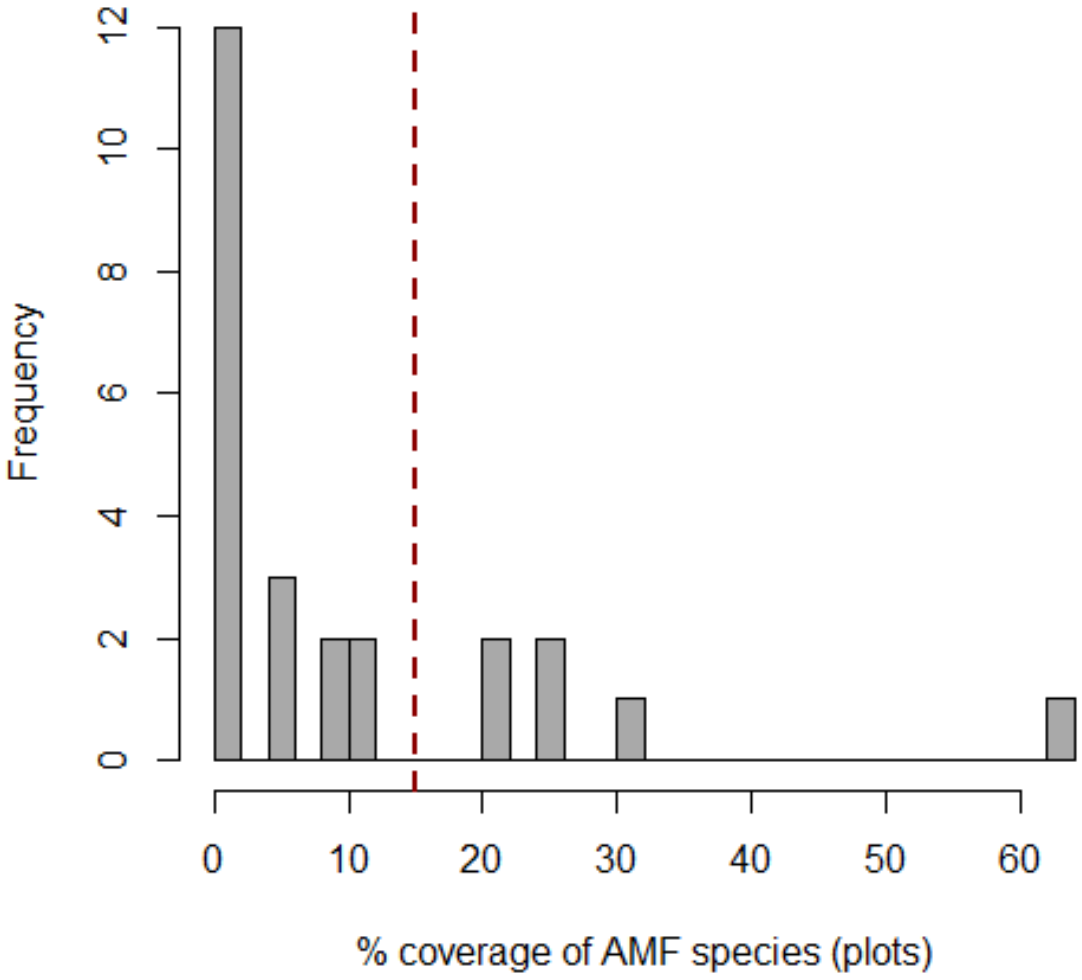


Figure S6 Histogram depicting the classification threshold of 15% in relation to AM-host coverage across plots. Plots with a relative coverage of AMF-associating woody species of over 15% were classified as *high* plots whereas those with a relative coverage of AMF-associating woody species of below 15% as *low*. The plot with a relative coverage of AMF-associating woody species of 64% was no outlier: That specific plot was only assayed for *Hedera* at the fourth harvest and the two samples had AMF richness of 11 and 9 respectively (richness estimates ranged between 4 and 17 OTUs per sample (median: 10 OTUs with the quartiles being 8 and 12). Additionally, in the ordination plots there was nothing special about the clustering patterns of these two samples.

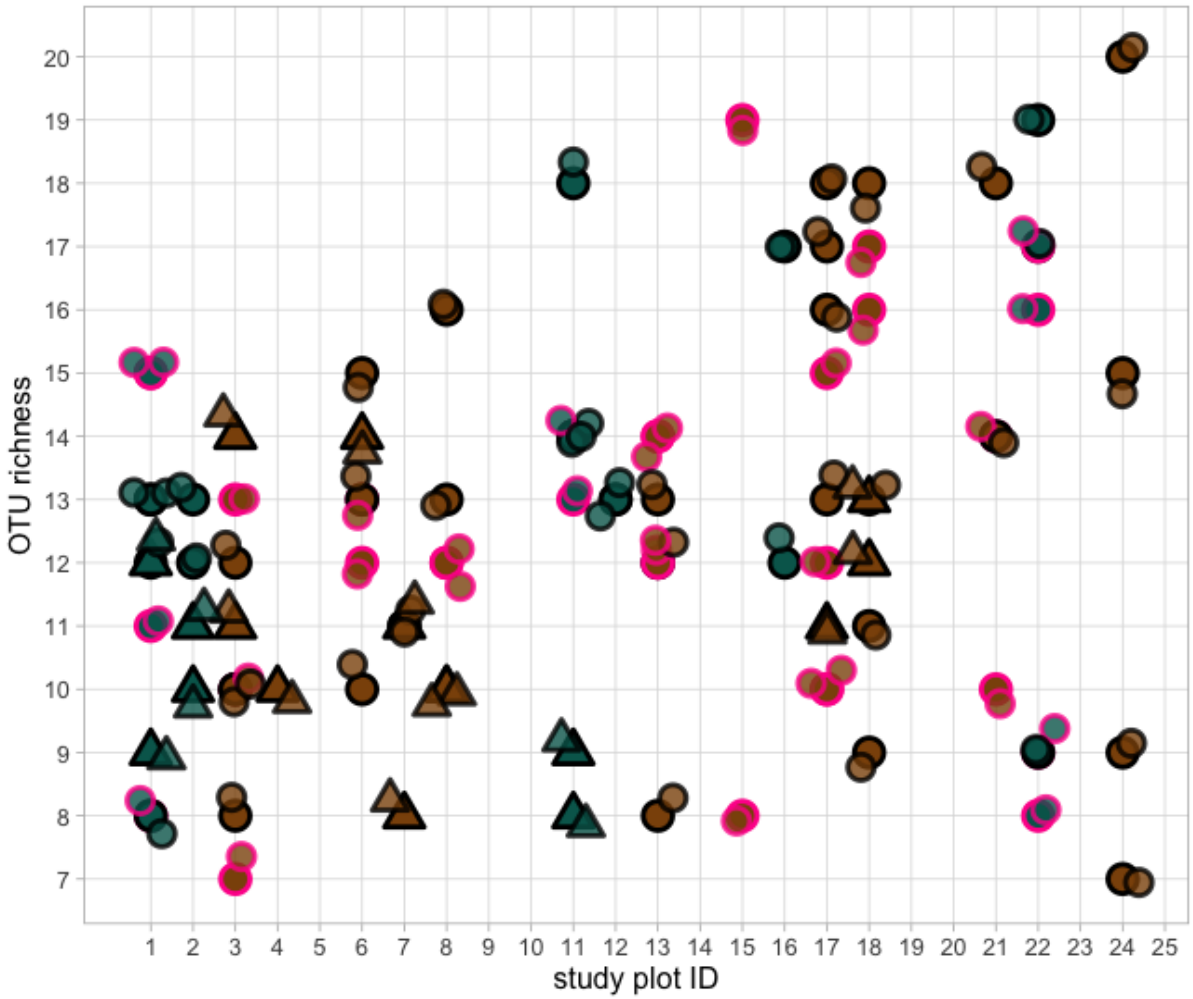


Figure S7 Richness based on numbers of OTUs from each harvest across sampling plots. Green symbols represent samples from *high* plots whereas brown represent *low* plots. A pink border was used for spring and a black border for autumn. Triangles represent samples of *Euonymus* whereas circles those of *Hedera*.

