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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.

1 | 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 and Grayston 2013). Tree roots also engage in specific symbiotic relationships with mycorrhizal fungi (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 and 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 et al. 2013). These functional differences can shape microbial guild composition, including saprotrophs, pathogens, and mutualists (Averill et al. 2014; Tedersoo and 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 type 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. 2016; Peay 2018; Singavarapu et al. 2022).

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. 2022). 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 forests for AM tree species (Yamamura et al. 2013). In subtropical ecosystems, community patterns specific to different mycorrhizal types and microbial taxa tend to converge as tree diversity increases (Singavarapu et al. 2022). Contrastingly, temperate forest ecosystems consistently demonstrate a strong connection between AM and EcM tree species and soil fungal diversity, including phylogenetic richness and composition (Nguyen et al. 2016; Peay et al. 2013). Generally, higher tree diversity in temperate forests leads to increased soil microbial diversity (Chen et al. 2017; Peay et al. 2013). 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 (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 and Ning 2017), while homogeneous selection occurs when consistent abiotic and biotic environmental conditions result in similar community composition (Zhou and 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 (Lerch et al. 2023; Zhou and Ning 2017). Dispersal limitation refers to the inability of certain species to colonize new habitats due to physical barriers or environmental conditions (Lerch et al. 2023; Liu et al. 2022; Zhou and Ning 2017), while drift involves random changes in species abundance over time due to chance events (Lerch et al. 2023; Zhou and Ning 2017), and deterministic processes influence the composition and abundance of microbial communities via their fitness (Li et al. 2019), 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 and Ning 2017; Liu, Zhu et al. 2021), 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; Zhou and Ning 2017). 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; Zhou and Ning 2017). Microbial taxa can broadly be classified into core, intermediate, and rare groups based on their prevalence and relative abundance across samples (Jiao and 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 (Wang et al. 2023; Jiao and Lu 2020; Zheng et al. 2021; Liao et al. 2017). 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 and Lu 2020). Similarly, in oil-contaminated soils (Shuo Jiao et al. 2017) and grassland soils (Ji et al. 2020), stochastic processes were found to play a larger role in assembling rare taxa than abundant ones (Ji et al. 2020). 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.

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 stands 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 (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 and 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 and 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 and Genre 2010; Smith and 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 and 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.

2 | Materials and Methods

2.1 | Experimental Sites

The study was carried out at the Experimental Research Station of the Helmholtz Centre for Environmental Research—UFZ, located in Bad Lauchstädt, Germany (51°23′ N, 11°53′ E), at an altitude of 115 m a.s.l. The site experiences a temperate climate with an average yearly temperature of 8.8°C and precipitation totaling 484 mm. The soil at the research site is identified as Haplic Chernozem formed from loess, with a pH ranging from 6.6 to 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 8×8 m (Figure S1). 140 plants were grown spaced 1 meter apart in each plot. To minimize weed intervention a water-permeable tarp was placed over all plots. 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 grown in mixtures of one, two, and four species (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). This analysis serves as a continuation and further exploration of the findings presented in (Haq et al. 2024).

2.2 | Sample Collection and Pre-Processing

Soil sampling from 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 Table S1. 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, Figure. S1) and then pooled together. The pooled soil samples were filtered using a 2 mm sieve and subsequently transferred to the field laboratory under controlled cool conditions. All samples were then transferred to the central lab and frozen at −20°C before conducting soil DNA analyses.

