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RESEARCH ARTICLE

Secondary forest succession drives differential responses of bacterial communities and interactions rather than bacterial functional groups in the rhizosphere and bulk soils in a subalpine region

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Abstract

Background and aims Community dynamics, functions and driving factors of rhizosphere and bulk soil bacteria during secondary forest succession remain poorly understood in subalpine regions.

Methods Three typical successional stages (grassland, shrubland and secondary forest) were selected to analyse bacterial communities, functions and interactions in the rhizosphere and bulk soils using high-throughput sequencing technology.

Results The results showed no significant difference in the bacterial α-diversity in the rhizosphere soil, whereas the bacterial α-diversity in the bulk soil of the grassland was significantly lower than that of the shrubland and secondary forest. Bacterial β-diversity in the rhizosphere soil differed significantly among the three succession stages, while the bacterial β-diversity in the bulk soil in the shrubland and secondary forest was significantly different from that in the grassland. However, the potential bacterial functions of the carbon, nitrogen and sulfate cycles revealed a consistent response in the rhizosphere and bulk soils to secondary forest succession. The soil total phosphorus, ammonium nitrogen, ratio of carbon to phosphorus and pH were the main factors affecting bacterial communities and potential functional groups. Bacterial network complexity was highest in the secondary forest rhizosphere soil and the shrubland bulk soil. Different keystone bacteria were detected in the rhizosphere and bulk soils among the three successional stages; they play major role in maintaining ecosystem function and community structure.

Conclusion Our results demonstrate that the bacterial communities and interactions in the rhizosphere and bulk soils respond differently to secondary forest succession, while the bacterial functional groups revealed a consistent response.

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Keywords Secondary forest succession · Rhizosphere · Bacterial communities · Bacterial functional groups · Co-occurrence network · High-throughput sequencing

Introduction

Soil microorganisms are important factors in plant–soil interactions and can regulate aboveground plant community composition and dynamics by participating in the nutrient cycle or through plant diseases (van der Heijden et al. 2008). Soil bacteria are essential components of soil microorganisms and play an important role in soil organic matter decomposition, the soil nutrient cycle and plant health (Hu et al. 2020; van der Heijden et al. 2008). Therefore, soil bacterial communities are regarded as an essential part of ecosystem restoration assessments (Jiang et al. 2021; Liu et al. 2020; van der Heijden et al. 2008). Previous studies reported that the soil bacterial communities followed different successional patterns during forest succession (Jiang et al. 2021; Liu et al. 2020). For example, bacterial α-diversity increased (Chai et al. 2019; Jiang et al. 2021), increased first and then remained stable (Liu et al. 2020), or showed no significant difference (Li et al. 2021) during forest succession. Moreover, the bacterial β-diversity also varied significantly during forest succession (Jiang et al. 2021; Liu et al. 2020; Qiang et al. 2021; Wang et al. 2019), although no significant difference was observed in forest succession in a karst area (Li et al. 2021; Xu et al. 2021b). In recent years, numerous studies have indicated that ecological networks can represent interactions between organisms and influence the response of microbial communities to environmental change (de Vries et al. 2018; Dini-Andreote et al. 2014). However, it is not clear how the relationship between soil bacteria changes during secondary forest succession. In addition, previous studies mainly focused on the variation in soil bacterial communities between a single ecosystem of trees (Cao et al. 2020; Krishna et al. 2020; Yan et al. 2020) or grassland (Song et al. 2019; Zhong et al. 2020), and the dynamics of bacterial functions and interactions during succession were usually neglected.

The variations in soil bacterial communities during forest succession are influenced jointly by plant communities and soil physicochemical properties. First, plant communities exert effects on bacterial communities (Qiang et al. 2021; Zhong et al. 2020) through the input of plant litter and root exudates during forest succession or can indirectly affect soil properties and then drive changes in soil bacterial communities (Qu et al. 2020; Vives-Peris et al. 2020; Zhalnina et al. 2018; Zheng et al. 2021). Second, previous studies found that soil organic carbon (SOC), soil total nitrogen (TN), soil available nitrogen (AN), soil available phosphorus (AP), soil water content (SM) and soil pH had significant influences on bacterial communities during secondary forest succession (Jiang et al. 2021; Wang et al. 2019; Xu et al. 2021b). In addition, Liu et al. (2015) found that soil texture changed significantly during secondary forest succession; the variations in soil texture have significant influences on plant properties, root exudate composition (Vieira et al. 2020), soil oxygen content (Ferreyra et al. 2008) and soil water (Li et al. 2014), thereby further affecting soil bacterial diversity and community composition (Karimi et al. 2018; Xia et al. 2020). However, the effects of soil texture on the soil bacterial community structure during secondary forest succession have often been overlooked (Jiang et al. 2021; Liu et al. 2020; Qiang et al. 2021; Xu et al. 2021b). Therefore, the driving factors of soil bacterial communities during long-term secondary forest succession remain unclear.

