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PART 1

General introduction

Temperate forests are of utmost importance for the global climate and play a vital role in supplying a large portion of the worlds' population with food and energy resources (Karjalainen et al., 2009; Tillman, 1978). They are characterized as being located between 25 and 55° N and S of the equator and span large portions of the current earths land masses. These forests are mainly located in Europe, North America, parts of South America (southern Chile and Argentina), north-eastern Asia and New Zealand and estimated to span 767 Mha land area (Lal & Lorenz, 2012). Their vegetation is commonly composed of broad-leaved deciduous trees and evergreen conifers, which are often found in mixed assemblages. Due to intense seasonality primary productivity fluctuates and phenological effects on observed diversity are common.

Humans rely on temperate forests for a variety of reasons. They significantly regulate local climates and can buffer extreme climatic events (Figueroa & Pasten, 2015). Forests plant, animal and microbial materials are used globally as medicinal and food sources (Karjalainen et al., 2009). They also vitally contribute to human health as they promote physical and mental health by reducing stress and function as sources for diverse natural pharmacies. Timber is of enormous economic value as it has many structural purposes and is an essential energy resource for large portions of humanity. As it is regenerative it does not inherently promote climate change such as the use of fossil fuels (Tillman, 1978).

During the ice ages of the Pleistocene ice shields covered large portions of land, which are currently home to most temperate forests. Tree community compositions in these regions exhibit legacy effects, meaning measureable impacts of historic influences. Models of species distributions compared with real world species distributions have shown that the realized extent of many European tree species do not reach the full potentially viable habitats, which would climatically suit them. It is likely that this pattern is due to legacies from the last glacial maximum and its glaciers which covered large parts of Europe north of the alps (Svenning & Skov,

2007). These dynamics need to be considered in any long-term predictions based on current observational data.

Anthropogenic influences have induced rapid changes severely impacting the future development of forests around the globe. Climate change and land use change threaten ecosystems globally and research into natural systems health and their projected future is of utmost importance. Temperate forests harbour a significant portion of terrestrial carbon stocks and moist temperate forests have been found to contain the highest carbon densities by area for terrestrial forests (Keith et al., 2009). Deforestation and degradation fuel climate change significantly and their reduction is key to climate change mitigation efforts. Current levels of global forest losses constitute a major threat to earths CO2 budget. Around 80% of deforestation is estimated to be driven by land conversion for agricultural purposes. Forests currently act as a major terrestrial carbon sink and absorb about 30% of anthropogenic CO2 excess (Canadell et al., 2007; Canadell & Raupach, 2008). Forests store more carbon in their biomass than the atmospheric stock holds and span 4 billion hectares, about 30% of the terrestrial land mass (Forestry Economics and Policy Division & Forest management Division, 2006). One direct consequence of climate change are extended drought periods and heat waves, which increase the risks of forest fires (Flannigan et al., 2009). It has been shown that increases in forest fires induce shifts in plant community compositions, leading to altered ecosystems and potential shifts in ecosystem functioning and stability (Fairman et al., 2015).

Four strategies have been proposed to utilize forest systems to mitigate effects of increasing atmospheric CO2 and consequent climate change: First, increasing forest coverage by reforestation; Second, increasing carbon density in extant forest systems; Third, expanding the use of forest products (such as timber); Fourth, reducing emissions caused by deforestation and degradation (Canadell & Raupach, 2008). It is clear that sustainable forest management is a necessary component of future efforts in climate change mitigation as they contain the capacity to store billions of tons of CO2 per year globally, which no other form of carbon capture and storage proposed to date could even hope to achieve (Canadell & Raupach, 2008; Clery et al., 2021).

An additional threat to forests is posed by invasive species. The most common and disruptive groups are insects, plants and microbial pathogens. Due to anthropogenic influences the so called ‘biotic homogenization’ has been altering the earths biosphere for centuries (Smart et al., 2006). Biological invasions are proposed to be the most significant environmental threat to the maintenance of natural forest ecosystems leading to permanent alterations in ecosystems community composition (Liebhold et al., 1995). Effects of environmental pollutants on forest health vary greatly in impact and intensity and have been reviewed and surmised in (W. H. Smith, 2012). Specific effects of nitrogen deposition will be further elaborated on in the final section.

All mentioned effects have the potential to alter forest composition, geographical extent and ecosystem functioning and need to be integrated into predictive models. As mentioned, the maintenance and expansion of forest systems is of utmost importance. Among others shifts in European forest types are currently observed with deciduous trees moving to higher elevations previously dominated by Norway spruce (Hanewinkel et al., 2013; Lexer et al., 2015).

A forests carbon storage does not only consist of tree (or vegetation) biomass. Soils contain a vast organismic and organo-chemical diversity and are inherently linked to aboveground carbon pools not only when it comes to decomposition of dead organic matter and nutrient remineralization but also through their direct interactions with vegetation. Belowground carbon stocks frequently exceed aboveground stocks, with certain estimates claiming them to harbour a three-fold stock of the earths’ vegetation (Kimble, 2003; Post et al., 1982). 60 gigatons of sequestered carbon, which nearly matches yearly terrestrial net primary productivity, are transferred to this belowground carbon pool, which stands in equilibrium with the amount of carbon respired in soils (Lal, 2008). Storage is especially pronounced in temperate cool forests where soils contain an average of 120 t C/ha (Pan et al., 2011). It is important to emphasize that forests soil organic carbon inputs are primarily plant derived (Drigo et al., 2008). The fate of this plant organic carbon input is heavily regulated by microbial activity (Drigo et al., 2008). Different forms of transformation processes lead to the accumulation of soil organic matter – globally the largest pool of terrestrial organic carbon (Jackson et al., 2017; Lal, 2008). The transformation and accumulation of this organic carbon is controlled by soil microbes. Organic matter

decomposition is only one facet of this process. Certain groups of root associated fungi play an important role in this accumulation of carbon, which will be explained in a subsequent section. Microbial decomposition of plant organic matter inputs can take many shapes. Different microbial groups show different saprotrophic capabilities. Impactful decomposers are often fungi and in the case of litter decomposition often Basidiomycetes. They generally show strong enzymatic capabilities producing extracellular enzymes capable of degrading plant tissues into polymers and further into oligo- and monomers (J. Schimel & Schaeffer, 2012). Their activity is heavily linked to litter properties such as moisture content and C/N stoichiometry (Parton et al., 2007; J. P. Schimel et al., 1999). Initial degradation of plant tissues, the primary form of organic matter transfer to soil microbial communities is often facilitated by bacterial groups and fungi such as Ascomycetes and Deuteromycetes which feed on less recalcitrant litter parts (Dilly et al., 2001; Kubartová et al., 2009). The decomposition of the bulk of litter tissues however is regulated by the saprotrophic capabilities of fungi that can access the lignocellulose matrix of litter which other microbes cannot (Dix & Webster, 1995; Ritz, 2004). This key step of litter degradation is mainly conducted by brown-rot and white-rot Basidiomycetes. Remaining recalcitrant components are thought to form belowground soil organic matter complexes. Litter decomposition is therefore a successional process. Additionally, it is likely that within these successional stages microbes of different metabolic capabilities form symbioses in the form of degrader cohorts (Lindemann, 2020).

Recently, a study was conducted investigating litter decompositions dependency on root presence and their interactions with fungi. Two treatments were chosen in a natural temperate forest, one excluding litter, the other excluding root access to decomposing litter. The goal was to investigate fungal biomass, community composition and community assembly and their relation to soil carbon. Only root activity (inferred through their presence) showed major impacts on communities and drove stochastic community assembly. It was associated with higher saprotrophic and ectomycorrhizal fungal biomass and soil carbon. This shows how fungi are impactful mediators in the conversion of aboveground and belowground plant inputs to soil organic matter and that plant root presence is a vital component of this transformation (Whalen et al., 2021). The activity of saprotrophic fungi is an essential

component of this step in nutrient cycling, but this study also emphasizes that symbiotic root fungal relationships heavily factor into temperate forests ecosystem functioning, as demonstrated by the significant increase in ectomycorrhizal biomass. Plant root exudations can have strong impacts on the microbial community in the rhizosphere leading to distinct microhabitats with distinct functional properties (Bertin et al., 2003). However, a great diversity has also been observed for non-environmental root-microbe symbioses, which can take different shapes with enormous impacts especially for temperate forest systems. In temperate forests most trees are known to partake in mycorrhizal symbiosis, where in exchange for energy rich photosynthates fungi enhance plant water and nutrient availability for their host. This symbiosis is known to greatly contribute to plant fitness and plays an important role in terrestrial ecosystem functioning. Ectomycorrhiza (ECM) is the dominant form of mycorrhiza in European temperate forests. In these soils it has been estimated that the majority of mycelial biomass comes from ECM fungi (Goldmann et al., 2015; Schröter et al., 2019). ECM receive around 20-30% of plant photo assimilates (Hobbie et al., 2012; Söderström & Read, 1987), which is thought to be the main flux of aboveground to belowground organic carbon (Godbold et al., 2006). The fact that ECM have evolved in multiple fungal and plant lineages (Tedersoo & Brundrett, 2017) and the fact that most tree species of temperate and boreal ecosystems are obligate partners in this symbiosis (Policelli et al., 2020a) emphasizes the importance of ECM in these systems. Ectomycorrhizal fungi inter-cellularly merge with plant root tips forming a matrix (the ‘Hartig net’), a physical structure with an outer mantel, from which extraradical hyphae or hyphal cords extend (Finlay, 2008). They have been shown to promote plant growth and health in a variety of different forms, which has been extensively reviewed by van der Heijden & Sanders (2003). In short, ECM fungi can have versatile enzymatic capabilities, mainly providing nitrogen compounds, an often limiting nutrient in these systems (Read & Perez-Moreno, 2003), to their hosts by scavenging and decomposing litter and soil organic matter (Pritsch & Garbaye, 2011). They are also known to protect plant roots from pathogen infections (J. A. Bennett et al., 2017; Marx, 1972).

Above I have mentioned how fungi, especially root associated fungi, heavily mediate carbon flux from aboveground to belowground pools. In temperate forest soils ECM are thought to be the most influential functional group effecting the accumulation of

soil carbon (Frey, 2019). Due to the large amount of carbon received by their plant symbionts (Hobbie et al., 2012; Söderström & Read, 1987) and the fact that they account for a large portion of microbial biomass in temperate and boreal systems it is not surprising that they strongly contribute to belowground carbon pools. Through the growth of extraradical hyphae photoassimilated carbon is transported throughout the soil matrix as mycelial tissues which then ultimately die. The resulting large amount of necromass enhances soil organic carbon matter input (Godbold et al., 2006; Schmidt et al., 2011). Additionally, ECMs capacity to decompose and scavenge for nitrogen containing compounds contributes to the stabilization of soil organic matter. Either by actively mining (through exoenzyme release) for these compounds or by passive absorption carbon rich compounds are left behind. Thereby the stoichiometry of the soil organic matter pool is shifted towards nitrogen depletion (Clemmensen et al., 2015; Lindahl & Tunlid, 2015; Nicolás et al., 2019). The remaining carbon rich organic matter is hard to degrade (due to its strong stoichiometric imbalance) and forms stable organo-mineral complexes increasing the overall recalcitrant soil carbon pool. The activity of ECM therefore controls the previously mentioned large soil carbon pools in temperate forests. It has been proposed that they might also benefit the capacity of forest soil carbon sinks in the face of climate change. To elaborate on the issue, a plants photosynthetic rate is constrained among others by the amount of atmospheric CO2. Rising CO2 levels therefore often show a fertilization effect on plant growth. As these levels rise with climate change other limiting factors such as nitrogen availability become restrictive. ECM have the capacity to supply nitrogen to their hosts liberating them from this limitation (Terrer et al., 2016). The inclusion of mycorrhizal processes has now found recognition in climate change models (Terrer et al., 2019). However a recent study has sowed doubt about this uniform effect correlating ECM and terrestrial carbon storage. In their meta-analysis Terrer and colleagues (2021) found a negative relationship between plant and soil carbon storage in ectomycorrhizal ecosystems. The underlying mechanism for this effect is debated and current global climate models do not account for it.

It is important to mention that ECM are a highly diverse group. Approximately 60 different fungal lineages have evolved from saprotrophic to ectomycorrhizal lifestyles. Certain similarities in genetic losses can be observed, but different groups

retain different enzymatic traits from their saprotrophic ancestors (Martin et al., 2016). Some ECM show little enzymatic activity altogether, while others can degrade a variety of tissues and soil organic matter compounds. There is debate as to which enzymes truly target which compounds and therefore it is hard to clearly characterize the nutrients a specific ECM species provides to its host (Zak et al., 2019). It has been proposed that proteases and laccases play an important role in soil organic matter decomposition (Rineau et al., 2016), while others highlight the importance of class II fungal peroxidases as they might be more potent in the degradation of soil organic matter complexes (Baskaran et al., 2017; Kyaschenko et al., 2017). It is likely that different ECM taxa exhibit vastly different functions in their provisions to their hosts and overall effects on nutrient pools and fluxes in their surroundings. A study conducted investigating transition between boreal forests and tundra suggested that ECM groups of two different exploration types (mycelial morphotypes) contributed do differing soil carbon stocks between the two ecosystems (Clemmensen et al., 2021). This suggests that ECM are not a functionally homogenous group and that a finer resolution for taxonomic groups is required to quantify ECMs effects on biogeochemical processes.

Aside their functional diversity ECM are also phylogenetically highly diverse. Plants colonized roots are characterized by diverse communities often with several dominant and many infrequent fungal species (Tedersoo et al., 2012). Certain taxa dominate high colonizer abundances such as Russulaceae and Thelephoraceae (Horton & Bruns, 2001) but this can vary depending on host species and ecosystem type. As for many symbiotic relationships a certain level of host preference is perceived in forests ectomycorrhizal symbioses (Ishida et al., 2007; Põlme et al., 2018). A strong host preference applies when two partners in a symbiotic relationship show very little variation in their potential interaction partners. It has been shown that ECM communities differ when assessed at higher levels of hosts phylogenetic relatedness, for example family level (Ishida et al., 2007). While this trend has been observed, ECM colonizers are generally extremely diverse. For four of the most common European temperate forests tree species (*Fagus sylvatica, Picea abies, Pinus sylvestris, Quercus spp.*) a meta-analysis has shown they can be colonized by 160 to 226 different ECM taxa (De Roman et al., 2005a). There are however major differences between tree species within a forest, with *Fagus sp.* being able to host up to eight times higher symbiont diversity compared to *Tilia spp.*

or *Carpinus betulus* (De Roman et al., 2005a; Timonen & Kauppinen, 2008). It is vital to understand how certain tree species and general forest tree composition interact with ECM and what diversity patterns certain groups produce. This requires investigation into symbiont community compositional differences within and between plant species as projected shifts in vegetation communities due to environmental change will change endemic mycorrhizal community composition which could impact ecosystem functioning and stability. On that note, effects primarily impacting ECM composition will likely also impact vegetation composition through these host-symbiont dependencies. Climate change is altering climate and vegetation zone shifts in communities towards tree species/genera with wider ecological amplitudes such as *Acer, Carpinus, Fraxinus* and *Tilia* (Marigo et al., 2000), for example due to lower summer precipitation (Geßler et al., 2007). In this regard, it is also necessary to investigate what environmental selective pressures could potentially alter host preference patterns and drive co-evolution of the ECM symbiosis. For example, it has been shown that severe habitats such as arctic climates do not promote host preference for mycorrhizal plants, which was interpreted as a reaction to present environmental conditions and implies benefits of more opportunistic, non-host-specific symbiotic relationships in harsh environments (Botnen et al., 2014a). Lastly, an altered vegetation community with increased plant diversity could also enhance ECM diversity due to an increase in potential hosts (Kernaghan et al., 2003). The question whether (and if so to what extent) ECM diversity is important for tree species and overall forest ecosystem functioning remains somewhat elusive (Leake, 2001), but as diversity is thought to correlate with functional redundancy and systems stability (i.e., resistance and resilience) certain benefits of increased system diversity come to mind.

While it is clear that the ECM symbiosis plays a central role in temperate forests, other forms of root fungal associations exist which can also greatly enhance plant fitness (Zuccaro et al., 2014). Although many root colonizers are obligate symbionts some can be facultative biotrophs even switching their functions entirely. Some ECM exhibit this trait but it is also seen in dark septate endophytes, an association with a paraphyletic group of Ascomycete fungi which can colonize roots intracelluclarly or intercellularly. They have been found in over 600 plant species (from 114 families) and have been shown to interact with ECM (Jumpponen & Trappe, 1998). They have been found globally and seem to occur in higher abundances in more stressed

environments but not much is known about the diversity and ecology of this symbiotic relationship (Mandyam & Jumpponen, 2005). A study has found that species from a common complex of dark septate endophytes showed very high host preference *Picea abies* over *Fraxinus Excelsior* and *Acer Pseudoplatanus* (Stroheker et al., 2021) indicating that host preference might play an important role in dark septate endophytes occurrence in temperate forest systems. Functionally studies have proven their capacity to break down organic substances (cellulose, starch, xylan and gelatine) and to protect against pathogens, herbivores and abiotic stress (heat) (Jumpponen & Trappe, 1998; Newsham, 1999, 2011).

Sebacinalean endophytes (Basidiomycota) are similarly elusive and produce a variety of associations be it biotrophic mycorrhizal, necrotrophic or symptomless endophytes. They can colonize an extremely diverse array of hosts and have been found globally producing no clear host or geographical preference patterns. They have been found to improve plant growth and resistance against biotic and abiotic stressors (Deshmukh et al., 2006; Waller et al., 2005; Weiß et al., 2011). While the mechanism has not been identified nutrient exchange between host and symbiont have been observed (Zuccaro et al., 2011, 2014). Due to their often plant growth promoting nature and sheer ubiquity it has been proposed that they 'may be a previously unrecognized universal hidden force in plant ecosystems' (Weiß et al., 2011). It is likely that further such elusive endophytic relationships exist benefitting plant fitness and ecosystem functioning in a multitude of ways. Apart from more understood variations of mycorrhiza (arbuscular-, arbutoid-, ecto-, ericoid-, monotropoid-, orchid- mycorrhiza) other endophytic association categories such as the previously mentioned dark septate endophytes, sebacinalean endophytes, fine root endophytes, fire associated and the non-colonizing symbiosis (also referred to as feremycorrhiza) have been described (Kariman et al., 2018).

Root-fungal associations can also be detrimental to plant fitness. Characterization of pathogenic and parasitic root fungal associations is also an important field of research as these pests can inflict major damage on plants and therefore entire ecosystems. Root rot, a disease in which roots gradually die off, is mostly caused by pathogenic microbes and heavily regulated by the humidity of the ambient soil. As this can potentially have major economic consequences for agricultural producers research has focused heavily on these systems. Common root infecting pathogens in forests are for example, the Armillaria root disease caused by species of the

eponymous genus (Basidiomycota) such as *Armillaria ostoyae*, the mainly spruce infecting *Inonotus tomentosus* and the pine infecting *Heterobasidion annosum*. The phylogenetic and functional diversity of this symbiotic relationship with tree roots has not been extensively studied. For an overview on root diseases in forest ecosystems see Laflamme (2010).

There is a vast unexplored diversity in root fungal associations and many of the known classifications of associations can actually vary in their ecology. Kairman and colleagues (2018) conducted an extensive literature review on this topic. Conventionally assumed saprotrophic fungal taxa have been found to form endophytic structures sometimes exhibiting plant growth promoting capabilities (Grelet et al., 2017; G. R. Smith et al., 2017). It has been proposed that taxa can produce intermediary strategies between saprotrophic and biotrophic (mycorrhizal) functions (Baldrian & Kohout, 2017; Selosse et al., 2009). Certain indicators for biotrophic lifestyles have been described such as the loss of genetic potential to degrade plant tissue. These genetic modifications are thought to enforce the switch from saprotrophic to symbiotrophic (Hacquard et al., 2016; Kohler et al., 2015; Martin et al., 2016). Another indicator for the symbiotic nature of an association can be the physical interface structure between host and endosymbiont. Large surface areas benefit nutrient exchange. Answers on the nature of root fungal associations require evidence, especially on the nutritional balance between both partners (Kariman et al., 2018; F. A. Smith & Smith, 1996). They can lie anywhere on the mutualism-parasitism spectrum and can even change depending on certain triggers (Klironomos, 2003).

Spatial analysis of the root fungal symbioses may enable the investigation and quantification of underlying neutral and ecological drivers. Molecular techniques have become much more affordable and allow the identification of diverse mycorrhizal colonizers from whole plants to root tips via amplicon sequencing of target marker regions. This enables the analysis of large community datasets which are required for detailed analysis into spatial community structures.

The distance decay of similarity, meaning the reduction in community similarity with increasing geographical distance, is a fundamental concept in biodiversity research (Whittaker, 1975). It can arise from deterministic processes such as environmental filtering (Cottenie, 2005) but also from neutral processes (Hubbell, 2011). The strength of these drivers in a given system determines the extent of observed

distance decay and is different between ecosystems and species (Soininen et al., 2007). It is imperative for studies aimed at investigating natural communities to consider the relevant scales of community similarity. Spatial autocorrelation - sample similarity merely due to spatial proximity – can lead to erroneous conclusions if it is neither taken into account for in sampling design, nor addressed and corrected for during analysis. Differences (changes in community composition) can be quantified by beta diveristy indices, such as the number of shared species, or dissimilarity measures and are mostly compared to the Euclidean distance between two sites. Rates and shape of the reduction in similarity are termed distance decay patterns and inform on community turnover. As mentioned, stochastic effects, for example random immigration events and following dispersal dynamics can also produce these patterns, which can be falsely interpreted as relevant species- or environmental relationships. To investigate to what extent drivers influence spatial patterns specific hypothesized processes and determinants need to be analyzed.

Tree species richness in temperate forests can be fairly low (for example compared to tropical systems) and individuals of species often span entire continuous forests so the description of tree community distance decay often makes more sense on larger geographic or global scales. The study of root fungal communities can be a lot more obscure. First, fungi can be highly diverse even on local scales, within the same plants and even root fragments. Hundreds of species each with potentially tens of individual genets can colonize a single tree individual (Bahram et al., 2011). This diversity can introduce noise into community analyses and impacts statistical power in species specific assessments. Second, unlike for macro-ecological surveys molecular methods for fungal community measurements do not capture species as fully distinct individuals. While detection of a species at two points one meter apart might be capturing the same connected mycelium this would not be the case for unicellular yeasts.

Distance decay patterns for soil microbial communities have been described for both prokaryotes and fungi (Barnes et al., 2016; Feng et al., 2019; Goldmann et al., 2016). Decay patterns of ECM have been extensively reviewed in scientific literature (Pickles et al., 2009; Pickles & Anderson, 2016; Wolfe et al., 2009). Here spatial organization and resulting community similarity distance decay have been observed from the smallest of scales to global scales (Ettema & Wardle, 2002). On a global scale evidence for typical island biogeographical distance decay patterns (commonly

found in macro ecological systems such as plant communities) have been found (Peay et al., 2012). These result from both enhanced dispersal capabilities via spores and selective pressures due to environmental variation in landscapes. Here ECM are primarily structured by host presence and abundance (Tedersoo et al., 2010, 2012), nutrient concentrations (Toljander et al., 2006) and climate (Bahram et al., 2012, 2013). Studies focused on local scales have also found significant spatial structuring (Lilleskov et al., 2004; Pickles et al., 2012). Spatial autocorrelation is highest in ranges from 0 to 3 meters (Lilleskov et al., 2004; Pickles et al., 2012). Co-occurrence analysis shows that species are segregated non-randomly, which could implicate competitional or environmental filtering effects impacting species spatial distribution (Pickles et al., 2012). Seasonal effects on ECM distance decay have also been observed suggesting an increase in decay towards the end of a growing season (Barnes et al., 2016). For non-ECM root associated fungi distance decay patterns are less clear. On continental and global scales a plants certain root endophytes seemed to be weakly structured with main effects likely driven by environmental variation. No clear distance decay pattern has been stated (Glynou et al., 2016; Jumpponen et al., 2017; Queloz et al., 2011). On few meter scales endophytes no obvious spatial clustering was perceived for a common complex of dark septate fungi (Grünig et al., 2002).
In a broader context, differences between soil inhabiting and root associated fungal distance decay patterns have been found to significantly differ with soil fungal communities showing stronger decay (Goldmann et al., 2016). Root fungal communities however were found to be mainly recruited from ambient soils (Goldmann et al., 2016).