2.3 | DNA Extraction, Sequencing, and Data Analysis

DNA extraction was done from soil samples utilizing the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) in accordance with the provided instructions. The DNA concentrations were subsequently quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) and alter to a range of 10-15 ng/µl. Established protocols were followed for the preparation of bacterial and fungal amplicon libraries (Bala Singavarapu et al. 2022). The bacterial 16S rRNA gene's V4 region was specifically targeted for amplification using the primer pair 515 f and 806r (Caporaso et al. 2011) which included Illumina adapter overhangs. For the fungal analysis, the ITS2 rDNA region was amplified through a semi-nested PCR approach. Initially, the primer set ITS1F and ITS4 was used (Gardes and Bruns 1993), followed by a second amplification with the fITS7 and ITS4 primer pair, both incorporating Illumina adapter sequence (Ihrmark et al. 2012). After amplification, the resulting amplicon libraries were purified using Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany). During the indexing PCR, Illumina Nextera XT Indices were attached to the ends of the fungal and bacterial fragments. The indexed products underwent a second round of purification with AMPure beads and were quantified using Qubit fluorometers (Thermo Fisher Scientific, Germany) with the dsDNA high-sensitivity assay. The amplicon libraries for fungi and bacteria were then pooled equimolarly, achieving a final concentration of 4 nM each. These pooled libraries were mixed in equal proportions (1:1) to create the final combined library, which was subsequently subjected to paired-end sequencing (2 × 300 bp) on an Illumina MiSeq platform using the MiSeq Reagent Kit v3. The sequencing was conducted at the Department of Environmental Microbiology, UFZ, in Leipzig, Germany.

The bioinformatic analysis was carried out using a series of specialized software tools to ensure high-quality data processing. QIIME 2.2020.2 (Bolyen et al. 2019) was the primary platform used to extract high-quality reads from the raw data generated by the Illumina MiSeq sequencing platform. Cutadapt was employed to trim primers after the forward and reverse reads were demultiplexed according to their index combinations. Following this, DADA2 was then used for denoising the reads and clustering them into Amplicon Sequence Variants (ASVs) (Callahan et al. 2016). The q2-feature-classifier, integrated with the classify-sklearn naive Bayes taxonomy classifier, facilitated the taxonomy assignment of ASVs for the bacterial 16S data set, using the silva-132-99-515-806-nb-classifier reference database (Bokulich et al. 2018). For the fungal ITS data set, the q2-ITSxpress Qiime2 plugin was utilized to identify and trim ITS2 sequences before they were denoised and clustered into ASVs using DADA2. The taxonomy assignment for fungal ITS ASVs was also conducted using the q2-feature-classifier (Bokulich et al. 2018) in conjunction with the classify-sklearn naive Bayes taxonomy classifier, relying on 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.

2.4 | Statistical Analysis

R (version 4.3.3) was used to import the bacterial and fungal ASV matrices, taxonomic tables, and representative sequences using the "phyloseq" package (McMurdie and Holmes 2013) for subsequent analysis. The fungal and bacterial ASV matrices were filtered and rarefied to ensure uniform sequencing depth, with fungal 5827 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-effects models were employed to assess the influence of mono- and mixed mycorrhizal types, tree species, and tree diversity as a fixed effect with alpha diversity as the response variable, and neighborhood composition was included as a random effect, employing the *lmerTest* function (Kuznetsova et al. 2017). The emtrends function from the "emmeans" package was applied to calculate unbiased marginal means (EMMs) and confidence intervals for the slopes associated with tree species in both mono and mixed mycorrhizal type conditions. A slope was deemed significant if its confidence interval did not include zero. The effects of mono (AM or EcM) and mixed (AE = AM and EcM) mycorrhizal type plots, tree species identity and tree diversity on the bacterial and fungal community compositions (based on Bray-Curtis distance dissimilarity) were analyzed through PERMANOVA using the *adonis2* function from the "vegan" package (Oksanen et al. 2013). We also employed Procrustes analysis (Ross 2004) to assess congruence in bacterial and fungal community composition across mono and mixed mycorrhizal type plots within each tree diversity level.

2.5 | 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 1000 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 sub-communities 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), 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.

3 | Results

3.1 | 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 (Figures 1a,c 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 (Figures 1d S5, Table S3).

