



Broad environmental adaptation of abundant microbial taxa in *Robinia pseudoacacia* forests during long-term vegetation restoration

Jiajia Li^a, Miaochun Fan^b, Zhenhao Wei^a, Kang Zhang^a, Xing Ma^b, Zhouping Shangguan^{a,*}

^a State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi, 712100, PR China

^b Department of Grassland Science, Northwest A&F University, Yangling, Shaanxi, 712100, PR China

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ABSTRACT

Vegetation restoration has significant impacts on ecosystems, and a comprehensive understanding of microbial environmental adaptability could facilitate coping with ecological challenges such as environmental change and biodiversity loss. Here, abundant and rare soil bacterial and fungal communities were characterized along a 15–45-year chronosequence of forest vegetation restoration in the Loess Plateau region. Phylogenetic-bin-based null model analysis (iCAMP), niche breadth index, and co-occurrence network analysis were used to assess microbial community assembly and environmental adaptation of a *Robinia pseudoacacia* plantation under long-term vegetation restoration. The drift process governed community assembly of abundant and rare soil fungi and bacteria. With increasing soil total phosphorus content, the relative importance of drift increased, while dispersal limitation and heterogeneous selection exhibited opposite trends for abundant and rare fungi. Rare soil fungal composition dissimilarities were dominated by species replacement processes. Abundant microbial taxa had higher ecological niche width and contribution to ecosystem multifunctionality than rare taxa. Node property values (e.g., degree and betweenness) of abundant microbial taxa were substantially higher than those of rare microbial taxa, indicating abundant species occupied a central position in the network. This study provides insights into the diversity and stability of microbial communities during vegetation restoration in Loess Plateau. The findings highlight that abundant soil fungi and bacteria have broad environmental adaptation and major implications for soil multifunctionality under long-term vegetation restoration.

Authors' contributions

Li Jiajia contributed to the concepts, design, and definition of intellectual content, literature search, data acquisition, data analysis, and manuscript preparation. Wei Zhenhao, Kang Zhang and Ma Xing provided assistances for sampling and data acquisition. Miaochun Fan assisted with typesetting. Zhouping Shangguan performed a manuscript review and approval of the version to be submitted.

1. Introduction

Forest restoration and afforestation of arable land are the most widely applied restoration activities for natural ecosystem biodiversity and function (Bongers et al., 2021; Zhang et al., 2021). In long-term vegetation restoration, biotic (e.g., plant) and abiotic factors (e.g., pH) indirectly or directly influence soil microbial community structure (Jiao

et al., 2018; Li et al., 2022, 2023a,b). Soil microbes exhibit skewed distribution, with rare species coexisting with abundant species locally (Jia et al., 2018). Recently, some studies have reported the distribution of rare and abundant species at the macro and micro scales (e.g., biogeographical patterns, soil aggregates) (Jiao et al., 2017; Zheng et al., 2021). However, distribution rare and abundant taxa under long-term vegetation restoration are unclear.

Soil microbes play crucial roles in the maintenance of ecosystem functionality (e.g., carbon cycling and nitrogen cycling) (Coban et al., 2022; Liu et al., 2020; Prescott and Grayston, 2013; Wang et al., 2017). The roles of abundant and rare taxa are different in different ecosystems. Rare bacterial taxa such as *Cyanobacteria* and *Glomeromycota* have been reported to be the major contributors to soil multifunctionality in agriculture ecosystems (Chen et al., 2020). In the Yellow River Delta, rare taxa exhibit broader environmental adaptation than abundant taxa, and play important roles in reforestation (He et al., 2022). Following

* Corresponding author. State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, PR China.

E-mail addresses: jiajia190203@163.com (J. Li), shangguan@ms.iswc.ac.cn (Z. Shangguan).

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external disturbance, microbial community structure is altered to adapt to environmental change. The numbers of taxa with high tolerance to stress typically increase their numbers to maintain community function under disturbance, and such groups are reportedly relatively stable in microbial communities (Xun et al., 2021; Hector and Bagchi, 2007). These relatively stable species are closely linked with ecosystem multifunctionality via species interactions, resource utilization, or other processes (Xue et al., 2022). Therefore, a comprehensive understanding of microbial community stability could facilitate cope with questions of sustainable development of ecosystem.

Forests have broad temporal dynamic states. Accumulation of disperse and selective pressure over time alter the directions and strengths of deterministic and stochastic processes (Dini-Andreote et al., 2015). In the early stages of subtropical forests, the deterministic processes dominate soil microbial community assembly, while stochastic processes play important roles in microbial community assembly in later stages (Liu et al., 2021). Microbial assembly processes (deterministic processes and stochastic processes) are a central issue in microbial ecology (Zhou et al., 2013; Stegen et al., 2013). Understanding of the assembly process is precondition for the knowing of the mechanism of community stability. However, the assembly processes of abundant and rare microbes remain uncertain during long-term vegetation restoration.

The Loess Plateau has a semi-humid and semi-arid climate with severe soil erosion and ecological fragility. *Robinia pseudoacacia* plantation plays an important role in ecological restoration in this region (Xu et al., 2019). However, there are concerns over the ability of reforestation using monoculture plantations to contain or reverse biodiversity loss (Zhang et al., 2021). Microbial diversity and richness are the results of selection for environmental adaptation (Jiao and Lu, 2020; Xue et al., 2022). Species with broader niches would obtain more resources and have an advantage over the competition (Jiao and Lu, 2020). High-throughput throughput sequencing was used to uncover the environmental adaptation and composition dissimilarity of abundant and rare microbial taxa in the present study. The experimental site was set up in Yongshou County, Shaanxi Province, which has a relatively complete restoration sequence of *R. pseudoacacia* vegetation over a period of 45 years. The aims of the present study were to investigate (i) the environmental adaptability and roles of abundant taxa and rare taxa in *R. pseudoacacia* plantation during long-term restoration and (ii) the stability of abundant and rare subcommunities. The findings of the present study could facilitate the prediction of the responses of soil microbial communities to forest development, in addition to enhancing our understanding of the mechanisms of maintenance of soil microbial diversity and functional stabilization in forest ecosystems.

2. Material and methods

2.1. Experimental site

The study area was located at the Huaiping Forest Farm, Yongshou County, Shaanxi Province, China (34°12′–34°50′N, 108°56′–108°51′W). The mean air temperature and annual precipitation were 11.3 °C and 569.9 mm, respectively. *Robinia pseudoacacia* is the dominant tree species. Samples were collected from four sites under restoration over different rehabilitation years (15, 25, 35, and 45 years, hereafter referred to as Y15, Y25, Y35, and Y45, respectively), and a farmland with wheat adjacent to the restoration sites (Y0). Wheat is the major crop in the study area. Wheat was grown under a single cropping system.

2.2. Soil sampling and ecosystem multifunctionality index calculation

Plots (20 × 20 m) with six replicates were selected for each forest age group to collect soil samples in July 2020. Six soil cores (0–20 cm depth) were collected along an S-shaped pattern and mixed into one soil sample. Each soil core was passed through a 2-mm sieve (Φ200 × 50 mm, 10 mesh) to remove roots and stones. A part of the samples was stored at

4 °C for determination of microbial biomass, and another part was used for determination of soil physical and chemical properties. Samples for molecular analyses were stored at –20 °C. Please see the Supplementary Materials (S1) for the soil physicochemical property measurement methods. Soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), Soil ammonium-nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N), microbial biomass nitrogen (MBN), microbial biomass carbon (MBN), dissolved organic carbon (DOC), dissolved organic nitrogen (DON) were used to calculate the soil multifunctional index after being normalized using the *decostand* function in R v4.3.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The standardized ecosystem functions were then averaged to obtain a multifunctional index (Jiao et al., 2019).