Different from bulk soil, the rhizosphere is defined as a narrow zone of soil influenced by plant roots (Hartmann et al. 2008). The rhizosphere provides a niche for plant-microorganism interactions and is considered one of the most dynamic interfaces on Earth (Philippot et al. 2013). Previous studies reported differences between the rhizosphere and bulk soil in different plant species. For example, herbaceous species (*Calamagrostis crispa*, *Nassella nardoides* and *Jarava frigida*) (Fernández-Gómez et al. 2019), halophytes (*Phragmites communis* and *Suaeda salsa*) (Zhao et al. 2022) and trees (beech and Norway spruce) (Uroz et al. 2016) revealed significant differences in bacterial communities in the rhizosphere and bulk soils. The differences in bacterial communities between the rhizosphere soil and bulk soil are mainly attributed to the effects of plant species through the input of different root exudates during their growth (Qu et al. 2020; Zhalnina et al. 2018). In addition, different dominant species can also affect the composition of rhizosphere bacterial

community by regulating soil ectomycorrhizal (ECM) fungi, which leads to the differential response of bacterial communities in the rhizosphere and bulk soil (Cumming et al. 2015; Wang et al. 2021a). However, Cui et al. (2019) and Zhao et al. (2022) reported that the bacterial communities of *Abies fabri* Mast and *Aeluropus sinensis* showed no significant difference between the rhizosphere soil and bulk soil. Xu et al. (2021b) also reported a similar change in the rhizosphere and bulk soils during secondary forest succession in a karst area. This indicated that in addition to plant species, the rhizosphere bacterial communities were also influenced by other factors (i.e., soil properties) (Song et al. 2019; Vieira et al. 2020). Therefore, the changes in dominant plant communities and soil properties during forest succession are bound to affect the bacterial communities and functions in the bulk and rhizosphere soil. However, it is still unclear how the bacterial communities, functional groups and interactions in the rhizosphere and bulk soils respond to secondary forest succession.

The southwestern subalpine forest, located in the upper reaches of the Yangtze River, is the main body of alpine forests on the eastern part of the Qinghai-Tibet Plateau. This area plays an important role in the regional economy, water resource conservation and ecological screening (Liu. 2002). Due to the combined influence of climate, geology, landform and other natural backgrounds and human activities, the subalpine coniferous forest vegetation ecosystem is in a relatively fragile environment and has an unstable system, which makes it extremely difficult to recover from damage (Liu et al. 2001; Liu 2002). Therefore, further research on community diversities and functions during subalpine forest restoration is of great significance to gain an in-depth understanding of vegetation restoration mechanisms. A few studies have investigated the responses of plant communities, soil properties and microbial communities at different succession stages in this region (Cao et al. 2020; Qiang et al. 2021; Sheng et al. 2021). However, these studies focused only on the variations in the bacterial communities of bulk soil. The bacterial communities, functional groups, interactions and driving factors in the rhizosphere and bulk soils during secondary forest succession have not been evaluated thus far. To complement our knowledge of soil bacterial communities and function during secondary succession in subalpine forests, the rhizosphere and bulk soils of three typical successional stages (grassland, shrubland and secondary forest) were sampled to investigate the soil bacterial communities, functional groups, interactions, and driving factors during secondary forest succession. We hypothesized that 1) bacterial communities and functional groups in the rhizosphere and bulk soils respond differently to secondary forest succession; 2) soil bacterial interactions reveal differential responses in the rhizosphere and bulk soils among the three successional stages; and 3) the key driving factor explaining the variation in soil bacterial community dynamics and functional groups is different in the rhizosphere and bulk soils during secondary succession.