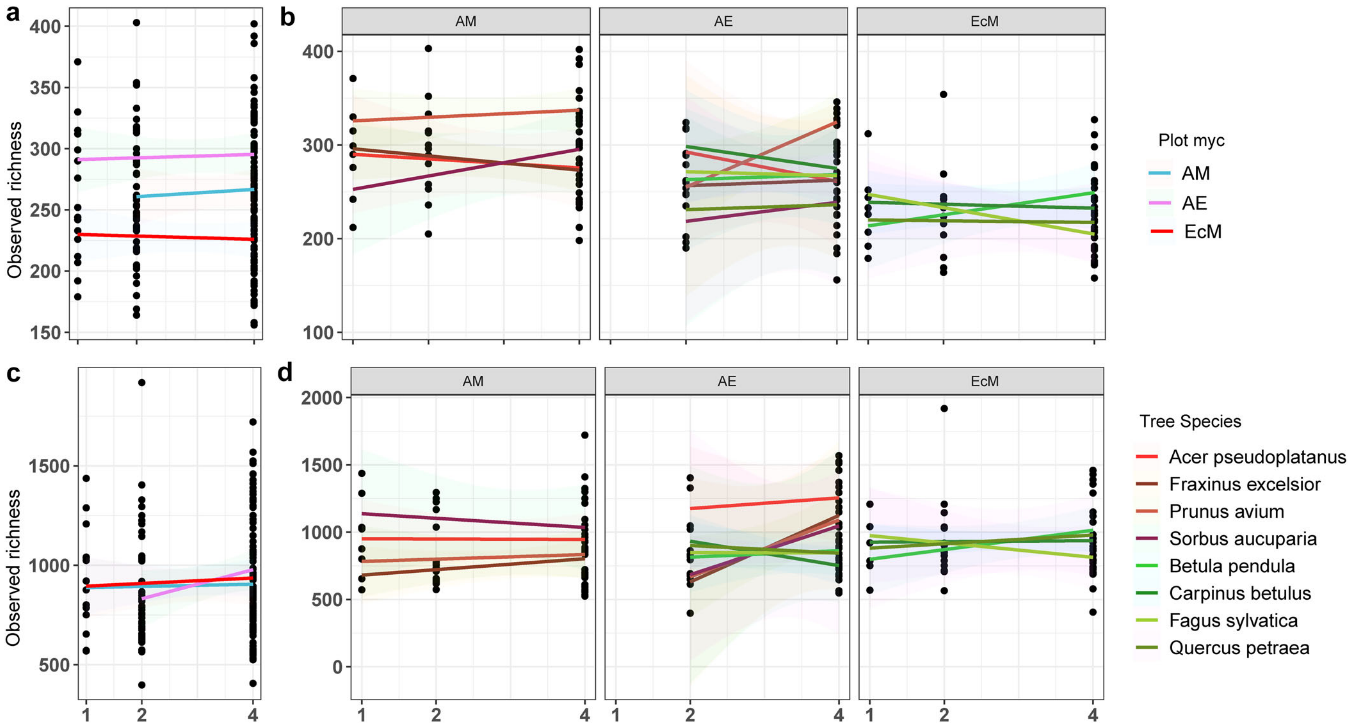

FIGURE 1 | Linear mixed model effects of mono (AM and EcM) and mixed (AE) mycorrhizal type plots tree species with increasing tree diversity (1, monoculture; 2, 2sp mixture; 4, 4sp mixture) on fungal (a) and bacterial (c) alpha diversity, represented via observed richness. Linear mixed model effects of tree species identity and tree species richness by plot mycorrhizal type, and tree species identity on fungal (b) and bacterial (d) observed richness.

3.2 | 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 tree 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).

3.3 | 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 microbial community correlations (Figure S6). 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 in 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.

3.4 | 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

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).

	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**

Note: Bold values are significant $p < 0.05$. 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); and significance (p).

