Received: 30 March 2024 | Accepted: 23 July 2024

DOI: 10.1111/1365-2435.14651

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Mycorrhizal Networks

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

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Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: 319936945/GRK2324 and Ei 862/29-1

Handling Editor: Tamir Klein

Abstract

1. 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.
2. 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).
3. 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, PO_4^-, NO_3^- were found to be equally important unlike in AM and EcM multi-species stands. Comparative analysis of the fungal taxa specialisation 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 specialised 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

4. 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.

KEYWORDS
generalist fungi, mycobiota, myco-type, rooting zone, specialist fungi, specialisation, tree diversity, trophic mode

1 | INTRODUCTION

Forest ecosystems contain a wide range of soil fungi that play a crucial role in regulating plant community dynamics (Averill et al., 2014; Molina & Horton, 2015) and biogeochemical processes (Clemmensen et al., 2013; Van Der Heijden et al., 2008). 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 (Kandlikar et al., 2019; Peay, 2016).

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 et al., 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., 2015). 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 (Teste et al., 2017; van Der Heijden et al., 2015; Wagg et al., 2011). 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 (Teste et al., 2017; van Der Heijden et al., 2015). 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 (Liang et al., 2020; 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 et al., 2013). Consequently, AM and EcM trees are characterised by acquisitive and conservative nutrient absorption mechanisms, respectively (Averill et al., 2019; Luo et al., 2018). 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., 2022), 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., 2024; 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., 2022). 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 et al., 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 et al., 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.

2 | MATERIALS AND METHODS

2.1 | 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 center 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).

2.2 | 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 center 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.

2.3 | 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 $CaCl_2$. Soil microbial biomass and basal soil respiration were measured on an automated O_2-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 autoanalyser (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. NH_4^+, NO_3^- and PO_4^{3-} were detected on ion exchange membranes (IEM), followed by subsequent processing as per the protocols outlined in (Duran et al., 2013; Rodríguez et al., 2009).

2.4 | 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., 2022). 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).

2.5 | 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., 2022). 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., 2018), 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., 2016) 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.

2.6 | Statistical analysis

All statistical analyses and figure plotting were carried out with R, (version 4.2.2). Data preprocessing and analysis were mainly performed using the 'phyloseq' package (McMurdie & Holmes, 2013).

All graphs were created using the 'ggplot2' package (Wickham et al., 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 (Kunzetsova et al., 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'.

3 | 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 6427 ASVs. The read coverage of the 320 samples was rarefied to the smallest read coverage of 11,273 reads per sample, yielding 5827 ASVs.

3.1 | Taxonomic and functional guild relative abundances

Comparative visualisation of the top 10 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, 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 symbiotrophs, with their relative abundance increasing with increase in tree diversity. In contrast, the AM stands were dominated by