A pioneer paper published in 2010 first confirmed the spatial structure and genetic evidence of the now accepted concept of common mycorrhizal networks (Beiler et al., 2010). The first postulations of its existence and evidential findings were put forward in the end of the 1990s (Graves et al., 1997; Robinson & Fitter, 1999; Simard et al., 1997) and showed labelled carbon flux between plant species. This has fuelled research into the ecology and overall impact these interconnecting networks have on overall forest ecosystem functioning. Also termed the wood-wide web this concept describes the fact that ectomycorrhizal symbionts (individual genets) can connect multiple plant individuals, even individuals of different plant

species, and have been found to mediate long distance interplant resource transfer (Simard & Durall, 2004) and interplant communication (Gilbert & Johnson, 2015; Gorzelak et al., 2015). Trees have also been found to promote seedling ECM colonization by either impacting the soils inoculum pool or possibly via these established common mycorrhizal networks (Dickie et al., 2002) and seedlings ECM diversity is related to their proximity to mature trees (Cline et al., 2005). First quantifications of carbon transfer showed how significant portions of assimilates were exchanged between tree individuals and species in a European temperate forest (Klein et al., 2016). In their similar follow up study Rog and colleagues (2020) conducted an experiment in a mixed forest in Switzerland, where they also labelled *Picea* trees with 13C and then examined surrounding organisms for the isotopic label. They found 13C enrichment in all surrounding tree species (*Fagus, Picea, Pinus, Larix*) tissues with levels of enrichment significantly higher for the phylogenetically more related the gymnosperms. When analyzing root tip diversity low host preference was found for most tree species although here too, phylogenetic conservation of community similarity was observed especially between *Picea* and *Pinus* trees. 50-70% of operational taxonomic units were associated with three or four of the host species and 90% of all at least with two. Sporocarp 13C signatures strongly indicated ECMs role in mediating this carbon transfer. It has become evident that common mycorrhizal networks play an important role in overall forest ecosystem functioning and as their activity relies on physical connections the scales at which they operate are likely impacted by ECM mycelium sizes. A related study subsequently was able to identify key mediators of the common mycorrhizal network between pines (*Pinus halepensis*) and oaks (*Quercus calliprinos*). Using DNA-SIP, a method to trace labelled carbon into synthesized DNA researchers identified different fungal groups involved in the transfer of photoassimilates. Interestingly, there were clear differences in the fungal taxa that were involved in inter- and intra- (plant) species transfer. *Tomentella ellisii* was dominant in mediation between oaks and pines, while *Pustularia spp.*, *Terfezia pini* and *Tuber oligospermum* facilitated transfer between pines (Cahanovitc et al., 2022).

Temperate forest ecosystems are a vital component of global biogeochemical element cycles and contribute greatly to earth's climate and health. A large component regulating these forests ecosystem functioning are located belowground.

Here trees undergo a variety of associations with fungi via their roots, which can greatly benefit plant fitness. These associations have co-evolved in many lineages and take a variety of different shapes, each form being specialized to a degree in their provision of certain resources, pathogenicity and in host preference. In general these diverse interactions lead to improved plant fitness and overall ecosystem functioning (Kariman et al., 2018). The ECM symbiosis is especially relevant for temperate forest ecosystems and ECM plant and fungal abundance is directly linked to forests soil carbon stocks (Soudzilovskaia et al., 2019). Anthropogenic influences have profound impacts on temperate forest ecosystems. Climate change is predicted to reduce these carbon stocks, potentially converting temperate forests to carbon sources (Liang et al., 2017; Steidinger et al., 2019). It induces shifts in extant forests geographic and elevational distribution (Lexer et al., 2015) and influences drought periods increasing wildfire frequency and severity (Flannigan et al., 2009), thereby reducing forest resilience (Stevens-Rumann et al., 2018). Increasing forest losses through land use change fragment habitats and reduce critical carbon stocks and the potential for the enhancement of sinks. These effects (aside others such as invasive species) massively influence vegetation community composition, which in itself can lead to hard to predict consequences. As reviewed above, temperate forest vegetation is critically linked to belowground root-fungal associations, which mediate a vast portion of the above- to belowground carbon flux and therefore their analysis is crucial to fully understand mechanisms and interdependencies shaping these ecosystems. From a microbial perspective too, human impact can also have devastating effects as is the case with nitrogen deposition: ECM (including their hosts) are predicted to massively reduce in global prevalence, which will lead to far reaching impacts on soil nutrient cycling and alter plant fitness, thereby impacting forest distributions and their residing communities (Lilleskov et al., 2019). It has also become clear that temperate forest ecosystems are highly interlinked and cannot be simplified to the study of their individual components. One key aspect of this complexity are common mycorrhizal networks, which are likely a key component in tree population dynamics and overall forest health. They highlight the importance of intra and inter plant species relationships, fungal host community patterns and the spatial distributions of either groups.

A temperate forest ecosystem consists of an intimately linked community of plant and fungal species. While each group can be effected by disturbances or environmental change in their own distinct way, knock on effects will always effect their counterparts. To gain a mechanistic understanding of these dependencies it is necessary to understand how plants and fungi react to perturbance as a whole system. Their association forms a highly dynamic complex system, which can only be truly understood by investigating its individual aspects in a holistic context. Central questions arise when considering this interface between these organism groups. How diverse are fungi on different tree species roots? How are they structured? How host specific (or preferential) are these associations? What are the spatio-temporal patterns and dynamics of these associations? How do they react to different forms of change? Investigating the underlying mechanisms of these associations will likely enable the prediction of shifts in central organism groups shaping forest communities in the light of environmental change or disturbance. Insights can help inform management and reforestation goals as it has been shown that the promotion of native mycorrhiza can restore the establishment of indigenous plants (Koziol et al., 2018) and consideration of ECM in plant and ecosystem restoration have effectively been used in areas effected by soil erosion, heavy metal contamination, wildfires and logging. Many studies have described the role ECM play in the establishment of invasive plant species (Nuñez & Dickie, 2014; Policelli et al., 2019; Pringle et al., 2009) and how these species effect existing plant fungal associations (Callaway et al., 2008). ‘A holistic view of forest management that considers a belowground perspective of forest restoration’ has been put forward amidst the growing body of evidence proving the importance of plant-microbial symbioses for forest health (Policelli et al., 2020a).

Investigating underlying plant-fungi association patterns and the rules which govern them will enable the prediction of forests changes in plant and fungal community composition. In the following manuscript I aim to investigate the root fungal communities of a temperate mixed beech forest from these different perspectives and associate the insights from different analyses. First I describe the spatial structure of trees and belowground roots and how they are related to each other. Then I relate locations dominated by different root species to ambient soil properties. Finally, I analyse root fungal diversity patterns in their relation to host preference and

spatial structure. Finally, I integrate the resulting insights and assess which drivers primarily influence a given roots fungal community structure.

PART 2

Manuscript

„Community composition and spatial structure of root associated fungi in a mixed temperate forest“

Introduction

Forests contain a substantial proportion of the terrestrial carbon stock and therefore play an essential role in the global carbon cycle. Their protection and proliferation is recognised as a key component in climate change mitigation efforts (Canadell & Raupach, 2008; Clery et al., 2021). Apart from tropical systems the majority of forest carbon is stored belowground and temperate forests can be especially dense in soil carbon (Post et al., 1982; Scharlemann et al., 2014). Processes controlling carbon sequestration, its chemical transformations and ultimate fate in either storage or release are highly interconnected and facilitated by both aboveground vegetation and belowground microbial communities. Symbiotic relationships between plant roots and fungi have been found critical in the extent of these processes and resulting nutrient fluxes and stocks. Only by investigating these plant microbe interactions can we understand the central mechanisms that shape forest ecosystems.

Ecotmycorrhiza (ECM) is a widespread symbiosis in temperate forests in which fungal hyphae encase host roots and provide a multitude of services in exchange for photosynthesized, energy rich carbon compounds. They promote plant growth by enhancing water and nutrient availability (especially nitrogen compounds) (LeBauer & Treseder, 2008; Pritsch & Garbaye, 2011; van der Heijden & Sanders, 2003); by providing protection from pathogens (Marx, 1972) and can facilitate interplant resource transfer and communication (Gilbert & Johnson, 2015; Gorzelak et al., 2015; Graves et al., 1997; Robinson & Fitter, 1999; Simard & Durall, 2004). The fact that the ECM symbiosis has evolved in at least 30 vascular plant- and 80 fungal lineages (Tedersoo & Brundrett, 2017; Tedersoo & Smith, 2017) and that plants are estimated to provide 20-30% of their photoassimilated carbon to their ECM symbionts signifies the importance of this association (Hobbie et al., 2012; Söderström & Read, 1987). This large flux of carbon feeds ECM mycelia, which permeate the soil matrix where their necromass then contributes to stabilised soil organic matter pools (Godbold et al., 2006; Schmidt et al., 2011). Additionally, through their scavenging and exoenzymatic nutrient mining activity they can degrade soil organic matter and deplete its nitrogen content (Clemmensen et al., 2015; Lindahl & Tunlid, 2015; Nicolás et al., 2019). Resulting carbon-rich compounds can

stabilize in organo-mineral complexes and increase the pool of recalcitrant soil organic carbon. Globally, soil carbon stocks have been positively related to the biomass fraction of ectomycorrhizal plants (Soudzilovskaia et al., 2019). Due to their immense impact on terrestrial soil carbon dynamics they have been included in climate change models (Terrer et al., 2019) and they could benefit the terrestrial carbon sink by alleviating nutrient constraints, which would dampen the CO2 fertilization effect (Terrer et al., 2016, 2019). Other root fungal symbioses can be diverse and extremely widespread, benefitting their plant host fitness such as dark septate endophytes (Jumpponen & Trappe, 1998; Mandyam & Jumpponen, 2005; Newsham, 2011) and sebacinalean endophytes (Weiß et al., 2011; Zuccaro et al., 2014) and it is suspected that there are more impactful associations undescribed to date (Kariman et al., 2018).

Most temperate and boreal tree species are ECM symbionts (Policelli et al., 2020b). As symbionts generally exhibit a degree of partner preference (the range of different partners a symbiont can engage with) it is important to understand how dependent both tree and fungal groups are on each other's specific presence or overall diversity and at which point perturbations might impair their individual or combined survival. Restoration measures have shown that native (arbuscular-) mycorrhizal inoculant can enable the establishment of indigenous plants after disturbance, signifying the importance and benefits of specific symbiont presence (Koziol et al., 2018). Studies have found ECM can co-invade with their hosts (Nuñez & Dickie, 2014; Policelli et al., 2019; Pringle et al., 2009) and how invading ECM impact local associations (Callaway et al., 2008). ECM can be extremely diverse even on single plants but hosts symbiont diversity can vary greatly (De Roman et al., 2005b; Timonen & Kauppinen, 2008). ECM host preference is generally assumed low (Põlme et al., 2018). Tree species in temperate forests share a large amount of symbionts (Rog et al., 2020) and trees fungal community distinction is best found at higher phylogenetic levels (Ishida et al., 2007). Host preference for endophytic groups is less studied but high colonizer diversity (also within the same plant) and significant preference has been found for a known dark septate endophyte complex (Stroheker et al., 2021).

The assessment of both host and symbiont diversity and their dependence on each other can greatly benefit from detailed analysis of an associations function. ECM can have varying exoenzymatic capabilities each with individual impacts on soil nutrient

cycling (Martin et al., 2016; Zak et al., 2019). Differing exploration types have been suggested to play a role in soil carbon dynamics between tundra and bordering shrub/forest regions (Clemmensen et al., 2021) and also correlate with forest stands age and plant functional traits (Wasyliw & Karst, 2020). Spatial analysis as well as co-occurrence analysis are tools for the assessment of species interactions, uncovering of functional cohorts, species/cohorts impacts or dependencies on environmental conditions and other ecological drivers (including neutral effects). Drivers can be analysed in the framework of a fundamental ecological concept: Community similarity distance decay (Soininen et al., 2007; Whittaker, 1975).

For ECM spatial autocorrelation has been analysed and found most pronounced between 0 and 3 meters and community co-occurrences imply non-random structuring (Lilleskov et al., 2004; Pickles et al., 2012). So far methods mostly sample ECM root tips only and focus on single plant species. Very few studies have investigated distance decay relationships on the entire mycobiome of roots. We know that root associated fungi show weaker spatial structuring than free living soil fungi on large scales (Goldmann et al., 2016) but how does the full mycobiome differ from the more abundant ECMs spatial structuring? And how do these relationships perform on small (forest stand) scales? More importantly, as it is known that root associated fungi are dependent on their hosts, research has been lacking, which includes host preferential effects in the analysis of spatial structuring as both drivers (space and host preference) clearly dictate community composition in unison. This study aims to investigate these two important drivers for different symbiotic fungi and integrate the findings into a broader picture, able to better characterize how these drivers interact and at which scales these drivers operate. Other potential drivers such as species sorting (either via species interactions or environmental filtering) have mainly been studied on continental and landscape scales and have received little attention on forest patch scales and the physical scales of tree roots and fungal mycelia.

Plant-fungal associations are at the core of forests being and they must be perceived as highly interconnected and interdependent systems. The discovery of the common mycorrhizal network makes this even more apparent (Beiler et al., 2010; Graves et al., 1997; Robinson & Fitter, 1999; Simard et al., 1997). Vast mycelial networks span the underground sharing nutrients and facilitating communication between plant

individuals and species (Gilbert & Johnson, 2015; Gorzelak et al., 2015; Simard & Durall, 2004). Host species phylogeny plays a role in interplant nutrient quantities transferred (Klein et al., 2016; Rog et al., 2020) and specific ECM species have been identified transferring nutrients depending on host species identities (Cahanovitc et al., 2022). This suggests that full understanding of forest ecosystem functions and dynamics requires a holistic and integrated plant symbiont perspective.

Many threats are expected to increase in the future and global forest cover is steadily declining (Curtis et al., 2018). Climate change and other anthropogenic influences invoke droughts (A. C. Bennett et al., 2015; Choat et al., 2012), forest fires (Flannigan et al., 2009), shifts in environmental conditions (Hanewinkel et al., 2013), pollution (Lilleskov et al., 2019; W. H. Smith, 2012), species invasions (Liebhold et al., 1995) and many more devastating threats. Not only must we understand the immediate mechanisms these effects might have on environments and individual organism groups but also what the consequences for their interacting counterparts will be and how these changes in communities will effect biogeochemical cycles in a broader context. This requires basic understanding of how these organisms connections are structured in their natural systems. Investigation into diversity- and spatial patterns on an integrated plant-fungal community level will help contextualize research into consequences of disturbances and environmental change. More broadly, it will identify scale dependencies of community drivers and in a more practical sense advise optimal sampling designs aimed at characterizing community structures and their alteration.

In this study we aim to describe the structure of root fungal associations of a temperate forest system from different perspectives. First, we assess the local edaphic properties and tree (stem and root) distribution patterns. We then analyse host effects on root associated fungal communities and investigate their spatial structuring on multiple scales. Our main research questions were: i) How are roots mycobiomes structured? ii) How are root fungal associations spatially organized and how does this differ for between tree species and fungal groups? iii) How much does host preference impact community structure? iv) At which scales do these drivers become impactful predictors of community composition? v) Does species sorting play an important role in community structure on local forest scales?

We integrate our findings and identify which factors play a dominant role in the observed root fungal distribution patterns and which scales are of particular interest for the analysis of root fungal communities.

Material and Methods

Study site and sampling design

The sampling site is located in the study region Zöbelboden, a mountainous (900 m.a.s.l.) mixed beech forest, which is part of the Nationalpark Kalkalpen situated in the south of the state of Upper Austria. Established in 1992, this site is part of the UNECE International Co-operative Program "Integrated Monitoring of Air Pollution Effects on Ecosystems" and has also become a study site for the Austrian long-term Ecosystem Research Network. Most of the produced data from research conducted at this site is publicly available. The mixed forest contains a variety of tree species (*Picea abies, Abies alba, Larix decidua, Fagus sylvatica, Fraxinus excelsior, Sorbus aria* and *Acer pseudoplatanus*) and has undergone minimal management. In our sampled plots only four tree species (and their roots) were reliably determined: *P. abies, L. decidua, F. sylvatica* and *A. pseudoplatanus*.

In total four large plots were selected along a transect with two additional sub-plots located in dense patches of young beech seedlings (Figure 1). One of the large plots was located on a slope (SA) and was the only plot to containing roots of *A. pseudoplatanus*. The other three large plots (TA, TB & TC) were located on the main plateau of the study region. Each large plot consisted of a 15 x 15 meter grid that was divided into nine 5 x 5 meter sub-plots. Sampling points were at the centre of each sub-plot, with two randomly selected sub-plots receiving an additional three sampling points at mid-diagonals as illustrated in Figure S1. The two small plots containing young beech seedlings (5 x 5 meters; Plots B1 & B2) were also sampled in this manner. For all three large plots on the plateau trees inside the grid and its surroundings (+ 5 meters) were assessed for their species, their diameter at breast height (dbh) as well as their exact geographic location. Sampling points of these plots were also georeferenced (Pseudopositions of SA and B1 & B2 plot were informed from notes). Sampling took place in mid-August 2019 (12th and 13th of August).

At every sampling point two soil cores (5 cm diameter, 4.5 cm depth) were taken directly next to each other. For either core washed roots were processed separately. While roots from one core were separated into fine and coarse roots (threshold of

roughly 1mm diameter) and their weights determined, five individual and continuous root-fragments of the other core were randomly selected and stored at -20°C for further DNA analysis. Soils of both cores were pooled and sieved through a 2mm mesh and kept at 4°C for subsequent measurements.

Measurements of soil parameters

Soils elemental composition was determined via Gas Chromatography Isotope Ratio Mass Spectrometer (CE Instruments, EA 1110 combined with mass spectrometer Finnigan, DeltaPlus, IRMS) from dried soils. Measurements revealed total soil Carbon and Nitrogen as a percentage of dry weight and their resulting ratio (Amt%C; Amt%N; C:N), as well as the isotopic ratios of 15N/14N and 13C/12C.

KCl soil extracts (2g soil shaken in 10ml 1M KCl for 30 minutes then filtered) were analyzed in a TOC/TN Analyzer (Shimadzu, TOC-VCPH /CPNTNM-1) from which dissolved organic carbon (DOC) and dissolved nitrogen (DN) was measured.

Soil pH was determined from soil water extracts (2g of soil shaken in 10ml ultrapure water for 30min then filtered). As enzyme activity heavily relies on the ambient pH, we decided to split samples into three groups to assess their enzyme activity in solutions representing their respective soil pH. The lowest pH group consisted of samples ranging from pH 4.4 to 5.3 (n = 10, mean = 4.7, std. dev. = 0.40; Adjusted to 4.5); the midrange group showed values between 5.7 and 6.9 (n = 21, mean = 6.3, std. dev. = 0.34; Adjusted to 6.5) and the highest pH group combined samples with values between pH 7 and 7.9 (n = 37, mean = 7.44, std. dev. = 0.282, Adjusted to 7.5). Enzyme activity was measured via photometry. Sample slurries (1g fresh soil, ultrasonicated in ultrapure water) combined with pH adjusted Acetate buffer (depending on respective samples pH group) were added to target fluorophore containing substrates. Enzyme activity degrading substrate complexes (releasing fluorophores) were measured for their fluorescence at two timepoints in a plate reader (TECAN Infinite M200 spectrometer). Enzymes of interest, their chemical function and targeted substances are listed in Table 1.

Table 1 | **Enzymes, their functions and the substrates used for measurement**

Enzyme	Enzyme function	Substrate
Exoglucanase (cellobiosidase)	Releases cellobioside from cellulose	4-Methylumbelliferyl β-D-cellobioside
Exochitinase (N-acetyl-β-glucosaminidase)	Chitin decomposition (releases glucosamine from the end of chitin)	4-Methylumbelliferyl N-acetyl-β-D-glucosaminide
Acid Phosphatase	Releases inorganic P from organic phosphorus	4-Methylumbelliferyl phosphate
Leucine-Aminopeptidase (Protease)	Degrades peptides/proteins	L-Leucine-7-amido-4-methylcoumarin

Previously mentioned water extracts were measured via High Performance Liquid Chromatography (ICS 5000; column: AS11-HC 2mm 15; eluent: KOH) and were analyzed for anions and cations. HPLC settings were as follows: 0-10 min 1mM KOH; 11-24 min increase to 15 mM; 25-33 min increase to 60 mM; 34-38 min held at 60 mM; 39-45 reduce to 1 mM. Anions were quantified by an internal standard mix and went as follows: acetate, butyrate, chloride, citrate, formiate, iso-citrate, lactate, malate, nitrate, nitrite, oxalate, phosphate, propionate, quinate and sulfate. Similarly, cations were measured on the same machine (ICS 5000; column: CS16 5mm 35; eluent MSA). HPLC settings here went as follows: 0-10 min 15mM MSA; 11-15 min increase to 30 mM; 16-25 min held at 30 mM; 26-30 min reduce to 15 mM; 31-35 held at 15 mM. The only cation quantified was ammonium. Quantification for all Ions was performed by manual peak integration on the Chromeleon software (Thermo Scientific).

Lipid detection was conducted on around 2g of freeze-dried soil and was extracted in a mixture of chloroform, methanol and pH 4 adjusted 0.15M citrate buffer (0.5:1:0.4 v/v/v) for >12 h. A re-extraction step with the same buffer solution was performed after the first liquid phase was separated. Phase separation was induced adding chloroform citrate buffer (1:1 v/v) and the lower organic phase was dried in a N2 atmosphere. The fractionation of Lipids was performed on silica columns (Phenomenex Strata SI-1 Silica; 55 µm, 70 Å). First, neutral lipids were eluted in 1 ml chloroform in two steps. Second, glycolipids were eluted in 1 ml acetone. Third,

phospholipids were eluted in 0.5 ml methanol. Both neutral lipids and phospholipids were converted to fatty acid methyl esters via alkaline methanolysis. Samples were dried and solubilized in 100 µl iso-octane. Nonadecanoic acid functioned as an internal standard enabling quantification. External, qualitative standards for determining bacterial and fungal fatty acids were BAME and FAME mixes (BAME CP mix, Supelco; 37 Component FAME mix, Supelco).NLFAs and PLFAs were measured on a gas chromatography-mass spectrometry (Trace GC Ultra, Thermo Scientific; column: DB5-MS - 60m length 250 µm internal diameter) with the following settings: 1 min initialization at 80°C; increase 30 °C/min then 1 min at 150°C; increase 2°C/min to 200°C; increase 4°C/min then 15 min at 230°C; increase 30°C/min then 6 min at 280°C (total time 58.83 min). Peak identification and quantification was performed with ChromaTOF (LECO) software. Lipid fatty acid biomarkers - assigned to certain microbial groups (G. T. Hill et al., 2000; Leckie, 2005; A. P. Smith et al., 2015; Willers et al., 2015) - were determined as follows: Actinomycetes (10Me17:0, 10Me18:0); general bacteria markers (C14:0, C15:0, C16:0, C18:0), gram positive bacteria (C14:0i, C15:0a, C15:0i, C16:0i, C17:0a, C17:0i), gram negative bacteria (C16:1w5, C16:1w7, C17:0cy, C19:0cy) and fungi (C18:1w9cis, C18:1w9trans, C18:2w6,9). Finally, lipids assigned to marker groups were summed to quantify total group lipid abundances for NLFAs and PLFAs.