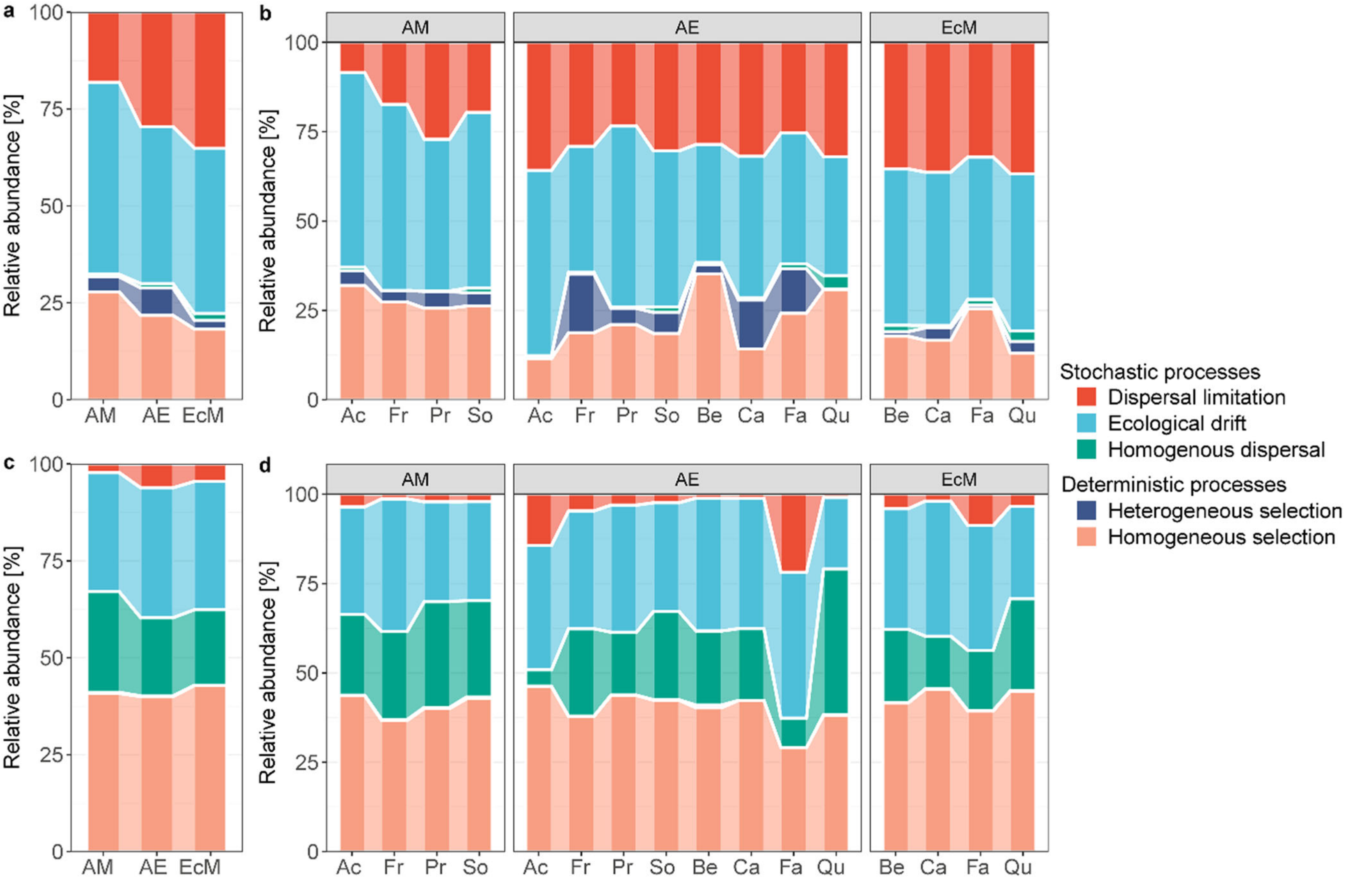

FIGURE 2 | Relative importance of various community assembly processes to soil fungal (A, B) and bacterial (C, D) communities across mono (AM, EcM) and mixed mycorrhizal type (AE) plots. Tree species abbreviation Ac, *Acer pseudoplatanus;* Pr, *Prunus avium*; So, *Sorbus aucuparia;* Fr, *Fraxinus excelsior*;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

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 are prominent for bacterial communities than fungal communities (Table S7).

3.5 | 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).

3.6 | Association Among Microbial Communities' 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

