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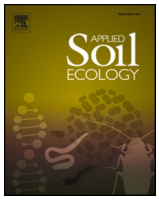

Assembly and enrichment of rhizosphere and bulk soil microbiomes in *Robinia pseudoacacia* plantations during long-term vegetation restoration

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ABSTRACT

Despite myriad studies on root–soil–microbe interactions, the assembly and enrichment dynamics of rhizosphere and bulk soil microbiomes remain poorly understood. Here, we characterized soil bacterial and fungal communities in rhizosphere and bulk soil along a 15–45-year chronosequence of forest vegetation restoration. The neutral model, modified stochasticity ratio, niche breadth index, co-occurrence network, and source tracker were used to assess microbial community assembly, species enrichment, and filtering processes in the rhizosphere and bulk soil of *Robinia pseudoacacia* plantations. The relative importance of deterministic processes in microbial community assembly differed markedly between the rhizosphere and bulk soil. Microbial network complexity was higher in the rhizosphere soil than in the bulk soil. Both rhizosphere and bulk soil microbial networks were dynamically associated with forest age. Overall, the rhizosphere microbial community was mainly derived from the bulk soil, and filtered by the soil environment and plant selection. The soil environment was less selective for bacteria than for fungi, thus increasing bacterial community migration ratio. Furthermore, soil microbes formed distinct clusters in different niche compartments, with greater interaction and niche sharing potential in the rhizosphere soil than in the bulk soil.

1. Introduction

Soil microbes are the main decomposers in terrestrial ecosystems and they play essential roles in ecosystem maintenance during long-term ecological restoration (Jiao et al., 2018, 2019). Afforestation is one of the major ecological restoration strategies of returning farmland to forest, and its influence on soil microbial communities has increasingly attracted the attention of researchers (Liu et al., 2018a, 2018b; Jia et al., 2019; Xu et al., 2019). Soil microbial community structure varies across time and space, and the activities of community members are impacted by associated plants, animals, and habitats (Jiang et al., 2018; Jiao et al., 2018). Therefore, core and frontier issues in ecological restoration research are the factors driving soil microbial community assembly and the underlying mechanisms (Zhou and Ning, 2017; Tripathi et al., 2018; Liu et al., 2020). An understanding of soil microbial community assembly and species coexistence dynamics in the course of ecological restoration is crucial for ecosystem service and function maintenance, effective environmental and agricultural management, and soil-borne disease control (Chase, 2010; Zhou et al., 2014; Fukami, 2015; Zhou and Ning, 2017).

Community assembly processes include deterministic (heterogeneous and homogeneous selection) and stochastic (homogeneous diffusion, diffusion limitation, and ecological drift) processes. A myriad of studies reported the equal importance of deterministic and stochastic processes in governing the community assembly and species distribution of bacteria and fungi in soils, termite mounds, and bioreactors (Fukami et al., 2010; Zhou et al., 2013; Liu et al., 2015; Tripathi et al., 2018; Chen et al., 2021). However, more experimental studies are required to validate the previous conclusions, because the relative importance of such ecological processes depends on the time scale, environmental gradient, and soil fertility considered (Stegen et al., 2012; Tripathi et al., 2018; Liu et al., 2020). In this regard, time series experiments combining micro and macro theory are necessary to identify the mechanisms that allow microbial communities to maintain equilibrium states during ecosystem restoration, and thereby facilitate ecosystem stabilization.

Microbes coexist in environments with limited resources and exhibit distinct biogeographic patterns from regional to global scales (Nemergut et al., 2013; Ji et al., 2020). Microbe coexistence patterns are mediated

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by species interactions, dispersal limitation (Jiao et al., 2021; Li et al., 2021), and various environmental factors. The rhizosphere is a micro zone much more complex than initially thought. Dynamic life activities, such as the exchange of information and matter between plants and microbes, occur in the rhizosphere soil (Canarini et al., 2019). Plants at different developmental stages can release distinct root exudates to meet the nutrient requirements of associated microbes, and some microbial species can in turn facilitate plant growth (Zhao et al., 2021). Such interactions inevitably lead to the differentiation of soil microbial communities in different ecological niches. The rhizosphere, a habitat zone of only a few millimeters, harbors great species interaction and niche sharing potential (Shi et al., 2016; Yuan et al., 2018a). Nonetheless, many studies have focused on only one or a few of the factors, and our understanding of how different niches, soil factors, and stand age jointly affect the assembly processes of soil microbial communities in forests remains poor (Zhou and Ning, 2017).

Black locust (*Robinia pseudoacacia*) is a fast-growing tree native to North America. The tree species was planted over a large area in the Yellow River Basin in China during the 20th century. *R. pseudoacacia* plantations are of practical significance for soil and water conservation and ecological restoration in the Loess Plateau region in China. In recent years, improving the quality and efficiency of degraded and inefficient *R. pseudoacacia* forests in the plateau region has become an urgent and challenging issue. Microbes are vital for material circulation and energy flows in terrestrial ecosystems, and they can facilitate the remediation of polluted soil (Sasse et al., 2018). Rhizosphere microbes are considered the second plant genome, with diverse roles in nutrient acquisition, growth hormone production, and defense against diseases (Li et al., 2019). However, the underlying mechanisms of the assembly of soil microbial communities, especially those in the rhizosphere during vegetation restoration, are not yet comprehensively understood.

The present study, conducted using *R. pseudoacacia* plantation stands of different ages, had the following objectives: (i) to investigate variation in soil bacterial and fungal community structure across different niche compartments (rhizosphere vs. bulk soil) along a vegetation restoration chronosequence, (ii) to uncover the assembly processes and common microbial community species between rhizosphere soil and bulk soil and (iii) to explore the microbial co-occurrence trends with change in forest age. The results of the present study could reveal the community assembly processes of soil bacteria and fungi, in addition to their species coexistence and enrichment trends, in *R. pseudoacacia* plantations. This research could also enhance our understanding of belowground ecological processes in the course of long-term vegetation restoration activities.

