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Tree and shrub richness modifies subtropical tree productivity by regulating the diversity and community composition of soil bacteria and archaea

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Abstract

Background Declines in plant biodiversity often have negative consequences for plant community productivity, and it becomes increasingly acknowledged that this may be driven by shifts in soil microbial communities. So far, the role of fungal communities in driving tree diversity-productivity relationships has been well assessed in forests. However, the role of bacteria and archaea, which are also highly abundant in forest soils and perform pivotal ecosystem functions, has been less investigated in this context. Here, we investigated how tree and shrub richness affects stand-level tree productivity by regulating bacterial and archaeal community diversity and composition. We used a landscape-scale, subtropical tree biodiversity experiment (BEF-China) where tree (1, 2, or 4 species) and shrub richness (0, 2, 4, 8 species) were modified.

Results Our findings indicated a noteworthy decline in soil bacterial α-diversity as tree species richness increased from monoculture to 2- and 4- tree species mixtures, but a significant increase in archaeal α-diversity. Additionally, we observed that the impact of shrub species richness on microbial α-diversity was largely dependent on the level of tree species richness. The increase in tree species richness greatly reduced the variability in bacterial community composition and the complexity of co-occurrence network, but this effect was marginal for archaea. Both tree and shrub species richness increased the stand-level tree productivity by regulating the diversity and composition of bacterial community and archaeal diversity, with the effects being mediated via increases in soil C:N ratios.

Conclusions Our findings provide insight into the importance of bacterial and archaeal communities in driving the relationship between plant diversity and productivity in subtropical forests and highlight the necessity for a better understanding of prokaryotic communities in forest soils.

Keywords Archaea, Bacteria, BEF-China, Shrub competition, Tree species richness, Tree growth

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Background

Anthropogenic activities have resulted in the loss of biodiversity worldwide [1, 2], in altered ecosystem functioning [3] and services [4]. This has fostered a large research field that aims at understanding the relationship between biodiversity and ecosystem functioning [5]. Much of the work originates from grassland systems [6–9], where it has been found that plant species diversity generally increases plant community productivity, and that this relationship is driven by shifts in the soil microbial community [10]. Although recent studies found similar biodiversity-ecosystem functioning (BEF) relationships in forests [11–14], it is still poorly understood how changes in soil biodiversity contribute to increased productivity in diverse tree stands. Moreover, the presence of shrubs in forests can interfere with the diversity effects of trees. In general, shrubs in the understory may reduce tree productivity [15, 16], but these effects may be weakened at higher levels of shrub richness [16]. Therefore, to fully understand BEF relationships in forests it will be of importance to test how tree and shrub diversity in forest ecosystems drive soil community composition, and consequently influence tree productivity.

Microbial diversity may underlie positive effects of plant diversity on productivity, since higher plant species richness may lead to an increased availability of plant-derived resources, resulting in improved niche optimization and complementary use of subsistence resources [17, 18]. As a result, the diversity across multiple trophic levels is enhanced [19–21], ultimately improving ecosystem functioning [20–22]. Evidence from long-term diversity experiments in grassland support the idea [23, 24] that plant diversity drives the structure and functioning of soil microbial communities through the bottom-up (resource control) effects [7, 23]. Forests host a diverse array of microbial communities, including fungi, which often form symbiotic relationships with plants in forest soils [25–27]. The composition of fungal communities has been shown to be closely linked to tree species richness [28–31]. In addition, forest soils also harbor abundant prokaryotic communities [32], with certain groups, such as bacteria and archaea, playing important roles in carbon fluxes, nutrient cycling, and decomposition [33–40]. However, the extent to which shifts in bacterial and archaeal diversity, community composition, and complexity (e.g., network structure, connectedness) underlie BEF relationships in forests remains unclear. In addition, there is limited information on whether the mycorrhizal types of the focal tree species shape soil prokaryote communities in the context of changing tree and shrub species richness levels, although there has been extensive research demonstrating that it has a significant effect on fungal communities [31, 41, 42].

As plant diversity increases, it leads to the development of complex interactions among plants, which subsequently increases the complexity of interactions among plants and associated microbes [43–45]. Given that non-random community assembly may be a general characteristic for microorganisms [46], a correlation-based network of cooccurring microorganisms based on strong and significant correlations (non-parametric Spearman's) [47] was widely used to reveal microbial co-occurrences and the connectivity among community members [48–53]. A pioneer research in experimental grassland ecosystems observed that microbial network complexity positively influences multiple ecosystem functions [22]. It would be of great interest to examine whether changes in plant diversity could influence the microbiome complexity, such as diversity and interconnectedness among co-occurring microbes, and whether it could have an impact on the way in which microbe communities influence ecosystem function. To investigate how soil prokaryotic community composition, diversity, and cooccurrence networks respond to tree and shrub species richness and how this in turn affects tree productivity, we conducted an experiment in BEF-China platform: a subtropical forest in southeast China where tree and shrub diversity are experimentally varied [54]. We used Illumina amplicon sequencing of small subunit ribosomal RNA markers to determine the communities of bacteria and archaea in bulk soils under the canopy of focal trees. Along the three tree species richness levels (1, 2, 4) with four shrub species richness levels (0, 2, 4, 8), we investigated the relationships between plant diversity with bacterial and archaeal diversity, composition, and co-occurrence relationships, furthermore, how microbes respond to changes in aboveground plant diversity and thus regulate stand-level tree productivity. We hypothesized that (H1) the diversity, composition, and network complexity of bacterial and archaeal communities would be positively influenced by tree species richness due to resource complementarity and microenvironments [55]. (H2) the impact of shrub species richness on bacterial and archaeal communities would vary depending on the level of tree species richness due to the interactions between tree and shrub species richness [16]; (H3) plant species richness would have a cascading effect on community-level plant productivity by regulating the bacterial and archaeal communities.

Methods

Study area

The BEF-China platform (https://bef-china.com/) has been set up to investigate the relationship between subtropical plant diversity and ecosystem functioning in Xingangshan, Jiangxi Province in southeast China

(29°08′–29°11′ N, 117°90′– 117°93′ E) [54]. The main experimental sites of the BEF-China platform were established over a two-year period from 2009 to 2010, and the study site is located in the subtropical climate zone. The mean annual temperature is 16.7 °C, with the coldest temperature 0.4 °C occurred in January, and the warmest 34.2 °C in July [56], while mean annual precipitation is 1821 mm. The vegetation in natural ecosystems surrounding the BEF-China platform is an evergreen and deciduous broad-leaves mixed forest [57]. The soils belong to Regosols, Cambisols, Acrisols, Gleysols, and Anthrosols [58].

Experimental setup and sampling

The design of BEF-China main experiment was described by Bruelheide et al. [54]. In brief, two experimental sites, A (18.4 ha) and B (20 ha), were respectively set up after clear-cutting the *Cunninghamia lanceolata* plantation, where some *Pinus massoniana* individuals were scattered only at the border of site A. In both study sites, there were 32 super-plots measuring 4 mu each, which were further divided into four plots with dimensions of 25.8 × 25.8 m (equivalent to 1 mu of Chinese area unit). Within each plot, there were 400 trees randomly planted in 20 × 20 grids, with a 1.29-m interval between tree individuals along the cardinal compass directions. A species pool containing 40 broadleaved tree species and 18 shrub tree species was first established, to minimize the confounding effects of a particular species combination on diversity effects [54]. Based on the species pool, tree and shrub species were randomly selected to build a crossed tree and shrub species richness gradient. The super-plots represented five tree species richness levels: one- (16 super-plots), two- (8 superplots), four- (4 super-plots), eight- (2 super-plots), and sixteen- (1 super-plot) and twenty-four species richness (1 super-plot). There were 32 super-plots in total, with 128 1 mu plots. Within each 4 mu super-plot, there were four 1 mu plots where shrubs were planted, with 0, 2, 4, or 8 shrub species richness randomly assigned in these plots.

In this study, a total of 16, 8, and 4 plots with three tree species richness levels of 1, 2, and 4 were selected in both sites A and B in October 2018, respectively (Fig. 1a, b). Within each plot, four tree individuals per species were randomly selected, resulting in 4, 8, and 16 individuals per plot of tree species richness of 1, 2, and 4, respectively.

A composite soil sample was collected per individual, as illustrated in Figure S1. Specifically, four soil cores at 0-10 cm depth, where the greatest microbial diversity was found [59–61], were collected from different directions within 1/2 of the canopy projection area of each tree individual, and well mixed to avoid spatio-temporal autocorrelation. Therefore, a total of 64 soil samples were collected from each of three tree richness levels, resulting in 192 samples in site A or B, as presented in Table S1. These soil samples were further divided into two parts: (1) the air-dried for soil physicochemical properties measurement; (2) the stored at -80 °C for DNA extraction and subsequent microbiome analyses.