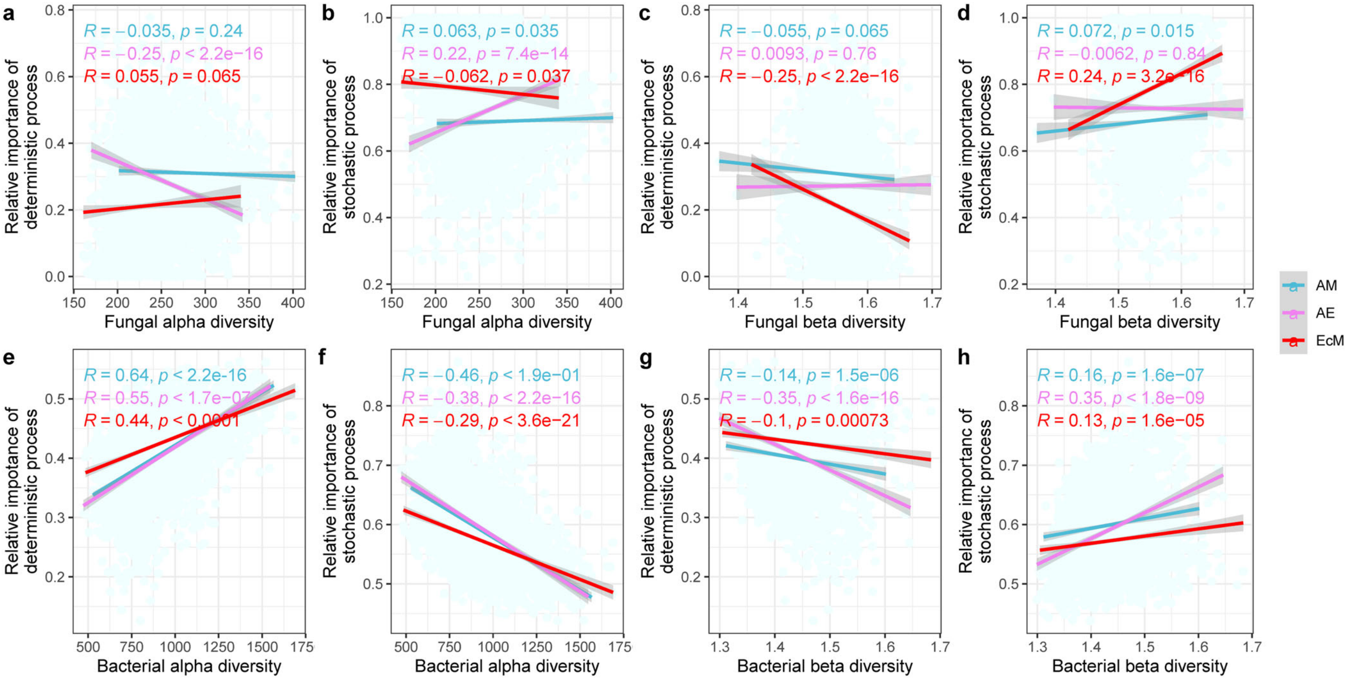

FIGURE 3 | Relative importance of deterministic and stochastic processes as a function of fungal alpha (a, b) and beta (c, d), and bacterial alpha (e, f) and beta diversity (g, h) within mono (AM or EcM) and mixed (AE) mycorrhizal type plots. Spearman correlation models were employed to calculate R^2 and p-values of the linear fitted lines.

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).

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).

4 | 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 of 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.

4.1 | 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 have demonstrated a stronger alpha diversity