2.3. Microbial bioinformatics analysis

Soil total DNA was extracted from 0.5-g soil samples. DNA purity and concentration were determined on 1% agarose gels (Muarry and Thompson, 1980). The internal transcribed spacer (ITS1) regions of fungal ITS rRNA genes were amplified using the primers ITS5-1737 F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTCGCTCTTC ATCGATGC), whereas the V4–V5 regions of bacterial 16 S rRNA genes were amplified using the primers 515 F (GTGCCAGCMGCCGCGG) and 907 R (CCGTCAATTCMTTTRAGTTT). Sequencing was conducted on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). 16s rRNA and ITS gene sequences were analyzed using the QIIME2 pipeline (2020.8) (Callahan et al., 2016). The DADA2 module of QIIME2 software (<https://docs.qiime2.org/2020.8/install/native/>) was used to trim and denoise the sequence data, which were used to infer Amplicon Sequence Variants (ASVs). Assignment of representative sequences to taxonomic lineages was performed according to the method of Chen et al. (2021).

Here, ASVs were divided into “rare” and “abundant” categories, which correspond to relative abundances of >0.1% and <0.01% of the total sequence, respectively (Jiao and Lu, 2020; Li et al., 2019). Microbial diversity, niche breadth index, and network analysis are provided in the Supplementary Materials. β diversity reflected changes in microbial composition during long-term vegetation restoration, which may be the result of species replacement or richness difference (Shen et al., 2020). Species replacement indicates species replacement in different communities, whereas richness difference is caused by species loss or gain (Stéphane et al., 2017). Microbial compositional dissimilarities (β diversity) among groups were partitioned into replacement and richness difference components (Jaccard dissimilarities) using the *adespatial* package in R (Stéphane et al., 2017). Microbial community stability was estimated using average variation degree (AVD), which is calculated as described in Supplementary Materials (S3). Lower AVD indicated higher stability (Xun et al., 2021).

To infer the microbial assembly process, beta Net Relatedness Index (βNRI) and Raup-Crick Index (RCI) were calculated using the “iCAMP” package in R platform (Ning et al., 2020). βNRI >1.96 or βNRI <–1.96 indicated heterogeneous and homogeneous selection, respectively, |βNRI| ≤ 1.96 and |RCI| > 0.95 indicated dispersal limitation, |βNRI| ≤ 1.96 and RCI <–0.95 indicated homogenizing dispersal, |βNRI| ≤ 1.96 and RCI >0.95 indicated drift (Ning et al., 2020). Random Forest (RF) model analysis was also performed to evaluate the key environmental factors governing microbial community dissimilarity. The relationships between key environmental factors and ecological processes were analyzed via linear regression.

3. Results

3.1. The distribution of abundant and rare bacteria and fungi along a vegetation restoration chronosequence

Across the 30 samples, a total of 26,643 and 8367 ASVs were obtained for bacteria and fungi, respectively. Abundant soil bacteria and

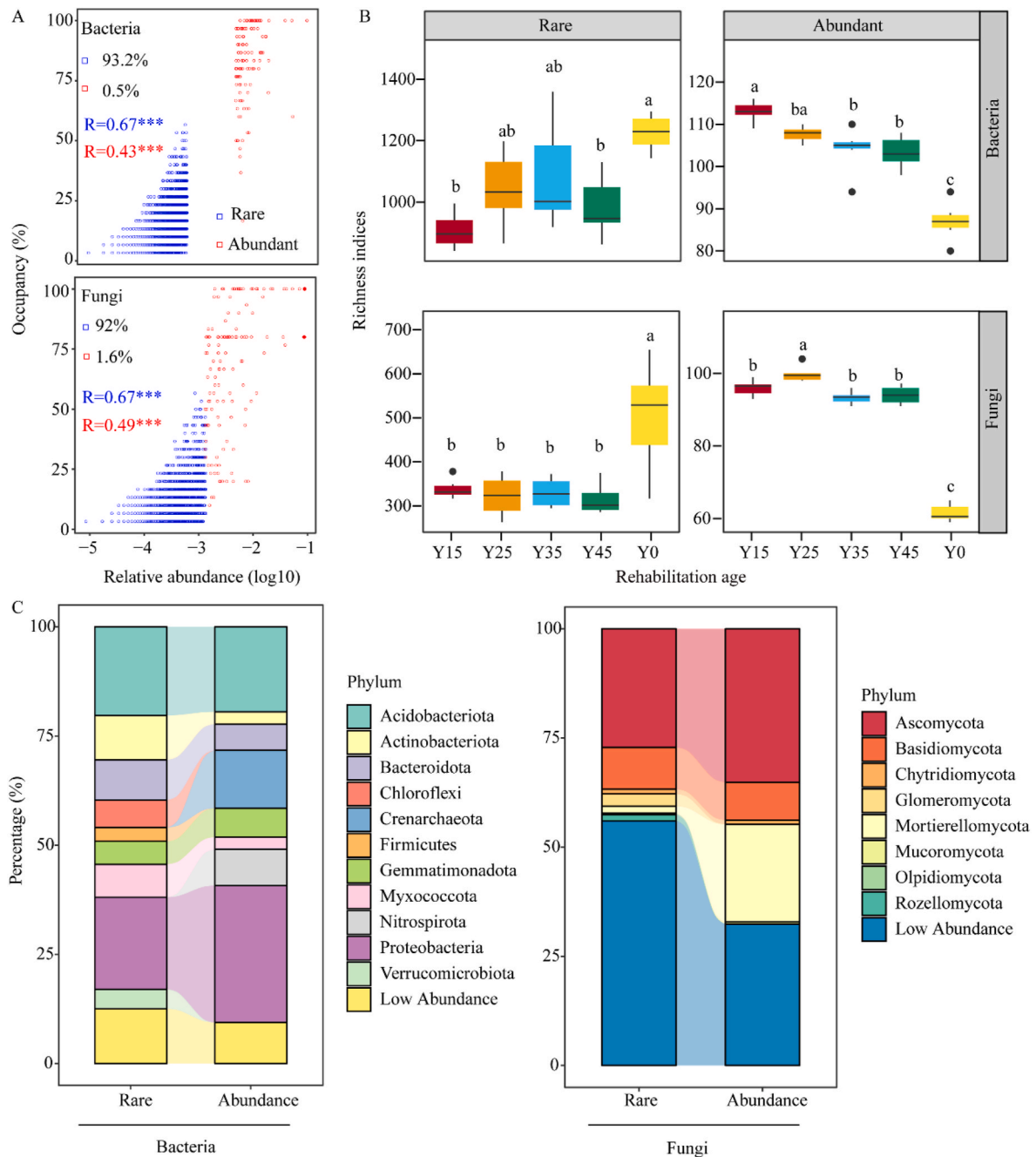


Fig. 1. Analysis of microbial structure. (A) Abundance-occupancy relationships of rare and abundant taxa, estimated via regression analysis. $***$, $P < 0.001$. (B) Richness index of rare and abundant taxa in each site. Y15, Y25, Y35, and Y45 represent 15, 25, 35, and 45 years, respectively. Wheat field was used as the control (Y0). (C) Dominate species composition of rare and abundant microbial sub-communities.

fungi have 98 and 50 shared ASVs during the long-term vegetation restoration, respectively (Fig. S1). Among the rare taxa, there were 26 fungal shared ASVs, and 127 bacterial shared ASVs (Fig. S1). Number of unique ASVs decreased over time under long-term vegetation restoration (Fig. S1). A very low proportion of the ASVs (Bacteria: 0.5%, Fungi: 1.6%, Fig. 1A) were identified as abundant taxa; about 90% of the ASVs were classified as rare taxa (Bacteria, 93.2%; Fungi, 92%, Fig. 1A). Abundant taxa had lower ASV richness than rare taxa in both bacteria and fungi (Fig. 1B). The richness index of abundant species (bacteria and fungi) in the cropland field was lower than that in forest land, and rare species exhibited an opposite trend (Fig. 1B). The compositions of bacterial and fungal sub-communities varied in the course of the vegetation restoration, according to the PCoA plots based on Bray-Curtis distances

(Fig. S2). Permutational Multivariate Analysis of Variance results showed that the influence of vegetation restoration on rare microbial community dissimilarity (Fig. S2, $P < 0.001$, $R_{Bacteria}^2 = 0.31$, $R_{Fungi}^2 = 0.32$) was less than that on abundant taxa (Fig. S2, $P < 0.001$, $R_{Bacteria}^2 = 0.84$, $R_{Fungi}^2 = 0.71$). At the phylum level, *Proteobacteria* and *Acidobacteriota* were the dominant taxa in abundant and rare bacteria. The rare fungi subcommunity was dominated by *Ascomycota* (27.1%), and the abundant fungi subcommunity was dominated by *Ascomycota* (35.1%) and *Mortierellomycota* (22.4%) (Fig. 1C).