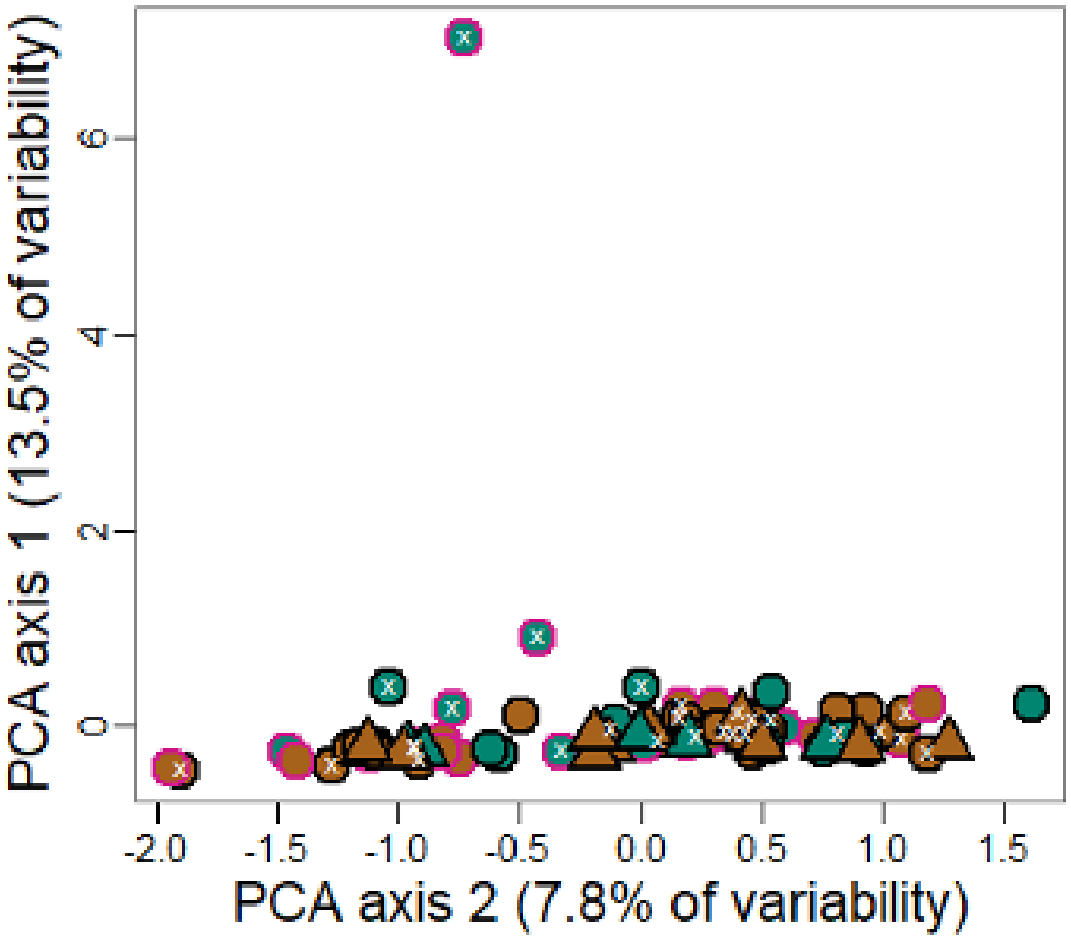


Figure S8 Principal component analysis of Hellinger transformed AMF community data with axis one explaining 13.5% of variability. Green points represent samples from high plots whereas brown represent low plots. A pink border was used for spring and a black border for autumn. We used white "x" symbols to highlight samples taken over the first year. Triangles represent samples of *Euonymus* whereas circles those of *Hedera*. In main text Fig. 4a we display axes two and three because after excluding the outlier on axis one the rescaled variances for the three axes are 1.03% - axis 1, 7.84% - axis 2 and 6.88% - axis 3.

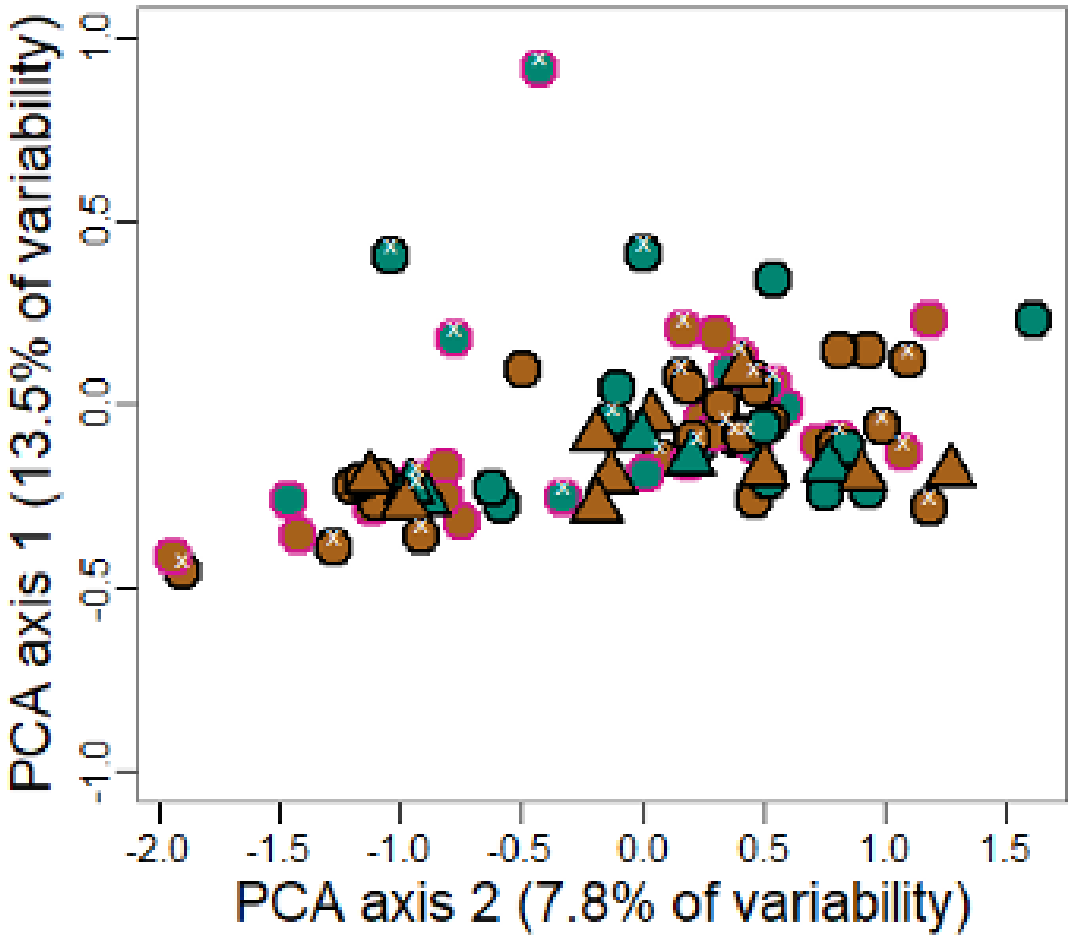


Figure S9 Principal component analysis of Hellinger transformed AMF community data with axis one explaining 13.5% of variability (see Fig. S4) after the exclusion of outliers. Green points represent samples from high plots whereas brown represent low plots. A pink border was used for spring and a black border for autumn. We used white "x" symbols to highlight samples taken over the first year. Triangles represent samples of *Euonymus* whereas circles those of *Hedera*. In main text Fig. 4a we display axes two and three because after excluding the outlier in axis one the rescaled variances for the three axes are 1.03% - axis 1, 7.84% - axis 2 and 6.88% - axis 3. The take home message of this figure is that with the outlier included, the scores on axis 1 are driven by that outlier resulting in a strong correlation on that axis.

Table S4 Visually assessed AMF-associating plant cover across the 25 plots presented in Fig. S1. We used a % cover threshold of 15% to classify them into *high* and *low* plots (see Fig. S2). Roots were collected from 17 of the 25 plots. The four harvests took place on 31 May 2017, 27 September 2017, 31 May 2018 and 25 September 2018. We progressively made the harvests from plots where the two species had a high relative abundance. This was done to avoid issues arising from the two plants becoming locally extinct.

plot within site	% cover of AM-associating woody plants	classification AM plant cover	harvests where *Hedera helix* was sampled	sampling *Euonymus europaea* in 4th harvest
1	26	High	1-4	Yes
2	31	High	4	Yes
3	10	Low	1-4	Yes
4	1	Low	NA	Yes
6	6	Low	2-4	Yes
7	11	Low	4	Yes
8	5	Low	2-3	Yes
11	22	High	2-4	Yes
12	21	High	4	No
13	12	Low	1-4	No
15	1	Low	1	No
16	64	High	4	No
17	1	Low	1-4	Yes
18	2	Low	2-4	Yes
21	1	Low	3-4	No
22	26	High	1-4	No
24	2	Low	2,4	No

Table S5 Soil pH (measured in 0.01m $CaCl_2$) and nutrient levels of phosphorus, potassium, calcium and magnesium at the sampling site. Measurements were taken at three different soil depths by Wulf (1992). The soil constitutes a humid to waterlogged pseudogley (Roeschmann, 1971). Because we worked at a relatively small spatial scale we assumed soil characteristics to be consistent across plots.

soil depth	pH	Exchangeable P [mg/100g]	K [mg/100g]	Ca [mg/100g]	Mg [mg/100g]
0 - 5 cm	4.6	2.8	11.2	139.6	9.8
5 - 10 cm	5.1	1.3	5.9	168.2	8.5
10 - 20 cm	5.6	0.9	3.6	188.5	7.8

Table S6 OTU ID information. We accepted OTUs as belonging to Glomeromycota when they had > 97.5 % similarity and > 99 % coverage to entries in MaarjAM.

