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Tree mycorrhizal type and tree diversity shape the forest soil microbiota

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Summary

There is limited knowledge on how the association of trees with different mycorrhizal types shapes soil microbial communities in the context of changing tree diversity levels. We used arbuscular (AM) and ectomycorrhizal (EcM) tree species as con- and heterospecific tree species pairs (TSPs), which were established in plots of three tree diversity levels including monocultures, two-species mixtures and multi-tree species mixtures in a tree diversity experiment in subtropical China. We found that the tree mycorrhizal type had a significant effect on fungal but not bacterial alpha diversity. Furthermore, only EcM but not AM TSPs fungal alpha diversity increased with tree diversity, and the differences between AM and EcM TSPs disappeared in multi-species mixtures. Tree mycorrhizal type, tree diversity and their interaction had significant effects on fungal community composition. Neither fungi nor bacteria showed any significant compositional variation in TSPs located in multi-species mixtures. Accordingly, the most influential taxa driving the tree mycorrhizal differences at low tree diversity were not significant in multi-tree species mixtures. Collectively, our results indicate that tree mycorrhizal type is an important factor determining the diversity and community composition of soil microbes, and higher tree diversity levels promote convergence of the soil microbial communities.

Significance statement

More than 90% of terrestrial plants have symbiotic associations with mycorrhizal fungi which could influence the coexisting microbiota. Systematic understanding of the individual and interactive effects of tree mycorrhizal type and tree species diversity on the soil microbiota is crucial for the mechanistic comprehension of the role of microbes in forest soil ecological processes. Our tree species pair (TSP) concept coupled with random sampling within and across the plots, allowed us the unbiased assessment of tree mycorrhizal type and tree diversity effects on the tree-tree interaction zone soil microbiota. Unlike in monocultures and two-species mixtures, we identified species-rich and converging fungal and bacterial communities in multi-tree species mixtures. Consequently, we recommend planting species-rich mixtures of EcM and AM trees, for afforestation and reforestation regimes. Specifically, our findings highlight the significance of tree mycorrhizal type in studying 'tree diversity – microbial diversity – ecosystem function' relationships.

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Introduction

Soil microorganisms, predominantly fungi and bacteria, are highly abundant and diverse living entities on earth (Fierer, 2017). Both fungi and bacteria play key roles in a wide range of processes like biogeochemical cycles and regulate plant diversity and productivity (Van Der Heijden *et al*., 2008; Bender and van der Heijden, 2015; Delgado-Baquerizo *et al*., 2016a, 2016a; Kappler and Bryce, 2017; Wei *et al*., 2019).

Notably, the diversity and composition of microbial communities are essential for the multifunctionality of ecosystems (Wagg *et al*., 2014; Delgado-Baquerizo *et al*., 2016b). As an essential part of soil microbial communities, mycorrhizal fungi, form symbiotic associations with more than 90% of terrestrial plant species. Within this symbiosis, plants exchange carbon with mycorrhizal fungi to support their nutrient uptake, pathogen defence and environmental stress tolerance (Wang and Qiu, 2006; Smith and Read, 2010; Brundrett and Tedersoo, 2018). There are two dominant mycorrhizal types, namely, ectomycorrhiza (EcM) and arbuscular mycorrhiza (AM), that are associated with approximately 80% of all vascular plants. Ecto and arbuscular mycorrhizal fungi differ in resource acquisition, allocation strategies and plant–soil feedback relations (Aerts, 2003; Phillips *et al*., 2013; Bennett *et al*., 2017; Kadowaki *et al*., 2018). For example, ectomycorrhizal fungi have relatively greater access to the organic nitrogen in the soil than arbuscular mycorrhizal fungi (Tedersoo and Bahram, 2019). The fungal mycorrhizal partners can mediate the interactions between plants and the soil microbial community through the mycorrhizosphere (i.e., the area of soil under the combined influence of the plant root and the mycorrhizal fungal community) and the hyphosphere (i.e., the soil zone under the influence of mycorrhizal extraradical hyphae; Rambelli, 1973; Buee *et al*., 2009; Churchland and Grayston, 2014). The extraradical hyphae can form belowground networks connecting numerous plant roots, known as hyphal networks or common mycorrhizal networks (Simard *et al*., 2012). In addition, free-living soil fungi and bacteria respond to changes in the mycorrhizosphere and surrounding soil processes such as rhizodeposition and organic matter decomposition (Fitter and Garbaye, 1994; Johansson *et al*., 2004; Bardgett and Wardle, 2010). In an observational study from boreal and temperate regional sites, Bahram *et al*. (2020) described differentiated microbial communities between sites dominated by AM and EcM type plants. In addition, Weißbecker *et al*. (2018) reported a significant correlation of EcM fungal community structure with EcM type trees. Despite these studies, the influence of a plant's mycorrhizal type on the diversity and composition of soil microbial communities, including bacteria, remains unclear, especially at the local scale.

As tree diversity increases, different tree–tree interactions develop, and so does the complexity of the associated plant–plant, plant–microbe and microbe–microbe interactions (Bonfante and Anca, 2009; Schuldt *et al*., 2017). Previous research has shown positive tree diversity effects on soil microbial diversity (Gao *et al*., 2013; Barberan *et al*., 2015; Hiiesalu *et al*., 2017) but also no or small effects were reported (McGuire *et al*., 2012; Rivest *et al*., 2019). Tree species richness effects may develop in some cases and may not in others. These inconsistent findings might also result from a strong context-dependency of tree diversity effects on the soil microbial community (Tedersoo *et al*., 2016), which calls for an experimental setting with a controlled environmental context. Such controlled settings facilitate the systematic testing of how the tree mycorrhizal type in tree-tree interactions affects soil microbiota in forest ecosystems and how these relations are shaped by different levels of tree species diversity. In this way, context-dependency can be reduced to diversity effects, in addition to the effects of the identity of the target tree species and their neighbours, and environmental variation that differs between sampling locations. While in a field experiment, environmental variation cannot be fully excluded, it can be accounted for when being measured. The knowledge of how the tree mycorrhizal type of focal trees, their neighbour tree species and tree species diversity affect the soil microbiota of the tree-tree interaction zone would shed light on microbial community assembly and ecosystem functioning.

To address this knowledge gap, we used the BEF-China experimental research platform, where trees were grown with tree diversity levels of 1, 2, 4, 8, 16 and 24 species (Bruelheide *et al*., 2014). We employed the tree-species pair (TSP) concept wherein, two adjacent trees were selected as a target sampling unit (Trogisch *et al*., 2021). The TSP design provides a focal TSP partner and also facilitates uniform soil sampling to capture the focal tree–tree soil interaction zone. Combined with random sampling, this would further facilitate the unbiased identification and comparison of tree mycorrhizal type effects on the soil microbiota across tree diversity levels. The interaction zone soil microbial communities were assessed using paired-end Illumina sequencing targeting the bacterial 16S (V4 region) and the fungal internal transcribed spacer (ITS2) regions.

We hypothesized that: (H1) soil microbial alpha diversity is affected by the tree mycorrhizal type and that within the mycorrhizal type of EcM and AM TSPs, microbial alpha diversity increases with increasing tree species diversity. Given the anatomical and ecophysiological differences of the two mycorrhizal types (Bonfante and

Genre, 2010), their effect on the soil nutrient cycling (Cheeke *et al*., 2017), and differential capability to mobilize organic (EcM fungi) and inorganic (AM fungi) compounds (Read and Perez-Moreno, 2003; Smith and Read, 2008), we expected a lower microbial diversity in EcM TSPs than in AM TSPs. Furthermore, since higher plant diversity can enrich the microbial communities through increased carbon inputs into the rhizosphere (Lange *et al*., 2015; Eisenhauer *et al*., 2017), we expect an increase in microbial diversity with increasing tree diversity in both AM and EcM TSPs. Likewise, we hypothesized that (H2) tree mycorrhizal type, tree diversity levels and the site-specific environmental conditions influence the microbial community composition. Through promoting the diversity of nutrient resources and increasing microhabitat complexity (Hooper *et al*., 2000; Prober *et al*., 2015) a high plant diversity facilitates the coexistence of diverse microbial communities. More specifically, we tested the hypothesis that (H2a) microbial community composition depends on tree mycorrhizal type, because different mycorrhizal type trees provide different types of resources (Tedersoo and Bahram, 2019). Furthermore, we expected (H2b) microbial communities to become more similar with increasing tree diversity because the more diverse resources provided by the host species should allow the coexistence of a larger part of the total pool of bacteria and fungi (Lange *et al*., 2015; Kaspari *et al*., 2017). Consequently, with increasing tree diversity, we expected that the most influential microbial taxa driving the differences between mycorrhizal types would be reduced. Besides, since plant diversity influences the local edaphic and microclimatic environment (Bruelheide *et al*., 2014), while some environmental variation (such as topography) is independent of plant diversity, we expected (H2c) abiotic and biotic environmental factors to contribute to shaping the soil microbial community composition in addition to tree diversity and the mycorrhizal type effects.