2. Materials and methods

2.1. Study area and soil sampling

The study region was located at 34°12′–34°50′ N and 108°5′6″–108°5′11″ E in the range of distribution of dominant *R. pseudoacacia* plantations in Yongshou County, Shaanxi Province, China (Fig. S1). The mean annual temperature in the area is 11.30 °C with a mean annual precipitation of 569.90 mm. The region has a semi-arid climate and an elevation of 1368.3–1393.6 m. Four *R. pseudoacacia* plantation stands (15, 25, 35, and 45 years in age, with gentle slopes of 18°, 15°, 10°, and 36°, respectively) were selected for the present study. The dominant understory plants at the selected sites included *Rubus*, *Panicum*, *Aster*, *Equisetum*, *Leonurus*, *Carpesium, Artemisia*, and *Reineckia* (15 years); *Carpesium*, *Humulus*, and *Iris* (25 years); *Humulus*, *Artemisia*, and *Chenopodium* (35 years); and *Humulus*, *Chenopodium*, and *Carpesium* (45 years).

Field sampling was carried out in July 2020. The sampling locations within the four plantation stands were >50 m apart, with six 20-m × 20-m plots set up and sampled in each site. Each plot was divided into six 2-m × 2-m subplots around a tree. In each subplot, soil cores were obtained from the 0–20-cm depth along an S-shaped pattern. Bulk soil was obtained from the soil sampling points at the outside edge of the tree crown (Ridder-Duine et al., 2005). Rhizosphere soil was collected from fine roots (diameter < 2 mm) of *R. pseudoacacia* (Liu et al., 2018a, 2018b) by gently removing the bulk soil of the roots and obtaining the soil attached to them (Chaparro et al., 2014; Yuan et al., 2018b).

Six soil samples were combined per subplot for a given tree and then mixed thoroughly to generate a composite sample. According to the ages of the *R. pseudoacacia* plantations, the rhizosphere soil samples collected from different forest sites were marked as RS15, RS25, RS35, and RS45, and the bulk soil samples were similarly denoted as nRS15, nRS25, nRS35, and nRS45, respectively. All soil samples were sieved through a 2-mm mesh to remove any plant litter and rocks and were transported to the laboratory in sterile cryogenic tubes on dry ice. A portion of each soil sample was stored at 4 °C for use in physicochemical analysis; another portion was stored at −20 °C for use in subsequent DNA extractions.

2.2. Data acquisition and analysis

Total genomic DNA was extracted from each soil sample using the CTAB method (Yi et al., 2018). The primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the V4–V5 regions of bacterial 16S rRNA genes, whereas 1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and 2043R (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the internal transcribed spacer (ITS1–5F) regions of fungal rRNA genes (Chen et al., 2016; Chen et al., 2017; Zhao et al., 2014). High-throughput sequencing was performed on a Nova Seq600 platform (Illumina Inc., San Diego, CA, USA) by the Novogene Bioinformatics Institute (Beijing, China). After the acquired sequences were denoised and dereplicated, a feature table of amplicon sequence variants (ASVs) was generated using the QIIME2 platform (Supplemental Material Method S1) (Callahan et al., 2016).

Common testing methods were used to determine the pH, soil organic carbon (SOC), soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), nitrate-nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N), available phosphorus (AP), and available potassium (AK; Supplemental Material Method S2). The mean values of soil properties at each sampling site were calculated based on the site coordinates. Differences in soil properties among the four forest sites and between the two niche compartments were tested using Analysis of Variance (ANOVA; Table S1).

The Chao1 and Shannon indices of bacteria and fungi were calculated using the 'vegan' package in R v4.0.4 (Oksanen et al., 2020). Differences in microbial α-diversity were tested using Tukey's method, with the *MuiaovMcomper* function in the 'EasyStat' package in R v4.0.4 (Wood, 2011). Bacterial and fungal β-diversity were assessed using computed Bray-Curtis distance matrices and then ordinated using an unconstrained Principal Coordinate Analysis (PCoA). The community dissimilarity associated with forest age and niche compartment was tested using Permutational Multivariate Analysis of Variance (PERMANOVA), and this analysis was performed based on Bray-Curtis distances using the *adonis* function of the 'vegan' package in R v4.0.4 (Oksanen et al., 2020). To estimate β-diversity changes with soil environments across different niche compartments, the relationships between soil environmental heterogeneity (Euclidean distance) and microbial β-diversity in the rhizosphere and bulk soil were analyzed using the Mantel test.

Redundancy Analysis (RDA) was used to analyze the effects of environmental factors on microbial community structure, based on proportions of variation in community structure explained by environmental factors. Furthermore, the Mantel test was performed (9999 permutations) to explore correlation between each environmental factor and microbial community structure based on the 'Pearson' method.

A neutral model was used to estimate the relative contribution of stochastic processes to microbial community assembly. The model fits the observed abundance–frequency relationship with a β-distribution, and examines the dynamics of each abundant taxon (Ofiteru et al., 2010; Zhou and Ning, 2017). Generally, a higher m value indicates a higher community migration ratio that is less affected by dispersal limitation (Supplemental material Method S3). Migration ratios of bacteria and fungi were computed based on the software code of Burns et al. (2016).

A general mathematical framework was used to quantify the relative importance of deterministic and stochastic processes in microbial community assembly between the rhizosphere and bulk soil (Ning et al., 2019). We used a unique form of the normalized stochasticity ratio, namely, the modified stochasticity ratio (MST), with 0.5 as the threshold for distinguishing deterministic (MST < 0.5) and stochastic (MST > 0.5) processes. Bray-Curtis similarity matrices were constructed in combination with the neutral models of fixed taxa richness and proportional taxa occurrence frequency (Chen et al., 2021). Bootstrapping ($n = 1000$ replicates) was used to test MST in bacterial and fungal communities. The MST analysis was executed in R v4.0.4 using the 'NST' package (Ning et al., 2019). The Wilcox test was used to test differences in MST between rhizosphere and bulk soil.

Niche breadth refers to the total sum of resources utilized by a particular species in a community (Pandit et al., 2009). Levin's niche breadth index was used to reveal the patterns of species sorting and dispersal limitation, and their influence on microbial community structure, in rhizosphere and bulk soil. Levin's niche breadth indices (B) for bacteria and fungi were calculated using the following equation:

$$B_j = 1 \Big/ \sum_{i=1}^{N} P_{ij}^2$$

where B_j indicates the habitat niche breadth of ASV_j in a meta-community and P_{ij} is the proportion of ASV_j in community i (Pandit et al., 2009; Wu et al., 2018). A high B-value for an ASV indicates that it has a broad habitat niche. The community-level B-value (*Bcom*) was calculated as the average of B-values from all ASVs (Pandit et al., 2009). The calculation of niche breadth was performed using the *niche.width* function of the 'spaa' package in R v4.0.4 (Zhang, 2016). Differences in niche breadth between the two niche compartments were tested using the Wilcoxon rank-sum test.