OTU ID	Accession	Description	Max score	Total score	Query coverage	E-value	Max identity
OTU5	HF568031	Glomeraceae Glomus Varela-Cervero15 BG9	154	218	90.10%	5.20E-80	85.50%
OTU6	HF568033	Glomeraceae Glomus Varela-Cervero15 BG9	158	222	90.30%	2.50E-82	84.70%
OTU11	HF568087	Glomeraceae Glomus Varela-Cervero15 BG21	135	200	89.00%	5.10E-69	82.30%
OTU14	LT723796	Glomeraceae Glomus sp. VTX00342	34	34	15.70%	1.40E-10	82.90%
OTU19	JN009464	Diversisporaceae Diversispora sp. VTX00380	516	516	100.00%	0	99.40%
OTU22	HF568033	Glomeraceae Glomus Varela-Cervero15 BG9	158	222	90.30%	2.50E-82	84.70%
OTU24	KC665641	Glomeraceae Glomus Lopez-Garcia14 Glo166	76	76	33.30%	6.80E-35	83.80%
OTU26	HF568087	Glomeraceae Glomus Varela-Cervero15 BG21	173	238	90.10%	5.20E-91	85.90%
OTU27	LT723796	Glomeraceae Glomus sp. VTX00342	34	34	15.70%	1.40E-10	82.90%
OTU28	KJ959950	Glomeraceae Glomus sp. VTX00219	479	479	100.00%	0	97.70%
OTU32	HF568038	Glomeraceae Glomus Varela-Cervero15 BG9	169	228	87.60%	1.10E-88	85.40%
OTU34	KC708365	Claroideoglomeraceae Claroideoglomus sp. VTX00193	427	427	100.00%	0	94.70%

OTU36	FN869852	Archaeosporaceae Archaeospora Aca VTX00338	473	473	100.00%	0	98.80%
OTU37	HF568087	Glomeraceae Glomus Varela-Cervero15 BG21	149	216	91.50%	4.10E-77	83.70%
OTU39	LT934587	Glomeraceae Glomus sp.	158	225	93.90%	2.50E-82	83.30%
OTU41	HF568087	Glomeraceae Glomus Varela-Cervero15 BG21	176	238	89.40%	9.60E-93	85.80%
OTU42	HF568058	Glomeraceae Glomus Varela-Cervero15 BG13	159	221	92.60%	6.60E-83	84.00%
OTU45	HF568021	Glomeraceae Glomus Varela-Cervero15 BG7	122	190	87.00%	1.80E-61	81.50%
OTU47	KC665641	Glomeraceae Glomus Lopez-Garcia14 Glo166	82	82	33.30%	2.40E-38	85.10%
OTU50	HF568040	Glomeraceae Glomus Varela-Cervero15 BG9	174	238	86.50%	1.40E-91	87.70%
OTU52	HF568040	Glomeraceae Glomus Varela-Cervero15 BG9	183	247	86.50%	8.70E-97	88.70%
OTU54	KC665641	Glomeraceae Glomus Lopez-Garcia14 Glo166	85	85	33.30%	4.50E-40	85.80%
OTU55	HF568087	Glomeraceae Glomus Varela-Cervero15 BG21	165	230	88.80%	2.20E-86	85.30%
OTU58	HF568034	Glomeraceae Glomus Varela-Cervero15 BG9	164	220	87.90%	8.50E-86	84.80%
OTU59	HF568038	Glomeraceae Glomus Varela-Cervero15 BG9	152	152	73.90%	7.40E-79	83.90%
OTU60	FN429106	Glomeraceae Glomus VeGlo13 VTX00153	495	495	100.00%	0	98.30%

OTU62	AB746997	Claroideoglomeraceae Claroideoglomus Yoshimura13b Glo17	435	435	100.00%	0	94.30%
OTU63	JQ246044	Glomeraceae Glomus sp. VTX00359	79	79	67.60%	1.30E-36	76.70%
OTU64	LT934587	Glomeraceae Glomus sp.	152	214	92.10%	7.50E-79	82.70%
OTU65	LN900648	Glomeraceae Glomus sp. VTX00096	477	477	100.00%	0	97.30%
OTU66	HF568040	Glomeraceae Glomus Varela-Cervero15 BG9	166	227	87.00%	6.00E-87	87.50%
OTU67	AJ854097	Archaeosporaceae Archaeospora sp. VTX00006	450	487	100.00%	0	99.30%
OTU70	EU417640	Glomeraceae Glomus Sciaphila ledermannii symbiont VTX00166	498	498	100.00%	0	98.50%
OTU71	LN623488	Paraglomeraceae Paraglomus MO-P2 VTX00433	125	195	87.60%	3.20E-63	81.40%
OTU72	LT983627	Glomeraceae Glomus SS-G1 VTX00448	248	313	91.20%	2.00E-134	92.30%
OTU73	LN827079	Glomeraceae Glomus sp. VTX00344	466	466	100.00%	0	96.90%
OTU74	HF568033	Glomeraceae Glomus Varela-Cervero15 BG9	160	221	90.10%	1.70E-83	84.90%
OTU75	HF569099	Gigasporaceae Scutellospora Varela-Cervero15 BS2	140	205	91.50%	6.50E-72	82.30%

Notes S3 Defining stochasticity

The term stochasticity often has been used in the literature synonymously with variability (Shoemaker *et al.*, 2020). Shoemaker *et al.* (2020) propose narrowing the definition of stochasticity to processes that can be represented in a probabilistic way defined by their parameters (e.g., mean, variance, and skew) and those authors distinguish three forms of stochasticity: demographic stochasticity, environmental stochasticity, and measurement error. Based on this definition it is possible that the aggregate of community variance that is explained by deterministic processes and stochasticity falls below 100%.

In our study we define stochasticity in relation to the proportion of variance that is not explained by deterministic processes (i.e., deterministic processes and variance together explain 100% of community variance). We do so because arbuscular mycorrhizal associations represent complex systems which we do not understand sufficiently to effectively model processes such as demographic stochasticity (priority effects and succession, for example, have been poorly defined for AMF systems) which could have led us to seriously underestimate stochasticity. We thus describe stochasticity here as the fraction of community variance that is not explained by deterministic processes (de Vrieze *et al.*, 2020).

To show a few examples of the proportion of stochasticity that authors often find in AMF communities we carried out a literature search. We used the key words "deterministic" AND "arbuscular" in the Web of Knowledge on 30 June 2020. The search yielded 9 results which we screened for studies that contained figures on variance explained following a variance partitioning exercise. There were four such studies, which we list below:

study	variance explained by deterministic factors
Maciel Rabelo Pereira et al. 2020 Journal of Biogeography	9%
Rasmussen et al. 2018 New Phytologist, (Caruso 2018 New Phytologist Commentary)	7-25%
Kohout et al. 2015 Molecular Ecology	39.3%
Dumbrell et al., 2010 ISME Journal	68.5%

Notes S4: Limitations of existing multivariate techniques in addressing spatio-temporal designs and how we addressed them

Most studies addressing the relative role of deterministic and stochastic processes in structuring communities engage in variance partitioning with the aim to explain as much variance as possible (e.g., Horn et al., 2014). Accuracy of such estimates depends strongly on the degree to which the underlying ordination model captures the experimental design. For example, assaying a plot at multiple instances in time represents a violation of independence and potentially can be addressed through a repeated-measures design in which case plot will be the unit of analysis (i.e., *Subject*) whereas time is the within-subject factor (e.g., Palmer et al., 2008). Determining between-subject and within-subject sources of variance is critical in the case of variance partitioning because through this step it becomes apparent that each group will be compared against a unique fraction of unexplained variance (e.g., within-subjects unexplained variance). Many of the studies addressing spatio-temporal designs, however, incorrectly assume that iterative harvests of a single plot are independent of each other.