Results

Sequence data processing

From 4 648 777 and 11 720 448 raw sequencing reads, after quality filtering through denoising, merging, chimera and non-target taxa removal, we obtained 3 678 803 (79.1%) ITS and 8 939 606 (76.3%) 16S sequence reads, which were then clustered into 12 813 fungal and 25 928 bacterial amplicon sequence variants (ASVs) respectively. Rarefaction followed by removing low abundant and potentially spurious ASVs in both fungal and bacterial datasets at a threshold of 5% sample abundance, resulted in 8041 fungal and 15 913 bacterial taxa respectively. The alpha diversity indices after removal of low abundant taxa were well fitted (*adj.*R^2 values range: 0.98–1) with that of the indices before filtering (Appendix S2: Fig. S2). Also, the Mantel tests using Bray–Curtis distance on data matrices before and after removal of low abundant taxa showed high congruence (for fungi $R = 1$, $P = 0.001$; for bacteria $R = 0.99$, $P = 0.001$), therefore, suggesting that the removal of rare taxa had no significant impact on the microbial community analysis. Thus, we used the latter dataset to test our hypotheses.

Tree mycorrhizal type and tree diversity effects on microbial alpha diversity

The alpha diversity measures observed richness, Shannon diversity, Pielou's evenness and Gini dominance indices showed significant differences between tree mycorrhizal types for fungal but not for soil bacterial communities (Fig. 1). Further, Wilcoxon rank-sum tests within the tree diversity levels revealed that for fungal communities, the differences between mycorrhizal types were present in monocultures and two-species mixtures but were absent at multi-tree species mixtures (Fig. 1B, D, F and H). A two-way ANOVA analysis on fungal alpha diversity metrics showed strong effects of tree mycorrhizal type and significant interaction with tree diversity levels (Appendix S3: Table S2). Furthermore, pairwise analysis of EcM and AM TSP soil fungal communities along the tree diversity levels also confirmed that the fungal alpha diversity increased only for EcM TSPs, and the differences between EcM and AM TSPs disappeared at multi-tree species mixtures (Appendix S2: Fig. S3). In contrast, within the tree diversity levels, no significant differences were found in bacterial communities except for Pielou's evenness in two species mixtures (Fig. 1J, L, N and P). Comparison of the effect of tree mycorrhizal types at the multi-tree species mixtures, including also the Mycomix-TSPs along with EcM and AM TSPs, showed no significant differences among these different types (Appendix S2: Fig. S4).

Tree mycorrhizal type and tree diversity effects on taxonomic and functional groups

Fungal communities were dominated by Basidiomycota in EcM TSPs, while both Ascomycota and Basidiomycota were in nearly equal proportions in AM TSPs. In contrast, bacterial communities were dominated by the phylum Acidobacteriota followed by Proteobacteria, although these proportions were not distinctly different between EcM and AM TSPs (Appendix S2: Fig. S5). Visualization of the taxonomic compositions at the order level indicated that the soil fungal communities differed in their relative abundances of taxa between tree mycorrhizal types and along the tree diversity levels (Fig. 2A and B), whereas bacterial communities displayed relatively less conspicuous differences (Fig. 2E and

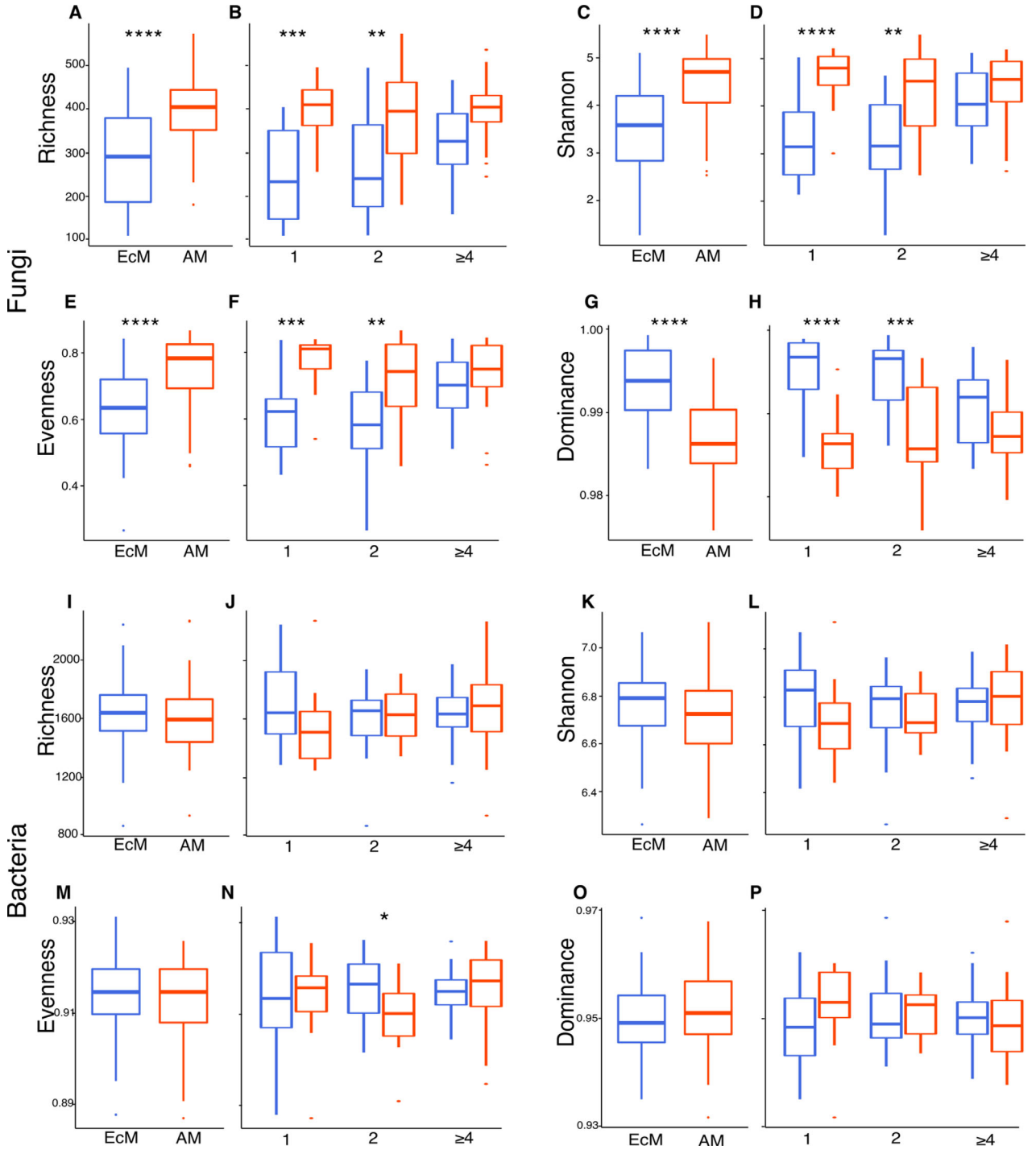

Fig. 1. Fungal and bacterial alpha diversity indices, namely, observed ASV richness (i.e., 'Richness'), Shannon diversity (i.e., 'Shannon'), Pielou evenness (i.e., 'Evenness') and Gini dominance (i.e., 'Dominance'). On the x-axis, EcM (blue colour) and AM TSPs (red colour) and the tree diversity levels (1 – monocultures, 2 – two-species mixtures and ≥ 4 – multi-tree species mixtures).
A, C, E and G. Comparison of soil fungal alpha diversity between all EcM and AM TSPs.
B, D, F and H. Within the tree diversity level differences between EcM and AM TSPs for the respective fungal alpha diversity measures.
I, K, M and O. Comparison of soil bacterial alpha diversity between all EcM and AM TSPs.
J, L, N and P. Within the tree diversity level differences between EcM and AM TSPs for the respective bacterial alpha diversity measures. The asterisks show the *P*-value significance level, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$.