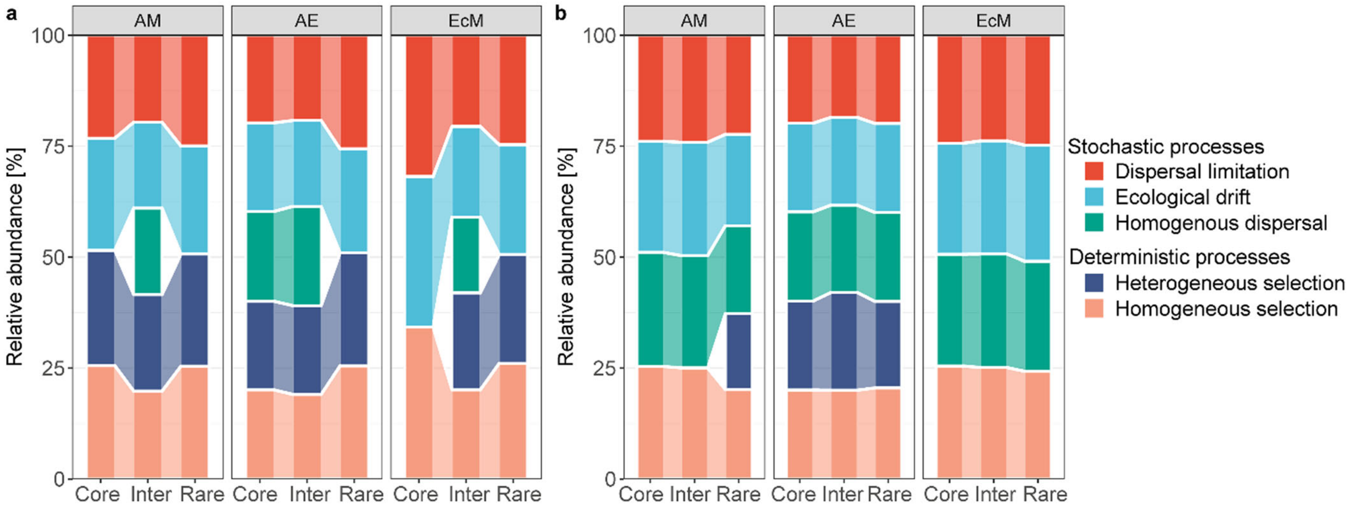

FIGURE 4 | Relative importance of different community assembly processes to core to rare fungal (a) and bacterial (b) subcommunity assemblies in mono (AM, EcM) and mixed mycorrhizal type (AE) plots. Core = core taxa, Inter = intermediate taxa, and Rare = rare taxa.