Root sample DNA extraction and sequencing

Individual root fragments ranged in weights from 0.02mg to 71mg and were stored at -20°C after harvest. All subsequent DNA extraction and sequencing were conducted by the Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna and generally aligned with the method presented in Pjevac et al. (2021) unless specified otherwise. Sample homogenization was performed in ‘Lysis matix E’ tubes which were shaken for 40 seconds at 6 m/s. DNA extraction was then followed by Power Soil Pro (Qiagen) according to the manufacturers’ protocol. As host specific effects were of interest for this study two sequencing runs were performed. One primer pair targeting the plant chloroplast RbcL gene (Ribulose bisphosphate carboxylase large chain), the other targeting the fungal marker region ITS (Internal Transcribed Spacer). RbcL amplification was performed in a single run.

Utilized primer sequences can be found in Table 2. ITS amplification was performed with an additional nested PCR run (ITS2 then ITS7) and a prior cleaning step of the new template with SequelPrep (as mentioned in Pjevac et al., 2021). Each PCR with MM Dream Taq PCR MM 2x (lot: 02241) was performed in triplicates. Primer pairs are listed in table 2. RbcL amplification used rbcLa-F and rbcLa-R primerpairs, ITS first amplification used ITSOF-T and ITS4 primerpairs and the ITS nested PCR used gITS7 and ITS4 primerpairs.

Settings for amplification went as follows: RbcL - Initial denaturation 94°C for 4 min; 35 cycles of (Cycle denaturation 94°C for 30 s, Annealing 49°C for 30 s, Cycle elongation 72°C for 1 min); final elongation 72°C for 10 min. ITS2 - 94°C for 4 min; 35 cycles of (94°C for 45 s, 48°C for 45 s, 72°C for 1 min); final elongation 72°C for 10 min. ITS7 - 94°C for 4 min; 20 cycles of (94°C for 30 s, 53°C for 30 s, 72°C for 30 sec); final elongation 72°C for 10 min. Amplicons were sequenced on the Illumina platform.

Table 2 | **List of primers used in amplifications. Added headers are highlighted in bold.**

Primer ID	Primer sequence	References
rbcLa-F (fwd.)	**GCTTGCGCGAGCTGC** ATGTCACCACAAACAGAGACTAAAGC	Fazekas et al., 2012
rbcLa-R (rev.)	**TAGCGCACACCTGGTA**GTAAAATCAAGTCCACCRCG	Fazekas et al., 2012
ITSOF-T (rev.)	ACTTGGTCATTTAGAGGAAGT	Nilsson et al., 2019
ITS4 (fwd.)	**TAGCGCACACCTGGTA**TCCTCCGCTTATTGATATGC	Nilsson et al., 2019
gITS7 (rev.)	GTGARTCATCGARTCTTTG	Nilsson et al., 2019

Processing of sequencing data

Sequenced reads were assessed for their quality and ASVs were determined with the DADA2 pipeline (Callahan et al., 2016). Fungal ASV taxonomy was further ascertained with the UNITE database (Nilsson, Larsson, et al., 2019).

For the RbcL-amplified samples root species classification was conducted with two criteria. Firstly, the most abundant ASVs were required to be classified to at least a genus level corresponding to one of the four tree species observed aboveground. Secondly, the most abundant ASV was required to contain at least 50% relative abundance in its sample. 286 of 340 samples were successfully classified by these criteria.

For the ITS sequencing data singletons and doubletons (ASVs, which only have one or two reads in the entire dataset) were deleted. This reduced the amount of ASVs from 1939 to 1933 across 340 samples. Total read counts were highly heterogeneous between samples. 246 samples showed lower total reads than 50. 27 samples produced total reads between 50 and 500, leaving 67 with more than 500 total reads. Of these a lot of samples (53) had a more common higher read count of more than 10.000 total reads. Two datasets were constructed: One referred to as 'Higher than 50 sample reads data' where the dataset was trimmed to exclude samples with fewer than 50 reads (reducing samples from 340 to 94), the other termed 'Higher than 500 sample reads data' was trimmed to exclude samples with fewer than 500 reads (reducing samples from 340 to 67). Data was then trimmed for likely contaminated samples. For a total of 12 samples the dominant ASV (90-100% relative abundance) was assigned *Phialemoniopsis curvata*, a species found in humans and therefore a likely contaminant. All samples were also required to be assigned to a tree root species as mentioned above. Together, this left 72 samples for the 'Higher than 50 sample reads data', which was used for analyzing effects of the most abundant ASVs as a 'top down' abundance threshold was deemed more appropriate in the analysis for samples with varying library sizes. Analyses on this dataset was generally presence absence based and relative abundances were not utilized. Any analyses based on the 'n most abundant ASVs' (considering the most abundant ASVs in a sample by rank) was conducted on this data and whenever a sample would not reach the required amount of ASVs this sample was excluded from analysis. For this dataset *Acer pseudoplatanus* had 3 samples, *Fagus sylvatica* had 48 samples, *Larix decidua* had 1 sample and *Picea abies* had 20 samples. 'Higher than 500 sample reads data' (sample size = 55) was used in co-occurrence network construction. For this dataset *A. pseudoplatanus* had 3 samples, *F. sylvatica* had 38 samples, *L. decidua* had 1 sample and *P. abies* had 13 samples.

Data analysis and statistics

1. Software platforms

All statistical analysis was conducted in R (4.1.1). Graphics were also created in R if not explicitly stated otherwise. When referring to (statistical) significance an alpha threshold of < 0.05 is meant. Functional assignments were performed using the FUNGuild database (Nguyen et al., 2016). A georeferenced map containing sampling points and the location of tree stems was constructed in QGIS 3.16 (QGIS Development Team, 2021. QGIS Geographic Information System. Open-Source Geospatial Foundation Project).

2. Spatial analyses of stems and roots

As *F. sylvatica* and *P. abies* were by far most dominant found in both stem and root data the investigation focused on those species. The two-species ratios for stems (r_s) were calculated taking the number of *P. abies* assigned stems (n_p) and dividing them by the number of *F. sylvatica* assigned stems (n_f)

$$r_s = \frac{n_p(stems)}{n_f\,(stems)}\,.$$

The same was done for *P. abies* assigned roots and *F. sylvatica* assigned roots (r_r)

$$r_r = \frac{n_p(roots)}{n_f\,(roots)}\,.$$

The resulting stem ratio (= fraction of how much fewer *P. abies* occurred compared to *F. sylvatica*) was divided by the roots ratio and gives a percentage of how much more *F. sylvatica* occurred in root samples than would be expected if the composition of both trees by their stem numbers were exactly the same (r_{excess}). This excess was defined by the following equation (1):

$$r_{excess} = \frac{r_s}{r_r}\,. \qquad (1)$$

Calculations were conducted for each plot individually and for all plots combined.

A simple nearest neighbor approach was chosen to assess whether tree stem proximity could inform on root species identity. For every root the closest tree stem

was located and their species identities were compared. The number of comparisons for which stem and root identity matched was summed for all tree species (m_{Acer} + m_{Fagus} + m_{Larix} + m_{Picea} = m_{total}). This total amount of all matching comparisons (m_{total}) was then divided by the total amount of comparisons made (c_{total}). The resulting value gave the probability of the nearest neighboring stem species identity accurately predicting the root species identity (prediction value = m_{total} / c_{total}). This was conducted for all plots individually and for all plots combined. Additionally, a permutation test functioned as a null model. Root species were randomly shuffled and analyzed by the same method for a null-prediction score (10.000 times). Actual prediction scores were then assessed for their significance compared with all null-prediction scores.

To investigate same-core root species similarity data was separated by sample points (cores) and individually analyzed. To assess the number of all possible pairs we can use the expression $\binom{n}{k}$ from combinatorics, where *n* is the number of species roots (subscripts: *f = F. sylvatica, p = P. abies, l = L. decidua, a = A. pseudoplatanus*) and *k* signifies the number of objects combined ('k-combination'; in the case of pairs *k* = 2). This is then calculated by $\frac{n!}{(n-k)!*k!}$. The sum of matching pairs for which any two root species were the same was calculated (*Number of matching species pairs*) and divided by the number of all possible pairs (*Total number of possible pairs*) and is noted by a_i as defined in the following equation (2):

$$a_i = \frac{Number\ of\ matching\ species\ pairs}{Total\ number\ of\ possible\ pairs} = \frac{\binom{n_a}{2}+\binom{n_f}{2}+\binom{n_l}{2}+\binom{n_p}{2}}{\binom{n_a+n_f+n_l+n_p}{2}} \quad (2)$$

where *i* represents the *i*th core.

This was repeated for all cores (a_i) and their results were averaged by the total number of cores (*N*):

$$a_{mean} = \frac{\sum_{i=1}^{N} a_i}{N}. \quad (3)$$

The result gave a measure of the likelihood of species originating from the same core being assigned to the same species (a_{mean}). In a permutation test we shuffled all root species across all cores (10.000 times) and analyzed the data by the same

method. This resulted in a null distribution, which was used to assess significance of the calculated prediction value.

To assess whether cores, which were dominated by either an individual root species or cores showing multiple root species were linked to certain soil properties significant relationships were determined via Kruskal Wallis test and subsequent post-hoc Dunn test and visualized with boxplots.

The number of root samples matching our defined quality criteria per sampling point (core) (as defined in the section ‘processing of sequencing data’) was correlated with the cores soil properties to investigate whether certain properties might have impacted root fungal colonization. A spatially referenced map was also constructed to investigate whether a spatial clustering mechanism could be assumed driving low colonization rates.

3. Host-symbiont diversity and preference analysis

To investigate the variation of diversity across samples two methods were used. First simple boxplots show species richness for our data. Second, species accumulation curves were constructed with the ‘specaccum’ function of the vegan package (Oksanen et al., 2020). These curves indicate how much diversity (species richness) is covered by a single sample and how much this diversity is increased by including additional samples into the richness estimate. Accumulation curves were applied once for *F. sylvatica* and *P. abies* samples individually. A second graph was created in which in which *F. sylvatica* was restricted to 20 samples to match the sample size of *P. abies*. Subsampling of *F. sylvatica* was repeated 100 times and results were averaged.

To analyze the preference of fungal taxa to their hosts we apply two methods. For the first method we trim the data to a level of *n* most abundant ASVs for every sample (*n* = a certain number of ASVs ranked highest to lowest by their abundance). For each trimmed dataset (*n* takes values from 1-100) we calculate how many ASVs are shared between samples of *F. sylvatica* and *P. abies* and how many ASVs are unique to either plant species. ASVs are only counted once, so multiple occurrences of identical ASVs in the shared or either plants unique communities are only counted a single time. As *F. sylvatica* contained many more samples than *P. abies* a

subsampling approach was chosen. At every level of calculations for data of the *n* most abundant ASVs we randomly subsampled *F. sylvatica* assigned samples to the amount of *P. abies* samples. Results for the number of shared ASVs and ASVs unique to either root species were averaged across 100 runs of this subsampling. This gave us the amount of shared and unique ASVs from only high abundance taxa and shows how the inclusion of less abundant ASVs alters these relationships. This analysis was repeated for ECM assigned ASVs (assigned by the FUNGuild database) only too (*n* takes values 1-30).

Ordinations were also performed to assess community dissimilarity between root species. First, a detrended correspondence analysis (DCA; (M. O. Hill & Gauch, 1980)) was performed on presence absence data using the default settings of the 'decorana' function of the vegan package as implemented by Botnen et al. (2014). Second, a Bray-Curtis dissimilarity based non-metric multidimensional scaling ordination was performed (NMDS; Kruskal, 1964). Ordinations were performed on data trimmed to the 5 most abundant species and to the 100 most abundant ASVs. For the data of the 5 most abundant analysis of the DCA two samples (TC9_c_3 and TC_9_c_1) were excluded as outliers.

4. Spatial community similarity analysis

We conducted spatial analysis on data trimmed to identical species richness (number of ranks by abundance). Here, the Sørensen similarity index, a metric for beta diversity is analogous to the percentage of species that are shared between two samples. Sample pairs Sørensen similarity indices plotted against their pairwise Euclidian distance was the basis for the analysis of community distance decay relationships. Detailed analysis focused on the relationship of pairs within plots as decay was assessed strongest at this scale. All pairs within a plot were assessed and similarity-distances were analyzed together for Spearman correlation coefficient and significance. In this analysis too we iteratively investigated similarity for each rank of the n most abundant ASVs (n = 1 – 100) and relationships were investigated for all samples and for *F. sylvatica* and *P. abies* only. The shape of the distance decay relationship, so the function describing the reduction in similarity (y) with distance (x) was fit via non-linear least squares, which is implemented in base R with the nls() function. Both exponential (y = *a* * exp(*b* * x) + *c*) and power law (y = *a* * x^*b*

+ *c*) relationships were investigated for their fit via residual plots and residual sum of squares. Both of these functions have been used to describe distance decay relationships and their applicability is tied to the spatial scales and environmental heterogeneity in sampling. Distance decay curves were analyzed and shifts in function parameters when including more ASVs (ranked by their abundance) were investigated to assess how the most abundant root colonizers performed in comparison to a more complete community. The analysis was conducted once for all samples and once for *F. sylvatica* samples only. The nls() function was not able to fit *P. abies* data only due to the lack of datapoints. To investigate distance decay relationships for both *F. sylvatica* and *P. abies* transect scale data was separated by species and analyzed individually, as these datasets contained sufficient pairs similarity-distance data points for analysis.

Spatial networks were constructed and visualized using the R packages igraph (Csardi & Nepusz, 2005) and ggraph (Lin Petersen, 2021). All sample pairs were assessed for the number of ASVs they shared. Nodes were embedded in a spatial context, so their positions reflected sampling points (soil cores) geographic locations. As sample points contained multiple root samples node size reflected the number of samples included in analysis. As all sample pairs were analysed, two samples from the same core could share self-edges and multiple edges between nodes composed of multiple samples were also possible. Edge weights reflected a sample pairs number of shared ASVs. Not only were the transects spatial networks assessed for range in which spatial autocorrelation was most pronounced. They were also analyzed for network properties potentially indicating spatial structuring. Here edge establishment was binary, so two samples sharing a single ASV received an edge. Apart from a network encompassing the whole transect scale – so all samples in all plots - networks were also created for all plots individually. Network creation was conducted at three levels (5, 20 & 50 most abundant ASVs). For each network at each level the mean connectance and mean clustering coefficient was calculated and compared.

To investigate whether root species in the same cores also tend to share the same ASVs all pairs containing either *F. sylvatica* and or *P. abies* were compared. The percentage of shared ASVs (analogous here to the Sørensen similarity index) at each rank of the n most abundant ASVs was averaged for same species pairs

(termed '*Fagus sylvatica x Fagus sylvatica'* and '*Picea abies x Picea abies'*) and mixed species pairs ('*Fagus sylvatica x Picea abies'*). The average of shared ASVs within the same cores were contrasted to the average of all random pairwise sample comparisons (therefore spatially indiscriminate).

5. Co-occurrence network analysis

Module analysis was performed on the 'Higher than 500 sample reads data', which was trimmed by a relative abundance threshold of 1%. As there are many methods for inferring co-occurrence networks from amplicon sequencing data we selected a SPIEC-EASI like approach which has been recommended for plant microbiome research (Birt & Dennis, 2021). The network was constructed using the graphical lasso (GLASSO) algorithm as implemented in SPIEC-EASI (Friedman & Tibshirani, 2019; Kurtz et al., 2015). The settings for the function used went as follows: GLASSO [out.glasso = huge(data, method = "glasso", nlambda=30, lambda.min.ratio = 0.1 , cov.output = TRUE). 30 networks (lambda = 30) were created by GLASSO and the network with lambda 9 was selected for analysis. The final network contained 336 nodes and a total of 1370 edges. Modules were determined via the 'cluster_louvian' function form the igraph package. Modules compositions were then investigated on a phylum and functional level. Nodes (ASVs) shapes were assigned by the trimmed data used for network construction (Higher than 500 sample reads data' with a relative abundance threshold of 1%). Host affiliation was deemed species specific if an ASV only occurred in root samples of the same species or were deemed 'host unspecific' If they occurred on at least two samples of different species. Descriptive analysis investigated how spatial (or sample) heterogeneity led to module formation.

A second co-occurrence network was constructed on the 'Higher than 50 sample reads data' and restricted samples to their first 100 most abundant ASVs by rank (44 samples remained). Associations were calculated via presence-absence based checkerboard scores as provided by the 'ecospat' package (Broennimann et al., 2021). The checkerboard score, or C-score, is a statistic calculating the degree of randomness in the distribution between species (Stone & Roberts, 1990). Conventionally all pairwise C-scores are averaged for a community. Here pairs C-scores are tested with a null model for their significance (10.000 permutations as

recommended). Positive associations with p-values below the significance threshold of 0.05 were determined edges and the resulting network contained 1368 nodes and 26659 edges. Over proportional group associations were then assessed. For this, phylogenetic and functional grouping categories (f. ex. phylum) were selected and all edges within and between nodes of the levels of a given category (for example: Ascomycetes, Basidiomycetes, etc.) were quantified producing a group edge matrix. To correct for the differences in node level counts (f. ex. Basidiomycetes make up majority of nodes) null model networks were constructed. For this the network was randomly rewired 1.000 times and the amount of randomly appointed edges between nodes of each group – so the average degree for nodes within a group – were used to test for significance as a null distribution. Then, for all significant results the mean value in this null distribution and the true value observed in the network were used to calculate a fold de-/increase between the null model and the observed values. Resulting values show the significant disproportionally represented associations between certain groups (taxonomic and functional).

Results

Tree stem species and root species compositions do not match distinctly within plots (Table 3). While *Larix decidua* generally had low occurrence in most sites it is noteworthy that it made up a substantial proportion of tree stems for the TA plot but was extremely underrepresented in the plots root samples. About every fifth stem in the plots was either dead or had been logged. A georeferenced map of plots for which tree stems were assessed can be seen in Figure 1.

Table 3 | **Tree species root and stem counts and their abundance ratios for individual plots and all plots combined (total).**

	Abundance ratios	TA	TB	TC	Total
Stems	*Fagus:Picea:Larix* (counts)	20:18:7	27:10:1	23:12:0	70:40:8
	Fagus:Picea:Larix (ratios)	0.44:0.40:0.16	0.71:0.26:0.03	0.66:0.34:0	0.59:0.34:0.07
	Alive:Dead (counts)	45:9	38:6	35:9	118:24
	Alive:Dead (ratios)	0.83:0.17	0.86:0.14	0.80:0.20	0.83:0.17
Roots	*Fagus:Picea:Larix* (counts)	32:25:2	56:10:1	56:12:0	144:47:3
	Fagus:Picea:Larix (ratios)	0.54:0.42:0.03	0.84:0.15:0.01	0.82:0.18:0	0.74:0.24:0.02

Generally, *Picea abies* tends to be underrepresented in the root samples when compared to the tree stem composition. *Fagus sylvatica* disproportionally dominates the root sample composition when compared to the aboveground tree stem composition. This excess (Eq. 1) was not consistent and varied between 15% and 143% (Table 4).

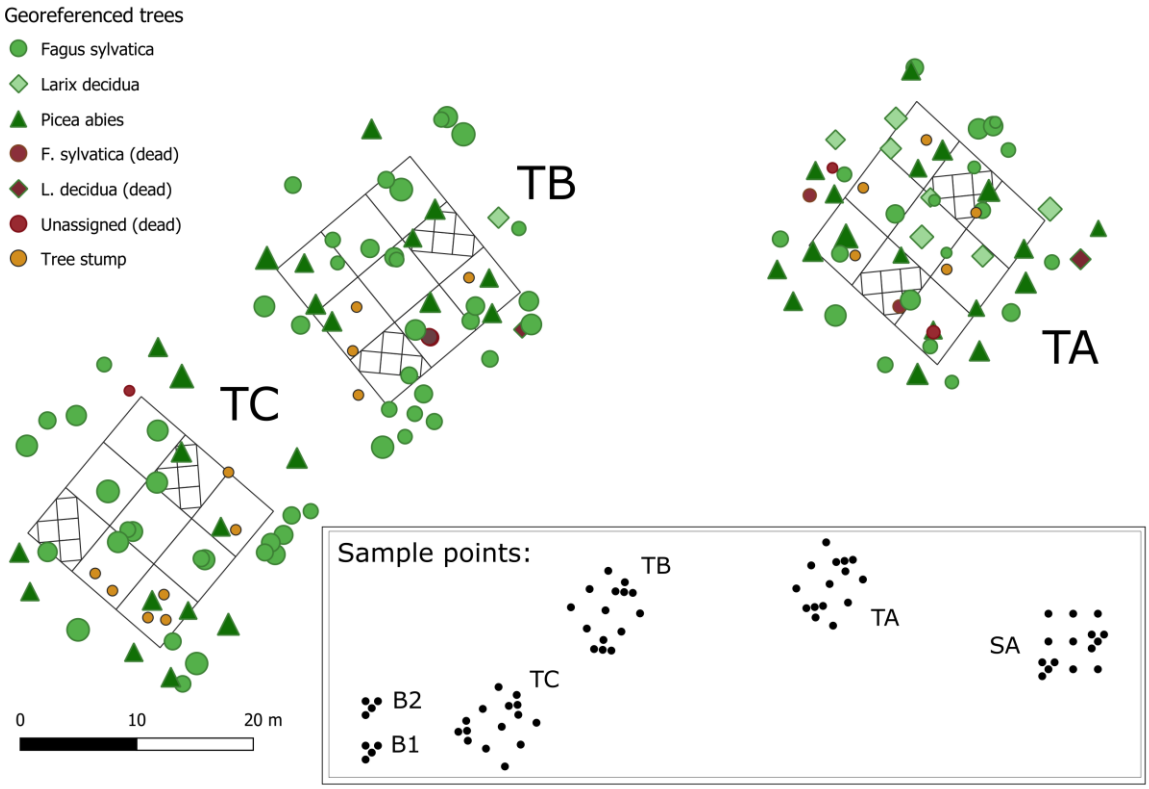

Figure 1 | **Georeferenced data on tree stem and sample point locations. Tree stems of plots TA, TB and TC were mapped and assessed for their species and diameter at breast height (represented by symbol shape/colour and symbol size respectively). Plots were 15 x 15 meters and divided into nine (5 x 5m) squares, two of which recieved additional sampling (divided into four subplots as seen above). At the center of each square soil cores were harvested (sampling points). The box on the lower right shows the relative locations of all sample points at which soil cores were taken. Plots B1, B2 and SA positions were not precisely mapped and are represented with pseudopositions.**

Table 4 | **Tree stem to root ratio relationships for *Picea abies* and *Fagus sylvatica* by plots and combined. *P. abies* to *F. sylvatica* ratios for stems were divided by *P. abies* to *F. sylvatica* ratios for roots. The results show that *F. sylvatica* root samples exceed *P. abies* when compared to the observed species stem ratios.**

	TA	TB	TC	Total
Stem ratios (r_s)	0.9	0.37	0.52	0.57
Root ratios (r_r)	0.78	0.18	0.21	0.33
r_{excess}	1.15	2.07	2.43	1.75

Comparison with a permutation test based null model and resulting significance levels show that given the local forest tree densities a nearest neighbor approach does not enable root identity prediction (Table 5). Not only are results insignificant

but the root-stem prediction value also differed from null model distribution by showing lower prediction values in two cases and one higher ratio.

Table 5 | **To assess whether the nearest tree stem could predict a roots species all roots and their closest located tree stem were compared for matching species. The number of accurate predictions were divided by the total amount of comparisons made. Random predictions were generated by a null model where root samples were randomly shuffled (10.000 times) and resulting predictions were averaged. p-values were then calculated from the resulting null distribution via one tailed significance test.**

	Obs. prediction value	Mean null prediction value	p-value
TA	0.41	0.43	0.3642
TB	0.57	0.52	0.1777
TC	0.57	0.60	0.3701

Investigation of root similarity in single sampling units (as defined in Eq. 2 & 3) produced an average prediction score of 0.78 exceeding the prediction score of 0.57 of the randomized predictions, indicating stronger clustering of similar root species. This result was highly significant as the null model could not explain such high score values.

Soil properties differed slightly between cores either dominated by single root species or in mixed assemblages (Supplementary material, Figure S1). *P. abies* dominated cores seem to differ most from other core types. These differences can be attributed to plot level differences as most samples dominated by *P. abies* stem from B plots (B1 & B2) which showed the highest overall differences to the other four large plots.