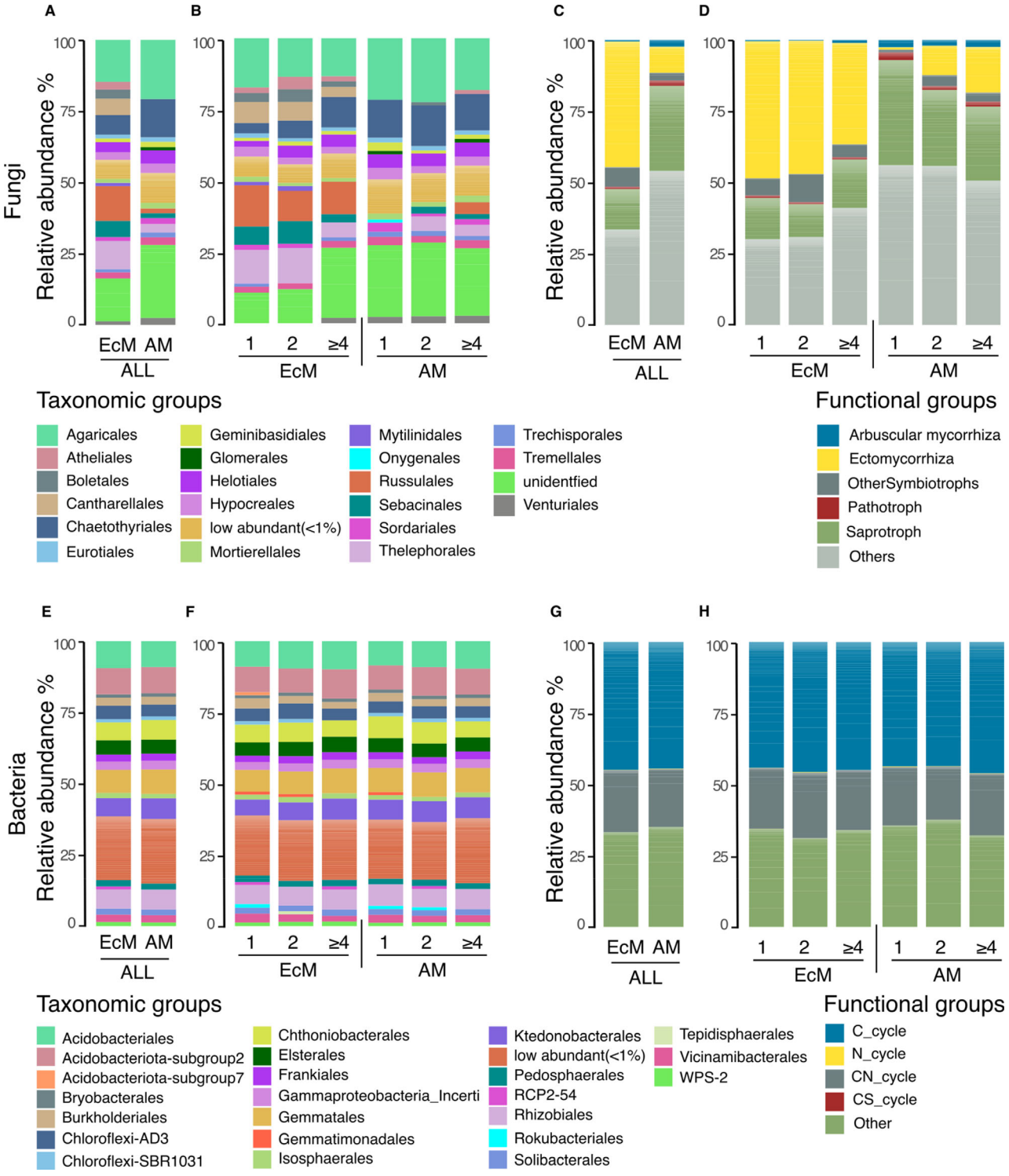

Fig. 2. Taxonomic and functional group composition of soil fungal and bacterial communities. On the x-axis EcM and AM TSPs and the tree diversity levels (All – combined dataset irrespective of tree diversity, 1 – monocultures, 2 – two-species mixtures and ≥4 – multi-tree species mixtures). Assigned functional groups were only shown here. Order-level taxonomic composition of fungal communities of (A) EcM and AM TSPs and (B) across diversity levels. Functional group composition of fungal communities of (C) EcM and AM TSPs and (D) across diversity levels. Order-level taxonomic composition of bacterial communities of (E) EcM and AM TSPs and (F) across diversity levels. Functional group composition of bacterial communities of (G) EcM and AM TSPs and (H) across diversity levels.

F). For instance, in fungal communities, Cantharellales with a major proportion of ectomycorrhizal fungi (Appendix S3: Table S4) were distinctive in EcM TSPs but minuscule in AM TSPs. In contrast, Glomerales were relatively less abundant in EcM than in the AM TSPs. The relative abundances of Thelephorales and Sebacinales were decreased in EcM TSPs of multi-tree species mixtures compared with monocultures, while these taxa were trivial in AM tree monocultures. Whereas, in bacterial communities, the EcM TSPs of EcM tree monocultures had a higher relative abundance of Acidobacteriota|Subgroup_7, Chloroflexi|SBR1031, Gemmatimonadales, Rokubacteriales and Vicinamibacterales than that of multi-tree species mixtures.

Analysis of the functional group abundances of the soil fungal communities showed distinct patterns between the EcM and AM TSPs and among the different tree diversity levels. The EcM TSPs were dominated by symbiotrophs, mainly by ectomycorrhizal fungi (e.g., the genera *Inocybe, Russula, Clavulina*). In comparison, the AM TSPs were dominated by saprotrophs and displayed a lower proportion of symbiotrophs, mainly by arbuscular mycorrhizal fungi (e.g., the genera *Glomus, Rhizophagus, Diversispora*) in the monocultures and an increasing proportion of EcM and other symbiotrophs in the two and multi-tree species mixtures (Fig. 2C and D). The bacterial functional groups, however, showed no clear pattern between the tree mycorrhizal types and the diversity levels in both EcM and AM TSPs (Fig. 2 G and H).

Tree mycorrhizal type and tree diversity effects on microbial community composition

The dbRDA-based ordination analysis showed that EcM and AM TSPs soil fungal communities were significantly more distant in monocultures than in two-species mixtures, while they clustered closely together in the multi-tree species mixtures (Fig. 3A and B). In contrast, bacterial communities were relatively less distinct between EcM and AM TSPs and showed differences in the tree diversity levels, wherein the tree mycorrhizal types clustered closely in multi-tree species mixtures (Fig. 3C and D). The permutational analysis of variance (PERMANOVA) test also confirmed the significant main and interaction effects of tree mycorrhizal type and tree diversity levels on fungal community composition (explained variance = 6.9%). In contrast, there was only a significant main effect of tree diversity (explained variance = 2.8%) in bacterial communities (Table 1). The analysis of multivariate homogeneity of the groups' dispersion confirmed that the variances within groups did not differ among groups, thus indicating that the significant differences between group means as revealed by the PERMANOVA were not an artefact of heterogeneity among groups (Fungi, $F = 1.39$, $P = 0.22$; Bacteria, $F = 0.18$, $P = 0.98$).

Furthermore, pairwise comparisons along the tree diversity levels revealed that the EcM TSPs soil fungal communities differed in their composition between monocultures and multi-tree species mixtures and between two and multi-tree species mixtures (Appendix S3: Table S3). In contrast, no such differences were encountered for AM TSPs soil fungal communities between tree diversity levels. Differences between EcM and AM TSPs soil fungal communities along tree diversity levels were found in monocultures and two-species mixtures, but they disappeared at the multi-tree species level (Appendix S3: Table S3). For bacterial communities, the only significant difference was detected between the EcM tree monocultures and EcM multi-tree species mixtures. Comparison of the tree mycorrhizal types at the multi-tree species mixtures, including also the Mycomix-TSPs along with EcM and AM TSPs, showed no significant differences for both fungal and bacterial community compositions (Fungi, $F = 0.91$, $P = 0.78$; Bacteria, $F = 0.88$, $P = 0.63$).

Furthermore, to evaluate the effects of TSPs within the mycorrhizal type on the microbial community variation, PERMANOVA analysis was performed. The EcM TSPs ($F = 1.202$, $R^2 = 11.13\%$, $P = 0.009$) and the AM TSPs ($F = 1.263$, $R^2 = 11.63\%$, $P = 0.001$) had a similar effect on fungal community composition. Post-hoc pairwise analysis revealed that AM TSPs including *Nyssa sinensis, Liquidambar formosana, Choerospondias axillaris* and *Koelreuteria bipinnata* had significant TSP effects (Appendix S3: Table S5). While EcM TSPs did not show any significant effects in post-hoc pairwise analyses. Similar to fungi, both EcM TSPs ($F = 1.494$, $R^2 = 13.47\%$, $P = 0.005$) and AM TSPs ($F = 1.423$, $R^2 = 12.96\%$, $P = 0.025$) had a comparable strong effect on bacterial communities. Further post-hoc pairwise analyses revealed marginal significant effects ($P = 0.053$) only for EcM TSPs that included *Quercus fabri, Castanopsis sclerophylla* and *Cyclobalanopsis glauca*.

Random forest model based microbial predictors of tree mycorrhizal types across tree diversity levels

Random forest (RF) models further revealed the effects of tree mycorrhizal type and tree diversity by identifying the most influential microbial taxa (classifier taxa), differentiating the tree mycorrhizal types across the tree diversity levels except in multi-species mixtures (Fig. 4). The soil fungal communities exhibited a higher number (90) of classifier taxa for tree mycorrhizal type irrespective of the tree diversity [RF model, $P < 0.001$, Area under the ROC Curve (AUC) = 0.75; Fig. 4; Appendix S2: Fig. S6A and G]. The number of classifier fungal taxa was reduced to

