Effects of tree mycorrhizal types and their mixtures as well as tree diversity on soil microbial communities

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"***This is a story of extensive soil collection, pipetting until my tomb become cramp, scripting, writing with tons of revision, late nights sleep, frustration, homesickness, insomnia, and the long wait for article publication. throughout this arduous journey, there remained a steadfast belief and trust on Allah***".

Hafeez Ul Haq, on this dissertation

Table of Contents

Summary

Forests play a vital role in providing ecosystem services, including the supply of timber, nourishment, and fuel, while also regulating water quality, carbon sequestration, nutrient cycling, and climate. These ecological functions are underpinned by the soil microbiome, composed of predominantly bacterial and fungal communities. Mycorrhizal fungi, primarily ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) types, form symbiotic associations with over 90% of terrestrial plant species. Forest plant communities are defined by their diverse species compositions and mycorrhizal associations, the impact of mono (AM, EcM) and mixed (AM and EcM) mycorrhizal types and tree diversity on ecosystem services remains underexplored. This dissertation investigates how mono and mixed myco-type tree species, tree diversity, and target tree species rooting zone compartments influence the structure, community assembly processes, and genomic functional potential of soil microbial communities.

- Chapter 2 examines how tree mycorrhizal type (AM, EcM), plot myco-type mixture (mono: AM or EcM; and mixed: AM and EcM tree species plot), tree diversity and rooting zone compartments (rooting and interactive zone) influence soil fungal communities under experimentally controlled conditions. The results revealed consistent and significant impacts of tree mycorrhizal types, plot myco-type mixture and tree species richness on fungal diversity and community composition, while the target tree rooting zone compartment had no significant effects. With increasing tree species richness, fungal communities both in mono and mixed myco-type stands tend to converge. Soil nutrient concentrations exerted a higher influence on soil fungal communities in monoculture stands compared to four species mixtures, suggesting a complex interplay of tree diversity with soil nutrient concentrations. In addition, soil fungi demonstrated a high degree of specialization regarding tree myco-type and mixture, as seen in differences of fungal taxonomic and trophic groups between tree myco-types. This specialization results in clear differences in ecosystem functions and services provided by mono and mixed myco-type multi-species forest microbiome ecosystems.

- In addition, to the factors addressed in chapter 2, which only analysed fungi, chapter 3 focused on the comparison of fungi with bacteria, additionally taking tree species identity into account. The results indicated that AM plots had higher fungal richness compared to EcM and

mixed myco-type (AE) plots, with AE plots showing intermediate richness. The different tree myco-types did not affect bacterial alpha diversity, while tree diversity had no effect on both fungal and bacterial alpha diversity. Tree species identity, tree diversity and plot myco-type mixture significantly impacted fungal community composition, while tree species identity influenced bacterial community composition. In EcM and AE plots, the congruence of fungal and bacterial community compositions patterns increased with increasing tree diversity. The fungal and bacterial community assembly differed in the processes involved. Fungal communities were mainly shaped by stochastic processes, while bacterial communities were influenced by both stochastic and deterministic processes. Plot myco-type mixture and microbial abundance patterns impacted community assembly, with differing contributions of deterministic and stochastic processes to alpha- and beta-diversity in AM, EcM, and mixed myco-type plots. The relation of both the core and rare taxa were mostly controlled by both stochastic and deterministic processes in fungal subcommunities (specific groupings of microbial communities within the broader community e.g. Core, intermediate and rare taxa), whereas those of bacterial subcommunities were dominantly affected by stochastic processes.

- Chapter 4 explores how plot myco-types mixture, tree species identity, tree diversity, and microbial diversity and composition shape microbial functional potential in a controlled experimental condition. The results consistently demonstrated that both plot myco-type and tree species identity exerted significant impacts on the fungal and bacterial genomic functional potentials, whereas tree species diversity was not significant. Notably, I observed a distinct relationship between fungal diversity and genomic functional potentials in tree species richness in both mono or mixed mycorrhizal types while no significant effect was observed for bacterial diversity and bacterial genomic functional potentials. Further, plot myco-type emerged as a strong determinant of the compositional differences in the genomic functional potential of fungi and bacteria. In addition, the carbon, nitrogen, and phosphorus cycling genes as well as their relative ratios were found to shape soil microbial communities differently in mono and mixed myco-type and tree diversity levels. However, soil nutrient concentration did not exhibit a significant correlation with microbial functional potentials or community composition in plots myco-type mixture with varying tree species richness.

This thesis emphasizes the complex interactions among plot myco-type mixtures (mono: AM or EcM; and mixed: AM and EcM), tree myco-type, tree diversity, and target tree species rooting zone compartments (rooting and interactive zones) in shaping the structure, community

assembly, and genomic functional potentials of the forest soil microbiota. This knowledge is essential for comprehending the ecological and functional mechanisms that sustain these microbial communities. The findings show that a more resilient belowground microbial community with a broad range of genomic functional potential can be produced by diversifying tree species with various mycorrhizal partners. This information is useful for forest management strategies.

Zusammenfassung

Wälder spielen eine entscheidende Rolle bei der Bereitstellung von Ökosystemdienstleistungen, einschließlich der Versorgung mit Holz, Nahrung und Brennstoff, sowie der Regulierung der Wasserqualität, der Kohlenstoffspeicherung, des Nährstoffkreislaufs und des Klimas. Diese ökologischen Funktionen werden von der Bodenmikrobiota getragen, die überwiegend aus bakteriellen und pilzlichen Gemeinschaften besteht. Mykorrhizapilze, vor allem ektomykorrhizale (EcM) und arbuskuläre Mykorrhiza (AM), bilden symbiotische Assoziationen mit über 90 % der terrestrischen Pflanzenarten. Waldpflanzengemeinschaften zeichnen sich durch ihre vielfältige Artenzusammensetzung und Mykorrhiza-Assoziationen aus. Der Einfluss von mono- (AM, EcM) und gemischten (AM und EcM) Mykorrhizatypen sowie der Baumdiversität auf Ökosystemdienstleistungen ist jedoch noch weitgehend unerforscht. Diese Dissertation untersucht, wie mono- und gemischte Mykorrhiza-Baumarten, Baumdiversität und Wurzelzonenkompartimente der Zielbaumarten die Struktur, die Prozesse der Gemeinschaftsassemblierung und das genomische funktionale Potenzial von Bodenmikrobengemeinschaften beeinflussen.

- Kapitel 2 untersucht, wie Baum-Mykorrhizatypen (AM, EcM), Mykorrhiza-Mischungen der Parzellen (mono: AM oder EcM; und gemischt: AM- und EcM-Baumartenparzellen), Baumdiversität und Wurzelzonenkompartimente (Wurzel- und Interaktionszone) die Bodenpilzgemeinschaften unter experimentell kontrollierten Bedingungen beeinflussen. Die Ergebnisse zeigen konsistente und signifikante Auswirkungen der Baum-Mykorrhizatypen, der Mykorrhiza-Mischungen der Parzellen und der Baumartenvielfalt auf die Diversität und Zusammensetzung der Pilzgemeinschaften, während die Wurzelzonenkompartimente der Zielbäume keine signifikanten Effekte hatten. Mit zunehmender Baumartenvielfalt neigen Pilzgemeinschaften sowohl in mono- als auch in gemischten Mykorrhizabeständen dazu, sich anzugleichen. Die Nährstoffkonzentrationen im Boden hatten in Monokulturparzellen einen stärkeren Einfluss auf die Bodenpilzgemeinschaften als in Artenmischungen, was auf ein komplexes Zusammenspiel von Baumdiversität und Bodennährstoffkonzentrationen hinweist. Darüber hinaus zeigten Bodenpilze eine hohe Spezialisierung in Bezug auf Baum-Mykorrhizatyp und -mischung, was sich in Unterschieden zwischen pilzlichen taxonomischen und trophischen Gruppen zwischen

den Mykorrhizatypen widerspiegelt. Diese Spezialisierung führt zu deutlichen Unterschieden in den von mono- und gemischten Mykorrhiza-Multi-Arten-Waldmikrobiomen erbrachten Ökosystemfunktionen und -dienstleistungen.

- Zusätzlich zu den in Kapitel 2 untersuchten Faktoren, die ausschließlich Pilze analysierten, konzentriert sich Kapitel 3 auf den Vergleich von Pilzen mit Bakterien und berücksichtigt dabei auch die Baumartenidentität. Die Ergebnisse zeigen, dass AM-Parzellen eine höhere Pilzdiversität aufwiesen als EcM- und gemischte Mykorrhiza-Parzellen (AE), wobei AE-Parzellen eine mittlere Diversität zeigten. Die unterschiedlichen Baum-Mykorrhizatypen beeinflussten nicht die bakterielle Alpha-Diversität, während die Baumdiversität weder einen Effekt auf die pilzliche, noch auf die bakterielle Alpha-Diversität hatte. Die Baumartenidentität, die Baumdiversität und die Mykorrhiza-Mischung der Parzellen hatten jedoch signifikante Auswirkungen auf die Zusammensetzung der Pilzgemeinschaften, während die Baumartenidentität die bakterielle Gemeinschaftszusammensetzung beeinflusste. In EcM- und AE-Parzellen nahm die Übereinstimmung der pilzlichen und bakteriellen Gemeinschaftszusammensetzungen mit zunehmender Baumdiversität zu. Die Prozesse der Gemeinschafts-Assemblierung von Pilzen und Bakterien unterschieden sich. Pilzgemeinschaften wurden hauptsächlich durch stochastische Prozesse geformt, während bakterielle Gemeinschaften sowohl durch stochastische als auch deterministische Prozesse beeinflusst wurden. Die Mykorrhiza-Mischung der Parzellen und die Mikrobendichte-Muster beeinflussten die Gemeinschafts-Assemblierung, wobei deterministische und stochastische Prozesse in unterschiedlichem Maße zur Alpha- und Beta-Diversität in AM-, EcM- und gemischten Mykorrhizaparzellen beitrugen. Die Beziehungen sowohl der Kern- als auch der seltenen Taxa wurden hauptsächlich durch stochastische und deterministische Prozesse in pilzlichen Subgemeinschaften (spezifische Gruppierungen von Mikroben innerhalb der breiteren Gemeinschaft, z. B. Kern-, Zwischen- und seltene Taxa) kontrolliert, während bakterielle Subgemeinschaften überwiegend von stochastischen Prozessen beeinflusst wurden.

- Kapitel 4 untersucht, wie Mykorrhiza-Mischungen der Parzellen, Baumartenidentität, Baumdiversität sowie die mikrobielle Diversität und Zusammensetzung das funktionale Potenzial von Mikroben in einem kontrollierten experimentellen Rahmen beeinflussen. Die Ergebnisse zeigen, dass sowohl die Mykorrhiza-Mischung der Parzellen als auch die

Baumartenidentität signifikante Auswirkungen auf das funktionale genomische Potenzial von Pilzen und Bakterien hatten, während die Baumartenvielfalt keinen signifikanten Einfluss ausübte. Bemerkenswert ist, dass eine deutliche Beziehung zwischen pilzlicher Diversität und funktionalem Potenzial in Baumartenreichtum sowohl bei mono- als auch gemischten Mykorrhizatypen gefunden wurde, während für bakterielle Diversität und deren funktionales Potenzial keine signifikanten Effekte festgestellt wurden. Darüber hinaus erwies sich die Mykorrhiza-Mischung der Parzellen als starker Einflussfaktor für die Zusammensetzung des funktionalen genomischen Potenzials von Pilzen und Bakterien. Die Gene des Kohlenstoff-, Stickstoff- und Phosphorkreislaufs sowie deren relative Verhältnisse prägten die Bodenmikrobengemeinschaften in mono- und gemischten Mykorrhiza- und Baumdiversitätsniveaus unterschiedlich. Bodennährstoffvariablen zeigten jedoch keine signifikante Korrelation mit den mikrobiellen funktionalen Potenzialen oder der Gemeinschaftszusammensetzung in Mykorrhiza-Mischungen der Parzellen mit unterschiedlicher Baumartenvielfalt.

Diese Dissertation betont die komplexen Interaktionen zwischen Mykorrhiza-Mischungen der Parzellen (mono: AM oder EcM; und gemischt: AM und EcM), Baum-Mykorrhizatypen, Baumdiversität und Wurzelzonenkompartimenten (Wurzel- und Interaktionszonen) bei der Gestaltung der Struktur, der Assemblierung der Gemeinschaftein und des funktionalen genomischen Potenzials der Bodenmikrobiota im Wald. Dieses Wissen ist entscheidend, um die ökologischen und funktionalen Mechanismen zu verstehen, die diese mikrobiellen Gemeinschaften unterstützen. Die Ergebnisse zeigen, dass durch die Diversifizierung von Baumarten mit verschiedenen Mykorrhizapartnern eine widerstandsfähigere unterirdische Mikrobengemeinschaft mit einem breiten Spektrum an funktionalem genomischem Potenzial geschaffen werden kann. Diese Informationen sind nützlich für Strategien des Waldmanagements.

CHAPTER 1
General Introduction

Forests are vital ecosystems that deliver a wide range of essential services. These include provisioning services which encompass the production of food and medicine; regulating services such as moderating climate, purifying water, and controlling pests; cultural services which offer opportunities for recreation and education; and supporting services which involve processes like nutrient cycling and the formation of soil (Balloffet *et al.* 2012; Jenkins & Schaap 2018). Regardless of their significance, forests are under severe threat from human activities, including deforestation and urbanization. Since 1990 about 420 million hectares of forest have been lost highlighting a critical need for rapid and effective forest restoration efforts. Research indicates that forests with high tree species diversity exhibit enhanced functionality (Liang *et al.* 2016). Over 90% of terrestrial plants engage in symbiotic relationships with mycorrhizal fungi, particularly ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) types (Brundrett & Tedersoo 2018). Therefore, it is crucial to understand the relationships between tree diversity and mycorrhizal associations to implement successful afforestation and reforestation strategies that preserve these invaluable ecosystem services.

Forests host a significant portion of global biodiversity, with over 80% of terrestrial species residing belowground (Stohr 2013). This belowground biodiversity is essential for facilitating critical forest ecosystem functions such as sequestering carbon and facilitating nutrient cycles (Itps, Scbd & Sofo 2020). Soil fungal and bacterial communities not only perform vital soil biogeochemical processes but also enhance plant development which positively influence overall plant productivity and community dynamics (Almario *et al.* 2022). Additionally, both biotic and abiotic factor influence these belowground microbial communities, which in turn shape the ecosystem services provided by forests. However, our understanding of how plot myco-type mixture (mono: AM, EcM; and mixed: AM and EcM), tree myco-type, tree diversity and target tree species rooting zone compartments (rooting and interactive zones) impact these belowground microbial communities remain limited.

In this thesis, I examined the effects of plot myco-type mixture, tree myco-type, tree diversity, and rooting zone compartment (rooting and interactive zones) on the structure, community assembly processes, and microbial genomic functional potentials of soil fungal and bacterial communities. The initial chapter establishes the context for the research, outlines the study's

purpose and objectives, presents the thesis structure, and explains the experimental methodology employed.

Soil biodiversity

Soil biodiversity encompasses the extensive variety and abundance of organisms residing within the soil, which include a diverse array of taxa such as bacteria, fungi, archaea, protozoa, nematodes, mites, springtails, earthworms, insects, and vertebrates like rodents. This belowground diversity often exceeds that found aboveground in terrestrial ecosystems (Swift *et al.* 1979). It was estimated that the Earth is home to between 100 billion to 1 trillion microbial species (Locey & Lennon 2016). Among these, bacteria and fungi are particularly diverse, forming the dominant groups in soil with biomass levels vastly exceeding those of other major soil groups like archaea and viruses (Fierer 2017). Prokaryotes including archaea and bacteria which do not have a nucleus or other organelles enclosed by membrane are believed to have first appeared about 3.5 billion years ago (Woese, Kandler & Wheelis 1990). These microorganism have been participating in biogeochemical cycles on earth for at least 3.2 billion years (Homann *et al.* 2018). In contrast, fungi, which are eukaryotic organisms containing membrane-bound organelles such as a nucleus, emerged roughly 1.5 billion years ago (Wang, Kumar & Hedges 1999) and have significantly contributed a crucial role in facilitating the establishment of land plants approximately 450 million years ago (Humphreys *et al.* 2010). In summary, the complex ecosystems we observe today have been significantly shaped by the coevolution of land plants with soil microorganisms.

Soil serves as a foundational element for delivering a range of ecosystem services in forests and other environments, encompassing provisioning, regulating, supporting, and cultural services. The critical role of soil microbes including bacteria and fungi cannot be overstated. These microorganisms drive biogeochemical cycles by decomposing organic materials and are integral to soil carbon sequestration through mechanisms such as fungal mycelial networks (Clemmensen *et al.* 2013; Li *et al.* 2019b). Furthermore, bacteria and fungi significantly influence soil structure, fertility, and water quality, which in turn affect the associated ecosystem services (Bender & van der Heijden 2015; Nagy *et al.* 2017). These microorganisms are also essential for plant nutrition with functions such as nitrogen fixation and mycorrhizal associations that effect the diversity and functionality of plant thus supporting ecosystem services (Baldrian 2017). Recent studies have highlighted that soil microbial diversity is crucial for ecosystem multifunctionality (Delgado-Baquerizo *et al.* 2016; Wagg *et al.* 2019),

emphasizing the need to explore the factors driving microbial diversity structure, community processes, and genomic functional potential.

Ecological theories posit that belowground microbial diversity is shaped by principal environmental factors which include resource availability and soil nutrient stoichiometry (Bardgett & Van Der Putten 2014; Delgado-Baquerizo & Eldridge 2019). Resource availability encompasses both aboveground inputs like vegetation litter and belowground organic matter, which typically correlates with increased microbial diversity. The ratio of carbon, nitrogen, and phosphorus in the soil, known as soil nutrient stoichiometry, is shaping microbial communities. This elemental ratio significantly impacts essential soil functions, including the breakdown of organic matter, the conversion of organic compounds into inorganic forms, and the uptake of nutrients by microorganisms (Hooper *et al.* 2000). Research grounded in nutrient stoichiometry has unveiled various mechanisms that delineate patterns within belowground microbial communities on local to global scales.

Tree mycorrhizal type and their mixture influence on the soil microbial communities

Forests rely on the mutually beneficial associations between trees and mycorrhizal fungi for their well-being and long-term viability. These symbiotic interactions are generally categorized into four primary groups: arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM), and orchid mycorrhiza (OrM) (Brundrett & Tedersoo 2018). Among these, AM and EcM are the most widespread with nearly 80% of all vascular plants associating with either AM or EcM type (Aerts 2003; Blackwell 2011). AM fungi penetrate root the cortical cells to form arbuscules and vesicles serving as sites for nutrient exchange with the plant. Their hyphal network extends inside and outside the root without forming an external fungal sheath around root tips. In contrast, EcM fungi surround root tips with a thick hyphal mantle and create a Hartig net between epidermal and cortical cells for nutrient exchange. They form an external hyphal network in the soil, encapsulating root tips with a dense fungal sheath. (Figure 1.1, (Idowu, Pietrasiak & Hoellrich 2023)).

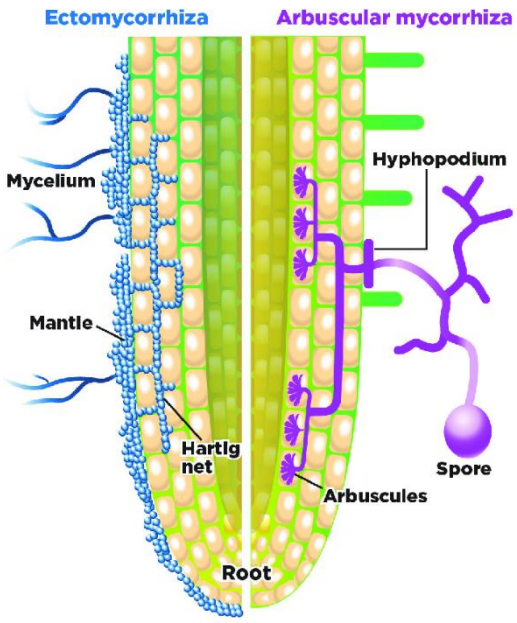

Figure 1.1 Illustration depicting the structural variations in root colonization between ectomycorrhizal (light blue) and arbuscular mycorrhizal (purple) associations. Modified from (Idowu, Pietrasiak & Hoellrich 2023).

Plants and mycorrhizal fungi engage in a mutually beneficial relationship called mycorrhizal symbiosis, where plants provide carbon in exchange for essential nutrients like phosphorus and nitrogen supplied by the fungi (Brundrett & Tedersoo 2018). This interaction primarily takes place in the mycorrhizosphere, a zone where plant roots and fungal hyphae jointly influence soil processes and are crucial for nutrient cycling within ecosystems (Johansson, Paul & Finlay 2004). EcM and AM plants differ in their strategy for nutrient acquisition (Aerts 2003; Phillips, Brzostek & Midgley 2013b; Bennett *et al.* 2017). For instance, EcM fungi specialize in mobilizing organic compounds; contrasting with AM fungi's proficiency in accessing inorganic nutrients (Read & Perez-Moreno 2003; Smith & Read 2010). These differing strategies significantly influence the physicochemical properties of the mycorrhizosphere and consequently the availability of nutrients; which in turn affects the microbial community composition in these environments.

AM fungi are known for their capacity to improve plants' phosphorus absorption and facilitate nitrogen-fixing bacteria, thus playing a crucial role in the nutrient acquisition process of plants (Amora-Lazcano, Vazquez & Azcon 1998; Phillips, Brzostek & Midgley 2013b). In contrast, EcM fungi are known for fostering bacterial communities that possess a high potential for mineral weathering (Phillips, Brzostek & Midgley 2013b; Churchland & Grayston 2014). These divergent interactions between AM and EcM fungi with their microbial communities result in distinct belowground microbial functionalities especially in terms of nutrient cycling dynamics

within forest ecosystems (Cheeke *et al.* 2017). The soil microbial community composition in forest ecosystems is significantly influenced by the diversity of tree species and their associated mycorrhizal associations (van Der Heijden *et al.* 2015a). These mycorrhizal associations play a crucial role in key ecosystem functions, such as nutrient cycling, the breakdown of organic materials, and the storage of carbon.

The understanding of mycorrhizal community diversity and composition has been greatly improved by recent progress in molecular methods. Techniques such as DNA sequencing and polymerase chain reaction, which utilize phylogenetic markers like small subunit ribosomal RNA genes, have played a crucial role in this advancement (Taylor & Bruns 1997; Lee Taylor & Bruns 1999). The research investigations cover a wide range of forest ecosystems, including boreal, temperate, tropical, and sub-tropical zones. These studies aimed at examining the connections between different types of host plants (EcM or AM) and the fungal communities associated with them, particularly focusing on mycorrhizal symbiotic relationships (Öpik *et al.* 2008; Tedersoo *et al.* 2012; Gao *et al.* 2013). Researchers have observed positive correlations between the prevalence of plants with specific mycorrhizal types and the composition of their respective mycorrhizal communities. EcM plants typically associate with ectomycorrhizal fungi (EMF), whereas AM plants form partnerships with arbuscular mycorrhizal fungi (AMF), influencing both soil and root environments (Gao *et al.* 2013; Neuenkamp *et al.* 2021). Moreover, investigations have highlighted the significant influence of tree mycorrhizal partners including both single and dual mycorrhization (where trees associate with multiple mycorrhizal types simultaneously), on the diversity and composition of fungal communities within root systems (Heklau *et al.* 2021), emphasizing the pivotal role of plant mycorrhizal type in shaping microbial community dynamics. Although considerable advancements have been made, there is still a lack of clarity regarding how these mycorrhizal types in mono (AM, EcM) or mixed (AM and EcM) specifically affect soil fungal and bacterial communities.

Impact of tree species diversity on soil microbiota

Current research consistently supports a positive relationship between plant diversity and ecosystem functionality. For instance, mixed tree species stand demonstrate higher productivity compared to monocultures (Tilman 1999; EM 2000). Additionally, a comprehensive analysis of experimental grassland research indicates that plant diversity's impact on soil organisms intensifies with time (Eisenhauer, Reich & Scheu 2012). This synthesis highlights the beneficial

role of soil biota such as AMF and rhizobacteria in promoting plant growth in diverse grasslands. Similarly, increasing tree diversity fosters complex interactions among trees and between trees, microbes, and soil (Bonfante & Genre 2010; Schuldt *et al.* 2017). Positive outcomes of tree diversity have been documented in both soil (Barberán *et al.* 2015; Hiiesalu, Bahram & Tedersoo 2017) and root-associated microbial communities (Gao *et al.* 2013; Ferlian *et al.* 2021), highlighting the strong interconnections between above- and belowground ecological systems.

The beneficial impacts of plant diversity on ecosystems are supported by various underlying mechanisms, with two primary theories currently under discussion (Tilman, Isbell & Cowles 2014). The first theory, known as mass ratio effects, posits that diverse plant assemblages are more likely to contain species that significantly influence ecosystem functions, such as enhancing microbial diversity (Grime 1998). This presence can lead to positive outcomes for the community that extend beyond the mere presence of multiple species (Cardinale *et al.* 2006). Conversely, The second theory, known as functional complementarity, suggests that increased interactions and resource partitioning among different species improve the availability and utilization of resources like nutrients, water, and space, thereby enhancing ecosystem functionality (Loreau & Hector 2001). For example, research has shown that mixed forest stands exhibit higher root productivity and more efficient soil exploitation compared to monocultures (Brassard *et al.* 2013) likely due to both complementarity and net biodiversity effects, which arise from the overall diversity of the community. Additionally, a meta-analysis has highlighted the beneficial impacts of plant species diversity on soil microbe mass and respiration (Van Der Heijden, Bardgett & Van Straalen 2008). This finding reinforces the significance of species complementarity in enhancing ecosystem functions beyond mere species composition (Chen *et al.* 2019).

The relationship between plant species diversity and microbial communities in soil and roots is intricate and often unpredictable. Some studies have shown that plant richness does not always have a significant effect on fungal communities associated with roots (Navrátilová *et al.* 2019; Otsing *et al.* 2021). Similarly, some research has shown no or minimal effects of tree diversity on soil microbial diversity (Rivest, Whalen & Rivest 2019). The impacts of tree species richness may vary depending on specific conditions, suggesting a context-dependent nature. These inconsistencies likely arise from various unaccounted factors, such as the age of the ecosystem (Eisenhauer, Reich & Scheu 2012) and the combined influence of environmental factors on both plant and belowground microbial communities (Tedersoo *et al.* 2016). This underscores

the necessity for controlled experimental settings and comprehensive analyses that consider multiple factors when examining the impacts of plant diversity. However, despite this extensive research there is significant knowledge gap that how tree diversity effect either in mono or mixed myco type effect below ground soil microbial communities.

Community assembly processes impacts on the soil microbial communities

Community assembly processes significantly influence soil microbial communities through a combination of deterministic and stochastic mechanisms, shaping their composition and function within ecosystems (Liu *et al.* 2021b). Deterministic processes are primarily driven by selection pressures, which can be categorized into homogeneous and heterogeneous selection based on environmental conditions (Zhou & Ning 2017; Zhao *et al.* 2022). Homogeneous selection occurs in environments where abiotic and biotic factors are relatively uniform, resulting in microbial communities with similar structures and compositions (Zhou & Ning 2017; Zhao *et al.* 2022). This uniformity arises because competitive interactions and functional traits are more decisive in determining which microbial species thrive under consistent resource availability. Microbes with comparable metabolic capabilities or physiological traits often dominate these environments due to their competitive advantages (Zhou & Ning 2017; Zhao *et al.* 2022). Conversely, heterogeneous selection operates in environments characterized by diverse abiotic and biotic conditions (Zhou & Ning 2017; Zhao *et al.* 2022). Acidic soils may favour acidophilic bacteria, while nutrient-rich soils may support microbial communities specialized in nutrient cycling. This environmental filtering plays a crucial role in structuring microbial communities by limiting the establishment and persistence of species not adapted to prevailing conditions. Stochastic processes are due to dispersal (e.g., homogenizing dispersal and dispersal limitation) or drift (Stegen *et al.* 2013). Homogenizing dispersal involves a high rate of microbial movement among communities, leading to similar community structures across different locations (Zhou & Ning 2017; Zhao *et al.* 2022). Microbes dispersed evenly throughout soil environments establish communities through random encounters rather than specific environmental preferences, contributing to spatial variation in microbial composition and diversity. In contrast, dispersal limitation restricts the movement and colonization of microbes to new locations, resulting in dissimilar community structures (Stegen *et al.* 2013). Factors such as geographic barriers hinder the exchange of microbial species between soil habitats, while dispersal vectors like wind, water, and animals increase the exchange. Consequently, isolated microbial populations evolve independently, developing unique genetic

and functional traits over time. In ecological communities, random fluctuations in the relative abundance of different species over time are known as ecological drift. This phenomenon occurs due to the unpredictable nature of fundamental life processes such as birth, death, and reproduction, which affect species composition without regard to their specific identities (Zhou & Ning 2017; Zhao *et al.* 2022). Understanding soil microbial community assembly mechanisms is crucial for elucidating microbial roles (Liu *et al.* 2021a). Both deterministic and stochastic processes influence microbial community assembly, with their importance varying based on factors such as host specificity, dispersal ability, and soil physiochemical properties (Langenheder & Székely 2011). These factors can significantly impact fungal and bacterial community assemblies across different forest ecosystems, including responses to forest conversion, nitrogen supplementation, and soil acidification (He *et al.* 2017; Liu *et al.* 2018). Recent studies have explored how tree species with different mycorrhizal types, such as arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) species, influence soil fungal and bacterial communities. They have investigated structural and functional differences between AM- and EcM-dominated natural forests, as well as the effects on microbial biomass and metabolic activity (Bahram *et al.* 2020; Heděnec *et al.* 2020). Despite these advancements, it remains unclear how shifts in the mono and mixed myco-type with varying tree diversity levels impact the community assembly processes of soil fungal and bacterial communities in forest ecosystems.

Functional potential of belowground microbial communities

Genomic functional potential potentials reflect the genetic blueprint within soil microbial communities that enables essential nutrient cycling processes, encompassing the carbon (C), nitrogen (N), and phosphorus (P) cycles. This genomic potential is fundamental for assessing the functionality of microbial communities which directly impacts soil fertility and broader ecosystem processes. The abundance of carbon cycle associated genes like Beta-glucosidase provides microbes with the ability to decompose plant-derived organic matter, thus contributing to the conversion of complex organic compounds into simpler forms that enrich soil organic matter and support nutrient turnover (Fierer, Bradford & Jackson 2007b). This process not only drives carbon flow within the ecosystem but also influences the availability of other nutrients tied to soil health. Nitrogen cycle-related genes like Nitrogenase and Hydroxylamine reductase play crucial roles in mediating key biochemical pathways, such as nitrogen fixation, nitrification, and denitrification. These processes are pivotal for microbial growth and

ecosystem functioning as they regulate the transformation and movement of nitrogen, a key nutrient that limits plant productivity in many terrestrial ecosystems (Philippot *et al.* 2013; Fierer 2017). The genomic capacity for nitrogen processing affects microbial metabolic activities and helps maintain the nitrogen balance necessary for ecosystem stability. Phosphorus is a vital nutrient that often limits plant growth. Phosphorus is cycled through microbial processes facilitated by phosphorus-related genes involved in solubilization and mineralization. These genes enable the conversion of organic and mineral forms of phosphorus into bioavailable forms, and thus, not only support their own metabolism but also enhance plant nutrient uptake (Richardson & Simpson 2011; Frossard *et al.* 2016). The potential of these phosphorus-cycling associated genes like Exopolyphosphatase has profound implications for nutrient accessibility and soil productivity. The interactions and collective abundance of these C, N, and P cycling genes shape the composition, metabolic capabilities, and functional resilience of microbial communities. Such genetic diversity and functional potential directly impact soil health, productivity, and the capacity of an ecosystem to respond to changes, whether natural or anthropogenic (Fierer *et al.* 2012; Cleveland *et al.* 2013). Understanding these complex genomic interactions is essential for developing sustainable soil management practices that can enhance nutrient cycling, promote soil fertility, and ensure long-term ecosystem functionality.

Arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) trees have distinct impacts on the microbial genomic functional potentials of soil microbial communities. AM-associated trees are linked to microbial communities that support faster nutrient cycling and favor genes involved in carbon and phosphorus mineralization, enhancing processes such as organic matter decomposition and phosphorus solubilization (Chagnon *et al.* 2013). Conversely, EcM trees promote microbial communities enriched associated with nitrogen cycling related genes, facilitating processes like nitrification and denitrification, and contributing to nutrient retention (Stark 2010; Averill, Turner & Finzi 2014). The involvement of microbial organisms in these different processes constitutes the functional genetic potential of microbial communities, impacting soil nutrient dynamics and carbon storage (Read and Perez-Moreno, 2003). However, there is limited knowledge on how mono and mixed myco-type stands across tree diversity gradients influence microbial genomic functional potentials. This gap underscores the need for more research to understand the interactions between tree diversity, plot myco-types mixture, and the genomic capacity for nutrient cycling in soil microbial communities.

The aim of this thesis, objectives and outline

This dissertation seeks to fill the aforementioned research gaps by studying the effects of mono (AM, EcM) and mixed (AM and EcM) myco-type, tree diversity, tree species identity, and rooting zone compartment (rooting and interactive zones) effects on the structure, community assembly processes, and microbial genomic functional abundance of belowground bacterial and fungal communities. To accomplish this goal, the research is organized into three main objectives, which are presented in chapters 2, 3, and 4, as illustrated in (Figure 1.2).

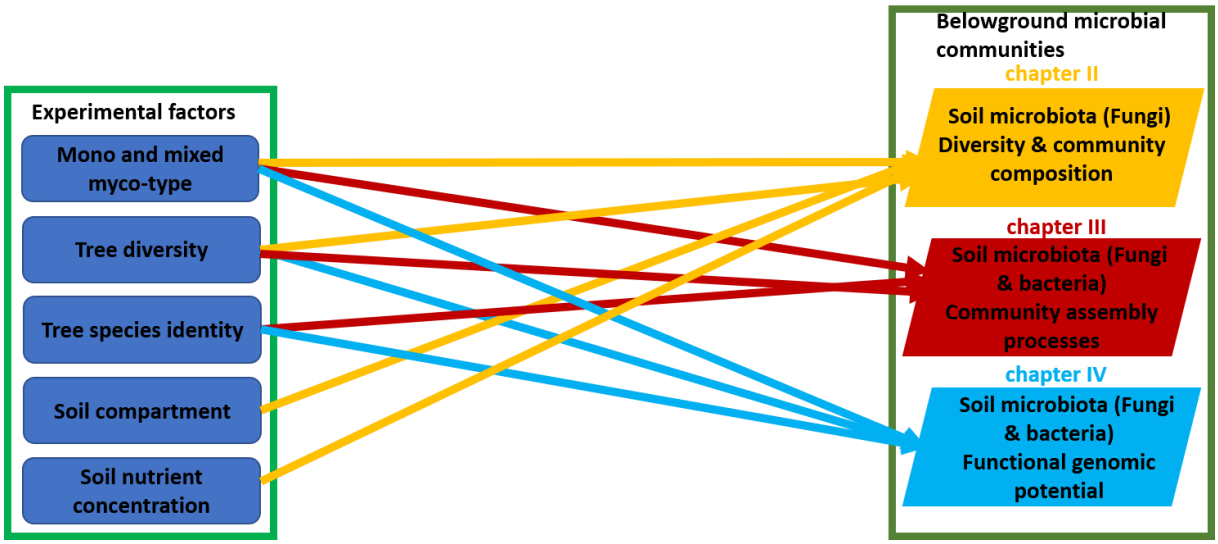

Figure 1.2: Outline of the thesis, depicting the interconnections among variables, utilizing distinct solid-colored arrows to represent the relationships examined in the research objectives outlined in Chapters 2 through 4.

In chapter 2, I conducted research to characterize soil microbiota and evaluate the impact of various experimental factors on fungal communities. Specifically, I investigated how mono (AM, EcM) and mixed (AM and EcM) myco-types, tree diversity, and target tree rooting zone compartments influence the diversity and composition of fungal communities.

In chapter 3, I investigated the effects of mono (AM, EcM) and mixed (AM and EcM) myco-types, tree species identity, and tree diversity levels on microbial diversity, community composition, and microbial community assembly processes. Additionally, I assessed the congruence between fungal and bacterial communities across mono and mixed myco-type plots at varying tree diversity levels. I also analysed the differential impacts of ecological processes on core and rare fungal and bacterial sub-communities in both mono and mixed myco-type plots as well as tree diversity levels.

In chapter 4, I determined the microbial genomic functional potentials with regard to the cycling of three major nutrients carbon (C), nitrogen (N) and phosphorus (P). I explored the influence of plot myco-types mixture, tree species identity, tree diversity on microbial genomic functional abundances. Additionally, I investigated the relationship between microbial genomic functional potentials and microbial diversity and composition in mono and mixed myco-type and tree diversity levels.

In the last chapter, I presented an overview and analysis of the primary outcomes derived from the aforementioned research objectives. This section synthesizes the crucial findings, explores their implications, addresses the study's limitations, and outlines potential avenues for future research.

Experimental design

The research for my thesis was conducted at the MyDiv experimental platform, located at the Experimental Research Station of the Helmholtz Centre for Environmental Research – UFZ in Bad Lauchstädt, Saxony-Anhalt, Germany (latitude 51°23' N, longitude 11°53' E) (Ferlian *et al.* 2018). The initiative began in March 2015 on a former agricultural land. The experiment involved the arrangement of 80 plots, each measuring 11 × 11 meters, with a central core area of 8 × 8 meters (Figure 1.3). Each plot was planted with 140 trees one meter apart in a consistent species arrangement, and covered with a water-permeable weed tarp to minimize weed growth. A total of ten tree species were used, divided equally between those associated with arbuscular mycorrhizal (AM) fungi and those with ectomycorrhizal (EcM) fungi (Ferlian *et al.* 2018). The trees were planted in monocultures, two and four species mixtures. Specifically, the design differentiated plots by mycorrhizal type either exclusively AM or EcM, or a mixture of both. This methodological setup was aimed at examining the ecological dynamics between different mycorrhizal communities (Ferlian *et al.* 2018) (Figure 1.3).

For my thesis, eight of the ten planted tree species comprising an equal number of arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) species were selected for detailed analysis as outlined in Table 1.1. Chapter II of my study focused on the compartment effects between a target tree and its neighboring trees. Soil sampling occurred in September 2021 around a target tree encircled by eight other trees. Sampling locations included the "target tree rooting zone" immediately adjacent to the tree trunk (10 cm away) and the "interaction rooting zone," located centrally within the area where four tree species intersect (50 cm from the target tree). Four soil cores were collected from each rooting zone, both the target (Rz1-Rz4) and interaction zones

(Int1-Int4), with each core measuring 10 cm in depth and 5 cm in diameter. These samples were subsequently pooled according to tree species for both the rooting and interaction zones, as depicted in Figure 1.3. In subsequent chapters, Chapters 3 and 4, analysis was confined to the rooting zones alone due to the absence of significant microbiota differences between the rooting zones and the interaction zones noted in Chapter 2.

Table 1.1 List of tree species and their mycorrhizal types studied in this thesis

Tree Species	Mycorrhizal type	Reference
Betula pendula	EcM	(Newman, Harley & Harley 1988; Atkinson 1992)
Carpinus betulus	EcM	(Newman, Harley & Harley 1988)
Fagus sylvatica	EcM	(Newman, Harley & Harley 1988)
Quercus petraea	EcM	(Newman, Harley & Harley 1988)
Acer pseudoplatanus	AM	(Newman, Harley & Harley 1988)
Fraxinus excelsior	AM	(Newman, Harley & Harley 1988)
Prunus avium	AM	(Newman, Harley & Harley 1988)
Sorbus aucuparia	AM	(Newman, Harley & Harley 1988)

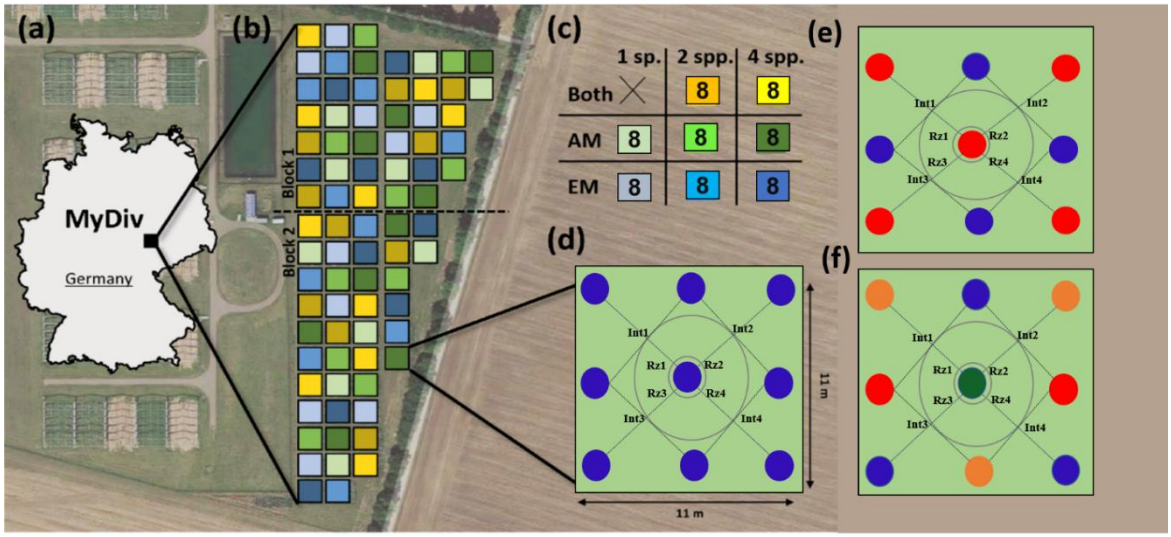

Figure 1.3 MyDiv experimental (A) platform between- and within plot spatial design (B) and mycorrhizal type, mycorrhizal type mixtures, and tree richness (C) (modified after Ferlian et al. (2018)). Sampling scheme of the soil sampling with the target tree (green circle) surrounded by eight neighbor trees across the tree diversity levels. Pooled samples of four soil cores were taken at two scales: 1) target tree rooting zone samples (close to the target species, Rz1-Rz4) and 2) interaction rooting zone soil samples (at the center of each of the four-tree species surrounding the target tree species, Int1-Int4). Circles with different colors represent different tree species (single species mixture, D), (two species mixture, E), and (four species mixture, F).

CHAPTER 2

The interactive effect of tree mycorrhizal type, mycorrhizal type mixture and tree diversity shapes rooting zone soil fungal communities in temperate forest ecosystems

This chapter has been published in the Functional Ecology journal as

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Abstract

The underlying processes of plant-microbe associations particularly their interactions with their mycorrhizal fungal partners have been extensively studied. However, considerably less is known about the consequences of tree-tree interactions on rooting zone soil microbiota when tree species of different mycorrhizal type (myco-type) grow together as mono and mixed myco-type mixtures along a tree diversity gradient.

Using the MyDiv tree diversity experiment, where arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) trees and their mixtures were planted in monocultures, two-species and four-species mixture plots, we investigated the interplay of target tree myco-type, myco-type mixture, tree diversity and rooting zone compartment (target tree dominated and its interaction zones with neighbour trees) on the rooting zone soil mycobiota employing meta-barcoding of the ITS2 rDNA fragment of the fungal internal transcribed spacer (ITS).