Materials and methods

Experimental site

The study site is located in the Miyaluo Nature Reserve (31°42′ to 31°51′N, 102°41′ to 102°44′E, 2200 m-5500 m), Sichuan Province, China. This area is in the alpine valley transition area between the Qinghai-Tibet Plateau and the Sichuan Basin. The area is characterized by a snow climate with a dry winter and warm summer (Dwb) according to the Koppen-Geiger climate classification. The average annual temperature is 8.7 °C (from 0.6 °C in January to 16.4 °C in July, and the annual precipitation ranges from 600 mm/y to 1100 mm/y. The soil is classified as mountain brown soil based on Chinese soil taxonomy (Cao et al. 2020). This area suffered from large-scale deforestation from the 1918s to 1998s (Liu et al. 2001) and have gradually formed a natural and continuous succession process from grasslands and shrublands to secondary forests with the implementation of national natural forest restoration programs (Liu. 2002; Qiang et al. 2021), therefore providing a natural setting for studying bacterial community composition and function during secondary forest succession.

Sample preparation

The method of ‘space-for-time substitution’ was adopted in this experiment, and we selected three different habitat types, grassland, shrubland and secondary forest to represent the different stages of the

typical vegetation successional process in this area. All the selected stands had similar slope direction, soil types, climates and geologies, and the selected stands of each successional stages revealed a similar slope (Table S1). Grassland was dominated by *Poa annua*, followed by *Rumex nepalensis*, *Hydrocotyle sibthorpioides*, *Halenia elliptica*, *Fragaria moupinensis*, *Clinopodium chinense* and *Potentilla fulgens*, and the mean vegetation coverage was 98%, the. Shrubland was dominated by *Quercus aquifolioides*, and the main undergrowth plants were *Lonicera japonica*, *Picea asperata*, *Sorbus koehneana*, *Betula platyphylla* and *Betula albosinensis*. The secondary forest was dominated by *Picea asperata*, and the main undergrowth plants included *Quercus aquifolioides*, *Sorbus koehneana* and *Betula platyphylla*. The grassland, shrubland and secondary forest in this study were approximately 0–20, 30 and 60 years, respectively (Chen et al. 2002; Sheng et al. 2021). In August 2018, one approximate 100 m × 100 m sampling site was established in each succession stage. Five plots grassland (1 m × 1 m), shrubland (5 m × 5 m) and secondary forest (10 m × 10 m) plots were established for soil sampling during the three succession stages. The plots were spaced more than 20 m apart to possible avoid spatial autocorrelation and edge effects in each succession stage (Chen et al. 2019). The plot information is described in Fig. 1. The root of the dominant plant was identified by the root-tracing method (Xu et al. 2021a). Rhizosphere soil is defined as the soil that remains attached to the plant roots after shaking the roots (Bell et al. 2014). Briefly, the roots of dominant plants at the three successional stages were dug out with a hoe, and then the roots were gently shaken; cotton swabs were used to collect rhizosphere soil that remained on the roots. The bulk soil between plants was sampled by a soil drill (9 cm in diameter and 10 cm in depth). Due to an extremely high herb coverage (98%), we collected the soil that dropped off the dominant herb roots after gentle shaking as bulk soil in the grassland. In each plot, five rhizosphere and bulk soil samples were collected randomly and then mixed into one composite sample. These soil samples were passed through a 2 mm sieve to remove debris and fine roots, and then the soil samples were divided into two parts. One part was stored at -40 °C until DNA extraction, and the other part was stored at 4 °C to determine the soil physicochemical properties.