When investigating root samples ITS sequencing data quality (‘quality samples’ defined as samples matching the criteria described in the material and methods section) no relationships with relevant soil properties could be found. Visual assessment of the geographic location of ‘quality samples’ hints at a certain spatial clustering of quality samples (Supplementary material, Figure S2).

Investigating those samples data matching the criteria defined for the ‘Higher than 50 sample reads data’ the relative abundances by their rank perform very similarly. The most abundant ASVs tend to produce a mean relative abundance of around 40% which rapidly declines with the following ranks (Supplementary material, Figure S3a). For example, ASVs at rank 20 tend to produce relative abundances of around 0.4%.

These results are consistent for both *F. sylvatica* and *P. abies* samples. It can also be seen that *F. sylvatica* tends to produce higher species richness than *P. abies* and that this richness varies greatly between samples (Supplementary material, Figure S3c & S4). As a top down abundance threshold for assessing a set amount of ASVs ranked by their abundance was implemented, the higher the amount of most abundant ASVs were analysed, the fewer samples were taken into account (Supplementary material, Figure S3b, S3d).

When investigating the measured ITS diversity a gradual shift in community composition can be observed (Supplementary material, Figure S5). High abundance ASVs tend to be assigned Basidiomycetes (especially Agaricomycetes) and are functionally classified as symbiotrophs (ectomycorrhizal). With decreasing ranks in relative abundance ASVs are more likely assigned Ascomycetes (or other less frequent groups) and tend to be assigned more diverse functions.

Root specific sample sizes were heterogeneous (Figure 2a) and therefore comparative analysis investigating shared ASVs was conducted only on *P. abies* and *F. sylvatica*, the latter being repeatedly subsampled and resulting comparisons averaged. *F. sylvatica* and *P. abies* do not differ strongly in the amount of their unique associations (Figure 2b-e). The amount of unique ASVs are substantially higher between either sample type than the amount of shared ASVs. Ectomycorrhizal ASVs tend to be more unique when they show high abundance and tend to become less unique the more are included by their abundance. Even including a lot of ECM ASVs (n most abundant > 30) unique associations still surpass shared associations (Figure 2e).

When increasing the amount of ASVs by their abundance ranks *F. sylvatica* produces more unique association partners than *P. abies*. In both cases shared ASVs increase in a linear fashion.

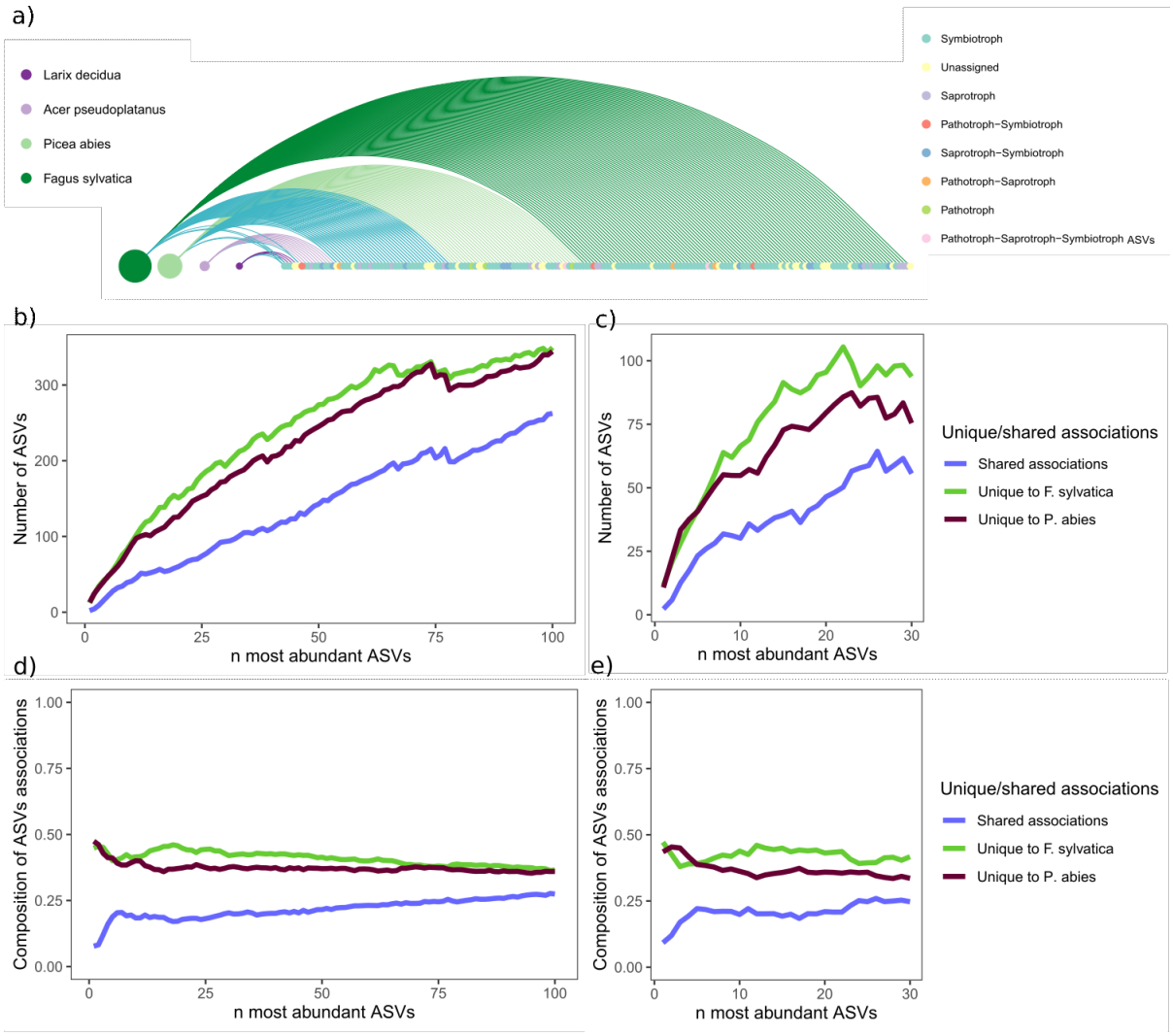

Figure 2 | **a) Bipartite network of all samples 5 most abundant ASVs and their co-occurrences with plant roots. On the left nodes represent all ASVs coloured by their functional assignments. On the right node colours represent the four root species and node size is defined by the square root of the number of samples included in construction (L. decidua = 1, A. pseudoplatanus = 3, P. abies = 19, F. sylvatica = 48). If edges between ASVs and root species are unique, they are coloured by the root species, if nodes are shared by multiple roots they are coloured blue. b & c) Counts of shared and unique ASVs for either root sample type considering a given amount of ASVs ranked by their abundance (n most abundant). To account for differing sample sizes for every rank *F. sylvatica* samples were subsampled to the number of *P. abies* samples. Subsampling was bootstrapped for 100 repetitions and resulting assessment of unique and shared ASVs were averaged. b) Includes all ASVs. c) Only ECM ASVs. d & e) Give the relative composition ASV associations. d) All ASVs. e) Only ECM ASVs.**

To investigate levels of host preference between either root species ordinations were performed. The DCA plots show no clear separation between groups (Figure 3). NMDS ordinations were also constructed and could not find any substantial distinctions between root species' colonizing communities (Supplemental material, Figure S6). NMDS plots stress for the 5 most abundant ASVs was just low enough

for evaluation (0.048) but stress for the 100 most abundant ASVs was unsuitable (0.18).

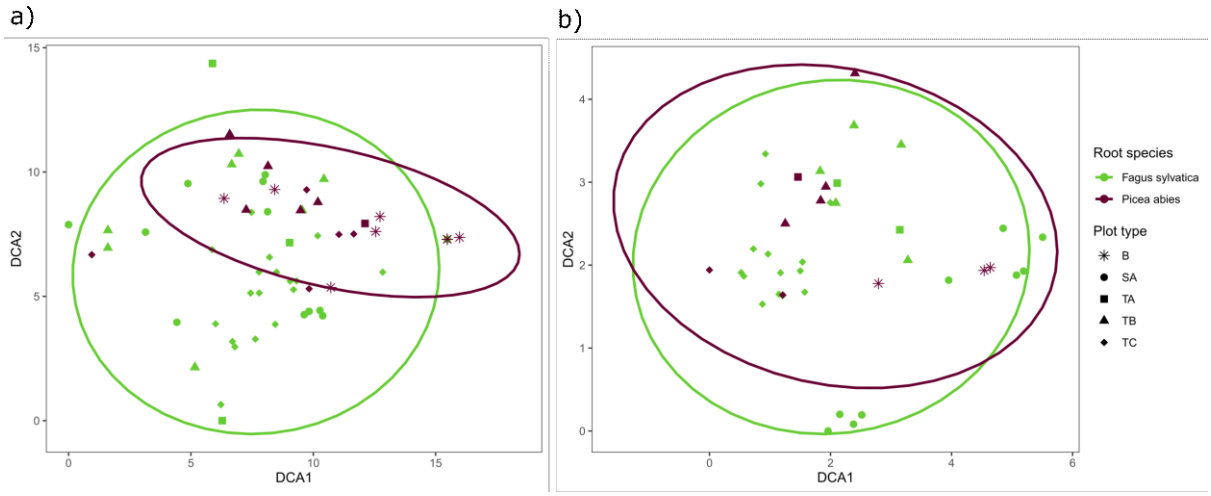

Figure 3 | **DCA ordination plots on presence absence data. A samples root species is represented by colour and its plot origin by shapes. a) For the 5 most abundant ASVs. b) For the 100 most abundant ASVs.**

A clear decay in community similarity with distance can be seen for both the 5 and 100 most abundant ASVs on the transect scale (Figure 4). Visual investigation of the relationship points towards a more pronounced distance decay for distances between 0 and around 20 meters. After this distance a certain level of similarity persisted irrespective of distance. With this insight from the transect scale in mind the subsequent analysis for plot scale community similarity distance decay was conducted. To allow comparison between analyses the Sørensen similarity index was chosen, which basically mirrors the percent of shared ASVs between two samples in the following analyses. A distance decay relationship is defined here as the reduction in sample pairs Sørensen similarity with increasing Euclidean distance. Similarity-distance Spearman's correlations were highly significant when considering all samples and *F. sylvatica* samples only (Supplementary material, Figure S7). Distance decay relationships were investigated by fitting both exponential and power law functions to the data. Both exponential and power law functions were able to describe the decay in community similarity to a similar extent with residual sum of squares giving no hints at either being better fit (Supplementary material, Figures S8 & S9). Exponential functions were fit to data trimmed to multiple ranks of n most abundant ASVs (Figure 5).

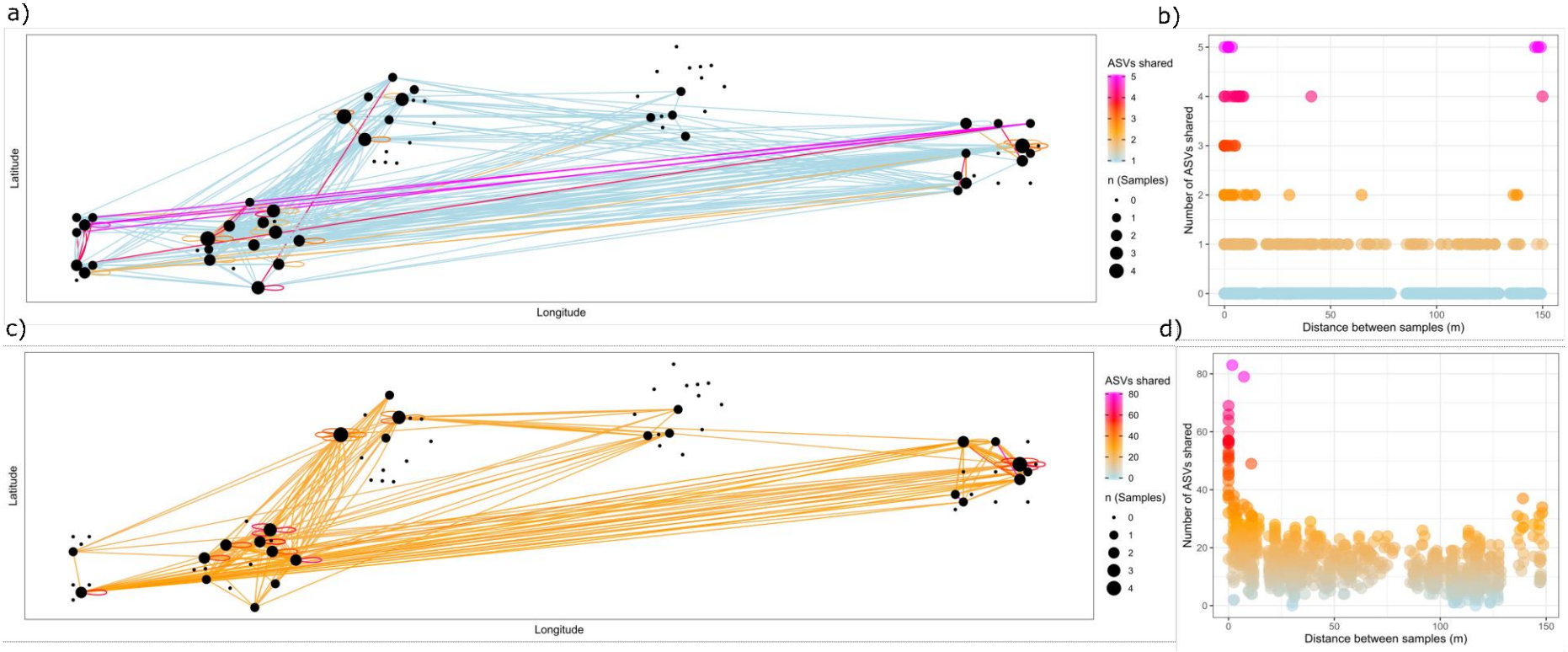

Figure 4 | **Transect scale: a & c) Spatial network visualization of community similarity defined as the amount of shared ASVs between two samples. Nodes represent sampling points and can be made up of multiple samples symbolized by a nodes size. Edges between nodes are produced when two samples share ASVs. As nodes can contain multiple samples they can produce self-edges (two or more samples from the same sampling point share ASVs). Likewise, two nodes can be connected by two or more edges when multiple samples between the sampling points produce edges. Sampling points with no samples included in the analysis are visualized by small dots (size zero). Edge weight reflects the number of shared ASVs between samples. a) Network considering the 5 most abundant ASVs. c) Network considering the 100 most abundant ASVs (edge weights below 20 are excluded from plot). b & d) Euclidean distance (meters) between two samples and the amount of ASVs they share (including zero values) for b) 5 most abundant ASVs. d) 100 most abundant ASVs.**

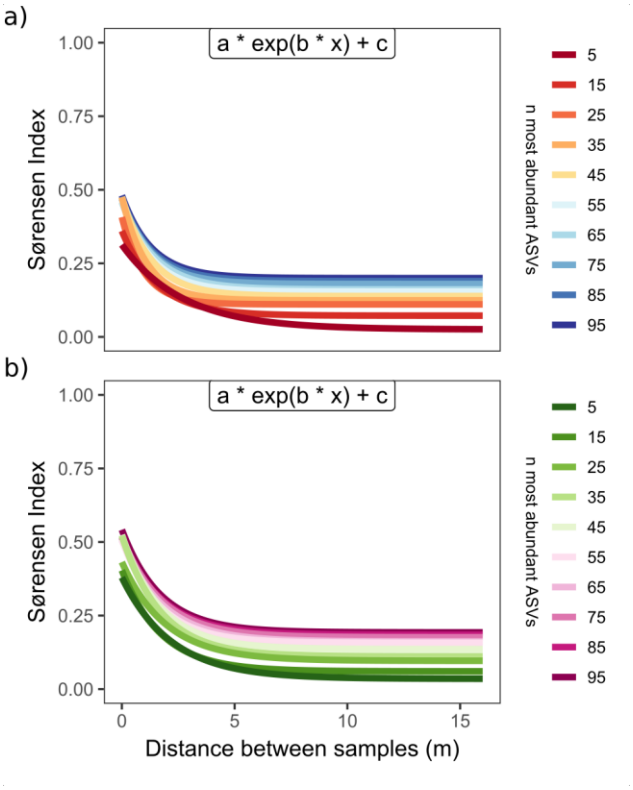

Figure 5 | **Distance decay relationship curves in community similarity over distance (m) at different levels of community data trimmed to the n most abundant ASVs. The exponential function used to fit the data is shown above the curves. a) Distance decay curves for all samples. b) Distance decay curves when including F. sylvatica samples only. Functions coefficient values can be found in Figure 6.**

The shape of these functions define the distance decay relationships and the three parameters *a*, *b* and *c* of the exponential function (f(x) = *a* * exp(*b* * x) + *c*; where x is distance) quantify this relationship. Parameter c can be seen as the level of "baseline similarity" for the relationship below which similarity does not fall even at far distances (Figure 6 upper plots). When looking at the most abundant ASVs we see that at greater distances there is very little similarity. The more ASVs we include in this analysis by their abundance, the more this overall similarity increases in a somewhat linear fashion. While similar to *c*, *a* does increase initially when including ASVs ranked by their abundance in the analysis a decreases after about 30 ASVs. The sum of coefficients a & c gives the functions Sørensen similarity at distance zero (x = 0). This similarity for the most adjacent roots (*a* + *c*) actually stays at fairly consistent levels when increasing the amount of ASVs above around 35 ASVs (Figure 5). Parameter *b* gives the rate of distance decay. It is comparatively low for the most abundant ASVs and decay increases strongly when including more ASVs by their abundance. The rate of distance decay is strongest at around 25 ASVs and becomes less pronounced when including more ASVs ranked by their abundance. Similar results were found for power law functions, although the interpretation of parameters *a*, *b* and *c* were not as easily interpretable as for the exponential function (Supplementary material, Figure S10). When investigating how curves and their

coefficients behave including *F. sylvatica* samples only similar basic patterns can be seen, which makes sense as they make up the majority of samples (Figure 5b; Figure 6 lower plots). However, the similarity for adjacent roots (coefficient *a* + *c*) is slightly higher than for all samples combined. Coefficient *b*, the rate of distance decay, does not show the same increase at 25 most abundant ASVs. It produces a jittering exponential decline (eveining out around 50 most abundant ASVs) and rates are not as strong as they are for the combined dataset.

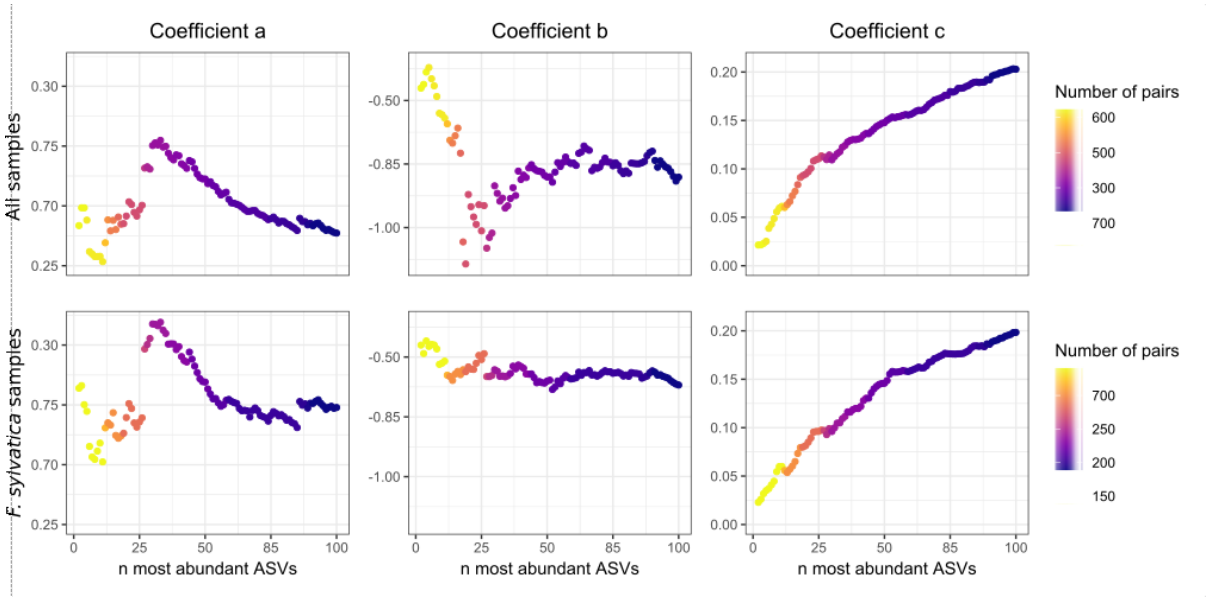

Figure 6 | **Exponential function coefficients of the distance decay relationship for data trimmed to n most abundant ASVs (n = 1 to 100) for the function: f(x) = *a* * exp(*b* * x) + *c*. Columns represent the functions coefficient values when data is trimmed to n ASVs ranked by their abundance. Coefficient *a* gives the initial value of the Sørensen similarity index at x = 0 minus coefficient *c*. Coefficient *b* gives the decay constant (as it is negative). The lower this value the stronger the in similarity with distance is. Coefficient *c* gives the shift of the function along the y-axis and can be seen as a "baseline similarity", a level under which similarity does not drop. The first row shows the exponential distance decay coefficients when all samples are included. The second row shows the coefficients when only *F. sylvatica* samples are included.**

The transect scale was also investigated for distance decay relationship curves of the two dominant root species. As the number of data points (sample pairs) differ quite substantially and different distances were not as widely covered by *P. abies* data the interpretability of the comparison is limited. Interpreting the available data it seems like distance decay for *F. sylvatica* is stronger than it is for *P. abies* (Supplementary material, Figure S11). Parameter b (the rate of exponential decay) was -0.42 for *F. sylvatica* and -0.18 for *P. abies* when fitting the data with the

exponential decay function. *F. sylvatica* also shows a higher initial similarity than *P. abies*.

Spatial networks, as presented in Figure 4 were also analyzed for certain network properties, which would hint at spatial community structuring (note that an edge weight threshold was implemented in the visualization of Figure 4c). Mean degrees and mean clustering coefficients both were generally higher for all plots compared to the transect scale network (Table 6). The TA plots network contained very few samples, which likely skewed the calculated results. Differences in mean degree and mean clustering coefficient were strongest for the networks produced considering the 5 most abundant ASVs and differences between the transect scale networks and plot scale networks became less pronounced when increasing the amount of ASVs considered in network construction.

Table 6 | **Spatial network properties. Networks were created once for all samples (All = transect scale) and then for all individual plots (B = Combined B1 & B2, SA, TA, TB and TC). Additionally these spatial networks were constructed for data trimmed to the 5, 20 and 50 most abundant ASVs. For all resulting networks the connectivity (mean node degree divided by all possible connections) and the mean clustering coefficient were calculated.**

	n(ASVs)	All	B	SA	TA	TB	TC
Mean connectivity	5	0.12	0.53	0.16	0.67	0.22	0.22
	20	0.59	0.71	0.70	0.67	0.73	0.84
	50	0.94	1.00	0.96	1.00	0.99	1.00
Mean clustering coefficient	5	0.64	0.92	0.74	0.00	0.72	0.64
	20	0.72	0.84	0.81	0.00	0.81	0.87
	50	0.95	1.00	0.97	1.00	0.99	1.00

The likelyhood for samples from the same cores to share the same ASVs is much higher than compared to any two pairs (Figure 7a). This is most pronounced for *F. sylvatica*, which has a likelyhood to share the same most abundant ASV of more than 40%. Increasing the amount of 'most abundant ASVs' only marginally reduces this value from here. Interestingly *P. abies* is fairly unlikely to share this first most abundant ASV (12%) however including the second most abundant it immediately reaches levels of *F. sylvatica*. The amount of shared ASVs between the different root types also reaches a similar level of around 40% but requires consideration of more ASVs ranked by their abundance. For the spatially independent comparison similarity is far lower (Figure 7b). *F. sylvatica* and *P. abies* behave similarly in this

regard. The probability of any two samples of either species sharing the same ASVs is substantially reduced compared to same species comparisons.