Our results revealed significant individual and interaction effects of tree myco-type, myco-type mixture and tree diversity but not rooting zone compartment on the fungal taxonomic and functional alpha and beta diversity. This implies intermingling of roots of target and neighbouring tree species there by reducing the target tree species effect in its rooting zone. As tree diversity increases, we found convergence of the fungal community in general, where the fungal community dissimilarity varies depending on the co-existing tree species myco-type and tree species diversity. Furthermore, the fungal community composition in the two and four species mixtures were consistently influenced by soil pH, whereas in the mixed multi-species stands basal respiration, N, $PO4^{-}$, $NO3^{-}$ were found to be equally important unlike in AM and EcM multi-species stands. Comparative analysis of the fungal taxa specialization between mono and mixed myco-type multi-species stands revealed that the mixed myco-type plots shared 23.5% (AM) and 19.7% (EcM) of the generalist fungal communities However, the percentage of specialized fungal community in mixed myco-type plots (13.2%) was significantly higher as compared to EcM (9.5%), and significantly lower (9%) as compared to AM (11.7%) plots, resulting in myco-type and myco-type mixture specific fungal communities and functional guild patterns

Our results provide novel insights on the significance of tree species and its co-existing trees preferred mycorrhizal association in shaping the target tree rooting zone soil mycobiome along a tree diversity gradient. Furthermore, it highlights the significance of generalist and specialist

fungal communities in mono and mixed myco-type stands in modulating tree-tree interaction, tree species co-existence and regulating soil properties and ecosystem functions.

Introduction

Forest ecosystems contain a wide range of soil fungi that play a crucial role in regulating plant community dynamics (Averill, Turner & Finzi 2014; Molina & Horton 2015) and biogeochemical processes (Van Der Heijden, Bardgett & Van Straalen 2008; Clemmensen *et al.* 2013). Fungi are responsible for driving numerous classical ecological phenomena in forests including modulation of soil fertility and plant community structure relationships (Qin *et al.* 2021). Plants engage in mutualistic partnerships with soil fungi, which can have a significant impact on the host plant's fitness and competitive ability, ultimately determining the growth, survival and productivity of the host plant (Peay 2016; Kandlikar *et al.* 2019).

Mycorrhizal fungi establish a symbiotic relationship with plants by directly associating with their roots, improving the absorption of water and enhancing the plant's resistance to pathogens, while in exchange, fungi obtain carbohydrates from the plant (Wei, Song & Jia 2021). Arbuscular mycorrhiza (AM) and ectomycorrhiza (EcM) are the two primary types of mycorrhizal associations found in most plants worldwide (van der Heijden *et al.* 2015b). They have been identified as potential regulators of plant–soil feedback mechanisms and drivers of plant diversity (Bennett *et al.* 2017; Tedersoo & Bahram 2019). Arbuscular mycorrhizal fungi mainly scavenge inorganic forms of P and N that are released by saprotrophic microbes (Wagg *et al.* 2011; van Der Heijden *et al.* 2015a; Teste *et al.* 2017). In addition to acquiring inorganic P and N, the majority of ectomycorrhizal fungi can also utilise organic P and N sources through secretion of extracellular enzymes to degrade complex organic compounds, such as proteins, chitin, and inositol phosphates (van Der Heijden *et al.* 2015a; Teste *et al.* 2017). Furthermore, AM fungi have a low host specificity and spatially complement each other during soil nutrient scavenging, thus facilitating plant productivity and coexistence (Wagg *et al.* 2011). Conversely, though EcM fungi exhibit a high host specificity some are host generalists and can form fungal hyphal networks that coordinate flow of nutrients and signals between conspecific tree species, which generally promote EcM fungal dominance (van der Linde *et al.* 2018). Apart from variations in nutrient acquisition, there

are distinct suites of nutrient-use characteristics in AM and EcM tree species that impact the availability of nutrients. Typically, EcM tree species produce low-quality litter that decomposes slowly and suppresses nutrient mineralisation, while AM trees produce high-quality litter that decomposes faster and enhances nutrient mineralisation (Phillips, Brzostek & Midgley 2013b). Consequently, AM and EcM trees are characterised by acquisitive and conservative nutrient absorption mechanisms, respectively (Luo *et al.* 2018; Averill *et al.* 2019). These distinctions of AM and EcM trees and their fungal partners underscores their potential in shaping their co-occurring soil microbial community composition and degree of specialisation. Tree myco-type have been shown to influence the rhizosphere soil microbial community composition of conspecific AM and EcM tree species pairs in subtropical forest ecosystems (Singavarapu *et al.* 2022b), while mixing trees of different myco-types has been shown to increase the richness of root-associated Ascomycota and to decrease that of Glomeromycota in AM trees where the effect was less/not prominent in EcM trees in temperate forests (Heklau *et al.* 2021). Little is known about the effect of tree myco-type on the rooting zone soil mycobiome community composition of mono (AM or EcM trees) and mixed myco-type (AM and EcM trees) forest stands. Besides tree mycorrhizal type effect on soil fungi, it has been reported that tree communities with higher tree diversity also support higher soil fungal diversity (Weißbecker *et al.* 2019). A higher tree diversity enhances mycorrhizal fungal activity fostering diverse carbon compounds through increased root exudates (Heidi-Jayne *et al.* 2023). Consequently, mycorrhizal fungi transfer carbon from trees to soil thereby enriching the soil carbon pool (Simard *et al.* 1997), facilitating carbon sequestration (Domeignoz-Horta *et al.* 2021) and influence the soil microbial community composition (Lange *et al.* 2023; Wu *et al.* 2023). Studies based on two temperate forest diversity experiments revealed that the richness of soil fungal communities in Estonia and EcM fungi in Finland were positively associated with tree diversity (Tedersoo *et al.* 2014). However, these effects became insignificant with increasing tree diversity in subtropical forests (Singavarapu *et al.* 2022a). Therefore, further studies are needed to better understand the interplay of tree species myco-type, co-existing tree species myco-type mixtures and tree diversity in shaping the rooting soil mycobiome composition. Soil fungal community composition can also be influenced by various biotic and abiotic factors, such as soil properties (Glassman, Wang & Bruns 2017; Schappe *et al.* 2017) and co-existing tree species (Cheng & Yu 2020). Soil characteristics, including soil pH and nutrient levels are known to be important variables influencing soil fungal community composition (Dumbrell *et al.* 2010;

Glassman, Wang & Bruns 2017). The soil properties of a target tree species rooting zone are modified by the tree species and its neighbour trees through for example, secretion of tree species-specific root exudates (Kong *et al.* 2021; Neuenkamp *et al.* 2021) that changes the soil chemical conditions in the vicinity of plant roots and variation in litter cover that changes the soil temperature (González-García *et al.* 2023). Consequently, they exert direct or indirect effects on the soil fungal communities (Huang *et al.* 2021; Sweeney *et al.* 2021). Additionally, neighbour trees may function as fungal nurseries (Facelli *et al.* 2018) or alternative sources of carbon (Moeller *et al.* 2015), which helps maintain the local fungal species pool. While the target tree species have more influence on its own rooting zone close to its stem, which is defined here as the 'target tree rooting zone', the neighbour trees could equally affect the 'interaction rooting zone', where the roots of a target tree species meet with those of its neighbours. Thus, comparative analysis of the fungal communities of these rooting zone soil compartments and assessing the relative contribution of the soil biotic and abiotic conditions might shed light on the role of soil microbiota in tree-tree interactions and plant species co-existence in forest ecosystems. To fill the above-mentioned knowledge gaps, we used the MyDiv experimental platform to investigate how tree mycorrhizal type (myco-type), specifically arbuscular mycorrhiza (AM) or ectomycorrhiza (EcM), influences soil fungal community composition and their ecological functions. Additionally, we explored the impact of mixture of tree mycorrhizal types (myco-type mixture) in co-occurring trees distinguishing between a mono myco-type (all trees having either AM or EcM) and a mixed myco-type (trees having a combination of both AM and EcM mycorrhizal types). We also examined different tree-rooting zone compartments, including the target tree and interaction rooting zones. The investigation was conducted along a gradient of tree species richness of 1, 2 and 4 species mixture stands. Specifically, we hypothesised that (H1) tree myco-type, myco-type mixture and target tree rooting zone compartments impact the soil fungal diversity and community composition individually and interactively, where AM trees contributing most both in the mono and mixed myco-type stands. Furthermore, we tested the hypothesis (H2) that the expected effects in H1 are modulated by tree diversity and soil conditions. Specifically, we expected that (H2a) the tree myco-type effects in H1 become less prominent with increasing tree diversity due to co-existence of AM and EcM trees in high-diversity plots and consequently (H2b) the contribution of soil abiotic conditions in mono and mixed myco-type stands changes with increasing tree diversity. Finally (H3), we hypothesised that the proportion of shared generalist and specialised fungal

communities of mixed myco-type stands to be higher with AM than EcM tree species in high diversity plots.

Materials and Methods

Study site

The MyDiv experimental platform is located in Saxony-Anhalt, Germany, at the Experimental Research Station of the Helmholtz Centre for Environmental Research—UFZ in Bad Lauchstädt (51°23′ N, 11°53′ E; (Ferlian *et al.* 2018). The altitude is 115 m a.s.l. and the climate is characterised by an annual mean temperature of 8.8°C and precipitation of 484 mm. The type of soil is Haplic Chernozem formed from Loess with a pH of 6.6–7.4. The experiment was established on a former crop field in March 2015, and 80 plots of 11 × 11 meters were set up, with each plot having a core area at the centre of 8 × 8 m (Figure S1). In each plot, 140 trees were planted at a distance of 1 m between trees in a regular species pattern. All plots were covered with a water-permeable weed tarp to reduce weed establishment. The trees for each plot were selected from a total of 10 different species, with five species belonging to the arbuscular mycorrhizal (AM) group and the other five belonging to the ectomycorrhizal (EcM) group. Tree species belonging to the two primary mycorrhizal types were planted in monocultures, two-species and four-species mixtures. In the plots with species mixtures, the design incorporated a treatment based on mycorrhizal types, consisting of communities comprising only AM and only EcM trees, or a mix of AM and EcM trees (Ferlian *et al.* 2018).

Soil sampling and processing

Among the 10 planted tree species, we selected eight species, four AM and EcM tree species, represented by equal numbers of trees in the experimental design for our analysis. A list of the chosen species, their mycorrhizal type (AM, EcM) and the number of processed samples per plot diversity level is provided in the (Table S1). Soil was sampled from these selected tree species in September 2021, using them as a target tree species that is surrounded by eight tree individuals. Soil samples were taken from target tree rooting zones close to the stem of the target tree (20 cm),

referred to as the ‘target tree rooting zone’ and at the centre of the four-tree species interaction zone in the four corners defined as ‘interaction rooting zone’ (70 cm away from the target tree; Figure S1). We sampled four soil cores of each 10 cm depth using 2 cm diameter soil corers of the target tree rooting zone (Rz1-Rz4) and the interaction rooting zones (Int1-Int4), where the respective samples were pooled per target tree species into rooting and interaction rooting zone soil samples. The pooled soil samples were then sieved using 2 mm mesh size sieves and transported using a cooled box to the field laboratory. All samples were frozen at −20°C before measuring soil physicochemical properties and extracting DNA. Obtaining permissions for our fieldwork is not required.

Measurements of soil physicochemical properties

A subsample of all soil samples was ground and 10 g per sample were used for pH measurements by adding 0.01 m CaCl2. Soil microbial biomass and basal soil respiration were measured on an automated O2-micro- compensation apparatus using 6 g of fresh soil (Scheu 1992). Soil water content was calculated as percentage of water from the fresh soil weight by drying the soil samples for 3 days at 75°C. Soil total nitrogen was determined on an autoanalyzer (SEAL Analytical GmbH, Norderstedt, Germany) using the Kjeldahl methods. Soil total carbon was measured using a TOC analyser (Liqui TOC II; Elementar Analyses system GmbH, Hanau, Germany). The soil C/N ratio was calculated using the soil carbon and total nitrogen values generated in this study. NH4+, NO3− and PO43− were detected on ion exchange membranes (IEM), followed by subsequent processing as per the protocols outlined in (Rodríguez *et al.* 2009; Durán *et al.* 2013).

Nucleic acid extraction & amplicon sequencing

The genomic DNA extraction of the soil samples was performed using the DNeasy PowerLyzer PowerSoil Isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of genomic DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, United States), and the extract was adjusted to 10–15 ng/µL template concentration. The fungal amplicon libraries were prepared as described by (Singavarapu *et al.* 2022b). In short, the ITS2 rDNA region was amplified using semi-nested PCR with the initial primer combination of ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), accompanied by the primer pair fITS7 (Ihrmark *et al.* 2012) and ITS4

comprising the Illumina adapter sequences. The fungal amplified products were purified with Agencourt AMPure XP beads (Beckmann Coulter, Krefeld, Germany). The fungal fragments were indexed using Illumina Nextera XT indices at both ends during the indexing PCR. The indexed products were subsequently purified with AMPure beads and then measured by dsDNA high sensitivity assay using the Qubit fluorometers (Thermofisher Scientific, Germany). The amplicon libraries were equimolarly pooled to achieve a final concentration of 4 nM. Finally, paired-end 2 × 300 bp Illumina MiSeq sequencing was performed at the Helmholtz-Centre for Environmental Research-UFZ (Leipzig, Germany) using a MiSeq Reagent kit v2 on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States).

Bioinformatics workflow

Using the Quantitative Insights into Microbial Ecology, QIIME 2 version 2022.2 software (Bolyen *et al.* 2019), high-quality reads were extracted from the raw reads obtained from the Illumina MiSeq Sequencing platform as described by (Singavarapu *et al.* 2022b). The forward and reverse reads were demultiplexed based on their respective index combinations, followed by primer sequence trimming and denoising. Subsequently, the sequences were grouped into Amplicon Sequence Variants (ASVs) using Cutadapt (q2-cutadapt) and DADA2 (via q2-dada2) (Callahan *et al.* 2016), respectively. The analyses of the fungal ITS dataset was performed using the q2-ITSxpress QIIME2 plugin, by which ITS2 sequences were detected and trimmed, followed by denoising and grouped into ASVs using the DADA2 plugin (Rivers *et al.* 2018). Taxonomy was assigned to fungal ITS ASVs using the q2-featureclassifier (Bokulich *et al.* 2018b), which employed the classify-sklearn naive Bayes taxonomy classifier against the unite-ver8-99-classifier-04.02.2020 database. The fungal ASV matrices, taxonomic tables, and representative sequences were imported into R (version 4.2.2) for further statistical analysis using the 'phyloseq' package (McMurdie & Holmes 2013). Fungal ASVs assigned at least at the Phylum level were filtered and to minimise noise and avoid potentially spurious taxa, taxa that were not detected in at least 5% of the samples were removed from fungal datasets (Cao *et al.* 2021). Then the fungal ASVs were rarefied to 11,273 fungal reads per sample. The fungal functional groups were defined using FUNGuild (Nguyen *et al.* 2016a) and Fungal Traits (Põlme *et al.* 2020) databases (Singavarapu *et al.* 2024). This rarefied dataset at ASV level, consisting of ASVs present in more than 5% of samples, was used for the statistical analysis to test our hypotheses.

Statistical analysis

All statistical analyses and figure plotting were carried out with R, (version 4.2.2). Data pre-processing and analysis were mainly performed using the 'phyloseq' package (McMurdie & Holmes 2013). All graphs were created using the 'ggplot2' package (Wickham, Chang & Wickham 2016). The relative abundances of the top 10 taxonomic groups at Family level and fungal functional guilds are illustrated with respect to myco-type mixture, tree diversity and tree rooting compartment with bar plots. Alpha diversity estimates of observed species richness and Shannon's diversity index were calculated using the alpha function of the 'microbiome' package (Leo & Sudarshan 2017). The individual and interactive effects of tree myco-type, myco-type mixture, soil compartment and tree diversity were tested for fungi with a type III ANOVA using the 'lmerTest' package (Kuznetsova, Brockhoff & Christensen 2017). We used linear mixed model with fungal alpha diversity as response variable, myco-type, myco-type mixture, soil compartment, tree diversity and their interaction as fixed effect and tree species and neighbourhood composition as a random effect. The emtrends procedure from the emmeans package (Lenth *et al.* 2018) was used to estimate unbiased marginal means (EMMs) and confidence intervals for the slopes of each species, considering slopes significant if their confidence intervals did not overlap zero. The effect of myco-type, myco-type mixture (mono or mixed-myco- types), tree diversity and soil compartment on the fungal community composition was tested with permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the 'vegan' package. Distance-based redundancy analysis (dbRDA) constrained by myco-type mixture (AM or EcM mono myco-type and mixed myco-type) was done using the capscale function from the 'vegan' package (Oksanen *et al.* 2013), using Bray-Curtis distance to test and visualise the pattern in fungal community composition of AM or EcM mono and mixed myco-type stands. Pairwise fungal community differences were tested using the pairwise.adonis function of the package 'pairwiseAdonis' (Martinez Arbizu 2017). Environmental variables (soil nitrogen and carbon content, C:N ratio, basal respiration, microbial carbon (Cmic), soil pH and soil water content) associated with the fungal community compositions were selected depending on their effect on fungal species composition using capscale models based on the Bray–Curtis distance (capscale function in 'vegan'). Explanatory variables were standardised to a constant mean and standard deviation (decostand function in 'vegan'). Before variable selection, environmental factors were

checked for auto-correlation using the Variance Inflation Factor (VIF) of the vifstep function of the 'usdm' package (Naimi *et al.* 2014). Highly correlated variables with a threshold over 10 were excluded from the dataset. The stepwise backward model selection function of the 'vegan' package was used, where significant factors ($p < 0.05$) were extracted from the environmental variables. The generalist and specialist fungal community dynamics between the different myco-type and myco-type mixtures was assessed by the multinomial species classification method using the 'vegan' package and the function clamtest, with individual test error threshold of alpha 0.05 and coverage limit of 10. This method compares the abundance of the microbial communities between different experimental predictors and classifies the microbes into different classes, namely specialists, generalists and those that are too rare (Pedrinho *et al.* 2020). The statistical significance of the number of specialised ASVs was tested by binomial test using the function binom.test of the 'stats package'.

Results

Initially, raw sequencing data of 16,488,491 reads were generated from a total of 320 samples. Subsequently, the sequence data underwent several filtering steps, including denoising, merging, chimera, and non-target taxa removal, resulting in the retention of 12,822,931 reads (78 % of the initial sequence reads). These quality-filtered reads were then clustered into 6,427 ASVs. The read coverage of the 320 samples was rarefied to the smallest read coverage of 11,273 reads per sample, yielding 5,827 ASVs.

Taxonomic and functional guild relative abundances

Comparative visualization of the top ten fungal families indicated that the soil fungal communities differed in their relative abundances between the mono (AM or EcM trees) or mixed myco-type (AE or AM and EcM trees) stands. These variations were further maintained along the three tree diversity levels (Figure 1a, b, Table S2). However, these patterns did not differ between the two-target tree rooting zone compartments (Figure S2a, b, Table S3), For instance, Hymenogastraceae and Piskurozymaceae were the two most abundant families in mono myco-type stands, whereas Mortierellaceae and Pyronemataceae being the abundant families in mixed myco-type stands

(Figure 1a, Table S2). Distinct distribution patterns of Hymenogastraceae and Piskurozymaceae emerged across tree diversity levels in the EcM and AM mono myco-type stands as compared to the mixed myco-type stands. The relative abundances of Piskurozymaceae decreased with increasing AM tree species diversity, while Hymenogastraceae showed an increase in the relative abundance with increasing tree diversity in mono EcM stands. We observed comparable trends in the mixed myco-type stands when tree diversity increased from two to four species mixture for both Piskurozymaceae and Hymenogastraceae (Figure 1b, Table S2).

Ecological function assignment of the fungal ASVs resulted in a total of seven fungal trophic modes, with saprotroph-symbiotroph (33.4%), symbiotroph (30.3%), pathotroph-saprotroph (20.3%), and saprotrophs (11.2%) representing 89.3% of the total relative abundances. Further comparative analysis indicated that the relative importance of the fungal trophic modes did not differ between mono (EcM and AM) and mixed (AE) myco-types (Figure 1c, Table S2). However, distinct distribution patterns emerged across tree diversity levels in the mono myco-type as compared to the mixed myco-type stands. The EcM tree stands were dominated by symbiotroph, with their relative abundance increasing with increase in tree diversity. In contrast, the AM stands were dominated by saprotroph-symbiotroph fungi (Figure 1d, Table S2) while these patterns did not differ between the two target tree species rooting zone compartments (Figure S2c, d, Table S3).

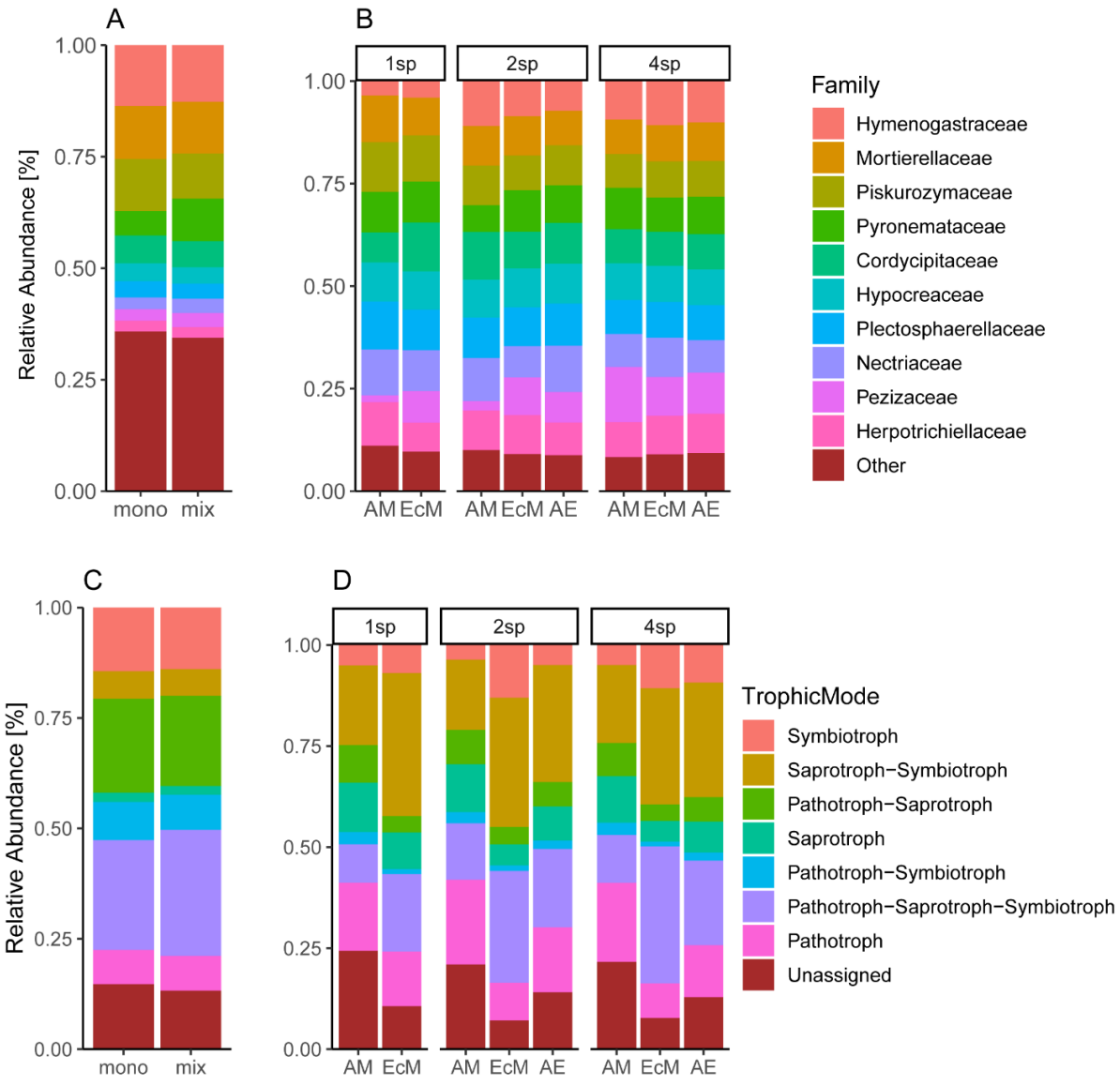

Figure 1. Relative abundance distribution of dominant fungal families and trophic modes as a function of the myco-type mixture (mono (AM or EcM) or mix (AE = AM and EcM)). Top 10 fungal Family relative abundance distribution as a function of tree myco-type mixtures (a) over all and (b) by tree diversity levels. Fungal trophic mode distribution patterns as a function of myco-type mixtures (c) over all and (d) by tree diversity levels. Abbreviations: 1sp = monocultures, 2sp = two species mixture, 4sp = four species mixtures, others = non-top 10 fungal Families, and unassigned = fungal ASVs not assigned to fungal functional guilds.

Fungal taxonomic and functional alpha diversity

Results of ANOVA indicated that observed fungal richness was found to be dependent on the target tree species myco-type ($F = 81.82$, $p < 0.0001$) and the interaction of tree myco-type and myco-type mixture ($F = 34.3$, $p < 0.0001$), but not on the rooting zone compartment, tree diversity and myco-type mixture. Functional guild-based analysis revealed that the richness of symbiotroph and saprotroph fungal communities were influenced by different factors. Saprotroph fungal richness was impacted by myco-type ($F = 88.5$, $p < 0.001$) and interactive effects of myco-type and myco-type mixture ($F = 39.1$, $p < 0.001$). Whereas symbiotroph fungal richness was mainly controlled by tree diversity ($F = 25.6$, $p < 0.001$), rooting zone soil compartment ($F = 15.4$, $p < 0.001$), and interaction effect of myco-type and myco-type mixtures ($F = 9.5$, $p < 0.002$). Comparable effects were found for fungal and functional guild-based Shannon diversity (Table S4).

Further linear mixed effect model analysis revealed myco-type and interactive effects of tree myco-type and tree diversity on fungal richness. The fungal diversity was significantly lower in EcM than in AM tree species growing in mono myco-type stands. The fungal diversity in mixed myco-type stands was higher than EcM tree species stands and lower than the AM tree species stands with increasing tree diversity (Figure 2, Table S5, S6). Further functional guild-based analysis indicated contrasting patterns in symbiotroph and saprotroph fungal communities. The saprotroph fungal communities follow the same pattern as the overall fungal communities. Whereas the symbiotroph fungal richness increased significantly with increasing tree diversity particularly in mixed myco-type stands (Figure 2, Table S5, S6). Comparable effects were found for fungal and functional guild-based Shannon diversity (Figure S3, Table S5, S6). We found no significant differences in fungal richness between the two compartments with increasing tree diversity (Figure S4, Table S7, S8)

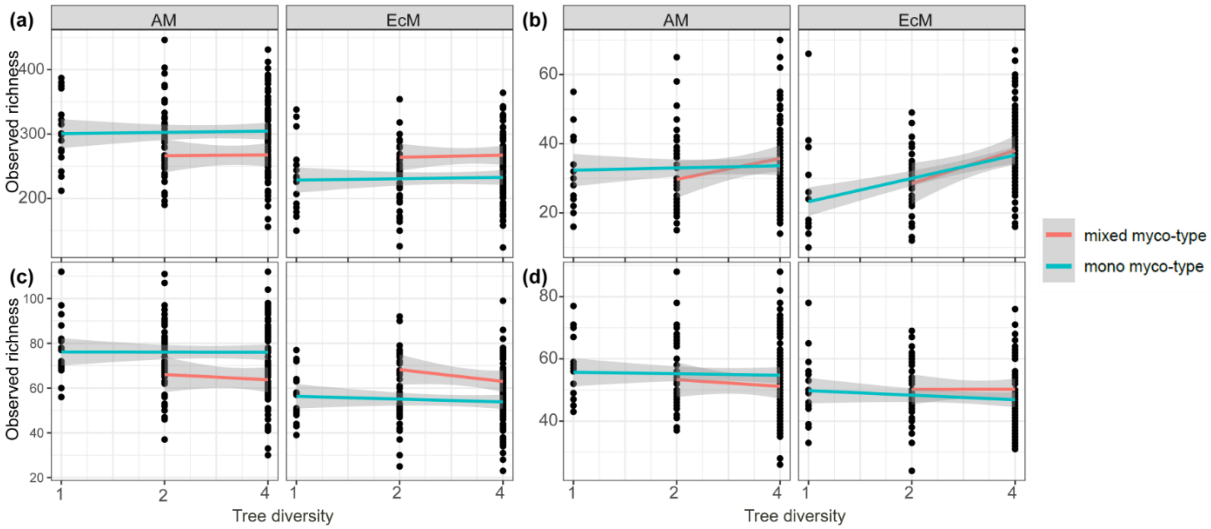

Figure 2. Linear mixed-effects models relating individual and interactive effects of tree myco-type (AM or EcM), myco-type mixture (mono myco-type stands (AM or EcM trees) or mixed types (AM and EcM trees) grow together and $\log_2$ transformed tree diversity on observed richness based on rarefication of all fungi (a), Symbiotroph (b), Saprotroph (c), and Saprotroph-Symbiotroph (d) observed richness.

Fungal community composition

Permutational analysis of variance (PERMANOVA) showed that 39.75% of the variance in fungal community composition was explained by the tested parameters and indicated the significant effects of myco-type (30.6%) and myco-type mixture (0.7%), tree diversity (0.73%) and the interaction of myco-type and myco-type mixture (7.5%). Further analysis of their contribution to the composition of each trophic mode confirmed significant effects of tree myco-type, myco-type mixture, tree diversity, and the interaction of myco-type and myco-type mixture for symbiotroph and saprotroph fungal communities, while in addition, the symbiotroph-saprotroph community was found to be shaped by myco-type and the interaction of myco-type and myco-type mixture. The tree myco-type followed by its interaction with myco-type mixture contributed the major portion of the explained compositional variance in all cases, while there were no significant effects of rooting zone compartment on the overall and trophic mode fungal community compositions (Table 1).

Table 1. Effects of myco-type mixture (MM), tree myco-type (MT), rooting zone compartment (C) and tree diversity (TD) on the compositional differences of soil fungal and three major fungal trophic mode communities based on PERMANOVA with 999 permutations

	All Fungi		**Symbiotrophs**		**Saprotrophs**		**Saprotroph_Symbiotroph**	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Myco-type (MT)	0.3055	**0.001****	0.261	**0.001*****	0.376	**0.001*****	0.217	0.001***
Myco-type Mixture (MM)	0.007	**0.031***	0.049	**0.001*****	0.054	**0.001*****	0.003	**0236**
Compartment (C)	0.0020	0.287	0.002	0.244	0.001	0.255	0.002	0.381
Tree Diversity (TD)	0.0073	**0.015***	0.014	**0.001*****	0.011	**0.001*****	0.002	0.203
(MT * MM)	0.0754	**0.001*****	0.087	**0.001*****	0.121	**0.001*****	0.042	**0.001*****
Residual	0.6025		0.583		0.433		0.732	

All significant P-values are highlighted in bold followed by significance level codes. *P ≤ 0.05. **P ≤ 0.01. ***P ≤ 0.001.

Subsequent dbRDA-based ordination constrained with myco-type and myco-type mixture (AE (mixed myco-type), AM (mono myco-type), and EcM (mono myco-type) levels showed increased similarity between the soil fungal communities of the mono AM and EcM stands with the mixed (AE) communities with increasing tree diversity and converged with increasing tree diversity. These effects were consistent within the target tree rooting and interaction zone compartments (Figure 3). However, pairwise comparisons among myco-types and myco-type mixture levels, overall and within tree species diversity levels, revealed significant differences among the tested pairs at all levels, pointing to the relevance of these factors for community composition although the communities converged with increasing tree diversity (Table S9). Consistently, similar dbRDA-based ordination carried out separately by each of the three fungal trophic communities also showed that the communities of the different trophic modes significantly differed between the tree myco-type and myco-type mixture levels in the overall data and within the three tree diversity levels (Figures S5-7 and Table S9).

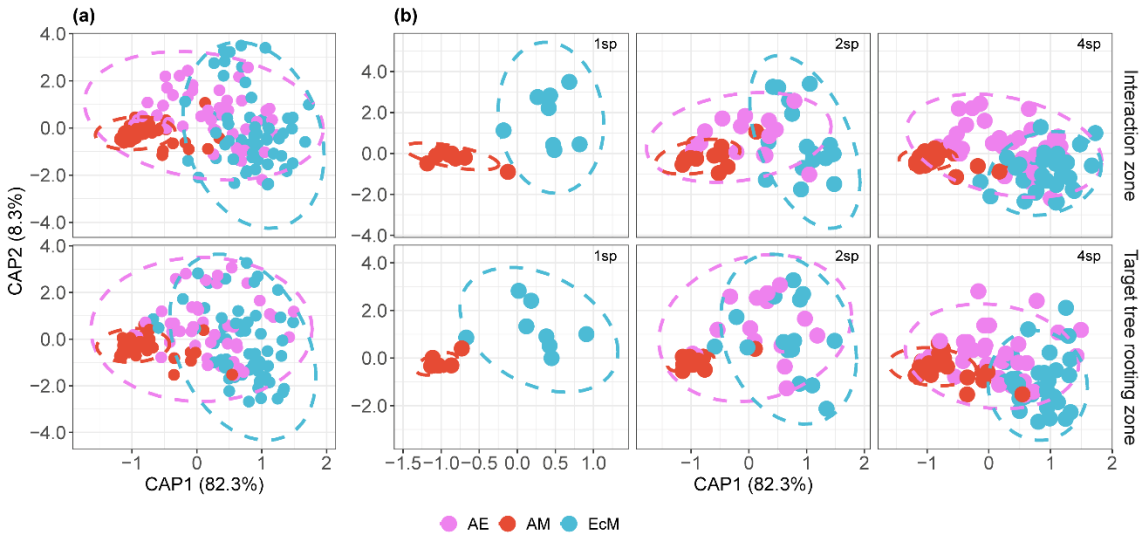

Figure 3. Distance-based RDA (dbRDA) ordination plots constrained with myco-type and myco-type mixture (AE (mixed myco-type), mono myco-type (AM and EcM)) and faceted by rooting zone soil compartment and tree diversity level. (a) Ordination of the overall dataset of fungal communities. (b) Ordination of fungal communities faceted by tree diversity level: 1sp (single species mixture), 2sp (two species mixture), and 4sp (four species mixture), and by the two soil compartments (target tree and interaction rooting zones).

Soil properties shaping the soil fungal community composition

Analysis of the contribution of soil abiotic parameters on the fungal community composition, revealed that pH, $NH4^+$, NO_3^-, and PO_4^{3-} were significant factors shaping the overall soil fungal community composition. The relative importance of the soil variables varied between the two myco-type mixture levels. In mono myco-types the soil water content, microbial biomass, and $NH4^+$ were the most important soil parameters, followed by soil pH, $PO_4^{3-,}$ and NO_3^- (Table S10). In contrast, significant factors in the mixed myco-type were the NO_3^-, PO_4^{3-}, $NH4^+$, pH, and carbon content (Table S10). Further assessment of the role of these soil properties on the fungal community composition of mono and mixed myco-types at the tree species diversity levels also indicated myco-type and myco-type mixture specific importance of soil properties in shaping the fungal community composition. In the mono AM and EcM myco-type one species stands Cmic, NH_4^+ and PO_4^3 significantly contributed in shaping the fungal community compositions, while C:N ratio and NO_3^- further contributed in the mono EcM tree species stands. Remarkably the fungal communities in the 2 and 4 species mixtures of the AM, EcM and mixed myco-type stands were consistently affected by soil pH. In the mixed myco-type plots, however, basal respiration,

N, PO_4^3, NO_3^- were also found to be important contributors, while Cmic and NH_4^+ were important in 2 and 4 EcM tree species stands (Table S11).

Differentiation of specialist and generalist soil fungal taxa and functional groups

The CLAMtest revealed a high number of specialized fungal ASVs with respect to myco-type mixtures (Figure 4a, Table S12) and tree myco-types (Figure S8a, Table S12). Among the 5,827 fungal ASVs identified in this study, 24% were categorized as generalists, while 11% and 6% were specialized to mono and mixed myco-type plots, respectively. In the mono myco-type stands (AM and EcM) including all tree diversity levels, 13.5% of the 4,991 ASVs were classified as generalists, while significantly higher numbers of specialized ASVs were found for AM myco-type (19.3%) than EcM myco-type (11.6%) fungal communities (Figure S8b). Consistently, we found a significantly higher degree of specialized community in AM than EcM myco-type soil fungal communities both in one species (17% AM and 9.5% EcM of 2,023 ASVs, Figure S8b) and multi (2 and 4) species mixtures (20% AM and 11.8% EcM of 4589 ASVs) (Figure 4b). Comparison of mono myco-type (AM or EcM) multi-tree species and mixed (AE) myco-type stands indicated that the mixed myco-type plots shared a higher proportion of generalists (23.5% with AM and 19.7% with EcM plots) (Supplementary Figure 8c and 8d, respectively). However, the percentage of specialized community in mixed myco-type plots was significantly higher (13.2%) as compared to EcM (9.5%) and significantly lower (9%) as compared to AM (11.7%) stands (Figure 4c, d).

Analysis of the top three fungal taxa at the family level and their trophic modes revealed myco-type specific and myco-type-mixture specific taxa and functional guild patterns. Comparison of multi-species stands of AM and mixed (AE) myco-type stands revealed higher proportion of specialized symbiotroph in AE, while AM multi-species plots had higher proportions of saprotrophs and pathotroph-saprotrophs. Whereas the EcM and AE multi species stand comparison showed higher proportion of Symbiotroph in EcM stands while the AE plots were mainly dominated by Symbiotroph and Saprotrophs. The top three fungal taxa behind these functions were also found to be members of different fungal families (Figure S9).

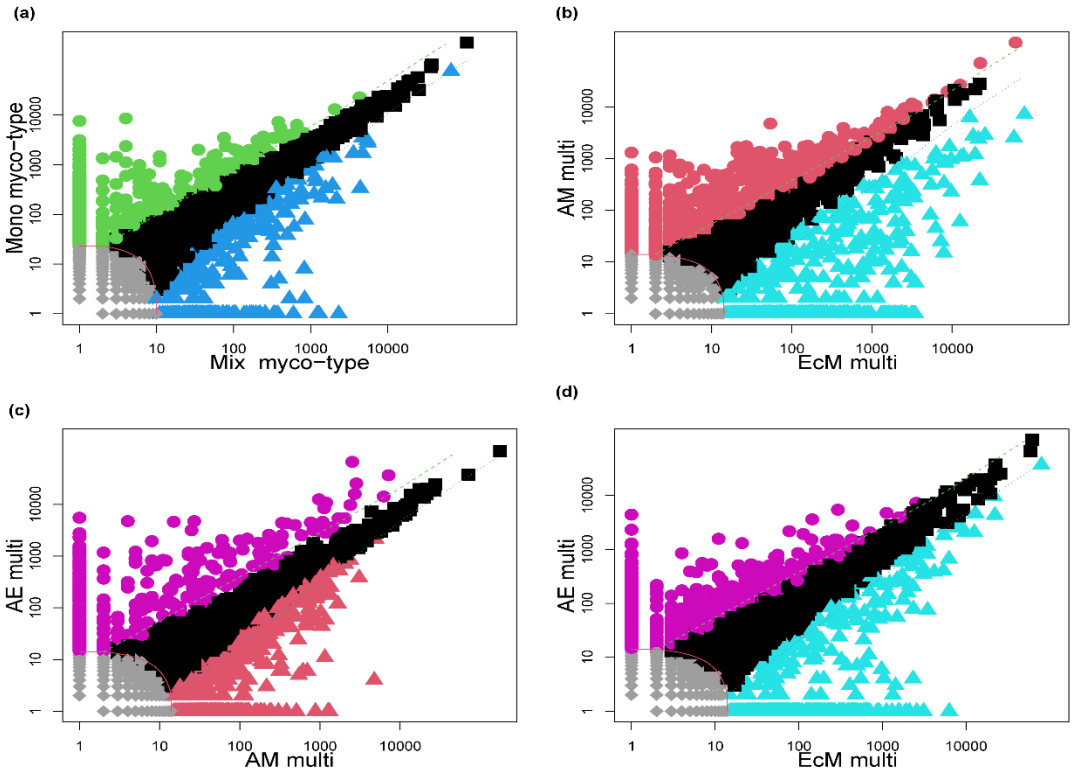

Figure 4. Multinomial species classification method (CLAM) for fungal species specialization of treatment pairs. The evaluated pairs were: mono myco-types versus mixed myco-types (a), AM multi (mixed myco-types) versus EcM multi (b), AE multi versus AM multi (c), AE multi versus EcM multi (d). Proportion of the fungal ASV specializations are represented as specialists of pair on the y axis (circles), specialists of pair on the x axis (triangles), generalist (black squares) and rare taxa (grey diamonds) across evaluated pairs (multi = 2 and 4 tree species mixture).

Discussion

In this study we investigated the interplay of tree myco-type, myco-type mixture, tree species diversity and rooting zone compartments in shaping the target tree rooting zone soil fungal communities under experimentally controlled platform. Our results revealed consistent and significant effects of tree myco-type, myco-type mixture, and tree species diversity on both fungal diversity and fungal community composition, whereas there was no significant effect of the target tree rooting zone compartments. In addition, we found a high degree of specialization of soil fungi with respect to myco-type and myco-type mixture characterized by different fungal taxonomic and

trophic mode distributions resulting in clear differences in the provision of ecosystem functions and services in mono and mixed myco-type multi species forest ecosystems.

Soil fungal alpha diversity

Our Anova results demonstrated that target tree rooting zone soil fungal alpha diversity was influenced mainly by tree myco-type, and the interactive effects of tree myco-type and myco-type mixture. Linear mixed effect model analysis also indicated tree myco-type and interactive effects of myco-type and tree diversity on fungal richness. Fungal diversity was significantly lower in EcM than in AM tree species when growing in mono myco-type stands, while fungal diversity in mixed myco-type stands showed intermediate diversity levels. Our findings are in line with other observational studies that reported an increase in fungal richness in mixed broadleaf conifer (Ji *et al.* 2021) and temperate (Wu *et al.* 2019) forests and contrasts with a global survey of soil fungi (Tedersoo *et al.* 2014), a tree diversity experiment with temperate mixed deciduous trees (Rivest, Whalen & Rivest 2019), and sub-tropical tree diversity experiment (Singavarapu *et al.* 2022b) that found no significant relationships between plant diversity and fungal richness. The observed difference in alpha diversity between AM and EcM mono myco-type stands in each of the tree diversity levels can be explained by the relatively higher host specificity of EcM trees and dual function of EcM fungi as mycorrhizal partners and saprotrophs as compared to AM trees which need both mycorrhizal partner and saprotrophic fungi to facilitate nutrient cycling and uptake in their rooting zones (Simard *et al.* 1997; Phillips, Brzostek & Midgley 2013b). Our finding of increased fungal richness in mixed myco-type stands as compared to pure EcM stands with increasing tree diversity could be due to the additional AM fungal partners and their associated saprotrophic fungal communities. We also found that the richness of symbiotroph and saprotroph fungal communities were influenced by different factors. In contrast to Saprotroph fungal richness that follow the overall fungal richness pattern, symbiotroph fungal richness was influenced by tree diversity, rooting zone soil compartment, and interactive effect of myco-type and myco-type mixtures. The symbiotroph fungal richness increased significantly with increasing tree diversity particularly in mixed myco-type stands These novel results underline the differential functional

significance of saprotroph and symbiotroph fungal communities across the target tree rooting zone compartment and neighboring tree diversity in a forest ecosystem.