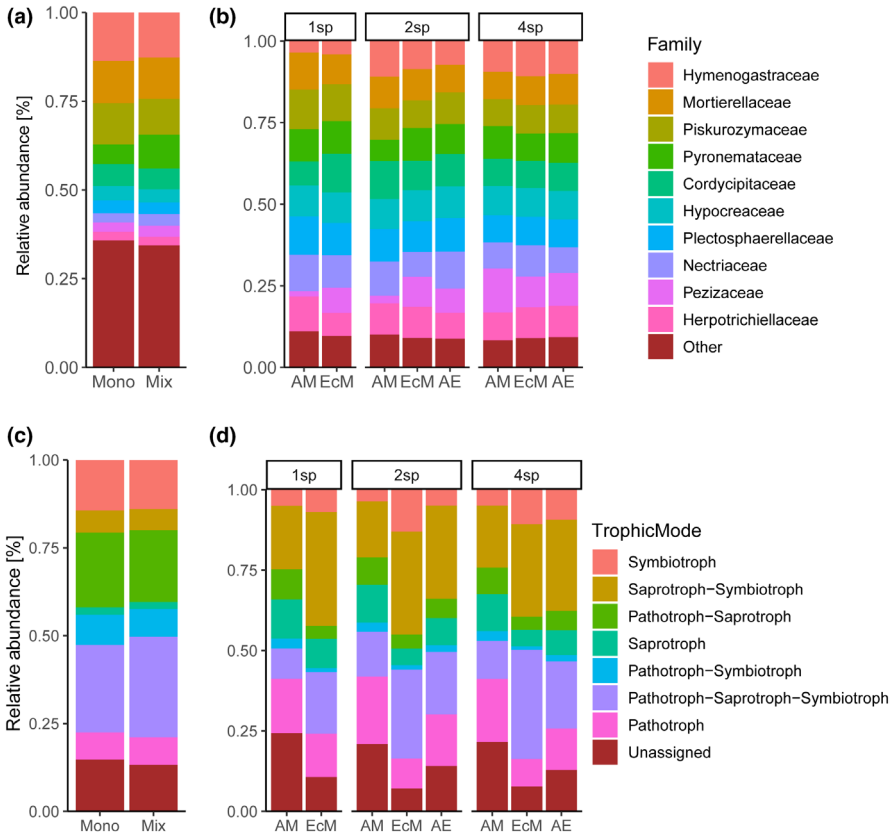

FIGURE 1 Relative abundance distribution of dominant fungal families and trophic modes as a function of the myco-type mixture (mono (AM or EcM) or mix (AE = AM and EcM)). Top 10 fungal Family relative abundance distribution as a function of tree myco-type mixtures (a) over all and (b) by tree diversity levels. Fungal trophic mode distribution patterns as a function of myco-type mixtures (c) over all and (d) by tree diversity levels. 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.

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).

3.2 | 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, Tables S5 and 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, Tables S5 and S6). Comparable effects were found for fungal and functional guild-based Shannon diversity (Figure S3, Tables S5 and S6). We found no significant differences in fungal richness between the two compartments with increasing tree diversity (Figure S4, Tables S7 and S8).

3.3 | 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

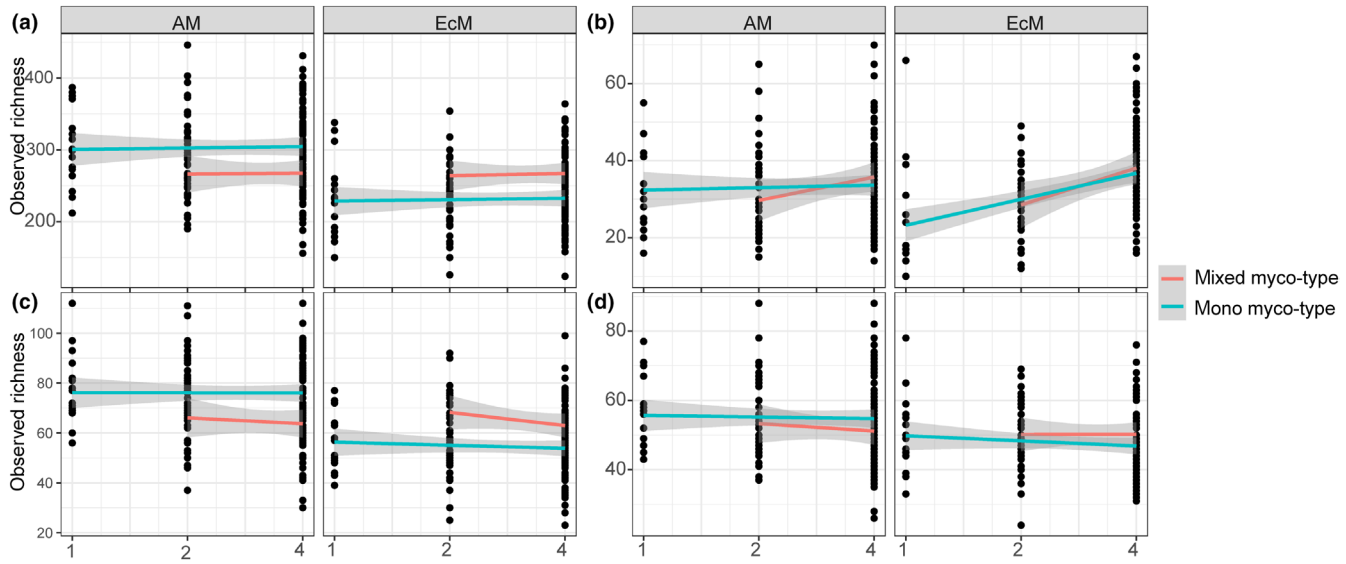

FIGURE 2 Linear mixed-effects models relating individual and interactive effects of tree myco-type (AM or EcM), myco-type mixture (mono myco-type stands [AM or EcM trees] or mixed types [AM and EcM trees]) grow together and $\log_2$ transformed tree diversity on observed richness based on rarefication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

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	

Note: All significant *p*-values are highlighted in bold followed by significance level codes.
$^*p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$.