Topographic and soil physicochemical properties

A digital elevation model was used to estimate mean plot aspect and inclination as explained in the BEF-China data portal [62, 63]. Two components, i.e., a north–south and an east–west slope aspect, were calculated based

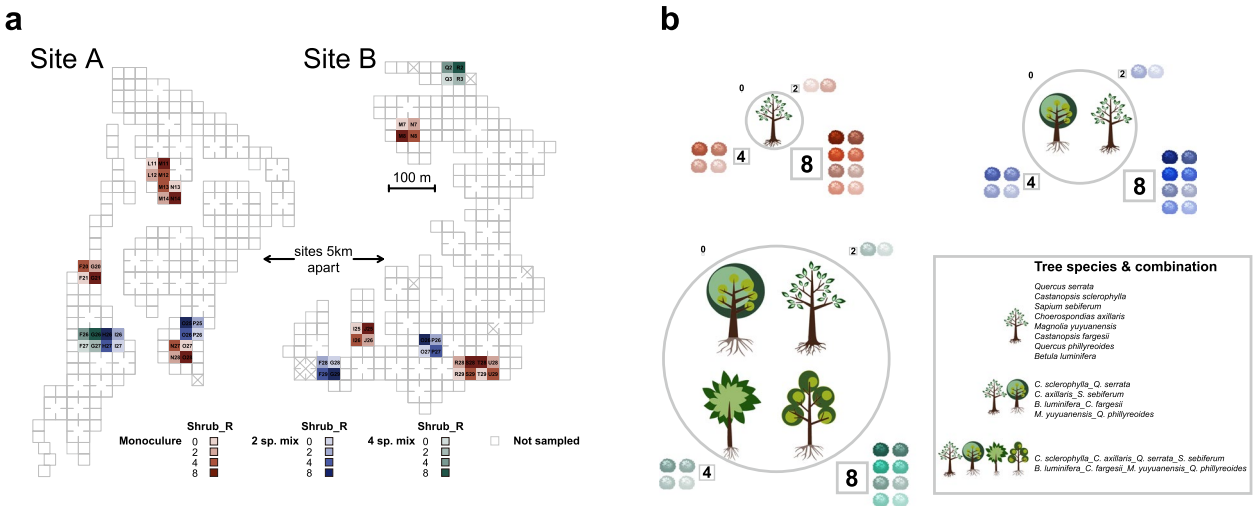

Fig. 1 Sampling and experimental design. **a** Plots with tree species richness gradients (1, 2, 4) and shrub species richness gradients (0, 2, 4, 8) selected from BEF-China platform (site A, site B). **b** The tree species and their combinations used in this study

on the mean plot aspect. Because there are also plots in flat areas and on small slopes that cannot be classified as a particular aspect, hence two identified components d.SLOPEnew and d.GRA_NS used in this study were estimated according to the following equations [64]:

$$\text{d.SLOPEnew} = \text{SLOPE} \times \text{pi}/180$$

$$\text{d.GRA\_NS} = \tan(\text{d.SLOPEnew}) \times \text{NS}$$

Fresh soil samples were sieved with a 2-mm sieve to measure soil properties. Soils were dried at 105 °C for 48 h for determine soil water content. Soil solutions with a 1: 2.5 soil to water ratio were used to measure soil pH with a glass electrode (Thermo Orion T20, USA). Air-dried soils were used to estimate soil organic C and total N with the CHNOS Elemental Analyzer (vario EL III, CHNOS Elemental Analyzer; Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil P and other chemicals, i.e., calcium (Ca), potassium (K), magnesium (Mg), and ferrum (Fe) were measured with inductively coupled plasma emission spectrometry (ICAP 6300 ICP-OES Spectrometer; Thermo Scientific, Waltham, MA, USA). Soil inorganic N including nitrate and ammonium N were measured using Continuous Flow Analyzer (SAN Plus, Skalar, Erkelenz, Germany).

Tree stand volume and increment

Tree stand volume and increment data were retrieved from a previous study [65] which estimated the stand-level tree productivity. Briefly, individual tree volume proxies were calculated as $H \times \pi\ (\text{BR})^2$ in which H is height and BR is basal radius at the ground, and then transformed to more accurate tree volume estimates by multiplying the proxies with a size-dependent correction factor based on a previous study [16]. The stand-level tree volume was calculated by aggregating the volumes of the surviving trees in the central 36 planting positions per plot and stand volume increment was calculated as the absolute differences in stand volume between two consecutive years. In our study, we used the tree stand volume data in 2018 and volume increment data between 2017 and 2018.

Soil microbial biomass

Microbial biomass was measured by the chloroform fumigation extraction method [66]. A pair of fresh soils per sample with 5 g weight of each were separately added into beakers, and then one of them was placed into a vacuum drier with 50 ml alcohol-free $CHCl_3$ to fumigate for 24 h, while the other was assigned as the control without fumigation. The paired fumigated and non-fumigated soils were both incubated at 25 °C for 24 h in the dark. The paired soils were extracted using 50 ml 0.5 M K_2SO_4 (1:2.5 w/v), and then C and N concentration in soil solutions were measured with TOC analyzer (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). The formula calculating microbial biomass C (MBC) and microbial biomass N (MBN) is as followed: $B_{c(n)} = F_{c(n)}/k_{c(n)}$. Here, $F_{c(n)}$ referes to difference value between amount of C or N extracted from fumigated and non-fumigated soil. $k_{c(n)}$ refers to the calibration coefficient of microbial biomass, where k_c is 0.38 for MBC [67] and k_n is 0.54 for MBN [66].

DNA extraction, PCR amplification and sequencing

Soil samples packed with dry ice were transferred and stored at -80°C in laboratory until DNA extraction. The extraction of microbial genomic DNA was conducted using the PowerSoil DNA Isolation Kit (Mobio, Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's protocols. The concentration of DNA extracts was determined using the NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and the quality of DNA extracts were examined using 1% agarose gel electrophoresis. The primer pairs 338F (5′-ACTCCTACGGGAGGCA GCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [68, 69] and the primer pairs 524F10extF (5′-TGYCAGCCG CCGCGGTAA-3′) and Arch958RmodR (5′YCCGGC G TTGAVTCCAATT-3′) [70] were used to amplify the hypervariable region V3-V4 of the bacterial 16S rRNA gene and V4-V5 of archaeal 16S rRNA gene, respectively.

The PCR amplification of 16S rRNA gene was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 10 °C. The PCR mixtures contain 5 × *TransStart* FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, *TransStart* FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH_2O up to 20 μL. The PCR reactions were performed in triplicate.

The PCR products were extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). The qualified PCR products were mixed, and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Bioinformatics analysis

All paired rRNA amplicon sequencing raw reads were processed via the Quantitative Insights into Microbial Ecology 2 (QIIME2) version 2020-2 [71]. Briefly, raw sequence data were imported into QIIME2 manually using the "qiime tools import" command. The quality trimming, denoising, merging and chimera detection and non-singleton amplicon sequence variants (ASVs) grouping were done using the plugin "qiime dada2 denoise-paired" in DADA2 [72] as implemented in QIIME2 v2020-2. The "-p-trimleft-f" and "-p-trim-left-r" parameters were set at 0 and the "-p-trunc-len-f" and "-ptrunc-len-r" parameters were set at 294 for bacteria and 298 for archaea, respectively, after reviewing the "Interactive Quality Plot tab" in the "demux.qzv" file. After the quality filtering steps, the ASV abundance tables were rarefied at 4337 for bacteria and 2083 for archaea, according to the "Interactive Sample Detail" in the "table.qzv" file, respectively to ensure even sampling depth. The α-diversity analyses were conducted from the rarefied ASV abundance tables through the core-metrics-phylogenetic method in the q2-diversity plugin. The bacteria AVSs were taxonomically classified using the qiime2 v2020-2 plugin "qiime feature-classifier classify-sklearn" with the pre-trained Naïve Bayes Greengenes classifier trimmed to the V3-V4 region of the 16S rDNA gene. The archaea ASVs were analyzed by RDP Classifier [73] against the SILVA Small Subunit rRNA Release v11.5 using a confidence threshold of 0.7. Furthermore, the taxa that were not present in at least 5% of total samples were removed from the matrices for both bacteria and archaea to reduce the noise [74]. The bacterial and archaeal ASVs were functionally annotated by FAPROTAX [75] and assigned to putative functional groups, i.e., microbial groups associated with carbon cycle, nitrogen cycle, or sulphur cycle.

Statistical analysis

All the statistical analyses and data visualization were performed in R statistical software (V. 3.6.3 [76]). To examine how tree and shrub species richness as well as tree mycorrhizal type impact microbial community diversity and composition, we used the vegan package [77] to calculate the Chao1 index for bacterial and archaeal richness and diversity, as well as unweighted UniFrac distance matrices to measure β-diversity.

The significance of different factors on community dissimilarity was tested with PERMANOVA by permutations of 999 in using the 'adonis2' function with the term "by=margin" of the vegan package [77] based on unweighted UniFrac distances. To investigate the impact of plant species richness on α-diversity, β-diversity, and volume growth, we utilized the Chao1 index, the unweighted Unifrac distance of bacterial and archaeal communities, and the volume increment between 2017 and 2018 as response variables, and tree species richness as the predictor variable to perform linear regression. The multiple *R*-squared value (r^2) and the significance of the model were used to evaluate the model fit.

To identify taxa with statistically significant differential abundant taxa across different levels of plant species richness, we utilized the DESeq2 package [78] to perform pairwise comparisons in a negative binomial generalized linear model in at an FDRadjusted p value of 0.05. To investigate the specific microbial groups under different levels of tree species richness, we conducted bipartite network analysis with the software Cytoscape [79] following the method described in a previous study [80]. The network association analysis was performed by igraph package [81] and visualized in Gephi [82] to explore the co-occurrence of microbial features from a holistic perspective. To evaluate the correlation of environmental/microbial properties with βdiversity distance matrices of bacterial and archaeal communities, we selected a set of fourteen predictor variables including eight soil physicochemical properties (pH, SM, NO_3^-, MBC, MBN, C, N, P, C/N, C/P), two soil microbial biomass predictors (MBC and MBN), and four topographic properties (aspect, altitude, d.SLOPEnew, and d.GRA_NS) to perform Mantel tests using ade4 package [83], all of which exhibited low multicollinearity (indicated by variance inflation factor values between 1 and 5) [84]. The correlation was visualized using the MatCorPlot package [85]. To tease apart the effects of tree and shrub species richness on bacterial or archaeal microbiome and the consequences on stand-level tree productivity, Structural Equation Modelling (SEM) were performed. The SEM models were built based on the conceptual model shown in Figure S2, using the "sem" function in lavaan package [86]. The path coefficient represents the direction and strength of the direct effect between two variables. The goodness of fit was estimated using three indices: (i) the root mean square error of approximation (RMSEA < 0.05) [87], (ii) the comparative fit index (CFI > 0.95) [88], and (iii) the standardized root mean squared residuals (SRMR < 0.08) [89].