Soil fungal community composition

The target tree rooting zone soil fungal community composition was found to be significantly influenced by tree myco-type, myco-type mixtures, tree diversity and the interactive effects of myco-type and myco-type mixture. These effects were consistent both for the symbiotroph and saprotroph fungal communities. Thus, the fungal community composition significantly varied between mono myco-type (AM or EcM) and mixed myco-type (AE) stands in each of the three tree diversity levels. These results are congruent with previous reports (Eagar *et al.* 2022a; Singavarapu *et al.* 2022b). Fungal community compositions of the EcM and AM tree species were reported to converge with increasing tree diversity in a sub-tropical tree experiment (Singavarapu *et al.* 2022b). In our study, however, even if the fungal communities start converging with increasing tree diversity the differences in the fungal community composition between the EcM and AM stands remained significantly different even in the four tree species mixtures. The underlying mechanism might be that different tree species can harbor both host-specific and generalist soil fungal communities (Phillips, Brzostek & Midgley 2013b; Peay, Kennedy & Talbot 2016). In mixed myco-type stands, these different soil fungal communities were all added to a common pool, leading to a converging microbiome (Singavarapu *et al.* 2022b). However, a higher host specificity in our study might have retained the differences found in the monospecific stands. In contrast to our expectation, no differences were found in soil fungal community composition between the target trees' rooting zone and the interaction rooting zone where the roots meet with those of the neighbor trees. The main possible reason for this finding could be a lack of clear rooting zone separation since in the studied experimental site trees are grown one meter away from each other, and eight years after planting, their root systems probably were already fully intertwined, leading to a target tree rooting and interaction zone continuum, where the microbial community composition was equally controlled by the target tree and its neighbors in both rooting zone compartments. Further analysis of the contribution of soil variables indicated soil pH, $PO4^{3-}$, NH4+, and $NO3^{-}$ as key soil properties shaping the overall fungal community composition, which confirms previous findings (Tedersoo *et al.* 2014; Weißbecker *et al.* 2018a). Our study, however, revealed that the relative signicance of the soil properties in shaping the rooting zone soil fungal

communities varies among the mono AM, EcM and mixed (AE) myco-type stands and tree diversity levels. For instance the fungal community composition of one species EcM tree stands were further influenced by C:N ratio and NO_3^- than AM tree stands, which might be attributed to the EcM tree-associated fungi, which can utilize organic matter, and thereby control the quality of the soil nutrients for other microbes, a capability not shared with AM fungi (Baldrian *et al.* 2022; Hicks Pries *et al.* 2023). Unlike the single species stands, soil pH was important in shaping the fungal community composition of 2 and 4 species mixtures regardless of the myco-type and myco-type mixture, which underlines the global role of soil pH in shaping soil fungal community composition (Zhang *et al.* 2016). Our results also indicated the significance of soil properties like basal respiration, N, PO_4^3, NO_3^- in shaping the fungal communities of multi species mixed myco-type stands as compared to mono myco-type stands. This could be explained by the increased niche opportunities of co-existing tree species, providing different resources, root exudates, and root structure (Schappe *et al.* 2017; Yang *et al.* 2021). Thus, our result indicates co-existence of tree species with different mycorrhizal preference will lead to even more diverse soil micro-habitats and properties than coexisiting mono myco-type tree species stands.

Taxonomic and functional guilds

Visualization of the relative abundance distribution of the dominant fungal families and functional guilds indicated that the observed differences in fungal community compositions are mediated by different fungal taxonomic and functional groups. Hymenogastraceae, Piskurozymaceae, Mortierellaceae, and Pyronemataceae were the families that showed differences in their relative abundance between mono and mixed myco-type stands. These fungal families are known to have important ecological functions as decomposers and symbionts (Telagathoti *et al.* 2022). For example, prominent fungal genera in the family Hymenogastraceae found in mono EcM myco-type stands were *Hebeloma* and *Hymenogaster*, which are ectomycorrhizal fungi (Ryberg, Kalsoom & Sanchez-Garcia 2022). The higher abundance of Nectriaceae, Pezizaceae, and Pyronemataceae in mixed myco-type stands as compared to mono AM and EcM myco-type stands might result in enhanced nutrient supply as they are efficient decomposers (Bödeker *et al.* 2014; Tedersoo *et al.* 2014), which facilitates the mobilization of nitrogen and phosphorus from complex organic matter in the immediate vicinity of fungal mycelium (Lindahl & Tunlid 2015; Kyaschenko

et al. 2017). The taxonomic relative abundance distribution patterns were in line with the observed differences in fungal ecological guilds of EcM and AM mono myco-type stands.

Specialization of soil fungal communities

Further fungal specialization analysis to disentangle the proportion of generalist and specialist fungal communities revealed more generalists than specialists in mono as compared to mixed myco-type stands. Zooming into the mono myco-type stands indicated more specialized fungal ASVs in AM stands as compared to EcM stands. This difference could be due to the fact that the different mycorrhizal types have distinct nutrient acquisition mechanisms, which lead to the recruitment of more specialized taxa adapted to the respective hosts (Phillips, Brzostek & Midgley 2013a; Tedersoo & Bahram 2019). Surprisingly, AM mono myco-type plots have shown the highest degree of specialized community, dominated by non-AM fungi, though AM fungi are generalists and show low diversity and have a broad host range (Chen *et al.* 2018). An explanation for this might be that in contrast to EcM trees, which associate with fungal partners with dual function of both symbiotroph and saprotrophs, AM trees need both mycorrhizal partners and saprotrophic fungi to facilitate nutrient cycling and uptake in their rooting zones (Phillips, Brzostek & Midgley 2013b).

Strikingly we found increased specialized fungal communities composed of dominantly symbiotroph in mixed myco-type stands as compared to mono EcM in contrast to mono AM multi species stands. This increase could be partly explained by the additional AM fungal partners and their associated saprotrophic fungal communities in the mixed myco-type stands and vice versa. Furthermore, the mixed myco-type stands had a higher prevalence of generalist fungal communities. This finding is consistent with the idea that mixed myco-type stands offer more ecological niche opportunities (Davison *et al.* 2022). Obviously, a wider range of resource supply and more different host characteristics in mixed myco-type stands can be used by a wide range of fungal taxa without requiring any specific capabilities (Pandit, Kolasa & Cottenie 2009; Wang *et al.* 2021). In contrast, specialists, due to their specific adaptations, may be more efficient at functions such as nutrient exchange with their specific hosts (Devictor, Julliard & Jiguet 2008). Thus, our results underline the need for further investigations to unravel the relative contribution

of generalist and specialist fungal communities in tree-tree interaction, species co-existence and functioning of forest ecosystems.

Conclusion

Our experimentally controlled study advances our understanding of tree-tree interactions and tree species co-existence and its interplay with the target tree rooting zone soil fungal community composition. The different composition of generalist and specialist rooting zone soil mycobiome composition coupled with the respective soil properties of mono (AM or EcM) and mixed (AM and EcM) myco-type tree species in forest ecosystems indicates enhanced ecosystem functioning in multi species stands of mixed myco-types. Further research is needed to address which particular ecosystem functions are promoted by mixing tree species of different myco-type preferences. In particular, it would be highly relevant for reforestation and afforestation projects to study, if mixed myco-type stands also lead to increased timber production. It is also necessary to study such comparative mixed myco-type effects across different geographical regions and climate zones to confirm that the observed patterns hold globally.

Appendix

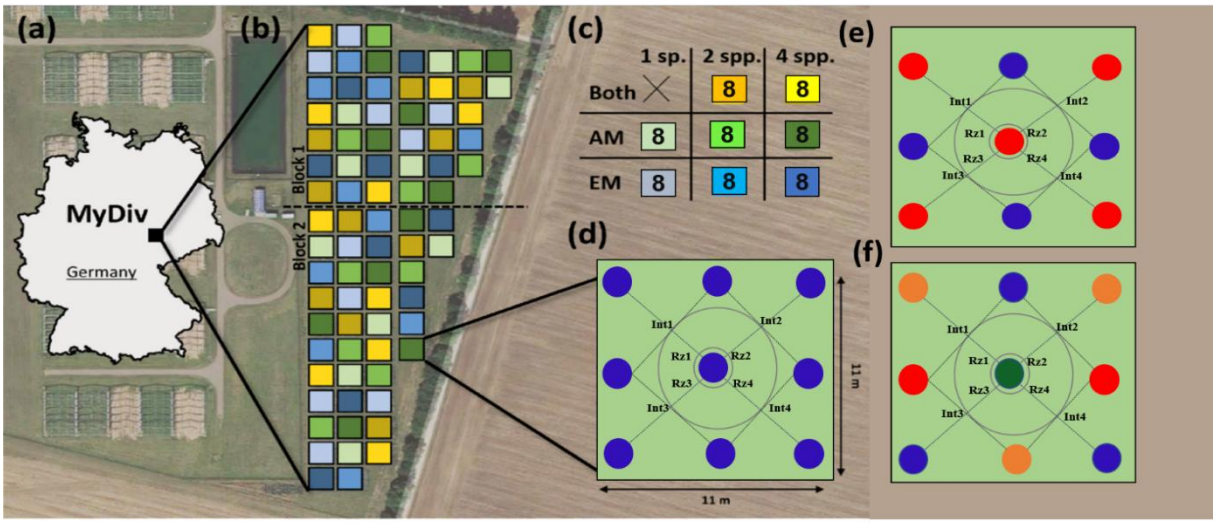

Figure S1. MyDiv experimental (A) platform between- and within plot spatial design (B) and mycorrhizal type, mycorrhizal type mixtures, and tree richness (C) (modified after Ferlian et al. (2018)). Sampling scheme of the soil sampling with the target tree (green circle) surrounded by eight neighbour trees across the tree diversity levels. Pooled samples of four soil cores were taken at two scales: 1) target tree rooting zone samples (close to the target species, Rz1-Rz4) and 2) interaction rooting zone soil samples (at the centre of each of the four-tree species surrounding the target tree species, Int1-Int4). Circles with different colours represent different tree species (single species mixture, D), (two species mixture, E), and (four species mixture, F).

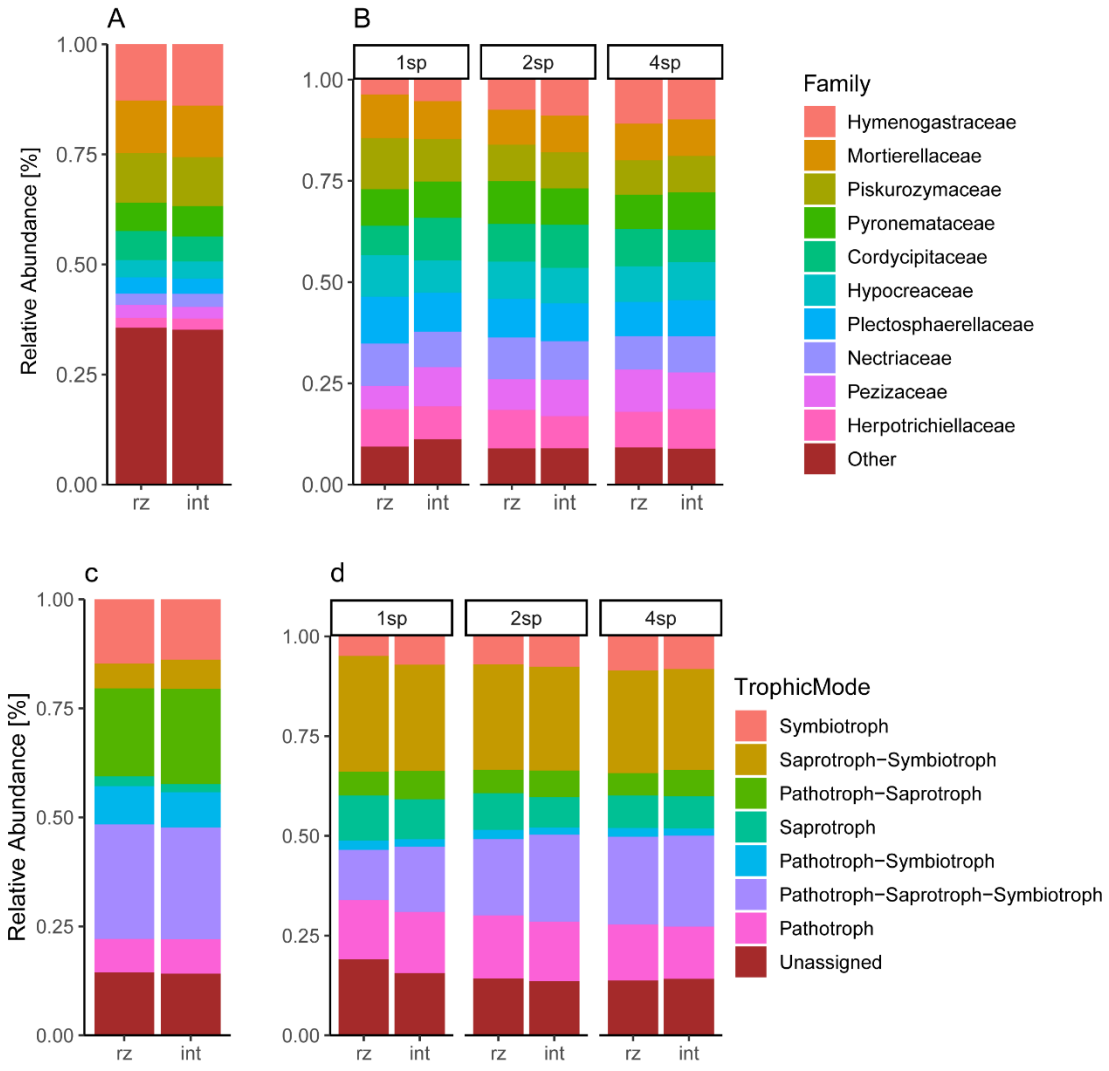

Figure S2. Relative abundance distribution of dominant fungal families and fungal trophic modes as a function of the two-target tree rooting zone compartment (rz: rooting zone) (int = Interaction zone)). Top 10 fungal Family relative abundance distribution (A) as a function of tree rooting zone compartment and (B) in addition by tree diversity levels. Fungal trophic mode distribution patterns (C) tree rooting zone compartment, and (D) in addition by tree diversity levels. Abbreviations: 1sp = monocultures, 2sp = two species mixture and 4sp = four species mixtures.

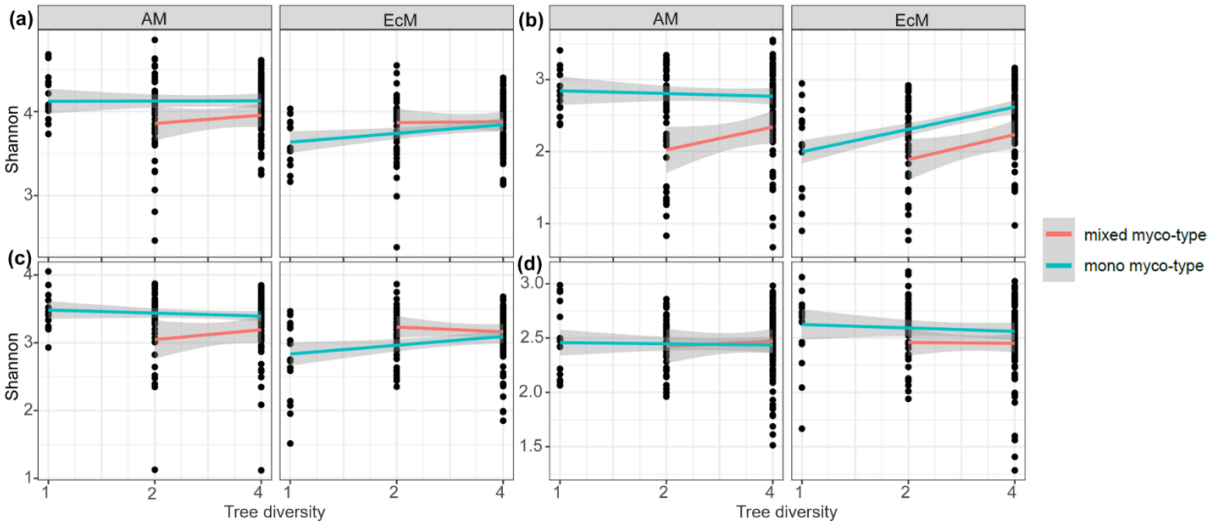

Figure S3. Linear mixed effects models using shannon diversity relating individual and interactive effects of tree myco-type (AM or EcM), myco-types mixture (mono-typic stands (AM or EcM trees) or mixed types (AM and EcM trees) and tree diversity of the fungi (A), Symbiotroph (B), Saprotroph (C), and Saprotroph-Symbiotroph (D)

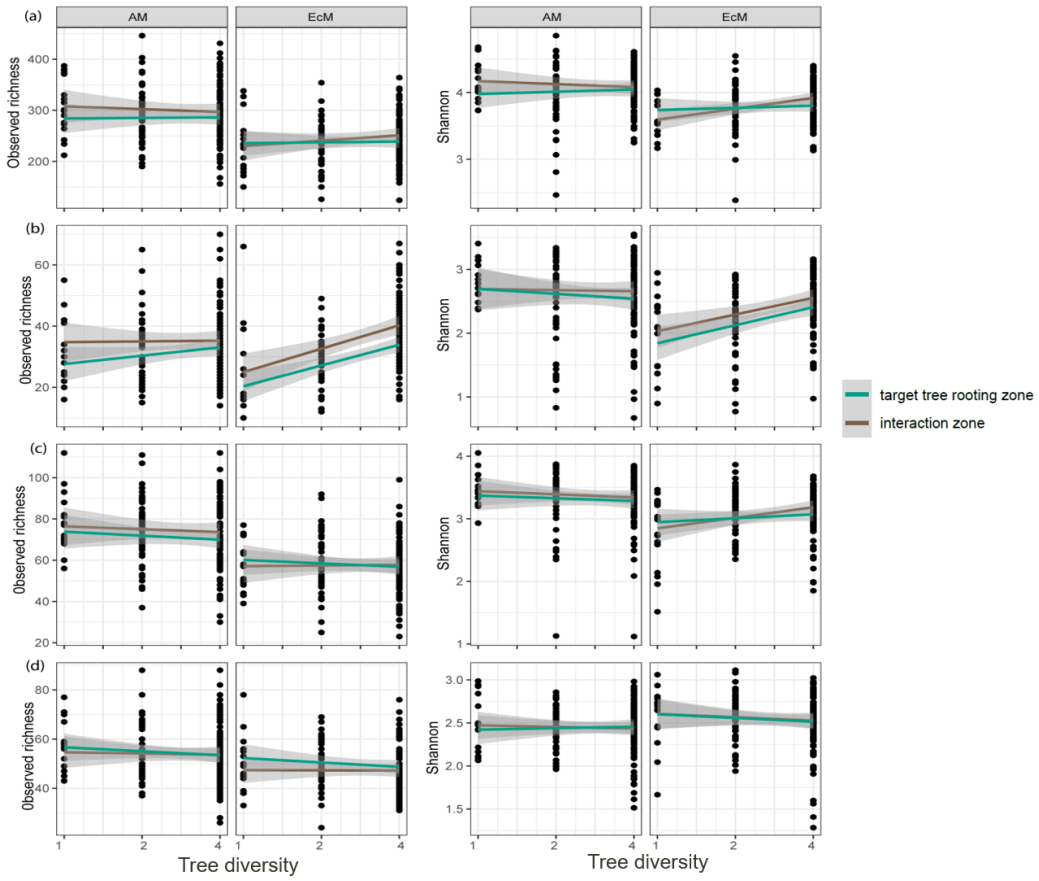

Figure S4. Linear mixed effects models relating individual and interactive effects of tree myco-type (AM or EcM), two target tree rooting soil compartment and tree diversity of the fungi (A), Symbiotroph (B), Saprotroph (C), and Saprotroph-Symbiotroph (D) observed richness and shannon diversity

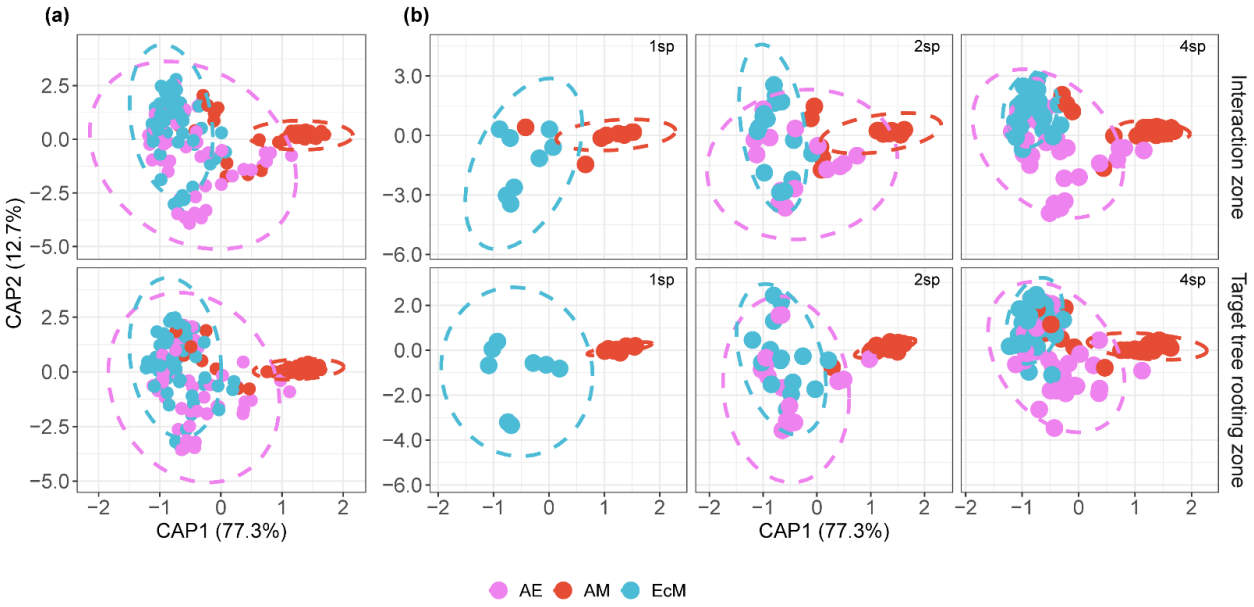

Figure S5. Distance-based RDA (dbRDA) ordination plots of Symbiotroph community constrained with myco-types and myco-type mixture (AE, AM and EM) and faceted by two soil compartment (target tree and interaction rooting zones) and tree diversity level, 1sp (single species mixture), 2sp (two species mixture) and 4sp (four species mixture)

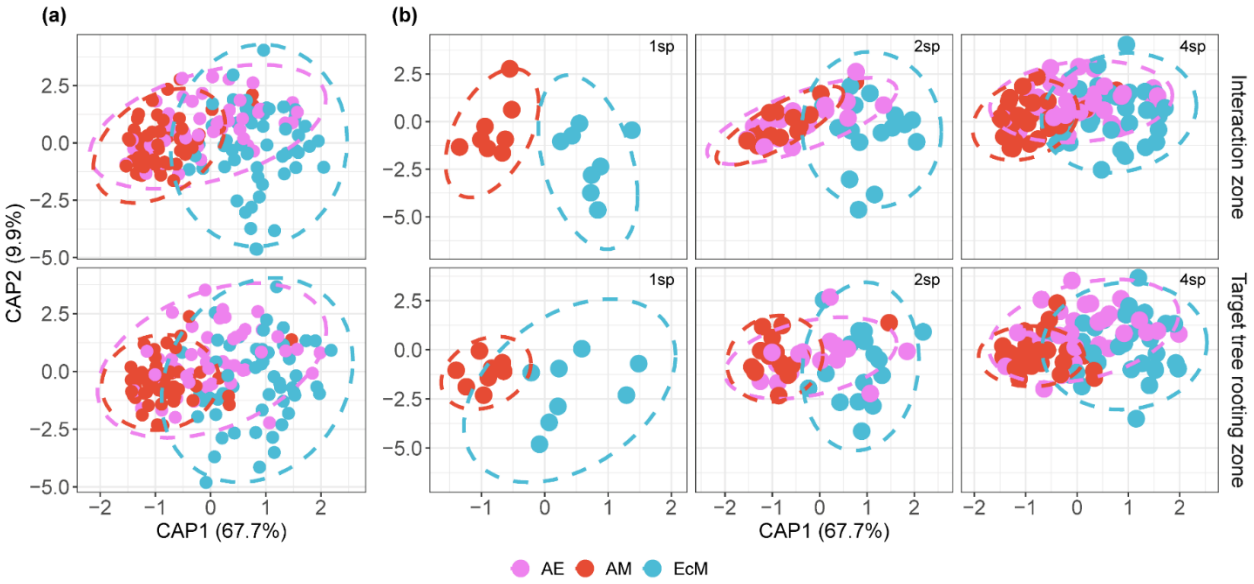

Figure S6. Distance-based RDA (dbRDA) ordination plots of Saprotroph community constrained with myco-types and myco-type mixture (AE, AM and EM) and faceted by two soil compartments (target tree and interaction rooting zones). and tree diversity level, 1sp (single species mixture), 2sp (two species mixture) and 4sp (four species mixture).

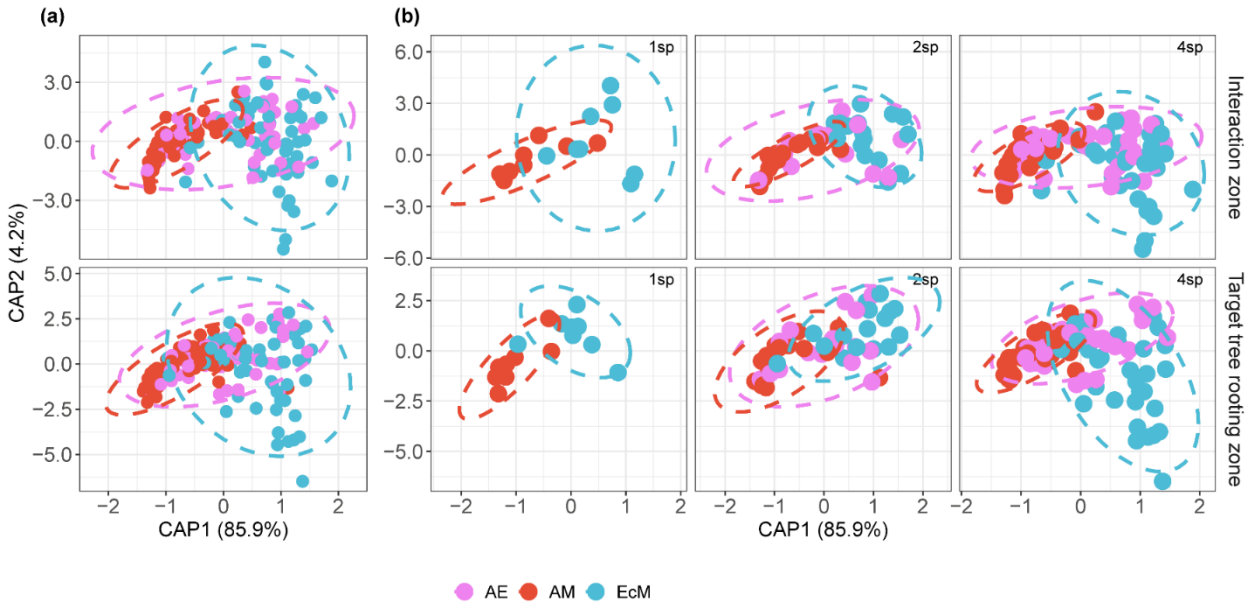

Figure S7. Distance-based RDA (dbRDA) ordination plots of Symbiotroph_Saprotroph community constrained with myco-types and myco-type mixture (AE, AM and EM) and faceted by two soil compartments (target tree and interaction rooting zones). and tree diversity level, 1sp (single species mixture), 2sp (two species mixture) and 4sp (four species mixture).

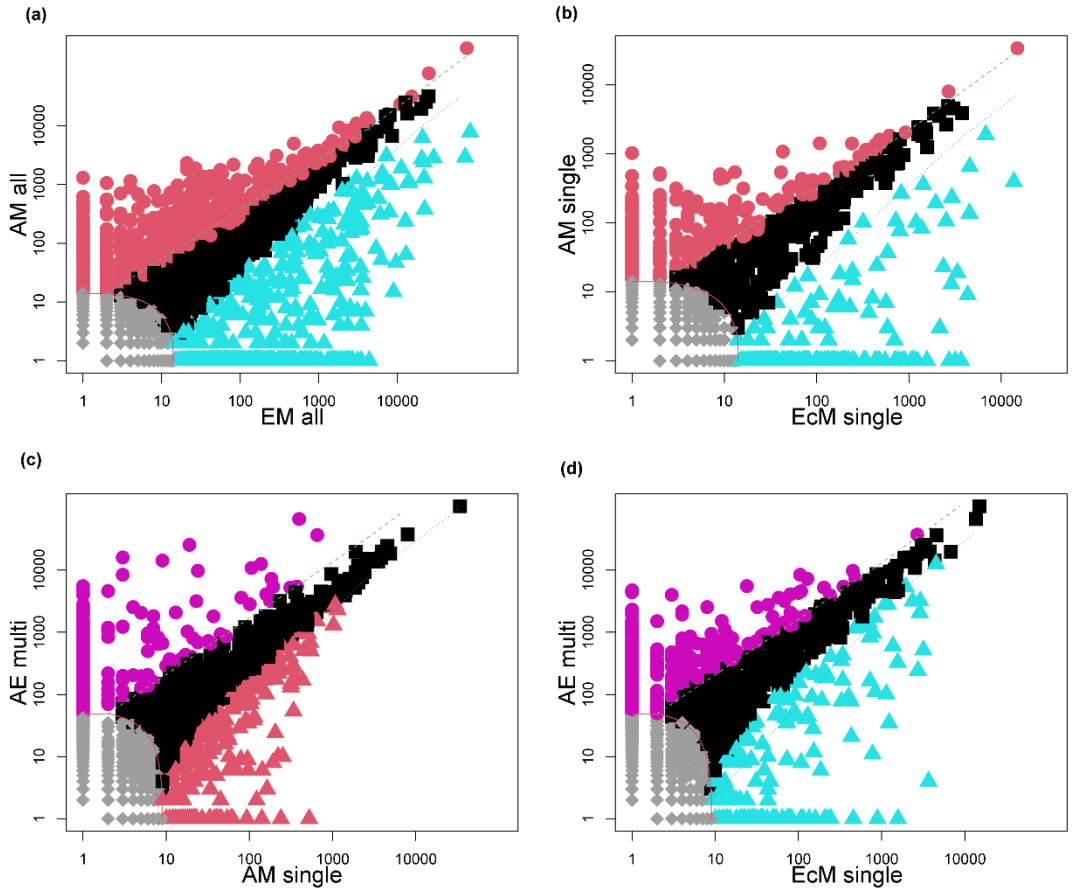

Figure S8. Multinomial species classification method (CLAM) for fungal species specialization of treatment pairs. The evaluated pairs were: mix of myco-types (A) AM all versus EcM all, (B) AM single species versus EcM single species, (C) mix myco-types multi species versus AM single species, (D) mix myco types multi species versus EcM single species. Proportion of the fungal ASV specializations are represented as specialists of pair on the y axis (closed circles), specialists of pair on the x axis (closed triangles), generalist (closed square with black colour squares) and rare taxa (closed grey diamonds with grey colour) across evaluated pairs (multi = 2 and 4 tree species mixture).

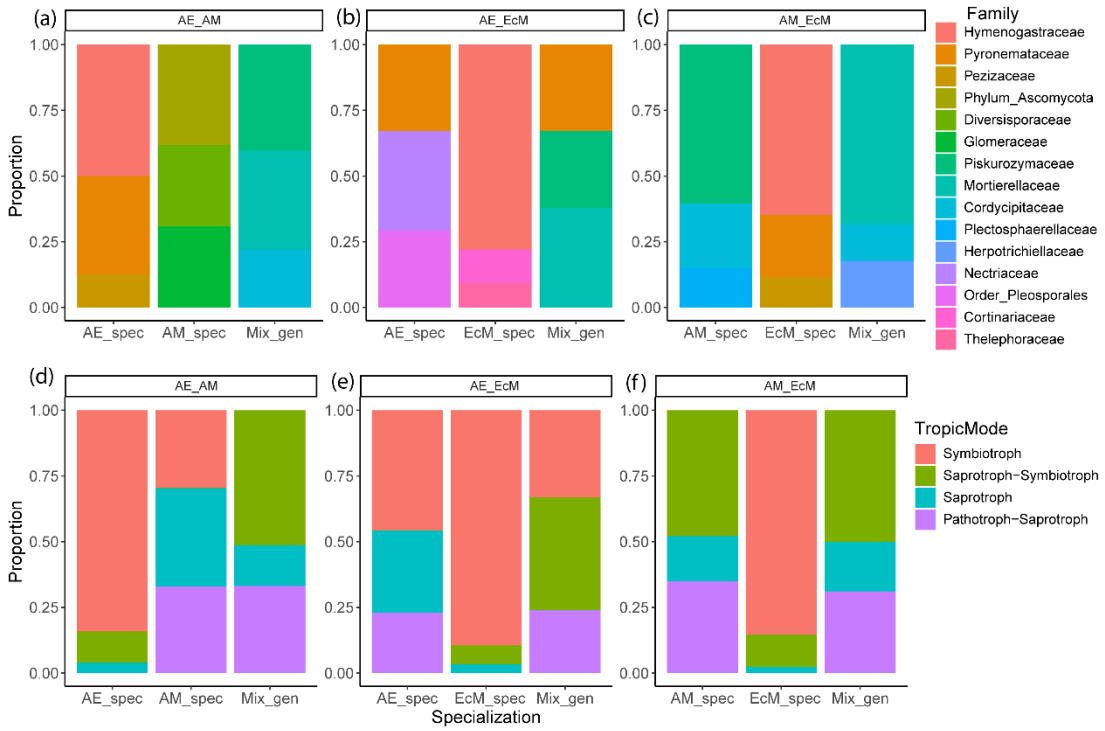

Figure S9. Relative abundance bar plots of the top 3 fungal families (a-c) and fungal trophic modes (d-f) of the specialized fungal communities of the mixed (AE), and mono (AM and EcM multi species mixtures) myco-types mixture levels (see also Figure 04 c-e).

Table S2. Relative abundance of fungal family and trophic mode trait in Effect of and myco-type mixture, mono (AM, EcM) and mixed (AE) myco-type and tree diversity levels.

Taxa	Mono	Mix	AM	EcM	AE	AM1	AM2	AM4	AE2	AE4	EcM1	EcM2	EcM4
Cordycipitaceae	6.256	5.896	8.484	4.028	5.898	5.828	9.930	8.425	6.647	5.524	5.790	4.009	3.598
Herpotrichiellaceae	2.432	2.435	2.708	2.155	2.435	2.696	2.620	2.755	2.202	2.551	1.841	2.267	2.177
Hymenogastraceae	13.61	12.70	2.425	24.80	12.70	0.796	2.663	2.713	10.38	13.86	12.14	23.44	28.65
Hypocreaceae	4.029	3.690	5.607	2.451	3.690	5.000	5.194	5.965	4.058	3.506	2.769	2.570	2.311
Mortierellaceae	11.91	11.59	14.17	9.647	11.59	15.09	13.70	14.18	11.07	11.85	10.67	10.22	9.100
Nectriaceae	2.654	3.269	3.499	1.810	3.269	3.673	3.699	3.355	4.190	2.808	2.193	1.522	1.859
Pezizaceae	2.553	3.114	0.359	4.747	3.114	0.054	0.082	0.574	2.601	3.370	4.393	4.821	4.799
Piskurozymaceae	11.70	10.10	17.21	6.190	10.10	19.52	16.72	16.87	11.14	9.585	8.447	5.788	5.827
Plectosphaerellaceae	3.617	3.307	4.913	2.320	3.307	5.349	4.851	4.835	3.828	3.047	2.742	2.422	2.164
Pyronemataceae	5.415	9.501	0.715	10.11	9.501	0.659	0.465	0.854	9.827	9.338	12.15	11.24	9.040
Others	35.80	34.37	39.89	31.72	34.37	41.31	40.05	39.45	34.03	34.53	36.84	31.67	30.46
Trophicmodes	**Mono**	**Mix**	**AM**	**EcM**	**AE**	**AM1**	**AM2**	**AM4**	**AE2**	**AE4**	**EcM1**	**EcM2**	**EcM4**
Pathotroph	14.40	13.93	19.58	9.48	13.93	16.84	20.96	19.57	16.09	12.87	13.48	9.33	8.56
Patho-Sapro	6.25	6.03	8.48	4.15	6.03	9.28	8.53	8.25	6.01	6.04	4.02	4.32	4.09
Patho-Sapro-Symbio	21.26	20.42	12.08	29.98	20.42	9.48	13.95	11.79	19.36	20.94	19.17	27.63	33.88
Patho-Symbio-Sapro	2.09	2.00	2.98	1.25	2.00	3.05	2.80	3.06	2.11	1.95	1.23	1.37	1.19
Sapro-Symbio	8.61	7.93	11.69	5.69	7.93	12.21	11.83	11.49	8.42	7.69	9.10	5.18	5.10
Symbiotroph	24.86	28.57	18.79	30.63	28.57	19.76	17.35	19.27	28.96	28.37	35.40	31.98	28.75
Symbiotroph	7.78	7.84	4.57	10.83	7.84	5.00	3.61	4.94	4.94	9.27	6.92	13.04	10.69
Unassigned	14.73	13.28	21.83	7.99	13.28	24.38	20.97	21.62	14.11	12.88	10.67	7.15	7.75

Table S3. Relative abundance of fungal family and trophic mode trait in compartment myco-type and tree diversity levels.

Taxa	rz	Int	rz1	rz2	rz4	Int1	Int2	Int4
Cordycipitaceae	6.595	5.703	5.281	6.964	6.630	6.338	6.760	5.069
Herpotrichiellaceae	2.311	2.553	2.362	2.481	2.219	2.176	2.246	2.771
Hymenogastraceae	12.75	13.93	5.168	10.70	15.04	7.776	13.62	15.11
Hypocreaceae	3.953	3.902	4.509	4.099	3.788	3.262	3.783	4.068
Mortierellaceae	11.93	11.70	14.25	11.55	11.73	11.51	11.79	11.69
Nectriaceae	2.661	3.016	3.085	3.093	2.375	2.782	3.181	2.974
Pezizaceae	2.838	2.605	1.804	2.410	3.226	2.645	2.594	2.604
Piskurozymaceae	11.30	11.14	15.72	11.47	10.48	12.25	10.96	11.04
Plectosphaerellaceae	3.633	3.415	4.646	3.892	3.335	3.446	3.510	3.363
Pyronemataceae	6.409	6.873	6.379	7.583	5.828	6.435	6.777	6.994
Others	35.60	35.15	36.79	35.74	35.33	41.37	34.76	34.30
Trophicmodes	**rz**	**Int**	**rz1**	**rz2**	**rz4**	**Int1**	**Int2**	**Int4**
Pathotroph	14.68	13.84	14.89	15.84	14.06	15.35	14.84	13.09
Pathotroph-Saprotroph	5.72	6.66	6.03	5.84	5.60	7.14	6.66	6.58
Patho-Sapro-Symbio	20.17	21.85	12.57	19.09	21.99	16.38	21.81	22.77
Pathotroph-Symbiotroph	2.26	1.87	2.37	2.33	2.20	1.85	1.83	1.90
Saprotroph	8.75	8.07	11.23	9.19	8.12	9.99	7.64	7.96
Saprotroph-Symbiotroph	26.30	25.66	28.96	26.46	25.76	26.60	25.98	25.34
Symbiotroph	7.69	7.90	4.90	6.98	8.51	7.09	7.62	8.18
Unassigned	14.43	14.16	19.05	14.25	13.74	15.60	13.63	14.19

Table S4. ANOVA of the individual and interactive effects of tree myco-type, myco-type mixture (mono-typic or mixed myco-types), soil compartment (target tree rooting and interaction zone) and tree diversity levels (mono, two and four tree species) on alpha diversity indices of the soil fungal and three major trophic modes.