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).

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–S7 and Table S9).

3.4 | 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

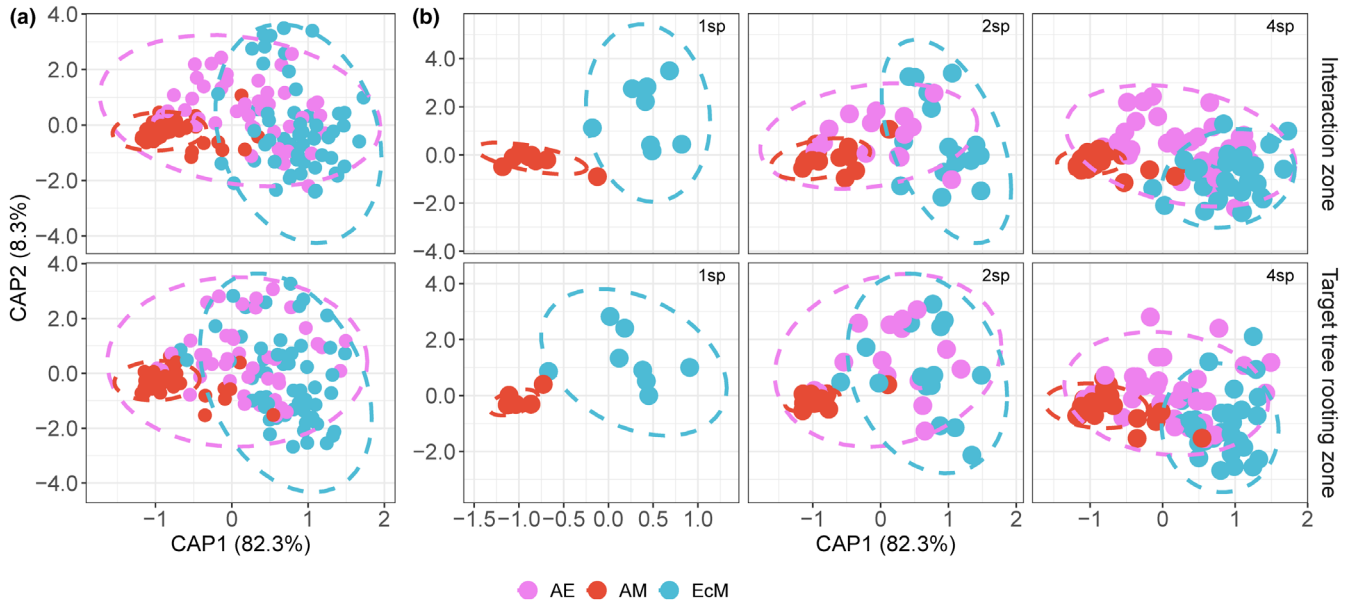

FIGURE 3 Distance-based RDA (dbRDA) ordination plots constrained with myco-type and myco-type mixture (AE [mixed myco-type], mono myco-type [AM and EcM]) and faceted by rooting zone soil compartment and tree diversity level. (a) Ordination of the overall dataset of fungal communities. (b) Ordination 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).

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 two and four EcM tree species stands (Table S11).

3.5 | Differentiation of specialist and generalist soil fungal taxa and functional groups

The CLAMtest revealed a high number of specialised fungal ASVs with respect to myco-type mixtures (Figure 4a, Table S12) and tree myco-types (Figure S8a, Table S12). Among the 5827 fungal ASVs identified in this study, 24% were categorised as generalists, while 11% and 6% were specialised 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 4991 ASVs were classified as generalists, while significantly higher numbers of specialised 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 specialised community in AM than EcM myco-type soil fungal communities both in one species (17% AM and 9.5% EcM of 2023 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) (Figure S8c,d, respectively). However, the percentage of specialised 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 specialised 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).