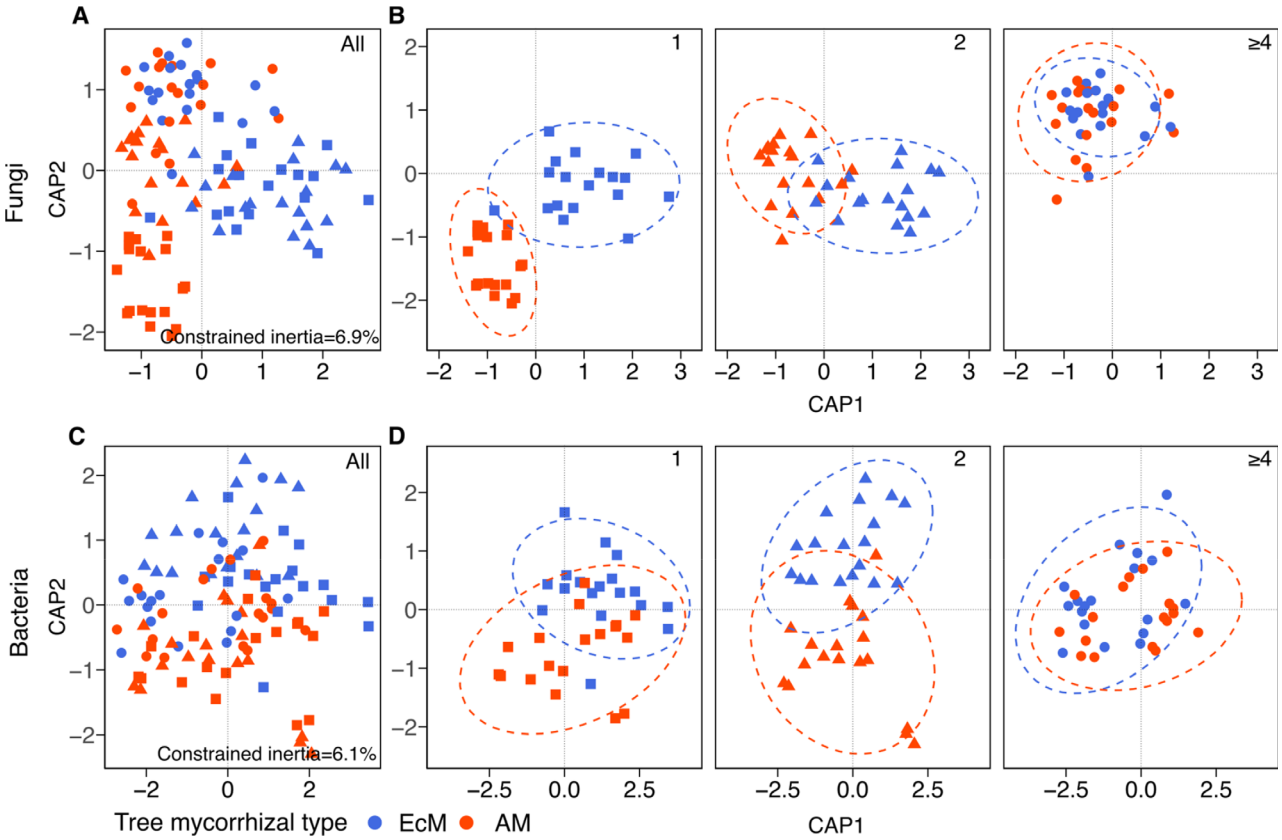

Fig. 3. Distance-based RDA (dbRDA) ordination plots constrained on the mycorrhizal type and tree diversity levels. EcM samples – blue colour and AM samples – red colour.
A. Fungal communities – combined dataset [both factors significant including the interaction between mycorrhizal type and tree diversity (permutest, $P = 0.01$)].
C. Bacterial communities – combined dataset [only tree diversity significant (permutest, $P = 0.03$)].
B and D. Ordination of fungal and bacterial communities faceted across mono (1), two (2) and multi-tree species mixtures (≥4) respectively. Ellipses represent 95% confidence intervals around mycorrhizal group centroids.

Table 1. Effects of tree mycorrhizal type and tree diversity level on the compositional differences of soil fungal and bacterial communities based on PERMANOVA with 999 permutations.

	Fungal communities				Bacterial communities			
	df	*F*	R^2	*P*	*df*	*F*	R^2	*P*
Mycorrhizal type (M)	1	2.522	0.023	**0.001*****	1	1.318	0.012	0.123
Tree diversity level (L)	2	1.228	0.022	**0.015***	2	1.529	0.028	**0.030***
Interaction (M × L)	2	1.290	0.024	**0.010****	2	1.111	0.020	0.236
Residual	102		0.931		102		0.939	

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

53 and 27 in monocultures (RF model, $P = 0.005$, AUC = 0.78) and two-species mixtures (RF model, $P = 0.008$, AUC = 0.74) respectively (Fig. 4; Appendix S2: Fig. S6B, C and G), while the RF model was not significant in multi-tree species mixtures ($P = 0.247$). In case of bacteria, the number of classifier taxa showed little variation among all TSPs in the combined dataset (RF model, $P = 0.001$, AUC = 0.75), monocultures (RF model, $P = 0.003$, AUC = 0.78) and two-species mixtures (RF model, $P = 0.001$, AUC = 0.74) (Fig. 4; Appendix S2: Fig. S6D–F, G). Similar to fungi, the RF model for bacteria was also not

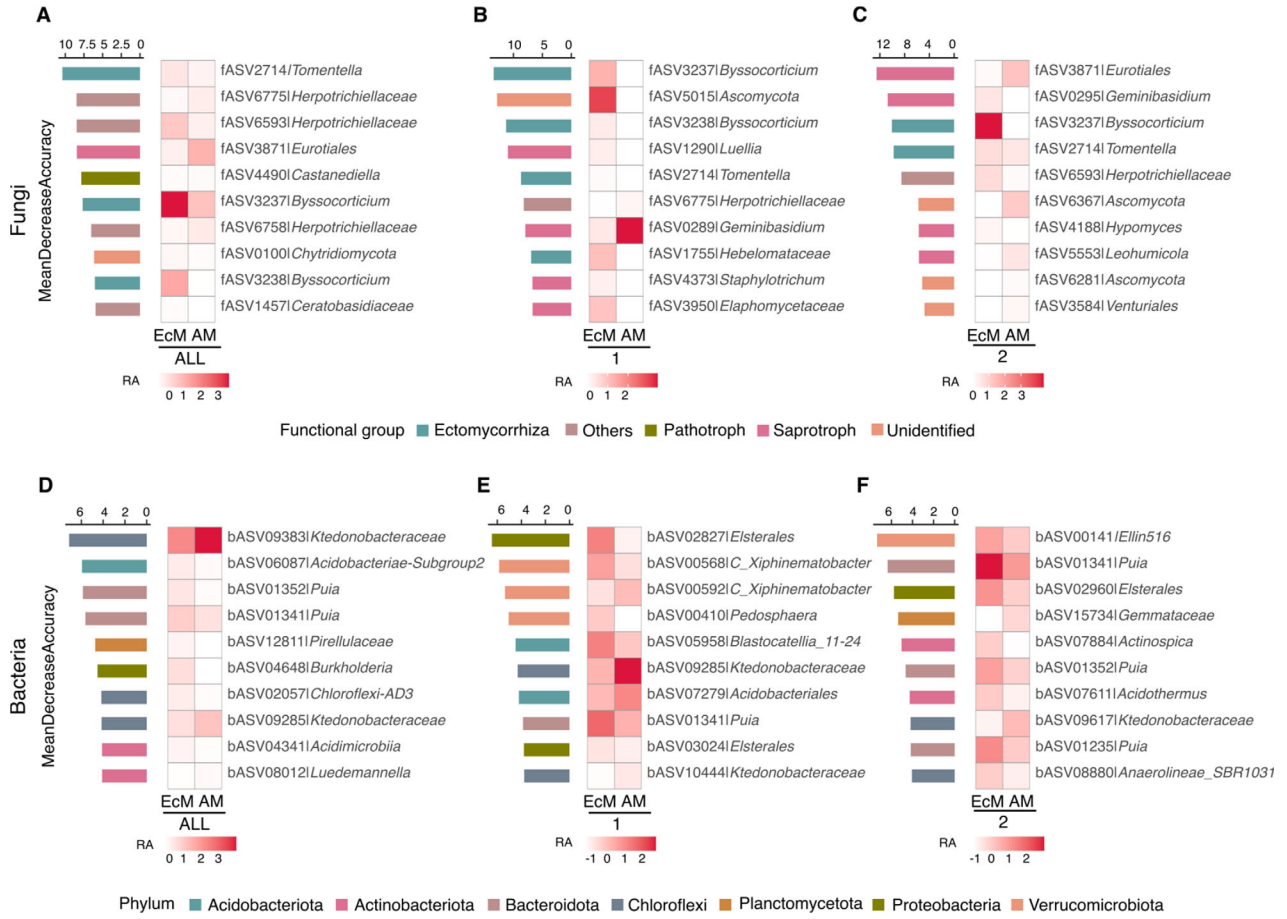

Fig. 4. Topmost influential soil microbial taxa driving the differences between tree mycorrhizal types. On the x-axis, EcM and AM TSPs and the tree diversity levels (All – combined dataset irrespective of tree diversity, 1 – monocultures, 2 – two-species mixtures) random forest (RF) model determined top 10 microbial taxa (ASVs) arranged in descending order of the mean decrease in accuracy. The left side panel of each subplot (i.e., A, B, C, D, E, F) shows the mean decrease in accuracy in bar graphs coloured by functional groups for Fungi (i.e., A, B, C) and phylum for Bacteria (i.e., D, E, F). Right side panel is the heatmap representation of the z-standardized percentage relative abundances (RA) of the respective taxa in EcM and AM TSPs. The taxa were named by respective ASV followed by its lowest taxonomic level up to the genus.
A. Combined fungal dataset of all TSPs.
B. Fungi in monocultures.
C. Fungi in two-species mixtures.
D. Combined bacterial dataset of all TSPs.
E. Bacteria in monocultures.
F. Bacteria in two-species mixtures. The RF models were not significant in multi-tree species mixtures.

significant in multi-tree species mixtures ($P = 0.701$). Furthermore, including also the Mycomix-TSPs along with EcM and AM TSPs at multi-tree species mixtures, as well resulted in no significant RF models for both fungi and bacteria (Fungi, $P = 0.179$; Bacteria, $P = 0.529$).