Results

Soil bacterial and archaeal α-diversity

For the soil bacterial community, α-diversity (expressed as Chao1) reduced from monocultures to 2-tree species mixtures (p < 0.001) and 4-tree species mixtures (p < 0.001) and was affected by an interaction between shrub and tree species richness (Fig. 2a). The interaction indicated that bacterial α-diversity increased with increasing shrub species richness for tree monocultures but decreased in 4-tree species mixtures. When tree species richness is 2, bacterial α-diversity increased generally

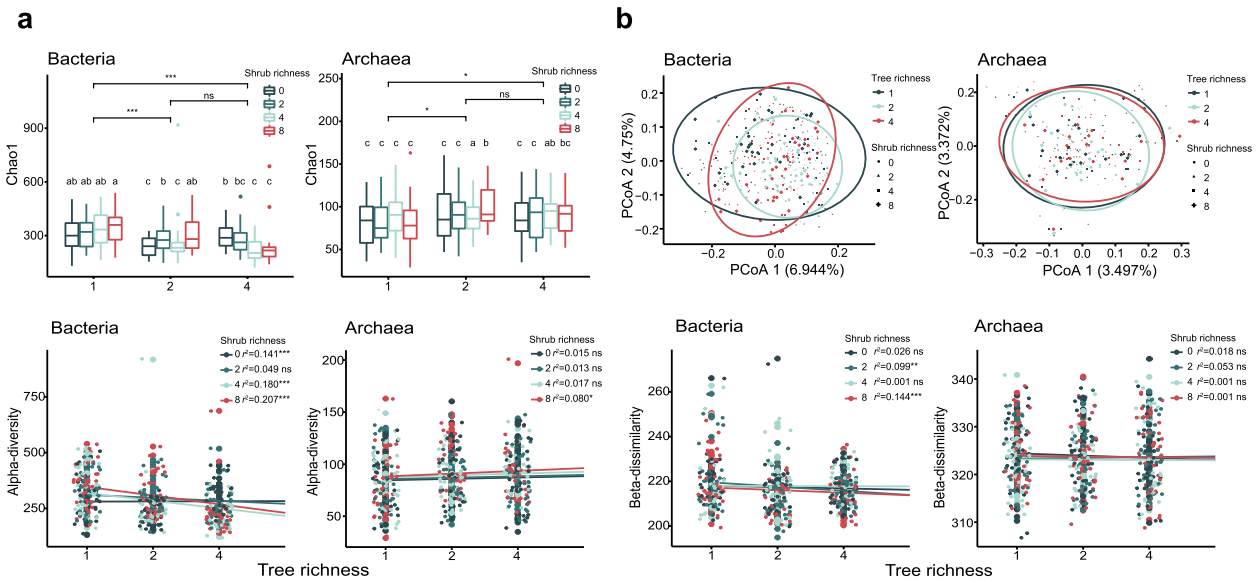

Fig. 2 Soil bacterial and archaeal α-diversity and community structure. **a** tree richness and shrub richness effects on soil microbial α-diversity; **b** tree richness and shrub richness effects on soil microbial β-dissimilarity. The asterisks showed the *p*-value significance level, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ and ns showed no significance

with increasing shrub richness, except for a significant decrease in shrub richness at 4. For archaea, Chao1 increased from monocultures to 2-tree species mixtures ($p < 0.05$) and 4-tree species mixtures ($p < 0.05$) (Fig. 2a). Moreover, shrub species richness enhanced archaeal α-diversity, especially in the context of 2- and 4-species tree mixtures (Fig. 2a). When considering the mycorrhizal types of the focal tree species, we found the bacterial diversity was higher for ectomycorrhizal (EcM) than for arbuscular fungi-colonized trees (AM) ($p < 0.05$). The bacterial α-diversity decreased from monocultures to polycultures for both EcM and AM trees ($p < 0.001$) (Figure S3a). However, no significant differences of archaeal diversity were found between EcM and AM tree species, only the archaeal α-diversity of EcM tree species increased with increasing tree species richness ($p < 0.01$), but not for AM tree species (Figure S3b).

We found that the ratio of MBC to soil organic C (MBC/C_{org}) and the ratio of MBN to soil organic N (MBN/N_{org}) were positively correlated to archaeal α-diversity. Soil organic C significantly negatively related to both bacterial and archaeal α-diversity (Table 1).

The composition bacterial and archaeal communities

We found that bacterial community composition differed between levels of tree species richness and shrub species richness, but the effects of tree species richness on bacterial community composition were stronger than effects of shrub species richness (Fig. 2b; Table 2). In contrast, soil archaeal communities were influenced by shrub species richness, but generally not by tree species richness (Fig. 2b; Table 2). In addition, bacterial community composition was influenced by the interaction between tree and shrub species richness (Table 2).

Soil bacterial community composition varied between EcM and AM trees (PERMANOVA test, $F = 1.68$ $p < 0.010$) (Figure S4a). In addition, tree species richness had a significant effect on bacterial community structure under both EcM and AM trees (Table S2). For archaeal community composition differences between tree mycorrhizal types were less pronounced (Figure S4b) and it was not affected by differences in tree and shrub species richness under the canopy of EcM or AM trees (Table S2).

We found that soil moisture (SM), pH, the soil C/N, and two topographical factors d.SLOPEnew and d.GRA_NS were positively associated with bacterial community composition ($p < 0.05$) (Fig. 3). However, there was no significant correlation between these factors and soil archaeal community composition (Fig. 3).

Bacterial and archaeal taxonomic and functional groups

For all 384 soil samples, we obtained a total of 70,836 ASVs for bacterial and 13,552 ASVs for archaeal communities. The dominant bacterial phyla across all samples were Acidobacteria (36.44% of the total bacterial sequences), Proteobacteria (27.99%), and Chloroflexi (6.37%) (Fig. 4a). As for the taxonomic abundance of the soil archaeal communities, the phyla Thaumarchaeota (56.97% of the total archaeal sequences), Euyarchaeota (29.00%), and Crenarchaeota (12.78%) dominated the

Table 1 Pearson correlation of bacterial and archaeal α-diversity with environmental variables

	Bacterial abundance		Archaeal abundance		Bacterial Chao1 index		Archaeal Chao1 index	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
pH	-0.084	0.099	-0.017	0.742	-0.060	0.241	-0.033	0.523
SM	-0.006	0.900	-0.037	0.465	-0.010	0.850	-0.057	0.267
NH_4^+	-0.040	0.434	0.048	0.351	-0.015	0.762	0.048	0.344
$N0_3^-$	0.067	0.193	0.113	**0.026**	0.060	0.237	-0.085	0.096
N	-0.010	0.851	0.084	0.099	0.009	0.854	0.010	0.847
MBC	0.047	0.360	0.104	**0.042**	0.072	0.161	0.030	0.557
MBN	0.022	0.668	-0.012	0.811	0.003	0.947	-0.009	0.856
MBC/MBN	0.004	0.934	0.060	0.243	0.026	0.607	0.006	0.901
MBC/C_{org}	0.081	0.115	0.176	**0.001**	0.115	**0.025**	0.121	**0.017**
MBN/N_{org}	0.047	0.358	0.113	**0.027**	0.047	0.360	0.107	**0.037**
P	-0.052	0.309	-0.051	0.316	-0.065	0.203	-0.034	0.513
C	-0.125	**0.014**	-0.165	**0.001**	-0.142	**0.005**	-0.189	**0.000**
C:N	-0.002	0.976	0.137	**0.007**	0.025	0.631	0.059	0.252

SM, MBC, MBN, C and N respectively refers to soil moisture, microbial biomass C, microbial biomass N, soil organic C and N. *Note*: Bold indicates the significant values

Table 2 The effects of tree and shrub richness on the compositional variances of soil bacterial and archaeal communities based on PERMANOVA with 999 permutations. *Note*: Bold indicates the significant values

	Bacterial community			Archaeal community		
	df	F	*p* value	df	F	*p* value
Tree richness (TR)	2	3.405	**0.001**	2	0.767	0.992
Shrub richness (SR)	3	1.424	**0.001**	3	1.168	**0.028**
Interaction (TR × SR)	6	1.254	**0.001**	6	0.942	0.831