Soil TP and pH were the main drivers of fungal and bacterial sub-community distribution, respectively, according to the results of RF analysis (Fig. S3). The dissimilarities in rare subcommunity compositional were primarily driven by species replacement processes,

Table 1

The table shows results of β diversity decomposition analyses. β diversity comparisons (using Jaccard similarity) for bacterial and fungal among all samples.

	Bacteria		Fungi	
	Rare	Abundant	Rare	Abundant
Species replacement processes	87%	16%	83%	12%
Richness difference processes	8%	9%	12%	23%

accounting for 87% and 83% of the variations for bacteria and fungi, respectively. Whereas richness difference process only contributed on average of 8% and 12%, respectively (Table 1). The relative contribution of species replacement to abundant taxa beta diversity was 16% and 12% in bacteria and fungi, respectively, whereas richness difference process contributed 9% and 23% on average, respectively (Table 1). In addition, the variation in ecosystem multifunctionality was explained by both abundant and rare microbial diversity (Bacteria: $R^2 = 71.2\%$, Fungi: $R^2 = 69.8\%$). Among them, the contribution of abundant taxa diversity to ecosystem multifunctionality was higher than that of rare taxa (Fig. 2A and B). Moreover, abundant taxa had a significantly broader niche breadth index compared to rare taxa (Fig. 2B–D).

3.2. Assembly processes of abundant and rare soil bacteria and fungi

Our study employed iCAMP analysis to infer microbial assembly process during long-term vegetation restoration. The findings revealed that drift process exhibited greater significance compared to other processes in both abundant and rare soil fungal and bacterial sub-communities (Fig. 3). Moreover, the importance of homogenizing

dispersal decreased from cropland soil to forest (Fig. 3). Linear regression was utilized to estimate the impact of environmental factors on microbial community assembly processes (Fig. 4). The importance of homogeneous selection within the bacterial community (abundant and rare taxa) exhibited a stronger correlation with soil pH ($R^2 = 0.18$, $P < 0.001$), while the rare bacterial taxa displayed a weak correlation with pH (Fig. 4A–B, $R^2 = 0.02$, $P < 0.001$). The relative influence of drift on the fungi community significantly increased with an elevation in soil TP, while heterogeneous selection displayed a contrasting trend (Fig. 4C–D, $P < 0.001$).

3.3. Co-occurrence network of abundant and rare microbial taxa

The entire bacterial co-occurrence network consisted of six major modules, with I–VI accounting for 21.22%, 20.78%, 19.07%, 15.4%, 10.12%, and 11.16% of the whole network, respectively (Fig. 5A). Conversely, in the fungal network, modules I–VI accounted for 18.58%, 15.71%, 13.68%, 13.01%, 12.67%, and 21.12% of the whole network, respectively (Fig. 5B). Six sub-modules were extracted from the whole network (bacteria and fungi) for rare taxa (Fig. S4). Three sub-modules of abundant bacteria were extracted from the whole bacterial network (Fig. S4B). In the cases of abundant fungi taxa, two sub-modules were extracted from the whole fungal network (Fig. S4C). The relationships between relative abundance of microbial network modules and ecosystem multifunctionality were used to infer the contributions of network modules. Bacterial module 2 (M2) and fungal module 5 (M5) were identified as important ecological clusters for ecosystem multifunctionality (Fig. 5). Moreover, the modules included more rare taxa (Fig. S4).

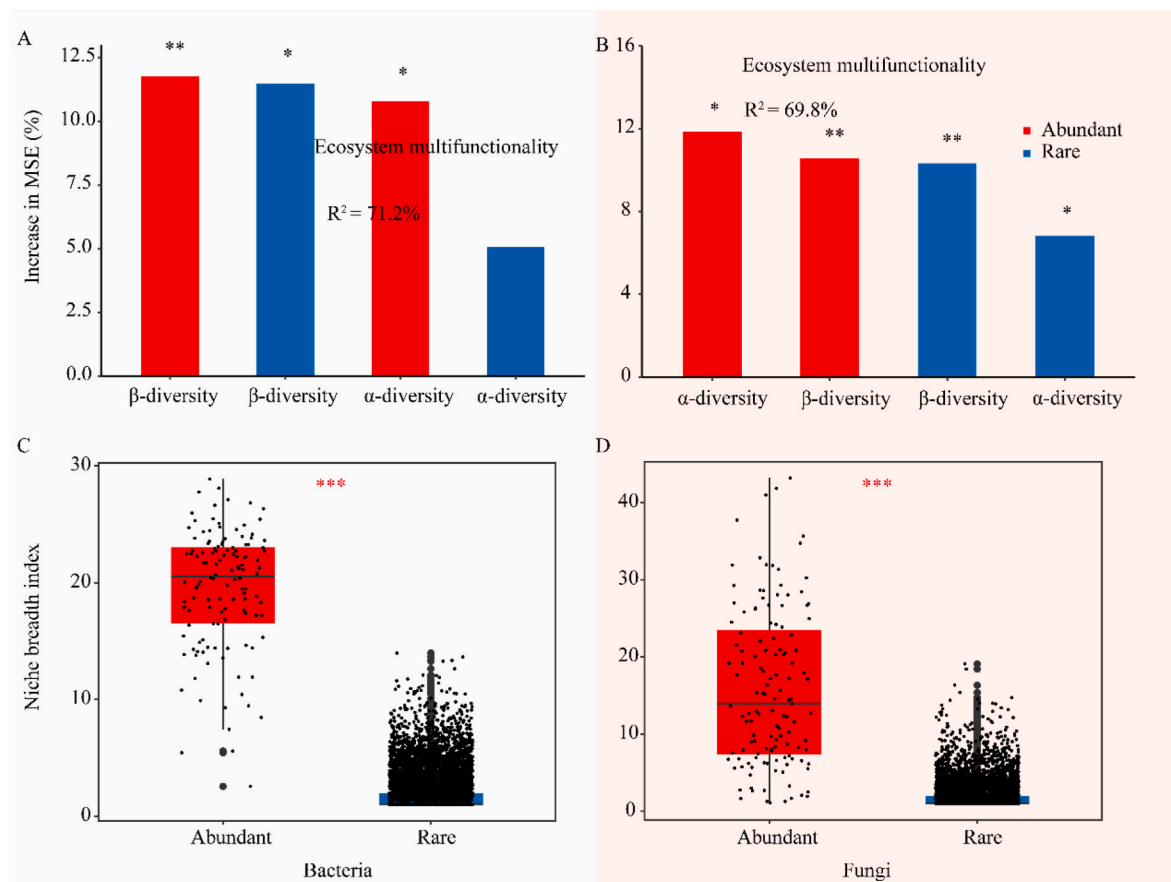


Fig. 2. Random forest analysis and niche breadth index calculation of rare and abundant taxa. (A–B) The contributions of rare and abundant taxa to ecosystem multifunctionality soil multi-nutrient index (Permutation test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (C–D) Difference of niche breadth index between abundant and rare taxa (Wilcox test *** $P < 0.001$).