	Observed richness			**Shannon**		
All fungi	Sum Sq	F value	P	Sum Sq	F value	P
Myco-types (MT)	205487	81.829	**<0.001*** **	5.13	43.28	**<0.001*** **
Myco-types Mixture (MM)	853	3.021	0.054	0.21	1.774	0.183
Soil compartment (C)	234	3.677	0.166	0.31	2.643	0.105
Tree Diversity (TD)	969	3.386	0.053	0.49	4.110	**0.043***
MT*MM	84483	33.643	**<0.001*** **	1.45	12.254	**0.0005*** **
Symbiotroph	Sum Sq	F value	P	Sum Sq	F value	P
Myco-types (MT)	0	0.004	0.949	6.171	25.32	**<0.001*** **
Myco-types Mixture (MM)	104	0.916	0.339	12.89	52.88	**<0.001*** **
Compartment (C)	1739	15.39	**0.0001*** **	1.20	4.917	**0.027***
Tree Diversity (TD)	2894	25.60	**<0.001*** **	4.05	16.63	**<0.001*** **
MT*MM	1076	9.520	**0.002****	2.68	11.09	**0.001*** **
Saprotroph	Sum Sq	F value	P	Sum Sq	F value	P
Myco-types (MT)	18000	88.46	**<0.001*** **	5.70	35.37	**<0.001*** **
Myco-types Mixture (MM)	32	0.159	0.691	0.20	1.262	0.262
Compartment (C)	224	1.103	0.294	0.28	1.728	0.189
Tree Diversity (TD)	142	0.696	0.405	0.17	1.024	0.312
MT*TM	7952	39.08	**<0.001*** **	3.27	20.29	**<0.001*** **
Symbiotroph_Saprotroph	Sum Sq	F value	P	Sum Sq	F value	P
Myco-types (MT)	2531	23.20	**<0.001*** **	0.727	7.72	**0.005****
Myco-types Mixture (MM)	07	0.06	0.804	0.210	2.23	0.135
Compartment (C)	143	1.31	0.252	0.001	0.10	0.918
Tree Diversity (TD)	109	1.00	0.317	0.043	0.45	0.502
MT*MM	537	4.92	**0.027***	0.325	3.45	**0.0**64

Table S5. Statistical analysis of tree mycorrhizal type (AM or EcM), myco-types mixture (mono-typic stands (AM or EcM trees) or mixed types (AM and EcM trees) and tree diversity effects on the fungal and three major tropic mode trait observed richness and Shannon diversity

	Observed richness			**Shannon**		
All. Fungi	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Mono	1.59	-7.67	10.9	0.044	-0.031	0.120
Mix	1.83	-19.27	22.9	0.055	-0.135	0.247
Symbiotroph	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Mono	3.42	0.64	6.2	0.132	-0.009	0.273
Mix	7.74	1.63	13.9	0.354	0.045	0.663
Saprotroph	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Mono	-0.903	-3.69	1.89	0.048	-0.031	0.128
Mix	-4.086	-10.97	2.80	0.034	-0.156	0.225
Symbio_Sapro	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Mono	-1.04	-3.04	0.95	0.016	-0.084	0.051
Mix	-1.19	-5.90	3.52	0.016	-0.157	0.189

Table S6. Linear mixed effects models relating individual and interactive effects of tree mycorrhizal type (AM or EcM), myco-types mixture (mono-typic stands (AM or EcM trees) or mixed types (AM and EcM trees) and tree diversity on the fungal and three major tropic mode trait observed richness and Shannon diversity

	Observed richness		**Shannon**	
All Fungi	**df**	***P***	**df**	***P***
Intercept	126.1	**<0.0001*** **	102.8	**<0.0001*** **
Log2_div (TD)	172.3	0.956	111.1	0.344
Myc.typEMF (MT)	143.7	0.904	94.82	0.602
Myc.mixmono (MM)	187.9	0.201	126.2	0.055
(MM * MT)	285.6	0.918	276.3	0.383
(TD * MM)	140.1	0.955	103.2	0.345
(MT * TD)	294.9	0.075	304.4	**0.011***
(TD * MT * MM)	267.9	0.918	288.4	0.135
Symbiotroph	**df**	***P***	**df**	***P***
Intercept	124.1	**0.0005*** **	97.23	**<0.0001*** **
Log2_div (TD)	102.1	0.964	77.86	**0.041***
Myc.typEMF (MT)	244.9	0.611	250.6	0.914
Myc.mixmono (MM)	102.9	0.183	74.01	**0.0003*** **
(TD * MT)	277.8	0.511	274.8	0.793
(TD * MM)	74.78	0.172	53.78	**0.046***
(MT * MM)	246.4	0.548	188.9	**0.032***
(TD * MT * MM)	166.4	0.540	103.9	0.083
Saprotroph	**df**	***P***	**df**	***P***
Intercept	121.8	**<0.0001*** **	170.7	**<0.0001*** **
Log2_div (TD)	142.2	0.503	143.1	0.277
Myc.typEMF (MT)	128.6	0.705	265.8	0.174
Myc.mixmono (MM)	162.7	0.394	158.3	**0.017***
(TD * MT)	283.5	0.685	281.5	0.205
(TD * MM)	130.8	0.572	115.6	0.198
(MT * MM)	306.1	**0.024***	285.2	**0.0009*** **
(TD * MT * MM)	301.1	0.891	235.4	**0.035***
Saprotroph-Symbiotroph	**df**	***P***	**df**	***P***
Intercept	181.5	**<0.0001*** **	115.9	**<0.0001*** **
Log2_div (TD)	166.2	0.460	111.4	0.804
Myc.typEMF (MT)	258.2	0.460	113.7	0.982
Myc.mixmono (MM)	186.7	0.984	125.9	0.854
(TD * MT)	282.7	0.578	277.5	0.859
(TD * MM)	141.4	0.603	104.1	0.759
(MT * MM)	292.5	0.981	303.5	0.487
(TD * MT * MM)	260.6	0.478	285.2	0.968

Table S7. Statistical analysis of tree myco-type (AM or EcM), two target tree rooting soil compartments and tree diversity on the fungal and three major tropic mode trait observed richness and Shannon diversity

	Observed richness			**Shannon**		
All. Fungi	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Rz	-0.87	-5.89	9.80	-0.430	-0.065	0.220
Int	-0.81	-9.72	19.5	-0.570	-0.091	0.198
Symbiotroph	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Rz	2.21	0.89	-5.1	-0.246	-0.091	0.349
Int	5.39	0.98	-3.6	-0.397	0.032	0.593
Saprotroph	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Rz	-0.783	-2.80	2.90	0.051	-0.045	0.181
Int	-0.089	-9.89	2.98	0.045	-0.159	0.237
Symbio_Sapro	**Slop**	**lower.CL**	**Upper.CL**	**Slope**	**lower.CL**	**Upper.CL**
Rz	-1.14	-4.81	1.94	0.028	-0.075	0.049
Int	-1.29	-4.78	3.78	0.026	-0.162	0.179

Table S8. Linear mixed effects models relating individual and interactive effects of tree myco-type (AM or EcM), two target tree rooting soil compartments and tree diversity on the fungal and three major tropic mode trait observed richness and Shannon diversity

	Observed rich		**Shannon**	
All Fungi	**df**	***P***	**df**	***P***
Intercept	34.22	**<0.0001*** **	26.93	**<0.001*** **
Log2_div (TD)	129.89	0.231	67.25	0.287
Myc.typEMF (MT)	34.01	**0.002** **	26.14	**0.005** **
Compartment rhz (CR)	247.5	0.192	230.8	0.071
(TD * MT)	181.8	**0.047***	116.4	**0.006** **
(TD * CR)	247.5	0.551	230.8	0.223
(MT * CR)	247.5	0.253	230.8	**0.026***
(TD * MT * CR)	247.5	0.323	230.8	**0.024***
Symbiotroph	**df**	***P***	**df**	***P***
Intercept	73.67	**<0.001*** **	91.21	**<0.001*** **
Log2_div (TD)	94.04	0.861	80.64	0.902
Myc.typEMF (MT)	77.10	0.054	124.0	0.056
Compartment rhz (CR)	233.0	**0.043***	231.7	0.988
(TD * MT)	147.9	**0.011***	179.7	0.185
(TD * CR)	233.0	0.247	231.7	0.529
(MT * CR)	233.0	0.610	231.7	0.390
(TD * MT * CR)	233.0	0.269	231.7	0.548
Saprotroph	**df**	***P***	**df**	***P***
Intercept	35.02	**<0.001** **	97.64	**<0.001*** **
Log2_div (TD)	97.61	0.187	121.3	0.413
Myc.typEMF (MT)	34.34	**0.010***	100.5	0.001**
Compartment rhz (CR)	225.1	0.592	230.3	0.637
(TD * MT)	155.8	0.141	156.5	**0.016***
(TD * CR)	225.1	0.858	230.3	0.932
(MT * CR)	225.1	0.414	230.3	0.424
(TD * MT * CR)	225.1	0.730	230.3	0.379
Saprotroph-Symbiotroph	**df**	***P***	**df**	***P***
Intercept	80.36	**<0.0001*** **	32.47	**<0.001*** **
Log2_div (TD)	165.3	0.653	199.0	0.847
Myc.typEMF (MT)	80.96	0.091	31.07	0.449
Compartment rhz (CR)	239.3	0.602	236.4	0.593
(TD * MT)	185.4	0.728	268.1	0.600
(TD * CR)	239.3	0.637	236.4	0.531
(MT * CR)	239.3	0.597	236.4	0.726
(TD * MT * CR)	239.3	0.854	236.4	0.617

Table S9. Pair-wise PERMANOVA of the myco-types and myco-types mixture soil fungal and tree major tropic mode trait communities along the tree diversity levels. Abbreviation: (AE (mixed)), AM (mono/ mixed) and EcM (mono/mixed), 1sp (single species mixture), 2sp (two species mixture) and 4sp (four species mixture)

	Fungi		**Symbiotroph**		**Saprotroph**		**Sap-Sym**	
pairwise-comparison	**R^2**	**p.adj**	**R^2**	**p.adj**	**R^2**	**p.adj**	**R^2**	**p.adj**
AE vs EcM	0.158	**0.001****	0.0761	**0.001****	0.048	**0.001****	0.087	**0.001****
AE vs AM	0.331	**0.001****	0.447	**0.001****	0.090	**0.001****	0.160	**0.001****
EcM vs AM	0.629	**0.001****	0.646	**0.001****	0.253	**0.001****	0.388	**0.001****
EcM_1sp vs AM_1sp	0.600	**0.0014***	0.554	**0.0015***	0.044	**0.0046***	0.294	**0.0018***
AM_2sp vs EcM_2sp	0.629	**0.0014***	0.673	**0.0015***	0.286	**0.0023***	0.434	**0.001***
AE_2sp vs AM_2sp	0.257	**0.0014***	0.462	**0.0015***	0.061	0.0404*	0.143	**0.0018***
AE_2sp vs EcM_2sp	0.204	**0.0014***	0.075	0.0154*	0.080	0.0120*	0.120	**0.002***
AM_4sp vs EcM_4sp	0.647	**0.0014***	0.678	**0.0015***	0.244	**0.0023***	0.409	**0.001***
AE_4sp vs AM_4sp	0.352	**0.0014***	0.429	**0.0015***	0.111	**0.0023***	0.169	**0.001***
AE_4sp vs EcM_4sp	0.169	**0.0014***	0.139	**0.0015***	0.0391	0.016*	0.102	**0.001***

All significant P values are highlighted in bold followed by significance level codes. *P ≤ 0.05. **P ≤ 0.01. ***P ≤ 0.001.

Table S10. Environmental factors associated with the fungal community compositional variation based on capscale model selection across myco-types mixture (mono-typic stands (AM or EcM trees) or mix types (AM and EcM trees). Significant factors were stepwise selected by ordistep function in R. Abbreviation: Basal resp= basal respiration, Cmic= carbon microbial biomass, SWC= soil water content, C= carbon, N= nitrogen, NH4+ = Ammonium, NO_3^-= Nitrate, PO_4^{3-} = Phosphate.

	Overall		Mono		Mix	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
pH	2.685	0.002**	1.697	0.042*	2.517	0.002**
SWC	3.299	0.001***	2.534	0.005**	-	-
Cmic	6.8	0.001***	7.678	0.001***	-	-
Basal resp	-	-	-	-	-	-
C	-	-	-	-	1.899	0.009**
N	1.549	0.042*	1.53	0.040*	-	-
C:N ratio	-	-	-	-	-	-
PO_4^{3-}	1.611	0.034*	2.935	0.001***	2.292	0.002**
NH_4^+	3.779	0.001***	4.076	0.001***	1.742	0.020*
NO_3^-	2.148	0.004**	2.995	0.003**	2.849	0.001***

Table S11. Environmental factors associated with the fungal community compositional variation based on capscale model selection across myco-types (AM, EcM) and myco-type mixture (AE) with in tree diversity levels (B). Significant factors were stepwise selected by ordistep function in R. Abbreviation: **Basal resp= basal respiration,** Cmic= carbon microbial biomass, SWC= soil water content, C= carbon, N= nitrogen, NH4+ = Ammonium, NO_3^-= Nitrate, PO_4^{3-} = Phosphate, 1 (single species mixture), 2 (two species mixture) and 4 (four species mixture).

	AM1		**AM2**		**AM4**		**AE2**		**AE4**		**EcM1**		**EcM2**		**EcM4**	
	F	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**	**F**	**Pr(>F)**
pH	-	-	1.608	0.033*	1.88	0.007**	2.435	0.003**	2.359	0.001***	-	-	2.033	0.002**	1.707	0.003**
SWC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.377	0.001**
Cmic	1.802	0.040*	2.518	0.003**	-	-	-	-	-	-	1.916	0.014*	1.799	0.019*	1.718	0.008**
Basal resp	-	-	-	-	-	-	2.723	0.001**	2.538	0.001***	-	-	-	-	-	-
C	-	-	-	-	-	-	1.691	0.039*	-	-	-	-	-	-	-	-
N	-	-	-	-	-	-	2.621	0.002**	2.689	0.001***	-	-	-	-	-	-
C:N ratio	-	-	-	-	-	-	-	-	-	-	1.890	0.016*	-	-	-	-
PO_4^{3-}	1.847	0.017*	-	-	2.668	0.001***	1.591	0.039*	3.772	0.001***	1.693	0.048*	-	-	3.028	0.001***
NH_4^+	1.648	0.025*	-	-	1.974	0.001***	2.087	0.004**	-	-	1.665	0.031*	2.126	0.001***	2.888	0.001***
NO_3^-	-	-	1.927	0005**	-	-	2.965	0.001***	1.906	0.006**	1.797	0.042*	1.878	0.006**	-	-

Table S12. Effect of myco-type and myco-type mixture on fungal community specialization across tree diversity levels.

	AM_12	AM_14	AM_24	EcM_12	EcM_14	EcM_24	AMAE_12	AMAE_14	AMAE_22	AMAE_24	AMAE_42	AMAE_44	EcMAE_12	EcMAE_14	EcMAE_22	EcMAE_24	EcMAE_42	EcMAE_44
Specialist_AM1	0.0737	0.0535	0.0000	0.0000	0.0000	0.0000	0.1100	0.0969	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Specialist_AM2	0.0871	0.0000	0.0684	0.0000	0.0000	0.0000	0.0000	0.0000	0.1139	0.1163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Specialist_AM4	0.0000	0.0767	0.0795	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1176	0.1177	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Specialist_EcM1	0.0000	0.0000	0.0000	0.0811	0.0731	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0722	0.0573	0.0000	0.0000	0.0000	0.0000
Specialist_EcM2	0.0000	0.0000	0.0000	0.1023	0.0000	0.0672	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0953	0.0668	0.0000	0.0000
Specialist_EcM4	0.0000	0.0000	0.0000	0.0000	0.1339	0.1203	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1215	0.1041
Specialist_AE2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0980	0.0000	0.0914	0.0000	0.0699	0.0000	0.1371	0.0000	0.1455	0.0000	0.1277	0.0000
Specialist_AE4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0975	0.0000	0.1007	0.0000	0.0900	0.0000	0.1213	0.0000	0.1345	0.0000	0.1317
Generalist	0.2545	0.2210	0.2659	0.1877	0.1560	0.1999	0.2030	0.1774	0.2393	0.2095	0.2092	0.2279	0.1740	0.1480	0.1737	0.1740	0.1657	0.1929
Too_rare	0.5847	0.6487	0.5861	0.6289	0.6370	0.6126	0.5891	0.6282	0.5555	0.5736	0.6032	0.5644	0.6167	0.6734	0.5855	0.6248	0.5851	0.5713

CHAPTER 3

Temperate forest soil microbiomes and their assembly processes are modulated by the interplay of co-existing tree species identity, diversity and their mycorrhizal type

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Abstract

Recent studies have highlighted the significant role of tree species' mycorrhizal traits on forest soil microbial communities and their associated ecosystem functions. However, our understanding of how tree species richness in mono-mycorrhizal (arbuscular mycorrhiza (AM) or ectomycorrhiza (EcM)) or mixed-mycorrhizal (AM and EcM = AE) stands affects the rooting zone microbial community assembly processes remains limited.

We investigated this knowledge gap using the MyDiv tree diversity experiment, which comprises plantings of AM and EcM tree species and their mixture in one-, two-, and four-species plots. Soil microbiomes in the target tree rooting zone were analyzed using meta-barcoding of the fungal ITS2 and bacterial 16S V4 rRNA regions. We examined the effects of plot mycorrhizal type, tree species identity and richness on microbial diversity, community composition, and microbial community assembly processes.

We found that AM plots exhibited higher fungal richness compared to EcM and mixed mycorrhizal type (AE) plots, whereas tree species identity and diversity showed no significant impact on fungal and bacterial alpha diversity within mono and mixed mycorrhizal type plots. The soil fungal community composition was shaped by tree species identity, tree diversity, and plot mycorrhizal type, while bacterial community composition was only affected by tree species identity. EcM tree species significantly impacted both soil fungal and bacterial community compositions. Plot mycorrhizal type and tree species richness displayed interactive effects on the fungal and bacterial community composition, with AM and EcM plots displaying contrasting patterns as tree diversity increased. Our results suggest that both stochastic and deterministic processes shape microbial community assemblage in mono and mixed mycorrhizal type tree communities. The importance of deterministic processes decreases from AM to EcM plots primarily due to homogeneous selection, while stochastic processes increase, mainly due to dispersal limitation. Stochastic processes affected fungal and bacterial community assembly differently, through dispersal limitation and homogenous dispersal, respectively. In fungi, the core, intermediate and rare abundance fungal taxa were mainly controlled by both stochastic and deterministic processes whereas bacterial communities were dominantly shaped by stochastic processes.

These findings provide valuable insights into the role of tree species identity, diversity and mycorrhizal type mixture on the soil microbiome community composition and assembly processes, highlighting the differential impacts on core and rare microbial taxa. Understanding the balance between deterministic and stochastic processes can help forest ecosystem management by predicting microbial community responses to land-use and environmental changes and influencing ecosystem functions critical for ecosystem health and productivity.

Introduction

The soil microbiome is a complex and dynamic system of microbial communities and plays a fundamental role in nutrient cycling (Bahram *et al.* 2018), plant health (Compant *et al.* 2019), and overall ecosystem functioning (Bahram *et al.* 2020). Plant roots exert a profound influence on the diversity and composition of the rhizosphere microbiota through physical modification of the rooting zone, changing the nutrient dynamics, secretion of root exudates and signals to recruit beneficial organisms and deter pathogens (Prescott & Grayston 2013). Tree roots also engage in specific symbiotic relationships with mycorrhizal fungi (Phillips, Brzostek & Midgley 2013b; Pan *et al.* 2024), primarily with arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) fungi (Deng *et al.* 2023). Due to the high litter quality and stimulation of free-living decomposers by AM fungi through provision of carbon (Keller & Phillips 2019). forests dominated by AM tree species exhibit an inorganic nutrient economy, characterized by fast litter decomposition and enhanced carbon and nitrogen mineralization. In contrast, forests with EcM tree species generally maintain an organic nutrient economy, expressed in slow litter decomposition and reduced carbon and nitrogen turnover (Phillips, Brzostek & Midgley 2013b). These functional differences can shape microbial guild composition, including saprotrophs, pathogens, and mutualists (Averill, Turner & Finzi 2014; Tedersoo & Bahram 2019). For instance, ECM associations may suppress saprotrophic activity altering fungal interactions and carbon cycling, whereas AM systems may promote distinct microbial communities through rapid nutrient turnover (Steidinger *et al.* 2019). However, the relative importance of the fungal functional guild was found to be comparable between mono (EcM and AM) and mixed (AM and EcM) mycorrhizal types plots although distinct patterns were found among tree diversity levels in AM and EcM mono mycorrhizal stands (Haq *et al.* 2024). To date, there are contrasting reports of the effect of tree mycorrhizal types and their co-occurring tree

species on the forest soil microbiome composition (Nguyen *et al.* 2016b; Peay 2018; Singavarapu *et al.* 2022a).

Research in subtropical regions have revealed that the mycorrhizal type and diversity of co-existing tree species significantly influence fungal and bacterial diversity and community composition in forest soils (Chen *et al.* 2017; Singavarapu *et al.* 2023). However recent studies suggest that soil microbial diversity and composition may not directly correspond to tree diversity, as observed in subtropics for EcM tree species (Chen *et al.* 2019) and in tropical forest for AM tree species (Yamamura, Schwendenmann & Lear 2013). In subtropical ecosystems, community patterns specific to different mycorrhizal types and microbial taxa tend to converge as tree diversity increases (Singavarapu *et al.* 2022a). Contrastingly, temperate forest ecosystems consistently demonstrate a strong connection between AM and EcM tree species and soil fungal diversity, including phylogenetic richness and composition (Peay, Baraloto & Fine 2013; Nguyen *et al.* 2016b). Generally, higher tree diversity in temperate forests leads to increased soil microbial diversity (Peay, Baraloto & Fine 2013; Chen *et al.* 2017). Some studies suggest that soil microorganisms may be more influenced by specific traits of AM tree species rather than tree species richness alone (Scheibe *et al.* 2015; Tedersoo *et al.* 2016). Recent studies have shown that the richness of EcM and AM fungi in tree species increases with tree diversity, and AM tree species have been observed to host a higher diversity of fungal species in their roots (Heklau *et al.* 2021)) and rooting zone soils (Haq *et al.* 2024) compared to EcM tree species. Despite these findings, there remains a gap in our understanding of the individual and interactive effects of co-existing mycorrhizal types in mono-mycorrhizal (AM or EcM) or mixed-mycorrhizal (AM and EcM) tree stands, as well as the impact of tree diversity levels on soil microbial community composition and their assembly rules in temperate forest ecosystems.

The soil microbial community assembly rules are mainly a blend of stochastic and deterministic processes (Ofiţeru *et al.* 2010; Stegen *et al.* 2016). Deterministic processes can be attributed to either heterogeneous or homogeneous selection (Chesson 2000; Stegen *et al.* 2016) . Heterogeneous selection, also known as variable selection, arises when diverse environmental conditions like soil pH and soil moisture drive greater differences in community composition (Zhou & Ning 2017), while homogeneous selection occurs when consistent abiotic and biotic environmental conditions result in similar community composition (Zhou & Ning 2017). In contrast, stochastic processes are brought about by dispersal e.g., homogeneous dispersal,

ecological drift and dispersal limitation (Stegen *et al.* 2016), which underscore the significance of birth, death, colonization, extinction, and speciation in shaping microbial community composition and dynamics (Chave 2004). Homogenizing dispersal occurs when organisms are uniformly distributed across space, reducing diversity between locations (Zhou & Ning 2017; Lerch *et al.* 2023). Dispersal limitation refers to the inability of certain species to colonize new habitats due to physical barriers or environmental conditions (Zhou & Ning 2017; Liu *et al.* 2022b; Lerch *et al.* 2023), while drift involves random changes in species abundance over time due to chance events (Zhou & Ning 2017; Lerch *et al.* 2023). and deterministic processes influence the composition and abundance of microbial communities via their fitness (Li *et al.* 2019a), stochastic processes result in unpredictable fluctuations within the community (Tilman 2004). Though, understanding the governing principles of soil microbial community assembly is pivotal in unveiling their significance in forest ecosystems (Tilman 2004; Zhou & Ning 2017). there is a limited knowledge with regard to how mycorrhizal type of co-existing tree species as well as the overall tree diversity influence the microbial community assembly processes.

Furthermore, the relative abundance of a microbial taxa within a community might have a key role in influencing the processes that drive community assembly (Zhao *et al.* 2022; Guo & Gong 2023). Furthermore, the relative abundance of a microbial taxa within a community might have a key role in influencing the processes that drive community assembly (Zhou & Ning 2017; Zhao *et al.* 2022). Microbial taxa can broadly be classified into core, intermediate, and rare groups based on their prevalence and relative abundance across samples (Jiao & Lu 2020; Zheng *et al.* 2021) or based on phylogenetic bin abundances (Ning *et al.* 2020; Ma *et al.* 2022). Core taxa are consistently present and often dominant across habitats; they are believed to carry out essential ecosystem functions and stabilize microbial communities. Rare taxa, though low in their relative abundance, contribute disproportionately to functional redundancy, resilience, and long-term ecosystem adaptability, whereas intermediate taxa may shift between core and rare states depending on environmental conditions (Jiao & Lu 2020; Zheng *et al.* 2021; Wang *et al.* 2023). For instance, research on agricultural soils has shown that stochastic processes predominantly shape the assembly of abundant fungal subcommunities (68.6%), whereas rare fungal communities are primarily governed by deterministic processes (86.7%) (Jiao & Lu 2020). Similarly, in oil-contaminated soils (Jiao, Chen & Wei 2017) and grassland soils (Ji *et al.* 2020), stochastic processes were found to play a larger role in assembling rare taxa than abundant ones. These

findings suggest that the assembly processes of bacterial and fungal communities in forest soils may differ across core, intermediate, and rare microbial communities and could further be influenced by tree species mycorrhizal type and diversity levels (Powell *et al.* 2015).

In this study our research focuses on exploring the interplay of mycorrhizal type and tree diversity of co-existing AM, EcM and mixed (AM and EcM) mycorrhizal type tree species communities in shaping the rooting zone soil microbial community composition and assembly processes in temperate forest ecosystem. We used the MyDiv forest experimental platform (Ferlian *et al.* 2018), where tree species of two mycorrhizal types (mycorrhizal type), AM and EcM, were grown in plots with one-, two-, and four-species mixtures. These plots were either mono (AM or EcM) or mixed (AM and EcM = AE) in their mycorrhizal type. We used target tree-specific rooting zone soil microbiome (fungal and bacterial) data generated by targeting the fungal ITS2 and bacterial 16S V4 fragments using paired-end meta-barcoding approaches (Haq *et al.* 2024)to address the following specific research questions and hypotheses (Figure S2). 1; Due to the high-quality litter and associated microbial decomposers in AM as compared to EcM stands (Keller & Phillips 2019), we expected that microbial alpha diversity (H1a) rises as tree diversity increases, with stronger increases in AM than in EcM and mixed (AE) tree species stands, and (H1b) a stronger relationship for fungi as compared to bacteria due to the differential contribution of the symbiotic and saprotrophic communities (Bonfante & Genre 2010). 2; We expected increasing convergence of fungal and bacterial community composition of both mono and mixed mycorrhizal type plots with increasing tree diversity. 3; We expected that (H3a) stands dominated by AM tree species, that mainly depend on the co-existing decomposer microbial communities for decomposition and nutrient availability (Bonfante & Genre 2010; Smith & Read 2010) are more driven by deterministic processes as compared to those stands dominated by EcM tree species which mainly depend on EcM fungi for decomposing and accessing organic nutrients in resource-limited soils (Smith & Read 2010). Moreover, (H3b) forests containing a mix of AM and EcM tree species were expected to exhibit a balance between deterministic and stochastic processes. Finally, we hypothesized (H3c) that microbial community assembly becomes more stochastic with increasing tree species richness (Hubbell 2005), due to a greater niche variability for soil microbes. 4; We hypothesized that core microbes play a key role in regulating community assembly processes both in mono and mixed mycorrhizal type plots, with their contribution increasing with tree richness.

Materials and methods

Experimental Sites

The research site is located at the Experimental Research Station of Helmholtz Centre for Environmental Research – UFZ in Bad Lauchstädt, Germany (51°23' N, 11°53' E) at 115 m a.s.l. The climate exhibits mean annual temperatures of 8.8°C with an annual precipitation of 484 mm. The soil type is Haplic Chernozem formed from Loess with a pH range of 6.6-7.4 (Ferlian *et al.* 2018). The experiment was initiated in March 2015 on a former crop field, and comprises 80 plots measuring 11 × 11 m, each with a core area at the center measuring 8 x 8 m (Supplementary Figure 01). In each plot, 140 trees were planted spaced 1 m apart. To minimize weed intervention, all plots were covered with a water-permeable weed tarp. The selected trees, totaling ten different species, were divided into two groups with five species preferentially associated with AM and five with EcM fungi. They were planted in mono, two, and four-species mixtures (Ferlian *et al.* 2018) Additionally, the experimental design included treatments based on mycorrhizal types comprising communities of only AM or EcM trees, or their mixture (AE) (Ferlian *et al.* 2018). For a comprehensive overview of the study site, design, and methodologies, please refer to Haq et al., 2024 (Haq *et al.* 2024). This analysis serves as a continuation and further exploration of the findings presented in Haq et al., 2024 (Haq *et al.* 2024).

Soil Sampling and Processing

Soil sampling of the selected eight tree species took place in September 2021, and a detailed list of the chosen species, their mycorrhizal associations and their physiological characteristics is provided in Supplementary Table 01. The sampling focused on a target tree species which was surrounded by eight tree individuals situated at one corner of four tree species quartets. In this configuration, four soil cores, each 10 cm deep and 2 cm in diameter with a sampling radius of 20 cm from the target tree stem, were collected from the rooting zone soil of the target tree species (designated as Rhz1-Rhz4, Supplementary Figure 01) and then pooled together. The pooled soil samples were subsequently sieved using a 2 mm sieve and transported under cool conditions to the field lab. All samples were then transferred to the central lab and frozen at -20°C before conducting soil DNA analyses.

DNA Extraction, Sequencing, and data processing

DNA extraction from soil samples was conducted utilizing the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, United States) following the manufacturer protocol. DNA concentrations were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and adjusted to a concentration range of 10-15 ng/µl. For the preparation of bacterial and fungal amplicon libraries we followed established protocols (Singavarapu *et al.* 2022a). Specifically, the V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 515f and 806r (Caporaso *et al.* 2011), incorporating Illumina adapter sequence overhangs. Amplification of the ITS2 rDNA region for fungi employed a semi-nested PCR approach, initially using the primer combination ITS1F and ITS4 (Gardes & Bruns 1993), followed by the primer pair fITS7 and ITS4, which included Illumina adapter sequences (Ihrmark *et al.* 2012). Following amplification, the amplicon libraries underwent purification with Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT Indices were added to both ends of the bacterial and fungal fragments during indexing PCR. Subsequently, the indexed products were purified once more with AMPure beads and quantified with Qubit fluorometers (Thermofisher Scientific, Germany) utilizing the dsDNA high sensitivity assay. The amplicon libraries were equimolarly pooled to a final concentration of 4 nM each for both fungi and bacteria. The fungal and bacterial libraries were then combined in a 1:1 ratio to form the final library, which underwent paired-end sequencing of 2 x 300 bp on an Illumina MiSeq platform (Illumina, San Diego, CA, United States) employing the MiSeq Reagent Kit v3 at the Department of Environmental Microbiology, UFZ, Leipzig, Germany.

Bioinformatic analysis was conducted to filter high-quality reads from the raw data generated by the Illumina MiSeq Sequencing platform, employing the Quantitative Insights into Microbial Ecology - QIIME 2.2020.2 software (Bolyen *et al.* 2019). The demultiplexing of forward and reverse reads based on index combinations, primer sequence trimming, was done using Cutadapt (Martin, 2011) and subsequent denoising and clustering of reads into Amplicon Sequence Variants (ASVs) was done utilizing DADA2 (Callahan *et al.* 2016). For bacterial 16S, ASVs taxonomy assignment was accomplished using the q2-feature-classifier (Bokulich *et al.* 2018a) in conjunction with the classify sklearn naive Bayes taxonomy classifier, utilizing the silva-132-99-

515-806-nb-classifier reference database. Regarding the fungal ITS dataset analysis employed the q2-ITSxpress Qiime2 plugin (Rivers *et al.* 2018) to identify and trim ITS2 fungal sequences followed by denoising and ASV clustering using DADA2. Taxonomy assignment for fungal ITS ASVs utilized the q2-feature-classifier (Bokulich *et al.* 2018a) in tandem with the classify-sklearn naive Bayes taxonomy classifier and the unite-ver8-99-classifier-04.02.2020 reference database. The fungal and bacterial phylogenetic tree was inferred using the q2-phylogeny plugin in QIIME2, which employs MAFFT for sequence alignment and FastTree for Maximum Likelihood tree construction. The tree was midpoint-rooted to ensure a robust and unbiased phylogenetic framework.

Statistical Analysis

The respective fungal and bacterial ASV matrices, taxonomic tables, and representative sequences were imported into R (version 4.3.2) using the "phyloseq" package (McMurdie & Holmes 2013) for further statistical analysis. The fungal and bacterial ASV matrices were filtered and rarefied to ensure uniform sequencing depth, with fungal 5,827 and bacterial 11,264 reads per sample. Alpha diversity represented by shannon and observed richness and was assessed using the "microbiome" package (version 2.0.1) (Lahti *et al.* 2017). Linear mixed effect models were used to evaluate the impact of mono and mixed myco-type, tree species and tree diversity as a fixed effect with alpha diversity as the response variable. Neighborhood composition was included as a random effect, employing the *lmerTest* function (Kuznetsova, Brockhoff & Christensen 2017). We used the emtrends procedure from the "emmeans" package to estimate unbiased marginal means (EMMs) and confidence intervals for the slopes of every single target tree species in mono and mixed myco-type. Slopes were considered significant if their confidence intervals did not overlap with zero. The effects of mono (AM or EcM) and mixed (AE = AM and EcM) myco-type plots, tree diversity, and tree species identity on the fungal and bacterial community compositions (based on Bray-Curtis distance dissimilarity) were tested using PERMANOVA using the *adonis2* function of the "vegan" package (Oksanen *et al.* 2013). We also employed Procrustes analysis to assess congruence in fungal and bacterial community composition across mono and mixed myco-type plots within each tree diversity level.

Assessment of Community Assembly Processes

The comparative significance of various community assembly processes of soil microbial community assembly in the target tree species rooting zone of AM, EcM and mixed (AE) mycorrhizal type plots, focusing mainly on the two and four-species mixtures, was assessed for the overall fungal, bacterial and mycorrhizal fungal communities employing the “iCAMP” package. Distinguished from other frameworks relying on null models, “iCAMP” as the capability to calculate ecological processes for specific taxonomic groups ("bins") instead of analyzing the entire community (Ning *et al.* 2020). The objective of phylogenetic binning is to obtain groups adequate within-group phylogenetic signal. These taxonomic groups typically consisting of 12 ~ 48 ASVs, are categorized based on phylogenetic relationships enhancing the accuracy of subsequent bin-level analyses for community assembly processes (Ning *et al.* 2020). This approach employs an absolute abundance of ASV table alongside a rooted phylogenetic tree to assess the relative significance of five community assembly processes. These processes are categorized as either deterministic, including heterogeneous and homogeneous selection, or stochastic, encompassing homogeneous dispersal, dispersal limitation and ecological drift. The phylogenetic bins were determined based on the phylogenetic signal of nitrogen concentration, using a bin size of 24 and 48 OTUs and a phylogenetic distance threshold of 0.05 for both fungi and bacteria respectively (Figure S3). The beta net relatedness index (bNRI) and the Raup–Crick metric (RC) were calculated using 1,000 randomizations of taxa across the phylogenetic tree tips, with both metrics employing the described threshold to classify community assembly processes (Figure S4) (Ning *et al.* 2020). Differences in community assembly processes between mono and mixed mycorrhizal types, along with tree species richness levels were assessed using the Wilcoxon rank sum test.

To investigate the association between community assembly processes and microbial rarity, fungal and bacterial communities were categorized into core, intermediate, and rare taxa based on the relative abundance of their phylogenetic bins. Unlike the classical abundance based arbitrary classification of the fungal and bacterial communities into core, intermediate and rare communities (Zheng *et al.* 2021), were divided into three groups: "we used the phylogenetic bin based null model in iCAMP (Ning *et al.* 2020) to define these microbial group as (core communities" for bins with relative abundances exceeding 0.85%, "rare communities" for bins with relative abundances

below 0.50%, and "intermediate communities" for bins that fell between these thresholds (Ma *et al.* 2022). We determined the proportion of community assembly processes within the three microbial sub-communities in relation to the overall ecological processes. By analyzing the bin contribution to each process data allowed us to determine the dominant process in each bin. In this way, we assessed the relative impact of deterministic processes (heterogeneous and homogeneous selection) and stochastic processes (homogeneous dispersal, dispersal limitation, and ecological drift) within these sub-communities. Spearman's rank correlation was used to examine the association among the relative impact of different community assembly processes influencing each bin and its relative abundance.

Results

Microbial alpha diversity

The linear mixed effect model analysis of mono (AM or EcM) and mixed (AE) mycorrhizal type plots revealed no significant impact of tree species richness on fungal and bacterial alpha diversity. However, AM plots displayed a higher fungal richness compared to EcM and AE plots (Figure 1a, c, Figure S5, Table S2). There were also no significant effects of tree diversity at tree species levels (Figure 1b). The same was true for fungal Shannon diversity (Figure S5, Table S3). Similarly, no notable differences were detected for bacterial richness and Shannon diversity for mono and mixed mycorrhizal type plots, with the exception of Betula pendula in pure EcM tree stands, which displayed significantly increasing bacterial Shannon diversity with increasing tree richness (Figure 1d, Figure S5, Table S3).

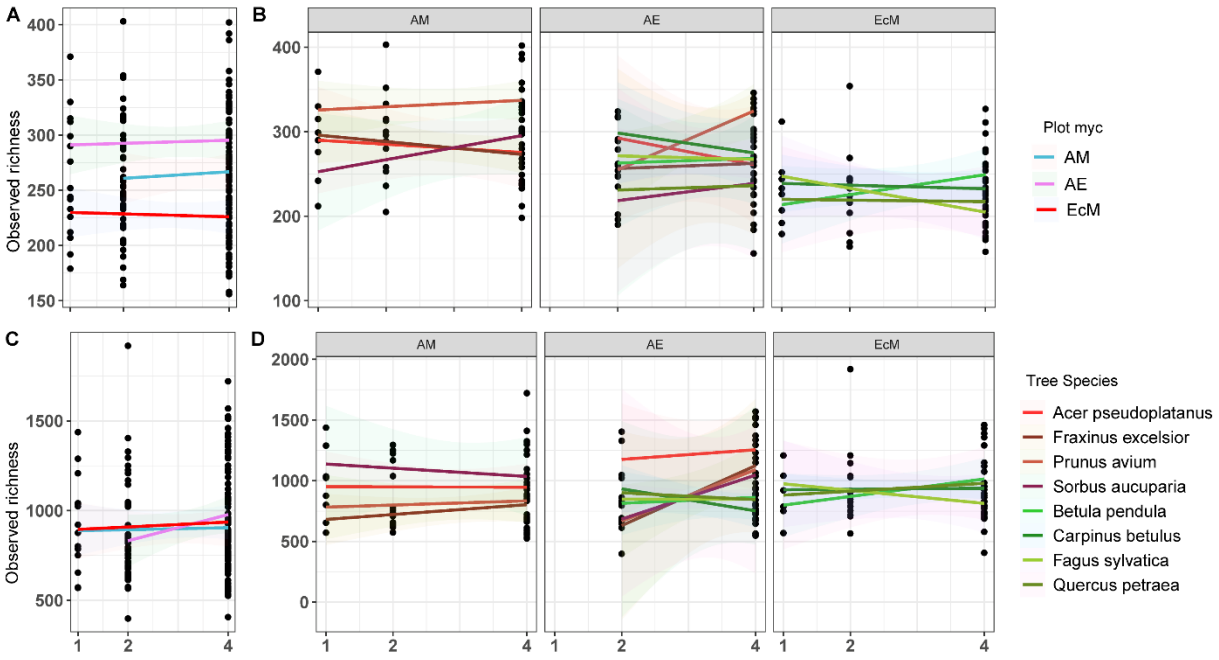

Figure 1. Linear mixed model effects of mono (AM and EcM) and mixed (AE) mycorrhizal type plots tree species with increasing tree diversity (1, monoculture; 2, 2sp mixture; 4, 4sp mixture) on fungal (A) and bacterial (C) alpha diversity, represented via observed richness. Linear mixed model effects of tree species and tree diversity by plot myco-type and tree species on fungal (B) and bacterial (D) observed richness.

Microbial community composition

The PERMANOVA based test of the effect of tree species, tree diversity and plot mycorrhizal type revealed significant effects of tree species (explained variance = 32.2%), tree diversity (explained variance = 1.2%), and plot mycorrhizal type (explained variance = 8.2%) on fungal community composition but no interactive effects (Table S4). In contrast, in bacterial communities, a significant effect was observed only for tree species (explained variance = 22.3%) (Table S4). Further PERMANOVA based test on the effects of tree species and treed diversity at the mono and mixed plot mycorrhizal type levels revealed significant effects of tree species identity both for AM and EcM plots for fungal communities and only for EcM plots for bacterial communities. However, in AM plot the bacterial communities were only marginally significantly affected by tree species identity (Table S5).

Concordance of fungal and bacterial communities

Comparison of bacterial and fungal community concordance in mono and mixed mycorrhizal type plots across tree diversity levels revealed interactive effects of plot mycorrhizal type and tree species richness on community correlations. The AM and EcM mycorrhizal type plots showed contrasting patterns of fungal and bacterial community correspondence with increasing tree diversity. Unlike in AM plots, EcM and the mixed AE mycorrhizal type plots the similarity of fungal and bacterial community composition patterns increases with increasing tree diversity (Table 1, Figure. S6). This means that the plots with co-existing tree species mixtures of AM and EcM or different EcM tree species lead to more congruent fungal and bacterial communities than plots with different AM tree species.

Table 1. Procrustes analysis comparing fungal and bacterial communities of mono (AM, EcM) and mixed (AE) mycorrhizal type plots at different tree diversity levels (1, 2 and 4 species). Procrustes sum of squares (m^2), i.e. the sum of squared distances between paired points of both communities, Correlation in a symmetric Procrustes rotation (C), significance (*p*).

	Overall	AM	AE	EcM
1 species	m^2=0.960, C = 0.199, *p* = 0.763	m^2 =0.402, C=0.772 ***p* = 0.017**		m^2 =0.961, C=0.578 *p* = 0.176
2 species	m^2 =0.980, C= 0.141 *p* = 0.532	m^2 =0.964, C=0.187 *p* = 0.881	m^2 =0.926, C=0.271 *p* = 0.047	m^2 =0.840, C=0.399 ***p* = 0.001**
4 species	m^2 =0.928, C=0.267 ***p* = 0.008**	m^2 =0.992, C=0.087 *p* = 0.927	m^2 =0.774, C=0.475 ***p* = 0.001**	m^2 =0.803, C=0.443 ***p* = 0.003**
2 & 4 sp	m^2=0.960, C=0.199 *p* = 0.756	m^2 =0.995, C=0.064 *p* = 0.969	m^2 =0.877, C=0.350 ***p* = 0.004**	m^2 =0.909, C=0.301 ***p* = 0.018**
All plots	m^2 =0.958, C=0.203 ***p* = 0.004**	m^2 =0.995, C=0.064 *p* = 0.969	m^2 =0.877, C=0.350 ***p* = 0.004**	m^2 =0.912, C=0.296 ***p* = 0.022**

Microbial community assembly processes

Analysis of the community assembly processes of fungal and bacterial communities of AM, EcM and mixed (AE) mycorrhizal type plots was tested focusing on the two- and four-tree species stands, indicated distinctive contribution of stochastic and deterministic processes in shaping the fungal and bacterial community assembly (Figure 2). The assembly patterns of fungal communities

were mainly driven by stochastic processes, with ecological drift contributing most, followed by dispersal limitation. Deterministic processes in fungal community assembly were primarily governed by homogeneous selection (26.6%, 21.5, 16.5%), which showed contrasting pattern to the increasing role of dispersal limitation (20.7%, 30.4%, 35.6%), as we compare AM with AE and EcM tree species stands (Figure 2a, b). Further analysis of the assembly rules of AM and EcM fungal communities also showed comparable assembly processes as the overall fungal community, where stochastic processes playing a major role regardless of the plot mycorrhizal type and tree species identity (Figure S8).

In contrast, bacterial communities were primarily driven by both stochastic and deterministic processes. The stochastic processes were mainly driven by ecological drift and homogenous dispersal, while the deterministic processes were solely contributed by homogenous selection. The contribution of homogeneous selection (40.2%, 40.3%, 42.8%), increased from AM to AE and EcM tree species stands, while the opposite trend was observed for homogeneous dispersal (26.1%, 20.2%, 19.5%) (Figure 2c, d). These contributions at the plot mycorrhizal type level remains consistent at the tree species richness levels (Figure S7). Additionally, pairwise comparison of the contributions of the different ecological processes at the plot mycorrhizal type level revealed significant differences in stochastic processes (dispersal limitation) in fungal communities between AM and EcM plots and deterministic processes (homogenous selection) in bacterial communities between AM and EcM, and EcM and AE plots (Table S6). However, we found tree species based significant pairwise correlation only within the mixed mycorrhizal type plots and the tree species pair-based variations on the contribution of the different ecological processes is prominent for bacterial communities than fungal communities (Table S7).