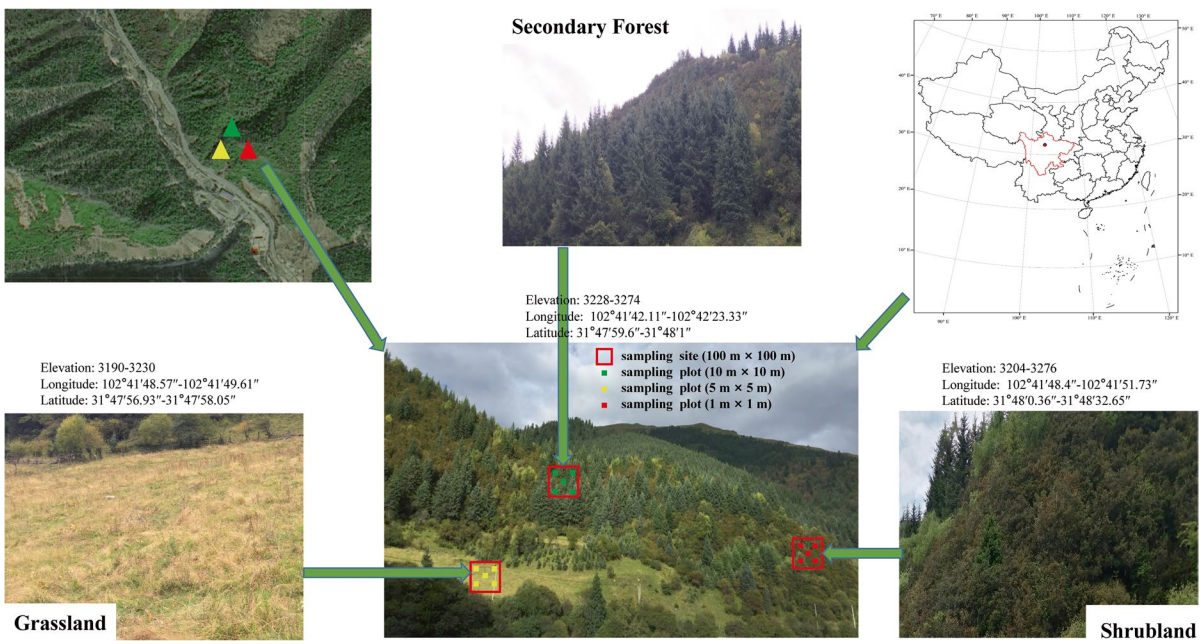


Fig. 1 Location of the study sites in the Miyaluo area of the western Sichuan subalpine forest

Soil physicochemical properties

Soil electrical conductivity (EC) and pH were measured at a soil-to-water ratio of 1:2.5 using a conductivity meter (Shanghai INESA & Scientific Instrument CO.LTD DDS307, China) and digital pH meter (Mettler-Toledo FE28, Switzerland), respectively. The soil moisture content (SM) was measured by oven-drying the soil samples at 65 °C for 72 h. SOC and TN were determined by a CHN elemental analyser (2400II CHN elemental analyser, PerkinElmer, Boston, MA, USA). Nitrate–N (NO_3^--N) and ammonium-N (NH_4^+-N) were extracted from soils with 2 M KCl and determined by the phenol disulfonic acid and indophenol blue methods, respectively. Total phosphorus (TP) was determined using molybdenum antimony blue colorimetry after the samples were digested with $HClO_4$-H_2SO_4. Soil texture was measured by the pipette method (sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (< 0.002 mm)). C:N indicates the ratio of SOC to TN; C:P indicates the ratio of SOC to TP, and N:P indicates the ratio of TN to TP. The soil properties (except for soil texture) were previously reported in Zhang et al. (2022).

DNA extraction, PCR amplification, and Illumina sequencing

Total genomic DNA was extracted from soil samples using MoBio PowerSoil DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA). The integrity and quality of the DNA extracts were checked by 1.0% agarose gel electrophoresis and a NanoDrop NC 2000 spectrophotometer (Thermo Scientific). Polymerase chain reaction (PCR) was used to amplify the V4-V5 region of the bacterial 16S rRNA gene using the primers 515 F 5′-TATCGCCTCCCTCGCGCCATCAG-3′ and 909 R 5′-CTATGCGCCTTGCCAGCCCGCT-3′ (Tamaki et al. 2011). The bacterial PCR amplification was carried out in a total volume of 25 μL reaction mixture, which included 12.5 μL 2×Es TaqMasterMix, 9.5 μL ddH_2O, 1 μL of each primer and 1 μL genomic DNA. PCR was performed at an initial annealing temperature of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s for denaturation, annealing at 56 °C for 1 min and extension at 72 °C for 1 min, followed by a final elongation at 72 °C for 10 min. The PCR products were verified and purified using 2% agarose gel electrophoresis and the Qiagen Gel Extraction Kit (Qiagen, Germany). Finally, the purified amplicons were pooled for paired-end sequencing on the Illumina HiSeq (PE 250×250) platform at Biomarker Technologies Corporation (Beijing, China). The raw sequences were trimmed and quality filtered by the QIIME2 pipeline according to their barcodes (Bolyen et al. 2019). Then, random resampling was carried out at an equal depth of 10,065 sequences per sample. The representative sequence of bacterial species was classified based on the Silva v138.1 database. The sequence data were deposited in the NCBI SRA database under accession number PRJNA835217. We further annotated the functions of bacterial OTUs using the FAPROTAX database, which is available online at http://www.zoology.ubc.ca/louca/FAPROTAX (Louca et al. 2016).