The majority of the top fungal classifier taxa belonged to EcM and saprotrophs, which consisted of 4, 8 and 6 ASVs out of the top 10 ASVs in the combined dataset, monocultures and two-species mixtures respectively (Fig. 4 A–C). Among the top fungal classifier taxa, all ectomycorrhizal ASVs (e.g., *Tomentella* fASV2714 and *Byssocorticium* fASV3237, fASV3238) had comparatively higher relative abundances in EcM TSPs than in AM TSPs in the overall dataset and across the monocultures and two-species mixtures. In contrast, saprotrophs did not show any distinct abundance pattern. For example, fASV0289 had a higher relative abundance in monocultures of AM TSPs, while fASV3950 had a higher abundance in EcM TSPs. In the case of top bacterial classifier taxa, the ASVs belonging to Bacteroidota (*Puia* bASV01352, bASV01341 and bASV01235) and Proteobacteria (Elsterales bASV02827, bASV03024, bASV02960 and *Burkholderia* bASV04648) had comparatively higher relative abundances in EcM TSPs than AM TSPs. In contrast, the ASVs belonging to the family Ktedonobacteraceae of the phylum Chloroflexi were relatively highly abundant in AM TSPs (Fig. 4D–F).

Interplay among environmental factors, tree mycorrhizal type and tree diversity in shaping soil microbial community composition

Analysis of the role of soil, plant, topography and spatial variables in shaping the soil microbiota using the dbRDA model revealed that the fungal communities of both EcM and AM TSPs were associated with a common set of environmental conditions (Appendix S3: Table S6). P, NO_3^-, NH_4^+ and pH were the significant edaphic variables along with topographic, spatial and tree community variables that mainly influenced the variation in fungal community composition. However, across the tree diversity levels, the environmental factors associated with EcM and AM TSPs fungal communities varied to some extent (Appendix S3: Table S7). In general, AM TSPs fungal communities were significantly associated with a greater number of environmental variables measured in this study. In monocultures, pH and TSP identity were common edaphic and tree variables respectively that were significantly associated with both the EcM and AM TSPs fungal communities, while P, NH_4^+ and tree community composition were only related to AM TSPs fungal communities. In two-species mixtures, pH ($F = 1.547$, $P < 0.001$) and N ($F = 1.317$, $P = 0.034$) were significant edaphic factors related to the AM TSPs fungal communities, while P ($F = 1.576$, $P = 0.009$) was the only significant soil factor related to EcM TSPs fungal communities. In multi-tree species mixtures, a relatively smaller number of environmental variables had significant associations with the variation in fungal community composition. NO_3^-, altitude and slope were common variables related to both EcM and AM TSPs fungal community composition. In addition to AM TSPs ($F = 1.478$, $P = 0.027$), fungal communities under the Mycomix-TSPs ($F = 1.620$, $P = 0.001$) in multi-tree species mixtures were significantly related to the tree community composition (Appendix S3: Table S7).

Bacterial communities of both EcM and AM TSPs were also associated with a common set of environmental variables, including NO_3^-, pH, moisture and tree community composition along with topographical and spatial variables (Appendix S3: Table S6). Soil pH ($F = 10.05$, $P < 0.001$) was the most influential factor for bacterial communities, irrespective of the tree mycorrhizal type and tree diversity level. In monocultures, pH ($F = 2.980$, $P < 0.001$) was the only soil variable significantly associated with EcM TSPs bacterial communities, while AM TSPs bacterial communities in addition to pH ($F = 4.961$, $P < 0.001$), were also affected by P ($F = 2.113$, $P = 0.031$) (Appendix S3: Table S8). In two-species mixtures, a relatively greater number of environmental variables displayed significant associations with bacterial community composition compared with monocultures and multi-tree species mixtures. In multi-tree species mixtures, AM TSPs bacterial communities were significantly related to NO_3^- ($F = 1.700$, $P = 0.041$) and moisture ($F = 1.913$, $P = 0.029$), as well as to pH ($F = 3.492$, $P < 0.001$). Similar to fungi, bacterial communities under the Mycomix-TSPs in multi-tree species mixtures were in addition significantly related to the tree community variables (tree community composition: $F = 2.317$, $P = 0.001$; TSP identity: $F = 1.784$, $P = 0.039$).

Discussion

Tree mycorrhizal type affects fungal rather than bacterial alpha diversity

We found that the mycorrhizal type of the TSPs affected the fungal alpha diversity confirming our hypothesis (H1). The soil fungal alpha diversity of EcM TSPs was significantly lower than that of AM TSPs in terms of taxa richness, evenness and diversity. These consistent differences in various aspects of fungal alpha diversity indicate an important role of the mycorrhizal partner of EcM and AM TSPs in the recruitment of the co-occurring fungal community. These results are in line with the negative impact of higher EcM plant abundance in the soil fungal richness reported in boreal and temperate sites, underlining the differences between EcM and AM tree dominated forests (Bahram *et al*., 2020). This is mainly because EcM and AM fungal partners of the EcM and AM TSPs differ in their resource acquisition, allocation and plant–soil feedback strategies, which affect the recruitment of the different microbes into their respective mycorrhizospheres (Bonfante and Anca, 2009). EcM fungi were reported to have slower decomposition rates and could limit the abundance of saprotrophs and other free-living fungi through competitive interactions for organic nutrients (Moore *et al*., 2015; Bödeker *et al*., 2016; Bahram *et al*., 2020). In contrast, AM fungal partners rely on coexisting saprophytic fungal partners to facilitate decomposition and nutrient cycling in AM tree dominated habitats (Midgley *et al*., 2015; Jacobs *et al*., 2018; Tedersoo and Bahram, 2019). Accordingly, the high fungal diversity and relative abundances of saprotrophs under AM TSPs, irrespective of the tree diversity levels considered, indicate the taxonomic and functional contribution of saprotrophic fungi in AM-dominated systems (Beidler and Pritchard, 2017). The overall soil bacterial alpha diversity of EcM and AM TSPs, however, was not significantly different, indicating no strong impact by the mycorrhizal type in this early-successional forest ecosystem. Bahram *et al*. (2020) documented that sites in which EcM plants dominated had significantly lower soil bacterial taxonomic richness. However, they found a small difference in the bacterial

richness among the sites dominated by deciduous EcM plants, as well as among both coniferous and deciduous AM plants dominated sites. It is known that there are only a few strong drivers of the soil bacterial diversity, mainly soil pH (Fierer and Jackson, 2006; Delgado-Baquerizo and Eldridge, 2019). One of the possible explanations for non-significant differences in soil bacterial alpha diversity between EcM and AM TSPs could be that the differences in environmental conditions brought about by the experimental treatments, such as tree diversity and tree species composition, were not large enough to result in large differences as were reported in other studies.

Tree diversity level affects fungal rather than bacterial alpha diversity

As part of our hypothesis (H1), we had postulated that the soil microbial alpha diversity of the EcM and AM TSPs increases with the increasing tree species diversity. Tree diversity *per se* had no significant effect, neither on the overall fungal richness nor on bacterial richness. Nevertheless, we found that EcM TSPs soil fungal alpha diversity but not that of AM TSPs increased with tree species diversity. Furthermore, we found significant interactions between tree mycorrhizal type and tree diversity levels, indicating that the tree mycorrhizal type effect on soil fungal communities was dependent on the tree diversity level. Previous research was inconclusive about the tree diversity effect on soil fungal communities. For instance, in their global observational study on soil fungi, (Tedersoo *et al*., 2014) found no significant relationship between plant diversity and fungal richness, except for ectomycorrhizal fungi. Recently, a 7-year-old tree diversity experiment with temperate mixed deciduous trees (Rivest *et al*., 2019) could not demonstrate any effect of tree diversity on the fungal alpha diversity. Conversely, studies in grassland (Lange *et al*., 2015; Chen *et al*., 2017), temperate (Hiiesalu *et al*., 2017), subtropical (Gao *et al*., 2013; Weißbecker *et al*., 2019; Chen *et al*., 2019b) and tropical (Peay *et al*., 2013) ecosystems have reported positive relationships between tree diversity and fungal alpha diversity. Instead, our findings underline the need to consider tree mycorrhizal type as an important factor in studying 'tree diversity – soil microbial diversity' relationships. Previous studies described plant diversity and guild-specific fungal relationships, especially the positive relationship of ectomycorrhizal fungi with plant richness, while non-significant or rather weak effects were reported in the case of saprotrophs (Peay *et al*., 2013; Nguyen *et al*., 2016b). We, however, found contrasting patterns for EcM fungal relative abundance in the EcM and AM TSPs with increasing diversity levels which could be justified based on the knowledge that EcM fungi competitive interactions (Moore *et al*., 2015; Bödeker *et al*., 2016; Bahram *et al*., 2020) and AM fungi co-operative interactions (Beidler and Pritchard, 2017) with other fungal communities in resource acquisition. The predominance of ectomycorrhizal fungi in monocultures and two-species mixtures of EcM TSPs compared with that of multi-tree species mixtures might be an explanation for the higher alpha diversity in the latter. In both AM and EcM TSPs, the relative contribution of the EcM and saprotrophic fungi decreases with increasing tree diversity as the alpha diversity of other fungal groups increases.