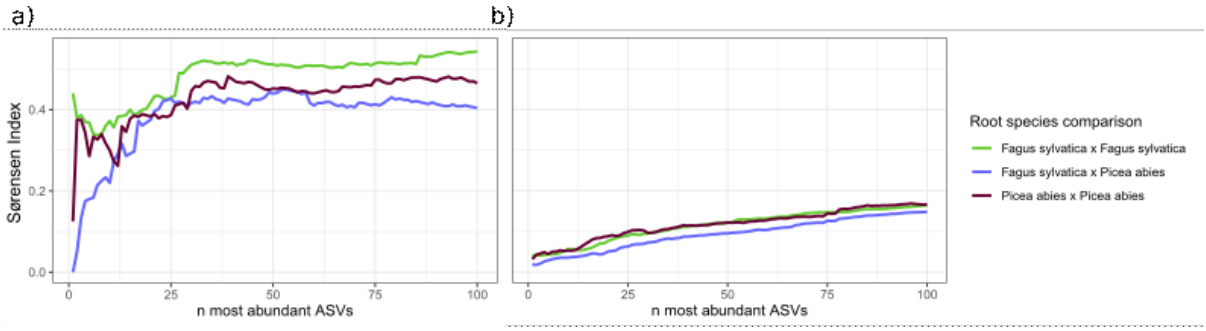

Figure 7 | **a) Pairwise similarity between samples from the same cores. Comparisons are made between samples belonging either to the same root species or between samples from differing species (*Fagus sylvatica* x *Picea abies*). The percentage of ASVs shared between samples is averaged for all observations in each category. b) Comparisons based on complete spatial randomness. All possible pairs are compared regardless of core association.**

Network analysis focussed on module description detected 19 individual modules in the inferred network. Figure 8a shows the constructed network and node colours represent their module affiliation (these same colour schemes are retained in Figure 8b & 8c and Figures S12 and S13 in the Supplemental material). Node shapes represent ASVs root host affiliations and many modules seen in the network are clearly dominated by host specific ASVs. Figure 8b & 8c show modules node composition (ASVs) on a functional and phylogenetic level. For almost all modules the dominant groups are symbiotrophs and Agaricomycetes. Modules vary in node sizes and on average contain 10 to 20 ASVs. Figures S12 and S13 found in the supplemental materials show modules ASV composition by samples. In these plots modules are ranked by average nodes mean degrees (within their module). Figure S12 consist of the first 1-10 and S13 of the 11-19 modules ranked by their internal mean degree. What can be consistently observed is that by the given network construction method and data trimming process (relative abundance threshold of 1%) modules mostly result from either single sample co-occurrences, or same core co-occurrences (multiple samples, originating from the same core). Very few modules - those with the least mean internal node degree – show a wider sample distribution.

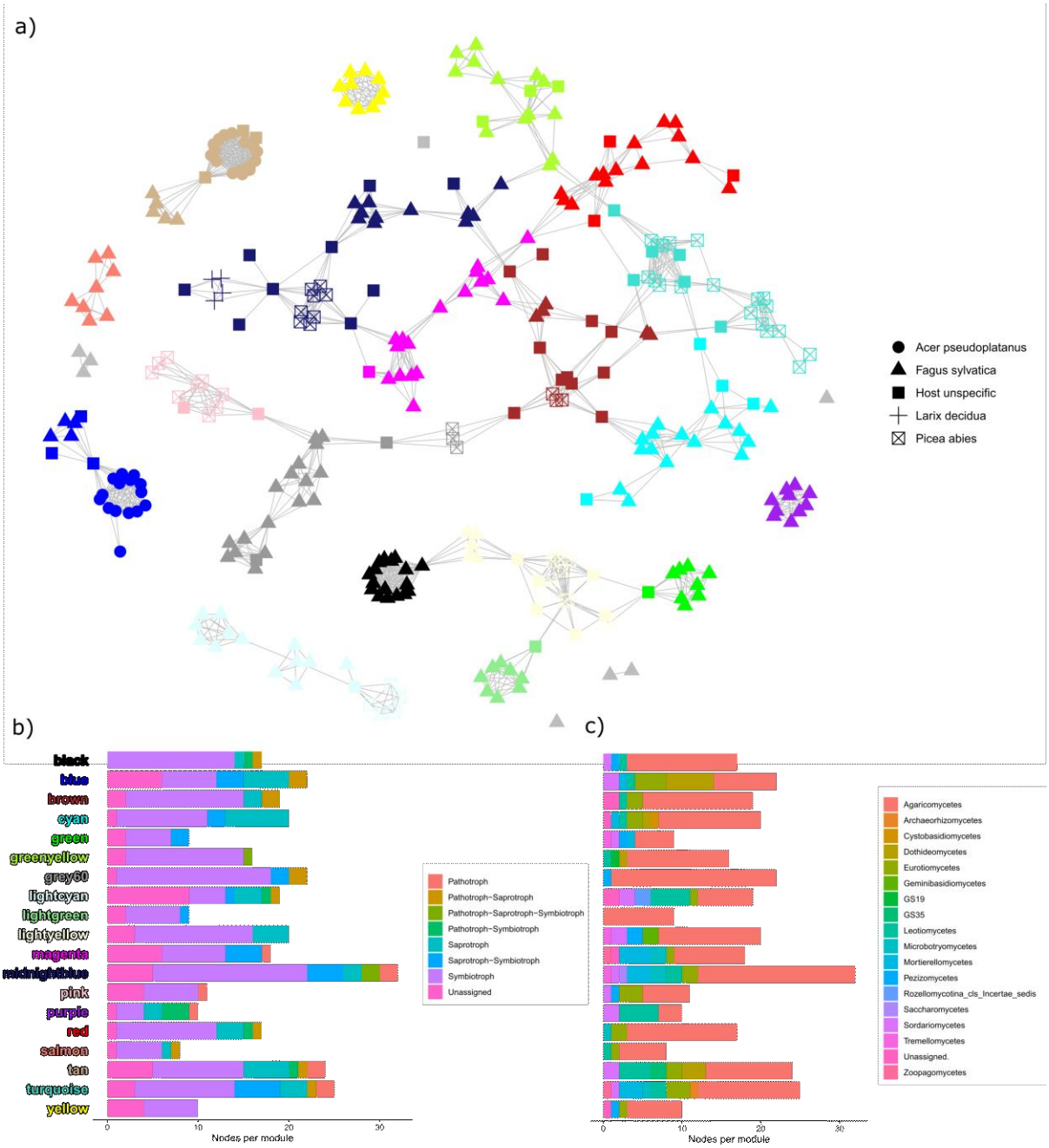

Figure 8 | **a) GLASSO network constructed from 'Higher than 500 sample reads data' trimmed by a relative abundance threshold of 1%. The network was selected for its distinct modular structure. Module assignment was performed using the 'Louvian method'. Node colours represent module affiliations; shapes represent root species affiliations. b & c) Community compositions of all modules. Y-axis colour labels match the colour assignments found in the network depicted a). b) ASVs functional composition (via FUNGuild assignment). c) ASVs taxonomic assignments at the class level.**

Groups co-occurrences were analysed based on individual C-scores calculated for all ASV pairs (The network of these associations can be found in the supplementary material, Figure S14). This analysis was aimed to investigate which phylogenetic or

functional groups would disproportionally co-occurr or co-exclude. As the C-score calculations are heavily impacted by two species prevalences (one species with a very high prevalence cannot achieve a significant C-score with a species with low prevalence) we investigated the prevalence distribution in the data used in C-score network construction. We found that prevalences were fairly similar between different groups (Supplementary material, Figure S15). Ascomycetes and Basidiomycetes tend to disprortionally co-occur with each other by a factor of around 0.3 (Supplemental material, Figure S16) but while there was a significant difference for the co-occurrence between both groups, the effect size of this difference was miniscule (0.035). The most strongly self co-occurring Phylum was Mucoromycota (with a factor of 2.24), followed by Rozellomycota (1.15) and Chytridiomycota (1.15). Mucoromycota and Rozellomycota had the strongest factor of co-exclusion observed (-0.86). Generally, intraphyla co-occurrences were higher than interphyla co-occurrences, which tended to be slightly negative. Investigating disproportionate levels of co-occurrence on a class level Agaricomycetes - by far the most abundant ASVs in the network – have a tendency to co-occur with themselves by a factor of 0.41 higher than compared to the null model (Figure 9). Ascomycete subgroups show strong tendencies for co-occurrence. For example Eurotiomycetes and Dothideomycetes disproportionally co-occur by a factor of 2.10 and 1.55 respectively. Both these groups also tend to co-occur 1.04 times more likely with each other than expected by chance. Sordariomycetes, another class in the phyla of Ascomycetes also tend to co-occur with Dothideomycetes and Eurotiomycetes (factors 0.65 & 0.43), less so with themselves (factor 0.25). In regard to disproportional co-occurrences between functional groups certain results parallel previous findings (Figure 10). Symbiotrophs have a 0.54 times higher co-occurrence likelihood than expected by the null model. Pathotrophs showed very similar levels of positive co-occurrences with each other. Saprotrophs also show this trend but to a much lower extent (0.16). Interestingly no significant relationships between these three groups could be found. Endophytes showed a large fold increase with each other compared to the null model (2.27). The increase for Ectomycorrhiza was comparatively lower (0.64). It must be mentioned that the most prevalent ‘group’ in these function based group analyses was the functionally unassigned and as here representatives of all groups are likely pooled to an extent interpretation of these results requires caution.

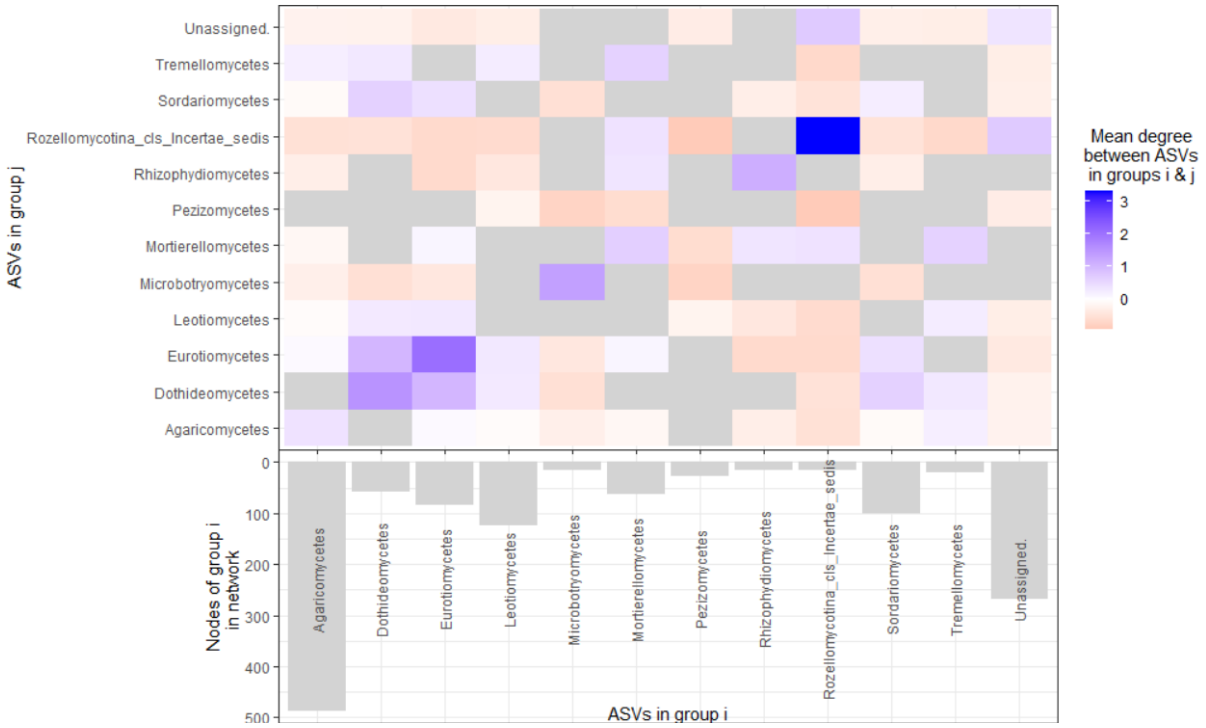

Figure 9 | **Disproportionate co-occurrences between groups in the C-score network (< 0.05 p-value). For the upper part of the plot (tiled matrix) all edges between nodes are quantified according to ASVs taxonomic assignment at class level. Edges within and between nodes of these groups are then counted. This results in the mean degree of nodes considering only those edges within this group, or between two groups. This was repeated for the null model - randomly rewired networks. This random rewiring was conducted 1000 times and the resulting null distribution was used to assess significance of the true measured value. Tile colour represents the fold increase or decrease in mean degrees, so the fold difference between the observed results values and the mean value of the null distribution. All tiles filled with values produced significant differences to the null distribution. Groups with less than 1% of total nodes assigned were excluded from the graphic.**

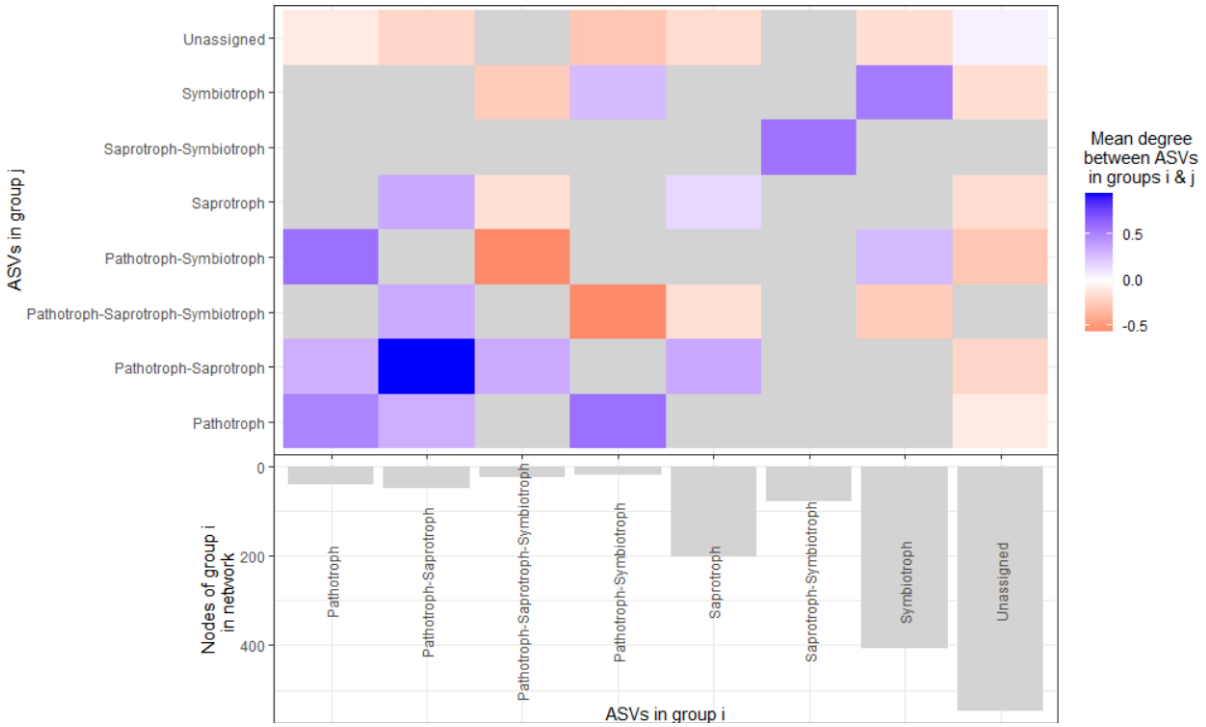

Figure 10 | **Disproportionate co-occurrence fold increase by node function affiliations in comparison with a null model. For the upper part of the plot (tiled matrix) all edges between nodes from the C-score network (< 0.05 p-value) are quantified according to nodes classification at functional level. All nodes assigned are classified at this level and edges within these groups and between these groups are counted. This represents the mean degree for nodes within this functional group, or between two functional groups. This was repeated for the null model - randomly rewired networks. Results of this random rewiring were used to assess significance between the true measured values and the random distribution. Tile colour represents the fold increase or decrease in these mean degrees and results from the difference between the observed results values and the mean value from the null model. Functions whose nodes made up less than 1% of the total number were excluded from this figure. All tiles filled with values produced significant differences to the null model. Groups with fewer than 1% of total nodes assigned were excluded from the graphic.**

Discussion

Our main objective was to identify the key structures that root fungal associations exhibit in temperate forest ecosystems. A general overview of tree species root distributions gave us some context to interpret further fungal community data by informing at which relevant scales this distribution might exhibit levels of structuring. On a plot level (15 x 15 m) general parallels in tree stem species composition and root species composition were seen (Table 3). At a soil core depth of 4.5 cm *F. sylvatica* tends to be overrepresented in comparison to *P. abies*, but to varying extents (Table 4). While *P. abies* tends to produce more shallow root systems in mixed beech forests, *F. sylvatica* has been shown enhanced radial growth in competition with *P. abies*, shifting fine root production towards shallower soil layers, which might explain these findings (Schmid & Kazda, 2001; Zwetsloot et al., 2019). On a shorter scale, nearest neighbor tree stem proximity played no role in root species prediction (Table 5). In their model Farrior (2019) shows how trees root growth constraints, for example by investment cost increase with distance and interspecies competitive effects, impacts root exploration patterns and thereby mixed forests root distributions. High nutrient availability and homogenous nutrient distribution increase intermingling. Real world observations show that nutrient rich forest soils such as temperate forests tend to produce a large overlap between trees roots (Rewald & Leuschner, 2009; Schenk et al., 1999). As supported by our data, at very small scales spatial segregation is widely observed. *F. sylvatica* has been found especially adept at outcompeting other species here (Rewald & Leuschner, 2009). Our findings supported these scale dependent concepts: Plots stem to root ratios were somewhat consistent (Table 3); nearest tree stem proximity did not indicate root species (Table 5) and we found highly significant small scale root segregation. To assess root species impacts on such small scale patches we separated cores which were dominated by single species and mixed assemblages. Cores containing mixed root species did not differ significantly in measured soil properties from monospecific cores (Supplementary material, Figure S1). The only significant differences were traced to *P. abies* dominating many cores in the young beech forest plots (B1 & B2), which showed strongest edaphic deviation from other plots. Why exactly this patch would be dominated by *P. abies* fine roots is unclear. Results on

fine root distribution in our sampling region informed us that an intentional sampling design aimed at capturing a targeted root species composition can be informed by plot scale broad characterization of stem abundances but not by the nearest tree stem species.

For the assessment of root fungal community structures surprisingly few samples yielded sufficient sample quality (as defined in methods). We were not able to trace the effect to relevant edaphic properties (Supplementary material, Figure 2a; Read & Perez-Moreno, 2003). While samples suitable for analysis tended to be more closely situated (Supplementary material, Figure S2b) other reasons for this might have been endogenous PCR inhibitors from the plant material, which could have especially impacted amplification in those roots with low colonization (as we selected roots at random). As samples varied strongly in sequencing depth (and ASV richness) we applied a ‘top down’ approach for the analysis sorting ASVs by their relative abundance. With this approach effects structuring the most abundant ASVs could be contrasted to those effects impacting the entire root fungal community. Contrasting these approaches made sense as the most abundant ASVs were heavily dominated by ECM (mostly Agaricomycetes) a highly impactful functional group in temperate forest soils (Supplementary material, Figures S5). The first few most abundant ASVs made up a substantial part of the total relative abundance (Supplementary material, Figures S3a). Less abundant ASVs tended to be functionally more diverse (more likely Ascomycetes) and were more likely saprotrophs and/or pathotrophs. As roots were washed with deionized water before DNA extraction it is highly likely that a portion of less abundant ASVs originated from environmental DNA of soil/rhizosphere associated fungi.

To investigate drivers shaping root fungal communities we investigated both host preference and spatial structuring. Host preference analysis was restricted to *F. Sylvatica* and *P. Abies*. The amount of unique and shared ASVs within and between samples of either root type showed similar levels of host preference (Figure 2). Minor differences possibly arise from *P. Abies* slightly lower ASV richness (Supplemental material, Figure S3c & S4). Methods of ordination were neither able to distinguish host community dissimilarity nor dissimilarity by transect or plots (Figure 3; Supplemental material, Figure S6). As our sampling design selected root fragments from cores at random irrespective of ECM tip structures our findings cannot be

equated to studies with such targeted sampling designs. Such studies have found community dissimilarity between the tree species studied in our research (Rog et al., 2020). Our results should encompass broad root associated fungal communities which were not distinguishable by the ordination methods applied.

While ordinations did not distinguish plots and their positions on the transect scale significant spatial structuring was found when applying targeted spatial analysis. Spatial network properties such as connectivity and clustering coefficients (indicating samples similarity) were higher for plots than they were for the entire transects network (Table 6, Figure 4a & c). Transect scale distance decay patterns showed high abundance ASVs of *F. sylvatica* showed more pronounced decay with distance than those of *P. abies* although sample pair numbers varied greatly and therefore curves fit differ strongly in evidential support (Supplementary material, Figure S8). It could be speculated that ECM colonizers on *P. abies* have more spatially extensive growth patterns which can connect the more sparsely distributed trees and underrepresented roots (Tables 2 & 3) but these claims cannot be made with the data provided in this study. While host preference effects could not be seen on a wide community scale comparisons between sample pairs from the same cores (so where similarity was maximal) showed that host preference clearly impacted community structure strongly. The most abundant ASV were rarely shared between roots of differing species at this scale (Figure 7a) but were shared between roots of the same species with a likelihood between 30% and 50%. This means host preference was a highly impactful predictor for similarity of the most abundant ASVs at the core scale.

Visual inspection of the relationship between the number of shared species and distance hinted at plot scales being most appropriate for detailed analysis of distance decay relationships (Figure 4b & d). Both exponential and power law functions fit equally well to describe the relationship at the observed scales (Supplementary material, Figures S8, S9). Analyzing exponential functions parameters, the most abundant ASVs (generally ECM) were more likely to be shared across wider spatial scales which could be due to ECMs mycelial physiology and exploration/foraging behavior. The level at which the distance decay started to stagnate was between 5 and 10 meters (Figure 5) and strongest within a few meters which fits studies investigating ECM spatial autocorrelations (Lilleskov et al., 2004; Pickles et al.,

2012). Investigating *F. sylvatica* distance decay relationships only (Figure 5b) an increase in decay strength was seen but the increase was much lower than for all samples included. Distance decay was always lowest for the most abundant ASVs. These ASVs were generally ECM whose mycelia are known to span multiple meters and can connect multiple hosts. The general increase in decay strength, meaning an increase in spatial structuring, seen when increasing the amount of ASVs (ranked by abundance) might be due to the inclusion of certain symbiotic groups such as fungi with narrow exploration ranges (possibly also ECM), non-mycelial fungi, fully endophytic or pathogenic fungi. These would be more likely shared only at short ranges due to dispersal and growth limitations. For low abundance ASVs two effects on sequenced communities might come into play. First, environmental fungi, so for example tissue from free living fungi of the rhizosphere might introduce eDNA into sequencing. Second, significant levels of 'false' diversity can be sequenced in the form of relic DNA, genomic residues of dead microbes (inactive and therefore biologically less relevant, Carini et al., 2016). Goldmann et al. (2016) show that environmental fungi exhibit stronger spatial structuring than root mycobiomes. However, their study assess distance decay on scales of hundreds of kilometers. For our data inclusion of very low abundance ASVs tends not to increase adjacent roots similarity (sum of coefficients *a* & *c*) but does increase background similarity (coefficient *c*). This is the case both for distance decay relationships of all samples included, as well as those of only *F. sylvatica* assigned samples. More abundant ASVs on adjacent *F. sylvatica* roots are more similar as can be seen in the fitted curves of Figure 5b (Values given by the sum of coefficients a & c in Figure 6) and the same core similarity calculations of Figure 7a. Distance decay rates also differ between these datasets (Figure 6, Coefficient *b*). Decay strength for all samples and *F. sylvatica* only is similar for the most abundant ASVs. Interestingly, it increases much stronger when comparing more ASVs ranked by their abundance in all samples than for *F. sylvatica* only. This means that the probability of roots from separate species sharing intermediate- and low abundance ASVs is relatively high for adjacent samples and becomes low for samples on few meter scales. The amount of unique ASVs on *F. sylvatica* roots rose even when including low abundance ASVs (Figure 2b-e). It seems that these ASVs are also more likely to be shared across few meter scales between F. sylvatica samples than between root species. 'Baseline similarity' (coefficient *c*), the level of overall similarity regardless of

distance was very similar between both datasets regardless of ASV abundance. This aligns with results from other methods applied (which did not account for small scale spatial similarity) that could not find strong host preferential effects (Figures 3 & 7b).