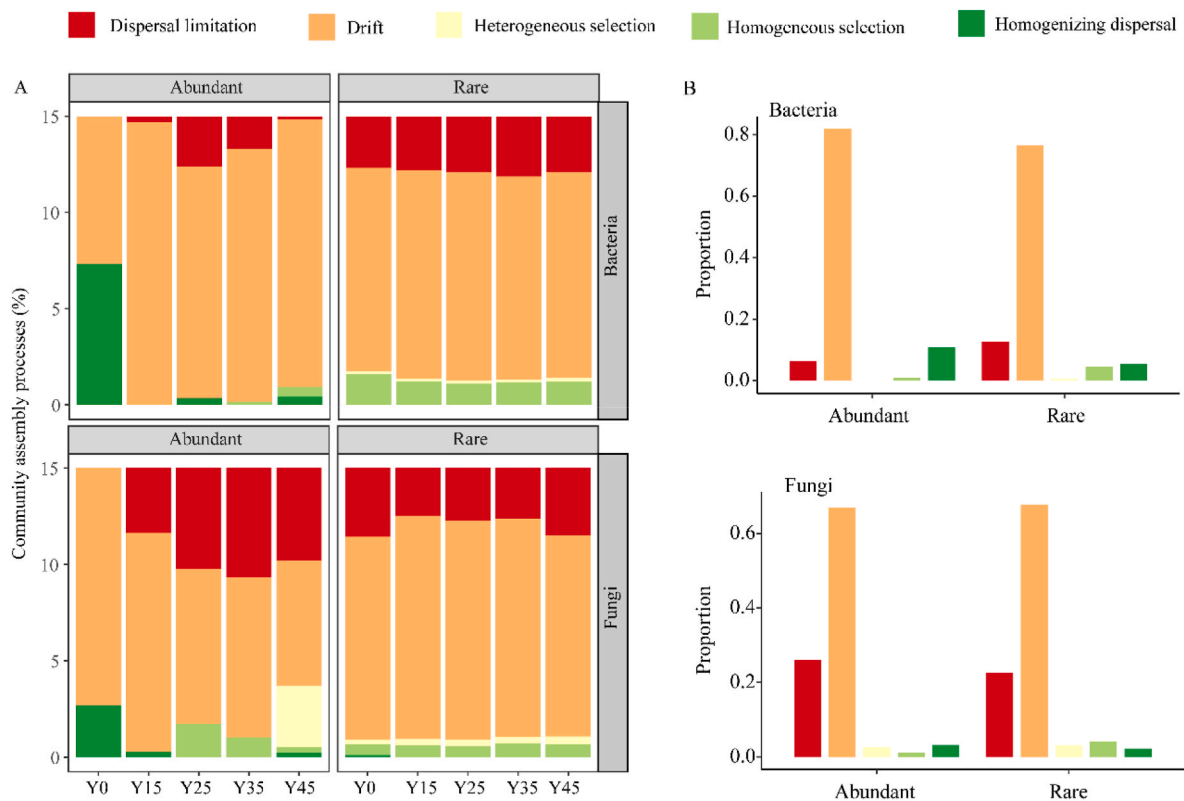


Fig. 3. Microbial assembly processes are detected from the values of two indices, beta Net Relatedness Index (β NRI) and Raup-Crick Index (RCD). (A) The relative importance of different ecological processes under long-term vegetation restoration. (B) The relative importance of different ecological processes in abundant and rare microbial assembly processes.

The interaction co-occurrence networks between rare and abundant community were constructed based on Spearman's rank correlation. Rare taxa had 1934 nodes and abundant taxa had 108 nodes (Fig. 6A). In the fungal network, rare taxa had 470 nodes, and abundant taxa had 122 nodes (Fig. 6C). The node properties (e.g., degree, betweenness, eigenvector, and closeness centrality) were compared to assess the relative importance of each node in the network (Fig. 6B and D). Node property values in the rare bacterial community were significantly lower than those in the abundant bacterial community (Fig. 6B), with similar results observed in fungi (Fig. 6D). As for bacteria, closeness coefficients for abundant subpopulations were substantially higher than those for rare subpopulations (Fig. 6B), which was not the case in fungi (Fig. 6D). Microbial community AVD exhibited no significant changes from Y15 to Y45. Moreover, the AVD values for rare subcommunities (bacteria and fungi) were comparatively lower than those observed for abundant subcommunities (Table 2).

4. Discussion

4.1. Abundant and rare sub-community compositional dissimilarities and the ecological drivers

Microbial community structure is an integrated indicator for assessing soil and ecosystem characteristics (Banning et al., 2011). The number of unique ASVs decreases with increasing stand age, suggesting that microbial community composition changes along vegetation restoration time series. In the present study, the rare subcommunity exhibited higher α -diversity and β -diversity than the abundant subcommunity, suggesting difference in species distribution (Fig. 1, Fig. S2). A significant and positive relationship was observed between species abundance and occupancy in the present study (Fig. 1A), indicating the influence of the balance between birth and death rate on

species distribution (Pedrós-Alió, 2012). Furthermore, soil pH and TP governed bacterial and fungal community dissimilarity, respectively (Fig. S3). In a study carried out on a mountain, soil pH was also the major factor influencing bacterial diversity, whereas mean annual temperature was the major predictor of fungal diversity (Shen et al., 2020). At the biogeographical scale, soil pH is reportedly a major factor influencing bacterial community compositional dissimilarity, because soil pH interacts with numerous other soil variables (Fierer and Jackson, 2006). In addition, we observed that soil TP was the most important predictor of fungal composition variance, which could be attributed to habitat preferences of species (Isabwe et al., 2022). Fungi are more sensitive to nutrient addition than bacteria; therefore, fungal community changes under long-term N and P addition are governed by soil P content in tropical montane forest soil (Ma et al., 2023). Previous studies have reported that fungi have a broader optimal growth pH range than bacteria based on pure culture and gene sequencing studies (Nevarez et al., 2009; Rousk et al., 2010). Therefore, it is reasonable that fungal composition dissimilarity is less responsive than bacteria to local-scale shifts in soil pH.

In the present study, average variation degrees for abundant communities were higher than those in rare communities, indicating relative stable rare microbial communities. The relative stability of rare taxa were the results of combined effects of various exogenous factors (e.g., soil factors and climate factors) as well as endogenous factors (e.g., biological interactions in microbial communities) (Hastings, 2010). We observed rare taxa composition dissimilarity was dominated by species replacement processes, which indicated that rare species were more vulnerable to replacement by species during vegetation restoration (Storch et al., 2018; Wang et al., 2021). Slight decreases in rare taxa abundance could lead to their extinction (Pedrós-Alió, 2006). But it is undeniable that rare taxa as microbial seed banks have potential importance for community stability and function (Jiao et al., 2017;