In contrast to our expectation, the bacterial alpha diversity did not significantly increase with tree diversity. In a 10-year-old tropical tree experimental site, (Yamamura *et al*., 2013) were not able to detect any significant differences in bacterial richness among plots with differing tree species richness. Likewise, no significant relationship between plant alpha diversity and bacterial alpha diversity was reported in grasslands (Prober *et al*., 2015). Evidence shows that the plant diversity effects are relatively stronger for fungi than that of bacteria (Lange *et al*., 2015; Eisenhauer *et al*., 2017; Vieira *et al*., 2020), probably as a result of their morphological and ecophysiological differences (Barberan *et al*., 2015; Dassen *et al*., 2017) which could be a possible reason for the observed non-significant differences in bacterial diversity in our study. Alternatively, the effect of tree diversity on soil bacterial diversity as well as on the fungal diversity of AM trees at our study site might become more important in the long term (Eisenhauer *et al*., 2010; Chen *et al*., 2019a; Xu *et al*., 2020). A noteworthy outcome of the positive tree diversity effects was the absence of soil microbial diversity differences in multi-tree species mixtures as a result of less diverging communities, irrespective of which tree species were involved.

Higher tree diversity levels neutralize the tree mycorrhizal type effects on soil microbial community composition

Mycorrhizal fungi are known to influence the surrounding soil microbiota composition through the mycorrhizosphere and extraradical mycelium by controlling resource allocation and chemical signalling (Wallander *et al*., 2006; Finlay, 2008; Tedersoo *et al*., 2009; Tedersoo *et al*., 2020a). We had hypothesized (H2a) that the microbial community composition depends on tree mycorrhizal type, and in line with this expectation, the tree mycorrhizal type had a significant effect on the fungal community composition. In contrast, bacterial community composition was not significantly impacted by the tree mycorrhizal type. Likewise, Bahram *et al*. (2020) also reported that bacterial community composition was not driven by the tree mycorrhizal type. Our data showed a strong impact of the environmental variables,

such as soil chemistry, topographical variables and spatial variables, on the bacterial community compositional differences rather than by tree community variables, which explains the relatively weaker effect of the tree mycorrhizal type.

Both soil fungal and bacterial community compositions of the EcM and AM TSPs became less dissimilar with increasing tree species diversity, confirming the second statement of our second hypothesis (H2b) tested by PERMANOVA and dbRDA analyses. Tree species (host) can select the soil microbiota, for instance, by the effects of tree species identity (Wubet *et al*., 2009; Weißbecker *et al*., 2018) and the genotype (Karliński *et al*., 2020). These effects can be mediated through modulating the soil chemistry resources (Urbanová *et al*., 2015; Wu and Yu, 2019). Assuming that each tree species to some extent can have species-specific and generalist soil microbial communities, one would expect an increasing number of microbial species with increasing tree diversity covering more and more taxa of the local microbial species pool. In addition, plants can both recruit from and contribute to the surrounding soil microbial species pool (Compant *et al*., 2019), and therefore, may explain the more similar microbial community composition in multi-tree species mixtures in this study. This view is supported by the ASV richness patterns both in fungi (here in particular under the EcM TSPs) and in bacteria (here in particular under the AM TSPs). However, it is important to consider that the observed neutralizing effect at higher tree diversity level is driven by either tree diversity regardless of the tree mycorrhizal type in bacterial communities, or the presence of different mycorrhizal type trees in the high diversity plots in the case of fungal communities. The fungal taxonomic and functional group relative abundance distributions of both EcM and AM TSPs in multi-tree species mixtures resembles a 'give-and-take' relationship (for example, Chaetothyriales abundance got increased in EcM TSPs of multi-tree species mixtures which were relatively abundant in AM TSPs, while ditto was the case for Thelephorales in AM TSPs of multi-tree species mixtures which were relatively abundant in EcM TSPs). These patterns might explain the maintenance of the local soil microbial species reservoir at the higher tree diversity levels.

The role of classifier taxa in driving the differences between tree mycorrhizal types

The discriminatory power of RF models confirmed the second statement of our second hypothesis (H2b), as the most influential microbial taxa driving the differences between tree mycorrhizal types were reduced to non-significant at the multi-tree species mixtures for both fungi and bacteria. This finding shows that at high tree species richness the presence of strong indicator taxa does not exclude the presence of other strong indicator taxa, thus allowing their coexistence. This is in concordance with the results from ordination and PERMANOVA analyses, as with lower dissimilarity in the microbial community composition also fewer microbial taxa should determine the differences. Moreover, RF models highlighted the differences between monocultures and two-species mixtures in bacterial communities, which were not reflected by the PERMANOVA. We observed that the bulk of the top fungal classifier taxa belonged to EcM and saprotrophs. This can not only be expected with regards to their respective relative abundance distributions under EcM and AM TSPs but also, more importantly, manifests the differential patterns in their nutrient acquisition and processing strategies (Tedersoo and Bahram, 2019). We found higher relative abundances of EcM as top fungal classifier taxa in EcM TSPs compared with that of AM TSPs, which was expected, but interestingly, saprotrophs did not show a similar pattern. Some saprotrophic taxa (e.g., fASV3871, fASV1290) were relatively either abundant or rare under AM TSPs, while the same was the case for other saprotrophic taxa under EcM TSPs (e.g., fASV3950, fASV5553). This pattern suggests that some saprotrophic taxa have an exclusive preferential association with either EcM or AM TSPs, which might indicate the role of tree mycorrhizal partners in the assembly of other taxonomic groups by modulating the microenvironment surrounding the hyphosphere. In a study by (Liu *et al*., 2018) characterizing relationships between macro-fungi and bacteria, the authors reported more Bacteroides in ectomycorrhizal hyphosphere soils, whereas they found more Chloroflexi in hyphosphere soils of saprotrophic fungi. We noticed a similar preferential pattern also in the top bacterial classifier taxa in which the ASVs belonging to Bacteroidota were relatively abundant in EcM than AM TSPs. Whereas, Ktedonobacteraceae of the phylum Chloroflexi were relatively abundant in AM than EcM TSPs. This pattern highlights the essential role of fungal-bacterial interactions in the soil interaction zone of trees in forest ecosystems.

Recently, microbial taxa have been more frequently used as potential predictors of various aspects of ecosystem status like pathogen suppression (Trivedi *et al*., 2017) and soil quality and physicochemical variables (Hermans *et al*., 2020). Similarly, we presented the soil microbial classifier taxa for EcM and AM mycorrhizal type TSPs at the local scale.

The additional contribution of environmental factors explaining microbial community composition

Investigation of the environmental factors across tree diversity levels revealed their significant contribution in

shaping the microbial communities besides the tree mycorrhizal type and tree diversity level, confirming the last part of our expectation (H2c). Furthermore, we found that most of the edaphic and tree community variables selected by our models were common ones, except neighbourhood abundance, for both AM and EcM TSPs soil fungal communities, while bacterial communities were differentially regulated by total organic carbon (TOC) and TSP identity. Nevertheless, AM TSPs soil fungal communities were more strongly affected by the topographic and spatial variables compared with that of EcM TSPs. These results are in accordance with earlier reports on the impact of common edaphic, floristic and spatial variables on fungal communities and their differential effect on different taxonomic and functional groups such as saprotrophs, EcM and arbuscular mycorrhizal fungi (Tedersoo *et al*., 2014; Nguyen *et al*., 2016b; Weißbecker *et al*., 2018).

We found soil pH (Rousk *et al*., 2010; Tedersoo *et al*., 2020b) and host identity (Tedersoo *et al*., 2016), which were known to impact fungal communities, were important factors in both EcM and AM TSPs in monocultures. Also, in bacterial communities, soil pH known as the strong factor driving bacterial community composition in different ecosystems (Fierer and Jackson, 2006; Delgado-Baquerizo and Eldridge, 2019; Jiao and Lu, 2020), was found to have a consistently strong effect irrespective of the mycorrhizal type and tree diversity levels. Since our study site is a tree diversity experiment in which tree composition was manipulated, the variation in soil conditions may have been caused, at least in part, by the differences between EcM and AM trees. This variation could be induced by different mechanisms such as litter inputs and mycorrhizal partner-mediated microbe–microbe interactions. It was reported that generally, AM trees produce high-quality litter (e.g., low C:N) and higher nutrient content compared with EcM trees (Midgley *et al*., 2015). This was evident, for example, that EcM TSPs bacterial communities were significantly impacted by TOC and the fungal communities of AM TSPs in monoculture were significantly impacted by NH_4^+. We observed in the multi-tree species mixtures for both fungi and bacteria that the number of significantly associated environmental factors decreased in comparison to lower diversity tree stands. This is expected as with the increasing tree diversity, the co-occurrence of tree species increases, yielding more similar environmental conditions. Microbes can also change the soil environment through their interactions by promoting or impeding processes like mineralization or nitrification. Soil chemical properties, including NO_3^-, N, pH and moisture, were the significant factors in the multi-tree species mixtures, whose significance might imply the microbe-regulated processes like mineralization or nitrification at higher tree diversity levels. Altogether, our findings confirm a tripartite interplay of tree mycorrhizal type, tree diversity and environmental factors in modulating the microbiota of the tree–tree soil interaction zone. Nevertheless, there might be potential unknown legacy effects at the site from the previous conifer plantations, which would be very difficult to quantify. However, the experiment was already 10 years old at the time of sampling, making legacy effects of the previous vegetation on the microbial community less likely.