Microbial co-occurrence networks were constructed using 'igraph', 'WGCNA', and 'stats' packages in R v4.0.4 (Csardi and Nepusz, 2006; Langfelder and Horvath, 2008). Spearman's correlation coefficients between the relative abundances of ASVs were calculated, and only robust (i.e., Spearman's $r > 0.07$) and significant ($P < 0.05$) correlations were retained. Network complexity was defined as described previously (Wagg et al., 2019; Xiong et al., 2021). The properties of each network (i.e., node number, betweenness, average length path, and average degree) were computed using the bootstrapping method, with 10,000 iterations (Banerjee et al., 2019). Subsequently, we performed two-sample Kolmogorov-Smirnov test to compare network topological features between the two niche compartments using the *ks.test* function in the stats package in R v4.0.4. The *DGEList* function in the 'edgeR' package in R v4.0.4 was used to identify significantly enriched ASVs in different niche compartments (Robinson et al., 2010), namely, the most sensitive ASVs ($P < 0.05$) between the rhizosphere and bulk soil.

Source tracker, based on the Bayesian approach, was used to identify the sources of microbial communities in the rhizosphere compartment (Knights et al., 2011).

3. Results

3.1. Microbial community structure associated with niche compartment and forest age

Soil niche compartment affected both bacterial and fungal species richness substantially. The Chao 1 index was higher in the bulk soil than in the rhizosphere soil, for both the bacterial and fungal communities (Fig. S2A—D). Bacterial α-diversity did not differ significantly in both the rhizosphere and bulk soil along the forest site time series (Fig. S2B); however, significant differences in the α-diversity of rhizosphere fungi were observed among the RS15, RS35, and RS45 treatments (Fig. S2D). The results showed that there was greater microbial species richness in the bulk soil than in the rhizosphere soil (Fig. S2).

Soil bacterial communities from 15, 25, 35, and 45-year forest stands displayed spatial divergence along the first principal coordinate of PCoA biplot (Fig. 1A). PERMANOVA of pairwise distances between bacterial communities indicated that the community composition differed significantly over time ($R^2 = 0.26$, $P < 0.001$; Table S2). Soil fungal communities from the mature forest stands (15 and 25 years) and highly mature forest stands (35 and 45 years) were separated along the second principal coordinate of PCoA biplot (Fig. 1D). PERMANOVA of pairwise distances between fungi communities indicated that the community composition differed significantly between the rhizosphere and bulk soil ($R^2 = 0.29$, $P < 0.001$; Table S2, Fig. 1D). The β-diversity of the rhizosphere bacterial community decreased with an increase in forest age, with an opposite trend observed for the bulk soil bacterial community (Fig. 1A). In the case of fungi, the β-diversity decreased with an increase in forest age in both niche compartments (Fig. 1D). In addition, environmental distance had a significant positive correlation with microbial community dissimilarity, excluding in the case of rhizosphere fungi (Fig. 1B-C; E-F).

Basidiomycota, *Mucoromycota*, and *Ascomycota* were the dominant phyla in the soil fungal community. *Basidiomycota* relative abundance was higher in nRS15 than in nRS35, whereas *Ascomycota* relative abundance increased with an increase in forest age in different niche compartments (Fig. S3B). The relative abundances of *Moriterellomycota* increased with forest age in the rhizosphere and were generally higher in the bulk soil than in the rhizosphere soil (Fig. S3B).

Subsequently, we determined which major soil environmental factors influenced microbial community structure (Fig. S4). Soil pH was the key soil variable influencing microbial community structure in the rhizosphere soil and bulk soil (Fig. S4A—D).

3.2. Microbial community assembly processes in different niche compartments

We estimated the relative importance of microbial community assembly processes using the neutral model, MST, and niche breadth. When fitted to the neutral model, the degree of fit for the bulk soil bacterial community surpassed that for the rhizosphere bacterial community, with similar trends for the fungal community (Fig. 2). Migration ratios of the bulk soil bacterial community exceeded those of the rhizosphere bacterial community (rhizosphere: $m = 0.012$; bulk soil: $m = 0.015$; Fig. 2A-B), suggesting the latter community was more limited by dispersal than the former community. Similar results were obtained for fungi in the two niche compartments (rhizosphere: $m = 0.003$; bulk soil: $m = 0.009$; Fig. 2C-D).

MST was used to quantify the roles of deterministic and stochastic processes in microbial community assembly (Fig. 3). In most cases, bacteria MST values were close to 0.5, indicating that deterministic processes might dominate bacterial community assembly when compared with stochastic processes, and the relative contribution of deterministic processes in the rhizosphere soil was significantly higher than that in the bulk soil (Fig. 3A). In contrast, fungal MST values were generally below 0.5, implying that deterministic processes were prominent in fungal community assembly. The MST values exhibited significant differences between rhizosphere and bulk soil (Fig. 3C).

Moreover, we estimated the importance of deterministic and stochastic processes at the community level by evaluating niche breadth. Higher mean niche breadths (*Bcom* values) were observed in bacterial communities than in fungal communities across different niche