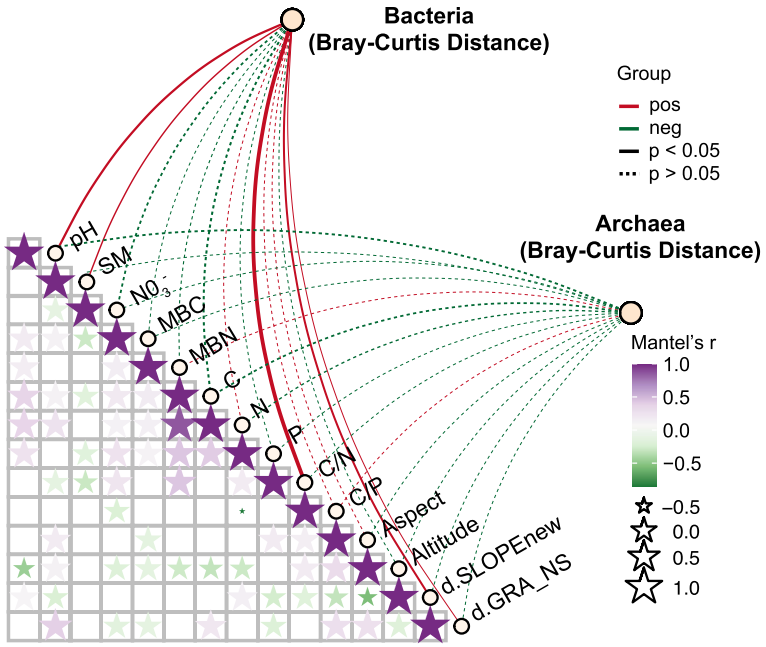

Fig. 3 Pairwise correlation matrix of environmental factors with Mantel tests of bacterial and archaeal communities. Red and blue lines indicate positive and negative correlations, respectively, while solid and dashed lines indicate the significant correlations ($p < 0.05$) and insignificant correlations ($p > 0.05$)

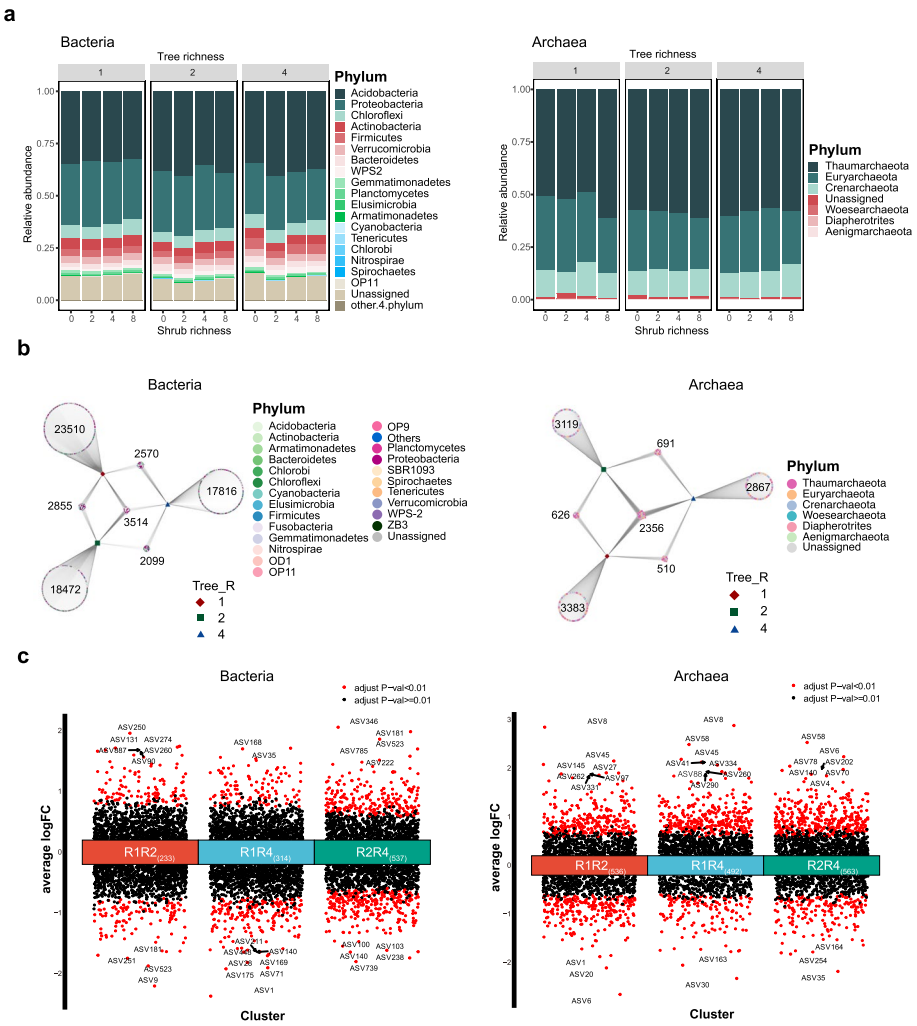

Fig. 4 A general overview of changes in taxonomic composition and species abundance for bacterial and archaeal communities affected by increased plant species richness. **a** the relative of phylum-level taxa dominated across tree species richness and shrub species richness levels; **b** bipartite networks illustrating the specific and conserved amplicon sequence variants (ASVs) assigned to monoculture, two-species mixtures, and four-species mixtures and their combinations, respectively for soil bacterial and archaeal community; **c** volcano plots showing up- and down-regulated ASVs in three comparisons: tree richness of 1 and 2, tree richness 1and 4, tree richness of 2 and 4. An adjusted *p* value < 0.01 is indicated in red, while an adjusted *p* value < 0.01 is indicated in black. The top ten ASVs with the most significant differences in abundance were indicated by their ID numbers and the numbers of ASVs with significantly differences in abundance for the three comparisons are indicated in bracket

archaeal communities (Fig. 4a). We found that tree species richness affected the relative abundance of certain bacterial taxonomic groups (Table S3). For example, the relative abundances of Chloroflexi increased with increasing tree species richness, while the relative abundances of Acidobacteria and Firmicutes were lower in 4-species mixtures compared to monocultures and 2-species mixtures. The abundance of the phylum Proteobacteria was lower in 2-species mixtures than monocultures (Table S3). In addition, the relative abundance of bacterial phyla was more likely to change with increasing shrub species richness, as tree species richness increased (Table S3). When tree species richness was at level 1, the relative abundance of bacterial phyla did not differ between shrub monocultures and other shrub diversity levels. However, the relative abundance of Acidobacteria decreased significantly from shrub richness level 2 to 4 when tree species richness level increases to 2, and increased significantly from shrub richness level 2 to 4 (or 8) when tree species richness increased to 4 (Table S3). For archaea, we found little effects of tree and shrub species richness on relative abundances (Table S3). Furthermore, we did not find significant differences in the taxonomic composition of bacterial or archaeal

communities in soils collected under EcM and AM trees (Figure S5).

Two bipartite association networks were used to assess the contribution of different microbial populations to the overall community structure (Fig. 4b). For this purpose, we obtained a specific subset of ASVs with their taxonomical assignments at each tree species richness level and core ASVs in their combinations (Fig. 4b; Table S4). We found that the abundance of ASVs in specific subset for either bacteria or archaea decreased with increasing tree species richness (Fig. 4b; Table S4). In addition, we found that the tree and shrub species richness significantly altered the abundance of microbial ASVs (Fig. 4c; Figure S6), with increasing tree species richness leading to a gradual increase in the number of bacterial differential ASVs, however, no such trend was observed for archaea (Table S5).

Our results showed that a large proportion of bacterial and archaeal ASVs were assigned to C-cycle and N-cycle groups (Figure S7). The relative abundance of bacteria was significantly lower in the C-cycle and S-cycle groups but higher in the N-cycle group in 4-tree species mixtures compared to monocultures and the relative abundance of archaeal C-cycle group was significantly higher in monocultures than 2-tree species mixtures (Table S6).

Bacterial and archaeal network complexity

Tree species richness decreased network complexity for bacteria (Fig. 5a), indicated by a decline in the average degree, network density, modularization, the number of nodes, and the number of edges. Network complexity did not change for archaea (Fig. 5b) and interactive effects of tree and shrub species richness on soil bacterial and archaeal co-occurrence networks were limited (Figure S8-S9).

The role of soil bacteria and archaea modifying BEF relationship

This study found a significant increase in stand-level volume growth with increasing tree species richness from monoculture to 2-species mixtures and 4-species mixtures, and a significant superimposed effect at the 0, 2, and 4 shrub species richness levels (Fig. 6a). We also found significant effects of plant diversity on soil key elements and MBC/C_{org} (Figure S10), some of which were further identified as key factors regulating the relationship between aboveground plant richness and belowground microbial communities, and then determining plant productivity (Fig. 6b). Specifically, soil C/N was positively correlated with MBC/C_{org}, and consequently increased tree productivity (Fig. 6b). Tree species richness exhibited a significantly positive effect on MBC/C_{org} as well (Fig. 6b). Most interestingly, we found that tree species richness positively linked to bacterial diversity, and modulate bacterial community composition, which then contributed to the increase in stand-level tree productivity (Fig. 6b). Impacts of tree species richness on bacterial community composition were modulated via altered soil C/N (Fig. 6b). Here, we note that the bacterial composition rather than diversity was a direct positive driver on MBC/C_{org}, thereby contributing to an increase in stand-level tree productivity (Fig. 6b). Neither tree nor shrub species richness directly altered the diversity and composition of archaeal community (Fig. 6b). However, we found that tree species richness influenced archaeal diversity via regulating soil C/N (Fig. 6b). Archaeal diversity was positively associated with MBC/C_{org} ratio, which then increased stand-level tree productivity (Fig. 6b).

Discussion

In our study, we explored how tree and shrub species richness affected the diversity, complexity, and composition of bacterial and archaeal communities in a large subtropical tree biodiversity experiment. In addition to earlier work on fungal communities [90, 91], we now show for the first time that tree species richness drives shift in bacterial and archaeal α-diversity and bacterial community composition (H1). In addition, we found significant interactions between tree and shrub species richness levels, indicating that the shrub species richness effect on bacterial α-diversity was dependent on tree species richness (H1). The complexity of the bacterial networks was found to decrease significantly with increasing tree species richness but was not altered by shrub species richness (H2). The complexity of archaeal co-occurrence network was not correlated with either tree or shrub species richness (H2). Contrary to the view that the presence of shrub competition in forests may reduce tree productivity [16], we found that both tree and shrub species richness contributed to tree productivity and highlighted soil bacterial and archaeal communities as vital linkages between plant richness and stand-level tree productivity in the context of plant-created soil chemical properties (H3). In summary, our study provided novel insights that diversity and composition of prokaryotic communities are responsive to tree species richness and appear to play a role in driving tree productivity; hence, the inclusion of them in forest soil community analyses is therefore important for better understanding the functioning of these ecosystems.