Conclusions

Here, we provided an unprecedented empirical evidence for the interactive effects of the tree mycorrhizal type and tree diversity on the soil fungal and bacterial communities. We also demonstrated that these effects varied with environmental conditions. Furthermore, differences in microbial species composition disappeared with increasing tree species richness. For bacterial communities, this effect was caused by the different tree species irrespective of their mycorrhizal type, while for fungal communities the effect was the result of the interactive effects of the coexistence of tree species of different mycorrhizal types at higher tree species richness. Overall, this led us to the generalized conclusion that microbial community differences among tree mycorrhizal types disappear in multi-tree species mixtures. Our results show that tree mycorrhizal type is an important factor to disentangle the mechanisms underlying positive, negative and/or neutral effects of tree diversity on soil microbial diversity in tree diversity experiments. This knowledge is crucial in light of the on-going and much-needed research on the biodiversity-ecosystem function (BEF) relationships. Moreover, we encourage further research to get a deeper understanding of the causal relationships among environmental variables, tree mycorrhizal type, soil microbial communities and the forest ecosystem functioning using controlled experiments. It is known that higher fungal and bacterial diversity enhances the soil ecosystem functioning (Wagg *et al*., 2019), but context-dependent effects need further exploration (Eisenhauer *et al*., 2019). Finally, using the tree species pair (TSP) approach, we have identified that planting AM and EcM mycorrhizal type trees together in higher tree diversity levels may promote high soil microbial diversity with converging community composition, which in turn might contribute to the stable and better forest soil ecosystem functioning.

Experimental procedures

Study site and experimental design

The study site is one of the BEF-China tree diversity experimental sites (Site A) with a diversity gradient

ranging from monocultures to 24-species mixtures, hosting native subtropical tree species (Bruelheide *et al*., 2014). The site was established in 2009, in a hilly area with a subtropical monsoon climate, located in Southeast China, Jiangxi Province. The previous land use was a plantation of *Pinus massoniana* and *Cunninghamia lanceolota*, which were cut before the trees were planted in the experiment. The plots had a size of 25.8 m × 25.8 m, spanned an area of 18.4 ha and were planted with 400 trees each, spaced on a regular grid at 1.29 m. Further information about the general design and establishment of the BEF-China experiment can be found in (Yang *et al*., 2013) and (Bruelheide *et al*., 2014).

Following the TSP concept, two adjacent trees were selected as a target-sampling unit and the 10 surrounding trees of a TSP were considered as the neighbourhood (Trogisch *et al*., 2021). Accordingly, six EcM and six AM type TSPs (Appendix S1: Table S1, Fig. S1) were randomly selected across 57 plots, with each three replicates in monocultures (denoted by '1') and two-species mixtures (denoted by '2'), and one replicate in each 4, 8 and 16 or 24 multi-tree species mixtures (denoted by '≥4'). We obtained the following six combinations: 'EcM|1' ($n = 18$), 'EcM|2' ($n = 18$), 'EcM| ≥ 4' ($n = 18$), 'AM|1' ($n = 18$), 'AM|2' ($n = 18$) and 'AM| ≥ 4' ($n = 18$). Besides, to study the heterotypic combination of mycorrhizal type (i.e., combination of EcM tree and AM tree), we included six pairs of AM and EcM tree species, each with three replicates ($n = 18$) as heterotypic pairs (referred to as 'Mycomix-TSPs') only in the multi-tree species mixtures but not in the two-species mixtures. This resulted in a total of 126 soil samples.

Soil sampling and processing

Soil samples were randomly collected from mid-August to the end of September. Before taking soil cores, litter and any other debris were cleared from the soil surface. Four cores of each 10 cm depth and 5 cm diameter were collected along the horizontal axis of the two partner trees of a TSP with distances of 5 cm from the centre for the first two cores and further 20 cm away for the other two cores (Appendix S1: Fig. S1D). The obtained four soil cores were pooled, mixed and root fragments were removed by sieving the mixed soil through 2-mm mesh size sieves to yield a composite soil sample. These soil samples were then aliquoted for soil chemistry (50 g) and microbiota analyses (30 g) into sampling tubes and immediately placed on dry ice in a cool box and transported to the field lab. Then the samples for the microbiota analysis were freeze-dried (Weißbecker *et al*., 2017) and stored at −80°C until further analyses.

Soil chemical properties

Each soil sample was divided into two parts used in the analysis of soil moisture and soil nutrients respectively. For the first portion, soil moisture was measured by recording the mass lost after drying the soil at 105°C for 24 h. The other sub-sample was air-dried. NH_4^+ and NO_3^- were extracted with 2 M KCl and measured by the colorimetric method with a Smart Chem 200 Discrete Auto Analyser (AMS, Italy) (Talbot *et al*., 2014). Soil TOC was measured by a TOC Analyser (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). Soil total nitrogen (TN) was measured on an auto-analyser (SEAL Analytical GmbH, Norderstedt, Germany) using the Kjeldahl method (Bradstreet, 1954). Soil total phosphorus (TP) was measured after wet digestion with H_2SO_4 and $HClO_4$ by a UV–VIS spectrophotometer (UV2700, SHIMADZU, Japan). Soil pH was measured in a 1:2.5 soil–water solution (pH meter Thermo Scientific Orion Star A221) after air-drying the soil at 40°C for 2 days.

DNA extraction, amplicon library preparation and sequencing

Microbial genomic DNA was extracted from freeze-dried soil samples using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, United States) according to the manufacturer's instructions. DNA concentrations were measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracts were adjusted to 10–15 ng μl^{-1} template concentration. The bacterial and fungal amplicon libraries were prepared as previously described (Schöps *et al*., 2018; Nawaz *et al*., 2019). Briefly, the V4 region of the bacterial 16S rRNA gene was amplified using the universal primer pair 515f and 806r (Caporaso *et al*., 2011) with Illumina adapter sequence overhangs. Semi-nested PCR was performed for fungi to amplify the ITS2 rDNA region using the initial primer combination of ITS1F (Gardes and Bruns, 1993) and ITS4 (White *et al*., 1990) followed by the primer pair fITS7 (Ihrmark *et al*., 2012) and ITS4 containing the Illumina adapter sequences. The amplicon libraries were purified with Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT Indices were added to both ends of the bacterial and fungal fragments in the indexing PCR. The indexed products were purified again with AMPure beads and then quantified by PicoGreen assay. The amplicon libraries were pooled equimolarly to a final concentration of 4 nM each for fungi and bacteria. Then fungal and bacterial libraries were pooled in 1:3 ratio to make the final library and paired-end sequencing of 2 × 300 bp was performed on an Illumina MiSeq platform (Illumina, San Diego, CA,

United States) using MiSeq Reagent kit v3 at the Department of Environmental Microbiology, UFZ, Leipzig, Germany.

Bioinformatics analysis

Bioinformatic analysis was performed to filter out high-quality reads from the raw reads generated by the Illumina MiSeq Sequencing platform using the Quantitative Insights into Microbial Ecology – QIIME 22020.2 (Bolyen *et al*., 2019) software. The forward and reverse reads were demultiplexed according to the index combinations, primer sequences were trimmed, followed by sequence denoising and grouping into ASVs using cutadapt (Martin, 2011) (q2-cutadapt) and DADA2 (Callahan *et al*., 2016; via q2-dada2) respectively. Taxonomy was assigned to 16S bacterial ASVs using the q2-feature-classifier (Bokulich *et al*., 2018), using the classify-sklearn naive Bayes taxonomy classifier against the silva-132-99-515-806-nb-classifier. The fungal ITS dataset was analysed using the q2-ITSxpress Qiime2 plugin (Rivers *et al*., 2018), where the ITS2 fungal sequences were identified and trimmed, followed by denoising and grouping into ASVs using the DADA2. Taxonomy was assigned to fungal ITS ASVs using the q2-feature-classifier (Bokulich *et al*., 2018), using the classify-sklearn naive Bayes taxonomy classifier against the unite-ver8-99-classifier-04.02.2020.

The respective fungal and bacterial ASV matrices, taxonomic tables and representative sequences were imported into R (version 4.0.2) using the phyloseq package (McMurdie and Holmes, 2013) for further statistical analysis. The fungal and bacterial ASVs were filtered, and the ASV matrices were rarefied to 16,542 and 28,897 reads per sample respectively. Furthermore, to avoid the potentially spurious taxa and to reduce the noise, taxa that were not present in at least 5% of the samples were removed in both fungal and bacterial datasets (Cao *et al*., 2021). Liner regression ('lm' function) and Mantel tests ('mantel' function in vegan) were used to test the effect of removal of low abundant taxa on alpha diversity indices and microbial community composition analyses respectively. Fungal and bacterial ASVs were annotated for potential functional groups using FUNGuild (Nguyen *et al*., 2016a) and FAPROTAX (Louca *et al*., 2016) respectively. Saprotrophs and pathotrophs were considered one functional group each. Symbiotrophs were further classified to distinguish ectomycorrhizae and arbuscular mycorrhizae as functional groups, and the remaining guilds of symbiotrophs were named as 'other symbiotrophs'. The fungal taxa with more than one trophic mode were classified as 'others'. We assigned the putative bacterial functional groups to broad ecological processes, namely carbon cycle, nitrogen cycle and sulphur cycle. Bacterial taxa were assigned to the respective aforementioned nutrient cycles if that particular taxon was assigned to at least one functional group within that particular category. If a taxon was associated with two or more functional groups belonging to different nutrient cycles (e.g., carbon cycle and nitrogen cycle), then it was assigned to a combined category (e.g., carbon & nitrogen cycle). Functional groups that did not fall under these preceding categories were grouped as 'other'.