Even though there are several techniques to address spatial autocorrelation in ordination analyses, to the best of our knowledge the only multivariate technique that works for temporal constraints is that of Palmer *et al*. (2008), which was specifically proposed for split plot designs. To minimize the assumptions of our analyses we plot our data with a PCA (i.e., meaning that we do not propose any underlying model; main text Fig. 4a) and then calculate distributions of pairwise Bray-Curtis distances (Fig. 4b). To back up our analysis we fit a redundancy analysis model (Notes S5) in which we address temporal constraints by restricting permutations (and thus calculation of resulting *P* values) to only be within plots. This approach may be an improvement compared to assuming full independence.

Our study was subject to some limitations which we raise here. First, we did not assay soil properties and assumed that the abiotic parameters throughout the site were homogenous. To this end we included in our analysis spatial corrections which should have accounted for spatial autocorrelations in soil properties. Second, to simultaneously address all factors we sacrificed the number of levels we considered. We worked with two plant species over two years and two seasons. Some of the results may be idiosyncratic because of this choice. Third, we only assayed two AM plant species, which were those that showed sufficient abundance across the target forest site. One of them (*Euonymus)* could only be collected at the last harvest. This was done because there were only a few individuals of *Euonymus* in the forest site and their destructive harvest could modify meta-community dynamics of AMF species. As a result, to a certain degree the variance that we allocated to plant species was nested within the variance explained by season and year. To address this point, we only tested spatio-temporal parameters for *Hedera*, and in the cases where we included *Euonymus* in analyses we formulated the models we fitted so that there were several alternative ranks of predictors.

Notes S5: Statistical analyses

Most of our statistics were descriptive. Ordination analyses were carried out after Hellinger transforming community data (for example, main text Fig. 4a) to avoid distortions of distances in the Euclidean space. To assay the distributions of Bray Curtis distances we randomly paired samples 9999 times (Fig. 4b). Relative abundance figures of AMF families were descriptive (Fig. 4c).

We carried out an Analysis of Variance on the RDA models that we fitted. The default settings of the command anova.cca in R, test hierarchically the terms of the RDA/CCA models and yield different results from when the predictors are fitted "simultaneously". Additionally, the command does not offer possibilities to fully distinguish between within-factor and between factors variability (i.e., does not address repeated-measures designs). An alternative is to constrain permutations so that they occur within the subjects of the analysis (i.e., here the plots), which we applied. To address that our experimental design was not balanced and that predictors were fitted hierarchically we tested alternative formulations of the models after changing the rank of the predictors. We additionally included models with and without the predictor *plant species*. To address spatial constraints, we used the Principal Coordinates of Neighbourhood Matrix (PCNM) approach to summarize space into three spatial axes. The parameters *year*, spatial PCNM axes and *plant species* (in the cases when it was included) were significant regardless of their rank in the models. Season was never significant, whereas the predictor *AM coverage* was only occasionally significant. We attach specific results:

Redundancy Analysis Statistics

```
> anova(myrda, by="terms", permutations=9999, strata=as.factor(field$plot))
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Blocks:  strata
Permutation: free
Number of permutations: 9999

Model: rda(formula = data2 ~ AM_coverage + year + season + plant_species + PCNM1 + PCNM2 +
PCNM3, data = field, scale = T)
              Df Variance    F Pr(>F)
AM_coverage    1   0.6071 1.5900 0.0002 ***
year           1   0.8247 2.1600 0.0009 ***
season         1   0.5360 1.4040 0.1974
plant_species  1   0.5651 1.4801 0.0203 *
PCNM1          1   0.5948 1.5580 0.1529
PCNM2          1   0.5199 1.3616 0.5799
PCNM3          1   0.5720 1.4982 0.0351 *
Residual      78  29.7804
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


> anova(myrda, by="terms", permutations=9999, strata=as.factor(field$plot))
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Blocks:  strata
Permutation: free
Number of permutations: 9999
```

```
Model: rda(formula = data2 ~ year + plant_species + AM_coverage + season + PCNM1 + PCNM2 +
PCNM3, data = field, scale = T)
              Df Variance    F Pr(>F)
year           1   0.7991 2.0929 0.0011 **
plant_species  1   0.6027 1.5786 0.0132 *
AM_coverage    1   0.6111 1.6005 0.1137
season         1   0.5200 1.3619 0.2276
PCNM1          1   0.5948 1.5580 0.1459
PCNM2          1   0.5199 1.3616 0.5852
PCNM3          1   0.5720 1.4982 0.0344 *
Residual       78  29.7804
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> myrda<-rda(data2~year+season+AM_coverage+PCNM1+PCNM2+PCNM3, data=field[1:71,], scale=T)
> anova(myrda, by="terms", permutations=9999, strata=as.factor(field$subplot)[1:71])
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Blocks:  strata
Permutation: free
Number of permutations: 9999

Model: rda(formula = data2 ~ year + season + AM_coverage + PCNM1 + PCNM2 + PCNM3, data =
field[1:71, ], scale = T)
              Df Variance    F Pr(>F)
year           1   0.7230 1.5365 0.0364 *
season         1   0.5508 1.1706 0.3986
AM_coverage    1   0.7495 1.5929 0.0459 *
PCNM1          1   0.7039 1.4961 0.6544
PCNM2          1   0.4805 1.0212 0.6495
PCNM3          1   0.6796 1.4445 0.0937 .
Residual       64  30.1127
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> anova(myrda, by="terms", permutations=9999, strata=as.factor(field$plot)[1:71])
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Blocks:  strata
Permutation: free
Number of permutations: 9999

Model: rda(formula = data2 ~ year + season + PCNM1 + PCNM2 + PCNM3 + AM_coverage, data =
field[1:71, ], scale = T)
              Df Variance    F Pr(>F)
year           1   0.7230 1.5365 0.0338 *
season         1   0.5508 1.1706 0.4004
PCNM1          1   0.7231 1.5369 0.7505
PCNM2          1   0.4844 1.0294 0.1324
PCNM3          1   0.9037 1.9206 0.0587 .
AM_coverage    1   0.5023 1.0676 0.9381
Residual       64  30.1127
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> anova(myrda, by="terms", permutations=9999, strata=as.factor(field$subplot)[1:71])
Permutation test for rda under reduced model
Terms added sequentially (first to last)
Blocks:  strata
Permutation: free
Number of permutations: 9999
```

```
Model: rda(formula = data2 ~ PCNM1 + PCNM2 + PCNM3 + AM_coverage + season + year, data =
field[1:71, ], scale = T)
              Df Variance    F Pr(>F)
PCNM1          1   0.7513 1.5968 0.0788 .
PCNM2          1   0.4173 0.8869 0.0788 .
PCNM3          1   0.8174 1.7372 0.0788 .
AM_coverage    1   0.5426 1.1533 0.0788 .
season         1   0.5270 1.1201 0.4780
year           1   0.8316 1.7674 0.0321 *
Residual      64  30.1127
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Repeated-measures ANOVA

```
> summary(aov(S~AM_coverage+plant_species+season+year+
Error(as.factor(subplot)/as.factor(sampling)), data=field))

Error: as.factor(subplot)
             Df Sum Sq Mean Sq F value Pr(>F)
AM_coverage   1   4.88   4.881   1.323 0.2725
plant_species 1  12.50  12.501   3.388 0.0905 .
season        1   2.57   2.575   0.698 0.4198
year          1  20.35  20.346   5.515 0.0368 *
Residuals    12  44.27   3.689
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: as.factor(subplot):as.factor(sampling)
             Df Sum Sq Mean Sq F value Pr(>F)
plant_species 1  51.06   51.06   7.281 0.0138 *
season        1   7.73    7.73   1.103 0.3062
year          1   4.21    4.21   0.601 0.4474
Residuals    20 140.25    7.01
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
             Df Sum Sq Mean Sq F value Pr(>F)
plant_species 1  27.34  27.337   4.483 0.0398 *
Residuals    45 274.41   6.098
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Warning message:
In aov(S ~ AM_coverage + plant_species + season + year +
Error(as.factor(subplot)/as.factor(sampling)),  :
  Error() model is singular
```

Indicator Species Analysis

```
> ind.sp = multipatt(data2, as.factor(paste0(as.factor(field$plant_species),
as.factor(field$AM_coverage))), control = how(nperm=9999), max.order=2, restcomb=c(1:6, 9:10))