Tree-shrub species richness affected the bacterial and archaeal diversity and community composition under the canopy of focal tree species

In contrast with our first hypothesis that soil microbial α-diversity increases with the increasing tree species

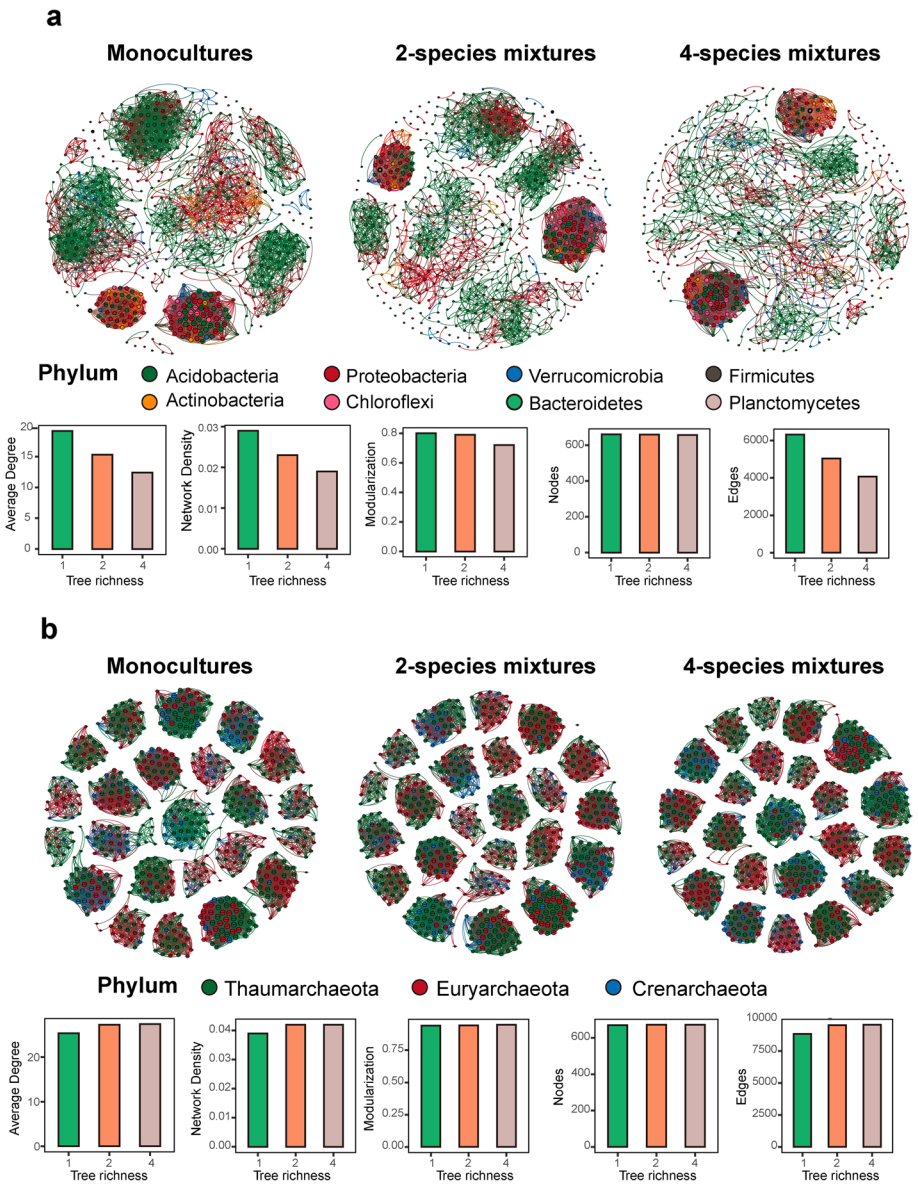

Fig. 5 The co-occurrence networks of bacterial communities (**a**) and archaeal communities (**b**) in three tree species richness levels, monocultures, two-species mixtures and four species-mixtures, respectively. The nodes in the networks are colored according to the taxonomic assignments at phylum level and the size of each node is proportional to the relative abundance

diversity, we found that plant community richness had a negative effect on soil bacterial diversity under the canopy of focal trees, indicating that the most diverse bacterial communities in our study occurred in monocultures and that diversity decreased with increasing community-level tree richness. This is in contrast with earlier work in grassland [10] and on fungal communities in the BEF-China experiment [29]. Unlike these earlier studies, we have collected soil samples underneath individual trees rather than at the community level, and it is therefore reasonable to suggest that the decline in soil bacterial diversity may point to a 'dilution effect' [26, 92]. From this perspective, the tree species richness gradient from 1, 2 to 4 resulted in reducing densities of conspecific tree species in the focal tree species, so that some focal treespecific bacteria may be restricted. In addition to the sampling strategy, we also speculated that the soils in tree monocultures with low-diversity resources may amplify bacterial competitive pressures, resulting in highly antagonistic bacterial communities, while higher diverse plant communities that provided diverse resources to the soil may reduce microbial

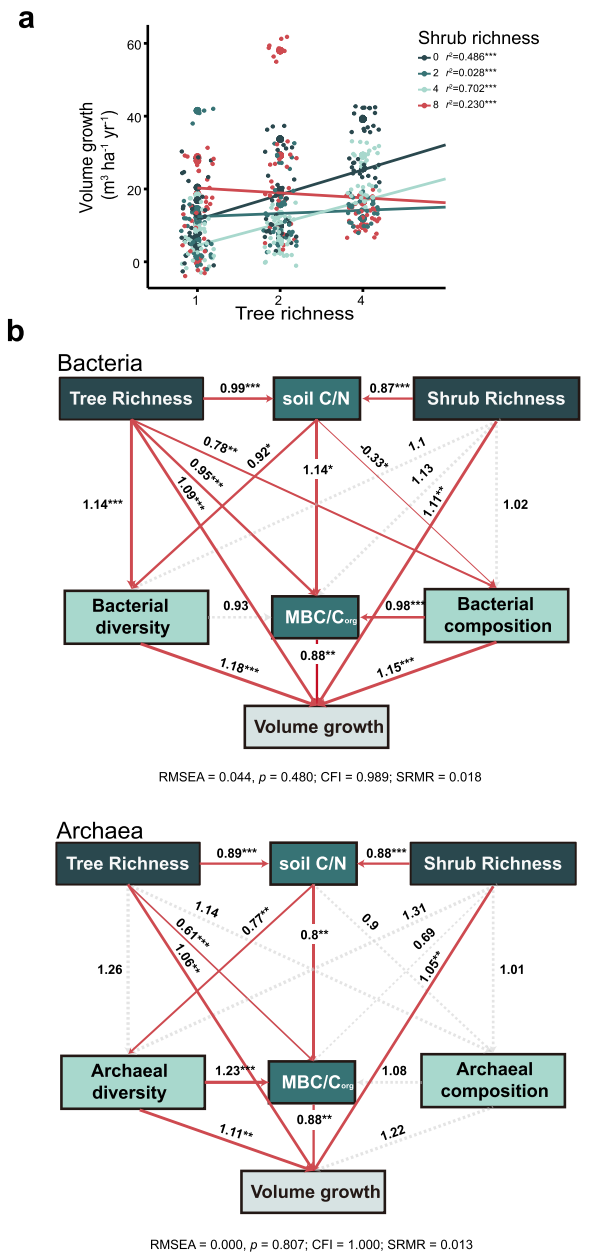

Fig. 6 Plant richness affects tree productivity by regulating soil properties and microbial communities. **a** Stand-level tree volume increment as a function of aboveground plant richness from 2017–2018. **b** Structural equation models demonstrating the direct and indirect effects of aboveground plant richness on soil nutrient contents, microbial communities and community-level tree productivity, red arrows indicate significant and positive relationships ($p < 0.05$), and dashed arrows indicate connections with insignificant relationship ($p > 0.05$). TC: total carbon, TN: total nitrogen, TP: total phosphorus, SM: soil moisture, C/N: the ratio of soil organic C and N, MBC/C_{org}: the ratio of microbial biomass C and soil organic C

competitive pressure and generate less diverse bacterial communities [55, 93, 94].

In line with our first hypothesis, we found that tree and shrub species richness resulted in shifts in bacterial community composition. In addition, we found that bacterial community composition became more similar with increasing tree species richness, which is in line with earlier findings from the BEF-China study that fungal community composition was more similar in multi-tree species mixtures [31]. The community composition and diversity can be pronouncedly changed by modulating the soil chemistry resources, which can promote or inhibit the relative abundance of specific microbial taxa [95].

As part of our hypothesis (H1), we postulated that the α-diversity of soil archaea increases with increasing tree species richness but decreases with increasing shrub species richness. Our results are partly consistent with this hypothesis that α-diversity of the soil archaea consistently increased with increasing tree species richness, likely due to changes in the abundance of ammonia-oxidizing archaea resulting from increasing tree species richness [96, 97]. However, we found that the effect of shrub species richness on archaeal diversity was rather weak. One possible explanation is that the changes in nitrogen content brought about by the changes in shrub species richness were not sufficient to cause a significant difference in archaeal diversity. Furthermore, neither tree species richness nor shrub species richness showed a significant effect on soil archaeal compositional variation, unlike bacteria, which may be related to their large differences in environmental adaptations, cellular structure, or cellular metabolisms [98, 99]. Despite the key role archaea play in soil biogeochemical cycles, studies on how their abundance is influenced by plant diversity remain extremely sparse [100].