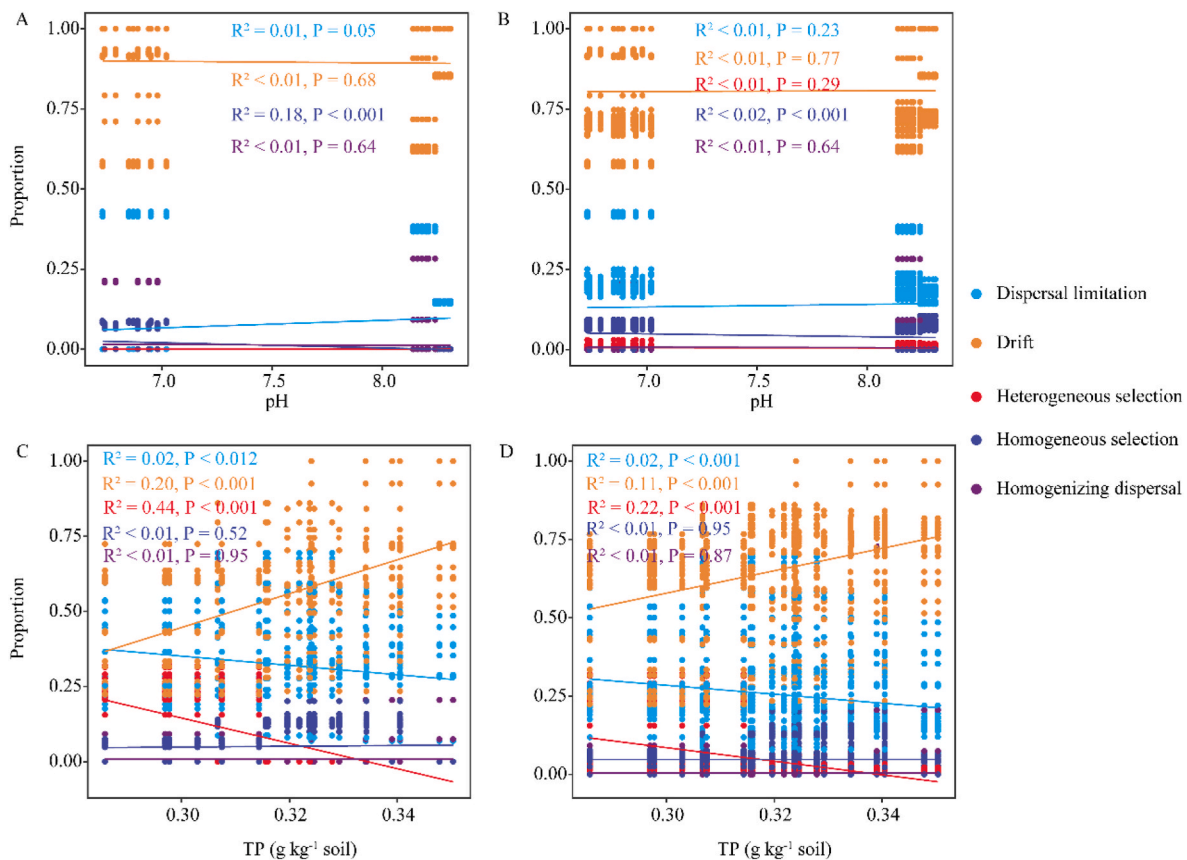


Fig. 4. Relationship between soil factor and microbial assembly processes. Linear regression models and associated correlation coefficients are provided in each panel (A. abundant bacteria, B. rare bacteria, C. abundant fungi, C. rare fungi).

Liang et al., 2020). Therefore, the research of rare taxa should be further explored in future work.

4.2. Niche breadth and assembly mechanism of abundant and rare subcommunities

According to the results of the present study, abundant taxa had broader niche breadths than rare taxa, suggesting abundant taxa had broader environmental adaptation under the long-term vegetation restoration (Fig. 2). Similar observations have been made in a soybean field and in cadmium-contaminated soil obtained from abandoned farmland (Xu et al., 2021; Zhang et al., 2018). Abundant species occupy a broader niche, competitively exploit resources, and are highly adaptive (Jiao et al., 2017); conversely, rare species with narrower niche breadths, face intense competition from abundant communities and are more vulnerable to environmental selection pressures (He et al., 2022; Wan et al., 2021; Yang et al., 2022).

Furthermore, our results showed that drift governs soil bacterial and fungal community assembly. Soil TP and pH primarily drove bacterial and fungi community assembly processes, respectively. The homogeneous selection varied with soil pH in abundant bacterial taxa, indicating that soil pH governs homogeneous selection in abundant bacterial sub-communities (Fig. 4A). Previous studies have suggested that soil pH mediates the balance between stochastic processes and deterministic processes along a glacier chronosequence and at the continental scale (Tripathi et al., 2018; J.M. Wang et al., 2021), highlighting the effect of soil pH on microbial assembly processes at multiple scales (Luan et al., 2022). Although extreme pH conditions would exert more stringent limits on survival and fitness, imposing strong selective pressures, pH conditions close to neutral lead to phylogenetically less clustered with more stochasticity (Tripathi et al., 2018). This explains why more

stochasticity occurred in bacterial community assembly processes under long-term vegetation restoration.

The relative influence of heterogeneous selection (deterministic processes) was negatively correlated with soil TP both in the rare and abundant fungal taxa (Fig. 4). However, the relative influence of drift processes (stochastic processes) increased gradually with an increase in soil TP content (Fig. 4). The results suggested that soil TP mediates the balance between stochastic and deterministic processes in fungal sub-community assembly. Fungi exhibit more sensitive responses to nutrient availability than bacteria (Ma et al., 2023), which could be explained by nutrient limitation and differences in microbial energy metabolism (Cui et al., 2018, 2019). Abundant taxa and rare taxa comprise the total biodiversity in the ecosystem, and abundant taxa exhibit faster growth and more active predation than rare taxa (Pedrós-Alió, 2006). Therefore, abundant taxa exhibited broader niche breadth and higher variance degree in the present study. In a previous study, increasing soil available nutrient decreased environmental selection pressure, resulting in the dominance of stochastic processes surpassing that of the deterministic processes (Wang et al., 2013). Furthermore, microbes exploit available resources and adapt to the environment through stochastic dispersal (Barberán et al., 2014; Jousset et al., 2017). Available soil nutrient concentrations might enhance microbial metabolic potential, and in turn, reduce environmental selection for microbes (Cui et al., 2019). Therefore, stochastic processes dominated abundant and rare soil bacterial and fungal assembly in the present study.

4.3. Network centrality of abundant microbial taxa

Unravelling the interactions of microbial taxa (abundant and rare) could facilitate the understanding of the complex structures of resident microbial communities, in addition to the ecological principles guiding