 Association function: IndVal.g
 Significance level (alpha): 0.05

 Total number of species: 34
 Selected number of species: 5
 Number of species associated to 1 group: 2
 Number of species associated to 2 groups: 3
 Number of species associated to 3 groups: 0
```

```
 List of species associated to each combination:

 Group Euonymus europaea  #sps.  1
       stat p.value
OTU70 0.826   2e-04 ***

 Group Hedera helix  #sps.  1
       stat p.value
OTU19 0.644  0.0383 *

 Group Euonymus europaea+Euonymus europaea  #sps.  1
      stat p.value
OTU2 0.854  0.0349 *

 Group Hedera helix+Hedera helix  #sps.  2
       stat p.value
OTU8  0.904  0.0007 ***
OTU13 0.880  0.0027 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix III: Chapter 4a

Supplementary information on Chapter 4a can be found at https://doi.org/10.1007/s00572-022-01087-0

Appendix IV: Chapter 4b

Experiment One

Abbreviations in the code:

Treatment: categorical variable with three levels (low, intermediate and high connectance)

Distance: Categorical variable describing whether the pair of samples was proximal ("*same*") to each other or distant ("*dif*") to each other

Test 5.1 Comprehensive analysis

```
> summary(aov(Jaccard~Treatment + Distance+ Error(save3$pair), data=save3 )) Error: save3$pair
          Df  Sum Sq  Mean Sq F value Pr(>F)
Treatment  2 0.00061 0.000305   0.034  0.966
Distance   1 0.00752 0.007521   0.845  0.385
Residuals  8 0.07122 0.008902
Error: Within
          Df  Sum Sq  Mean Sq F value Pr(>F)
Treatment  2 0.01106 0.005529   1.340 0.2714
Distance   1 0.02618 0.026177   6.342 0.0151 *
Residuals 49 0.20225 0.004128
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Test 5.2 Test between samples belonging to the "same" vs "different" patch

```
> t.test(save3$Jaccard[save3$Distance=="dif"], save3$Jaccard[save3$Distance=="same"])

Welch Two Sample t-test
data: save3$Jaccard[save3$Distance == "dif"] and save3$Jaccard[save3$Distance == "same"] t =
1.9085, df = 26.79, p-value = 0.0671
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.004040162  0.111055343
sample estimates:
 mean of x  mean of y
-0.2709194 -0.3244270
```

Experiment Two

Abbreviations in the code:

Treatment: categorical variable with three levels (low, intermediate and high connectance)

Distance: Categorical variable describing whether the pair of samples was proximal ("*same*") to each other or distant ("*dif*") to each other

Test 6.1: Comprehensive analysis

```
> summary(aov(Jaccard~Treatment + Distance+ Error(save3$pair), data=save3 )) Error: save3$pair
          Df  Sum Sq Mean Sq F value Pr(>F)
Treatment  2 0.01158 0.00579   0.176  0.842
Distance   1 0.00314 0.00314   0.096  0.765
Residuals  8 0.26295 0.03287
```

```
Error: Within
          Df  Sum Sq  Mean Sq F value Pr(>F)
Treatment  2 0.00425 0.002126   0.918  0.406
Distance   1 0.00490 0.004896   2.115  0.152


Residuals 49 0.11343 0.002315
```

Test 6.2: test between samples belonging to the "same" vs "different" patch

```
> t.test(save3$Jaccard[save3$Distance=="dif"], save3$Jaccard[save3$Distance=="same"])
```

Welch Two Sample t-test

```
data: save3$Jaccard[save3$Distance == "dif"] and save3$Jaccard[save3$Distance == "same"] t =
1.0821, df = 29.941, p-value = 0.2879
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.02376243  0.07731167
sample estimates:
 mean of x  mean of y
-0.3200010 -0.3467756
```

Experiment Three

Abbreviations in the code:

***Treatment*:** categorical variable with three levels ("*control*", "*aggregated*" and "*overdispersed*")

Mypredictor*:* Categorical variable with three levels describing whether the pair of samples was from *fertilized habitats*, *unfertilized habitats* or belonged to *different habitats*

Test 7.1: comprehensive test

```
> summary(aov(Jaccard ~ factor(save3$treatment) + factor(save$mypredictor) + Error(save$pair)
))
Error: save3$pair
            Df Sum Sq Mean Sq F value Pr(>F)
treatment    2 0.1819 0.09095   1.756  0.194
mypredictor  2 0.0149 0.00743   0.143  0.867
Residuals   24 1.2430 0.05179
factor(save3$mypredictor)+ Error(save3$pair) ))
Error: Within
```

Test 7.2: test exclusively between aggregated and overdispersed samples

```
> t.test(save3$Jaccard[save3$treamentt=="aggregated"],
save3$Jaccard[save3$treatment=="overdispersed"])
#### Note, this test is quite liberal because it does not discriminate between distances
within and between experimental units

 Welch Two Sample t-test
data: Jaccard [save3$treatment == "aggregated"] and save3$ratio[save3$treatment ==
"overdispersed"] t = -2.0318, df = 144.03, p-value = 0.04401
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.0674768330 -0.0009298034
sample estimates:
mean of x mean of y
0.4921048 0.5263081
treatment
```

```
mypredictor
Residuals
---
Df Sum Sq  Mean Sq F value Pr(>F)
 1 0.0037 0.003711   0.921 0.3385
 2 0.0323 0.016133   4.002 0.0197 *
            207 0.8345 0.004032
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

References

Aragón R, Oesterheld M, Irisarri G, Texeira M. **2011**. Stability of ecosystem functioning and diversity of grasslands at the landscape scale. *Landscape Ecology* **26**: 1011–1022.

Augé RM. **2001**. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.

Avio L, Njeru EM, Oehl F, Turrini A, Bocci G, Bàrberi P, Giovannetti M, Sbrana C. **2020**. Small-scale soil heterogeneity affects the distribution of arbuscular mycorrhizal fungal species in a hot-spot field in a Mediterranean site. *Applied Soil Ecology* **154**: 103631.

Azcón-Aguilar C, Barea J. **1996**. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens–an overview of the mechanisms involved. *Mycorrhiza* **6**: 457–464.

Bahadur A, Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q, Pan J, Liu Y, Feng H. **2019**. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *International Journal of Molecular Sciences* **20**: 4199.

Barbier S, Gosselin F, Balandier P. **2008**. Influence of tree species on understory vegetation diversity and mechanisms involved-A critical review for temperate and boreal forests. *Forest Ecology and Management* **254**: 1–15.

Bar-Massada A. **2015**. Complex relationships between species niches and environmental heterogeneity affect species co-occurrence patterns in modelled and real communities. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20150927.

Barto EK, Weidenhamer JD, Cipollini D, Rillig MC. **2012**. Fungal superhighways: Do common mycorrhizal networks enhance below ground communication? *Trends in Plant Science* **17**: 633–637.

Bjornstad ON. **2018**. ncf: Spatial Covariance Functions. R package version 1.2-6.

Boeraeve M, Honnay O, Jacquemyn H. **2019**. Local abiotic conditions are more important than landscape context for structuring arbuscular mycorrhizal fungal communities in the roots of a forest herb. *Oecologia* **190**: 149–157.

Bonfante P, Anca IA. **2009**. Plants, mycorrhizal fungi, and bacteria: A network of interactions. *Annual Review of Microbiology* **63**: 363–383.

Brundrett MC, Tedersoo L. **2018**. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* **220**: 1108–1115.

Bruns TD, Taylor JW. **2016**. Comment on "Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism." *Science* **351**: 826.

de Cáceres M, Legendre P. **2009**. Associations between species and groups of sites: Indices and statistical inference. *Ecology* **90**: 3566–3574.

Cadotte MW. **2007**. Competition-colonization trade-offs and disturbance effects at multiple scales. *Ecology* **88**: 823–829.

Camenzind T, Hempel S, Homeier J, Horn S, Velescu A, Wilcke W, Rillig MC. **2014**. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology* **20**: 3646–3659.

Caruso T. **2018**. Disentangling the factors shaping arbuscular mycorrhizal fungal communities across multiple spatial scales. *New Phytologist* **220**: 954–956.

Caruso T, Powell JR, Rillig MC. **2012**. Compositional divergence and convergence in local communities and spatially structured landscapes. *PLoS ONE* **7**: 1115–1124.

Chagnon PL, Bradley RL, Klironomos JN. **2014**. Plant-fungal symbioses as ecological networks: The need to characterize more than just interaction patterns. *Fungal Ecology* **12**: 10–13.

Chaudhary VB, Lau MK, Johnson NC. **2008**. Macroecology of Microbes-Biogeography of the Glomeromycota. In: Mycorrhiza. Berlin, Heidelberg: Springer, 529–563.