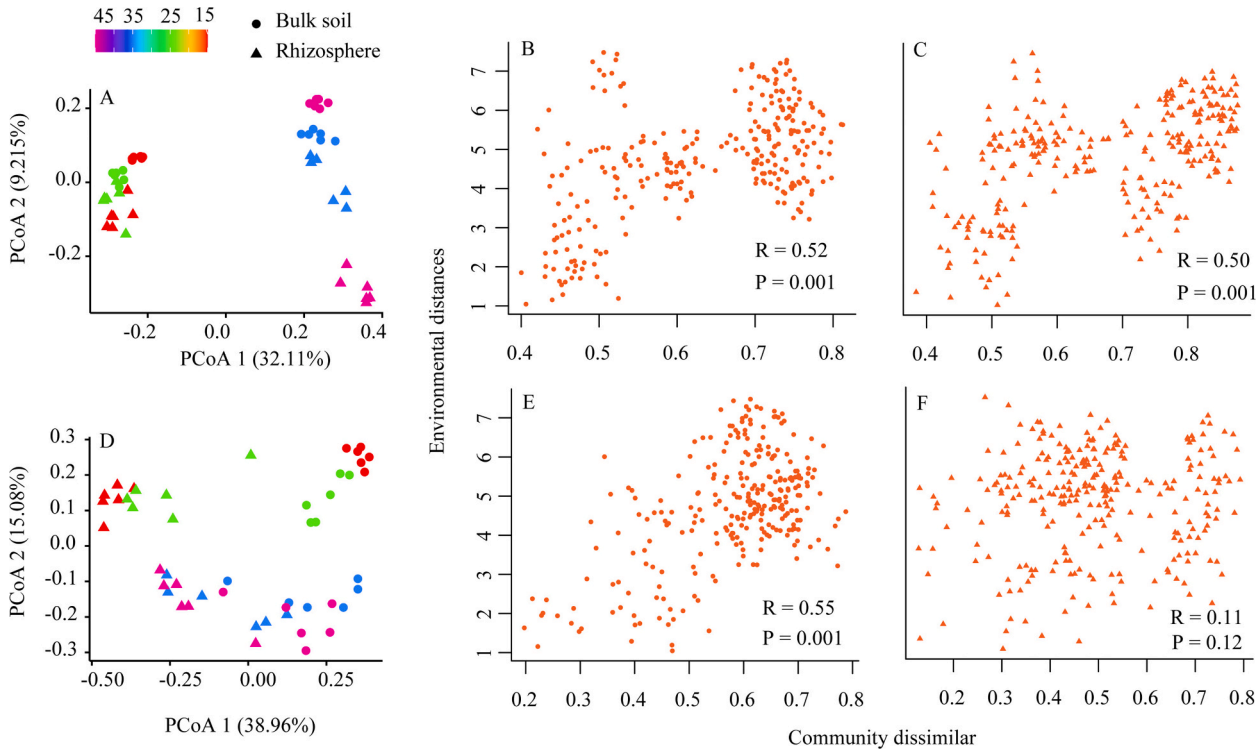

Fig. 1. Soil microbial community dissimilarity and responses to environmental distance in different niche compartments. Principal coordinate analysis (PCoA) biplots of microbial β-diversity (A: bacteria; D: fungi). Mantel test correlation between microbial community dissimilarity and soil environmental distance for different niche compartments (B, C: bacteria; E, F: fungi; B, E: bulk soil; C, F: rhizosphere).

compartments (Fig. 3B, Fig. 3D), indicating a broader habitat niche for bacteria. Overall, the results demonstrated that both stochastic and deterministic processes drove bacterial community assembly, although deterministic processes were dominant. As for fungi, deterministic processes were dominant in both the rhizosphere and bulk soil.

3.3. Microbial co-occurrence patterns in different niche spaces along the time series

To further characterize the niche compartment and time series (forest age) effects on soil bacterial and fungal communities, we assessed their co-occurrence patterns using network analysis. Niche compartment affected microbial network patterns significantly, and the topological features of bulk soil microbial networks differed significantly between the mature (15 and 25 years old) and highly mature (35 and 45 years old) forest stands (Fig. 4, Tables S3–S4). The network complexity of bacteria and fungi, as indicated by their average degrees, increased from the bulk soil to the rhizosphere soil (Table S3). Bacterial network complexity also increased significantly along the time series in both the rhizosphere and bulk soil, whereas fungal network complexity decreased along the time series.

In the time series, node number, edge number, and network diameter for bacterial communities all increased; conversely, the clustering coefficient decreased significantly (Fig. 4, Table S3). In the case of fungal communities, excluding network diameter, other network properties decreased over time in the bulk soil. The results suggested that highly connected ASVs were grouped in the rhizosphere, but they changed markedly with an increase in forest age. Rhizosphere and bulk soil microbial network properties displayed significant differences across the time series (Tables S3-S4).

We further analyzed microbial co-occurrence and enrichment patterns in different niche compartments. Overall, 896 bacterial and 117 fungal ASVs were significantly enriched in the rhizosphere soil, with 1137 bacterial and 469 fungal ASVs enriched in the bulk soil (Fig. 5A-C, Table 1). Unique bacterial taxa between the rhizosphere and bulk soil were identified at the phylum level (Fig. 5B). For example, *Cyanobacteria* and *Fibrobacteria* only occurred in the rhizosphere bacterial community (Fig. 5B). The relative abundance of common fungal taxa varied between the niche compartments. Fungal taxa were less abundant in the rhizosphere soil than in the bulk soil, with *Mucoromycota*, *Olpidiomycota*, and *Zoopagomycota* only observed in the bulk soil (Fig. 5D). The relative abundances of other common fungal taxa were considerably different between the rhizosphere and bulk soil. Generally, the microbes formed distinct clusters in different niche compartments. Compared with bacteria, fungi exhibited more distinct changes in different niche spaces. According to source tracker analysis results, 71.2 % of rhizosphere bacteria originated from the bulk soil, with 28.8 % having an unknown origin; the corresponding percentages for rhizosphere fungi were 76.7 % and 23.3 % (Fig. 5E).

4. Discussion

In the present study, a holistic survey of bulk soil and rhizosphere bacterial and fungal communities was carried out in field-grown *R. pseudoacacia* plantations. We identified the potential sources, assembly processes, co-occurrence patterns, and dominant taxa of soil microbiomes associated with *R. pseudoacacia* plantation forests. According to the results, the complexity of *R. pseudoacacia*-associated microbial networks was markedly lower in the bulk soil than in the rhizosphere soil. The main source of microbes in the rhizosphere was the bulk soil community. Bacterial and fungal enrichment from the bulk soil to the rhizosphere soil was primarily driven by deterministic processes. The findings present empirical evidence on potential sources and enrichment process of forest soil microbiomes during long-term ecological restoration. The results provide critical information that could facilitate the regulation of forest soil microbiomes toward sustainable ecosystems.