4 | DISSCUSSION

In this study, we investigated the interplay of tree myco-type, myco-type mixture, tree species diversity and rooting zone compartments

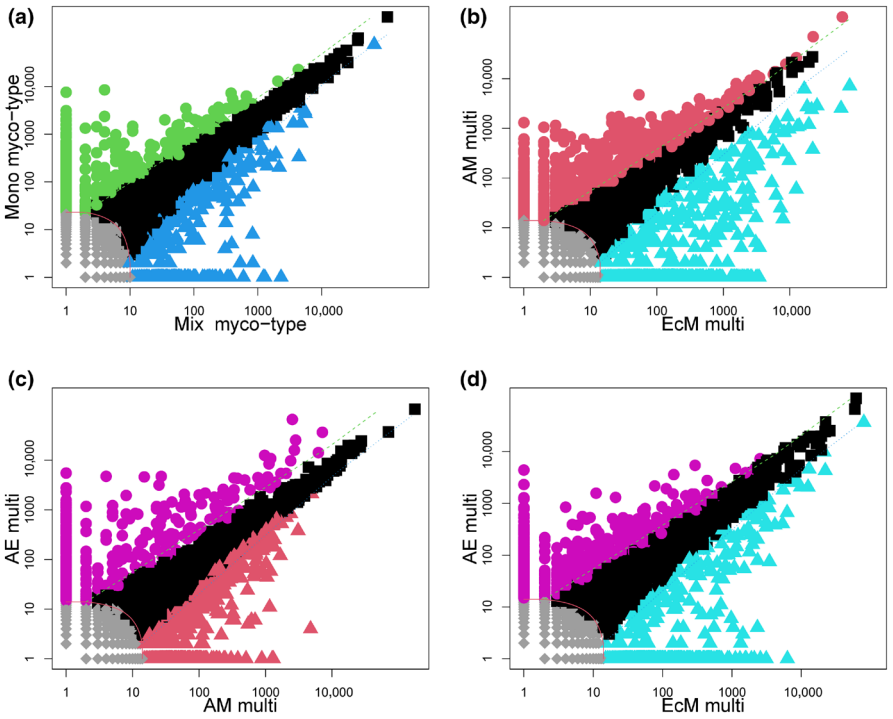

FIGURE 4 Multinomial species classification method (CLAM) for fungal species specialisation of treatment pairs. The evaluated pairs were: Mono myco-types versus mixed myco-types (a), AM multi (mixed myco-types) versus EcM multi (b), AE multi versus AM multi (c), AE multi versus EcM multi (d). The x and y axis values refer to the ASV richness values (ASVs units) in that particular treatment. Proportion of the fungal ASV specialisations 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).

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 specialisation of soil fungi with respect to myco-type and myco-type mixture characterised 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.

4.1 | 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 et al., 2019) and sub-tropical tree diversity experiment (Singavarapu et al., 2022) 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 (Phillips et al., 2013; Simard et al., 1997). 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 neighbouring tree diversity in a forest ecosystem.

4.2 | 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., 2022; Singavarapu et al., 2022). 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., 2022). 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 harbour both host-specific and generalist soil fungal communities (Peay, 2016; Phillips et al., 2013). 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., 2022). 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 neighbour 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 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.

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., 2018). 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 utilise 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 two and four 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.

4.3 | Taxonomic and functional guilds

Visualisation 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 et al., 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 mobilisation of nitrogen and phosphorus from complex organic matter in the immediate vicinity of fungal mycelium (Kyaschenko et al., 2017; Lindahl & Tunlid, 2015). 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.

4.4 | Specialisation of soil fungal communities

Further fungal specialisation 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 specialised 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 specialised taxa adapted to the respective hosts (Phillips et al., 2013; Tedersoo & Bahram, 2019). Surprisingly, AM mono myco-type plots have shown the highest degree of specialised 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 et al., 2013).

Strikingly we found increased specialised 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 et al., 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 et al., 2008; Wang et al., 2021). 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.

5 | 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.