Chaudhary VB, Nolimal S, Sosa-Hernández MA, Egan C, Kastens J. **2020**. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. *New Phytologist* **228**: 238–252.

Chaudhary VB, Rúa MA, Antoninka A, Bever JD, Cannon J, Craig A, Duchicela J, Frame A, Gardes M, Gehring C, *et al.* 2016. Data Descriptor: MycoDB, a global database of plant response to mycorrhizal fungi Background & Summary. *Scientific data* **3**: 1–10.

Chen YL, Xu ZW, Xu T Le, Veresoglou SD, Yang GW, Chen BD. **2017**. Nitrogen deposition and precipitation induced phylogenetic clustering of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry* **115**: 233–242.

Chiarello M, McCauley M, Villéger S, Jackson CR. **2022**. Ranking the biases: The choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold. *PLoS ONE* **17**.

Christian N, Whitaker BK, Clay K. **2015**. Microbiomes: Unifying animal and plant systems through the lens of community ecology theory. *Frontiers in Microbiology* **6**: 1–15.

Cordero RD, Jackson DA. **2019**. Species-pair associations, null models, and tests of mechanisms structuring ecological communities. *Ecosphere* **10**: e02797.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman D a. **2012**. The application of ecological theory toward an understanding of the human microbiome. *Science* **336**: 1255–62.

Davison J, Ainsaar L, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, Johnson NC, Kane A, Koorem K, Kochar M, *et al.* 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very lowendemism. *Science* **349**: 970–973.

Davison J, Moora M, Semchenko M, Adenan SB, Ahmed T, Akhmetzhanova AA, Alatalo JM, Al-Quraishy S, Andriyanova E, Anslan S, *et al.* 2021. Temperature and pH define the realized niche space of arbuscular mycorrhizal fungi. *New Phytologist*.

Davison J, Öpik M, Daniell TJ, Moora M, Zobel M. **2011**. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology* **78**: 103–115.

Davison J, Öpik M, Zobel M, Vasar M, Metsis M, Moora M. **2012**. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. *PLoS ONE* **7**: e41938.

Deveautour C, Donn S, Bennett AE, Power S, Powell JR. **2021**. Variability of arbuscular mycorrhizal fungal communities within the root systems of individual plants is high and influenced by host species and root phosphorus. *Pedobiologia* **84**: 150691.

Dickie IA, Fitzjohn RG. **2007**. Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: A methods review. *Mycorrhiza* **17**: 259–270.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T. **2011**. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist* **190**: 794–804.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. **2010a**. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal* **4**: 337–345.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. **2010b**. Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: Is there a role for stochastic processes? *Journal of Ecology* **98**: 419–428.

Edgar RC. **2013**. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Egan C, Li DW, Klironomos J. **2014**. Detection of arbuscular mycorrhizal fungal spores in the air across different biomes and ecoregions. *Fungal Ecology* **12**: 26–31.

Egerton-Warburton LM, Johnson NC, Allen EB. **2007**. Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. *Ecological Monographs* **77**: 527–544.

Emmett BD, Lévesque-Tremblay V, Harrison MJ. **2021**. Conserved and reproducible bacterial communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. *ISME Journal* **15**: 2276–2288.

Evelin H, Kapoor R, Giri B. **2009**. Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. *Annals of Botany* **104**: 1263–1280.

Facelli E, Facelli JM. **2002**. Soil phosphorus heterogeneity and mycorrhizal symbiosis regulate plant intra-specific competition and size distribution. *Oecologia* **133**: 54–61.

Fodelianakis S, Valenzuela-Cuevas A, Barozzi A, Daffonchio D. **2021**. Direct quantification of ecological drift at the population level in synthetic bacterial communities. *ISME Journal* **15**: 55–66.

Frey-Klett P, Garbaye J, Tarkka M. **2007**. The mycorrhiza helper bacteria revisited. *New Phytologist* **176**: 22–36.

Gange AC, Bower E, Stagg PG, Aplin DM, Gillam AE, Bracken M. **1999**. A comparison of visualization techniques for recording arbuscular mycorrhizal colonization. *New Phytologist* **142**: 123–132.

Garbaye J. **1994**. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *Acta Botanica Gallica* **128**: 197–210.

Gilliam FS. **2007**. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* **57**: 845–858.

Goldmann K, Boeddinghaus RS, Klemmer S, Regan KM, Heintz-Buschart A, Fischer M, Prati D, Piepho HP, Berner D, Marhan S, *et al.* 2020. Unraveling spatiotemporal variability of

arbuscular mycorrhizal fungi in a temperate grassland plot. *Environmental Microbiology* **22**: 873–888.

Gotelli NJ. **2000**. Null model analysis of species co-occurrence patterns. *Ecology* **81**: 2606–2621.

Gotelli N, Hart E, Ellison A, Maintainer]. **2015**. Package 'EcoSimR' - Null Model Analysis for Ecological Data. *R package*.

Grünfeld L, Mola M, Wulf M, Hempel S, Veresoglou SD. **2021**. Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest. *Mycorrhiza* **31**: 589–598.

Grünfeld L, Skias G, Rillig MC, Veresoglou SD. **2022**. Arbuscular mycorrhizal root colonization depends on the spatial distribution of the host plants. *Mycorrhiza*: 1–9.

Grünfeld L, Wulf M, Rillig MC, Manntschke A, Veresoglou SD. **2019**. Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. *New Phytologist* **227**: 10–13.

Hammer EC, Pallon J, Wallander H, Olsson PA. **2011**. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology* **76**: 236–244.

Hart MM, Antunes PM, Chaudhary VB, Abbott LK. **2018**. Fungal inoculants in the field: Is the reward greater than the risk? *Functional Ecology* **32**: 126–135.

Hart MM, Reader RJ. **2005**. The role of the external mycelium in early colonization for three arbuscular mycorrhizal fungal species with different colonization strategies. *Pedobiologia* **49**: 269–279.

Hart MM, Trevors JT. **2005**. Microbe management: Application of mycorrhyzal fungi in sustainable agriculture. *Ecology and the Environment* **3**: 533–539.

Hausmann NT, Hawkes C v. 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. *New Phytologist* **183**: 1188–1200.

Hazard C, Gosling P, van der Gast CJ, Mitchell DT, Doohan FM, Bending GD. **2013**. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. *ISME Journal* **7**: 498–508.

Van Der Heijden MGA. **2004**. Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. *Ecology Letters* **7**: 293–303.

van der Heijden MGA, Bardgett RD, van Straalen NM. **2008**. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Van Der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. **1998**. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. **2015**. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist* **205**: 1406–1423.

Hein CL, Öhlund G, Englund G. **2014**. Fish introductions reveal the temperature dependence of species interactions. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20132641.

Helgason T, Fitter AH, Young JPW. **1999**. Molecular diversity of arbuscular mycorrhizal fungi colonising Hyacinthoides non-scripta (bluebell) in a seminatural woodland. *Molecular Ecology* **8**: 659–666.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. **2002**. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**: 371–384.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, *et al.* 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394–407.

Horn S, Caruso T, Verbruggen E, Rillig MC, Hempel S. **2014**. Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. *ISME Journal* **8**: 2231–2242.

Horn S, Hempel S, Verbruggen E, Rillig MC, Caruso T. **2017**. Linking the community structure of arbuscular mycorrhizal fungi and plants: A story of interdependence? *ISME Journal* **11**: 1400–1411.

Hu Y, Veresoglou SD, Tedersoo L, Xu T, Ge T, Liu L, Chen Y, Hao Z, Su Y, Rillig MC, *et al.* 2019. Contrasting latitudinal diversity and co-occurrence patterns of soil fungi and plants in forest ecosystems. *Soil Biology and Biochemistry* **131**: 100–110.

Hubbell SP. **2001**. The unified neutral theory of biodiversity and biogeography. In: Monographs in population biology. Princeton University Press.

Janos DP. **2007**. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza* **17**: 75–91.

Johnson DW, Grorud-Colvert K, Sponaugle S, Semmens BX. **2014**. Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. *Ecology Letters* **17**: 743–755.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. **2010**. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences* **107**: 2093–2098.

Joos L, Beirinckx S, Haegeman A, Debode J, Vandecasteele B, Baeyen S, Goormachtig S, Clement L, de Tender C. **2020**. Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. *BMC Genomics* **21**: 1–17.