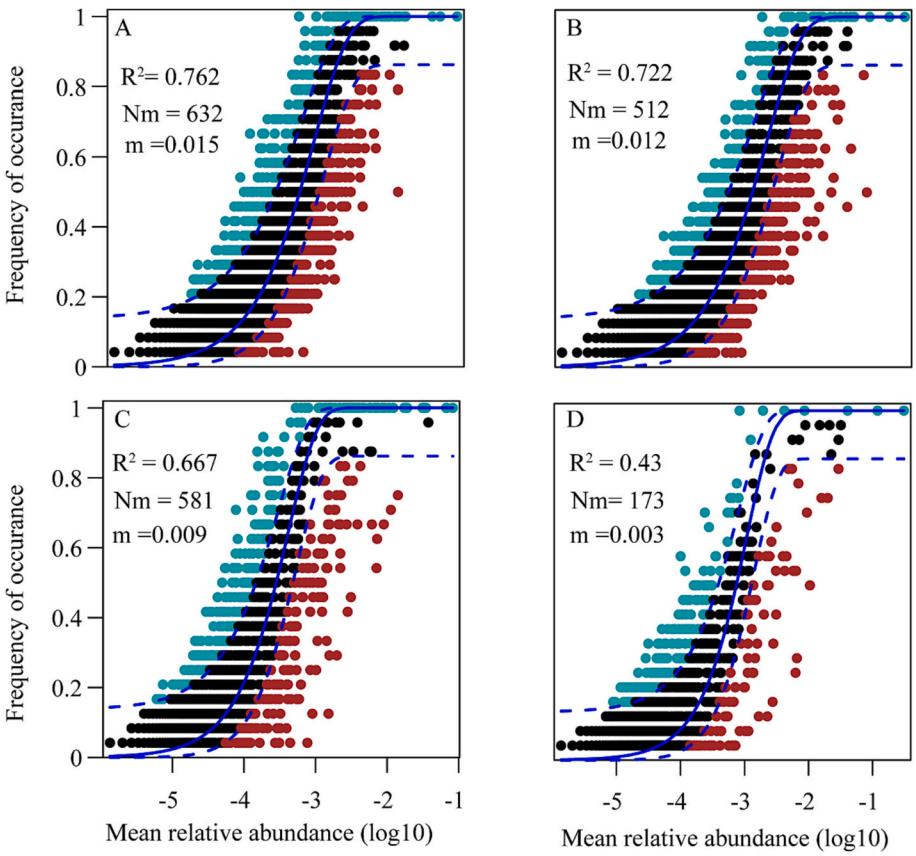

Fig. 2. The microbial community assembly processes as assessed by the neutral model. Rsqr is the R^2 value: the higher the R^2, the better the fit of the neutral model. Nm is the product of meta-community size (N) and migration ratio (m). The results of bulk soil and rhizosphere bacteria are in panels A and B, respectively; the results of bulk soil and rhizosphere fungi are in panels C and D, respectively.

4.1. Environmental responses of soil microbiomes in different niche compartments

The results of the present study showed the complex effects of long-term reforestation on soil and microbiome across time and space. Such effects are mainly reflected in increased difference in microbial community dissimilarity and environmental distance, and their significant positive correlation with each other (Fig. 4C-D). Soil microbial β-diversity showed substantial variation based on both the time series and niche compartments (rhizosphere vs. bulk soil). The bacterial communities in soil compartments exhibited significant positive relationship with environmental distance in the course of *R. pseudoacacia* restoration. The slope for rhizosphere was similar to that for bulk soil, suggesting that the influence of vegetation restoration was similar between the two niche compartments.

The fungal communities showed distinct environmental response patterns in different niche compartments, unlike those in the bacterial communities. Fungal communities had a steeper environmental distance slope in the bulk soil than in the rhizosphere soil. This result indicates that the influence of environmental factors on fungi was higher in the bulk soil than in the rhizosphere soil, which addresses and fulfils the first objective of the present study. A previous study has shown a steeper distance–decay relationship of fungal communities in rhizosphere than in bulk soil in soybean fields across China (Zhang et al., 2018). The discrepancy between the results of the previous study and the present study could be attributed to our research being based on small-scale *R. pseudoacacia* plantations, where pH is the major factor driving soil microbial (bacterial and fungal) community structure in the rhizosphere and bulk soil (Fig. S4A—D).

The impact of soil pH on soil microbial communities is widely documented (Jackson-Blake et al., 2012; Lauber et al., 2009; Zarraonaindia et al., 2015). For example, Hartman et al. (2008) reported that *Acidobacteria* and *Actinobacteria* abundances changed along a pH gradient at the phylum level; hence, they proposed pH as the best predictor of bacterial community variation. However, Lauber et al. (2009) considered soil pH a factor with indirect influence, because other soil properties (e.g., nutrient availability, ion exchange, and organic carbon content) are directly or indirectly affected by pH, which in turn influences soil microbial community structure. Therefore, pH may be a key soil variable in the structuring of soil microbial communities. In addition, pH drives bacterial system development and exerts stress on both community survival and selection beyond tolerable limits (Tripathi et al., 2018). We also inferred that pH influenced soil community structure in different soil compartments following statistical analyses. Collectively, the findings enhance our understanding of the environmental drivers of rhizosphere bacterial and fungal community structure during *R. pseudoacacia* forest restoration, although there is still a lack of specific observable direct evidence. Furthermore, microbial responses to soil factors are divergent between bacteria and fungi, as reported at the global scale (Bahram et al., 2018). The rhizosphere soil fungal community, which comprises vital consumers of labile organic compounds, is influenced by carbon flow from plants (Hannula et al., 2012). In addition, fungal community diversity can be explained based on SOM (Hannula et al., 2017; Holtkamp et al., 2008; Malik et al., 2016).

4.2. Assembly processes of both bulk soil and rhizosphere microbiomes

Mechanisms driving soil microbial community assembly dynamics have been reported in other studies (Guo et al., 2019; Liu et al., 2018a, 2018b; Xiao et al., 2021; Xu et al., 2019; Zhou and Ning, 2017). Here, we