Statistical analysis

All the statistical analyses were done in R (version 4.0.2) using the phyloseq package (McMurdie and Holmes, 2013). In both fungal and bacterial datasets, sequencing data of each replicate collected from 4, 8 and 16 or 24 tree species richness levels were combined into a 'multi-tree species mixtures' group after checking for the homogeneity of variance of sequence library sizes (Levene's test; for Fungi $P = 0.42$; for Bacteria $P = 0.22$). EcM and AM TSPs ($n = 108$) were used in the following statistical analyses of mycorrhizal type across tree diversity levels. The heterotypic TSP combination, i.e., Mycomix-TSPs ($n = 18$), were included in the analyses only to compare different mycorrhizal types (EcM, AM and Mycomix) at tree diversity level of multi-tree species mixtures unless otherwise stated. The ecosystem state variables, i.e., observed richness, Shannon diversity, Pielou evenness and Gini dominance were calculated as measures of alpha diversity using the microbiome package (Lahti *et al*., 2017). Wilcoxon rank sum-tests were used to test for group differences in alpha diversity. The interaction between mycorrhizal type and tree diversity was tested for fungi with two-way ANOVA, and for this purpose, the data was tested for normality and homogeneity of variance using Shapiro–Wilk test and Levene's test respectively. Fungal observed richness and Pielou evenness, each was Box–Cox transformed with a lambda value of 1.45 to meet the normality and homogeneity of variance assumptions using the car package (Fox and Weisberg, 2018). Taxonomic and assigned functional group relative abundances were calculated and visualized with bar charts. Distance-based ordination (dbRDA) constrained by tree mycorrhizal type and tree diversity was done with the 'capscale' function in the vegan package (Oksanen *et al*., 2019), using Bray–Curtis distance to test and visualize the patterns in microbial community compositions. The differences in compositions were tested for the effect of mycorrhizal type and tree diversity with PERMANOVA using the vegan package. Multivariate homogeneity of variances of groups was checked with 'betadisper' function before PERMANOVA. Pairwise community compositional

differences were tested using the function 'pairwise.adonis' from the pairwiseAdonis package (Martinez Arbizu, 2017).

We used RF models to determine the most influential microbial taxa driving the differences between tree mycorrhizal types. RF is a robust machine-learning tool with high prediction accuracy befitting for the microbiome data (Statnikov *et al*., 2013; Kim *et al*., 2020). All taxa with an abundance of > 3% mean total sequencing reads and a frequency of at least 2/3rd of the samples (≥33%) were considered for RF analysis in both fungal (728 taxa) and bacterial (798 taxa) datasets. The fungal and bacterial ASV relative abundance matrices were z score standardized and then RF classification models were constructed over 3001 decision trees using the rfPermute package (Archer, 2016). The RF models were assessed for statistical significance with 999 permutations using 'rf.significance' function in the rfUtilities package (Evans and Murphy, 2015). Further, the significance of the importance metrics of each microbial taxon was measured using 999 permutations of the response variable in the 'rfPermute' function in the rfPermute package. The microbial taxa responsible for significant ($P < 0.05$) mean decrease in accuracy and mean decrease in Gini impurity index of the RF models were selected as the most influential microbial taxa (here, referred to as classifier taxa). The top 10 taxa in RF models with high mean decrease in accuracy were identified as the top classifier taxa and their relative abundances among EcM and AM TSPs were visualized with heatmaps. Subsequently, the performance of the RF models was evaluated by the receiver operating characteristic curve (ROC curve) and AUC metrics using ROCR package (Sing *et al*., 2005).

Significant biotic and abiotic factors associated with the microbial (fungi and bacteria) community compositions were selected using distance-based redundancy analysis (dbRDA) models based on the Bray–Curtis distance ('capscale' function in vegan). Explanatory variables were standardized to a constant mean and standard deviation ('decostand' function in vegan). Prior to variable selection, multi co-linearity was checked using the 'vifstep' function in usdm package (Naimi *et al*., 2014), and then stepwise model selection ('ordistep' function in vegan) was carried out with permutation tests. Four groups of environmental components were considered for analysis, including tree community variables (tree community composition, TSP identity, tree and shrub species richness, tree Shannon and Simpson diversity indices, abundance and richness of tree neighbourhood, abundance and richness of neighbour AM and EcM TSPs) as biotic factors, soil parameters (C, N, P, C/N, C/P, N/P, TOC, SOM, NH_4^+, NO_3^-, pH and moisture) and topographical variables (altitude, slope, northness and eastness) as abiotic factors and sampling locations (latitude and longitude) as a spatial component. Vectors from principal coordinates of neighbourhood matrices (Dray *et al*., 2006) were used to represent the spatial component (vegan package). Tree community composition and TSP identity were characterized by principal components ('prcomp' function) on the Hellinger-transformed incidence data. Subsequently, the selected variables were used in the dbRDA models and their significance was tested with permutational test ('anova.cca' function in vegan).

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Authors contributions

Conceptualization – Tesfaye Wubet, Helge Bruelheide, Bala Singavarapu; secured relevant funds – Tesfaye Wubet, Helge Bruelheide, Simone Cesarz, Nico Eisenhauer; provided lab facilities – Tesfaye Wubet, Simone Cesarz, Nico Eisenhauer, Kai Xue, Yanfen Wang; field sampling – Bala Singavarapu, Rémy Beugnon; data generation – Bala Singavarapu, Rémy Beugnon, Jianqing Du; bioinformatic analysis and data curation – Bala Singavarapu, Ali Nawaz, Tesfaye Wubet; statistical analysis and visualization – Bala Singavarapu; writing the original manuscript draft – Bala Singavarapu; review and editing of the manuscript – Tesfaye Wubet, Helge Bruelheide, Rémy Beugnon, Simone Cesarz, Nico Eisenhauer, Ali Nawaz, Liang-Dong Guo, Jianqing Du, Kai Xue, Yanfen Wang, Bala Singavarapu; project administration and supervision – Tesfaye Wubet. All authors have read and agreed to this version of the manuscript.

Data availability statement

The datasets generated for this study can be found in the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under bioproject numberPRJNA702024.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 A schematic diagram of the study site, plot, study design and sampling strategy.

Detailed descriptions of components of this figure can be found in the following supplementary text. A. Study site showing the topography and arrangement of the plots (modified from Bruelheide *et al*., 20142014, Fig. 4). B. A schematic of a plot within the site showing individual trees, tree species pair (TSP) and its neighbourhood. C. An illustration of study design with tree species pairs (TSPs) categorized based on their mycorrhizal type shown at their neighbourhood level. Here, the focal TSP is represented by a pair of black line-hatched colour-filled dots (*light green – EcM TSPs; light orange – AM TSPs*). The brown bar bridging the two trees of a TSP represents the tree-tree interaction zone. The 10 colour-filled dots surrounding a TSP represent the 10-tree species in the neighbourhood (different colours represent different tree species). The vertical dotted line (blue colour – ECM; red colour – AM) in this schematic depicts the comparison of one EcM and 1 AM TSP across tree diversity. Mycomix-TSPs i.e. a pair of one EcM and 1 AM tree were shown in the multi-species mixtures. D. A cartoon portraying the soil sampling from the interaction zone of TSPs.

Fig. S2 Fungal and bacterial alpha diversity indices after filtering the low abundant (rare taxa) regressed on the diversity indices before removal of the low abundant taxa (X-axis). Four indices viz. observed ASV richness (i.e., 'Richness'), Shannon diversity (i.e., 'Shannon'), Pielou evenness (i.e., 'Evenness') and Gini dominance (i.e., 'Dominance')

Fig. S3. Pair-wise Wilcoxon tests among EcM and AM TSPs soil fungal communities along the tree diversity levels. (A–D) Fungal ASV richness, Shannon diversity, Pielou's evenness and Gini dominance index respectively. The asterisks above

the boxplots show the p-value (for multiple testing correction) significance level; ns.: $P > 0.05$, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$

Fig. S4. Comparison of soil microbial alpha diversity indices of tree species pairs (TSPs) in multi-tree species mixtures. A. Fungal communities. B. Bacterial communities

Fig. S5. Phylum-level taxonomic composition of TSP soil fungal and bacterial communities. A. Fungal composition under EcM TSPs; B. Fungal composition under AM TSPs; C. Bacterial composition under EcM TSPs; D. Bacterial composition under AM TSPs

Fig. S6. RF model performance metrics. A–C. ROC curves of RF models of soil fungal communities; B–F. ROC curves of RF models of soil bacterial communities; G. Bar plots showing the number of Significant classifier taxa determined by RF models. F – Fungi; B – Bacteria; All – Combined dataset; 1 – monocultures; 2 – two-species mixtures. The RF models were not significant in multi-species mixtures.

Table S1: List of Tree species and their mycorrhizal type

Table S2 Two-way-ANOVA effects of tree mycorrhizal type and tree diversity on alpha diversity metrics of the soil fungal communities

Table S3: Pair-wise PERMANOVA of the EcM and AM TSPs soil microbial communities along the tree diversity levels

Table S4: EcM and non-EcM fractions of fungal orders Agaricales, Cantharellales, Russulales, Sebacinales & Thelephorales

Table S5: Pair-wise analysis of TSP effects on soil microbial communities

Table S6: Significant factors associated with the fungal and bacterial community compositional variation based on dbRDA model selection

Table S7: Significant factors associated with the fungal community compositional variation across tree diversity levels based on dbRDA

Table S8: Significant factors associated with the bacterial community compositional variation across tree diversity levels based on dbRDA