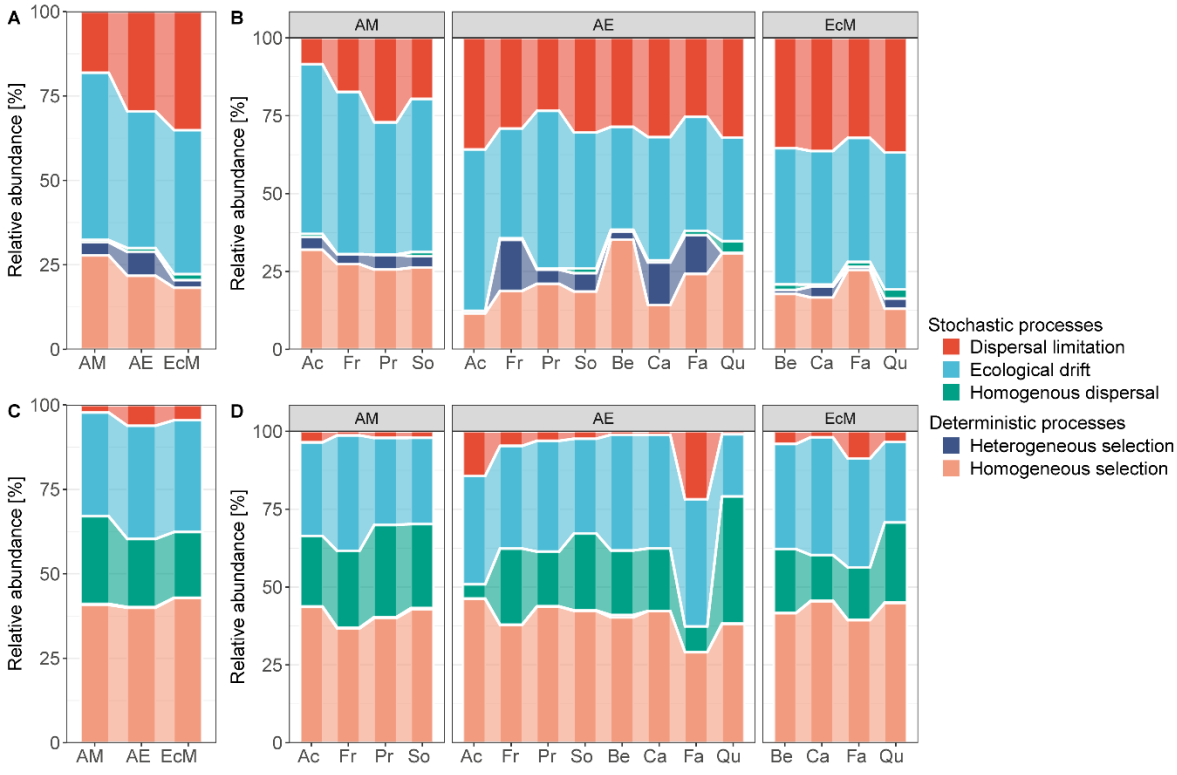

Figure 2. Relative importance of different ecological processes to soil fungal (A, B) and bacterial (C, D) communities across mono (AM, EcM) and mixed myco-type (AE) plots. Tree species abbreviation; Ac, *Acer pseudoplatanus;* Fr, *Fraxinus excelsior*; Pr, *Prunus avium*; So, *Sorbus aucuparia*; Be; *Betula pendula*, Ca; *Carpinus betulus*; Fa, *Fagus sylvatica*; Qu, *Quercus petraea*.

Relationships between microbial diversity and community composition with community assembly processes

The analysis of the contribution of different ecological processes with increasing fungal alpha diversity of AM, EcM and mixed mycorrhizal type multi species stands revealed contrasting effects. In AE plots, with increasing fungal alpha diversity the importance of deterministic processes decreases, while the importance of stochastic processes increases. AM and EcM plots, however, showed opposing patterns (Figure 3a, b). On the other hand, in EcM plots with increasing fungal beta-diversity the importance of deterministic processes decreases and that of stochastic processes increases significantly, while no significant relationship was found in AM and AE plots (Figure 3c, d). The relationships between bacterial alpha diversity and ecological processes, however, were much more consistent in all three plot mycorrhizal types, where with increasing bacterial alpha diversity the importance of deterministic processes increases and the contribution

of stochastic processes decreases significantly (Figure 3e, f). Contrasting relationships were observed for bacterial beta-diversity (Figure. 3g, h).

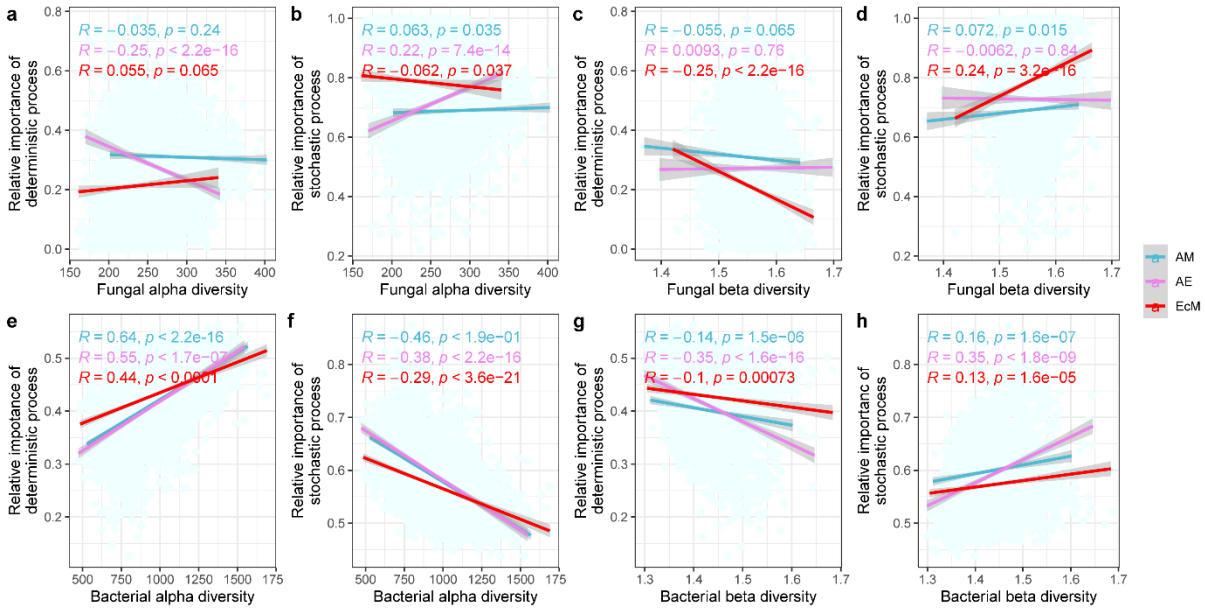

Figure 3. Relative importance of deterministic and stochastic processes as a function of fungal alpha (A, B) and beta (C, D), and bacterial alpha (E, F) and beta diversity (G, H) within mono (AM or EcM) and mixed (AE) mycorrhizal type plots. Spearman correlation models were employed to calculate R^2 and p-values of the linear fitted lines.

Association among microbial subcommunities abundance and community assembly processes

Assessment of the effects of community assembly processes on distinct groups of microbial communities encompassing core, intermediate, and rare microbes in the two- and four tree species plots of mono and mixed mycorrhizal type plots indicated that stochastic processes dominantly influenced core fungal communities by ecological drift (98.4%) and dispersal limitation (96.3%), whereas rare fungal communities were driven by deterministic processes, specifically by homogenous selection (51.1%) (Figure 4a). In contrast, in all classes of bacterial communities, stochastic processes were dominant. Compared to fungal communities, bacterial assemblies were driven by a much stronger contribution of homogenous dispersal (90.0%, 79.5%) (Figure 4b).

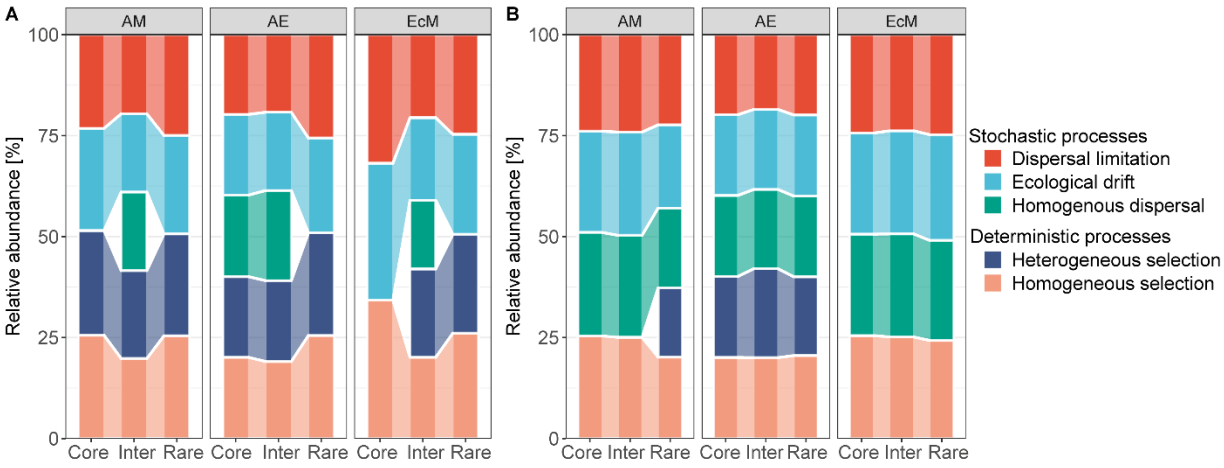

Figure 4. Relative importance of different ecological processes to core fungal (A) and bacterial (B) subcommunity assemblies in mono (AM, EcM) and mixed myco-type (AE) plots. Core = core taxa, Inter = intermediate taxa and Rare = rare taxa.

Furthermore, we determined the dominant processes that drove the relative abundance of each bin of fungal and bacterial communities. For fungal communities, both deterministic and stochastic processes with the exception of dispersal limitation, revealed a significant relationship ($p < 0.05$) with bin abundance in both mono and mixed mycorrhizal type plots (Figure 5a). This suggests that core fungal communities in AM and EcM plots are selectively shaped by stochastic processes, particularly ecological drift and homogenous dispersal. In contrast, mixed mycorrhizal type plots are more influenced by deterministic processes, with homogenous selection playing a key role (Figure 5a). For bacterial communities, both stochastic and deterministic processes were significantly affecting the abundance of bins ($p < 0.05$). There was a tendency of a stronger influence on the core community compared to those composed of intermediate or rare taxa, both in mono and mixed mycorrhizal type plots (Figure 5b).

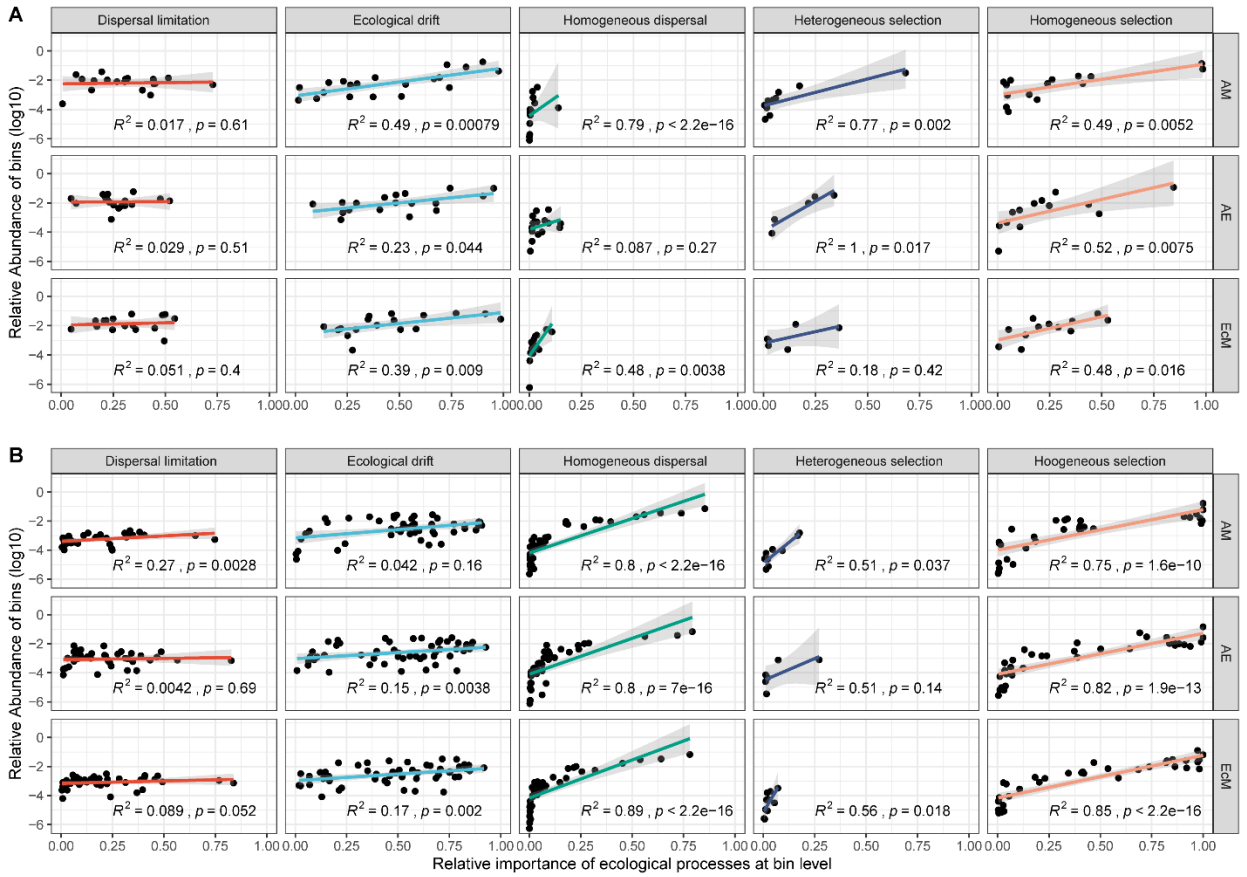

Figure 5. The relationship between relative abundance of taxon bins as a function of the ecological assembly processes driving them in mono (AM, EcM) and mixed myco-type (AE) plots in fungal (A) and bacterial (B) communities. The horizontal axis represents the relative importance of assembly processes per bin's abundance, while the vertical axis depicts the log10-transformed relative abundance of bins

Discussion

In this study, we analyzed the interplay of target tree mycorrhizal type (AM or EcM), mycorrhizal type mixture (mono or mixed), tree species identity, and tree diversity in shaping the rooting zone soil microbiome diversity and community composition and evaluated the driving microbial community assembly processes. Our results indicate that AM plots had higher fungal richness compared to EcM and mixed mycorrhizal type (AE) plots, with AE plots showing intermediate richness, but no effect plot mycorrhizal type on bacterial alpha diversity. We found no major effect of tree diversity on both fungal and bacterial alpha diversity of the target tree species both in mono and mixed mycorrhizal plots. The fungal community composition was significantly affected by tree species, tree diversity, and plot mycorrhizal type, whereas, bacterial communities were

affected only by tree species identity. Interestingly interactive effects of plot mycorrhizal type and tree diversity on fungal and bacterial community correlations were observed. In contrast to AM plots, in EcM and AE plots the congruence of fungal and bacterial community composition increased with increasing tree diversity. The fungal and bacterial community assembly processes were found to be predominantly driven by stochastic processes, where our results also revealed distinct contribution of dispersal limitation and homogenous dispersal for fungal and bacterial community assembly processes. Remarkably we found plot mycorrhizal type and microbial relative abundance related patterns of fungal and bacterial community assembly processes, where relative importance of stochastic and deterministic processes differs in relation to the fungal and bacterial alpha- and beta-diversity patterns in AM, EcM and mixed mycorrhizal type plots.

Microbial alpha diversity – differential effect of plot mycorrhizal type on fungal diversity

Corresponding with our hypothesis, AM tree species plots showed higher fungal richness as compared to EcM plots, where the mixed mycorrhizal types exhibited intermediate fungal richness. This finding corroborates with previous reports, which has demonstrated a stronger alpha diversity effect of AM tree species in subtropical (Singavarapu *et al.* 2022a) and mixed central European forest soils (Lang, Seven & Polle 2011). AM and ECM trees exhibit distinct root exudation patterns and resource partitioning strategies in their rhizospheres, driven by differences in nutrient acquisition and microbial interactions. Though both AM and EcM trees underscore the critical role of mycorrhizal symbioses in bridging the rooting zone plant-microbe interactions, AM plants primarily influence their microbiomes via chemical signalling and nutrient-focused partnerships, while EM plants rely on structural filtering and environmental resilience (Phillips & Fahey 2006). Consequently, the observed pattern is likely to be attributed to the host specificity of EcM trees and the dual function of EcM fungi as mycorrhizal partners and saprotrophs, as compare to AM trees which depend on both mycorrhizal fungal partners for nutrient uptake and saprotrophic fungi for nutrient cycling in their rooting zones (Phillips, Brzostek & Midgley 2013b). Thus, co-existence of AM and EcM trees could lead to intermediate fungal diversity as the saprophytic function could be partly compensated by the EcM fungal partners of the co-existing EcM tree species in contrast to AM plots. Moreover, our findings showed no significant effects of tree species richness on fungal richness in both mono and mixed mycorrhizal type plots. This could be due to a shift in the community composition shaped by co-existing tree species while

maintaining the fungal richness. The absence of tree species richness effects within plot mycorrhizal type on bacterial alpha diversity in our study, however, is in line with finding from subtropical (Singavarapu *et al.* 2022a) and tropical tree experimental sites (Yamamura, Schwendenmann & Lear 2013) who reported no significant differences in bacterial richness among AM and EcM plots across tree diversity levels. This could be due to the dominance of stochastic processes in community assembly, as indicated by our findings, which results in lower bacterial diversity. This occurs because the impact of specific environmental factors is diminished, leading to less organized and more uniform microbial communities (Xun *et al.* 2019; Aguilar & Sommaruga 2020).

Our finding also revealed that Betula pendula from EcM stand exhibited a positive correlation between bacterial diversity and tree species richness. This suggests that the interaction between tree diversity and soil microbial communities may not be uniform across all tree species, but rather depends on the specific traits of individual tree species (Prescott & Grayston 2013). For instance, *Betula pendula* is known as a fast-growing pioneer species that release substantial amounts of carbon into the rhizosphere due to higher rate of root exudation compared to slow-growing EcM tree species (Sell *et al.* 2024). Our results contradict previous literatures that reported a positive relationship between tree species richness of AM and/or EcM tree species with microbial diversity (Peay, Baraloto & Fine 2013; Hiiesalu *et al.* 2014; Tedersoo *et al.* 2016). This discrepancy may stem from field-based tree diversity gradients that include covarying factors with plant diversity or unrelated factors influencing soil microorganisms (Waldrop *et al.* 2006). Future studies should incorporate higher tree species gradients to better observe this relationship. The non-significant relationship between tree species and microbial diversity suggests that variation in resource availability might be low and independent of tree diversity levels in our study sites.

Microbial community composition– effects of tree mycorrhizal type, species identity and diversity

The fungal and bacterial community composition was found to be mainly influenced by tree species identity followed by mycorrhizal type and tree diversity effects in the fungal community. This strong influence of tree species identity on the microbial composition is mainly due to their differences in their litter, mycorrhizal fungal partners, root traits and exudates they release into the

rhizosphere (De Vries *et al.* 2012; Prescott & Grayston 2013). The effect of tree mycorrhizal type and tree diversity on the fungal community composition agrees with previous studies in subtropics (Eagar *et al.* 2022b; Singavarapu *et al.* 2022b). Additionally, we observed a significant impact of AM tree species identity on the fungal community and EcM tree species identity on both fungal and bacterial community compositions, with no significant contribution of tree species richness. This emphasizes previous studies suggesting the impact of tree species identity (Weißbecker *et al.* 2018b) and genotype identity (Karliński, Ravnskov & Rudawska 2020), on the rhizosphere soil microbial composition through their effect on soil chemistry and resource availability (Urbanová, Šnajdr & Baldrian 2015; Wu & Yu 2019). In diverse tree species plots of mono and mixed mycorrhizal type plant communities, however, plants interact with their neighbours in their rooting zones. There, they share the soil microbial pool, and interactively recruit and enrich their rooting zone microbial communities. This process potentially results in more shared or core taxa and consequently converging microbial communities with increasing tree diversity (Compant *et al.* 2019; Singavarapu *et al.* 2024).

Mycorrhizal type and tree diversity effects on the concordance of fungal and bacterial communities

Comparative analysis of the correlation of fungal and bacterial communities in mono mycorrhizal type plots revealed distinct patterns. In EcM plots, the correspondence between fungal and bacterial communities increased with higher tree diversity, whereas this relationship was weaker in AM plots. This difference arises because AM fungi, which are generalists, tend to promote more diverse microbial communities in the rhizosphere soil compared to the specialist EcM fungi. For instance, AM fungi (e.g., *Glomeraceae*) correlated weakly with bacterial taxa like *Acidobacteria* (Lahrach et al. 2024), while EcM fungi (e.g., *Cortinarius*) showed stronger links with bacteria like *Proteobacteria* (Guennoc et al., 2018). This suggests that co-existence of different AM tree species may exert differential influences on microbial community dynamics as compared to EcM trees with increasing tree diversity, resulting in lower Procrustes correlations in AM tree plots. Conversely, in mixed mycorrhizal type plots containing both AM and EcM tree species, the similarity between fungal and bacterial community composition patterns increased with increasing tree species richness. For instance, EcM-associated fungi like *Russula* and *Tomentella* and

bacterial taxa such as *Firmicutes* and Actinobacteria were found to converge in their community structures with increasing tree diversity (Singavarapu *et al.* 2022b). This implies that in mixed mycorrhizal type plots higher tree diversity fosters greater intra-kingdom and cross-kingdom convergence or similarity in microbial community dynamics within these plots. The variation in the observed fungal and bacterial community concordance in mono and mixed mycorrhizal type stands provide valuable insight into how mycorrhizal preference influences tree-tree interactions and co-existence in forest ecosystems.

Drivers of fungal and bacterial community assembly processes

Community assembly processes play a crucial role in shaping microbial community in a given ecosystem (Li & Hu 2021; Liu *et al.* 2022a). Our result indicated that fungal and bacterial community assembly processes were primarily driven by stochastic processes but with differential contribution of dispersal limitation in fungi and homogenous dispersal in bacteria. This aligns with previous findings indicating that the distribution patterns of microbial communities are influenced by assembly processes, with bacteria and fungi exhibiting distinct response patterns (Wang *et al.* 2023). However, the dominance of homogeneous dispersal in bacterial communities' contrasts with studies from agroecosystems, where deterministic processes often play a stronger role (Liu *et al.* 2020; Liu *et al.* 2021c; Xu *et al.* 2022). This difference may reflect the more stable environmental conditions and weaker anthropogenic filtering in temperate forests as compared to agricultural systems. The observed relatively high contribution of dispersal limitation in fungi as compared to bacteria could be due to the fact that fungi despite having spore-dispersal mechanisms, are often constrained by larger size, habitat specificity, and limited spore viability over long distances, leading to higher dispersal limitation (Chen *et al.* 2020; Wang *et al.* 2020). In contrast, Bacteria's small size and reliance on diverse vectors, such as wind, water, and animals, facilitate their widespread and uniform distribution across similar environments (Powell *et al.* 2015; Luan *et al.* 2020; Zhang *et al.* 2021). leading to homogenous dispersal and selection processes. Additionally, fungal communities showed a small but notable influence of heterogeneous selection which was absent in bacterial communities. This reflects the ecological specialization of fungi and their stronger interactions with environmental factors, such as soil chemistry and host species which create selective pressures (Wang *et al.* 2020). Conversely, Bacteria being more generalist and versatile in nutrient use may experience less selective pressure

in similar environments leading to the observed absence of contribution of heterogenous selection in their assembly processes (Liao *et al.* 2016).

Interestingly, we found that the importance of stochastic and deterministic processes in community assembly differs between fungal and bacterial alpha- and beta diversity patterns in AM, EcM and mixed mycorrhizal type plots. The contribution of stochastic and deterministic processes was significantly impacted by increasing bacterial alpha- and beta-diversity regardless of the plot mycorrhizal type. However, their importance was significantly affected by fungal alpha diversity in the mixed AE plots and by fungal beta-diversity in EcM plots. Generally, high fungal diversity is recognized for mitigating resource competition among individual taxa by increasing niche overlap and functional redundancy. This allows stochastic processes, such as random dispersal and ecological drift, to have a greater influence on the fungal diversity compared to deterministic mechanisms (Jiang & Patel 2008; Dumbrell *et al.* 2010). The observed negative relationship between fungal α-diversity and deterministic processes, particularly in mixed-species plots, suggests that the co-existing AM and ECM tree species provide a wide range of resources for diverse fungal communities. This intern reduces the impact of environmental filtering as niche saturation increases, ultimately amplifying priority effects (Fukami 2015).

Association among relative abundance of microbial bins and community assembly processes

Our analysis of community assembly processes of core, intermediate and rare taxa revealed distinct patterns, where the core microbial communities were predominantly influenced by stochastic processes while rare fungal communities were more driven by deterministic processes. This finding aligns with the previous studies that reported core taxa are often influenced by environmental constraints (Mohapatra *et al.* 2023), whereas rare taxa may face challenges in competing for limited resources, leading to the prevalence of deterministic processes in their community assembly (Zhu *et al.* 2023). Due to their higher relative abundance in the community, core taxa are less susceptible to dispersal constraints. In contrast rare taxa which exhibit low abundance, limited dispersal capacity, and heterogeneous distribution across mono and mixed

mycorrhizal type rendering them more susceptible to environmental filtering (Mohapatra *et al.* 2023).

To the best of our knowledge, our findings for the first time revealed that deterministic processes strongly impact rare taxa, whereas stochastic processes, and here mainly dispersal limitation, exhibit a larger tendency in regulating core taxa in temperate forest ecosystems. Comparative studies conducted in other terrestrial ecosystems, such as orchards (Zhao *et al.* 2022) and high-latitude wetland soils (Ma *et al.* 2022) indicated similar patterns, although some studies highlighted the significant influence of homogeneous selection on rare taxa (Jiao & Lu 2020; Zheng *et al.* 2021). The varying niche breadths of rare taxa across studies may account for these discrepancies. It can be argued that the narrow niche breadths of rare microbial taxa make them more susceptible to the effects of homogeneous selection (Jiao & Lu 2020).

Conclusion

Our results indicated that the fungal community composition was significantly influenced by tree species, tree diversity, and the plot mycorrhizal type, while the bacterial communities were affected mainly by tree species identity. Notably, interactive effects between plot mycorrhizal type and tree diversity were observed, impacting correlations between fungal and bacterial communities. Specifically, in ectomycorrhizal (EcM) and mixed mycorrhizal (AE) plots, the congruence between fungal and bacterial community composition increased with tree diversity, whereas this pattern was absent in arbuscular mycorrhizal (AM) plots. Our results also demonstrated that fungal and bacterial community assembly processes primarily differed in stochastic processes, mainly by the contribution of dispersal limitation and homogeneous dispersal. Moreover, our research reveals that core microbial taxa are more influenced by stochastic processes, primarily ecological drift, whereas rare taxa are more affected by dispersal limitation. By identifying the predominant ecological processes governing fungal and bacterial diversity and community assembly in mono and mixed mycorrhizal type plots, we have gained valuable insights into the mechanisms driving microbial diversity, community assembly and its implication in ecosystem functioning in diverse forest ecosystems. Understanding how different ecological processes contribute to shaping rooting zone soil microbial communities of co-existing tree species with the same or different mycorrhizal preference provides crucial information for

improving the accuracy of biodiversity indicators, assessing ecosystem function like nutrient cycling and decomposition as well as developing resilience-based management strategies that can adapt to future changes and uncertainties.

Appendix

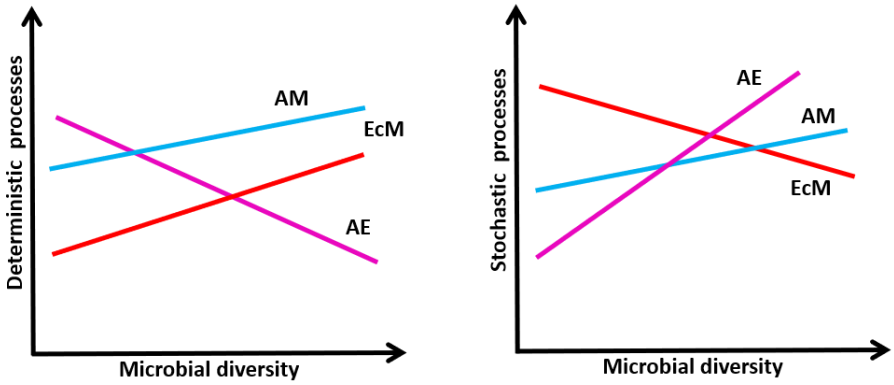

Figure S2. A conceptual figure depicting deterministic and stochastic processes on the y-axis as a function of microbial diversity on the x-axis, separated by mono and mixed mycorrhizal type tree communities. Different colors distinguish between mono (AM, EcM) and mixed (AE) plots.

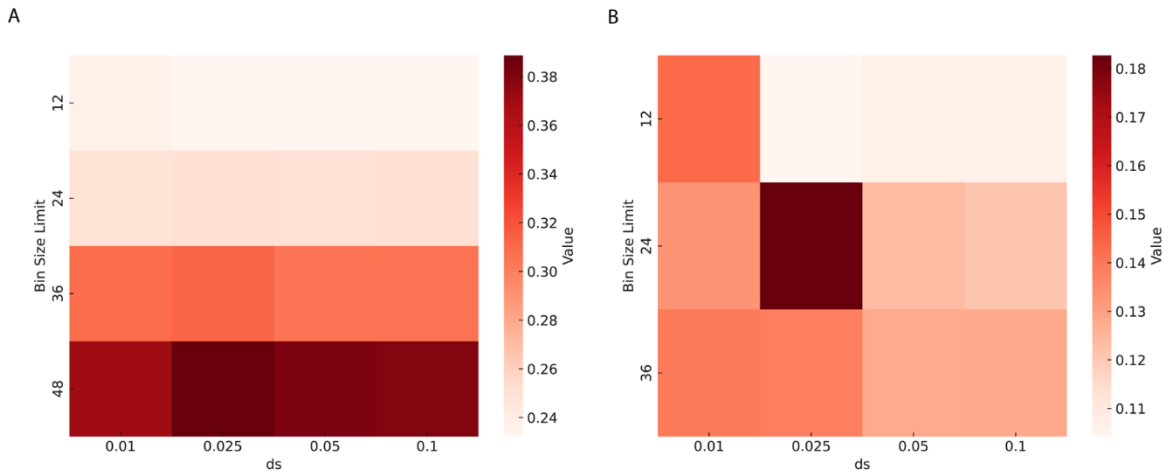

Figure S3: The phylogenetic signal is determined based on N concentration. The "ds" represents the phylogenetic distance, while "bin.size.limit" indicates the maximum number of ASVs contained within a single phylogenetic bin.

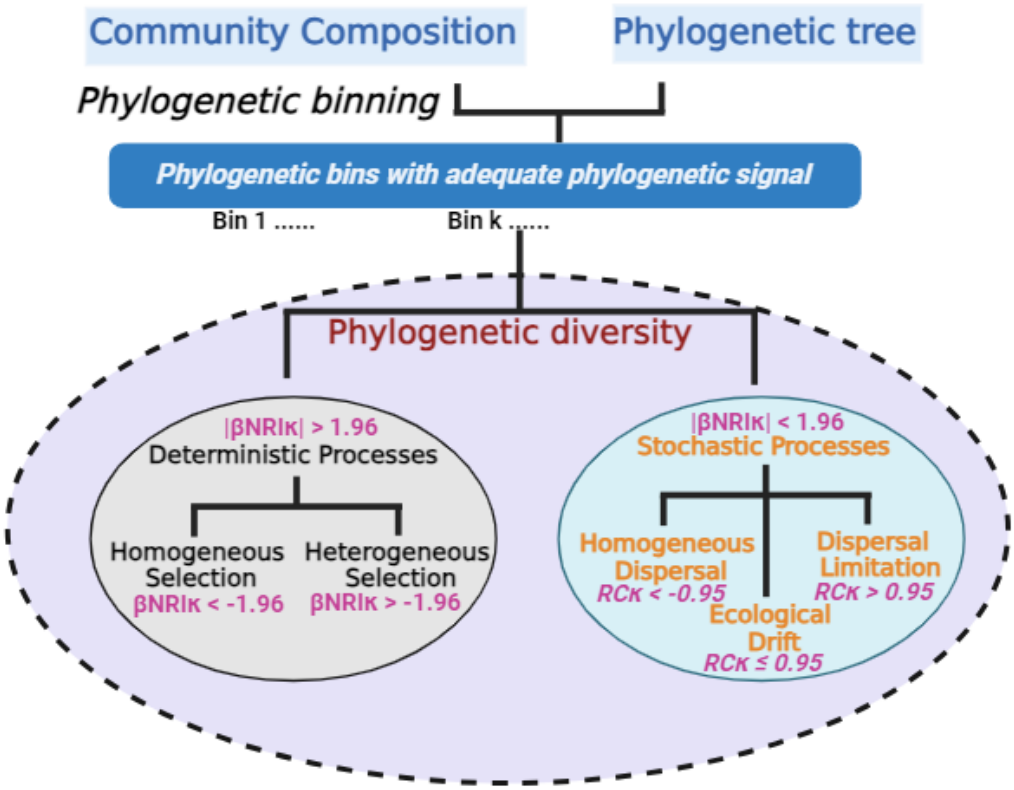

Figure S4: Overview of the ICAMP analysis highlighting key steps, including phylogenetic binning and bin-based null model simulations, to assess phylogenetic diversity and partition deterministic and stochastic processes (Adapted from Ning et al., 2020).

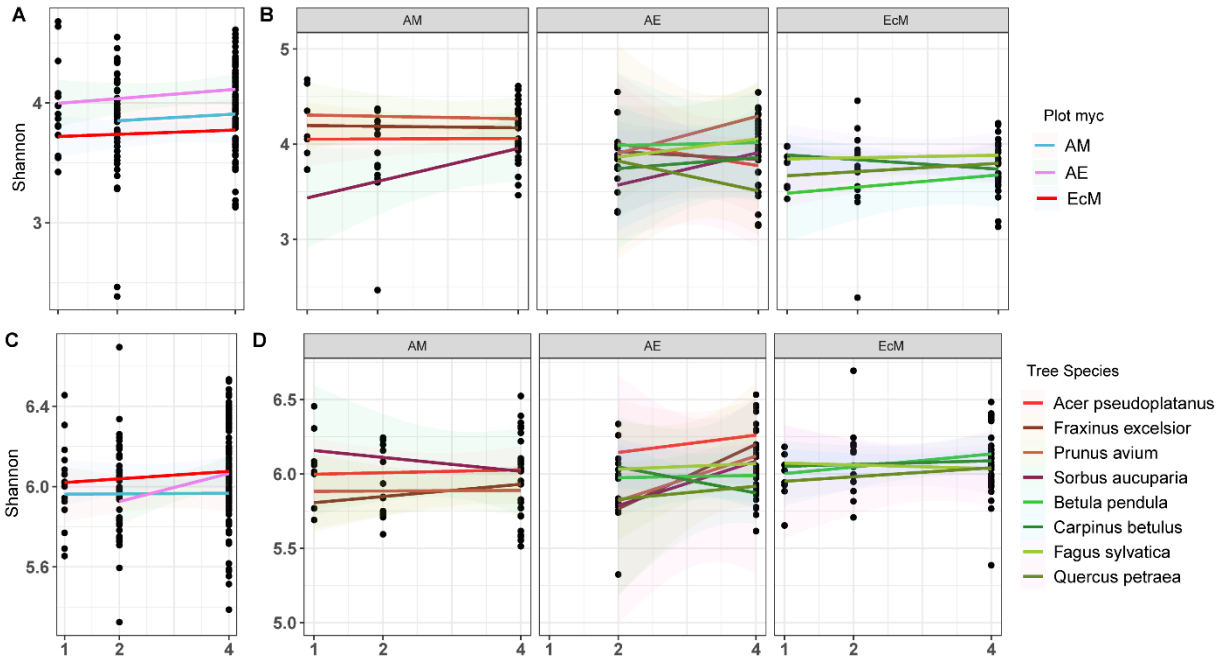

Figure S5: Linear mixed model effects of mono (AM, EcM) and mixed (AE) mycorrhizal type plots with increasing tree diversity (1, monoculture; 2, 2 species mixture; 4, 4 species mixture) on fungal (A) and bacterial (C) shannon diversity. Linear mixed model effects of tree species and tree diversity by the plot mycorrhizal type on fungal (B) and bacterial (D) shannon diversity.

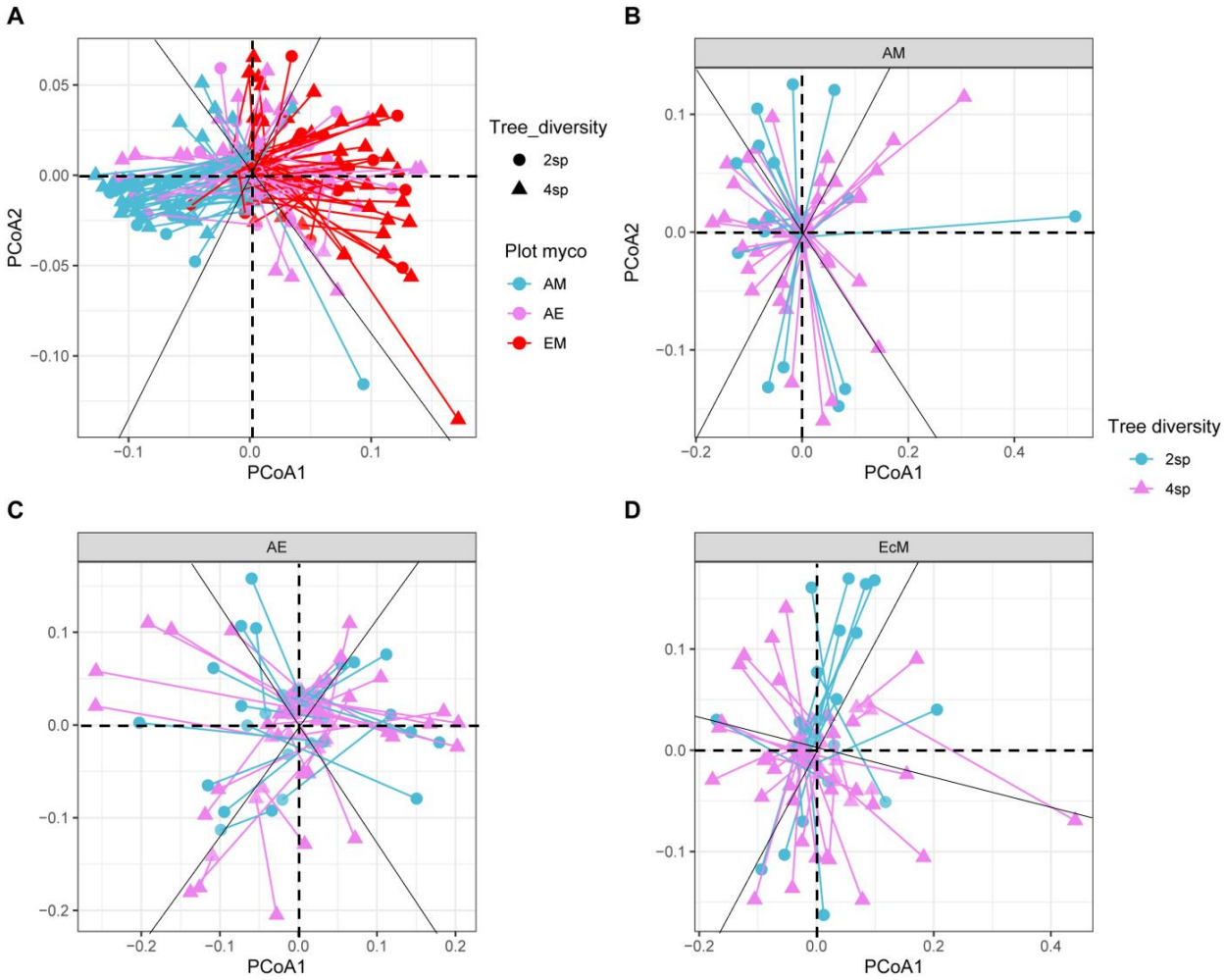

Figure S6: Procrustes analysis between fungal and bacterial community structure of mono- (AM, EcM) and mixed (AE) mycorrhizal type plots at different tree diversity levels (2sp, two species mixture; 4sp, four species mixture), based on PCoA using Bray-Curtis distances. Line connect corresponding points (samples) from the fungal PCoA (initial ordination) to the bacterial PCoA (final ordination), with arrow lengths indicating the distance differences between samples in the ordination space.

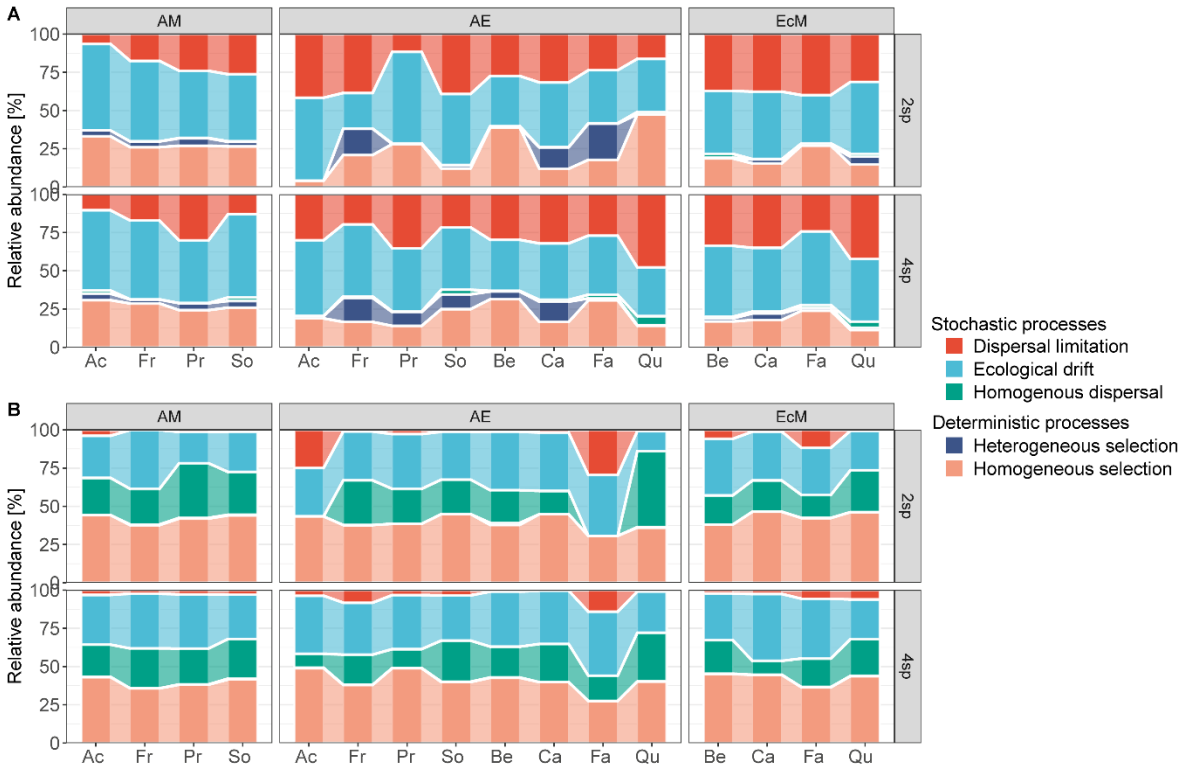

Figure S7: Relative importance of different ecological processes to soil fungal (A) and bacterial (B) communities in different mono (AM, EcM) and mixed mycorrhizal type (AE) plots tree species across tree diversity levels (2sp, two species mixture; 4sp, four species mixture). Tree species abbreviation; Ac, *Acer pseudoplatanus;* Fr, *Fraxinus excelsior*; Pr, *Prunus avium*; So, *Sorbus aucuparia*; Be; *Betula pendula*, Ca; *Carpinus betulus*; Fa, *Fagus sylvatica*; Qu, *Quercus petraea*.