AUTHOR CONTRIBUTIONS

Tesfaye Wubet, Helge Bruelheide and Hafeez ul Haq conceptualised the study. Tesfaye Wubet, Helge Bruelheide, Simone Cesarz and Nico Eisenhauer secured relevant funds. Tesfaye Wubet, Simone Cesarz and Nico Eisenhauer provided laboratory facilities. Hafeez ul Haq, Amelie Hauer and Henriette Christel performed the field sampling. Hafeez ul Haq, Amelie Hauer, Bala Singavarapu and Henriette Christel generated the data. Tesfaye Wubet and Hafeez ul Haq were responsible for bioinformatic analysis and data curation. Hafeez ul Haq, Tesfaye Wubet and Helge Bruelheide were responsible for statistical analysis and visualisation. Hafeez ul Haq, Tesfaye Wubet and Helge Bruelheide wrote the first manuscript draft. Tesfaye Wubet, Helge Bruelheide, Hafeez ul Haq, Bala Singavarapu, Amelie Hauer, Simone Cesarz, Nico Eisenhauer, Henriette Christel and Olga Ferlian reviewed and edited of the manuscript. Tesfaye Wubet was responsible for project administration and supervision. All authors have read and agreed to this version of the manuscript.

ACKNOWLEDGEMENTS

We would like to express our gratitude for the funding provided by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—319936945/GRK2324 and the International Research Training Group TreeDì. We also extend our thanks to the TreeDì coordinators and research group members for their support. Special acknowledgment goes to the student helpers who assisted in the field sampling campaign. We thank Fernando T. Maestre, Victoria Ochoa, Sergio Asensio and Beatriz Gozalo for the analysis of NH_4^+, NO_3^- and PO_4^{3-} Additionally, HU, TW, HB, HC, SC, OF and NE acknowledge the support from the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, funded by the DFG (FZT 118). N.E. acknowledges support by the DFG (Ei 862/29-1).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated for this research can be accessed in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) under the bioproject identifier PRJNA PRJNA1092870. The data supporting this study are available in the MyDiv digital data repository and can be accessed using the DOI: https://doi.org/10.25829/55P4-7F55.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Experimental site and sampling design.

Figure S2. Relative abundance distribution of dominant fungal families and fungal trophic modes as a function of the two-target tree rooting zone compartment.

Figure S3. Linear mixed effects models relating individual and interactive effects of tree myco-type, and myco-type mixture.

Figure S4. Linear mixed effects models relating individual and interactive effects of tree myco-type and two target tree rooting soil compartment.

Figure S5. Distance-based RDA (dbRDA) ordination plots of Symbiotroph community.

Figure S6. Distance-based RDA (dbRDA) ordination plots of Saprotroph community.

Figure S7. Distance-based RDA (dbRDA) ordination plots of Symbiotroph-Saprotroph community.

Figure S8. Multinomial species classification method (CLAM) for fungal species specialization of treatment pairs.

Figure S9. Relative abundance bar plots of the top 3 fungal families and fungal trophic modes.

Table S1. Summary of the selected tree species, their respective mycorrhizal type.

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.

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

Table S4. ANOVA of the individual and interactive effects of tree myco-type, myco-type mixture, soil compartment and tree diversity levels.

Table S5. Statistical analysis of tree mycorrhizal type, myco-types mixture and tree diversity effects.

Table S6. Linear mixed effects models relating individual and interactive effects of tree myco-type and myco-types mixture.

Table S7. Statistical analysis of tree myco-type (AM or EcM), two target tree rooting soil compartments and tree diversity.

Table S8. Linear mixed effects models relating individual and interactive effects of tree myco-type, soil compartments and tree diversity.

Table S9. Pair-wise PERMANOVA of the soil fungal and tropic mode communities.

Table S10. Environmental factors associated with the fungal community compositional variation.

Table S11. Environmental factors associated with the fungal community compositional variation across myco-types and myco-types mixture.

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

How to cite this article: Haq, H. u., Hauer, A., Singavarapu, B., Christel, H., Cesarz, S., Eisenhauer, N., Ferlian, O., Bruelheide, H., & Wubet, T. (2025). 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*, *39*, 1441–1454. https://doi.org/10.1111/1365-2435.14651