Linking root associated fungi by their abundance distribution, host preference and imprint on distance decay relationships to certain broadly assigned functions could be a promising tool to further investigate the root mycobiomes core drivers. For sequencing based community assessment the most abundant ASVs will be those with the highest biomass and thereby highest gene copy number. ECM dominate community composition and their contribution to biomass can clearly be seen under the microscope (Supplementary material, Figure S5d & S18). They are most likely host preferential and show low spatial structuring. A group of intermediate abundance will more likely be endophytic, saprotrophic or pathogenic as these groups build up substantial biomass on and within the roots they colonize without producing the root-symbiont organ seen with ECM root tips (Hartig-net). Different groups might show different levels of host preference and spatial structuring. The least abundant ASVs will more likely stem from eDNA and relic DNA. In our analysis including these ASVs substantial spatial structuring but this might be driven more by neutral effects. Reference databases are a valuable tool for community ecologists. Analysis was aided by the FUNGuild database, which showed critical but also limited utility. While it covered a significant portion fungal ASVs, especially many low abundance ASVs were functionally unassigned or assigned vague functional categories. This made dissemination of distance decay relationships by functional groups unreliable. Given sufficient data and context information distance decay relationship analysis could be applied to specifically targeted root fungal groups, as has been done in plant biogeographical studies (Nekola & White, 1999). It needs to be mentioned here that root fungal abundance metrics are unreliable predictors for fungis ecological impact and therefore any inferences made with such information require supporting evidence to make robust claims. Root tip ECM abundance does not strictly correlate with ECM mycelial abundance (Kjøller, 2006) and ECM root abundance does also not correspond to sporocarp abundance (Horton & Bruns, 2001).

While the analysis of communities subgroup structuring was deemed unfeasible for spatial and host preferential analysis we applied a broad analysis on subgroups co-

occurrence patterns to investigate whether specific fungal groups were prone to inhabit the same roots. Both phylogenetic and functional grouping characteristics were of interest. Analysis of C-score co-occurrences relied on a crude null model comparison to investigate significantly co-occurring ASVs by grouping categories. Therefore results can only implicate certain group affiliations. ECM and pathogens showed disproportionately low co-occurrences, which can be explained by the protective effect ECM have on roots from pathogen invasion (Marx, 1972). Fungal species from Ascomycete groups such as Eurotiomycetes, Dothideomycetes and Sordariomycetes can exhibit endophytic functions such as dark septate endophytes (Jumpponen & Trappe, 1998). These groups produced significantly disproportionate co-occurrences with themselves and each other, but as many ASVs of these groups were not well covered by FUNGuild classifications, these patterns might also be ascribed to other functional groups than endophytes. Only very few ASVs were actually assigned endophytic but a high positive co-occurrence was found within this group compared to the null model. This could implicate that specific endophytes tend to co-infect hosts and thereby possibly interact and co-depend on each other. A complex of dark septate endophytes has been shown to co-infect hosts and even showed high host preference (Stroheker et al., 2021), which would lead to significant co-occurrences in data such as ours. However investigation into the occurrences of endophytes in our data did not show strong host preference. Alternatively these groups also contain saprotrophic functional groups such as molds. Here too, a potential mechanism, the establishment of degrader cohorts may explain the observed potential excess in co-occurrences. As mentioned, any of these hints at groups functional potential require further in depth investigation.

Our network analysis approach was focused on the identification of reoccurring modular structures for abundant fungi. The idea was to investigate whether certain associations would occur spatially independent from each other and therefore indicate a subgroups dependence on either similar environmental conditions or species interactions (together known as species sorting). As this analysis focused on potential fungal consortia results cannot be applied to single or pairwise ASV (co-) occurrence distributions and their potential hints at species sorting. All modules were primarily spatially associated and their construction either arose from single sample co-occurrences, or co-occurrences in samples from the same core, or in very few

cases neighboring cores. Modules were also generally composed of single host associated ASVs. These results clearly align with previous analyses and the fact that very few groups significantly reoccurred outside of spatially linked samples indicates that deterministic consortia sorting (either by environmental preference or species-species dependencies) on a community level may play a minor role in forests root associated fungal communities.

The multifaceted analysis into influences on root fungal community structure allowed a nuanced differentiation of drivers and uncovered their scale dependence. The approach of analyzing ASVs by their abundance showed that abundant root colonizers underlie different drivers than their less abundant neighbors. For more abundant ASVs distance decay relationships were found strongest at levels that could match ECM mycelial physiology but they differed between host root species. While conventional approaches in community dissimilarity analysis could not differentiate between either host species symbiont communities, host preference was extremely pronounced for the most abundant ASVs on smaller scales.

While roots of the same species tended to mingle with each other on the smallest scale we found no soil properties associated with either root species dominance which might influence (or be influenced by) colonizer composition. The synthesis of these findings paint a picture of highly diverse and spatially structured belowground root fungal communities in temperate forest soils. Few most abundant fungi make up a significant portion of colonizers overall abundance, can span multiple meters and show high local host preference. Network analysis gave no indications for the reoccurrence of community modules, which could indicate a high level of neutral effects and thereby low species sorting effects influencing the spatial structuring found in our data.

We were able to distinguish the relevant spatial scales for fungal colonizer similarity and host preference and determined that root associated fungi were generally impacted by different drivers depending on their abundance. Further characterization of the studied systems and more extensive databases on fungal species functions will allow more in depth anaylsis of root fungal ecology. For example, evidence of fungis participation in the common mycorrhizal network will enable more precise definition of these findings ecological impact and better dissemination of key groups

found in community tables. Are ECM of the common mycorrhizal network generally very host preferential and do they also tend to be the most abundant? Are they likely to connect roots over long distances? A study published recently was first able to describe exactly which fungal species were actually mediating nutrient flow between trees and differentiated specific groups connecting individuals between plant species and within species (Cahanovitc et al., 2022). More general questions can also be asked about how likely fungal ASVs found on two roots actually originate from the same individual mycelium (identical genotype). How are root fungal distance decay patterns linked to growth patterns such as mycelial physiology? Are more abundant root associated fungi those which receive most resources from their host and do they also provide the most benefits to their hosts?

Finding answers to these questions by determining in what way they imprint on community patterns, so with what confidence effect-pattern relationships can be generalized will immensely improve this field of research. Such answers and studies investigating the impact of environmental change or disturbances on forests tree composition and the drivers of root fungal communities will allow an integrated host-symbiont view on these systems. A major goal in forest ecological research should be to link in depth studies on underlying mechanisms of different root fungal symbiotic relationships (as well as how these interact with each other) with root and fungal community distribution patterns. Once these links are well established research such as conducted in this study could serve as a great tool for monitoring and quantifying community scale shifts in the face of environmental change, to aid specific hypothesis testing and to validate modeling approaches.

Supplementary Material

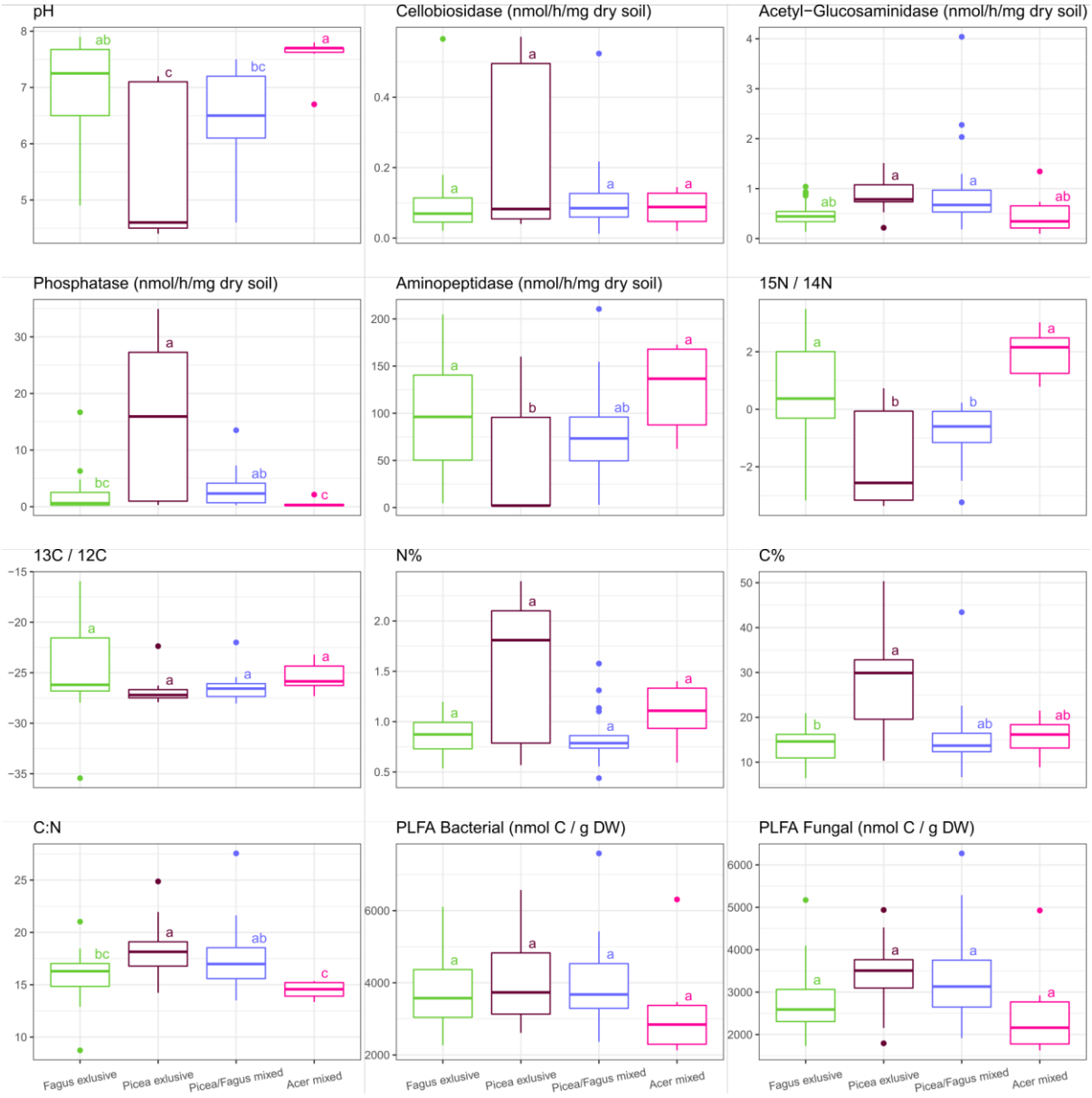

Figure S1 | **Soil property variation for cores containing either *F. sylvatica* (30 samples) or *P. abies* (9 samples) only, or mixed assemblages. 'Picea/Fagus mixed' (19 samples) contain those species only. 'Acer mixed' (6 samples) contained *A. pseudoplatanus* (an arbuscular mycorrhizal tree) and other root species. Letters represent significant differences between core types which were calculated by Kruskal Wallis Test ($p < 0.05$).**

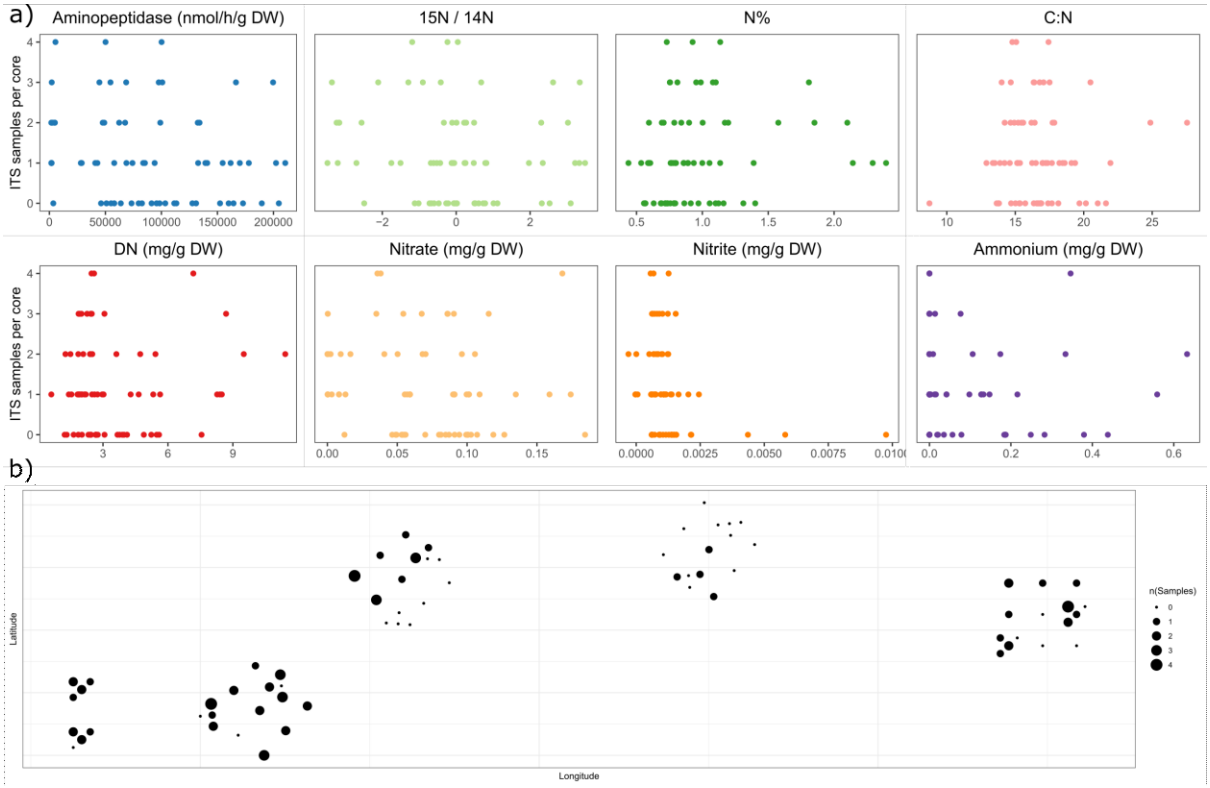

Figure S2 | **Assessment of DNA sequencing quality for ITS sequences. The term 'ITS samples per core' refers to the amount of samples, which reach the minimum requirement of 50 reads of the all collected samples (5) per core. Figures a) shows soil properties relevant to Nitrogen cycling in soils as it has been proposed that a soils nitrogen status dictates the levels of ECM colonization (the most abundant ASVs found in our samples). No significant correlations could be found. b) Represents the spatial distribution (pseudopositions for B and SA) of sampling points and the amount of samples matching the criteria mentioned above.**

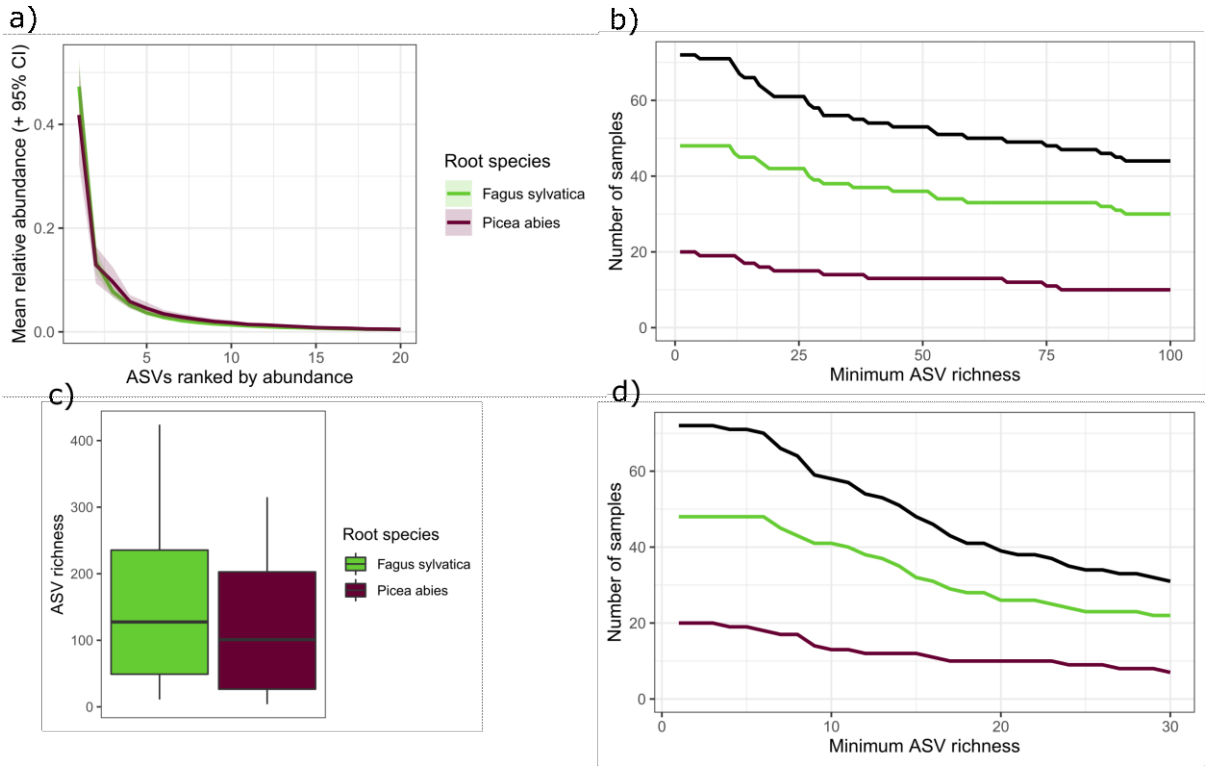

Figure S3 | **Fungal sequencing data distributions (72 samples). a) *F. sylvatica* and *P. abies* samples mean relative abundances of ASVs (y-axis) ranked by their relative abundance (x-axis). Confidence intervals show variability between samples. c) Boxplots show ASV richness (total number of ASVs) for all samples between *F. sylvatica* and *P. abies*. b) The amount of samples included in the analysis (y-axis) that contain a certain ASV richness (x-axis). Analysis conducted on the 'n-most abundant ASVs' will only consider those samples that contain the specified ASV richness. Line colours match species from a) and the black line contains both specie samples. d) The same plot with data trimmed to only contain ECM assigned ASVs.**

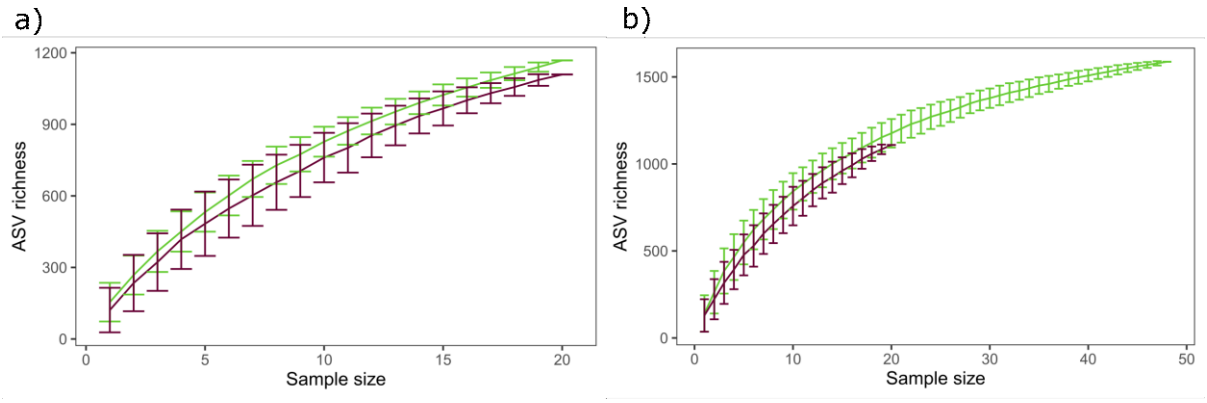

Figure S4 | **Species accumulation curves for *P. abies* and *F. sylvatica* as implemented in the specaccum function of the vegan package in R. Graphs show how species richness increases with the addition of samples (y-axis) and the saturation of the resulting curve hints at the level of unsampled diversity. a) Including all samples for either root species. b) Subsampled and averaged values for *Fagus sylvatica*. The same colour scheme as seen in Figure S3 is applied.**

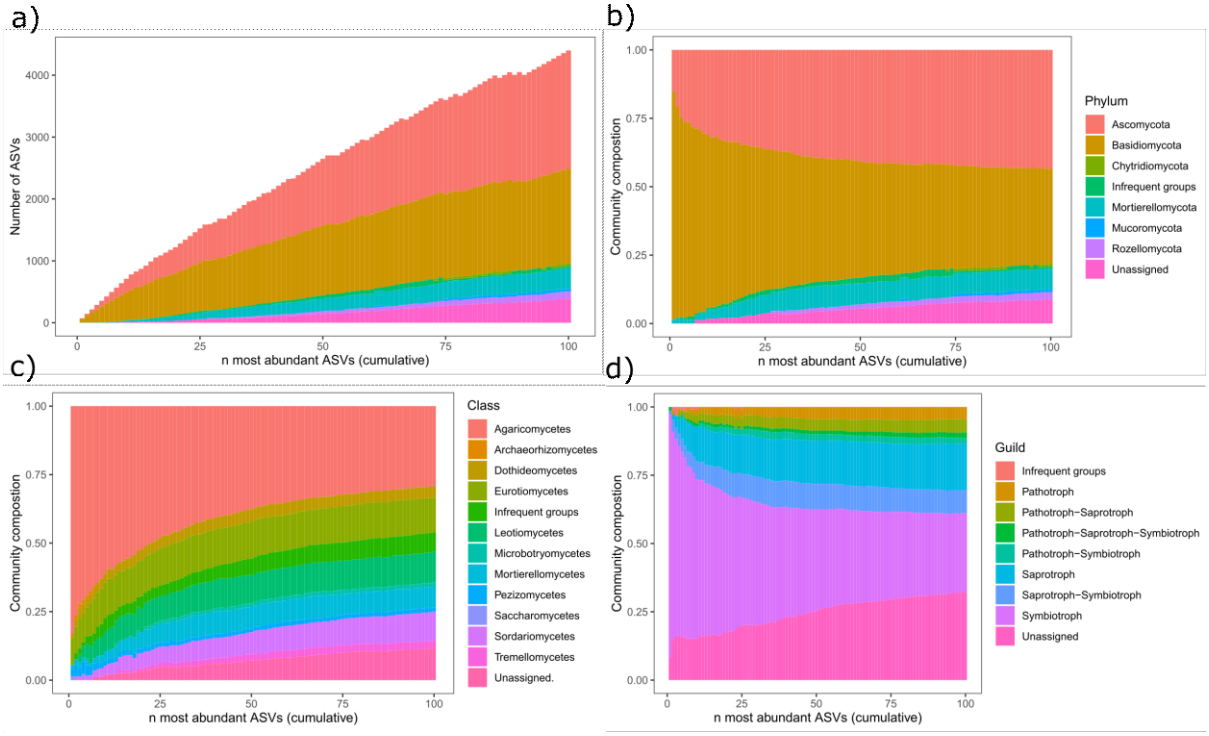

Figure S5 | **ASVs are ranked by their abundance in each sample and the data is trimmed to only include ASVs up to a certain rank (n most abundant ASVs = x-axis). The community composition for all ASVs down to this rank (= cumulative) is then assessed for all samples. All groups (taxa or guild) with a lower contribution to community composition than 1% are categorized as 'Infrequent groups'. a) Absolute number of ASVs by phylum. Community composition by: b) Phylum. c) Class. d) FUNGuild assigned Guilds.**