Notably, our findings also underline the need to consider the tree mycorrhizal types as important factor in studying 'tree-shrub diversity-soil prokaryotic community' relationships. We found that both bacterial and archaeal α-diversity showed significant differences between mycorrhizal types and the mycorrhizal type of the focal tree species influenced the microbial response pattern to tree species diversity. This is mainly because different mycorrhizal types-associated fungi differ in their strategies of resource acquisition, nutrient allocation, and plant-soil feedback, which could affect their recruitment of different microbes in the mycorrhizosphere [101]. In contrast to our results, a previous study examining the same field experiment showed no significant difference in soil bacterial α-diversity between EcM and AM trees [31]. This contrasting result may be because the study selected two adjacent EcM and AM

trees as a target sampling unit, making the difference in soil nutrient resources not significant enough to affect bacterial diversity.

The bacterial and archaeal communities under the canopy of focal tree species exhibited different co-occurrence patterns with increasing tree species richness

The shifts of topological characteristics in co-occurrence network inferred from soil bacteria along a tree species richness gradient suggests that tree species richness influences its complexity; however, contrary to our hypothesis (H2), the network complexity decreased progressively from monocultures to 2-tree species mixtures and 4-tree species mixtures. Bacterial network assembly has been found in many studies to be a deterministic process involving competitive interactions, non-overlapping niches, and thus follows a power-law distribution pattern when bacterial communities are constructed [48, 49, 102]. Therefore, we proposed the niche differentiation caused by the tree species richness could be the main reason for changes in bacterial network complexity, with plant monocultures providing a smaller variety of weaker niche differentiation than polycultures, and the weaker niche differentiation, the stronger microbial interactions would be [49, 102, 103].

The topological features of archaeal co-occurrence network are not influenced by tree species richness, contrary to our expectations (H2). One proposed explanation is that the archaeal interaction is structured as a random network following the ErdosRenyi model [49, 104], where the presence or absence of edges is a stochastic process, implying that all interactions between archaea are equally possible. This view is also supported in a recent study of archaeal biogeography showing that the diversity patterns of soil archaea are largely influenced by stochastic processes [105], that is, neutral processes are more important than deterministic factors for soil archaea.

The roles of bacterial and archaeal community in regulating the relationship between tree-shrub species richness and community-level tree productivity (BEF)

Both tree and shrub species richness contributed significantly to the increase in standlevel tree productivity, confirming our hypothesis (H3). Tree species richness can promote their productivity and thus accelerate C stock [106] and the underlying mechanism is often summarized as ecological niche complementarity [12]. Although shrub competition exists at low shrub species richness levels, but generally, diverse shrub communities positively contribute to stand-level tree productivity, suggesting that competition between shrubs and trees is reduced at higher shrub diversity, and indicating that complementarity effects extend from tree-tree interactions to tree-shrub interactions [16].

In addition, our study also provides insight into the potential role microbial communities play in this positive BEF relationship. The SEM model suggests that soil C/N is a critical linkage between plant diversity and tree productivity by influencing bacterial and archaeal communities. Bacteria and archaea inhabiting forest soil are important players in geochemical cycles and organic matter recycling, particularly in the C cycle [107]. The complexity of C cycling is often interlinked with the N cycle, influencing nitrification and denitrification processes and subsequently C/N [108]. Plant species richness significantly drove incremental soil C/N, which can be explained by increased carbon release from trees to the soil through litter production [109] and root exudates [110]. Both C and N are closely linked to microbial growth and development in biogeochemical cycles, and C/N has a direct effect on the relatively microbial biomass C (MBC/C_{org}), mainly because soil bacteria and archaea are predominately heterotrophic organisms that generally derive energy from the decomposition and mineralization of organic matter [39]. In a given ecosystem with high nutrient and resource availability, microbial biomass synthesis is prioritized over catabolism [111]. As a result, the stoichiometry (e.g., C/N) of soil organic matter is critical for regulating microbial communities and increasing microbial activity. Such increases can therefore induce biogeographical cycling of nutrients and maintain higher levels of functioning by increasing physiological potential of microorganisms, thus promoting tree volume growth at the community level. This view is supported by a study showing that plant diversity mediates the metabolic activity of soil microbes via higher root inputs and soil N status and C storage, which would be expected to lead to increased microbial activity [24]. However, in this study, the response of soil bacterial and archaeal communities was only investigated using amplicon sequencing. The response of microbial functions to increased plant species richness would be another intriguing exploration for future research.

Conclusions

Here, we provide pioneering empirical evidence for the interactive effects of tree and shrub species richness on soil bacterial and archaeal communities under the canopies of focal trees in our long-term biodiversity forest experiments. We demonstrate that α- diversity, co-occurrence networks, and community composition of bacteria and archaea follow different patterns towards increasing tree and shrub species richness. For bacterial communities, the α-diversity, and the complexity of co-occurrence network decreased with increasing tree species richness,

and the effect of shrub species richness on bacterial α-diversity varied across tree species richness levels. Our results highlight the dilution effect of tree species richness on soil bacterial diversity in tree diversity experiment. We also demonstrate that changes in bacterial community composition may be the result of the direct effects of plant species richness, or indirect effects of them via changing edaphic properties (e.g., C/N and pH). In contrast, for archaeal communities, the effects of tree and shrub species richness on α-diversity, microbial network complexity, and community composition were somehow ambiguous, while edaphic properties barely altered the archaeal community composition. Finally, we found that both tree and shrub species richness strongly increased the stand-level tree productivity through direct or indirect regulations on soil microbiota, however, their contributions and the roles of bacterial and archaeal communities in this process were content dependent. Tree species richness could indirectly accelerate bacterial diversity and modulate bacterial community composition via stimulating soil C/N, inducing a cascading effect on tree productivity. As for archaea, only the diversity of them increased with increasing soil C/N that may be attributable to tree species richness and thus contributed to stand-level tree productivity. Our findings highlight the important role of soil microbiome in modulating the relationship between tree and shrub species richness and productivity in subtropical forests.

Abbreviations

BEF	Biodiversity-Ecosystem Functioning
MBC	Microbial Biomass C
MBN	Microbial Biomass N
QIIME2	Quantitative Insights into Microbial Ecology 2
ASVs	Amplicon Sequence Variants
SEM	Structural Equation Modelling
RMSEA	Root Mean Square Error of Approximation
CFI	Comparative Fit Index
SRMR	Standardized Root Mean squared Residuals
Corg	Soil organic C
Norg	Soil organic N
SM	Soil moisture

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-023-01676-x.

Additional file 1: Figure S1. Flow chart of sampling, DNA extraction, microbial sequencing, and the detailed information of dissecting microbial community driven BEF relationships in a subtropical forest.

Additional file 2: Figure S2. *A priori* structural equation modeling (SEM) hypothesized causal pathways of how tree/shrub species richness and soil properties may influence stand-level tree productivity through modifying the bacterial and archaeal communities and microbial biomass carbon or nitrogen content.

Additional file 3: Figure S3. Soil bacterial and archaeal α-diversity under three tree species richness levels (1, 2, and 4) and four shrub species richness (0, 2, 4, and 8), respectively for both ectomycorrhizal fungi-colonized trees (EcM) and arbuscular fungi-colonized trees (AM).

Additional file 4: Figure S4. Principal coordinates analysis (PCoA) with unweighted unifrac distances matrices to visualize the bacterial and archaeal community composition for ectomycorrhizal fungi-colonized trees (EcM) and arbuscular fungi-colonized trees (AM). a. the effect of mycorrhizal types on community compositions of bacteria and archaea. b. the combined effects of tree species richness and shrub species richness on community compositions of bacteria and archaea, respectively for ectomycorrhizal fungi-colonized trees (EcM) and arbuscular fungi-colonized trees (AM).

Additional file 5: Figure S5. Taxonomic classifications of soil bacterial and archaeal community for ectomycorrhizal fungi-colonized trees (EcM) and arbuscular fungi-colonized trees (AM).

Additional file 6: Figure S6. Volcano plots showing up- and down-regulated ASVs in six comparisons between shrub richness levels at 0, 2, 4, 8 (R0R2, R0R4, R0R8, R2R4, R2R8, R4R8) under three tree species richness levels, respectively for bacteria and archaea. An adjusted p value < 0.01 is indicated in red, while an adjusted p value < 0.01 is indicated in black. The top ten ASVs with the most significant differences in abundance were indicated by their ID numbers and the numbers of ASVs with significantly differences in abundance for the three comparisons are indicated in bracket.

Additional file 7: Figure S7. Functional assignments with relative abundance of each functional groups in bacterial and archaeal community, including carbon cycling (C_cycle), nitrogen cycling (N_cycle), sulfur cycling (S_cycle), parasitism and others under the combined effects of tree species richness and shrub species richness.

Additional file 8: Figure S8. The co-occurrence networks of bacterial communities in three tree species richness levels (1, 2, and 4) coupled with four shrub species richness levels (0, 2, 4, and 8). The nodes in the networks are colored referred to the taxonomic assignments at phylum level and the size of each node is proportional to the relative abundance.

Additional file 9: Figure S9. The co-occurrence networks of archaeal communities in three tree richness levels (1, 2, and 4) coupled with four shrub richness levels (0, 2, 4, and 8). The nodes in the networks are colored by the taxonomic assignments at phylum level and the size of each node is proportional to the relative abundance.