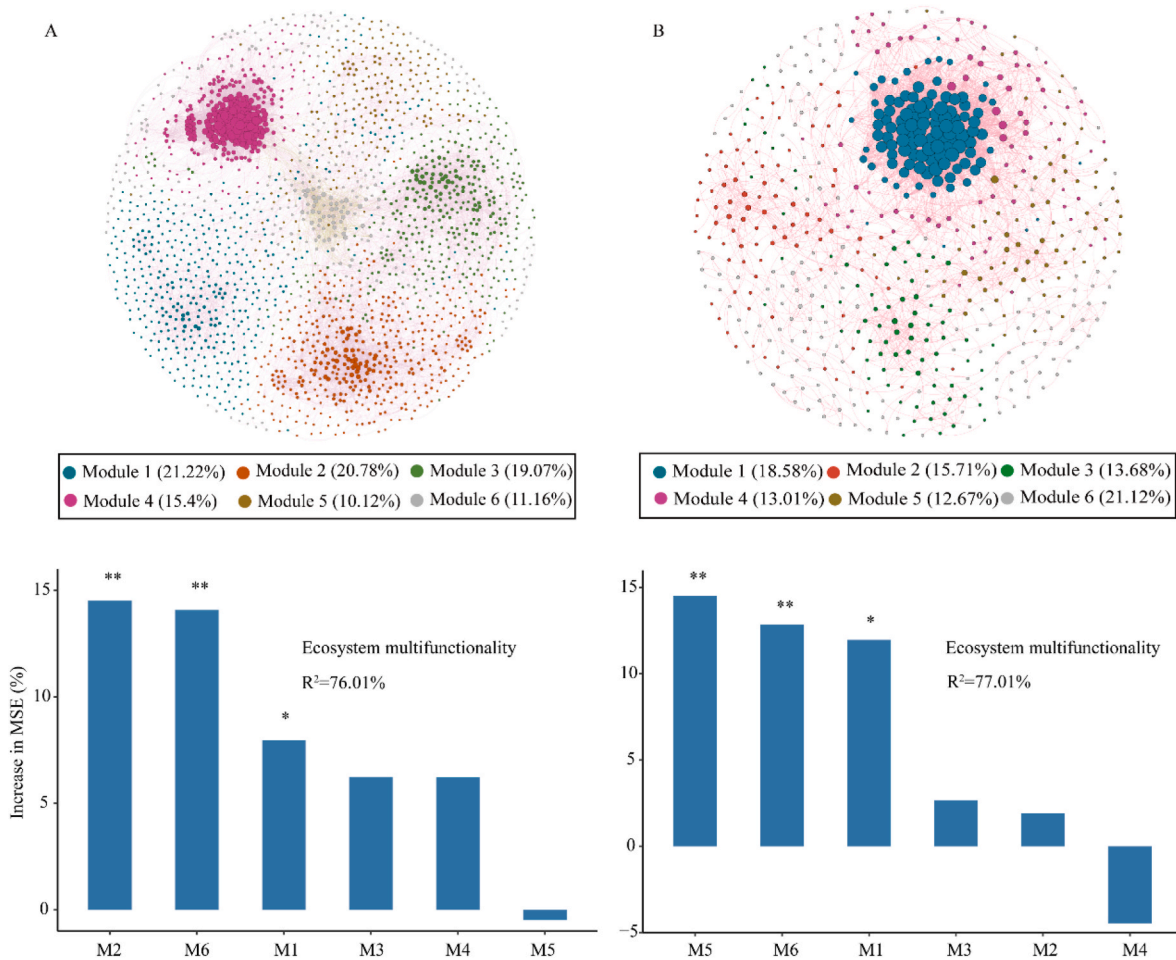


Fig. 5. Co-occurrence network during long-term vegetation restoration based on correlation analysis. (A–B) ASVs of bacteria and fungi colored by modularity class. The top six modules (module 1 to 6: M1–M6) were selected in the present study. Other modules are shown in gray color. The size of each node is proportional to the number of connections (i.e., degree). The important network module was identified by applying Random Forests regression of their scale relative abundances in bacteria (bottom panel on left) and fungi (bottom panel on right) against ecosystem multifunctionality during forest restoration.

community assembly (Barberán et al., 2012). The network properties and modularity in the present study were similar to those of diatom blooms observed in aquatic and wetland ecosystems (Yang et al., 2022; Zhang et al., 2020). The topology of the network indicates interactions between microbes, and node degree indicates the number of direct connections for a specific ASV. The potential impacts of one species on the networks of other nodes are expressed using the betweenness centrality of a node (Barberán et al., 2012). High betweenness centrality may indicate that a node occupies the central location in the network, and strongly influences other interactions (Li et al., 2022; Ma et al., 2016). For both bacteria and fungi in the present study, the abundant taxa had betweenness centrality values that were considerably higher than those of rare taxa, indicating that they were more centrally located in the network (Fig. 6).

Positive and negative interactions among microbial species are interpreted as either cooperative or mutualistic relationships, respectively, and either competitive or antagonistic relationships, respectively (Xue et al., 2018; Xun et al., 2017). The microbial mutualistic relationships exceeded competitive relationships during the long-term vegetation restoration (Fig. 6). In addition, the rare taxa had more modules in the network, in the cases of both bacteria and fungi, in the present study, and rare taxa had higher taxonomic diversity than abundant taxa. Key modules (bacteria, M2, fungi, M5) also included more rare taxa at the phylum level. Rare taxa have higher modularity and mediate more complex species interactions (Montoya et al., 2015; Olesen et al., 2007). Considering each function is performed by multiple

coexisting and taxonomically distinct species in nature (Louca et al., 2018), microbial communities with higher diversity have high functional redundancy (Jousset et al., 2017; Pedrós-Alió, 2012). The disconnect between function and diversity could be due to species replacing each other, interactions, and temporal heterogeneity (i.e., competition, predation) (Louca et al., 2018; Montoya et al., 2015). Despite rare taxa serve as a reservoir of extensive genetic functional diversity, their narrow ecological niche exposes them to competitive pressures from other species (Jiao et al., 2017; Kraft and Ackerly, 2010). Our results suggested that abundant microbes play core role in microbial interactions under long-term vegetation restoration of *Robinia pseudoacacia* plantations.

5. Conclusion

The findings suggested that the influence of vegetation restoration on rare microbial community dissimilarity was less than that in abundant taxa. Therefore, abundant microbes exhibit higher average variation degree than rare. Moreover, ecological drift dominated bacterial and fungal communities. Soil pH mediates bacterial community dissimilarity, which did not alter the stochasticity of bacterial community (rare and abundant taxa). Soil TP mediates balance between drift and heterogeneous selection processes. Abundant taxa have broader niche breadths, and become active under long-term vegetation restoration, so that they emerge as the major contributors to ecosystem multifunctionality and play roles in microbial interactions.