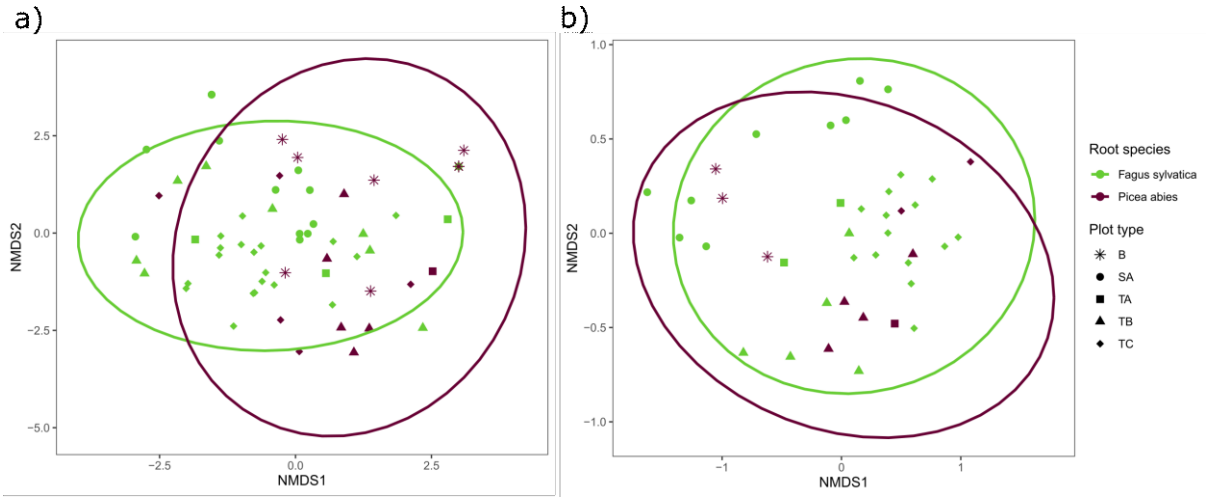

Figure S6 | **NMDS ordination plots based on Bray Curtis dissimilarity. Root species identities (colours) and site origin (shapes) indicate expected groups. a) For the 5 most abundant ASVs stress was 0.048 and therefore barely fits analysis; two outlier samples were deleted. b) For the 100 most abundant ASVs stress was 0.18 and therefore defined unfit for analysis.**

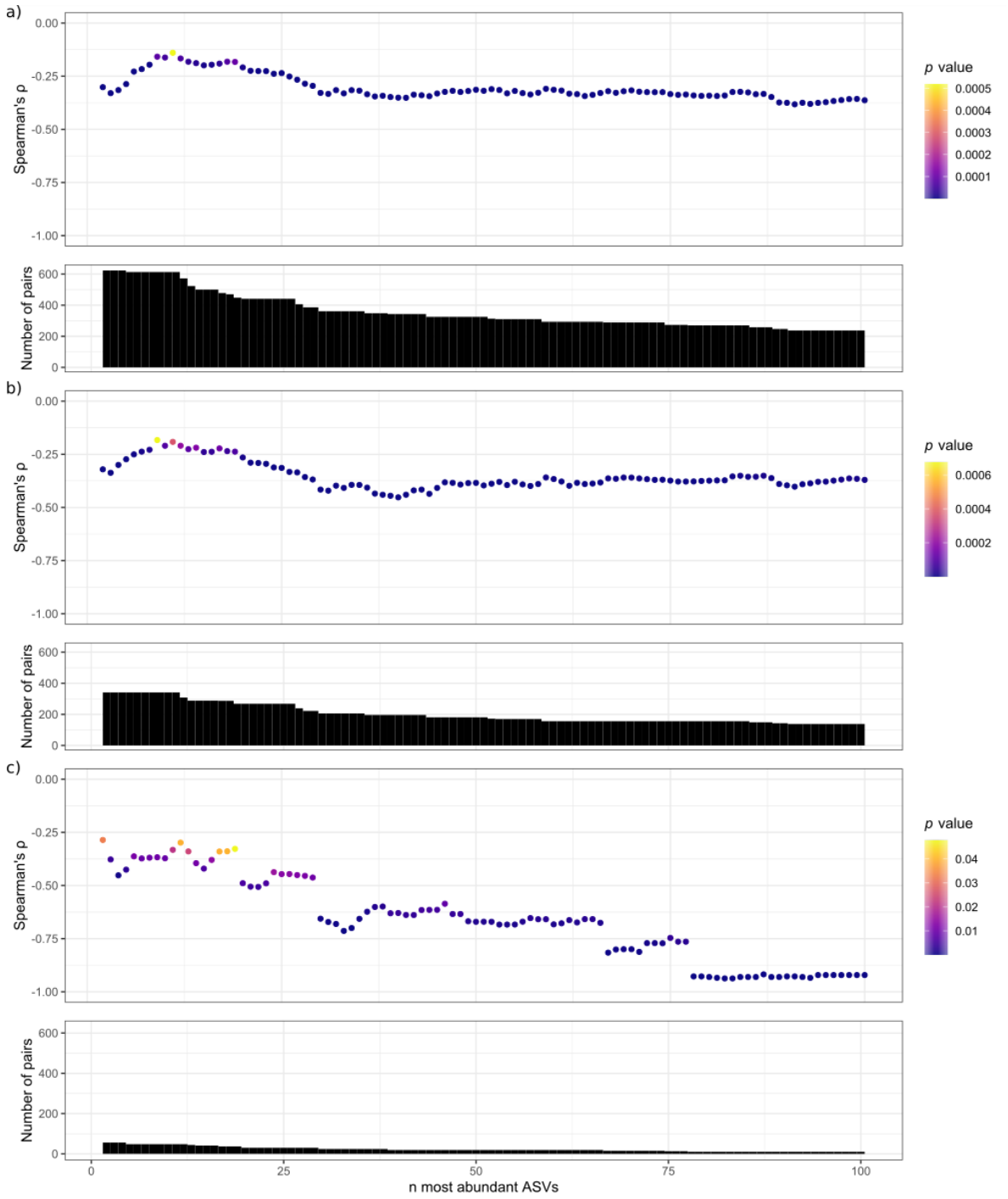

Figure S7 | **Spearman correlation coefficients for the relationship between Sørensen similarity and distance (m) for data trimmed to n most abundant ASVs (n = 1 to 100). The number of pairs included in calculations are given in barplots below each correlation plot. a) All roots included. b) Only roots assigned *F. sylvatica*. c) Only roots assigned *P. abies*. Significance values are represent by colours.**

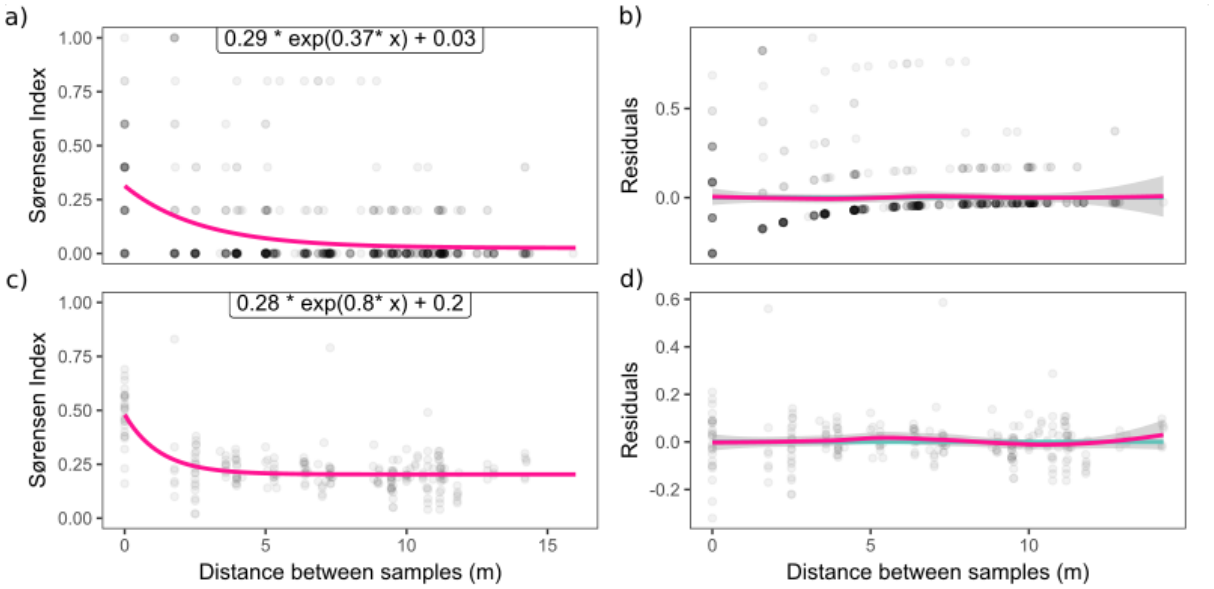

Figure S8 | **Distance decay relationship as an exponential function fit to Sørensen similarity index related to distance between sample pairs. a) Curve fit to data trimmed to 5 most abundant ASVs per sample. b) Residual plots (of a) with LOESS (locally estimated scatterplot smooting) overlayed (RSS = 18.36489). c) Curve fit to data trimmed to 100 most abundant ASVs. d) Residual plots (of c) with LOESS overlayed (RSS = 2.093832).**

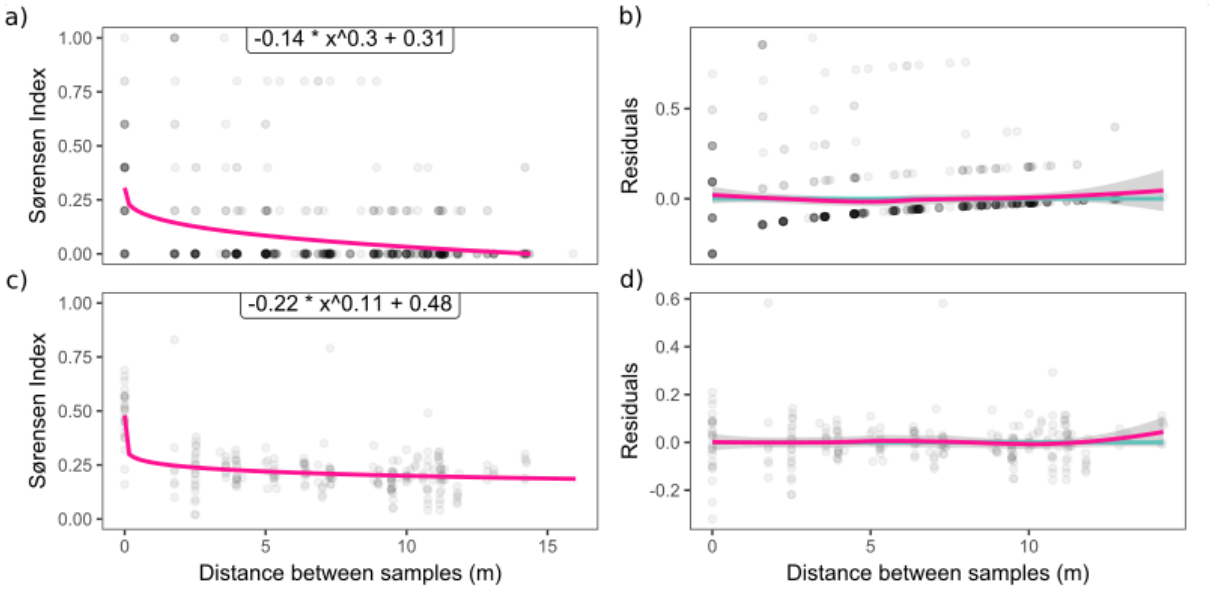

Figure S9 | **Distance decay relationship as an power law function fit to Sørensen similarity index related to distance between sample pairs. a) Curve fit to data trimmed to 5 most abundant ASVs per sample. b) Residual plots (of a) with LOESS (locally estimated scatterplot smooting) overlayed (RSS = 18.36489). c) Curve fit to data trimmed to 100 most abundant ASVs. d) Residual plots (of c) with LOESS overlayed (RSS = 2.093832).**

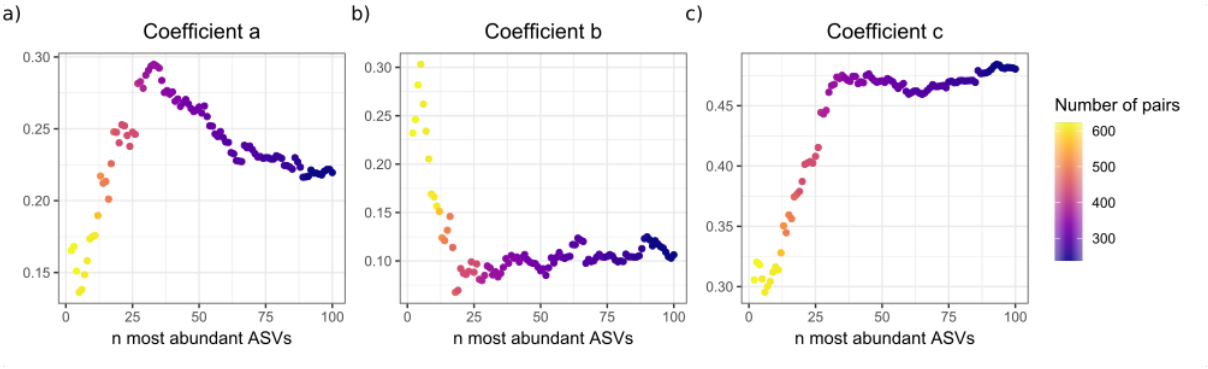

Figure S10 | **Power law function coefficients of the distance decay relationship for data trimmed to n most abundant ASVs (n = 1 to 100) for the function: f(x) = a * x1^b + c. a) Coefficient a can be seen as the slope of the initial decline of the curve. b) Coefficient b gives the decay constant. The lower this value the stronger the in similarity with distance is. c) Coefficient c gives the initial Sørensen similarity at x = 0.**

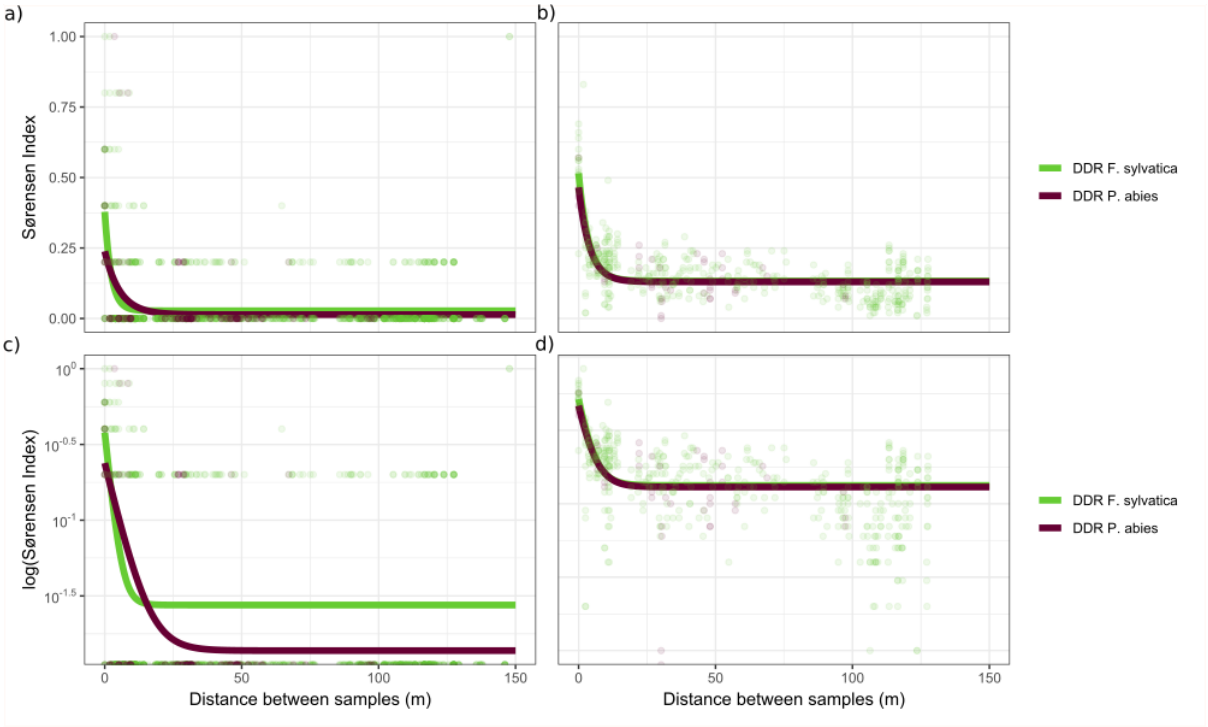

Figure S11 | **Power law function coefficients of the distance decay relationship for data trimmed to n most abundant ASVs (n = 1 to 100) for the function: f(x) = a * x1^b + c. a) Coefficient a can be seen as the slope of the initial decline of the curve. b) Coefficient b gives the decay constant. The lower this value the stronger the in similarity with distance is. c) Coefficient c gives the initial Sørensen similarity at x = 0.**

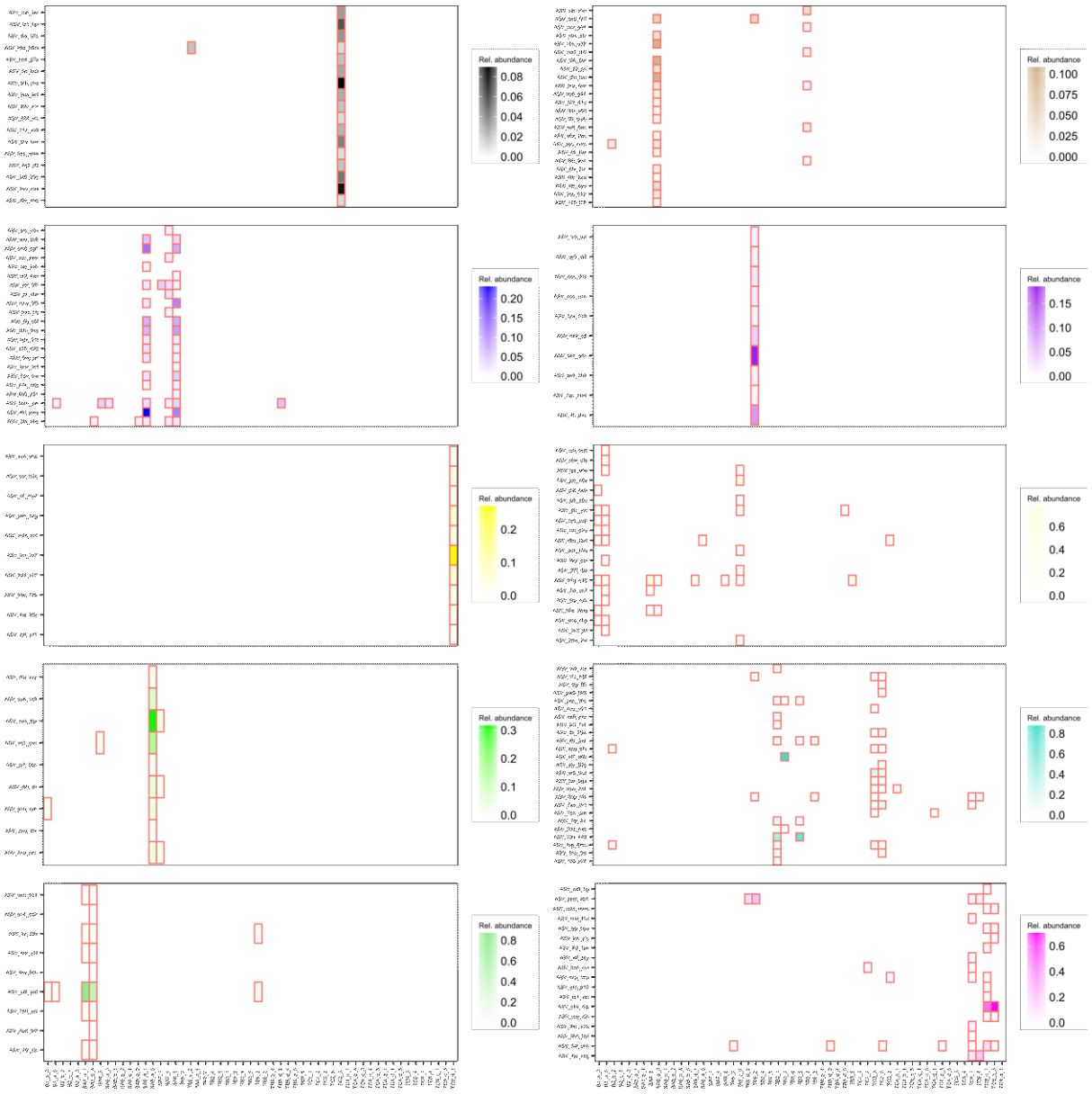

Figure S12 | **Part 1: Modules are coloured by the assignment seen in Figure 6 and sorted by their assortatitvity (internal mean degree) from left to right and top to bottom (Part 1 = Modules 1 to 10). ASVs (nodes) associated with either module are listed on the y-axis and samples in which they occur are listed on x-axis. Datapoints (signified by red boxes) represent occurrences above 0.01 relative abundance, as this threshold was implemented for network**

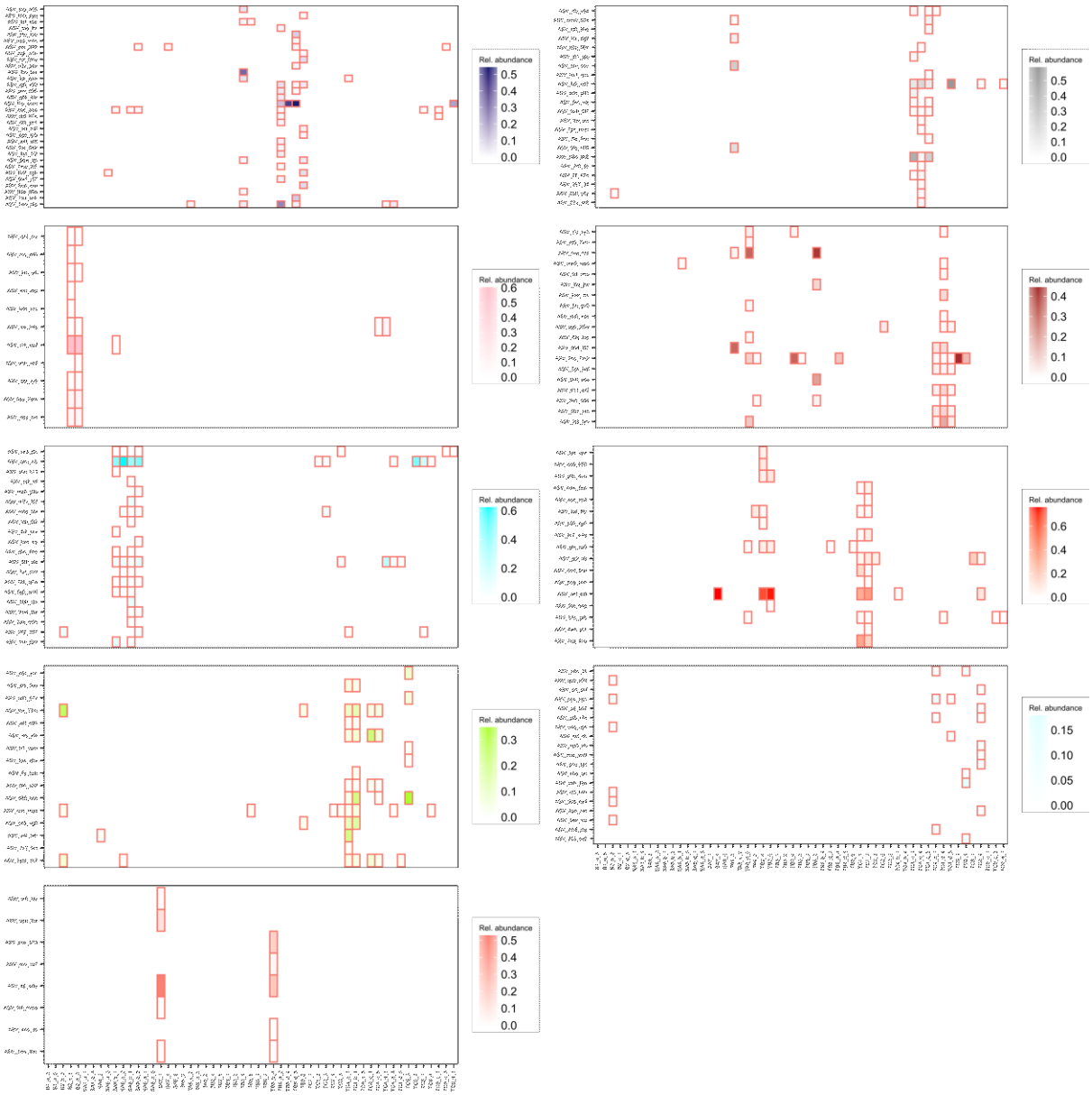

Figure S13 | **Part 2: Modules are coloured by the assignment seen in Figure 6 and sorted by their assortatitvity (internal mean degree) from left to right and top to bottom (Part 1 = Modules 11 to 19). ASVs (nodes) associated with either module are listed on the y-axis and samples in which they occur are listed on x-axis. Datapoints (signified by red boxes) represent occurrences above 0.01 relative abundance, as this threshold was implemented for network construction.**