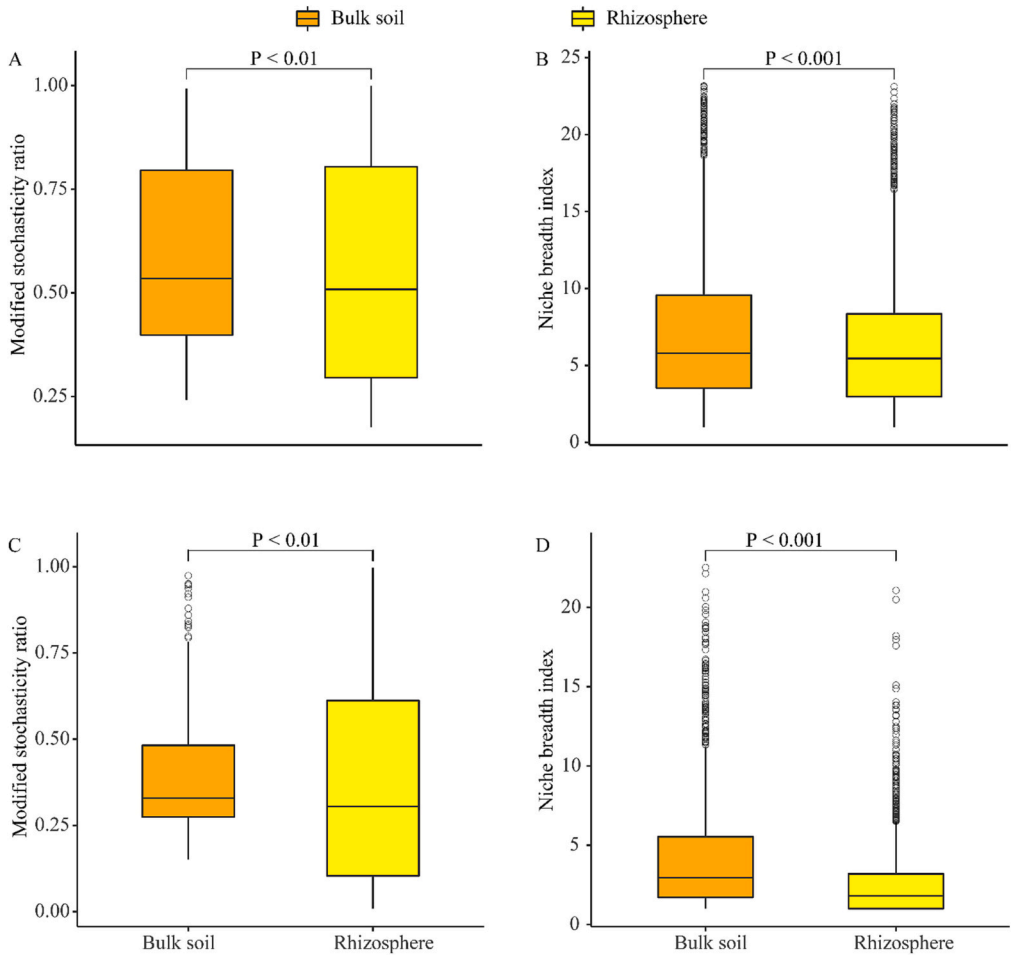

Fig. 3. Significant differences in the modified stochasticity ratio and niche breadth index of microbial communities between the rhizosphere and bulk soil (A: Bacteria, C: Fungi, and $P < 0.01$, Wilcox test). Significant differences in niche breadth were also observed between bacteria and fungi (B, D, and $P < 0.001$, Wilcox test).

identified the processes driving bacterial and fungal community assembly in different niche spaces during *R. pseudoacacia* forest restoration. We demonstrated that (1) the fungal community was governed more by environmental filtering (a deterministic process) than the bacteria community, (2) bacteria and fungi had higher migration ratio in the bulk soil compartment than in the rhizosphere compartment; and (3) the bacterial community was less limited by dispersal when compared with the fungal community.

Using a neutral model, we revealed that the effects of stochastic processes on bacterial community assembly were greater than the effects on fungal community assembly during long-term *R. pseudoacacia* restoration. An interesting finding was that deterministic processes had remarkable effects on fungal community assembly in the rhizosphere (Fig. 2A-D). In addition, our results suggested that rhizosphere fungi were limited by dispersal. To explain the observation above, we quantified the community-level habitat niche breadth and found that compared with fungi, soil bacteria had substantially broader niche breadths. Previous studies have reported that organisms with a wider niche breadth incur lower environmental filtering, so that they are more suitable for homogeneous environments and less influenced by deterministic processes (Pandit et al., 2009; Jiao et al., 2020). Furthermore, we observed considerably higher migration ratios for bacterial communities than for fungal communities based on the neutral community model (Fig. 2). This result could be attributed to 'body size'; that is, larger-sized microbes respond more easily to spatial distribution patterns than smaller-sized microbes (Liu et al., 2015).

We also observed low migration ratios, along with low MST and *Bcom* values for fungal communities in the bulk soil, consistent with the results of the neutral community model (Fig. 2C-D; Fig. 3D-D). One potential reason for the trend is that weak environmental selection could have enhanced dispersal and ecological drift (Chen et al., 2021), so that the relative contributions of deterministic process were lower for bacterial communities than for fungi. Another explanation is that microbes whose niche breadths are wider have greater metabolic plasticity and are less affected by deterministic processes (Pandit et al., 2009). While environmental variation and selection are deterministic processes in the absolute sense (Jiao et al., 2020), dispersal limitation can be either deterministic or stochastic and therefore may not be adopted as the sole evidence for deterministic processes (Zhou and Ning, 2017). Another study reported that in systems with less environmental variation or with environmental generalists, stochastic processes may overwhelm deterministic processes (Wang et al., 2013). In summary, the *R. pseudoacacia* plantation environment is less selective for bacteria than for fungi, thus increasing the migration ratio of soil bacterial communities.

4.3. Influence of niche filtering on microbial co-occurrence patterns during vegetation restoration

Although a microbial network cannot indicate the real microbial co-occurrence patterns in situ, it is still useful for understanding how microbial taxa coexist in environmental samples (Barberan et al., 2012) and for identifying their potential biotic interactions, habit affinities, or common physiological traits (Proulx et al., 2005). We observed that microbial network complexity increased from the bulk soil to