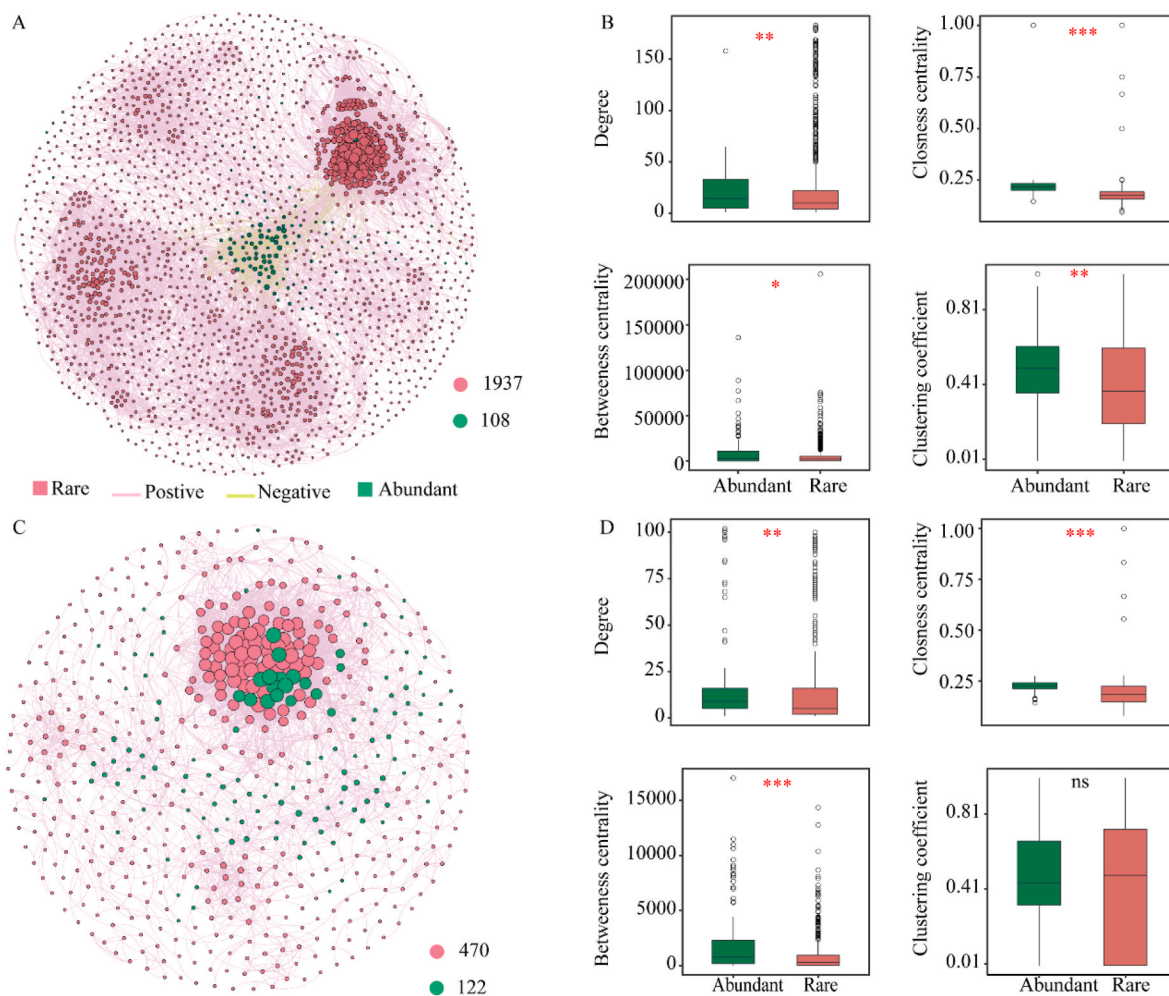


Fig. 6. Co-occurrence networks of abundant and rare amplicon sequence variants (ASVs) based on correlation analysis (A and C) and node features of the network (B and D). (A and C) The size of each node is proportional to the number of connections (i.e., degree). Number at the right represents node number. (B and D) Degree; closeness centrality; betweenness centrality; and clustering coefficient. *P < 0.05; **P < 0.01; ***P < 0.001. A, B show bacterial network (included abundant and rare taxa) and network topology property, respectively. C, D show fungal network (included abundant and rare taxa) and network topology property, respectively.

Table 2

The microbial stability analysis. The numbers in the table represent average variation degree (AVD). High AVD indicates low stability.

	Fungi		Bacteria	
	Rare	Abundant	Rare	Abundant
Y15	0.27 ± 0.01 b	0.61 ± 0.07 b	0.08 ± 0.00 b	16.74 ± 0.60 b
Y25	0.28 ± 0.03 b	0.65 ± 0.06 b	0.09 ± 0.01 b	17.53 ± 1.15 b
Y35	0.27 ± 0.01 b	0.63 ± 0.05 b	0.08 ± 0.00 b	16.99 ± 0.83 b
Y45	0.28 ± 0.02 b	0.61 ± 0.03 b	0.09 ± 0.01 b	17.15 ± 1.36 b
CK	0.37 ± 0.06a	0.89 ± 0.04a	0.11 ± 0.02a	22.72 ± 3.63a

The values are the mean ± SE (n = 6); different letters indicate significant differences at the 0.05 level.

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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

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.117720>.

References

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V., 2011. Soil microbial community successional patterns during forest ecosystem restoration. *Appl. Environ. Microbiol.* 77, 6158–6164.

- Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* 6, 343–351.
- Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., Fierer, N., Klironomos, J., Klironomos, J., 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecol. Lett.* 17, 794–802.
- Bongers, F.J., Schmid, B., Bruehlheide, H., Bongers, F., Li, S., von Oheimb, G., Li, Y., Cheng, A., Ma, K., Liu, X., 2021. Functional diversity effects on productivity increase with age in a forest biodiversity experiment. *Nat. Ecol. Evol.* 5, 1594–1603.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583.
- Chen, Q.L., Ding, J.Z., D. H., Hang, Wei, Delgado, B., Ma, Y.B., He, J.Z., Zhu, Y.G., 2020. Rare Microbial Taxa as the Major Drivers of Ecosystem Multifunctionality in Long-Term Fertilized Soils. *Soil Biology and Biochemistry* 141, 107687.
- Chen, Q., Hu, H., Yan, Z., Li, C., Nguyen, B.T., Sun, A., Zhu, Y., He, J., 2021. Deterministic selection dominates microbial community assembly in termite mounds. *Soil Biol. Biochem.* 152, 108073.
- Coban, O., De Deyn, G.B., van der Ploeg, M., 2022. Soil microbiota as game-changers in restoration of degraded lands. *Science* 375, eabe0725.
- Cui, Y., Fang, L., Guo, X., Wang, X., Zhang, Y., Li, P., Zhang, X., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. *Soil Biol. Biochem.* 116, 11–21.
- Cui, Y., Fang, L., Guo, X., Han, F., Ju, W., Ye, L., Wang, X., Tan, W., Zhang, X., 2019. Natural grassland as the optimal pattern of vegetation restoration in arid and semi-arid regions: evidence from nutrient limitation of soil microbes. *Sci. Total Environ.* 648, 388–397.
- Dini-Andreote, F., Stegen, J.C., van Elsland, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc. Natl. Acad. Sci. USA* 112, E1326–E1332.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* 3, 626–631.
- Hastings, A., 2010. Timescales, dynamics, and ecological understanding. *Ecology* 91, 3471–3480.
- He, Z., Liu, D., Shi, Y., Wu, X., Dai, Y., Shang, Y., Peng, J., Cui, Z., 2022. Broader environmental adaptation of rare rather than abundant bacteria in reforestation succession soil. *Sci. Total Environ.* 828, 154364.
- Hector, A., Bagchi, R., 2007. Biodiversity and ecosystem multifunctionality. *Nature* 448, 188–190.
- Isabwe, A., Yao, H., Zhang, S., Jiang, Y., Breed, M.F., Sun, X., 2022. Spatial assortment of soil organisms supports the size-plasticity hypothesis. *ISME Communications* 2, 1–11.
- Jia, X., Dini-Andreote, F., Salles, J.F., 2018. Community assembly processes of the microbial rare biosphere. *Trends Microbiol.* 26, 738–747.
- Jiao, S., Lu, Y.H., 2020. Abundant fungi adapt to broader environmental gradients than rare fungi in agricultural fields. *Global Change Biol.* 26, 4506–4520.
- Jiao, S., Chen, W.M., Wei, G.H., 2017. Biogeography and ecological diversity patterns of rare and abundant bacteria in oil-contaminated soils. *Mol. Ecol.* 26, 5305–5317.
- Jiao, S., Chen, W., Wang, J., Du, N., Li, Q., Wei, G., 2018. Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. *Microbiome* 6.
- Jiao, S., Xu, Y., Zhang, J., Hao, X., Lu, Y., Shade, A., 2019. Core microbiota in agricultural soils and their potential associations with nutrient cycling. *mSystems* 4, e313–e318.
- Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Kusel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G., Youssef, N.H., Zhang, X., Wei, Z., Hol, W.H., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME J.* 11, 853–862.
- Kraft, N.J., Ackerly, D.D., 2010. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecol. Monogr.* 80, 401–422.
- Li, L.J., Lin, Q., Li, X.Z., Li, T.Z., He, X.H., Li, D.P., Tao, Y., 2019. Dynamics and potential roles of abundant and rare subcommunities in the bioremediation of cadmium-contaminated paddy soil by *Pseudomonas chenduensis*. *Appl. Microbiol. Biotechnol.* 103, 8203–8214.
- Li, J., Yang, L., Fan, M., Shangguan, Z., 2022. Plantation vegetation restoration enhances the relationship between rhizosphere microbial diversity and soil multifunctionality. *Land Degrad. Dev.* 17, 3630–3640.
- Li, J., Fan, M., Yang, L., Yang, Z., Shangguan, Z., 2023a. Temporal Shifts in Root Exudates Driven by Vegetation Restoration Alter Rhizosphere Microbiota in *Robinia pseudoacacia* Plantations. *Tree Physiology*, tpad030.
- Li, J., Yang, L., Mao, S., Fan, M., Shangguan, Z., 2023b. Assembly and enrichment of rhizosphere and bulk soil microbiomes in *Robinia pseudoacacia* plantations during long-term vegetation restoration. *Appl. Soil Ecol.* 187, 104835.
- Liang, Y., Xiao, X., Nuccio, E.E., Yuan, M., Zhang, N., Xue, K., Cohan, F.M., Zhou, J., Sun, B., 2020. Differentiation strategies of soil rare and abundant microbial taxa in response to changing climatic regimes. *Environ. Microbiol.* 22, 1327–1340.
- Liu, W.J., Graham, E.B., Zhong, L.H., Zhang, J.W., Li, W.T., Li, Z.P., Lin, X.G., Feng, Y.Z., 2020. Dynamic microbial assembly processes correspond to soil fertility in sustainable paddy agroecosystems. *Funct. Ecol.* 34, 1244–1256.
- Liu, L., Zhu, K., Krause, S.M.B., Li, S., Wang, X., Zhang, Z., Shen, M., Yang, Q., Lian, J., Wang, X., Ye, W., Zhang, J., 2021. Changes in assembly processes of soil microbial communities during secondary succession in two subtropical forests. *Soil Biol. Biochem.* 154, 108144.
- Louca, S., Polz, M.F., Mazel, F., Albright, M., Huber, J.A., O'Connor, M.L., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. *Nat. Ecol. Evol.* 2, 936–943.
- Luan, L., Shi, G., Zhu, G., Zheng, J., Fan, J., Dini-Andreote, F., Sun, B., Jiang, Y., 2022. Biogeographical patterns of abundant and rare bacterial biospheres in paddy soils across East Asia. *Environ. Microbiol.* 2, 294–305.
- Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., Brookes, P.C., Xu, J., Gilbert, J.A., 2016. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME J.* 10, 1891–1901.
- Ma, X., Zhou, Z., Chen, J., Xu, H., Ma, S., Dippold, M.A., Kuzyakov, Y., 2023. Long-term nitrogen and phosphorus fertilization reveals that phosphorus limitation shapes the microbial community composition and functions in tropical montane forest soil. *Sci. Total Environ.* 854, 158709.
- Montoya, D., Yallop, M.L., Memmott, J., 2015. Functional group diversity increases with modularity in complex food webs. *Nat. Commun.* 6, 7379.
- Muarry, M., Thompson, W., 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 19, 4321–4326.
- Nevarez, L., Vasseur, V., Le Madec, A., Le Bras, M.A., Coroller, L., Leguérin, I., Barbier, G., 2009. Physiological traits of *Penicillium glabrum* strain LCP 08.5568, a filamentous fungus isolated from bottled aromatised mineral water. *Int. J. Food Microbiol.* 130, 166–171.
- Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., Yang, Y., Arkin, A.P., Firestone, M.K., Zhou, J., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* 11, 1417.
- Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination networks. *Proc. Natl. Acad. Sci. USA* 104, 19891–19896.
- Pedrós-Alió, C., 2006. Marine microbial diversity: can it be determined. *Trends Microbiol.* 6, 257–263.
- Pedrós-Alió, C., 2012. The rare bacterial biosphere. *Ann. Rev. Mar. Sci.* 449–466.
- Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *For. Ecol. Manag.* 309, 19–27.
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Rob, K., Fiere, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351.
- Shen, C., Gunina, A., Luo, Y., Wang, J., He, J.Z., Kuzyakov, Y., Hemp, A., Classen, A.T., Ge, Y., 2020. Contrasting patterns and drivers of soil bacterial and fungal diversity across a mountain gradient. *Environ. Microbiol.* 22, 3287–3301.
- Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7, 2069–2079.
- Stéphane, D., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Thibaut, J., Guillaume, L., Pierre, L., Naima, M., Hélène, H.W., Aurélie, S., 2017. Adespatial: Multivariate Multiscale Spatial Analysis.
- Storch, D., Bohdalková, E., Okie, J., 2018. The more individuals hypothesis revisited: the role of community abundance in species richness regulation and the productivity–diversity relationship. *Ecol. Lett.* 21, 920–937.
- Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. *ISME J.* 12, 1072–1083.
- Wan, W., Gadd, G.M., Yang, Y., Yuan, W., Gu, J., Ye, L., Liu, W., 2021. Environmental adaptation is stronger for abundant rather than rare microorganisms in wetland soils from the Qinghai-Tibet Plateau. *Mol. Ecol.* 30, 2390–2403.
- Wang, J., Shen, J., Wu, Y., Tu, C., Soininen, J., Stegen, J.C., He, J., Liu, X., Zhang, L., Zhang, E., 2013. Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *ISME J.* 7, 1310–1321.
- Wang, J., Gong, B., Huang, W., Wang, Y., Zhou, J., 2017. Bacterial community structure in simultaneous nitrification, denitrification and organic matter removal process treating saline mustard tuber wastewater as revealed by 16S rRNA sequencing. *Bioresour. Technol.* 228, 31–38.
- Wang, J., Li, M., Li, J., 2021. Soil pH and moisture govern the assembly processes of abundant and rare bacterial communities in a dryland montane forest. *Environ. Microbiol. Rep.* 13, 862–870.
- Wang, J.M., Li, M.X., Li, J.W., 2021. Soil pH and moisture govern the assembly processes of abundant and rare bacterial communities in a dryland montane forest. *Environ. Microbiol. Rep.* 13, 862–870.
- Xu, Y., Wang, T., Li, H., Ren, C., Chen, J., Yang, G., Han, X., Feng, Y., Ren, G., Wang, X., 2019. Variations of soil nitrogen-fixing microorganism communities and nitrogen fractions in a *Robinia pseudoacacia* chronosequence on the loess plateau of China. *Catena* 174, 316–323.
- Xu, M., Huang, Q., Xiong, Z., Liao, H., Lv, Z., Chen, W., Luo, X., Hao, X., 2021. Distinct responses of rare and abundant microbial taxa to in situ chemical stabilization of cadmium-contaminated soil. *mSystems* 6, e104021.
- Xue, Y., Chen, H., Yang, J.R., Liu, M., Huang, B., Yang, J., 2018. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. *ISME J.* 12, 2263–2277.
- Xue, R., Wang, C., Zhao, L., Sun, B.R., Wang, B.L., 2022. Agricultural intensification weakens the soil health index and stability of microbial networks. *Agric. Ecosyst. Environ.* 339, 108118.
- Xun, W., Huang, T., Li, W., Ren, Y., Xiong, W., Ran, W., Li, D., Shen, Q., Zhang, R., 2017. Alteration of soil bacterial interaction networks driven by different long-term fertilization management practices in the red soil of South China. *Appl. Soil Ecol.* 120, 128–134.
- Xun, W., Liu, Y., Li, W., Ren, Y., Xiong, W., Xu, Z., Zhang, N., Miao, Y., Shen, Q., Zhang, R., 2021. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* 9, 35.

- Yang, Y., Cheng, K., Li, K., Jin, Y., He, X., 2022. Deciphering the diversity patterns and community assembly of rare and abundant bacterial communities in a wetland system. *Sci. Total Environ.* 838, 156334.
- Zhang, J., Zhang, B., Liu, Y., Guo, Y., Shi, P., Wei, G., 2018. Distinct large-scale biogeographic patterns of fungal communities in bulk soil and soybean rhizosphere in China. *Sci. Total Environ.* 644, 791–800.
- Zhang, H., Hou, F., Xie, W., Wang, K., Zhou, X., Zhang, D., Zhu, X., 2020. Interaction and assembly processes of abundant and rare microbial communities during a diatom bloom process. *Environ. Microbiol.* 22, 1707–1719.
- Zhang, J., Fu, B., Stafford-Smith, M., Wang, S., Zhao, W., 2021. Improve forest restoration initiatives to meet sustainable development goal 15. *Nat. Ecol. Evol.* 5, 10–13.
- Zheng, W., Zhao, Z., Lv, F., Wang, R., Wang, Z., Zhao, Z., Li, Z., Zhai, B., 2021. Assembly of abundant and rare bacterial and fungal sub-communities in different soil aggregate size in an apple orchard treated with cover crop and fertilizer. *Soil Biol. Biochem.* 156, 108222.
- Zhou, J., Liu, W., Deng, Y., Jiang, Y., Xue, K., He, Z., Van Nostrand, J.D., Wu, L., Yang, Y., Wang, A., 2013. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. *mBio* 4, e00584-12.