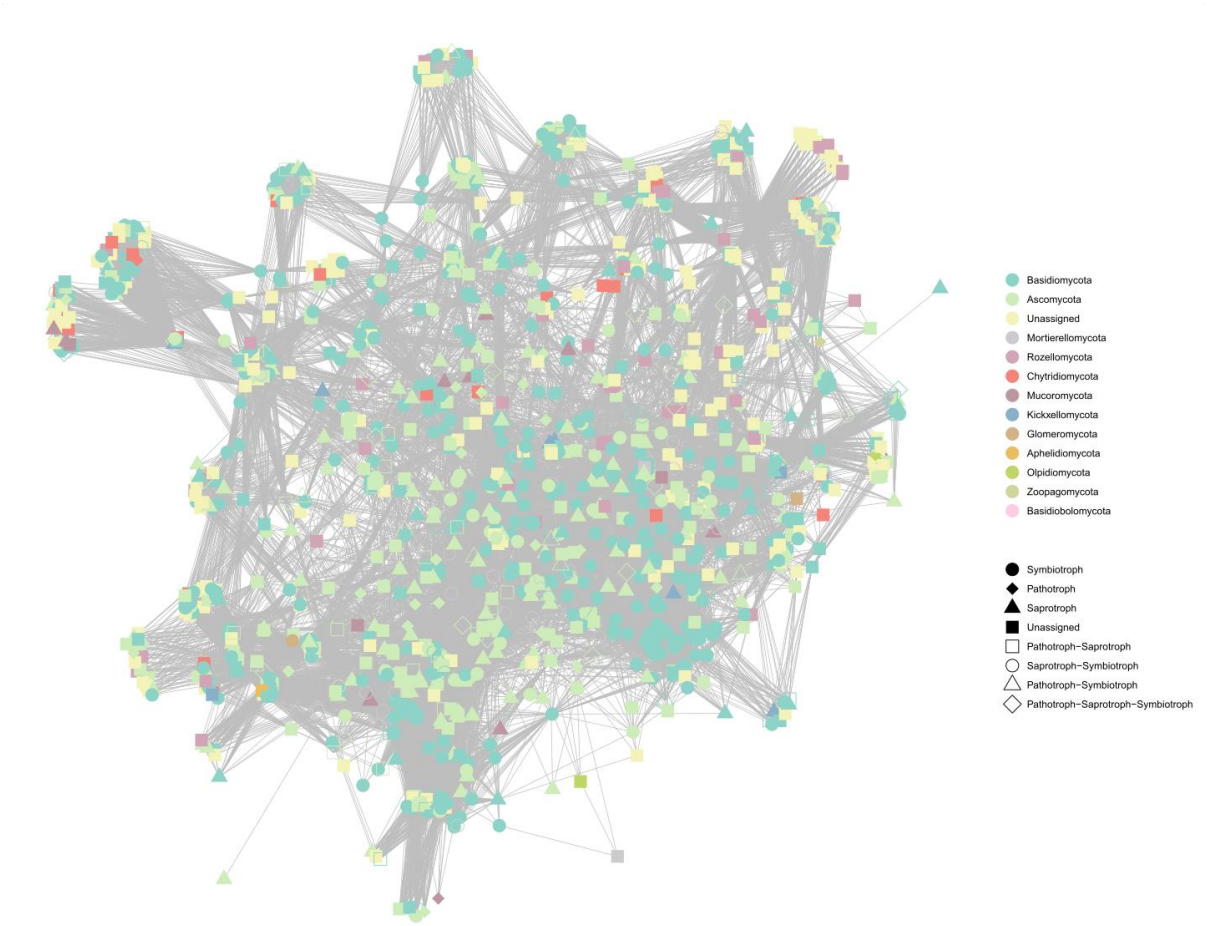

Figure S14 | **C-score network (hairball) constructed on the 100 most abundant ASVs in 44 samples. C-scores association significance threshold was set to <0.05. Nodes are coloured by an ASVs phylum and shapes reflect their functional classifications.**

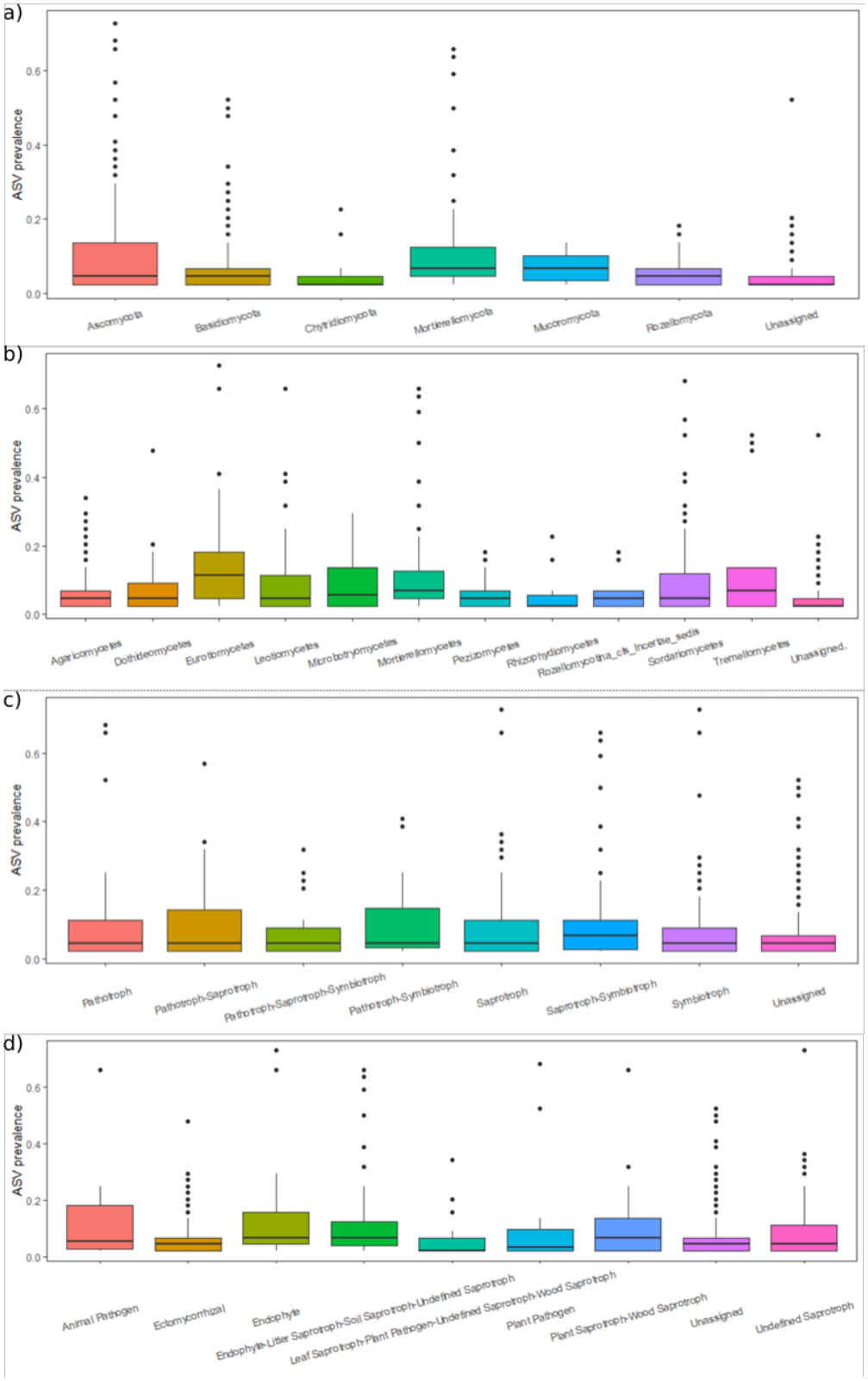

Figure S15 | **Prevalence distribution of ASVs within various grouping categories. Data was trimmed for the 100 most abundant ASVs (44 samples) and was used for group co-occurrence analysis. a) Grouping by phylum. b) Grouping by class. c) Grouping by function (broad). d) Grouping by function (specific).**

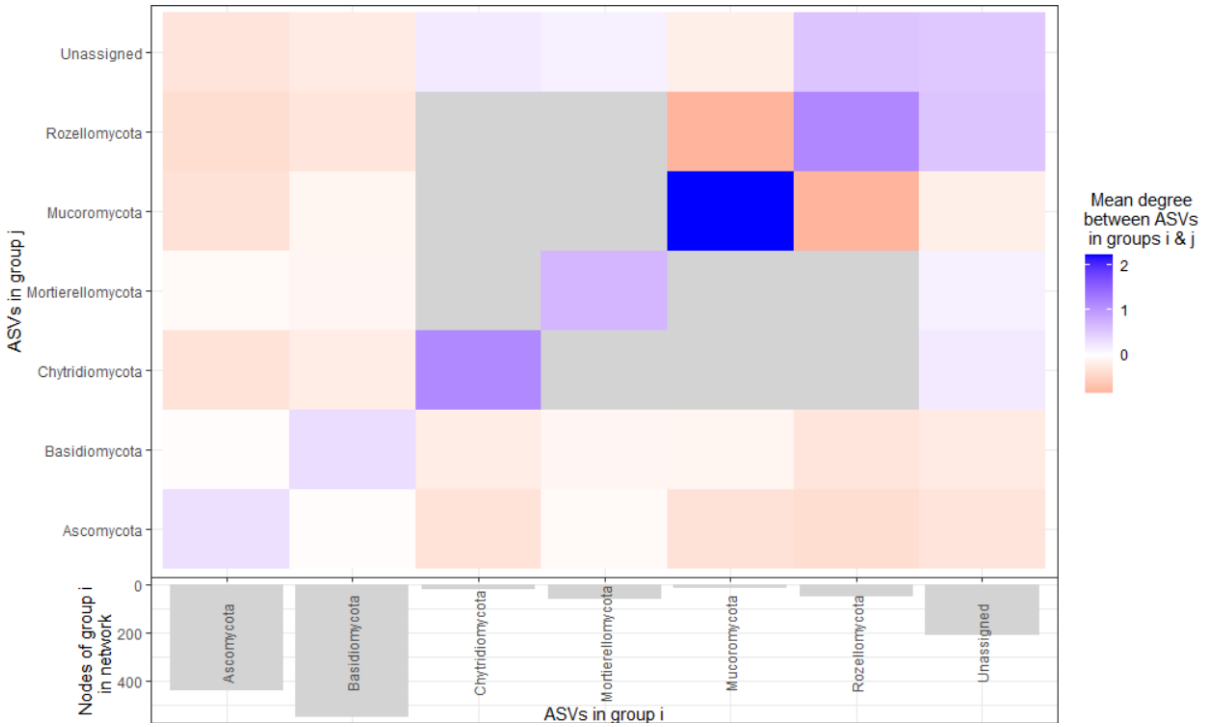

Figure S16 | **Disproportionate co-occurrence fold increase by node Phylum affiliations in comparison with a null model. For the upper part of the plot (tiled matrix) all edges between nodes from the C-score network (< 0.05 p-value) are quantified according to nodes taxonomic classification at phylum level. All nodes assigned are classified at this level and edges within these groups and between these groups are counted. This represents the mean degree for nodes within this phlyum, or between two phyla. This was repeated for the null model - randomly rewired networks. Results of this random rewiring were used to assess significance between the true measured values and the random distribution. Tile colour represents the fold increase or decrease in these mean degrees and results from the difference between the observed results values and the mean value from the null model. All tiles filled with values produced significant differences to the null model. Groups with fewer than 1% of total nodes assigned were excluded from the graphic.**

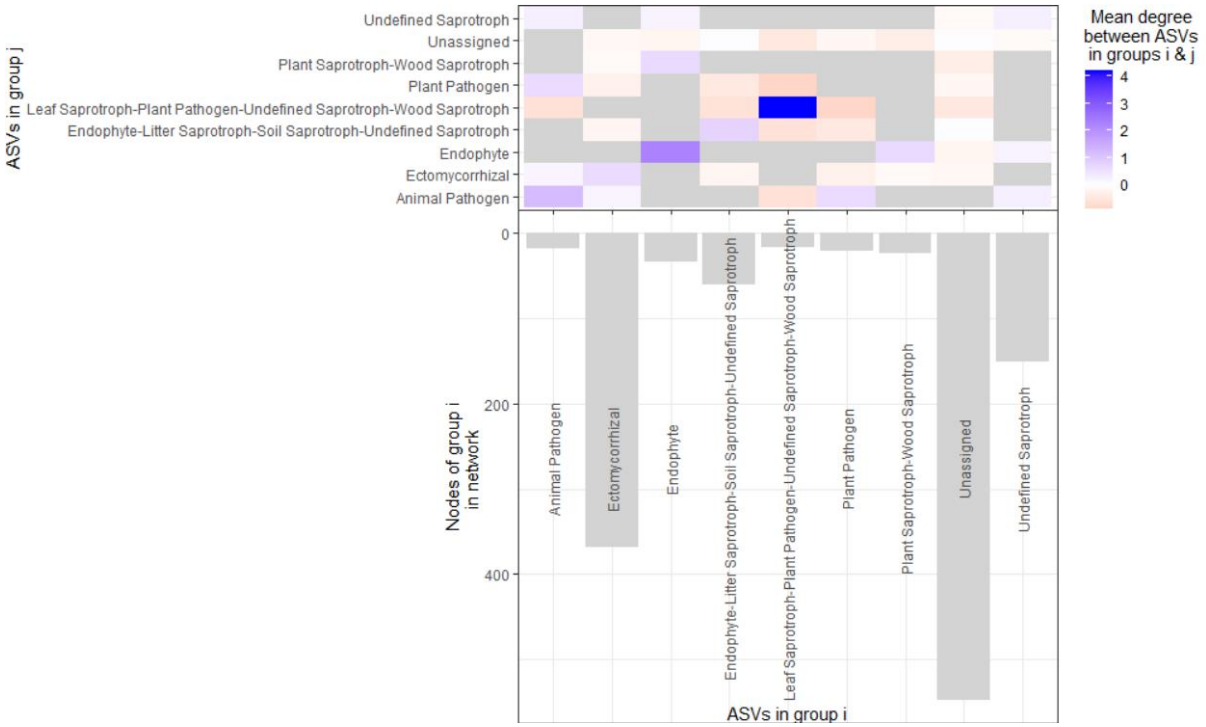

Figure S17 | **Disproportionate co-occurrence fold increase by node function affiliations in comparison with a null model. For the upper part of the plot (tiled matrix) all edges between nodes from the C-score network (< 0.05 p-value) are quantified according to nodes classification at functional level. All nodes assigned are classified at this level and edges within these groups and between these groups are counted. This represents the mean degree for nodes within this functional group, or between two functional groups. This was repeated for the null model - randomly rewired networks. Results of this random rewiring were used to assess significance between the true measured values and the random distribution. Tile colour represents the fold increase or decrease in these mean degrees and results from the difference between the observed results values and the mean value from the null model. Functions whose nodes made up less than 1% of the total number were excluded from this figure. All tiles filled with values produced significant differences to the null model. Groups with fewer than 1% of total nodes assigned were excluded from the graphic.**

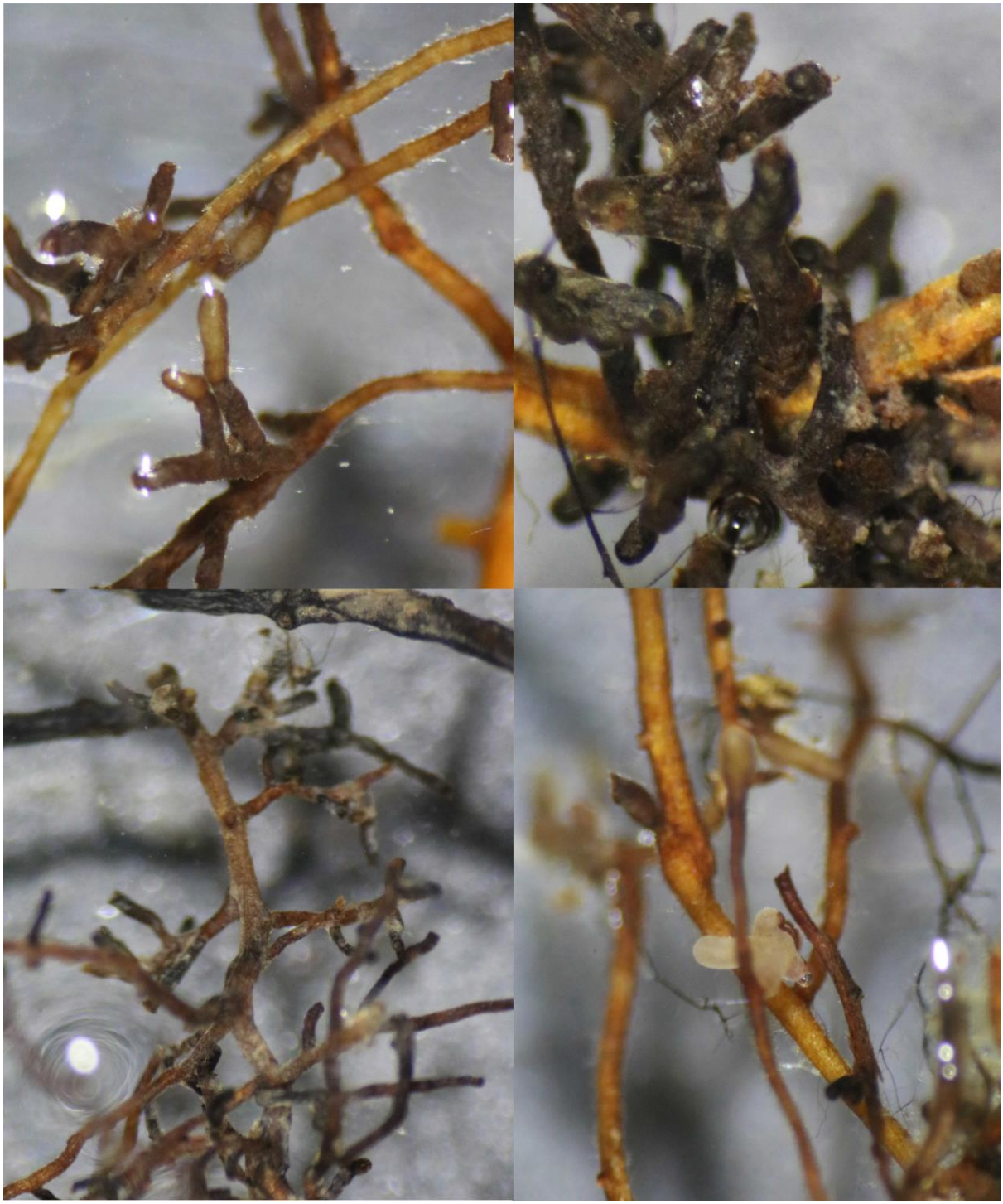

Figure S18 | **Photographs of root fungal associations taken during sampling. Pictures show a random assortment of structures found on roots. Morphology of root tips are most likely ectomycorrhizal.**

PART 3

Summary

Temperate forest systems provide crucial services to human and global health. Forests store the vast majority of terrestrial carbon and their protection and regrowth has been argued to be the most crucial component in mitigation efforts combating anthropogenic climate change. Root fungal associations such as ectomycorrhiza, endophytes and pathogens heavily impact nutrient cycles and tree fitness and thereby mediate forest ecosystem functioning. Describing their community composition and host-, soil property- and spatial dependencies can inform models and conservation policy.

This study investigated roots and their fungal colonizers from a mountainous mixed beech forest to assess these potential drivers. Significant differences between the dominant root species *F. sylvatica* and *P. abies* were not found for the entire community. However, communities were extremely diverse and our spatial analysis was able to discern a steep decline in community similarity with distance. At very short distances where communities were most similar the most abundant, mostly mycorrhizal fungi actually differed strongly between the two hosts. Low abundance fungi were more spatially structured and showed slightly lower host specificity. Network analysis supported these findings and showed that significantly co-occurring sub-communities were primarily driven by host preference and single sample or same core associations indicating low species sorting. Additionally, no significant dependencies on soil properties were found throughout the study. This means that root associated fungi are primarily structured at small scales and most likely by neutral effects. At these scales host preference plays an impactful role in community composition.

Zusammenfassung

Temperate Wälder haben nicht nur großen menschlichen Nutzen, sie sind auch essentieller Bestandteil globaler Stoffkreisläufe und damit globalen Klimas, da sie große Mengen Kohlenstoff speichern. Ihr Erhalt und Ausbau sind ein kritischer Baustein im Kampf gegen den Klimawandel. Wurzel assoziierte Pilze wie Ectomycorrhiza, Endophyten und Pathogene sind wichtige Komponenten temperater Waldökosysteme, da sie kritische Bodenprozesse regulieren und die Fitness von Bäumen beeinflussen. Um Klimamodelle und naturschutzfachliche Maßnahmen besser informieren zu können ist es wichtig Baumgemeinschaften und ihre Pilzbewohner, sowie ihre räumlichen und bodenbiologischen Abhängikeiten zu charakterisieren.

In dieser Studie wurden Wurzeln und deren besiedelnde Pilze aus einem montanen Buchen-Mischwald untersucht. Die Pilzgemeinschaten dominanter Baumarten *F. sylvatica* and *P. abies* konnten dabei nicht mit herkömmlichen Mitteln unterschieden werden. Die Gemeinschaften waren sehr divers und dabei stark räumlich strukturiert. Auf der kleinsten Ebene - Wurzeln die aus der selben Bodenprobe entnommen wurden – waren dominante Pilze sehr ähnlich, besonders zwischen Wurzeln der gleichen Art. Räumliche Strukturierung war stärker wenn Arten geringerer Abundanzen mit einbegriffen wurden. Aus dieser Analyse kann man Ableiten, dass die stärkste Organisationsebene von Wurzel-Pilz Assoziationen auf engstem Raum zu finden ist und auf dieser Ebene spielt Wirtsabhängigkeit eine bedeutende Rolle. Da kaum Abhängigkeiten von Bodenfaktoren, oder räumlich unabhängig erscheindende Pilz-gemeinschaften entdeckt werden konnten, könnte die räumliche Strukturierung ein Produkt neutraler Prozesse sein.

PART 4

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