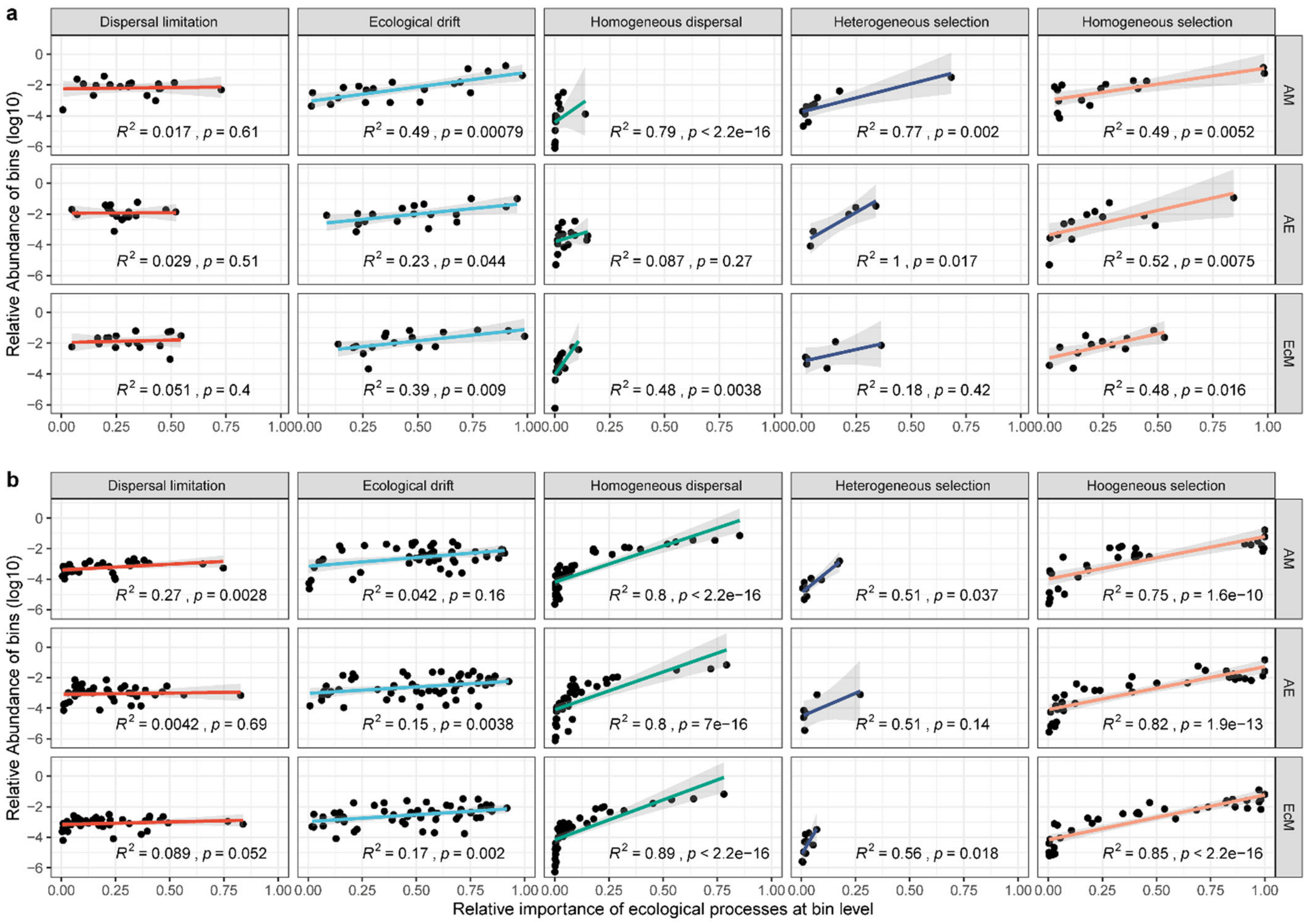

FIGURE 5 | The relationship between relative abundance of taxon bins as a function of the ecological assembly processes driving them in mono (AM, EcM) and mixed mycorrhizal type (AE) plots in fungal (a) and bacterial (b) communities. The horizontal axis shows the relative contribution of community assembly processes for each bin based on its abundance, while the vertical axis displays the log10-transformed values of bin abundances.

effect of AM tree species in subtropical (Singavarapu et al. 2022) and mixed central European forest soils (Lang et al. 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 signaling and nutrient-focused partnerships, while EM plants rely on structural filtering and environmental resilience (Phillips and 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 et al. 2013). Thus, coexistence 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. 2022) and tropical tree experimental sites (Yamamura et al. 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 (Aguilar and Sommaruga 2020; Xun et al. 2019).

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 and 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; Vakkari 2009). Our results contradict previous literatures that reported a positive relationship between tree species richness of AM and/or EcM tree species with microbial diversity (Hiiesalu et al. 2014; Peay et al. 2013). 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 nonsignificant 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.

4.2 | 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 and Grayston 2013). The effect of tree mycorrhizal type and tree diversity on the fungal community composition is in agreement with previous studies in subtropics (Eagar et al. 2022; Singavarapu et al. 2022). 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. 2018) and genotype (Karliński et al. 2020) on the rhizosphere soil microbial composition through their effect on soil chemistry and resource availability (Urbanová et al. 2015; Wu and Yu 2019). In diverse tree species plots of mono and mixed mycorrhizal type plant communities, however, plants interact with their neighbors 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. 2022).

4.3 | 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* (Miquel Guennoc et al. 2018). This suggests that coexistence 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. 2022). 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 provides valuable insight into how mycorrhizal preference influences tree-tree interactions and coexistence in forest ecosystems.

4.4 | Drivers of Fungal and Bacterial Community Assembly Processes

Community assembly processes play a crucial role in shaping microbial community in a given ecosystem (Yuanlong Li and Hu 2021; Liu et al. 2022; Vellend 2010). 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; Chen et al. 2020). 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, Graham et al. 2021; 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; Zhang et al. 2021). 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 (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 strong 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 and Patel 2008; Dumbrell et al. 2010). The observed negative relationship between fungal alpha-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)

4.5 | Association Among Relative Abundance of Microbial Bins and Community Assembly Processes

Our analysis of community assembly processes of phylogenetic bin-based 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 and 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 and Lu 2020).

5 | 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.

Author Contribution

Conceptual design: Tesfaye Wubet, Helge Bruelheide, and Hafeez Ul Haq. Funding acquisition: Tesfaye Wubet, Helge Bruelheide, and Nico Eisenhauer. Laboratory resources: Tesfaye Wubet. Field data collection: Hafeez Ul Haq and Amelie Hauer. Data production: Hafeez Ul Haq, Amelie Hauer, and Bala Singavarapu. Bioinformatics and data organization: Tesfaye Wubet and Hafeez Ul Haq. Statistical analysis and visualization: Hafeez Ul Haq, Tesfaye Wubet, and Helge Bruelheide. Initial manuscript drafting: Hafeez Ul Haq and Tesfaye Wubet. Manuscript review and revision: Tesfaye Wubet, Helge Bruelheide, Hafeez Ul Haq, Bala Singavarapu, Amelie Hauer, Olga Ferlian, and Nico Eisenhauer. Project management and oversight: Tesfaye Wubet. All authors have reviewed and approved this manuscript version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The bacterial and fungal sequence datasets are accessible through the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under the project number PRJNA1147626 and PRJNA1092870 respectively.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.