Kadowaki K, Yamamoto S, Sato H, Tanabe AS, Hidaka A, Toju H. **2018**. Mycorrhizal fungi mediate the direction and strength of plant–soil feedbacks differently between arbuscular mycorrhizal and ectomycorrhizal communities. *Communications Biology* **1**: 1–11.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–883.

Kircher M, Sawyer S, Meyer M. **2012**. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Research* **40**: 1–8.

Kivlin SN, Hawkes C V. 2016. Tree species, spatial heterogeneity, and seasonality drive soil fungal abundance, richness, and composition in Neotropical rainforests. *Environmental Microbiology* **18**: 4662–4673.

Klichowska E, Nobis M, Piszczek P, Błaszkowski J, Zubek S. **2019**. Soil properties rather than topography, climatic conditions, and vegetation type shape AMF–feathergrass relationship in semi-natural European grasslands. *Applied Soil Ecology* **144**: 22–30.

Klironomos JN, Moutoglis P. **1999**. Colonization of nonmycorrhizal plants by mycorrhizal neighbours as influenced by the collembolan, Folsomia candida. *Biology and Fertility of Soils* **29**: 277–281.

Kohout P, Doubková P, Bahram M, Suda J, Tedersoo L, Voříšková J, Sudová R. **2015**. Niche partitioning in arbuscular mycorrhizal communities in temperate grasslands: A lesson from adjacent serpentine and nonserpentine habitats. *Molecular Ecology* **24**: 1831–1843.

Koske RE, Gemma JN, Corkidi L, Sigüenza C, Rincón E. **2004**. Arbuscular mycorrhizas in coastal dunes. In: Martínez ML, Psuty NP, eds. Coastal dunes, Ecology and Conservation. Berlin Heidelberg: Springer-Verlag, 173–187.

Van der Krift TAJ, Berendse F. **2001**. The effect of plant species on soil nitrogen mineralization. *Journal of Ecology* **89**: 555–561.

LeBrun ES, Taylor DL, King RS, Back JA, Kang S. **2018**. Rivers may constitute an overlooked avenue of dispersal for terrestrial fungi. *Fungal Ecology* **32**: 72–79.

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, *et al.* 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology letters* **7**: 601–613.

Leifheit EF, Veresoglou SD, Lehmann A, Morris EK, Rillig MC. **2014**. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation-a meta-analysis. *Plant and Soil* **374**: 523–537.

Leinster T, Cobbold CA. **2012**. Ecological Society of America Measuring diversity: the importance of species similarity. *Source: Ecology* **93**: 477–489.

Lekberg Y, Rosendahl S, Olsson PA. **2015**. The fungal perspective of arbuscular mycorrhizal colonization in 'nonmycorrhizal'plants. *New Phytologist* **205**: 1399–1403.

Lekberg Y, Schnoor T, Kjøller R, Gibbons SM, Hansen LH, Al-Soud WA, Sørensen SJ, Rosendahl S. **2012**. 454-Sequencing Reveals Stochastic Local Reassembly and High Disturbance Tolerance Within Arbuscular Mycorrhizal Fungal Communities. *Journal of Ecology* **100**: 151–160.

Liang M, Johnson D, Burslem DFRP, Yu S, Fang M, Taylor JD, Taylor AFS, Helgason T, Liu X. **2020**. Soil fungal networks maintain local dominance of ectomycorrhizal trees. *Nature Communications* **11**: 1–7.

Liu Y, He J, Shi G, An L, Öpik M, Feng H. **2011**. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. *FEMS Microbiology Ecology* **78**: 355–365.

Luo Z, Wang E, Sun OJ. **2016**. A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. *Soil Biology and Biochemistry* **101**: 96–103.

van der Maarel E. **2007**. Transformation of cover-abundance values for appropriate numerical treatment – Alternatives to the proposals by Podani. *Journal of Vegetation Science* **18**: 767–770.

Maciel Rabelo Pereira C, López-García Á, da Silva DKA, Costa Maia L, Guldberg Frøslev T, Kjøller R, Rosendahl S. **2020**. Tropical forest type influences community assembly processes in arbuscular mycorrhizal fungi. *Journal of Biogeography* **47**: 434–444.

Madritch MD, Greene SL, Lindroth RL. **2009**. Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. *Oecologia* **160**: 119–127.

Maherali H, Klironomos JN. **2007**. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* **316**: 1746–1748.

Maitra P, Zheng Y, Chen L, Wang YL, Ji NN, Lü PP, Gan HY, Li XC, Sun X, Zhou XH, *et al.* 2019. Effect of drought and season on arbuscular mycorrhizal fungi in a subtropical secondary forest. *Fungal Ecology* **41**: 107–115.

Manoharan L, Rosenstock NP, Williams A, Hedlund K. **2017**. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. *Applied Soil Ecology* **115**: 53–59.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. **1990**. A new method which gives an objective measure of colonization of roots by vesicular- Arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

Mihaljevic JR. **2012**. Linking metacommunity theory and symbiont evolutionary ecology. *Trends in Ecology and Evolution* **27**: 323–329.

Mummey DL, Rillig MC. **2008**. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate g. *FEMS Microbial Ecology* **64**: 260–270.

Naaf T, Wulf M. **2010**. Habitat specialists and generalists drive homogenization and differentiation of temperate forest plant communities at the regional scale. *Biological Conservation* **143**: 848–855.

Newshan K, Fitter A, Watkinson A. **1995**. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Tree* **10**: 407–411.

Ning D, Deng Y, Tiedje JM, Zhou J. **2019**. A general framework for quantitatively assessing ecological stochasticity. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 16892–16898.

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK. **2013**. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environmental Microbiology* **15**: 1870–1881.

Olsson PA, Bååth E, Jakobsen I. **1997**. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. *Applied and Environmental Microbiology* **63**: 3531–3538.

Öpik AM, Metsis M, Daniell TJ, Zobel M, Moora M. **2009**. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424–437.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. **2010**. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Palmer MW, McGlinn DJ, Westerberg L, Milberg P. **2008**. Indices for detecting differences in species composition: Some simplifications of RDA and CCA. *Ecology* **89**: 1769–1771.

Pánková H, Lepinay C, Rydlová J, Voková A, Janoušková M, Dostálek T, Münzbergová Z. **2018**. Arbuscular mycorrhizal fungi and associated microbial communities from dry grassland do not improve plant growth on abandoned field soil. *Oecologia* **186**: 677–689.

Paudel S, Longcore T, MacDonald B, McCormicK MK, Szlavecz K, Wilson GWT, Loss SR. **2016**. Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. *Ecology* **97**: 605–614.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C. **2019**. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Paz C, Öpik M, Bulascoschi L, Bueno CG, Galetti M. **2021**. Dispersal of Arbuscular Mycorrhizal Fungi: Evidence and Insights for Ecological Studies. *Microbial Ecology* **81**: 283–292.

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelotto M. **2007**. A strong species-area relationship for eukaryotic soil microbes: Island size matters for ectomycorrhizal fungi. *Ecology Letters* **10**: 470–480.

Peay KG, Garbelotto M, Bruns TD, Bruns1 TD. **2010**. Evidence of dispersal limitation in soil organisms: Isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.

Phillips JM, Hayman DS. **1970**. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-IN18.

Pinheiro J, Bates D, DebRoy S, Sarkar D. **2007**. nlme: Linear and Nonlinear Mixed Effects Models. *R package version* **3**: 1–89.

Powell JR, Bennett AE. **2016**. Unpredictable assembly of arbuscular mycorrhizal fungal communities. *Pedobiologia* **59**: 11–15.

Powell JR, Rillig MC. **2018**. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist* **220**: 1059–1075.

Rasmussen PU, Hugerth LW, Blanchet FG, Andersson AF, Lindahl BD, Tack AJM. **2018**. Multiscale patterns and drivers of arbuscular mycorrhizal fungal communities in the roots and root-associated soil of a wild perennial herb. *New Phytologist* **220**: 1248–1261.

Redecker D, Kodner R, Graham LE. **2000**. Glomalean fungi from the Ordovician. *Science* **289**: 1920–1921.

Reiss J, Bridle JR, Montoya JM, Woodward G. **2009**. Emerging horizons in biodiversity and ecosystem functioning research. *Trends in Ecology and Evolution* **24**: 505–514.

Riaz M, Kamran M, Fang Y, Wang Q, Cao H, Yang G, Deng L, Wang Y, Zhou Y, Anastopoulos I, *et al.* 2021. Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. *Journal of Hazardous Materials* **402**: 123919.

Rillig M, Aguilar-Trigueros CA, Camenzind T, Cavagnaro TR, Degrune F, Hohmann P, Lammel DR, Mansour I, Roy J, van der Heijden MGA, *et al.* 2019. Why farmers should manage the arbuscular mycorrhizal symbiosis. *New Phytologist* **222**: 1171–1175.