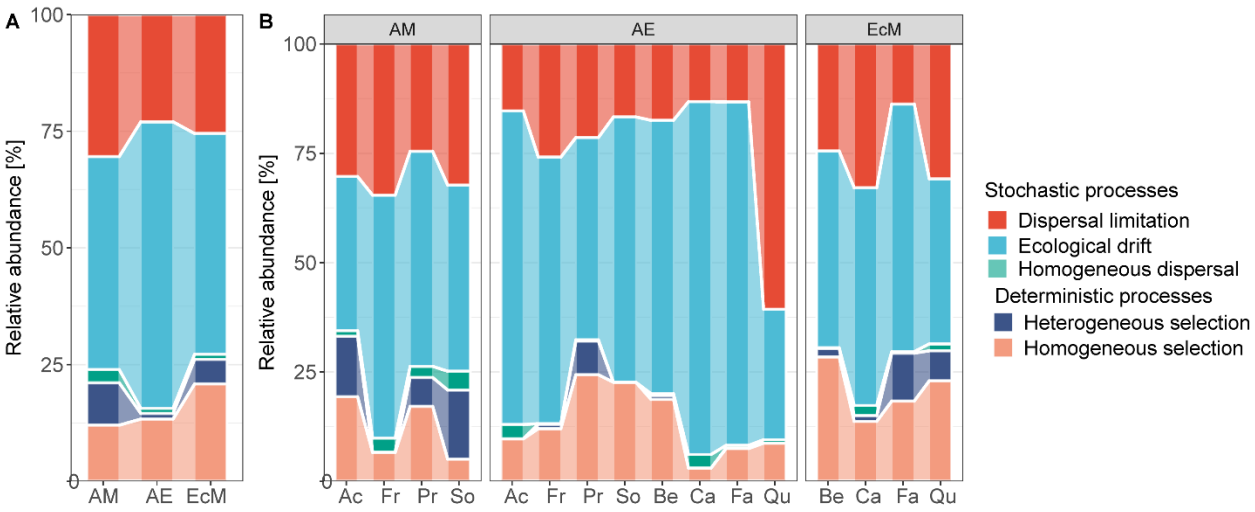

Figure S8: Relative abundance of various community assembly processes of AM and EcM fungal communities across mono (AM, EcM) and mixed mycorrhizal type (AE) tree species plots. Tree species abbreviation Ac, *Acer pseudoplatanus;* Pr, *Prunus avium*; So, *Sorbus aucuparia;* Fr, *Fraxinus excelsior*; Ca; *Carpinus betulus*; Qu, *Quercus petraea;* Be; *Betula pendula*, Fa, *Fagus sylvatica.*

Table S1. Mean diameter at ground and breast height and height for selected tree species in MyDiv experiment

	Tree species	Family	Diameter at 5cm (cm)	Diameter at breast height (cm)	Height (m)	Reference
AM	*Acer pseudoplatanus*	Sapindaceae	6.957	3.969	5.071	Yi et al., 2024
	Fraxinus excelsior	Oleaceae	6.135	3.583	4.342	Yi et al., 2024
	Prunus avium	Rosaceae	7.891	4.657	5.198	Yi et al., 2024
	Sorbus aucuparia	Rosaceae	5.963	3.176	4.317	Yi et al., 2024
EcM	*Betula pendula*	Betulaceae	7.618	4.559	5.508	Yi et al., 2024
	Carpinus betulus	Betulaceae	5.300	2.864	3.898	Yi et al., 2024
	Fagus sylvatica	Fagaceae	2.951	1.330	2.302	Yi et al., 2024
	Quercus petraea	Fagaceae	2.920	1.334	2.302	Yi et al., 2024

Table S2. Linear mixed effects models of mono (AM, EcM) and mixed myco-type (AE (AM and EcM)) plots with increasing tree diversity on the fungal bacterial observed richness and shannon diversity.

	Observed richness			**Shannon**		
Fungi	***slope***	***Lower CL***	***Upper CL***	***slope***	***Lower CL***	***Upper CL***
AE (Mixture)	5.94	-20.9	32.8	0.057	-0.163	0.277
AM	2.16	-14.2	18.5	0.035	-0.096	0.168
EcM	-2.23	-18.6	14.2	0.023	-0.108	0.155
Bacteria	***slope***	***Lower CL***	***Upper CL***	***slope***	***Lower CL***	***Upper CL***
AE (Mixture)	145.15	-34.8	325	0.139	-0.014	0.292
AM	-5.57	-119.4	108	-0.014	-0.111	0.083
EcM	27.49	-86.3	141	0.035	-0.062	0.132

Table S3. Linear mixed effects models relating tree species with increasing tree diversity on the fungal and bacterial communities observed richness and shannon diversity. Tree species abbreviation; Ac, Acer *pseudoplatanus;* Fr, *Fraxinus excelsior*; Pr, *Prunus avium*; So, *Sorbus aucuparia*; Be; *Betula pendula*, Ca; *Carpinus betulus*; Fa, *Fagus sylvatica*; Qu, *Quercus petraea*.

	Observed richness			**Shannon**		
Fungi	***slope***	***Lower CL***	***Upper CL***	***slope***	***Lower CL***	***Upper CL***
Ac	-2.58	-38.2	33.0	-0.096	-0.405	0.213
Fr	-11.13	-46.8	24.5	-0.064	-0.373	0.244
Pr	2.81	-32.8	38.4	-0.042	-0.352	0.266
So	19.54	-16.1	55.2	0.277	-0.031	0.587
Ac	-31.25	-112.9	50.4	-0.229	-0.905	0.446
Fr	6.00	-76.2	88.2	-0.080	-0.761	0.600
Pr	69.50	-12.2	151.2	0.394	-0.281	*1.070*
So	20.25	-62.1	102.6	0.341	-0.340	1.023
Be	5.50	-76.7	87.7	0.030	-0.650	0.711
Ca	-23.50	-105.7	58.7	0.116	-0.563	0.797
Fa	-4.25	-86.0	77.5	0.192	-0.484	0.868
Qu	5.25	-76.5	87.0	-0.315	-0.992	0.361
Be	18.56	-14.3	51.4	0.083	-0.167	0.334
Ca	-3.88	-36.7	29.0	-0.061	-0.312	0.189
Fa	-18.71	-51.6	14.2	-0.010	-0.261	0.240
Qu	-4.88	-37.7	28.0	0.063	-0.187	0.314
Bacteria	***slope***	***Lower CL***	***Upper CL***	***slope***	***Lower CL***	***Upper CL***
Ac	-24.5	-257	208	-0.003	-0.212	0.203
Fr	52.2	-180	284	0.054	-0.153	0.262
Pr	33.9	-198	266	0.003	-0.204	0.211
So	-97.6	-330	135	-0.116	-0.324	0.091
Ac	89.41	-415.1	594	0.149	-0.272	0.571
Fr	487.77	-20.1	996	0.407	-0.016	0.832
Pr	420.70	-83.7	925	0.292	-0.130	0.714
So	352.18	-156.3	861	0.265	-0.158	0.69-
Be	53.37	-454.6	561	0.037	-0.386	0.462
Ca	-178.66	-686.6	329	-0.165	-0.590	0.259
Fa	-3.11	-507.9	502	0.036	-0.385	0.458
Qu	-54.72	-559.3	450	0.088	-0.333	0.510
Be	120.3	-95.4	336	**0.080**	**0.054**	**0.106**
Ca	-1.1	-216.7	215	0.013	-0.156	0.184
Fa	-59.2	-274.8	156	-0.003	-0.174	0.164
Qu	51.5	-164.2	267	0.053	-0.108	0.214

Table S4. Effects of tree species in mono (AM, EcM) and mixed myco-type (AE (AM and EcM)) plots along tree diversity on the compositional differences of soil microbial communities based on PERMANOVA with 999 permutations

				Fungi	
Fungi	***df***	***Sumofseq***	***F***	R^2	***P***
Tree sp (TS)	7	93.97	11.87	0.322	**0.001*** **
Tree diversity (TD)	1	3.704	3.276	0.012	**0.022***
Plot mycorrhizal type (PM)	2	23.89	10.56	0.082	**0.001*** **
(TS*PM)	6	5.953	0.877	0.020	0.107
(TS * TD)	7	8.90	1.123	0.305	0.492
Residual	128	140.4		0.496	
Bacteria	***df***	***Sumofseq***	***F***	R^2	***P***
Tree sp (TS)	7	18.16	6.130	0.223	**0.001** **
Tree diversity (TD)	1	0.397	0.937	0.008	0.498
Plot mycorrhizal type (PM)	2	0.716	0.846	0.004	0.388
(TS*PM)	6	2.228	0.877	0.027	0.607
(TS * TD)	7	1.734	0,585	0.021	0.936
Residual	120	54.18		0.667	

Table S5. Effects of tree species and tree diversity on the compositional differences of soil fungal and bacterial communities with in mono and mixed myco-type based on PERMANOVA with 999 permutations

Fungi	***Fungi***	***df***	***Sumofseq***	R^2	***F***	***P***
AM	Tree sp (TS)	3	4.259	0.128	2.599	**0.002****
	Tree diversity (TD)	1	0.721	0.021	1.319	0.208
	TS*TD	3	1.882	0.056	1.149	0.289
	Residual	48	26.20	0.492		
AE	Tree sp (TS)	7	5.887	0.076	1.207	0.126
	Tree diversity (TD)	1	0.686	0.020	0.985	0.406
	TS*TD	7	4.562	0.136	0.935	0.624
	Residual	32	22.29	0.666		
EcM	Tree sp (TS)	3	4.536	0.113	2.270	**0.001****
	Tree diversity (TD)	1	1.766	0.044	2.651	0.052
	TS*TD	*3*	*1.864*	0.046	*0.933*	
	Residual	55	31.97	0.796		
Bacteria	**Bacteria**	***df***	***Sumofseq***	R^2	***F***	***P***
AM	Tree sp (TS)	3	0.792	0.085	1.554	0.056
	Tree diversity (TD)	1	0.085	0.009	0.502	0.936
	TS*TD	3	0.275	0.029	0.540	0.985
	Residual	48	0.876	0.876		
AE	Tree sp (TS)	7	3.978	0.025	2.673	0.116
	Tree diversity (TD)	1	0.259	0.023	1.363	0.322
	TS*TD	7	1.534	0.121	1.031	0.631
	Residual	32	6.801	1.000		
EcM	Tree sp (TS)	3	2.938	0.187	3.969	**0.003****
	Tree diversity (TD)	1	0.258	0.014	1.046	0.322
	TS*TD	3	0.598	0.038	0.808	0.631
	Residual	48	12.60	1.000		

Table S6. Pairwise comparison of different ecological processes among mono (AM, EcM) and mixed myco-type (AE (AM and EcM)) plots in fungal and Bacterial communities. different community assembly processes: DL, dispersal limitation; DR, ecological drift; HD, homogeneous dispersal; HeS, heterogeneous selection; HoS, homogeneous selection.

Fungi	**(HeS)adj. p**	**(HoS)adj. p**	**(DR)adj. p**	**(DL)adj. p**	**(HD)adj. p**
AM vs EcM	0.475	0.097	0.531	**0.045 ***	0.232
AM vs AE	0.469	0.209	0.462	0.095	0.764
EcM vs AE	0.367	0.310	0.709	0.303	0.502
Bacteria	**(HeS)adj. p**	**(HoS)adj. p**	**(DR)adj. p**	**(DL)adj. p**	**(HD)adj. p**
AM vs EcM	-	**0.0001*** **	0.180	0.632	0.559
AM vs AE	0.591	0.614	0.340	0.590	0.459
EcM vs AE	-	**0.0001*** **	0.312	0.632	0.967

Table S7. Pairwise comparison of different ecological processes among mono (AM, EcM) and mixed myco-type (AE (AM and EcM)) plots tree species in fungal and Bacterial communities. Tree species abbreviation Tree species abbreviation Ac, Acer pseudoplatanus; Fr, Fraxinus excelsior; Pr, Prunus avium; So, Sorbus aucuparia; Be; Betula pendula, Ca; Carpinus betulus; Fa, Fagus sylvatica; Qu, Quercus petraea. different community assembly processes: DL, dispersal limitation; DR, ecological drift; HD, homogeneous dispersal; HeS, heterogeneous selection; HoS, homogeneous selection. Only mentioned those who's significantly varied.

Fungi

Tree sp	**(HeS)adj. p**	**(HoS)adj. p**	**(DR)adj. p**	**(DL)adj. p**	**(HD)adj. p**
Ac_AE vs Ca_AE	0.006**	-	-	-	-
Be_AE vs Ca_AE	0.022*	-	-	-	-
Ca_AE vs Fa_AE	0.006**	-	-	-	-
Ca_AE vs Pr_AE	0.021*	-	-	-	-
Ca_AE vs Qu_AE	0.006**	-	-	-	-

(B) **Bacteria**

Tree sp	**(HeS)adj. p**	**(HD)adj. p**	**(DL)adj. p**	**(DR)adj. p**	**(HoS)adj. p**
Ac_AE vs Qu_AE	0.002**	0.0002***	-	0.031*	-
Be_AE vs Qu_AE	0.004**	0.003**	-	0.007**	-
Ca_AE vs Qu_AE	0.006**	-	-	0.031*	-
Fa_AE vs Qu_AE	0.002**	-	0.009**	0.007**	-
Fr_AE vs Qu_AE	0.002**	-	-	-	-
Pr_AE vs Qu_AE	0.002**	-	-	0.008**	-
Qu_AE vs So_AE	0.002**	-	-	-	-
Be_AE vs Fa_AE	-	-	0.009**	-	-
Ca_AE vs Fa_AE	-	-	0.009**	-	-
Fa_AE vs Fr_AE	-	-	0.012*	-	-
Fa_AE vs Pr_AE	-	-	0.011*	-	-
Fa_AE vs So_AE	-	-	0.009**	-	-
Ac_AE vs Ca_AE	-	0.023*	-	-	-
Ac_AE vs Fr_AE	-	0.026*	-	-	-

CHAPTER 4

Impacts of mycorrhizal types and tree diversity on the soil microbial genomic functional potential in temperate forests

This chapter is currently under peer review in the Microbiology Spectrum journal as

Hafeez ul Haq, Bala Singavarapu, Olga Ferlian, Henriette Christel, Simone Cesarz, Nico Eisenhauer, Helge Bruelheide, & Tesfaye Wubet (2025), Impacts of mycorrhizal types and tree diversity on the soil microbial genomic functional abundance in temperate forests

Abstract

Soil microbial genomic functional potential is the collective genetic capacity of the microbial community to carry out various functions, particularly those related to nutrient cycling (C, N, P), in soils. The microbial functional potential depends on the ability of a particular microbial taxon to perform a certain function in the soil and on the potential of this taxon in the microbial community. While the drivers of microbial community composition have been extensively studied, particularly in forest ecosystems, the influence of a tree's mycorrhizal type and tree diversity on the microbial genomic potential remains poorly understood.

We used soil microbiome data generated by meta-barcoding of fungal ITS2 and bacterial 16S V4 rRNA from samples of the tree rooting zone of eight target trees. The samples were collected in the MyDiv tree diversity experiment, which include AM and EcM tree species and their mixtures in one-, two-, and four-species mixture plots. The genomic functional potential was predicted using the MetaCyc database with respect to three major nutrients cycling carbon (C), nitrogen (N), and phosphorus (P) and their (C:N, C:P, N:P, C:N:P) potential ratio. We assessed the relationship of these different aspects of microbial functional potential with microbial diversity and community composition in mono (AM, EcM) and mixed (AM and EcM or AE) myco-type tree communities which also varied in tree richness.

Two-way ANOVAs showed that plot myco-type mixture predominantly influenced the fungal and bacterial taxa with genes involved in the C, N, and P cycles, while tree species richness significantly affected fungal taxa involved in the C and P cycle and bacterial taxa involved in the C cycle as well as the proportion of taxa involved in the C cycle to those involved in the P cycle. Additionally, tree species identity significantly influenced both fungal and bacterial genomic functional potential, with specific variation in C, N, P cycle and their ratios in AM, AE, and EcM plot. Fungal richness significantly decreased with C and P cyce in EcM plots, while N cycle significantly increased in AM plots. In contrast, bacterial richness showed no significant relationship with tree species diversity in either mono or mixed myco-type stands. Permanova analysis revealed a strong influence of plot myco-type mixture on fungal and bacterial genomic community composition. Furthermore, similar results were observed for the impact of tree species diversity on fungal genomic composition. For bacterial communities, tree species richness affected the potential of taxa involved in the P cycle as well as the ratio of the potential of taxa involved in

the N cycle relative to those involved in the P cycle as well as those involved in all three cycles (C:N:P). The PCoA analysis revealed that differences in the fungal and bacterial community composition between mono and mixed myco-types and different tree diversity levels is reflected in different proportions of taxa involved in C, N and P cycling. Soil nutrient concentrations showed no significant correlation with microbial functional potential or community composition at different levels of tree species richness and plot myco-type mixtures.

Our findings highlight that plot myco-type mixture, tree diversity and microbial diversity and composition shape the microbial functional potential for the cycle of essential nutrients. This suggest that forest management strategies fostering microbial community diversity through mixing species with different mycorrhiza type and increasing tree species richness could strengthen soil microbial functions, enhancing nutrient cycling and improving forest ecosystem resilience and productivity.

Introduction

Soil microorganisms play a crucial role in terrestrial ecosystems by regulating biogeochemical cycles, maintaining biodiversity, and supporting sustainable ecosystem functions (Zhang, Chen & Ruan 2018; Wang *et al.* 2022). These microbes influence nutrient uptake in plants by altering soil organic matter decomposition rates and nutrient cycling through the production of extracellular enzymes (Adamczyk *et al.* 2014; Qu *et al.* 2020). Soil stoichiometry of carbon (C) and its ratio with other nutrients like nitrogen (N) and phosphorus (P) in terrestrial ecosystems affects soil properties and ecosystem functions (Elser *et al.* 2007; Güsewell & Gessner 2009). This in turn shapes microbial community composition (Singavarapu *et al.* 2022a; Haq *et al.* 2024) and ensures nutrient equilibrium within plant-soil systems (Makino *et al.* 2003). Consequently, microbial metabolic processes are primarily influenced by the availability of C, N, and P in the soil (Ekblad & Nordgren 2002). For instance, higher soil C:N ratios indicate greater inputs of carbon-rich structural materials utilized by microbes, while lower N:P ratios suggest an abundance of P-rich ribosomes essential for protein synthesis (Delgado-Baquerizo *et al.* 2016). Additionally, soil microbial growth rates are inversely correlated with the C:N ratio (Trivedi *et al.* 2016). However, beyond soil nutrient concentrations and their ratios the concept of microbial genomic functional

potentials has gained importance (Singavarapu *et al.* 2023). This refers to the genetic potential of soil microbial communities to drive processes related to nutrient cycling, including C, N, and P cycles (Mackelprang *et al.* 2011; Fierer *et al.* 2012). An increased abundance of genes such as glycosaminidase and phosphatases which are involved in C, N, and P cycling can enhance the microbial community's ability to decompose organic matter, recycle these nutrients, and maintain ecosystem functioning (Prosser *et al.* 2007). For instance, genes associated with nitrogen fixation, phosphorus solubilization, or carbon degradation pathways directly influence the availability of these essential nutrients (N, P, and C respectively) in the soil (Philippot *et al.* 2013; Leff *et al.* 2015). Soils with diverse and abundant microbial genomic potentials can support nutrient cycling and ecosystem stability (Loreau *et al.* 2001). In this context, the genomic functional potential of microbial communities is closely linked to the availability of C, N, and P in soils. For example, soils with higher carbon content may harbor microbial communities with enhanced capabilities to break down carbon-rich organic matter (Fontaine *et al.* 2007), while soils with a more balanced C:N ratio might support microbial pathways for faster nitrogen and phosphorus cycling (Fierer *et al.* 2012; Delgado-Baquerizo *et al.* 2017). Though a number of studies showed that C, N, P and their ratios to shape the soil microbial community composition (Chen *et al.* 2016; Delgado-Baquerizo *et al.* 2017; Singavarapu *et al.* 2022a; Haq *et al.* 2024), there remains a significant knowledge gap on their relationship with the genomic potential of the soil microbial communities in temperate forest ecosystems.

In terrestrial ecosystems over 80% of plants engage in symbiotic relationships with fungi, predominantly with arbuscular mycorrhizal (AM) or ectomycorrhizal (EcM) fungi (Smith & Read 2010; van Der Heijden *et al.* 2015a). Plants associated with AM fungi tend to display enhanced acquisition of inorganic nutrients and accelerated growth rates. Conversely, those associated with EcM fungi often exhibit slower growth, as nutrient supply rates are lower when depending mineralization of organic matter (Sato *et al.* 2019; Chen, Chen & Chang 2022). Consequently, AM and EcM fungi exhibit distinct genomic adaptations that underpin their distinctive functional roles particularly in C, N, and P cycling (Averill *et al.* 2019; Gao *et al.* 2021). AM fungi are known for efficient P uptake, but they may rely on other co-occurring microbiota for the P cycling and availability in soil (Tisserant *et al.* 2013). Thus, one would expect that AM trees are associated with soil microbes that exhibit a higher genomic potential with respect to enzymes involved in the P cycle. In contrast, EcM fungi possess a complex genomic toolkit, including enzymes like

proteases and chitinases, enabling them to access organic N by decomposing complex organic matter (Kohler *et al.* 2015), Therefore, it can be expected that microbial communities associated with EcM trees exhibit a higher functional potential related to C and N cycling. Due to these functional differences, AM and EcM fungi contribute in unique but complementary ways to the C, N, and P cycling, ecosystem productivity, and soil health (Smith & Read 2008; Averill & Hawkes 2016). Despite extensive research, considering that soil functionality is closely linked to the dominant mycorrhizal type (Wagg *et al.* 2014; Singavarapu *et al.* 2022a), a substantial knowledge gap persists regarding how microbial genomic functional potentials varies in myco-type mixture and tree diversity levels.

To close this gap, we used the MyDiv experimental platform (Ferlian *et al.* 2018) where tree species of two mycorrhizal types AM and EcM, grow in one-, two- and four-species mixtures and in mono (AM or EcM) or mixed (AM and EcM or AE) myco-type plots. We employed PICRUSt2 (Douglas *et al.* 2020) to infer soil genomic functional potentials linked to soil nutrient cycling, using amplicon sequencing data of the target tree rooting zone soil microbiome (fungal and bacterial). The following hypotheses were evaluated: (H1) The microbial genomic functional potentials depend on mycorrhizal type and their mixture (H2) which is influenced by soil microbial diversity and composition (H3), although this (H1 & H2) relationship weakens as tree diversity increases. (H4), Further, we expect a strong relationship of soil nutrient concentration on the microbial genomic functional potentials.

Materials and methods

Experimental site

The MyDiv experimental platform is based at the Experimental Research Station of the Helmholtz Centre for Environmental Research in Bad Lauchstadt, Saxony-Anhalt, Germany (Ferlian et al. 2018). The climate features an average precipitation of 484 mm and annual temperature of 8.8°C. The soil type is Haplic Chernozem derived from Loess, with a pH 7.4 (Ferlian *et al.* 2018). The experimental site was established in March 2015 on a former crop field and the experiment comprises 80 plots measuring 11 × 11 meters each, with a core area of 8 × 8 meters at the center. Each plot contains 140 trees planted at 1-meter interval. The tree selection consists of ten species,

equally divided between AM and EcM groups, planted in one, two and four species mixture. Additionally, treatments based on mycorrhizal types are incorporated, including individual AM, EcM, or mixed AM and EcM tree species (Ferlian *et al.* 2018). Eight tree species representing both AM and EcM associations were selected for analysis with equal representation in the experimental design (Table S1). A target tree rooting zone soil sample was prepared by pooling and thoroughly mixing the four soil cores. The soil mixture was sieved through a 2-mm mesh to eliminate root fragments, and 30 g samples were stored at -20°C for subsequent DNA extraction.

Soil nutrient analysis

Soil nitrogen was quantified using the Kjeldahl method on an autoanalyzer (SEAL Analytical, Germany). Soil carbon was measured with a TOC analyzer (Liqui TOC II, Elementar Analyses system, Germany). Phosphorus was determined using ion exchange membranes (IEM), following protocols by (Durán *et al.* 2013).

DNA Extraction, Quantification, and Amplicon Library Preparation

Soil DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of the extracted DNA was then quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany). Bacterial 16S rRNA gene amplicons targeting the V4 region were generated for library preparation through PCR amplification using the universal primers 515f and 806r (Caporaso *et al.* 2011) both containing Illumina adapter overhangs. A two-step semi-nested PCR amplification process was used to prepare the fungal amplicon libraries. The initial amplification was done using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) followed by a second round of PCR using the fITS7 (Ihrmark *et al.* 2012) and ITS4 primers, which also included Illumina adapter sequences. Following amplification, AMPure XP magnetic beads (Beckman Coulter, Krefeld, Germany) were employed to purify the PCR products and eliminate residual primers and by-products. Illumina Nextera XT indexing was conducted in a second PCR amplification to introduce sample-specific barcodes, following another purification step with AMPure XP beads to ensure the indexed libraries quality. Quantification of the purified libraries was performed using the Qubit

dsDNA High Sensitivity Assay (Thermo Fisher Scientific) and then pooled in equimolar ratio aiming to achieve a target of 4 nM concentration. High-quality sequencing data for downstream analysis was obtained using the Illumina MiSeq platform with paired-end 300 bp reads, employing the MiSeq Reagent Kit v3 (Illumina, Inc., San Diego, CA, USA). Sequencing was conducted at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig, Germany.

Bioinformatics workflow

For processing the raw amplicon sequencing data, Quantitative Insights Into Microbial Ecology (QIIME 2 2020.2) (Bolyen *et al.* 2019) tool was employed for bioinformatics analysis. Initially, the raw sequences were filtered and trimmed using Cutadapt (Martin 2011) to remove any low-quality bases, adapters, and primers from the reads. Following filtration, sequences were denoised and grouped into amplicon sequence variants (ASVs) using the DADA2 (Callahan *et al.* 2016). For taxonomic assignment, the classify-sklearn naïve Bayes classifier (Bokulich *et al.* 2018a) was applied against the Silva-132-99-515-806-nb-classifier for bacterial sequence, while the fungal sequences were classified using the UNITE-ver8-99-classifier-04.02.2020 database. The resulting bacterial and fungal taxonomy and ASV abundance files were then imported into R for further analysis (v.4.0.2) using the phyloseq package (McMurdie & Holmes 2013). To predict the functional potential of the microbial communities, PICRUSt2 was employed, allowing for the inference of metagenome functional abundances based on the identified ASVs. To determine the microbial taxa, present in each mono- and mixed-myco-type combined with tree diversity levels (AM1, AM2, AM4, AE2, AE4, EcM1, EcM2, and EcM4), stringent filtering was applied to both fungal and bacterial datasets. The refined data served as input for the PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas *et al.* 2020) software to predict metagenomic functional profiles. In PICRUSt2, representative amplicon sequence variant (ASV) sequences from both bacteria and fungi were aligned to reference genome databases for 16S and ITS regions using hidden Markov models (HMMER) with a minimum alignment threshold of 0.7 to ensure inclusion of all taxa classified at the genus level. The aligned sequences were then assigned to a reference phylogeny using a maximum likelihood phylogenetic approach, implemented through the Gappa and EPA-ng tools. Number of genes per gene family for fungal and bacterial ASVs was predicted based on Enzyme Commission (EC) numbers, using

the MetaCyc database (Caspi *et al.* 2020). The resulting EC number predictions were further filtered to focus on genes involved in carbon, nitrogen, and phosphorus cycling, according to previous literature (Singavarapu *et al.* 2023).

Statistical analysis

Alpha diversity quantified by observed richness was evaluated using the "microbiome" package. The genomic functional potential for C, N, and P was calculated to create various ratios in the potential of microbial taxa involved in these different nutrient cycling ratios. Thus, in the following C:N potential refers to the potential of taxa involved in the C cycle relative to those in the N cycle. The same applies to C:P, N:P and C:N:P potential. To analyze the tree species richness, plot myco-type mixture (mono or mixed-myco-types), and tree species identity effect on microbial genomic functional potential, a two-way ANOVA was conducted with the "*aov*" function from the base package. The Pearson correlation between observed richness and the microbial genomic functional potential in tree species richness in both mono and myco-type was determined using the "ggpubr" package. The effect of tree species richness, plot myco-type mixture, and tree species identity on the shaping the compositional differences of fungal and bacterial communities with C, N, and P cycling genomic potential was tested with permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the ‘vegan’ package. Further, microbial genomic functional potential was fitted to the PCoA ordination of fungal and bacterial community composition across tree species richness and mono and mixed myco-types using the *envfit* function. Additionally, correlation between microbial functional potential and the relative abundances of dominant fungal and bacterial families were computed using the "*taxa.env.cor*" function within the microbiomeseq package. A linear regression analysis was conducted to examine the relationship between soil nutrient concentration and genomic functional potential in tree species richness and both mono and mixed myco-type.

Results

Influence of myco-type mixture, tree diversity and tree species on microbial functional gene potential

The two-way-Anova results revealed that fungal carbon (C), nitrogen (N) and phosphorus (P) cycling genomic functional potentials and their ratios were significantly related with the plot myco-type mixture, while significant effects of tree species richness were found for fungal C and P cycle. Similar effect of plot myco-type mixture was observed for bacterial genomic functional potential except for the C:N:P potential, where tree species richness effect was significant for C and C:P potential (Table S2).

Further two-way-Anova at the plot myco-type mixture levels indicated the contribution of tree species to the fungal and bacterial functional potential. The fungal C, N, P functional potential were significantly affected by AM and AE tree species, while C:N, C:P and N:P potential were significant for AE and EcM tree species (Table S3). In contrast, the bacterial C, N, and P genomic functional potential and their ratios were significantly related to AM, AE and EcM tree species diversity except C and C:N potential in AM tree species stands (Table S4).

Relationship between microbial diversity and their genomic functional potential

The correlation analysis between microbial diversity and genomic functional potential in tree species richness both in mono (AM, EcM) and mixed (AE) myco-type plots demonstrate distinct patterns. In AM and EcM monoculture plots, fungal observed richness showed a positive correlation with the fungal C, N, and P cycle. Additionally, a positive correlation with fungal N:P potential was observed in 2 species EcM plot. In four species mixture fungal N:P potential exhibited a significant positive relationship, while fungal C:N and C:P potential showed negative relationship in AE and EcM plots (Figure 1A). Conversely, no significant correlations were found between bacterial observed richness and bacterial genomic functional potential in the tree species richness levels in AM and AE stands although the importance of their genomic functional potential showed significant correlation in four species EcM plot (Figure 1B).

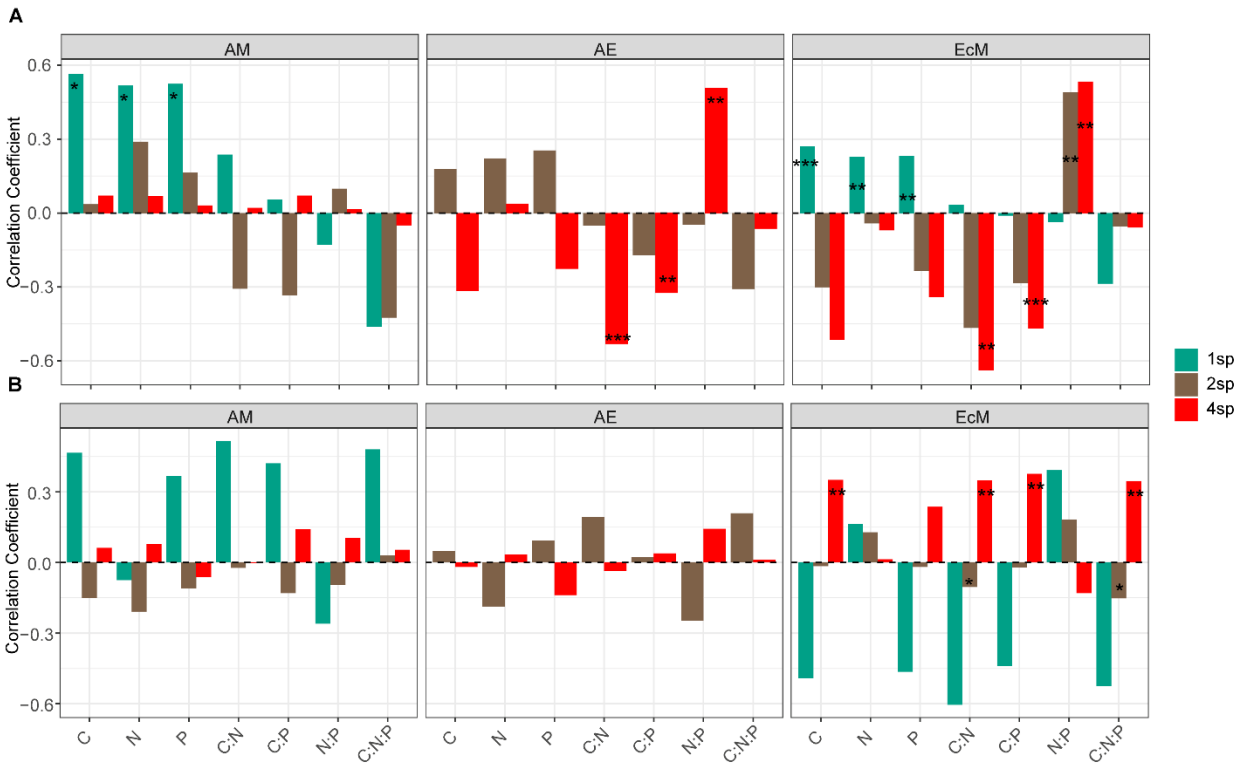

Figure 1. Relationships between microbial genomic functional potential and their ratios with fungal (A) and bacterial (B) observed richness among tree species richness in both mono (AM, EcM) and mixed (AE) myco-type plot. Correlation coefficient ρ and p values were calculated using a Pearson correlation model.

Furthermore, linear regression analysis revealed a significant increase with fungal N:P potential, and reduction in fungal C, P, C:N, and C:P functional potential in EcM plots with increasing fungal richness. Furthermore in AM plots the fungal N and C:N:P potential significantly decreased with increasing fungal richness. The fungal richness exhibit a significant and negative relationship with fungal C:N, and C:P potential, whereas significant and positive with N:P potential ratio in AE plot (Figure 2). In contrast, no significant relationship was found with bacterial observed richness and bacterial functional potential in mono and mixed myco-type stands (Figure S1).

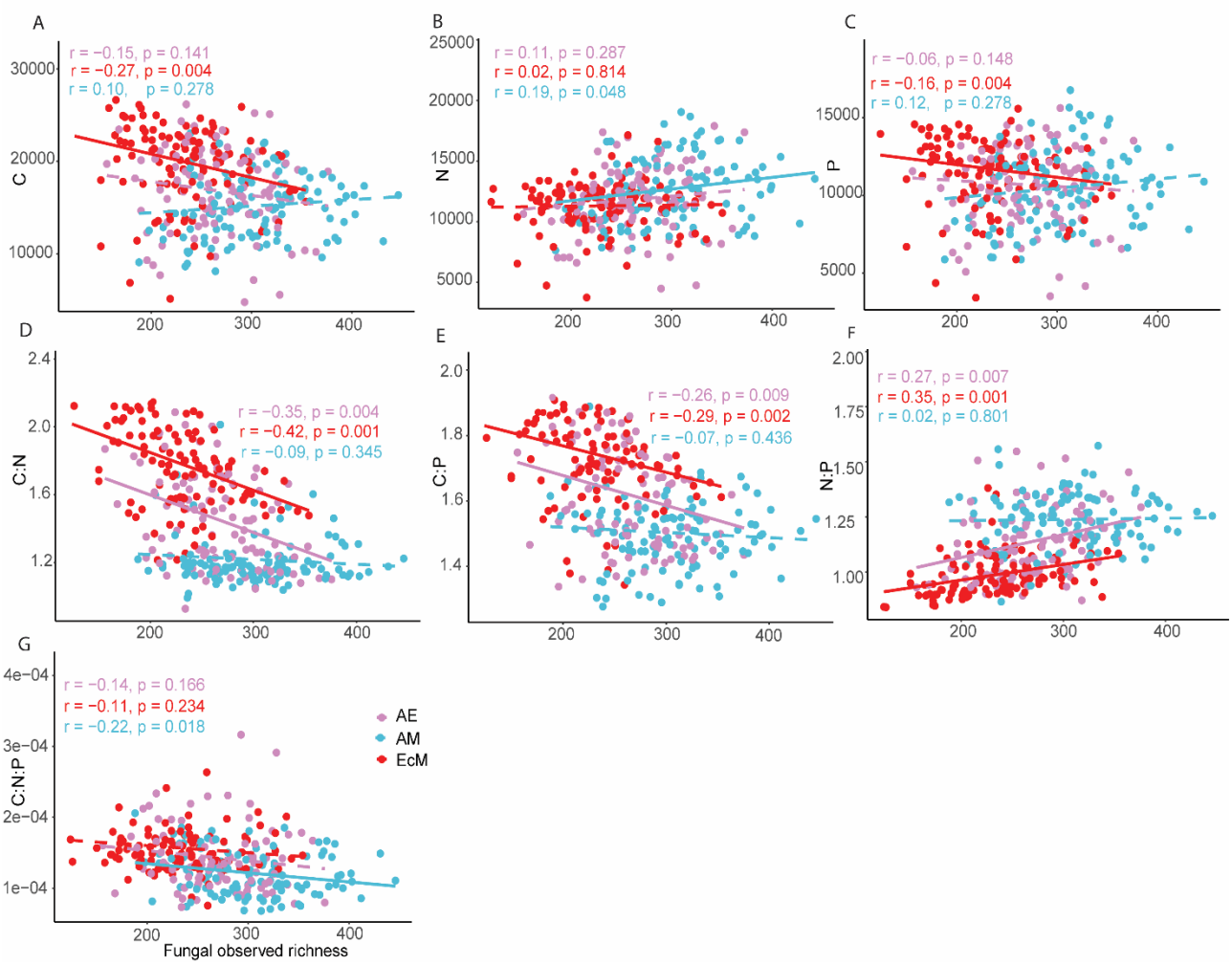

Figure 2. Relationships between fungal genomic functional potential with fungal observed richness within mono and mixed myco-type plots. The *r* and *p* values were calculated using a linear regression model. Regression lines are represented as solid for significant effects ($p < 0.05$) and dashed for non-significant effects ($p > 0.05$)

Effect of myco-type mixture, tree diversity and tree species on community composition of microbes with C, N, P cycling genomic potential

Permutational multivariate analysis of variance (PERMANOVA) to assess the factors shaping the compositional differences of fungal and bacterial communities with C, N, and P cycling genomic potential revealed significant and consistent effect of plot myco-type mixture, where tree diversity level being equally important in fungi but only for bacterial communities with N and P cycling genomic potential and their ratio. Interactive effects were also found for fungal and bacterial

communities with N genomic potential (Table S5). Similarly, PERMANOVA at the plot myco-type mixtures to evaluate the contributions of tree species and tree diversity in shaping the community composition of microbes with C, N, and P cycling genomic potential indicated the significant role of tree species in AM, AE and EcM plots for both fungal and bacterial communities with the exception of fungal P in AE and bacterial C:N potential in EcM stands where tree diversity level was found to play a significant role (Table S6. S7).

Investigation of the relationship between the overall microbial community composition and the microbial genomic functional potential using principal coordinate analysis (PCoA), revealed that the C, N, P cycle and their ratios contributed differently in shaping the soil microbial communities in mono and mixed myco-type stands and tree diversity levels (Figure 3, 4). It is worth to notice that the overall fungal C, P, C:N, C:P, and N:P functional potential were significantly influenced the fungal community differences between AM, EcM and AE stands (Figure 3A), while bacterial community composition was more influenced by bacterial N, C:N, N:P and C:N:P functional potential (Figure 3B). However, the contribution of both the fungal and bacterial C, N, P and their ratios genomic potential on the respective communities vary among the mono (AM and EcM) and mixed (AE) mycorrhizal type stands (Figure 4).

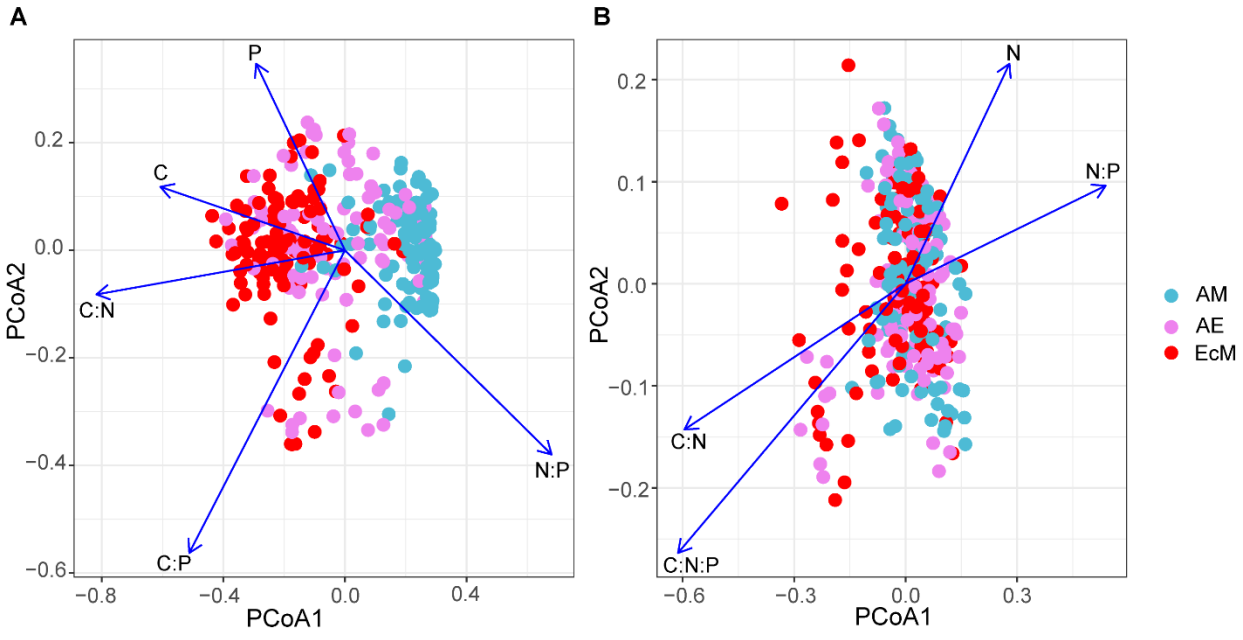

Figure 3. Principal coordinate analysis (PCoA) plots showing the relationship between overall fungal community composition (A) and bacterial community composition (B) and microbial genomic functional potential in different mono and mixed myco-type. Arrows show the significant ($p < 0.05$) post-hoc correlation between PCoA axis and microbial genomic functional potential.