Additional file 10: Figure S10. Direct effects of aboveground plant species richness on environmental factors.

Additional file 11: Table S1. The overview of sample information across the experimental plots.

Additional file 12: Table S2. The linear mixed effect model summaries for main effects of tree and shrub species richness on the community structure of bacteria and archaea, respectively for ectomycorrhizal fungi-colonized trees (EcM) and arbuscular fungi-colonized trees (AM).

Additional file 13: Table S3. The significantly differential abundances of bacterial and archaeal phyla under the combined effects of tree and shrub species richness by Generalized Linear Model tests.

Additional file 14: Table S4. The specific and shared amplicon sequence variants (ASVs) among the three levels of tree species richness.

Additional file 15: Table S5. Amplicon sequence variants (ASVs) with significantly different abundance between three levels of tree species richness and four levels of shrub specie richness.

Additional file 16: Table S6. The significantly differential abundances of bacterial and archaeal amplicon sequence variants (ASVs) assigned to functional groups under the combined effects of tree and shrub species richness by Generalized Linear Model tests.

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Authors' contributions
NZ and LQ planned and designed the research. TY collected the soil samples in BEF-China platform and soil physicochemical properties data. ST performed data analyses and wrote the draft of the manuscript in close consultation with NZ. GFV, NZ, ST and LQ contributed substantially to the revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data of seedling biomass, soil physicochemical parameters, and fungal community composition acquired in the study are all included in the manuscript and supplementary material. We submitted the representative sequences from Illumina MiSeq sequencing to NCBI Sequence Read Archive (SRA) database with the accession code PRJNA816566.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Pereira HM, Leadley PW, Proenca V, Alkemade R, Scharlemann JPW, FernandezManjarres JF, et al. Scenarios for global biodiversity in the 21st century. Science. 2010;330:1496–501.
2. Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. Impacts of climate change on the future of biodiversity. Ecol Lett. 2012;15:365–77.
3. Bardgett RD, van der Putten WH. Belowground biodiversity and ecosystem functioning. Nature. 2014;515:505–11.
4. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venaiol P, et al. Biodiversity loss and its impact on humanity. Nature. 2012;486:59–67.
5. Trumbore S, Brando P, Hartmann H. Forest health and global change. Science. 2015;349:814–8.
6. Mueller KE, Tilman D, Fornara DA, Hobbie SE. Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. Ecology. 2013;94:787–93.
7. Eisenhauer N, Dobies T, Cesarz S, Hobbie SE, Meyer RJ, Worm K, et al. Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N deposition in a long-term grassland experiment. Proc Natl Acad Sci USA. 2013;110:6889–94.
8. Ravenek JM, Bessler H, Engels C, Scherer-Lorenzen M, Gessler A, Gockele A, et al. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos. 2014;123:1528–36.
9. Chen YL, Xu TL, Veresoglou SD, Hu HW, Hao ZP, Hu YJ, et al. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. Soil Biol Biochem. 2017;110:12.
10. Mommer L, Cotton TEA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, et al. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytol. 2018;218:542–53.
11. Liang JJ, Crowther TW, Picard N, Wiser S, Zhou M, Alberti G, et al. Positive biodiversity-productivity relationship predominant in global forests. Science. 2016;354:6309.
12. Liu XJ, Trogisch S, He JS, Niklaus PA, Bruelheide H, Tang ZY, et al. Tree species richness increases ecosystem carbon storage in subtropical forests. P Roy Soc Bbiol Sci. 2018;285:20181240.
13. Paquette A, Hector A, Castagneyrol B, Vanhellemont M, Koricheva J, Scherer-Lorenzen M, et al. A million and more trees for science. Nat Ecol Evol. 2018;2:763766.
14. Jochum M, Fischer M, Isbell F, Roscher C, van der Plas F, Boch S, et al. The results of biodiversity–ecosystem functioning experiments are realistic. Nat Ecol Evol. 2020;4:1485–94.
15. Wagner RG, Little KM, Richardson B, McNabb K. The role of vegetation management for enhancing productivity of the world's forests. Forestry. 2006;79:57–79.
16. Huang YY, Chen Y, Castro-Izaguirre N, Baruffol M, Brezzi M, Lang A, et al. Impacts of species richness on productivity in a large-scale subtropical forest experiment. Science. 2018;362:80–3.
17. Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. Nature. 2017;1(546):145–7.
18. Debray R, Herbert RA, Jaffe AL, Crits-Christoph A, Power ME, Koskella B. Priority effects in microbiome assembly. Nat Rev Microbiol. 2022;20:109–21.
19. Hooper DU, Bignell DE, Brown VK, Brussard L, Dangerfield JM, Wall DH, et al. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. Biocience. 2000;50:1049–61.
20. Nielsen UN, Wall DH, Six J. Soil biodiversity and the environment. Annu Rev Env Resour. 2015;40:63–90.
21. Bender SF, Wagg C, van der Heijden MG. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol. 2016;31:440–52.
22. Wagg C, Schlaeppi K, Banerjee S, Kuramae E, van der Heijden MGA. Fungalbacterial diversity and microbiome complexity predict ecosystem functioning. Nat Commun. 2019;10:4841.
23. Scherber C, Eisenhauer N, Weisser WW, Schmid B, Voigt W, Fischer M, et al. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature. 2010;468:553–6.
24. Lange M, Eisenhauer N, Sierra CA, Bessler H, Engels C, Griffiths RI, et al. Plant diversity drives soil carbon storage by increased soil microbial activity. Nat Commun. 2015;6:6707.
25. Sterkenburg E, Bahr A, Brandström Durling M, Clemmensen KE, Lindahl BD. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytol. 2015;207:1145–58.
26. Ferlian O, Goldmann K, Eisenhauer N, Tarkka MT, Buscot F, Heintz-Buschart A. Distinct effects of host and neighbour tree identity on arbuscular and ectomycorrhizal fungi along a tree diversity gradient. ISME Commun. 2021;1:40.
27. Anthony MA, Crowther TW, van der Linde S, Suz LM, Bidartondo MI, Cox F, et al. Forest tree growth is linked to mycorrhizal fungal composition and function across Europe. ISME J. 2022;16:1327–36.
28. Gao C, Shi NN, Liu YX, Peay KG, Zheng Y, Ding Q, et al. Host plant genus-level diversity is the best predictor of ectomycorrhizal fungal diversity in a Chinese subtropical forest. Mol Ecol. 2013;22:3403–14.
29. Beugnon R, Du J, Cesarz S, Jurburg SD, Pang Z, Singavarapu B, et al. Tree diversity and soil chemical properties drive the linkages between