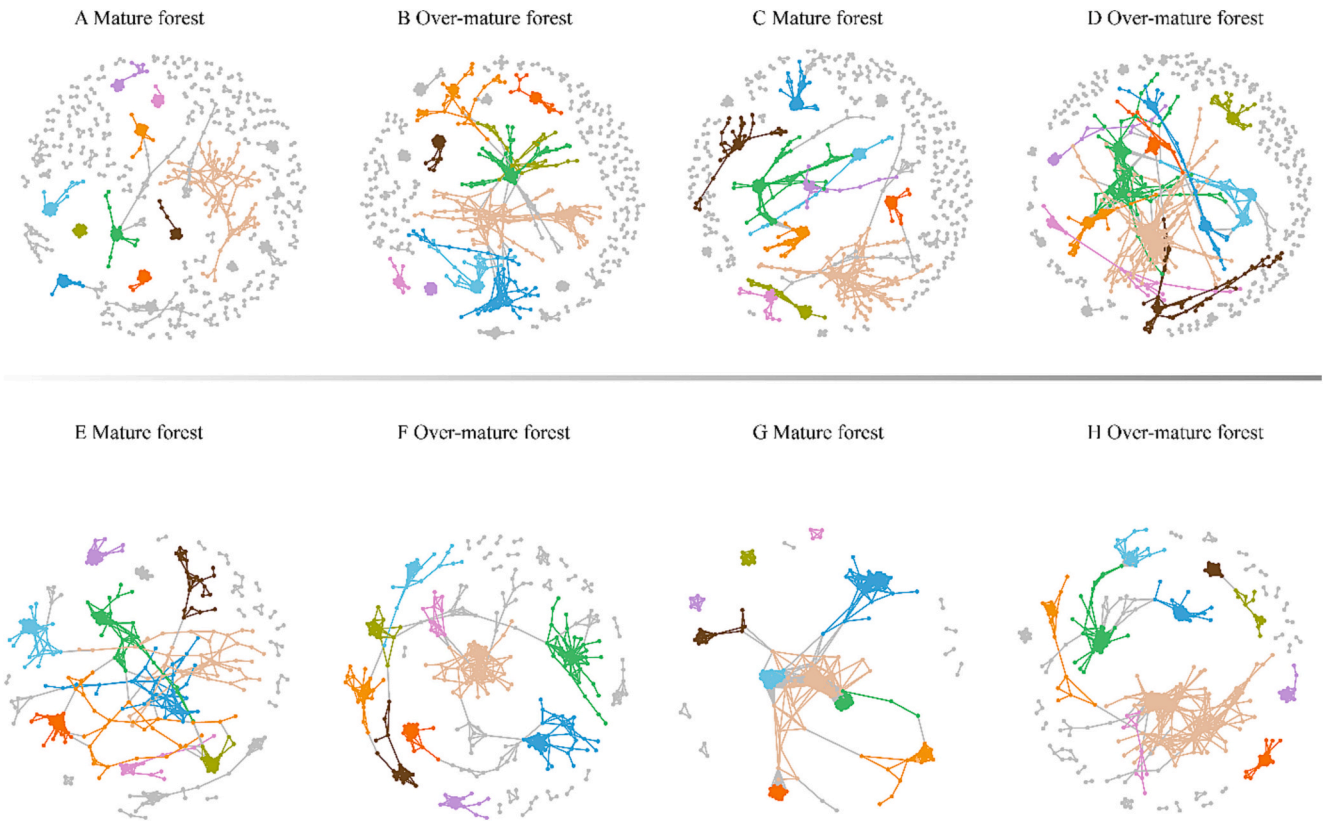

Fig. 4. Soil microbial network complexity based on time series and niche compartment (Spearman's $r > 0.07$, significant at $P < 0.05$). The top 10 modules that contain the largest number of nodes are selected and presented in different colors, and the remaining modules are colored in gray. (A–H) Microbial networks at different plant development sages. Panels A–D depict bacterial network development along the time series; the first pair shows the bulk soil bacterial network and the second pair shows the rhizosphere soil bacterial network. Panels E–H depict fungal community network development along the time series; the first pair shows the bulk soil fungal community network and the second pair shows the rhizosphere soil fungal network. Mature forest consisted of 15- and 25-year-old stands; over-mature forest stands were 35 and 45 years old.

rhizosphere soil (Fig. 2, Table S3); however, in the same direction, the enriched ASVs decreased. The high clustering coefficient in the rhizosphere indicated that highly connected ASVs, whose network topology shifted along with plantation development, were grouped in the neighborhood and clustered together (Barberan et al., 2012). The rhizosphere microbial network properties were unlike those of the bulk soil microbial networks, suggesting different niche compartments led to distinct bacterial and fungal co-occurrence patterns. The strength of microbial community differentiation is not only influenced by biotic factors (e.g., plant properties, microbial niche preference, and plant–microbe interactions) but also abiotic factors (e.g., nutrients and pH) (Leibold and McPeek, 2006; Shi et al., 2016; Guo et al., 2019; Fernandez-Baca et al., 2021). Our results indicate that the differentiation in bacterial and fungal communities was responsive to niche compartment. Soil microbes formed specific clusters and potentially interacted in the rhizosphere because of increasing host selection pressure.

Some studies have reported that rhizosphere effects alter microbial community composition (Li et al., 2022), with root exudates as key mediators of rhizosphere microbiome assembly for plants (Chaparro et al., 2014). Plant root exudates can meet the nutrient demands of certain microbes, inhibit the growth of pathogenic microbes, and recruit potentially beneficial microbes into the rhizosphere (Chaparro et al., 2014; Yuan et al., 2018a; Yuan et al., 2018b; Zhao et al., 2021). Consequently, host selection and root metabolic activity may cause microbial community differentiation between the rhizosphere and bulk soil. Moreover, we observed that both forest age and niche compartment influenced rhizosphere microbial community structure, suggesting rhizosphere microbiomes were shaped by both host plant growth/development and selection. This is plausible, considering roots are supposed to be the interface between host plant and the belowground environment (Bulgarelli et al., 2013; Philippot et al., 2013; Liu et al., 2018a, 2018b). Furthermore, our study showed that the network of rhizosphere microbial communities was more complex than bulk soil microbial network (Fig. 4, Table S3), which implied greater species interaction and niche sharing potential in the rhizosphere soil (Shi et al., 2016). The difference in network complexity also illustrates a fundamental difference between rhizosphere microhabitat and bulk soil. With regard to the bacterial community structure, despite no significant diversity differences between different niche compartments over time, its network complexity was greater in the rhizosphere soil than in the bulk soil. Fungal diversity, by contrast, decreased considerably from the bulk soil to the rhizosphere soil, yet fungal network complexity increased correspondingly. Overall, the results suggest that microbial diversity cannot comprehensively capture community-level organizational relationships (Shi et al., 2016) and microbial species have distinct ecological preferences (Jiao et al., 2021).