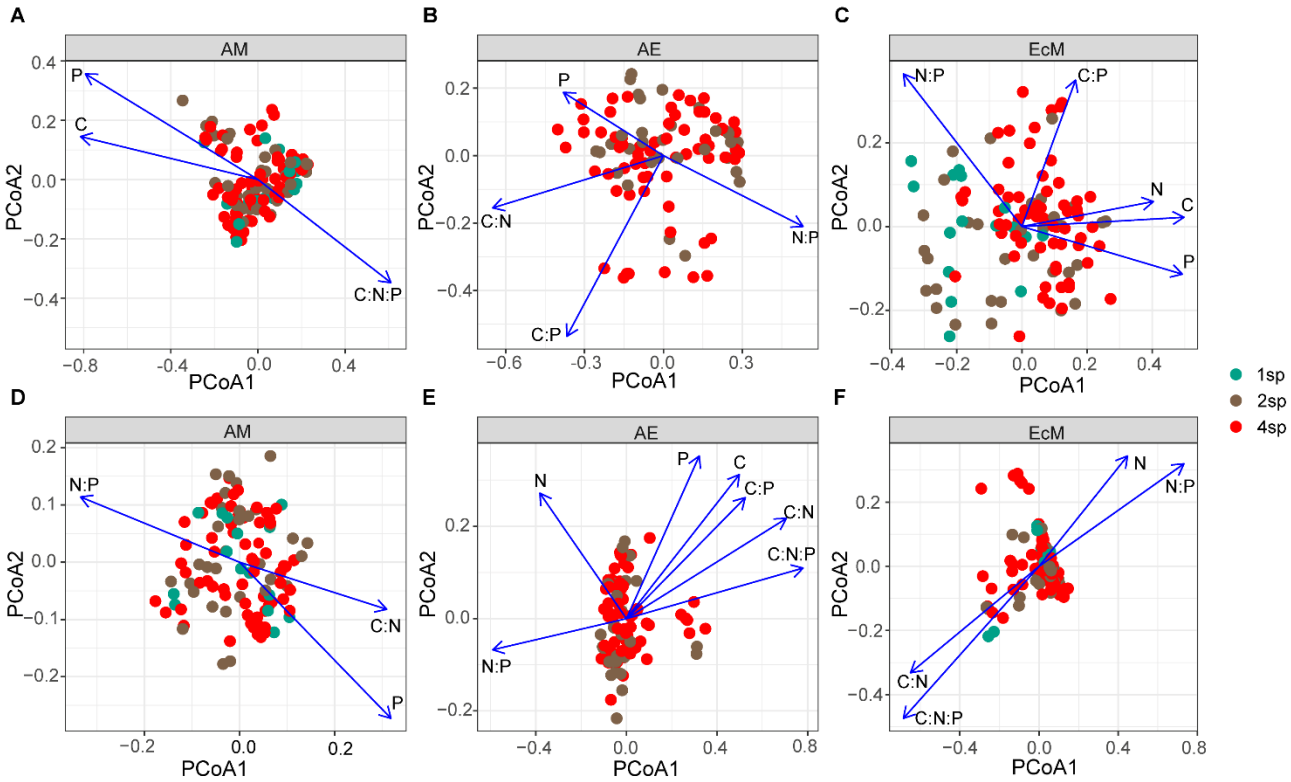

Figure 4. Principal coordinate analysis (PCoA) plots showing the relationship between the overall fungal (A, B, C) and bacterial community composition (D, E, F) and microbial genomic functional potential in tree communities composed either of mono (AM, EcM) or mixed (AE) myco-types. Arrows show the significant ($p<0.05$) post-hoc correlation between PCoA axes and microbial genomic functional potential.

Correlation between microbial genomic functional potential and relative abundance of microbial taxa

Correlation analysis of the top 10 fungal and bacterial families indicated plot myco-type related contributions. The relative abundance of the fungal family Hymenogastraceae showed positive correlation with all fungal genomic functional potential and their ratios except N:P, which exhibited a negative correlation in AM, AE, and EcM plots. Additionally, Nectriaceae displayed contrasting positive and negative correlation with fungal C genomic potential in AM and EcM

plots respectively, while no correlation was found in AE plots. Interestingly, the relative abundance of bacterial families was mostly positively correlated to bacterial genomic functional potential in all three plot myco-types. However, Chitinophagaceae showed negative correlation with N and N:P genomic potential in mono and mixed myco-type plot, Although the N:P correlation was absent in AM plot (Figure. 5). Further correlation analysis at different tree diversity also showed tree diversity level specific correlations (Figure S2).

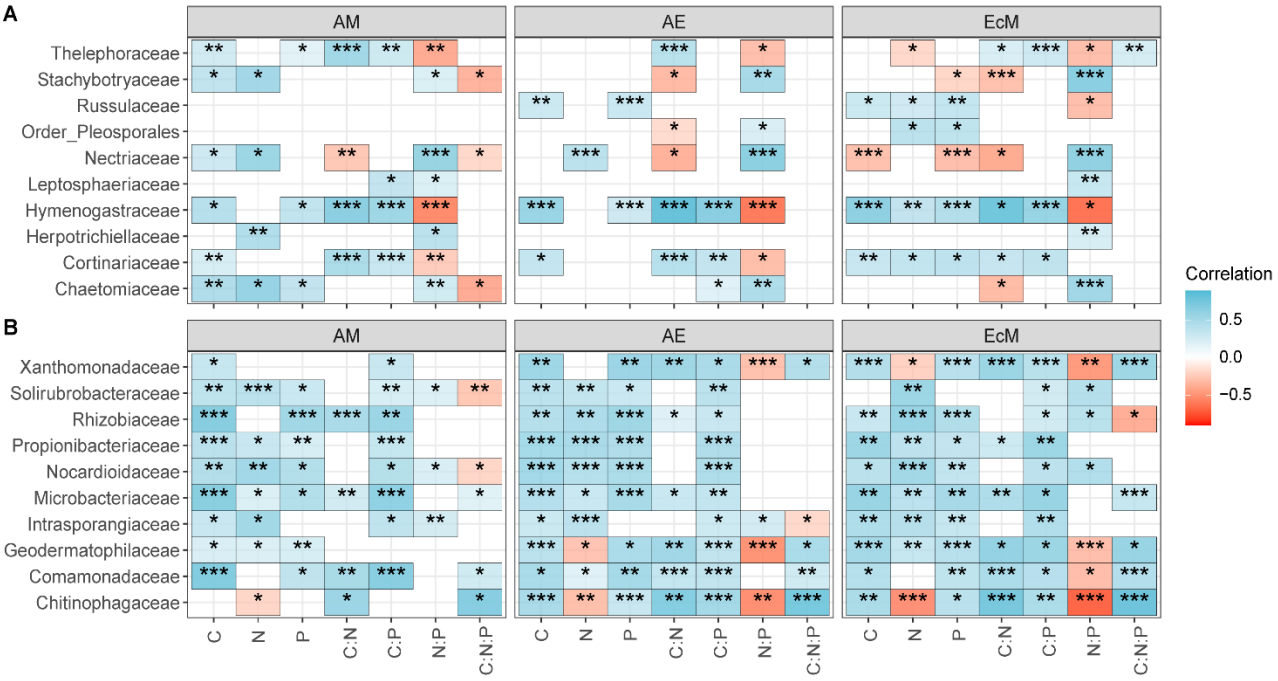

Figure 5. Correlations between the relative abundance of the dominant fungal (A) and bacterial (B) family and microbial genomic functional potential with in mono and mixed myco-type. The red color indicates negative correlations, while the sky-blue color indicates positive correlations as presented on the correlation coefficients scale to the right.

Relationship between soil C, N, P contents and their ratios with microbial genomic functional potential

The correlation analysis between soil C, N, P concentrations and microbial C, N, P genomic functional potential in tree species richness within the respective plot myco-type mixtures showed no significant correlation both within tree diversity levels and plot myco-types (Figure S3, S4). Regression analyses also confirmed the very weak relationships between soil nutrient

concentration and genomic functional potential of fungi and bacteria (Table S8, S9). Furthermore, principal coordinate analysis (PCoA) of fungi and bacteria with C, N, P genomic potential and post hoc analysis results revealed that no significant influence of soil C, N, P and their ratios contribution on the community composition of microbes with C, N, P genomic potential among tree species richness levels within mono and mixed myco-type stands (Figure S5, S6).

Discussion

In this study, we investigated the interplay of plot myco-type mixture, tree species identity, tree diversity, and microbial diversity and composition influence on microbial functional potential within an experimentally controlled platform. Our findings indicated consistent and significant effects of plot myco-type and tree species identity on the fungal and bacterial genomic functional potential, whereas tree species diversity showed no significant effect. We observed a distinct relationship between fungal diversity and composition and genomic functional potential within tree species richness in both mono- and mixed myco-type plots. Further, Plot myco-type strongly influenced the compositional differences in fungal and bacterial genomic community composition. Additionally, we found that C, N, P cycling and their ratios differentially shaped soil microbial communities in mono and mixed myco-type and tree diversity levels. Soil nutrient variables, however, did not significantly correlate with microbial functional potential or community composition in tree species richness in both mono and mixed myco-type.

Microbial genomic functional potential is related to tree myco-type mixture and tree diversity levels

Our results indicate that microbial genomic functional potential varies in mono AM, EcM and mixed AE myco-type plots. These variations may be influenced by distinct nutrient acquisition strategies, and specific functional adaptations within microbial communities (Phillips, Brzostek & Midgley 2013b). The potential reason is the distinct nutrient acquisition mechanism of AM and EcM tree associated fungi, which could lead to varied microbial genomic functional potential. (Smith & Read 2010; Phillips, Brzostek & Midgley 2013a). AM tree associated fungi primarily

enhance plant phosphorus uptake, utilizing inorganic phosphate, which aligns their functional potential closely with phosphorus cycling. This explains the significant P-related functional differences observed in AM plots. In contrast, EcM tree associated fungi possess complex enzyme systems that enable access to a broader nutrient spectrum, including organic forms of nitrogen and carbon (Phillips, Brzostek & Midgley 2013a; Lindahl & Tunlid 2015). Their capacity to decompose organic matter and release nitrogen and carbon results in notable differences in fungal C, N, and their ratios within EcM plots.

Further, our results indicate that tree species richness significantly influences fungal C and P cycle, and bacterial C and the C:P genomic potential. Higher tree diversity promotes niche differentiation within the soil microbiome potentially enhancing microbial specialization in nutrient cycling functions (Philippot *et al.* 2013; Lange *et al.* 2015). This diversity enables microbial communities to better adapt to varied resource inputs, such as different root exudates and litter types from diverse tree species (Bardgett & Van Der Putten 2014). Such functional compartmentalization can increase the abundance of genes involved in nutrient cycling. However, plant-microbe interactions may also introduce competitive dynamics that influence nutrient turnover rates and microbial efficiency in nutrient cycling (Van Der Heijden, Bardgett & Van Straalen 2008). Microbial communities broaden their functional capabilities as tree diversity increases, which can either accelerate or modulate nutrient cycling, depending on factors like litter composition and nutrient availability (Zheng *et al.* 2019). Further, diverse tree communities produce varied litter types, with those higher in lignin content slowing decomposition and potentially delaying nutrient release (Gessner *et al.* 2010). Thus, tree species richness has a complex impact on microbial functional potential, reflecting both the competitive interactions within the soil microbiome and the plant-microbe associations that shape nutrient cycling.

Microbial diversity and community composition predict microbial genomic functional potential

A growing number of studies demonstrated that soil C, N and P concentrations and their combination can be considered as the main driver for microbial diversity (Delgado-Baquerizo *et al.* 2016; Zhang *et al.* 2016; Yang *et al.* 2020). However, our study shifts this focus by examining the relationship between microbial genomic functional potential specifically C, N, and P cycling

and microbial diversity and composition. Our findings indicate the significant and positive correlation between fungal richness and fungal N and P cycle in AM plots emphasizes the critical role of fungal communities in the rooting zones of AM trees in contributing to the nutrient cycling, particularly in the decomposition of organic matter and the release of nutrients such as C, N, and P which enhancing soil microbial communities' ability to process and recycle these gene potential (Van Der Heijden, Bardgett & Van Straalen 2008). Additionally, soil properties like pH, and nutrient availability further influence fungal diversity, allowing these communities to exploit various soil niches and enhance their functional potential (Tedersoo *et al.* 2020). The negative correlation between fungal richness and fungal C:N and C:P functional gene ratios in EcM plot indicating a genomic potential imbalances, where high ratios indicate relative limitations in N and P compared to C cycling genes. Such genomic constraints can limit the functional potential for nutrient acquisition, subsequently affecting fungal growth and diversity (Chen *et al.* 2016). EcM trees related fungi exhibit a degree of stoichiometric flexibility, adapting to nutrient-limited conditions by modulating genomic pathways for nutrient processing, which can reduce fungal diversity and functional potential in EcM plots (Camenzind *et al.* 2021). In mixed mycorrhizal plot, genomic resource partitioning and competitive exclusion could influence fungal diversity. The soil microbiome associated with AM and EcM tree exhibit distinct functional adaptations to nutrient availability (Phillips, Brzostek & Midgley 2013a). Fungi associated with AM trees generally have genomic traits suited to low-resource conditions (Smith & Read 2010; van der Heijden *et al.* 2015b), whereas EcM trees associated fungi are more adapted to environments where their genomic capabilities supports the breakdown of complex organic matter (Lindahl & Tunlid 2015) and the acquisition of more diverse nutrient sources (Kohler *et al.* 2015). The high fungal C:N and C:P genomic potential may lead to competition, with AM tree-associated fungi potentially outcompeting EcM tree associated fungi for limited to N and P cycle, leading to a reduction in fungal richness as certain taxa dominate under genomic nutrient constraints.

The observed relationships between microbial genomic functional potential and fungal community composition highlight the distinct ecological roles (van Der Heijden *et al.* 2015a; Peay, Kennedy & Talbot 2016) and metabolic capabilities encoded in these fungi influencing nutrient cycling (Terrer *et al.* 2016) and soil ecosystem functions (Wagg *et al.* 2014). For example, Fungi associated with AM trees predominantly enhance genomic pathways related to phosphorus uptake, contributing to microbial genomic potential in phosphorus cycling through genes like alkaline

phosphatase, which is crucial for phosphorus transformation (Lang *et al.* 2020; Lu *et al.* 2023). Conversely, EcM trees associated fungi are specialized adept at decomposing complex organic matter, enhancing genomic potential for nitrogen and carbon cycling, which strongly correlates with gene pathways involved in these processes (Chuckran *et al.* 2021). Additionally, the presence of root litter and live root interactions can significantly modulate the expression of nitrogen-cycling genes, affecting genomic potential for nitrogen availability to AM and EcM trees (Sieradzki *et al.* 2023).

Our findings indicate that as tree species richness increases, the relationship between microbial diversity and functional potential weaken. Higher plant diversity promotes resource partitioning and niche differentiation, as diverse plant inputs such as root exudates and litter support more specialized microbial communities. This specialization reduces the direct link between microbial diversity and functional genes (Fierer, Bradford & Jackson 2007a). Additionally, increased tree diversity fosters functional redundancy within microbial communities, where multiple species perform similar function diminishing the relationship between diversity and functional gene abundance (Van Der Heijden, Bardgett & Van Straalen 2008). Additionally, as tree diversity increases, plant-microbe interactions (e.g., symbiosis, mycorrhizae) become more complex, potentially reducing the relationship between microbial diversity and functional genes (Zheng *et al.* 2019).

Relationship between soil nutrient variable with microbial genomic functional potential

We found no significant relationship between soil nutrient variable and fungal genomic functional potential, with weak regression and no influence on microbial functional community composition. This finding contrasts with our recent report on soil P and N as key factors shaping overall fungal community composition (Haq *et al.* 2024). Similarly, research from subtropical forests found P and NO_3 to be significant edaphic variables influencing fungal community composition (Singavarapu *et al.* 2022a). However, fungal community composition might react differently to soil nutrient compared to microbial genomic functional composition assessed in your study. This contrast may indicate varying microbial sensitivities to soil nutrients among different microbial taxa or functional genes. The observed results could be attributed to the resilience and functional redundancy within microbial communities (Allison & Martiny 2008). Microbial functions related

to C, N, and P cycling might be distributed across a diverse set of taxa, enabling these functions to persist even as individual community members fluctuate. This redundancy can buffer the functional composition against changes in these soil properties.

Conclusion

Our findings underscore the critical role of plot mycorrhizal mixtures, tree diversity, and microbial diversity and composition in shaping microbial functional potential essential for nutrient cycling. Specifically, our results suggest that the interactions between diverse tree species, mycorrhizal associations, and a rich microbial community enhance soil microbial functionality, leading to more efficient cycling of key nutrients. This in turn provides valuable insights for forest management practices by prioritizing diverse tree species and encouraging different mycorrhizal tree associations, along with fostering a robust microbial community, forest ecosystems can achieve improved resilience and productivity. Such strategies could enhance nutrient availability and cycling processes which can ultimately contributing to a healthier and more sustainable forest ecosystem.

Appendix

Table S2. Two-way-ANOVA effects of tree diversity levels and plot myco-type mixture on fungal and bacterial genomic functional abundance

		Fungi		Bacteria	
Genomic potential	**Variable**	**F Value**	**P**	**F Value**	**P**
C	Div_levels	16.29	**0.001*** **	13.77	**0.001 ** **
	Plot_myco	38.99	**0.001*** **	31.21	**0.001** **
	Plot_myco*div	5.245	0.056	3.167	0.083
N	Div_levels	8.079	0.180	3.558	0.060
	Plot_myco	1.804	**0.003*** **	6.610	**0.001 ** **
	Plot_myco*div	2.610	0.075	1.708	0.182
P	Div_levels	7.254	**0.008** **	8.321	0.078
	Plot_myco	7.673	**0.005** **	70.77	**0.001 ** **
	Plot_myco*div	2.513	0.082	4.644	0.103
C: N	Div_levels	5.950	0.172	8.522	0.141
	Plot_myco	166.30	**0.001*** **	21.16	**0.003 ** **
	Plot_myco*div	4.330	0.140	2.587	0.076
C:P	Div_levels	9.946	0.053	8.871	**0.003** **
	Plot_myco	91.417	**0.001** **	10.96	**0.001 ** **
	Plot_myco*div	4.824	0.161	1.828	0.162
N:P	Div_levels	8.499	0.062	1.539	0.216
	Plot_myco	103.8	**0.003 ** **	14.41	**0.001*** **
	Plot_myco*div	0.664	0.515	1.686	0.187
C:N:P	Div_levels	1.314	0.253	2.567	0.093
	Plot_myco	22.51	**0.001 ** **	2.814	0.058
	Plot_myco*div	2.248	0.107	1.323	0.267

Table S3. Two-way-ANOVA effects of tree diversity levels and mono (AM, EcM) and mixed (AE) myco-type tree species on fungal genomic functional abundance

		AM		**AE**		**EcM**	
Soil nutrient	**Variable**	**F value**	***P***	**F value**	***P***	**F value**	***P***
C	Div_levels	1.383	0.242	0.356	0.552	0.401	0.779
	Tree sp	10.61	**<0.001*** **	3.108	**0.005 ** **	0.517	0.671
	Tree sp*div	1.881	0.137	1.509	0.176	2.217	0.090
N	Div_levels	1.504	0.222	1.764	0.187	2.126	0.053
	Tree sp	23.37	**<0.001*** **	5.939	**<0.001*** **	4.127	**0.008** **
	Tree sp*div	2.621	0.054	1.835	0.091	0.606	0.612
P	Div_levels	1.763	0.187	0.028	0.868	4.855	0.059
	Tree sp	13.93	**<0.001*** **	4.374	**0.0003*** **	**2.084**	0.106
	Tree sp*div	2.392	0.072	1.459	0.194	1.600	0.193
C: N	Div_levels	0.230	0.632	3.323	0.072	2.490	0.056
	Tree sp	1.105	0.351	3.223	**0.004 ** **	5.940	**0.001** ***
	Tree sp*div	1.092	0.356	1.576	0.154	1.386	0.201
C:P	Div_levels	0.195	0.660	3.646	0.059	1.899	0.080
	Tree sp	1.566	0.202	3.574	**0.002 ** **	4.999	**0.002** **
	Tree sp*div	1.341	0.265	1.031	0.416	2.535	0.060
N:P	Div_levels	1.002	0.319	1.848	0.177	0.279	0.165
	Tree sp	0.502	0.681	2.925	**0.008 ** **	4.948	**0.002** **
	Tree sp*div	0.540	0.656	1.680	0.125	3.416	0.052
C:N:P	Div_levels	2.563	0.112	1.970	0.164	3.054	0.083
	Tree sp	19.50	**<0.001*** **	7.938	**<0.001*** **	4.181	**0.007** **
	Tree sp*div	2.767	0.055	1.789	0.101	0.304	0.822

Table S4. Two-way-ANOVA effects of tree diversity levels and mono (AM, EcM) and mixed (AE) myco-type tree species on bacterial genomic functional abundance

		AM		**AE**		**EcM**	
Soil nutrient	**Variable**	**F value**	***P***	**F value**	***P***	**F value**	***P***
C	Div_levels	0.759	0.385	3.869	0.052	4.806	0.055
	Tree sp	2.427	0.069	4.876	**0.001** ***	14.713	**0.001** ***
	Tree sp*div	2.292	0.082	1.014	0.820	3.950	0.060
N	Div_levels	1.112	0.294	0.317	0.574	2.448	0.067
	Tree sp	4.560	**0.004** **	4.370	**0.001** ***	4.668	**0.033** *
	Tree sp*div	0.278	0.841	1.818	0.052	1.145	0.334
P	Div_levels	0.485	0.487	1.481	0.0739	1.695	0.172
	Tree sp	10.78	**<0.001*** **	3.262	**0.004** **	20.95	**<0.001*** **
	Tree sp*div	2.978	0.054	2.099	0.052	2.266	0.085
C: N	Div_levels	0.012	0.912	2.228	0.087	4.676	0.054
	Tree sp	1.471	0.226	12.810	**<0.001*** **	12.781	**<0.001*** **
	Tree sp*div	0.249	1.326	4.047	0.076	3.116	0.093
C:P	Div_levels	0.661	0.418	2.518	0.116	6.466	0.050
	Tree sp	5.363	**0.001** **	5.588	**<0.001*** **	10.247	**0.001** **
	Tree sp*div	1.530	0.057	1.360	0.088	4.917	0.073
N:P	Div_levels	0.295	0.588	3.212	0.054	1.303	0.256
	Tree sp	8.984	**<0.001*** **	11.275	**<0.001*** **	6.431	**0.001** ***
	Tree sp*div	1.165	0.327	1.698	0.121	0.838	0.476
C:N:P	Div_levels	2.563	0.112	5.559	0.052	2.279	0.134
	Tree sp	19.50	**<0.001*** **	17.105	**<0.001*** **	18.04	**<0.001*** **
	Tree sp*div	2.767	0.055	4.197	0.079	2.333	0.078

Table S5. Effects of plot myco-type mixture and tree diversity level on the compositional differences of fungal and bacterial communities with C, N, P genomic potential based on PERMANOVA with 999 permutations

		Fungi		Bacteria	
Genomic potential	**Variable**	R^2	P	R^2	P
C	Div_levels	0.025	**0.001*****	0.005	0.413
	Plot_myco	0.265	**0.001** ***	0.055	**0.001*****
	Plot_myco*div	0.012	0.073	0.010	0.283
N	Div_levels	0.037	**0.001*****	0.020	**0.001*****
	Plot_myco	0.361	**0.001*****	0.136	**0.001*****
	Plot_myco*div	0.017	**0.015***	0.011	**0.037***
P	Div_levels	0.032	**0.002****	0.026	**0.005****
	Plot_myco	0.121	**0.001*****	0.150	**0.001*****
	Plot_myco*div	0.008	0.370	0.023	**0.004****
C: N	Div_levels	0.029	**0.001*****	0.144	0.168
	Plot_myco	0.289	**0.001*****	0.572	**0.001*****
	Plot_myco*div	0.013	0.057	0.034	0.384
C:P	Div_levels	0.028	**0.001*****	0.012	0.052
	Plot_myco	0.229	**0.001*****	0.094	**0.001****
	Plot_myco*div	0.010	0.141	0.014	0.075
N:P	Div_levels	0.036	**0.001*****	0.014	**0.028***
	Plot_myco	0.220	**0.001*****	0.084	**0.001*****
	Plot_myco*div	0.012	0.098	0.013	0.114
C:N:P	Div_levels	0.030	**0.001*****	0.012	0.048
	Plot_myco	0.252	**0.001*****	0.078	**0.001*****
	Plot_myco*div	0.011	0.083	0.012	0.056

Table S6. Effects of mono (AM, EcM) and mixed (AE) myco-type tree species and tree diversity level on the compositional differences of fungal communities with C, N, P genomic potential based on PERMANOVA with 999 permutations

		AM		**AE**		**EcM**	
Genomic potential	**Variable**	R^2	P	R^2	P	R^2	P
C	Div_levels	0.016	0.098	0.005	0.475	0.021	0.378
	Tree sp	0.256	**0.001** ***	0.184	**0.007** **	0.172	**0.001** ***
	Tree sp*div	0.035	0.133	0.085	0.228	0.025	0.074
N	Div_levels	0.008	0.221	0.018	0.077	0.070	0.181
	Tree sp	0.350	**0.001** ***	0.330	**0.001** ***	0.122	0.005 ***
	Tree sp*div	0.042	0.059	0.083	0.074	0.022	0.449
P	Div_levels	0.017	0.097	0.219	**0.002** **	0.052	0.061
	Tree sp	0.280	**0.001** ***	0.004	0.534	0.119	**0.001** ***
	Tree sp*div	0.041	0.081	0.086	0.170	0.035	0.172
C: N	Div_levels	0.015	0.120	0.006	0.439	0.030	0.224
	Tree sp	0.271	**0.001** ***	0.201	**0.001** ***	0.161	**0.001** ***
	Tree sp*div	0.037	0.128	0.085	0.182	0.045	0.101
C:P	Div_levels	0.016	0.109	0.005	0.509	0.028	0.266
	Tree sp	0.265	**0.001** ***	0.191	**0.005** **	0.159	**0.001** ***
	Tree sp*div	0.037	0.123	0.085	0.219	0.045	0.064
N:P	Div_levels	0.014	0.107	0.009	0.296	0.070	0.191
	Tree sp	0.306	**0.001*** **	0.255	**0.001** ***	0.106	**0.001** ***
	Tree sp*div	0.043	0.065	0.087	0.130	0.032	0.199
C:N:P	Div_levels	0.015	0.113	0.006	0.450	0.035	0.173
	Tree sp	0.275	**0.001** ***	0.205	**0.003** **	0.152	**0.001** ***
	Tree sp*div	0.038	0.095	0.086	0.177	0.043	0.090

Table S7. Effects of mono (AM, EcM) and mixed (AE) myco-type tree species and tree diversity level on the compositional differences of bacterial communities with C, N, P genomic potential based on PERMANOVA with 999 permutations

		AM		**AE**		**EcM**	
Genomic potential	**Variable**	***R²***	***P***	***R²***	***P***	***R²***	***P***
C	Div_levels	0.007	0.339	0.024	0.059	0.091	0.062
	Tree sp	0.099	**0.005 ****	0.222	**0.001 *****	0.104	**0.008 ****
	Tree sp*div	0.010	0.161	0.009	0.316	0.079	0.109
N	Div_levels	0.012	0.150	0.024	0.074	0.061	0.051
	Tree sp	0.176	**0.001*****	0.354	**0.001 *****	0.134	**0.001 *****
	Tree sp*div	0.041	0.041	0.091	0.050	0.041	0.074
P	Div_levels	0.006	0.414	0.029	0.057	0.098	0.050
	Tree sp	0.208	**0.001 *****	0.260	**0.004* ***	0.105	**0.001 *****
	Tree sp*div	0.056	0.061	0.024	0.052	0.042	0.084
C: N	Div_levels	0.009	0.271	0.002	0.189	0.120	**0.001 *****
	Tree sp	0.151	**0.001 *****	0.305	**0.001* ***	0.080	0.069
	Tree sp*div	0.055	0.271	0.028	0.110	0.057	0.096
C:P	Div_levels	0.006	0.359	0.028	0.091	0.064	0.081
	Tree sp	0.180	**0.001 *****	0.249	**0.001*****	0.107	0.001 ***
	Tree sp*div	0.061	0.359	0.020	0.074	0.052	0.098
N:P	Div_levels	0.008	0.298	0.028	0.156	0.084	0.052
	Tree sp	0.196	**0.001 *****	0.299	**0.001 *****	0.117	**0.001 *****
	Tree sp*div	0.052	0.057	0.094	0.079	0.042	0.063
C:N:P	Div_levels	0.008	0.325	0.083	0.101	0.083	0.058
	Tree sp	0.179	**0.001 *****	0.273	**0.002 *****	0.119	**0.001 *****
	Tree sp*div	0.056	0.009 **	0.092	0.092	0.049	0.089

Table S8. Comparative Regression Analysis of soil nutrient variable and microbial genomic functional abundance across mono (AM, EcM) and mixed (AE) myco-type. Soil indicate soil nutrient variable and Genomic indicate genomic functional potential processes.

	AM			AE			EcM		
Fungi	**Coefficient**	**R**	**P**	**Coefficient**	**R**	**P**	**Coefficient**	**R**	**P**
Soil C vs Genomic C	-2810.3	0.023	0.109	-2319.1	0.006	0.460	2257.1	0.014	0.208
Soil N vs Genomic N	-16587.3	0.009	0.322	-33714.4	0.026	0.115	1425.2	0.000	0.903
Soil P vs Genomic P	-295.7	0.004	0.526	762.5	0.018	0.192	31.122	0.000	0.929
Soil C:N vs Genomic C:N	-0.020	0.013	0.225	0.012	0.001	0.721	0.014	0.004	0.525
Soil C:P vs Genomic C:P	0.008	0.015	0.193	0.000	0.000	0.987	-0.003	0.002	0.640
Soil N:P vs Genomic N:P	-0.037	0.002	0.630	0.038	0.000	0.861	-0.059	0.009	0.321
Soil C:N:P vs Genomic C:N:P	0.000	0.008	0.358	0.000	0.001	0.727	0.000	0.000	0.908
Bacteria	**Coefficient**	**R**	**P**	**Coefficient**	**R**	**P**	**Coefficient**	**R**	**P**
Soil C vs Genomic C	-22.620	0.000	0.844	488.367	0.024	0.131	204.161	0.011	0.282
Soil N vs Genomic N	2253.3	0.032	0.059	2487.8	0.029	0.095	1032.0	0.004	0.502
Soil P vs Genomic P	-33.556	0.004	0.517	25.356	0.001	0.774	38.608	0.004	0.531
Soil C:N vs Genomic C:N	-0.006	0.021	0.127	0.004	0.002	0.672	0.012	0.029	0.071
Soil C:P vs Genomic C:P	-0.001	0.005	0.449	-0.004	0.022	0.149	0.000	0.001	0.790
Soil N:P vs Genomic N:P	-0.008	0.004	0.510	-0.016	0.004	0.524	0.017	0.018	0.163
Soil C:N:P vs Genomic C:N:P	0.000	0.002	0.653	0.000	0.005	0.499	0.000	0.000	0.899

Table S9. Comparative regression analysis of soil nutrient variable and microbial genomic functional abundance in tree species richness either in mono (AM, EcM) and mixed (AE) myco type. Soil indicate soil nutrient variable and Genomic indicate genomic functional potential processes.

Fungi						
AM	**1sp**		**2sp**		**4sp**	
	R^2	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C	0.29084	0.03109355	0.00977	0.59040	0.01535	0.32928
Soil N vs Genomic N	0.00240	0.85694760	0.01737	0.4721	0.00781	0.48725
Soil P vs Genomic P	0.00675	0.76224491	3.03E-05	0.97614	0.00033	0.88627
Soil C:N vs Genomic C:N	0.01097	0.69935628	0.02164	0.42163	0.00843	0.47052
Soil C:P vs Genomic C:P	0.32220	0.02181831	0.00877	0.61017	0.00942	0.44536
Soil N:P vs Genomic N:P	0.21474	0.07063614	0.00098	0.86457	0.01614	0.31713
Soil C:N:P vs Genomic C:N:P	0.00112	0.90188392	0.02850	0.35560	0.00836	0.47232
AE	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C			0.03516	0.30407	0.00035	0.88309
Soil N vs Genomic N			0.01602	0.48993	0.03146	0.16083
Soil P vs Genomic P			0.04564	0.24037	0.00554	0.55859
Soil C:N vs Genomic C:N			0.00037	0.91604	0.00335	0.64933
Soil C:P vs Genomic C:P			0.00055	0.89848	0.00305	0.66469
Soil N:P vs Genomic N:P			0.00049	0.90404	0.00762	0.49255
Soil C:N:P vs Genomic C:N:P			0.00066	0.88839	0.00054	0.85443
EcM	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C	0.02235	0.58046536	0.04859	0.22538	0.00224	0.70997
Soil N vs Genomic N	0.00909	0.72536495	2.04E-05	0.98044	0.00600	0.54276
Soil P vs Genomic P	0.05952	0.36250425	0.00839	0.61787	2.41E-0	0.99027
Soil C:N vs Genomic C:N	0.38190	0.01073323	0.08343	0.10885	0.00109	0.79540
Soil C:P vs Genomic C:P	0.00177	0.87690777	0.06296	0.16596	0.00592	0.54539
Soil N:P vs Genomic N:P	0.00977	0.71559849	0.01571	0.49420	0.01882	0.27966
Soil C:N:P vs Genomic C:N:P	0.00098	0.90833451	0.00024	0.93228	0.00319	0.65717
Bacteria						
AM	**1sp**		**2sp**		**4sp**	
	R^2	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C	0.00699	0.75815674	0.00202	0.80688	0.00635	0.53131
Soil N vs Genomic N	0.00065	0.92512646	0.04807	0.22794	0.03460	0.14108
Soil P vs Genomic P	0.07697	0.29819032	0.05762	0.18571	0.03536	0.13674
Soil C:N vs Genomic C:N	0.15294	0.13417353	0.00182	0.81655	0.03236	0.15486
Soil C:P vs Genomic C:P	0.04474	0.43163198	0.12176	0.05030	0.02191	0.24304
Soil N:P vs Genomic N:P	0.15116	0.13666402	0.0283	0.35738	0.01840	0.28514
Soil C:N:P vs Genomic C:N:P	8.52E-6	0.97294214	0.17384	0.01759	0.05853	0.05409
AE	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C			2.95E-05	0.97647	0.04722	0.08454
Soil N vs Genomic N			0.00027	0.92844	0.09001	0.01601
Soil P vs Genomic P			0.01884	0.45378	0.00384	0.62664
Soil C:N vs Genomic C:N			0.01096	0.56844	0.01361	0.35853
Soil C:P vs Genomic C:P			0.05582	0.31446	0.00026	0.89906

Soil N:P vs Genomic N:P			0.08516	0.10508	0.01777	0.29361
Soil C:N:P vs Genomic C:N:P			0.04545	0.24138	0.00178	0.74010
EcM	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**
Soil C vs Genomic C	0.0002	0.95266962	0.10085	0.07652	0.00446	0.59994
Soil N vs Genomic N	0.0320	0.50696734	0.07645	0.12552	0.00978	0.43676
Soil P vs Genomic P	8.44E-0	0.97306274	0.08956	0.09610	0.03786	0.12339
Soil C:N vs Genomic C:N	0.1074	0.21514820	0.0379	0.28564	0.01177	0.39340
Soil C:P vs Genomic C:P	0.1855	0.10256178	0.11120	0.06215	1.14E-0	0.99332
Soil N:P vs Genomic N:P	0.0041	0.81184376	0.11380	0.05900	0.01077	0.41441
Soil C:N:P vs Genomic C:N:P	0.2250	0.06338140	0.10226	0.07439	0.00335	0.64939

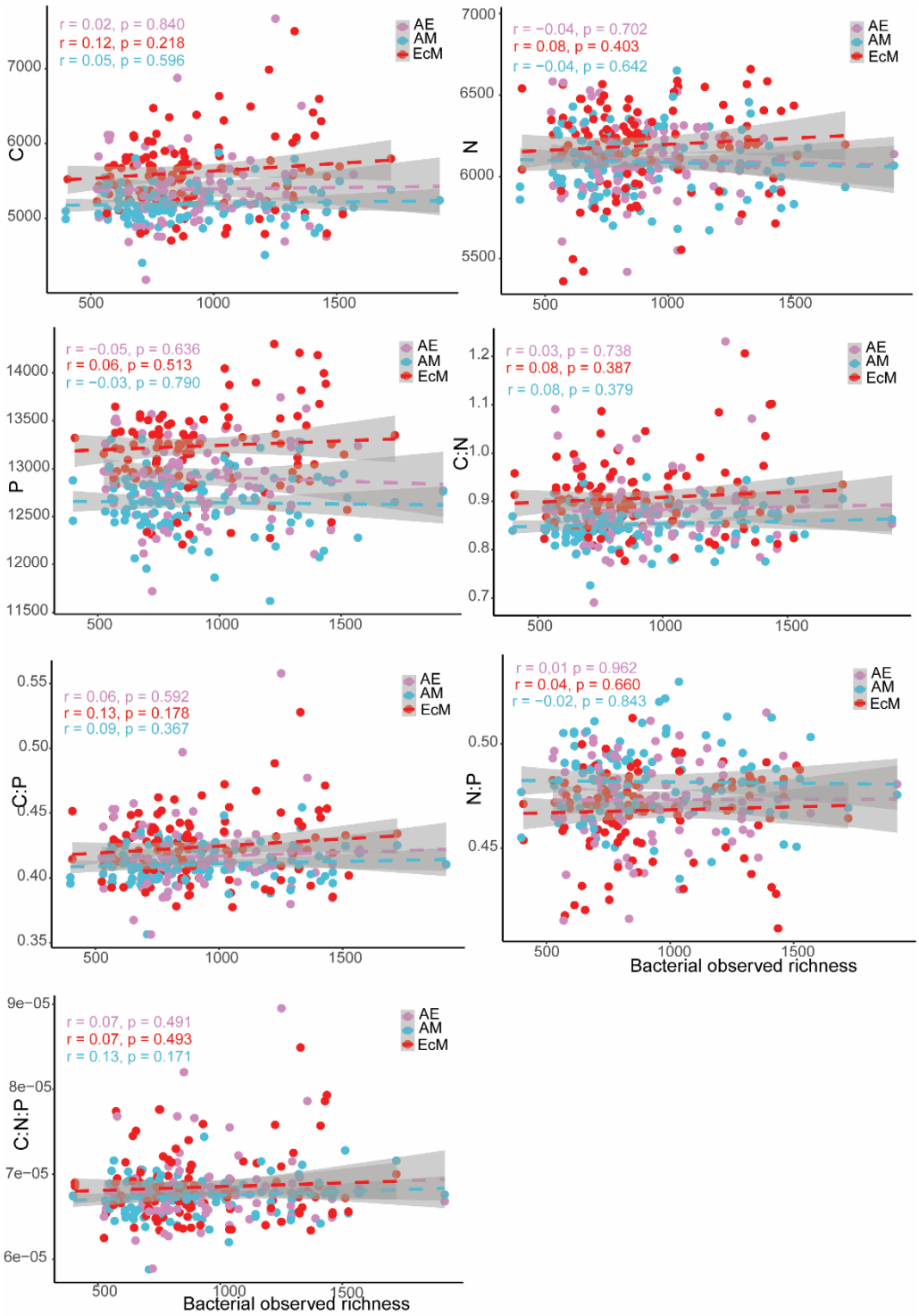

Figure S1. Relationships between bacterial genomic functional abundance with bacterial observed richness within mono (AM, EcM) and mixed (AE) myco-type plots. The *r* and *p* values were calculated using a linear regression model. Regression lines are represented as solid for significant effects ($p < 0.05$) and dashed for non-significant effects ($p > 0.05$)

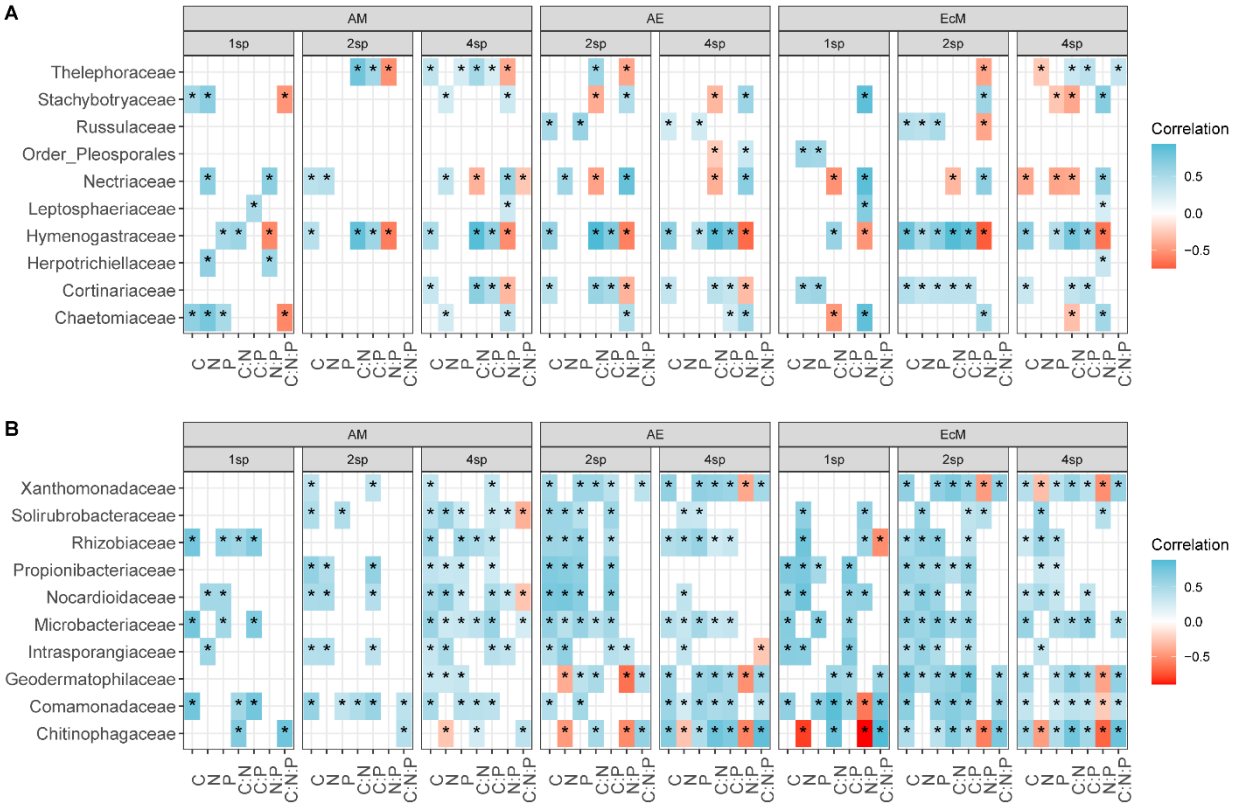

Figure S2. Spearman correlations between the relative abundance of the dominant fungal (A) and bacterial (B) families and microbial genomic functional abundance. The red color indicates negative correlations, while the sky-blue color indicates positive correlations.