soil microbial community and ecosystem functioning. ISME Commun. 2021;1:41.
30. Li XC, Qian X, Gao C, Seitz S, Scholten T, Wang YL, et al. Plant identity strongly structures the root-associated fungal community in a diverse subtropical forest. Basic Appl Ecol. 2021;55:98–109.
31. Singavarapu B, Beugnon R, Bruelheide H, Cesarz S, Du JP, Eisenhauer N, et al. Tree mycorrhizal type and tree diversity shape the forest soil microbiota. Environ Microbiol. 2022;24(9):4236–55.
32. Lladó S, López-Mondéjar R, Baldrian P. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiol Mol Biol R. 2017;81:e00063-e116.
33. van der Heijden MGA, Bardgett RD, van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 2008;11:296–310.
34. Reed SC, Cleveland CC, Townsend AR. Functional ecology of free-living nitrogen fixation: a contemporary perspective. Annu Rev Ecol Evol S. 2011;42:489–512.
35. Uroz S, Oger P, Lepleux C, Collignon C, Frey-Klett P, Turpault MP. Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. Res Microbiol. 2011;162:820–31.
36. Brown ME, Chang MC. Exploring bacterial lignin degradation. Curr Opin Chem Biol. 2014;19:1–7.
37. Berlemont R, Martiny AC. Genomic potential for polysaccharides deconstruction in bacteria. Appl Environ Microb. 2015;81:1513–9.
38. Tripathi BM, Kim M, Tateno R, Kim W, Wang J, Lai-Hoe A, et al. Soil pH and biome are both key determinants of soil archaeal community structure. Soil Biol Biochem. 2015;88:1–8.
39. Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, et al. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun. 2016;7:10541.
40. López-Mondéjar R, Zühlke D, Becher D, Riedel K, Baldrian P. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci Rep. 2016;6:25279.
41. Weißbecker C, Wubet T, Lentendu G, Kühn P, Scholten T, Bruelheide H, et al. Experimental evidence of functional group-dependent effects of tree diversity on soil fungi in subtropical forests. Front Microbiol. 2018;9:2312.
42. Bahram M, Netherway T, Hildebrand F, Pritsch K, Drenkhan R, Loit K, et al. Plant nutrient-acquisition strategies drive topsoil microbiome structure and function. New Phytol. 2020;227:1189–99.
43. Goberna M, Verdú M. Cautionary notes on the use of co-occurrence networks in soil ecology. Soil Biol Biochem. 2022;166:108534.
44. Hassani MA, Durán P, Hacquard S. Microbial interactions within the plant holobiont. Microbiome. 2018;6:58.
45. Shen C, Wang J, Jing Z, Qiao N, Xiong C, Ge Y. Plant diversity enhances soil fungal network stability indirectly through the increase of soil carbon and fungal keystone taxa richness. Sci Total Environ. 2022;818:151737.
46. Horner-Devine MC, Silver JM, Leibold MA, Bohannan BJ, Colwell RK, Fuhrman JA, et al. A comparison of taxon co-occurrence patterns for macro- and micro- organisms. Ecology. 2007;88:1345–53.
47. Junker BH, Schreiber F. Correlation Networks. In: Analysis of Biological Networks, Wiley Series on Bioinformatics. Hoboken, NJ: Wiley-Interscience; 2008. p. 346.
48. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 2011;6:343–51.
49. Ma B, Wang HZ, Dsouza M, Lou J, He Y, Dai Z, et al. Geographical patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. ISME J. 2016;10:1891–901.
50. Shi SJ, Nuccio EE, Shi ZJ, He ZL, Zhou JZ, Firestone MK. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol Lett. 2016;19:926–36.
51. Morriën E, Hannula SE, Snoek LB, Helmsing NR, Zweers H, de Hollander M, et al. Soil networks become more connected and take up more carbon as nature restoration progresses. Nat Commun. 2017;8:14349.
52. de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, et al. Soil bacterial networks are less stable under drought than fungal networks. Nat Commun. 2018;9:3033.
53. Banerjee S, Walder F, Büchi L, Meyer M, Held AY, Gattinger A, et al. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. ISME J. 2019;13:1722–36.
54. Bruelheide H, Nadrowski K, Assmann T, Bauhus J, Both S, Buscot F, et al. Designing forest biodiversity experiments: general considerations illustrated by a new large experiment in subtropical China. Methods Ecol Evol. 2014;5:74–89.
55. Bakker PA, Berendsen RL, Doornbos RF, Wintermans PC, Pieterse CM. The rhizosphere revisited: root microbiomics. Front Plant Sci. 2013;4:165.
56. Yang X, Bauhus J, Both S, Fang T, Härdtle W, Kröber W, et al. Establishment success in a forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China). Eur J Forest Res. 2013;132:593–606.
57. Bruelheide H, Boehnke M, Both S, Fang T, Assmann T, Baruffol M, et al. Community assembly during secondary forest succession in a Chinese subtropical forest. Ecol Monog. 2011;81:25–41.
58. Scholten T, Goebes P, Kuhn P, Seitz S, Assmann T, Bauhus J, et al. On the combined effect of soil fertility and topography on tree growth in subtropical forest ecosystems-a study from SE China. J Plant Ecol. 2017;10:111e127.
59. Eilers KG, Debenport S, Anderson S, Fierer N. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biol Biochem. 2012;50:58–65.
60. Ko D, Yoo G, Yun ST, Jun SC, Chung H. Bacterial and fungal community composition across the soil depth profiles in a fallow field. J Ecol Environ. 2017;41:34.
61. Zhao H, Zheng W, Zhang S, Gao W, Fan Y. Soil microbial community variation with time and soil depth in Eurasian Steppe (Inner Mongolia, China). Ann Microbiol. 2021;71:21.
62. Bruelheide H, Schmidt K, Seidler G, Nadrowski K. Site A plots: diversity treatments, coordinates, topography. BEF-China data portal, 2013a, http://china.befdata.biow.uni-leipzig.de/datasets/71
63. Bruelheide H, Schmidt K, Seidler G, Nadrowski K. Site B plots: diversity treatments, coordinates, topography. BEF-China data portal, 2013b, http://china.befdata.biow.uni-leipzig.de/datasets/46.
64. Roberts DW. Ordination on the basis of fuzzy set theory. Vegetatio. 1986;66:12331.
65. Bongers FJ, Schmid B, Bruelheide H, Bongers F, Li S, von Oheimb G, et al. Functional diversity effects on productivity increase with age in a forest biodiversity experiment. Nat Ecol Evol. 2021;5:1–10.
66. Brookes PC, Landman A, Pruden G, Jenkinson DS. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem. 1985;17:837–42.
67. Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem. 1987;19:703–7.
68. Huse SM, Dethlefsen L, Huber JA, Welch DM, Relman DA, Sogin ML. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. PLoS Genet. 2008;4:e1000255.
69. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone C, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA. 2011;108:4516–22.
70. Cao J, Pan H, Chen Z, Shang H. Bacterial, fungal, and archaeal community assembly patterns and their determining factors across three subalpine stands at different stages of natural restoration after clear-cutting. J Soil Sediment. 2020;20:2794–803.
71. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.
72. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
73. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microb. 2007;73:5261–7.
74. Cao Q, Sun X, Rajesh K, Chalasani N, Gelow K, Katz B, et al. Effects of rare microbiome taxa filtering on statistical analysis. Front Microbiol. 2021;11:607325.

75. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016;353:1272–7.
76. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2023. https://www.Rproject.org/. Accessed 18 May 2022.
77. Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: Community Ecology Package. R package version 2.5–6. 2019. Available at: https://cran.r-project.org/web/packages/vegan/index.html. Accessed in June 17 2022.
78. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
79 Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27:431–2.
80. Jiao S, Zhang Z, Yang F, Lin Y, Chen W, Wei G. Temporal dynamics of microbial communities in microcosms in response to pollutants. Mol Ecol. 2017;26:923–36.
81. Csardi G, Nepusz T. The igraph software package for complex network research. InterJ Complex System. 2006;1695:1–9.
82. Bastian M, Heymann S, Jacomy M. Gephi: an open-source software for exploring and manipulating networks. In: Third international AAAI conference on weblogs and social media. 2009;3:361–362.
83. Bougeard S, Dray S. Supervised multiblock analysis in R with the ade4 Package. J Stat Softw. 2018;86:1–17.
84. Mansfield ER, Helms BP. Detecting multicollinearity. Am Stat. 1982;36:158–60.
85. Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, et al. Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome. 2018;6:156.
86 Rosseel Y. Lavaan: an R package for structural equation modeling and more. Version 0.5–12 (BETA). J Stat Softw. 2012;48:1–36.
87. MacCallum RC, Browne MW, Sugawara HM. Power analysis and determination of sample size for covariance structure modeling. Psychol Methods. 1996;1:130–49.
88. Hu LT, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct Equ Modeling. 1999;6:1–55.
89. Diamantopoulos A, Siguaw JA. Introduction to LISREL: a guide for the uninitiated. London: SAGE Publications. Inc.; 2000.
90. Averill C, Turner BL, Finzi AC. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature. 2014;505:543–5.
91. van der Heijden MG, Bruin S, de Luckerhoff L, van Logtestijn RS, Schlaeppi K. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J. 2015;10:389–99.
92. Heklau H, Schindler N, Buscot F, Eisenhauer N, Ferlian O, Prada Salcedo LD, et al. Mixing tree species associated with arbuscular or ectotrophic mycorrhizae reveals dual mycorrhization and interactive effects on the fungal partners. Ecol Evol. 2021;11:5424–40.
93. Kinkel L, Bakker M, Schlatter D. A coevolutionary framework for managing disease-suppressive soils. Annu Rev Phytopathol. 2011;49:47–67.
94. Schlatter DC, Bakker MG, Bradeen JM, Kinkel LL. Plant community richness and microbial interactions structure bacterial communities in soil. Ecology. 2015;96:134–42.
95. Grigulis K, Lavorel S, Krainer U, Legay N, Baxendale C, Dumont M, et al. Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J Ecol. 2013;101:47–57.
96 He JZ, Wu HW, Zhang LM. Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biol Biochem. 2012;55:146e154.
97. Levy-Booth DJ, Prescott CE, Grayston SJ. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol Biochem. 2014;75:11–25.
98. Koga Y. Thermal adaptation of the archaeal and bacterial lipid membranes. Archaea. 2012;2012:789652.
99. Thion C, Prosser JI. Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying-rewetting stress. FEMS Microbiol Ecol. 2014;90:380–9.
100. Dassen S, Cortois R, Martens H, de Hollander M, Kowalchuk GA, van der Putten WH, et al. Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. Mol Ecol. 2017;26:4085–98.
101. Bonfant P, Anca IA. Plants, Mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol. 2009;63:363–83.
102. Faust K, Raes J. Microbial interactions: from networks to models. Nat Commun. 2012;10:538–50.
103 Li JB, Li CN, Kou YP, Yao MJ, He ZL, Li XZ, et al. Distinct mechanisms shape soil bacterial and fungal co-occurrence networks in a mountain ecosystem. FEMS Microbiol Ecol. 2020;96:fiaa030.
104. Newman M. The structure and function of complex networks. SIAM Rev. 2003;45:167–256.
105. Zheng YM, Cao P, Fu B, Hughes JM, He JZ. Ecological drivers of biogeographic patterns of soil archaeal community. PLoS ONE. 2013;8:e63375.
106. Poorter L, van der Sande MT, Thompson J, Arets EJMM, Alarcón A, ÁlvarezSánchez J, et al. Diversity enhances carbon storage in tropical forests. Global Ecol Biogeogr. 2015;24:1314–28.
107. Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, et al. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 2007;1:283–90.
108. Cardenas E, Orellana LH, Konstantinidis KT, Mohn WW. Effects of timber harvesting on the genetic potential for carbon and nitrogen cycling in five North American forest ecozones. Sci Rep. 2018;8:1–13.
109. Huang Y, Ma Y, Zhao K, Niklaus PA, Schmid B, He JS. Positive effects of tree species diversity on litterfall quantity and quality along a secondary successional chronosequence in a subtropical forest. J Plant Ecol. 2017;10:28–35.
110. Eisenhauer N, Lanoue A, Strecker T, Scheu S, Steinauer K, Thakur MP, et al. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. Sci Rep. 2017;7:44641.
111. Hao ZG, Zhao YF, Wang X, Wu JH, Jiang SL, Xiao JJ, et al. Thresholds in aridity and soil carbon-to-nitrogen ratio govern the accumulation of soil microbial residues. Commun Earth Environ. 2021;2:236.

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