The results of our source tracking analysis (Fig. 5E) indicated that the rhizosphere microbial communities were mainly derived from the bulk soil compartment, being enriched and filtered in the rhizosphere compartment. We also observed that unique and common microbial taxa occurred in both the rhizosphere and bulk soil compartments. These results are supported by a previous study that reported that the rhizosphere microbiome primarily originates from bulk soil (Ridder-Duine et al., 2005). Understanding the potential source and enrichment process is key to exploring and predicting plant–soil–microbiome interactions (Edwards et al., 2015; Bulgarelli et al., 2012). Despite existing knowledge on plant selection of microbial taxa colonizing the

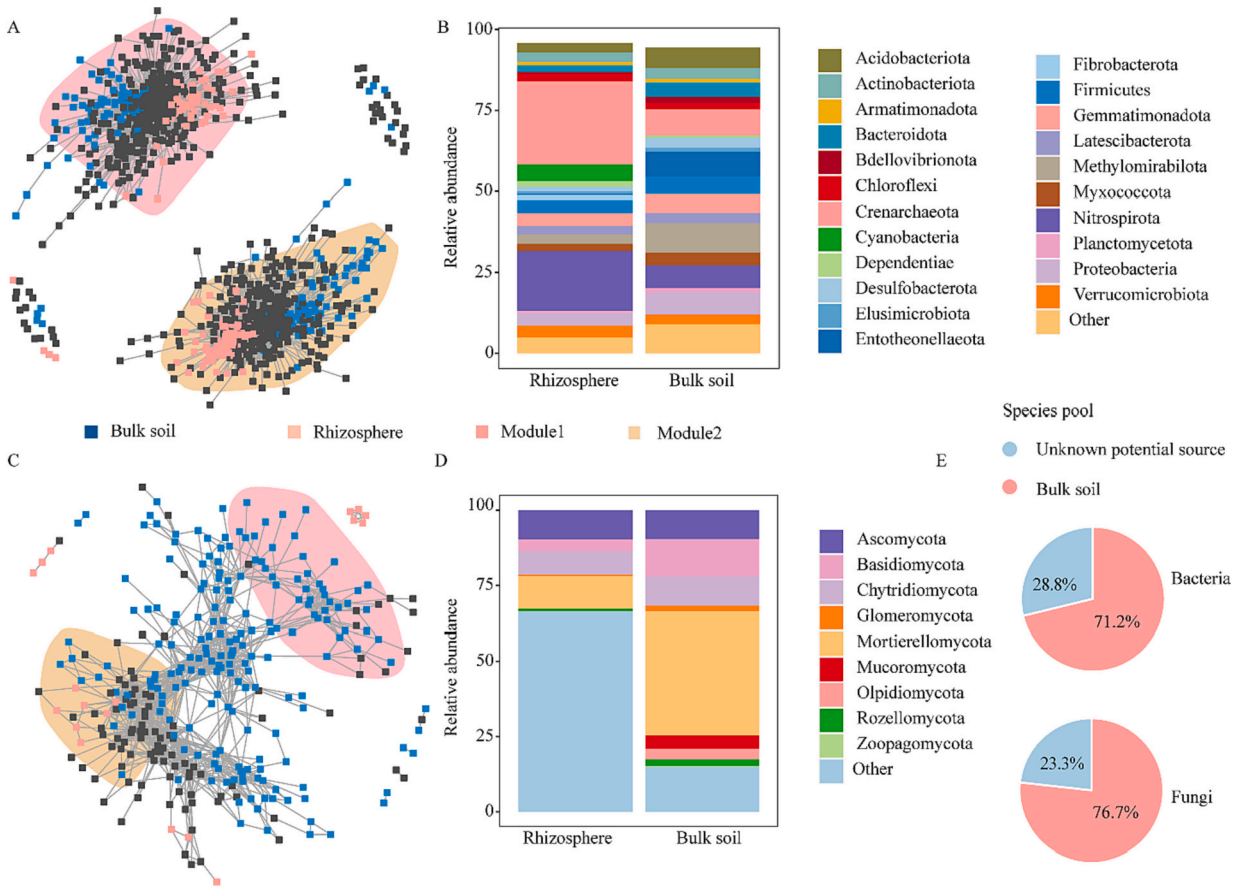

Fig. 5. Microbial co-occurrence (Spearman's r > 0.07, significant at P < 0.05) and enrichment in different niche compartments. (A–D) Bacterial and fungal co-occurrence patterns and common/unique taxa between the rhizosphere and bulk soil. (E) Potential source of rhizosphere microbes.

Table 1
Topological features of microbial co-occurrence networks both in the rhizosphere and bulk soil (Spearman's r > 0.07, significant at P < 0.05).

	Connection	Edge	Average. Degree	Cluster coefficient	Sig_ASVs^N	Sig_ASVs^R
Bacteria	0.03	16,506	30.29	0.52	1137	896
Fungi	0.03	1377	8.80	0.41	469	117

Node: N indicates bulk soil; R indicates rhizosphere soil.

rhizosphere (Bulgarelli et al., 2012; Hu et al., 2018; Yuan et al., 2018b), we still know little about the process of enrichment of forest soil microbiomes. Our work therefore advances the understanding of soil microbiome assembly in plantation forests by systematically evaluating the influence of host plant age and multiple environmental factors on soil bacterial and fungal community structures across different niche compartments.

5. Conclusions

The present study explored previously undocumented microbial community assembly in distinct soil niche compartments in the course of *R. pseudoacacia* forest restoration. Deterministic processes dominated rhizosphere bacterial community assembly compared with stochastic processes, and the relative importance of deterministic processes in fungal community assembly was greater in the rhizosphere than in the bulk soil. Niche breadth played a key role in microbial community assembly, whereas microbial network complexity became more pronounced over the course of vegetation restoration, with distinct topological features between the two niche compartments. The degree of bacterial or fungal community differentiation varied based on the niche compartment, with greater potential for microbial interactions and niche sharing observed in the rhizosphere. The differences in microbial networks suggest variation in soil biological properties at different plant development stages. Accordingly, our findings highlight the need to characterize microbial community organization, instead of simply focusing on change in microbial diversity. We propose that microbial coexistence and microbe–host interactions are crucial for microbial community assembly, ecological restoration, and ecosystem balance.

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Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Microbial raw data and all raw laboratory testing data used to support the findings of this study are available from the corresponding author upon request.

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