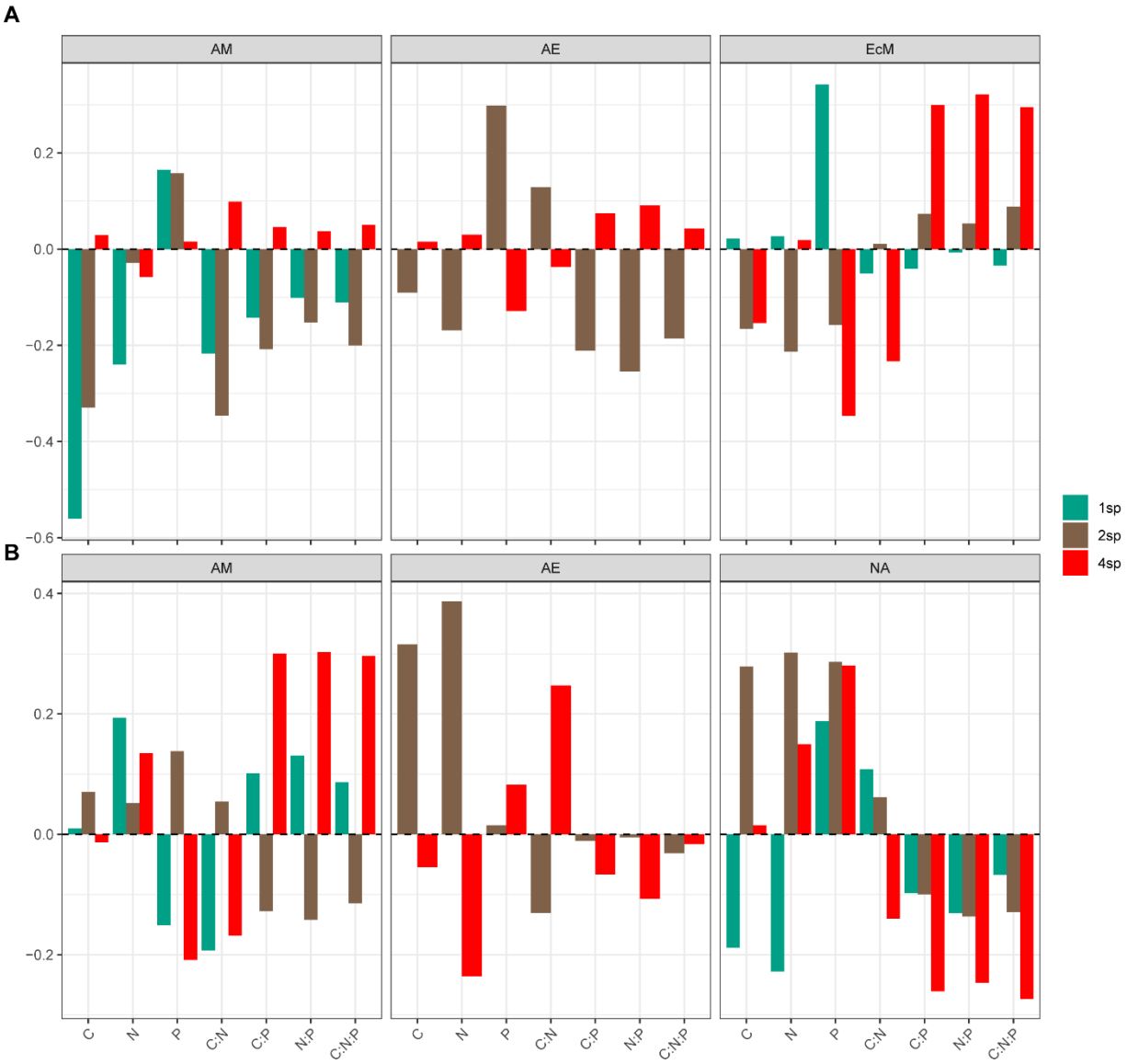

Figure S3. Relationships between soil nutrient variable with fungal (A) and bacterial genomic functional abundance across tree species richness levels either in mono (AM, EcM) and mixed (AE) myco-type plot. Correlation coefficient and p values were calculated using a Pearson correlation model.

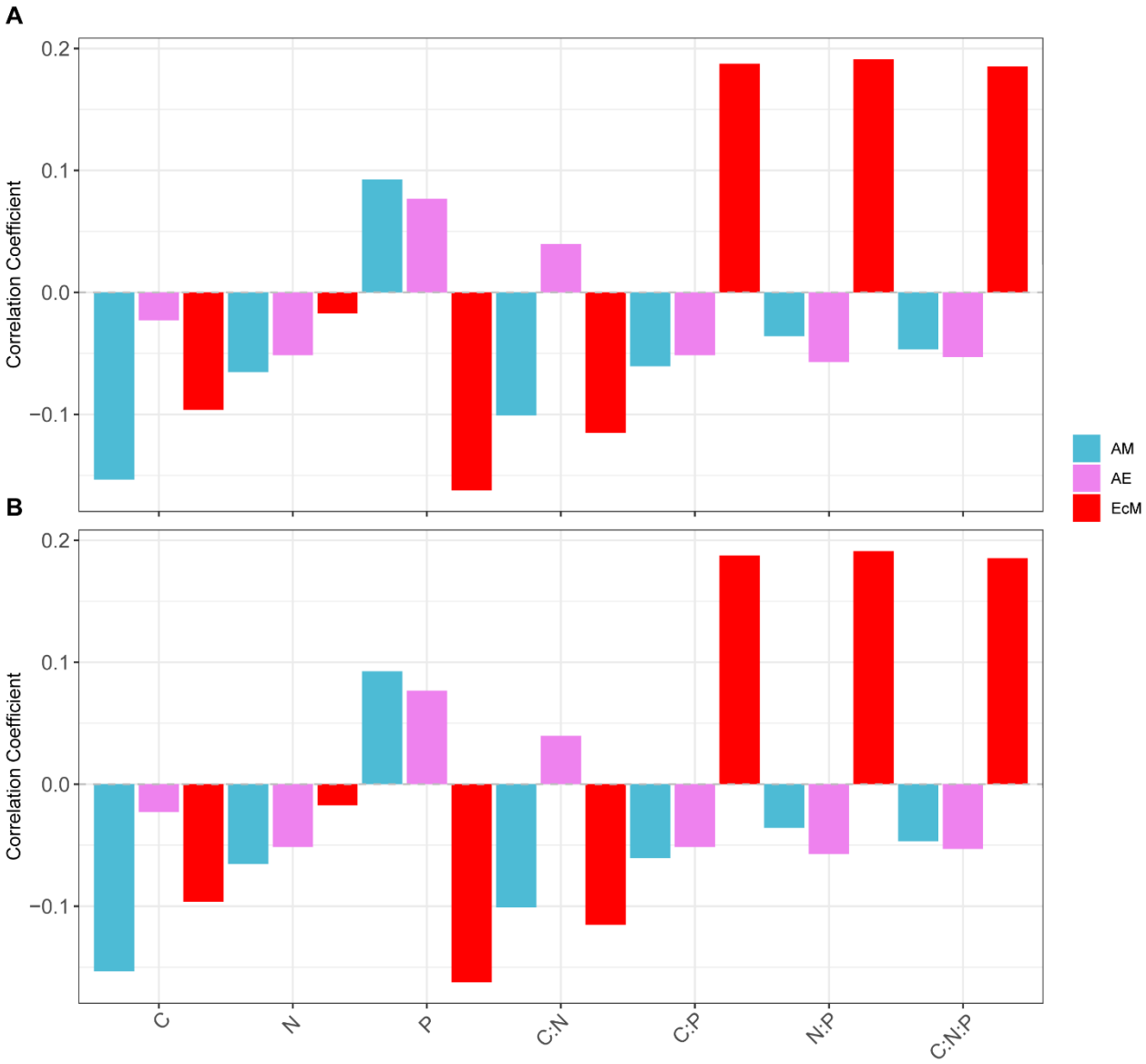

Figure S4. Relationships between soil nutrient variable with fungal (A) and bacterial genomic functional abundance between mono (AM, EcM) and mixed (AE) myco-type plot. Correlation coefficient and p values were calculated using a Pearson correlation model.

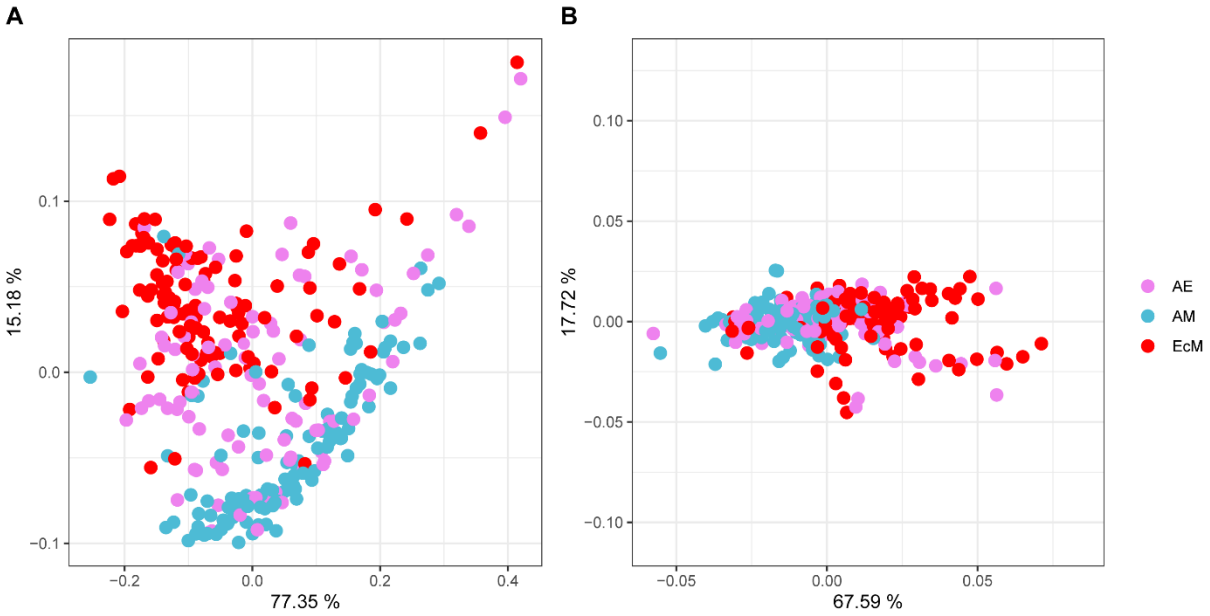

Figure S5. Principal coordinate analysis (PCoA) plots showing the relationship between the soil nutrient variable and fungal (A) and bacterial (B) genomic functional potential community composition in mono (AM, EcM) and mixed (AE) myco-types. Arrows show the post-hoc correlation between PCoA axes and microbial genomic functional abundance with only significant factors shown.

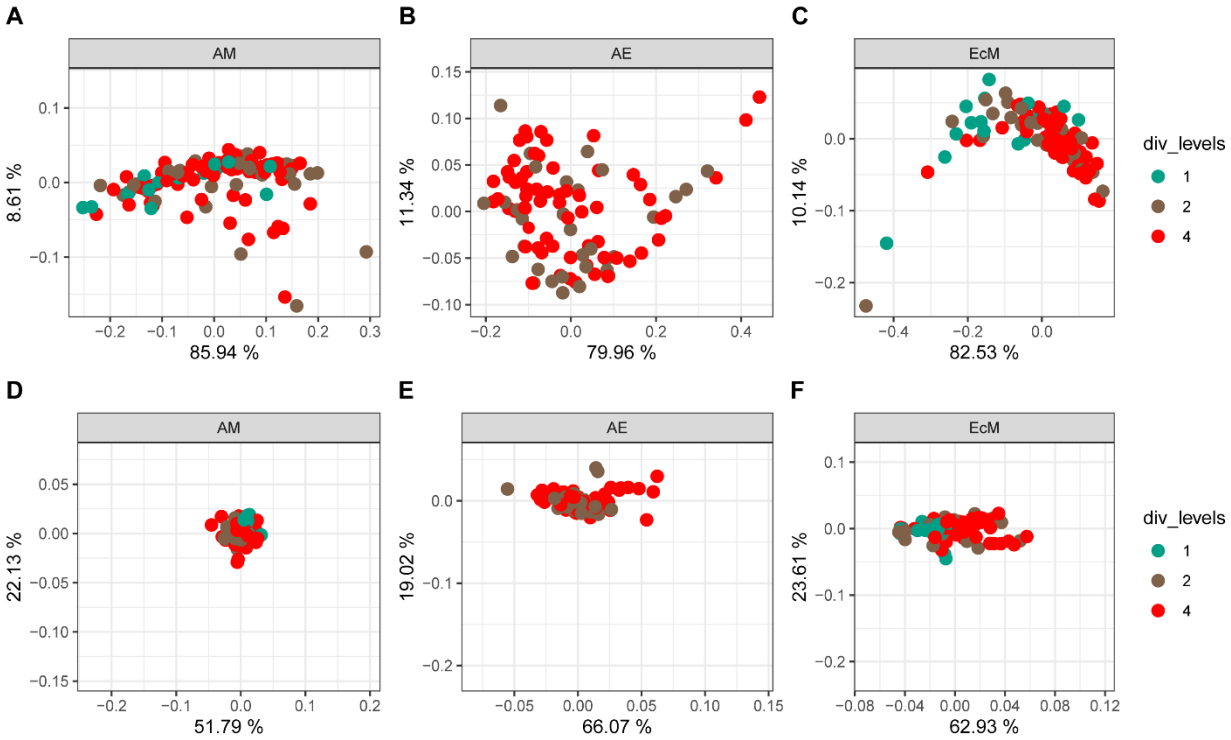

Figure S6. Principal coordinate analysis (PCoA) plots showing the relationship between the soil nutrient variable and fungal (A, B, C) and bacterial (D, E, F) genomic functional potential community composition in tree communities composed either of mono (AM, EcM) or mixed (AE) myco-types. Arrows show the post-hoc correlation between PCoA axes and microbial genomic functional abundance with only significant factors shown.

CHAPTER 5
General Discussion

The impact of mixing different mycorrhizal tree species at varying levels of tree diversity on belowground fungal and bacterial community structure, assembly processes, and functional potential remains largely unexplored. This thesis investigates how tree mycorrhizal type (myco-type), plot myco-type mixture (mono: AM, EcM; and mixed: AM and EcM), tree diversity and rooting zone compartment (rooting and interactive zones) influence the microbial community structure, community assembly processes, and microbial genomic functional abundance of soil fungal and bacterial communities. This final chapter provides a concise overview of the main results, explores their significance, acknowledges the study's constraints, and suggests avenues for subsequent investigations.

Summary of results

In **chapter 2**, I proposed that soil fungal diversity and composition are affected by tree myco type (AM or EcM), plot myco-type mixture (mono or mixed), tree diversity and rooting zone compartment. Specifically, I hypothesized that tree myco-type, myco-type mixtures, and rooting zone compartments independently and interactively impact soil fungal diversity, with AM trees contributing most in both mono- and mixed myco-type stands. I further proposed that plot myco-type mixtures, tree diversity, and environmental conditions shape fungal community composition, with these effects diminishing as tree diversity increases. Further, I hypothesized that mixed myco-type stands host a higher proportion of shared generalist and specialized fungal communities with AM than with EcM tree species in high-diversity plots. My findings revealed significant individual and interactive effects of tree myco-type, myco-type mixture, and tree diversity, with no effect from the rooting zone compartment. Fungal diversity is significantly lower in EcM than in AM tree species in mono myco-type stands, while in mixed myco-type stands, fungal diversity was intermediate with increasing tree diversity. Additionally, I found significant main effects of tree myco-type, myco-type mixture, and tree diversity, as well as interactive effects between myco-type and myco-type mixture on fungal community composition but no effect of the rooting zone compartment. As tree diversity increased, fungal community composition generally converged with community dissimilarity varying based on the co-existing tree species myco-type and tree species diversity. In addition to plot myco-type mixture, tree myco-type, and tree diversity, soil variables also significantly

influenced the fungal community composition. I also found that specialized fungal communities are more prevalent in mixed myco-type plots (13.2%) than in EcM stands (9.5%), but less so than in AM stands (11.7%). Moreover, AM multi-species mixtures (20%) exhibited a higher degree of specialization than EcM multi-species mixtures (11.8%).

In **chapter 3**, I investigated the examined the interactive effect of tree myco-type, myco-type mixture (mono or mixed) and tree diversity in shaping the rooting zone soil microbial diversity and composition and evaluated the drivers of the microbial community assembly processes. I found that AM plots exhibited higher fungal richness compared to EcM and mixed myco-type plots, whereas there is no effect of tree species identity on fungal and bacterial alpha diversity within mono and mixed myco-type plots. The soil fungal community composition is shaped by tree species identity, tree diversity, and plot myco-type mixture, while bacterial community composition is only affected by tree species identity. Further, I found that EcM plots fungal community dissimilarity increased with increasing tree diversity in contrast to AM and AE plots. Specifically, I observed significant effects only for tree species both for soil fungal and bacterial communities, and none of tree richness or of the interaction of tree species with tree richness. Plot myco-type mixture and tree diversity level displayed interactive effects on the fungal and bacterial community composition, while AM and EcM plots showed contrasting patterns with increasing tree diversity. Further, I found that that deterministic and stochastic processes together determine the assemblage of mono AM or EcM tree communities and those of mixed myco-types, while in the direction from AM to EcM plots deterministic processes, mainly due to homogeneous selection, decrease and stochastic processes increase, mainly due dispersal limitation. Further, the stochastic processes affected fungal and bacterial community assembly differently, through dispersal limitation and homogenous dispersal, respectively. Additionally, the core to rare taxa in fungal subcommunities are mostly controlled by both stochastic and deterministic processes, whereas bacterial subcommunities are dominantly affected by stochastic processes.

In **chapter 4**, I explored how plot myco-types mixture, tree species identity, tree diversity, and microbial diversity and composition shape microbial functional potentials. Specifically, I hypothesized that co-existing mono and mixed-myco types influence microbial genomic functional potential in tree diversity. I proposed that soil microbial diversity and composition might predict functional potential, with plot myco-type mixtures and tree diversity affecting this relationship. Specifically, trees with different mycorrhizal types likely shape microbial richness and composition in distinct ways due to their contrasting nutrient strategies. Finally, I

anticipated that soil nutrient availability (C, N, P concentrations), influenced by both mycorrhizal type mixture and tree diversity, would correlate strongly with microbial functional potentials. I found that tree species identity significantly influenced both fungal and bacterial functional potentials, with specific variation in C, N, P cycles and their ratios in mono and mixed myco-type plots. Fungal richness decreased with C and P cycling in EcM plots, while N cycling increased in AM plots. In contrast, bacterial richness showed no significant relationship with tree species diversity in either mono or mixed myco-type stands. In addition, I found a strong influence of plot myco-type mixture on fungal and bacterial genomic community compositional differences. Furthermore, Additionally, tree species diversity similarly influenced fungal genomic composition. For bacterial communities, tree species richness affected the potential of taxa involved in the P cycle and the N cycle ratio. The C, N, P cycles and their ratios contributed differentially to shaping the soil microbial communities in mono and mixed myco-types and tree diversity levels. Soil nutrient concentrations showed no significant correlations with microbial functional potentials or community composition in tree species richness and plot myco-type mixtures.

Discussion

To achieve a broad comprehension of the functionality of ecosystems, it is imperative to study a wide range of factors. My thesis demonstrated the importance of considering both mono (AM, EcM) and mixed (AM and EcM) mycorrhizal types, tree diversity, rooting zone compartment and soil nutrient variables together. This approach enhances my understanding of the structure, community assembly processes, and functional abundance and their relationship with microbial community structure of forest belowground communities. For example, AM plot exhibited higher fungal diversity than EcM plots, especially with increasing tree diversity (chapters 2 & 3). This aligns with earlier research findings from subtropical forests indicating that AM-dominated soils have higher fungal diversity relative to EcM plots (Singavarapu *et al.* 2022a). The observed difference in alpha diversity between AM and EcM mono myco-type stands at each tree diversity level can be attributed to the higher host specificity of EcM trees and the dual function of EcM fungi as both mycorrhizal partners and saprotrophs. In contrast, AM trees rely on both mycorrhizal and saprotrophic fungi to support nutrient cycling and uptake in their rooting zones (Phillips, Brzostek & Midgley 2013b). My finding of increased fungal richness in mixed myco-type stands compared to pure EcM stands with higher tree diversity may be due to the presence of additional AM fungal partners and their associated saprotrophic fungal communities. A research from (Peay, Baraloto & Fine 2013) revealed that AM trees

communities tended to have higher fungal diversity in mixed species forests compared to EcM trees communities, which are often more host-specific, potentially limiting diversity under higher tree diversity conditions. Further, my results indicate that fungal diversity was higher in mixed myco-types compared to mono EcM tree species with increasing tree diversity (chapters 2 & 3). This emphasizes the significance of plot myco-type mixtures. The results of earlier research on the connection between belowground microbial communities and tree diversity have been equivocal, with some suggesting that there is no meaningful relationship (Navrátilová *et al.* 2019; Rivest, Whalen & Rivest 2019; Otsing *et al.* 2021) and others showing significant relationships (Weißbecker *et al.* 2019; Ferlian *et al.* 2021). Specifically, (Rivest, Whalen & Rivest 2019) found no correlation between fungal diversity and tree diversity. However, they did not consider tree mycorrhizal type in their study. The results of my dissertation indicate that future research could gain more comprehensive insights into how tree diversity impacts underground microbial ecosystems by considering the combined effects of mono and mixed myco-types alongside tree diversity. This approach may help reconcile some of the current discrepancies in the field.

Plot myco-type mixture is a crucial determinant of the composition of microbial communities, as evidenced by PERMANOVA analyses on target rooting zone microbiome (chapters 2 & 3) and microbial functional potential (chapter 4). Previous research has primarily focused on either mycorrhizal communities (Gao *et al.* 2013; Neuenkamp *et al.* 2021) or entire fungal communities (Weißbecker *et al.* 2019; Heklau *et al.* 2021; Singavarapu *et al.* 2022a; Singavarapu *et al.* 2023). The influence of plot myco-type mixture in mono and mixed myco-type stands on fungal and bacterial communities' composition has not been extensively studied. For instance, (Singavarapu *et al.* 2022b) discovered that the bacterial communities in a subtropical forest were not significantly impacted by the plant mycorrhizal type. Although the PERMANOVA analyses in this thesis revealed no substantial impact on bacterial communities, but highlighted the influence of plot myco-types mixture on the bacterial and fungal genomic functional community composition (chapter 4). This suggests that AM and EcM tree mycorrhizal associations significantly affect C, N, and P cycle which in turn influence the composition and structure of microbial communities. These findings showed a close link between mycorrhizal activity and nutrient cycling.

Furthermore, my results indicate that increasing tree diversity reduces effects of the trees' mycorrhizal type on fungal community composition (chapter 2), microbial community assembly processes (chapter 3) and microbial functional potential (chapter 4). These findings are consistent with existing research that suggests that higher diversity promotes more

homogenized and resilient microbial communities. Previous literature from different ecosystem indicate that in diverse tree communities varied root exudates and broader nutrient inputs create overlapping niches that dilute the dominance of specific fungal species leading to reduced compositional specificity (Hoeksema *et al.* 2010; Singavarapu *et al.* 2024). This pattern shifts microbial assembly from deterministic processes where specific microbes are selected by host plants to stochastic processes where microbial colonization becomes more random due to weakened niche differentiation. (Stegen *et al.* 2012; Zhou *et al.* 2013) reported consistent shifts in microbial community assembly as tree diversity increased, leading to neutral interactions that decrease the predictability of microbial community composition. Additionally, the homogenization of microbial functional potential in high-diversity tree stands aligns with findings by (Delgado-Baquerizo *et al.* 2016). This supports the concept of functional redundancy where diverse microbial communities can provide similar ecosystem functions across various conditions, enhancing resilience to environmental changes. One other study showed that increased plant diversity promotes such microbial functional redundancy, thus reinforcing stability in ecosystem processes (van Der Plas *et al.* 2016). Together, these studies align with my findings, suggesting that increased tree diversity drives microbial communities toward generalized functional responses and stochastic assembly processes, leading to reduced compositional specificity and functional potential. Thus, my dissertation (chapters 2-4) demonstrates that with increasing tree diversity their microbial coexistence increased. indicating greater resource complementarity and facilitating a wider range of microbial interactions. This supports more stable and diverse microbial communities.

Moreover, findings from (chapter 3) emphasized that community assembly processes and genomic functional potential (chapter 4) showed different response to mono (AM, EcM) and mixed (AM and EcM) myco-type, which can be explained through differences in rooting structures and nutrient acquisition strategies of trees associated with different mycorrhizal types. Trees associated with AM and EcM fungi display distinct rooting structures that shape soil environments and influence microbial communities. AM trees develop fine shallow roots that enhance surface nutrient uptake, favouring fast-growing generalist microbes and promoting community assembly driven by stochastic processes (Smith & Read 2010). Conversely, EcM trees form thicker deeper roots that access nutrients in subsoil layers creating specialized niches and fostering selective, deterministic microbial communities adapted to slower nutrient cycling typical of EcM systems (Brzostek *et al.* 2015). AM fungi primarily absorb inorganic nutrients such as phosphate supporting microbial communities adapted to high inorganic nutrient fluxes (Phillips, Brzostek & Midgley 2013a). In contrast, EcM fungi specialize in obtaining complex

organic nutrients particularly nitrogen and phosphorus bound in organic matter selecting for microbes with genes that facilitate organic decomposition (Smith & Read 2010; Averill, Turner & Finzi 2014).

Surprisingly, the analysis from this thesis revealed no notable variations in the composition of fungal communities between the two studied soil compartments, that is the rooting zones of the target trees and the locations where their roots intersect with those of neighbour trees (chapter 2). The main possible reason for this finding could be a lack of clear rooting zone separation since in the studied experimental site trees are grown 1 m away from each other, and 8 years after planting, their root systems probably were already fully intertwined, leading to a target tree rooting and interaction zone continuum, where the microbial community composition was equally controlled by the target tree and its neighbours in both rooting zone compartments.

Additionally, the results in (chapter 2) revealed that soil nutrients have varying impact on the composition of belowground fungal communities in relation to mono and mixed myco-types as well as tree diversity. Soil fungal communities in mono myco-types (AM or EcM) were found to have a stronger relationship with soil nutrient concentrations, unlike those with mixed myco-types. This correlation is likely because in plots dominated by a single mycorrhizal type, nutrient availability and utilization patterns are more straightforward, leading to a clearer association between soil nutrients and fungal communities. For instance, AM fungi are known to increase soil phosphorus availability, directly influencing fungal community composition. In contrast, in mixed myco-type plots interactions between different mycorrhizal fungi and their varying nutrient uptake strategies create more complex and less predictable patterns. The presence of mixed myco-types can result in competing or synergistic effects, obscuring clear associations between fungal communities and soil nutrients.

Notably, as tree species diversity increased, the number of soil nutrient that showed significant associations with soil fungal communities decreased. This trend was observed for both mono and mixed myco-types compared to stands with less diverse tree diversity. This highlights that higher tree species richness can lead to functional redundancy in soil fungal communities, where different fungal species perform similar ecological roles. This redundancy implies that soil fungal communities might be less sensitive to changes in specific environmental variables, as many species or functional groups can fulfill similar ecological functions (Mangan *et al.* 2010).

A distinctive feature of this dissertation is the identification of soil microbial community assembly processes and different ecological processes drove the fungal and bacterial assembly

(chapter 3). The differences between bacteria and fungi align with previous reports indicating that microbial community distribution variability is linked to assembly processes, with distinct response patterns observed between bacteria and fungi (Wang *et al.* 2023). This is attributed to the larger size and lower dispersal potential of fungi, which restricts their spread over large distances (Chen *et al.* 2020; Zhang *et al.* 2021). In contrast, smaller-sized bacteria can disperse more easily through various vectors such as wind and animal movement (Powell *et al.* 2015; Luan *et al.* 2020; Zhang *et al.* 2021).

Further, I found that core microbial subcommunities are predominantly influenced by stochastic processes, while rare fungal subcommunities are more driven by deterministic processes. This finding contrast with previous reports that core taxa are often influenced by environmental constraints (Mohapatra *et al.* 2023), whereas rare taxa may face challenges in competing for limited resources, leading to the prevalence of deterministic processes among them (Zhu *et al.* 2023). Core taxa due to their higher relative abundance are less susceptible to dispersal constraints, while rare taxa with low abundance and limited dispersal capacity are more susceptible to environmental filtering and exhibit heterogeneous distribution across mono and mixed myco-types (Mohapatra *et al.* 2023).

Moreover, a comparative analysis of the correlation between fungal and bacterial communities in the rooting zone soils of mono and mixed myco-type plots in tree species richness levels revealed their intricate relationships within forest ecosystems. In mono myco-type plots, distinct patterns emerged between AM and EcM associations. This suggests that the co-existence of different AM tree species may exert differential influences on microbial community dynamics compared to EcM trees as tree diversity increases, resulting in lower Procrustes correlations in AM tree plots. Conversely, in mixed mycorrhizal-type plots comprising both AM and EcM tree species, the similarity of fungal and bacterial community composition patterns increased with rising tree richness. This implies that in mixed myco-type plots, higher tree diversity fosters greater intra-kingdom and cross-kingdom convergence or similarity in microbial community dynamics. The observed variation in fungal and bacterial community concordance in mono and mixed mycorrhizal-type stands provides insights into the role of mycorrhizal preference in tree-tree interactions within forest ecosystems.

My research yielded unexpected and fascinating results concerning the tree species diversity. Notably, the lack of a substantial effect of tree diversity on the belowground fungal diversity in AM tree species stands in contrast to the observations made for EcM tree species (chapters 2 & 3). The observed positive influence of EcM tree species on fungal diversity might be attributed

to complementarity effects arising from novel niche opportunities provided by coexisting tree species and their mycorrhizal associates (Trogisch *et al.* 2021). However, the absence of such an effect in AM tree species warrants additional exploration. My study concentrated on alpha diversities at the ASV level, but it is possible that the positive impact on richness occurs at a different taxonomic rank, such as order or genus levels, which I have not yet examined.

Furthermore, I noted that tree diversity had no substantial effect on the diversity of the belowground microbiome, their community assembly processes, or microbial genomic functional abundance in both mono and mixed myco-type plots (chapters 2, 3 & 4). This absence of effect on soil microbial diversity and nutrient dynamics may be explained by the fact that the trees in the MyDiv experiment were relatively young, having been established in 2015 and grown for only seven years when sampling occurred. This corresponds with research showing that the impact of tree species diversity on ecosystem functions tends to become more pronounced over time in both grassland and forest biodiversity studies (Guerrero-Ramírez *et al.* 2017; Huang *et al.* 2018). Changes in biogeochemical properties often require extended periods to become evident (Oelmann *et al.* 2007; Lange *et al.* 2023). Moreover, residual land-use legacies from previous agricultural activities may endure, indicating that soil microbial community development may not be significantly impacted by tree diversity within a short timeframe (Fichtner *et al.* 2014).

Secondly, the study revealed a surprising absence of significant effects on soil microbial community diversity and composition, even at higher levels of tree diversity. This finding was particularly unexpected given the strong host specificity typically observed in soil-associated microbiome and the anticipated influence of tree species identity on these communities. (Hoeksema *et al.* 2018). The interaction between soil nutrients, mono and mixed myco-types, and tree species diversity suggests that the two key ecological processes dispersal and selection are likely driving these observations. In plots where, multiple tree species with various mycorrhizal associations are combined their close proximity increases the likelihood of both active and passive dispersal, making microbial inoculum accessible to all tree species present. As time progresses trees engage in the selection of specific microbes through biotic and abiotic filtering processes which are influenced by various factors including tree species-specific root exudates (Jones *et al.* 2019).

The conclusions drawn from my thesis research and the mechanisms identified at the local level can be applied to various forest ecosystems, as comparable trends have been observed in studies across different biomes. For example, research conducted in temperate (Heklau *et al.* 2021),

boreal (Bahram *et al.* 2020), and tropical forests (Tedersoo & Nara 2010) has shown reduced diversity in belowground fungal communities associated with EcM tree species and EcM-dominated sites. Furthermore, investigations in temperate and boreal regions have revealed an inverse relationship between saprotrophs and the prevalence of EcM plants, as well as a positive correlation with AM plants (Bahram *et al.* 2020; Eagar *et al.* 2022b).

My thesis has shown that the highly diverse microbial communities converge both taxonomically and functionally with increasing tree species richness. This increased diversity may contribute to improved ecosystem functioning. Prior studies at our research site have indicated that greater tree diversity enhances forest productivity (Ray *et al.* 2023). Microbial diversity could be a key factor underlying this relationship. Collectively, my research suggests that combining tree species with different mycorrhizal associations at high diversity levels can promote varied belowground microbial communities. These communities exhibit distinct assembly processes and strong connections to genomic functional potential, potentially bolstering the preservation of forest ecosystem services. Given the increasing recognition of forests' crucial role in addressing climate change, a primary recommendation from my thesis is to incorporate tree species with diverse mycorrhizal types in both mono and mixed myco-type plantings at high diversity levels for afforestation and reforestation initiatives. Similarly, for commercial endeavours like timber production, planting diverse tree species mixtures could yield higher long-term productivity. Microbial taxa that are functionally diverse and resilient in nutrient cycling can boost tree growth, thereby enhancing overall productivity.

Limitations of the research

First, the study uses of universal primers (ITS2 region of 16S rRNA) successfully captured the taxonomic diversity of fungal and bacterial samples, as demonstrated by the distinct fungal taxa patterns observed in the experimental treatments. Nevertheless, employing AM fungal-specific primers based on the 18S rRNA large and/or small subunit could have provided a more detailed analysis of arbuscular mycorrhizal fungal diversity, offering a more comprehensive understanding of the AM fungal communities.

Second, the db-RDA analyses provided crucial insights into the intricate associations between microbial communities and soil nutrients concentration. However, these analyses were unable to establish the causality of the observed effects, which would have further clarified the underlying mechanisms at play.

Third, Chapter 2 offered a thorough insight into the structure and composition of fungal communities associated with the rooting zone. However, a more exhaustive characterization of underground communities could have been achieved by incorporating information on bacterial communities.

Fourth, PICRUSt2's predictions of functional potential proved highly valuable and economical in revealing new insights into the nutrient cycling capabilities of underground bacterial and fungal communities. Nevertheless, for a more comprehensive analysis of genomic potential and gene expression, metagenomic and metatranscriptomics studies would be necessary.

Fifth, throughout all chapters, the focus was on the soil microbiome within the rooting zone, aiming to provide a thorough understanding of the microbial community's structure, composition, assembly processes, and the microbial functional genomic potential. Nevertheless, a more exhaustive characterization of underground communities could have been obtained by including data related to roots as well.

Concluding remarks and future research directions

As climate change events intensify the significance of multifunctional forests in combating adverse effects like global warming and desertification becomes increasingly apparent. This doctoral research revealed that mono and mixed myco-types, as well as tree diversity, are critical in determining the community structure, community assembly processes, and genomic functional potentials of the forest belowground microbial communities. The findings indicated that mono and mixed myco-type is a fundamental factor in shaping the diversity and composition of these communities, in accordance with the functional requirements of the environment. The research also emphasized the importance of various ecological interactions between plants and microorganisms, including relationships in microbial diversity and composition. This investigation has made a substantial contribution to our overall comprehension of ecosystems function, and it emphasizes the importance of considering the interactions between mono and mixed mycorrhizal types as well as tree diversity in management of forest ecosystems. The findings from my thesis offer numerous avenues for future research to enhance our knowledge of forest ecosystem function. Soil ecosystems are influenced by a multitude of factors, both above and below the ground, which necessitates the use of interdisciplinary approaches to gain a more thorough understanding. My thesis was conducted as part of the international research training group (TreeDi), which integrating findings from above- and belowground studies to develop a mechanistic understanding of how

tree-tree interactions affect biodiversity-ecosystem functioning (Trogisch *et al.* 2020; Trogisch *et al.* 2021). The characteristics of soil microbial communities including their biomass, physiological capabilities, and taxonomic and functional compositions, are shaped by various factors such as the availability and quality of resources (Beugnon *et al.* 2021). Further investigation into the convergence mechanisms of fungal communities, as identified in my dissertation, could be conducted by correlating these characteristics with leaf chemical composition, litter decomposition rates, and soil chemical properties along a tree diversity gradient. Moreover, combining microbial community data with information on root exudates would improve our comprehension of plant-microbe biotic interactions. To accomplish this, I suggest utilizing state-of-the-art omics methodologies, including metagenomics, metatranscriptomics, metaproteomics, and metabolomics, which offer enhanced resolution for studying eco-evolutionary processes. Additionally, investigating the relationship between microbial community data and functional characteristics, such as root diameter and root length density, would shed light on the mechanisms underlying complementarity effects in mixed tree species, including spatial resource allocation. Subsequent research should aim to include underground microorganisms like protists and viruses to broaden our understanding of their organization and role within ecosystems. Incorporating other components of the soil food web, such as mesofauna and macrofauna, would further deepen our comprehension of belowground processes at multiple trophic levels. Although plot topography features can provide general insights into microclimatic conditions, future studies should incorporate direct measurements of environmental variables, such as soil temperature and light intensity, to gain a more comprehensive understanding of the factors influencing microbial community composition and functionality. Furthermore, examining aboveground characteristics like branching patterns, which reflect canopy structure, would enhance our ability to investigate how complementary mechanisms above the soil surface impact the organization and operation of belowground microorganisms.

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APPENDICES

Author contributions

Chapter 2: Haq, H. u., Hauer, A., Singavarapu, B., Christel, H., Cesarz, S., Eisenhauer, N., Ferlian, O., Bruelheide, H., & Wubet, T. (2024). The interactive effect of tree mycorrhizal type, mycorrhizal type mixture and tree diversity shapes rooting zone soil fungal communities in temperate forest ecosystems. Functional Ecology, 00, 1–14. https://doi.org/10.1111/1365-2435.14651

	HH[%]	AH[%]	BS[%]	HC[%]	SC[%]	NE[%]	OF[%]	HB[%]	TW[%]
Entwurf (Design)	80	0	0	0	0	0	0	10	10
Umsetzung (Implementation)	65	10	5	10	0	0	0	0	10
Auswertung (Analysis)	90	0	0	0	0	0	0	5	5
Schreiben (Writing)	68	1	3	1	1	1	1	7	17
Insgesamt (Overall)	70	3	3.5	4	1	1	1.5	6	10

Chapter 3: Haq, H.U., Singavarapu, B., Hauer, A., Eisenhauer, N., Ferlian, O., Bruelheide, H. and Wubet, T. (2025), Temperate Forest Soil Microbiomes and Their Assembly Processes are Modulated by the Interplay of Co-Existing Tree Species Identity, Diversity and Their Mycorrhizal Type. Journal of Sustainable Agriculture and Environment, 4: e70064. https://doi.org/10.1002/sae2.70064

	HH [%]	BS [%]	AH [%]	NE [%]	OF [%]	HB [%]	TW [%]
Entwurf (Design)	80	0	0	0	0	10	10
Umsetzung (Implementation)	90	0	0	0	0	0	10
Auswertung (Analysis)	90	0	0	0	0	5	5
Schreiben (Writing)	80	1	1	1	1	6	10
Insgesamt (Overall)	80	1	1	1	1	6	10

Chapter 4: Hafeez ul Haq, Bala Singavarapu, Olga Ferlian, Henriette Christel, Simone Cesarz, Nico Eisenhauer, Helge Bruelheide, & Tesfaye Wubet (2025). Impacts of mycorrhizal types and tree diversity on the soil microbial genomic functional potential in temperate forests. (Under review in Microbiology Spectrum).

	HH [%]	BS [%]	OF [%]	HC [%]	SC [%]	NE [%]	HB [%]	TW [%]
Entwurf (Design)	80	0	0	0	0	0	10	10
Umsetzung (Implementation)	90	50	10	0	0	0	0	10
Auswertung (Analysis)	90	0	0	0	0	0	5	5
Schreiben (Writing)	80	1	1	1	1	1	5	10
Insgesamt (Overall)	85	1	1	1	1	1	5	10

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Curriculum Vitae

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Education

08/2021 - present	**Ph.D. student**, at Helmholtz-Centre for Environmental Research - UFZ, Institute for Biology, Martin Luther University Halle, Germany. Research topic: "Tree mycorrhizal type and tree diversity effects on the structure and functional potential of forest belowground microbial communities"
09/2018-06/2021	**MS Molecular Biology & Biochemistry**, Collage of life sciences, Fujian Normal University Fuzhou, China Title master thesis: "Effect of manure amendment and crop rotation on rhizosphere microbiome and resistome"
09/2014-06/2018	**BS Biotechnology and Genetic Engineering**, Kohat university of science and technology, Pakistan Key subjects: Environmental Biotechnology, Molecular biology, Biostatistics, Bioinformatics.

Research stays

08/2023 - 11/2023	Institute of Microbiology, University of Chinese academy of science Beijing, China. Work on the project "Tree diversity interaction (TreeDi)"

Awards

09/2010-06-2021	Chinese government scholarship for Master study at China
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Publications and Conference Contributions

Publications

(1) **Haq, H.U.**, Singavarapu, B., Hauer, A., Eisenhauer, N., Ferlian, O., Bruelheide, H. and Wubet, T. (2025), Temperate Forest Soil Microbiomes and Their Assembly Processes are Modulated by the Interplay of Co-Existing Tree Species Identity, Diversity and Their Mycorrhizal Type. Journal of Sustainable Agriculture and Environment, 4: e70064. https://doi.org/10.1002/sae2.70064

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(10) Haq, E.U., Jianjun, H., **Ul Haq, H.,** Li, K. et al. An MRI-based deep learning approach for efficient classification of brain tumors. J Ambient Intell Human Comput 14, 6697–6718 (2023). https://doi.org/10.1007/s12652-021-03535-9.

Under review

(1) **Hafeez Ul Haq,** Bala Singavarapu, Olga Ferlian, Henriette Christel, Simone Cesarz, Nico Eisenhauer, Helge Bruelheide, & Tesfaye Wubet (2025) Iimpacts of mycorrhizal

types and tree diversity on the soil microbial genomic functional potential in temperate forests (Under review; Microbiology Spectrum)

(2) Henriette Christel, Georg J. A. Hähn, Rémy Beugnon, Yuanyuan Huang Olga Ferlian, **Hafeez Ul Haq**, Tesfaye Wubet, Nico Eisenhauer and Simone Cesarz (2025), Tree species richness effects on soil ecosystem multifunctionality vary with proximity to target trees (Under review: Journal of Ecology)

Conference contributions

(1) **Haq, HU.,** Bruelheide, H & Wubet, T. *The interactive effect of mycorrhizal type, and tree diversity shapes the soil fungal communities in forest ecosystems*. (Poster). ICOM12 Conference, England, 2024.

(2) **Haq, HU.,** Bruelheide, H & Wubet, T. *The interactive effect of mycorrhizal type, and tree diversity shapes the soil fungal communities in forest ecosystems*. (Poster and Oral contribution). TreeDi Conference, Germany, 2024.

(3) **Haq, HU.,** Wubet, T. *The influence of tree diversity and mycorrhizal type on soil fungal communities.* (Oral contribution). TreeDì-BEF China Seminar Series, Beijing, 2023.

(4) **Haq, HU.,** Wubet, T. *The influence of tree diversity and mycorrhizal type on soil fungal communities.* (Oral contribution). Seminar in Federal University of Rio Grande do Norte, Brazil 2022.

(5) **Haq, HU.,** Wubet, T. *Rhizosphere and root microbiome.* (Poster and Oral contribution). TreeDì Funding defense (DFG), Germany, online, 2021.

(6) **Haq, HU.,** Wubet, T. *Rhizosphere microbiome – progress report.* (Oral contribution). TreeDì Doctoral Conference, Germany, online, 2021.

Declaration of Independence / Eigenständigkeitserklärung

I hereby declare that I have written this dissertation entitled "Effects of tree mycorrhizal types and their mixtures as well as tree diversity on soil microbial communities" independently and without outside help and that I have not used any sources or aids other than those indicated in the text. Text passages, which were taken over from used works literally or in contents, were marked by me as such. I further declare that I have never applied for a doctoral degree before. This doctoral thesis has not been submitted to the Faculty of Natural Sciences I - Biosciences of the Martin Luther University Halle-Wittenberg or any other scientific institution for the purpose of a doctorate.

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Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel "Effects of tree mycorrhizal types and their mixtures as well as tree diversity on soil microbial communities" eigenständig und ohne fremde Hilfe verfasst sowie keineanderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommenwurden, wurden von mir als solche kenntlich gemacht. Ich erkläre weiterhin, dass ichmich bisher noch nie um einen Doktorgrad beworben habe. Die vorliegendeDoktorarbeit wurde bis zu diesem Zeitpunkt weder bei der NaturwissenschaftlichenFakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg nocheiner anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt.

Halle (Saale),

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Hafeez Ul Haq

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