Rillig MC, Mummey DL. **2006**. Mycorrhizas and soil structure. *New Phytologist* **171**: 41–53.

Rillig MC, Sosa-Hernández MA, Roy J, Aguilar-Trigueros CA, Vályi K, Lehmann A. **2016**. Towards an integrated mycorrhizal technology: Harnessing mycorrhiza for sustainable intensification in agriculture. *Frontiers in Plant Science* **7**: 1–5.

Roeschmann G. **1971**. Die Böden der nordwestdeutschen Geest-Landschaft. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft* **13**: 151–231.

Rożek K, Rola K, Błaszkowski J, Leski T, Zubek S. **2020**. How do monocultures of fourteen forest tree species affect arbuscular mycorrhizal fungi abundance and species richness and composition in soil? *Forest Ecology and Management* **465**: 118091.

Ruiz-Lozano JM, Porcel R, Bárzana G, Azcón R, Aroca R. **2012**. Contribution of arbuscular mycorrhizal symbiosis to plant drought tolerance: State of the art. In: Plant Responses to Drought Stress: From Morphological to Molecular Features. Springer-Verlag Berlin Heidelberg, 335–362.

Sangwan S, Prasanna R. **2021**. Mycorrhizae helper bacteria: Unlocking their potential as bioenhancers of plant–arbuscular mycorrhizal fungal associations. *Microbial Ecology*: 1–10.

Schmidt M, Kriebitzsch W, Ewald J. **2011**. *Waldartenlisten der Farn- und Blütenpflanzen, Moose und Flechten Deutschlands* (M Schmidt, Ed.). Bonn: BfN.

Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA. **2011**. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* **21**: 211–220.

Scotton M. **2019**. Mountain grassland restoration: Effects of sowing rate, climate and soil on plant density and cover. *Science of the Total Environment* **651**: 3090–3098.

Shoemaker LG, Sullivan LL, Donohue I, Cabral JS, Williams RJ, Mayfield MM, Chase JM, Chu C, Harpole WS, Huth A, *et al.* 2020. Integrating the underlying structure of stochasticity into community ecology. *Ecology* **101**: 1–17.

Silva MOD, Pernthaler J. **2020**. Biomass addition alters community assembly in ultrafiltration membrane biofilms. *Scientific Reports* **10**: 1–10.

Smith SE, Read D. **2008**. *Mycorrhizal symbiosis*. Academic Press New York.

Sowińska-Świerkosz BN, Soszyński D. **2014**. Landscape structure versus the effectiveness of nature conservation: Roztocze region case study (Poland). *Ecological Indicators* **43**: 143–153.

Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, *et al.* 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**: 1028–1046.

Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. **2003**. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C. *Science* **300**: 1138–1140.

Su Y-Y, Sun X, Guo L-D. **2011**. Seasonality and host preference of arbuscular mycorrhizal fungi of five plant species in the inner Mongolia steppe, China. *Brazilian Journal of Microbilogy* **42**: 57–65.

Sutherland WJ, Freckleton RP, Godfray HCJ, Beissinger SR, Benton T, Cameron DD, Carmel Y, Coomes DA, Coulson T, Emmerson MC, *et al.* 2013. Identification of 100 fundamental ecological questions. *Journal of Ecology* **101**: 58–67.

Toljander JF, Artursson V, Paul LR, Jansson JK, Finlay RD. **2006**. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. *FEMS Microbiology Letters* **254**: 34–40.

Tsunoda T, Kachi N, Suzuki JI. **2014**. Interactive effects of soil nutrient heterogeneity and belowground herbivory on the growth of plants with different root foraging traits. *Plant and Soil* **384**: 327–334.

Ulrich W, Piwczyński M, Maestre FT, Gotelli NJ. **2012**. Null model tests for niche conservatism, phylogenetic assortment and habitat filtering. *Methods in Ecology and Evolution* **3**: 930–939.

Unterseher M, Jumpponen A, Öpik M, Tedersoo L, Moora M, Dormann CF, Schnittler M. **2011**. Species abundance distributions and richness estimations in fungal metagenomics - Lessons learned from community ecology. *Molecular Ecology* **20**: 275–285.

Vályi K, Mardhiah U, Rillig MC, Hempel S. **2016**. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. *ISME Journal* **10**: 2341–2351.

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW. **2002**. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology* **11**: 1555–1564.

Vašutová M, Mleczko P, López-García A, Maček I, Boros G, Ševčík J, Fujii S, Hackenberger D, Tuf IH, Hornung E, *et al.* 2019. Taxi drivers: the role of animals in transporting mycorrhizal fungi. *Mycorrhiza* **29**: 413–434.

Verbruggen E, van der Heijden MGA, Weedon JT, Kowalchuk GA, Röling WFM. **2012**. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Molecular Biology* **21**: 2341–2353.

Veresoglou SD, Caruso T, Rillig MC. **2012**. Metacommunities and symbiosis: hosts of challenges. *Trends in Ecology and Evolution* **27**: 588–589.

Veresoglou SD, Caruso T, Rillig MC. **2013**. Modelling the environmental and soil factors that shape the niches of two common arbuscular mycorrhizal fungal families. *Plant and Soil* **368**: 507–518.

Veresoglou SD, Grünfeld L, Mola M. **2021**. Micro-landscape dependent changes in arbuscular mycorrhizal fungal community structure. *Applied Science* **11**: 5297.

Veresoglou SD, Halley JM. **2012**. A model that explains diversity patterns of arbuscular mycorrhizas. *Ecological Modelling* **231**: 146–152.

Veresoglou SD, Powell JR, Davison J, Lekberg Y, Rillig MC. **2014**. The Leinster and Cobbold indices improve inferences about microbial diversity. *Fungal Ecology* **11**: 1–7.

Veresoglou SD, Rillig MC. **2012**. Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. *Biology Letters* **8**: 214–217.

Veresoglou SD, Rillig MC. **2014**. Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant and Soil* **377**: 395–406.

Veresoglou SD, Sen R, Mamolos AP, Veresoglou DS. **2011**. Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. *Journal of Ecology* **99**: 1339–1349.

Veresoglou SD, Verbruggen E, Makarova O, Mansour I, Sen R, Rillig MC. **2019**. Arbuscular Mycorrhizal Fungi Alter the Community Structure of Ammonia Oxidizers at High Fertility via Competition for Soil NH4+. *Microbial Ecology* **78**: 147–158.

Veresoglou SD, Wulf M, Rillig MC. **2017**. Facilitation between woody and herbaceous plants that associate with arbuscular mycorrhizal fungi in temperate European forests. *Ecology and Evolution* **7**: 1181–1189.

de Vrieze J, de Mulder T, Matassa S, Zhou J, Angenent LT, Boon N, Verstraete W. **2020**. Stochasticity in microbiology: managing unpredictability to reach the Sustainable Development Goals. *Microbial Biotechnology* **13**: 829–843.

Weremijewicz J, Janos DP. **2013**. Common mycorrhizal networks amplify size inequality in Andropogon gerardii monocultures. *New Phytologist* **198**: 203–213.

Wilson DS. **1992**. Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. *Ecology* **73**: 1984–2000.

Winegardner AK, Jones BK, Ng ISY, Siqueira T, Cottenie K. **2012**. The terminology of metacommunity ecology. *Trends in Ecology and Evolution* **27**: 253–254.

Wolfe BE, Mummey DL, Rillig MC, Klironomos JN. **2007**. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. *Mycorrhiza* **17**: 175–183.

Wulf M. **1992**. *Vegetationskundliche und ökologische Untersuchungen zum Vorkommen gefährdeter Pflanzenarten in Feuchtwäldern Nordwestdeutschlands*. Stuttgart, Germany: Schweizerbart Science Publishers.

Zhang S, Lehmann A, Zheng W, You Z, Rillig MC. **2019**. Arbuscular mycorrhizal fungi increase grain yields: a meta-analysis. *New Phytologist* **222**: 543–555.

Zobel M, Öpik M. **2014**. Plant and arbuscular mycorrhizal fungal (AMF) communities - which drives which? *Journal of Vegetation Science* **25**: 1133–1140.

Zubek S, Rożek K, Stefanowicz AM, Błaszkowski J, Stanek M, Gielas I, Rola K. **2021**. The impact of beech and riparian forest herbaceous plant species with contrasting traits on arbuscular mycorrhizal fungi abundance and diversity. *Forest Ecology and Management* **492**: 119245.