Data analysis

One-way analysis of variance (ANOVA) and Tukey's HSD test were used to test the differences ($P < 0.05$) in soil properties, bacterial phyla and bacterial functional groups at different succession stages using SPSS software (Version 19.0, SPSS Inc., Chicago, IL, U SA). Principal coordinate analysis (PCoA) was performed to evaluate the variations in soil bacterial β-diversity based on Bray–Curtis distances at different successional stages. The significant differences in bacterial β-diversity during succession were examined by analysis of similarity (ANOSIM). Permutational analysis of variance (PERMANOVA) was implemented to evaluate the effect of successional stages and soil type on bacterial community composition. Venn diagrams were used to illustrate the shared and specific bacterial OTUs at the three succession stages. The Wilcoxon test was performed to analyse the differences in bacterial α-diversity in the rhizosphere and bulk soils at the three succession stages. Redundancy analysis (RDA) was performed to elucidate the influence of soil properties on the bacterial communities or functional groups among the three succession stages. Spearman correlation analysis was used to test the correlations between soil properties and bacterial α-diversity during secondary forest succession. Box, PCoA and heatmap plots were constructed by the Microbiome Database (http://egcloud.cib.cn/index-cn.html). The Molecular Ecological Network Analyses Pipeline (http://ieg2.ou.edu/MENA/main.cgi) was used to construct the molecular ecological network of soil bacteria and to calculate

their characteristic parameters among the three successional stages (Deng et al. 2012). First, the 10% most abundant OTUs at the three succession stages were selected to build individual networks. Second, an appropriate threshold was calculated according to random matrix theory as a filter for node associations. Third, we carried out module separation and modularity calculations on the basis of the topological properties of the network. The networks were visualized in Cytoscape (v. 3.8.2).

Results

Bacterial diversity and community composition in the rhizosphere and bulk soils during secondary forest succession

The bacterial α-diversity (Chao 1 and Shannon indexes) showed no significant difference in the rhizosphere soil at the three successional stages ($P > 0.05$), while the bacterial α-diversity (Chao 1 and Shannon indexes) in the bulk soil in the grassland was significantly lower than that in the shrubland and secondary forest ($P < 0.05$) and no significant changes between shrubland and secondary forest ($P > 0.05$) (Fig. 2). The bacterial β-diversity in the rhizosphere soil was significantly different among the three succession stages, while the bacterial β-diversity in the bulk soil in the shrubland and secondary forest was significantly different from that in the grassland, while the β-diversity in the shrubland and secondary forest revealed no significant differences ($R = 0.04$, $P = 0.296$) (Fig. 3, Table S2). More OTUs were shared between shrubland and secondary forest in the rhizosphere and bulk soils during succession (Fig. 4).

At the three successional stages, the bacterial communities were predominantly composed of the bacterial phyla Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Planctomycetes and Chloroflexi in the rhizosphere and bulk soils (Fig. S1, Table S3). Among them, the relative abundances of Proteobacteria and Chloroflexi showed no significant difference in the rhizosphere and bulk soils with succession ($F = 1.439$, $P = 0.246$; $F = 0.918$, $P = 0.486$) (Fig. S1, Table S3). The relative abundances of Acidobacteria and Planctomycetes in the rhizosphere and bulk soils of the grassland were higher than those in the shrubland and secondary forest, while Actinobacteria showed the opposite trend. The relative abundance of Bacteroidetes in the rhizosphere soil showed no significant difference ($P > 0.05$), while in the bulk soil of the grassland, it was lower than that in the shrubland and secondary forest.

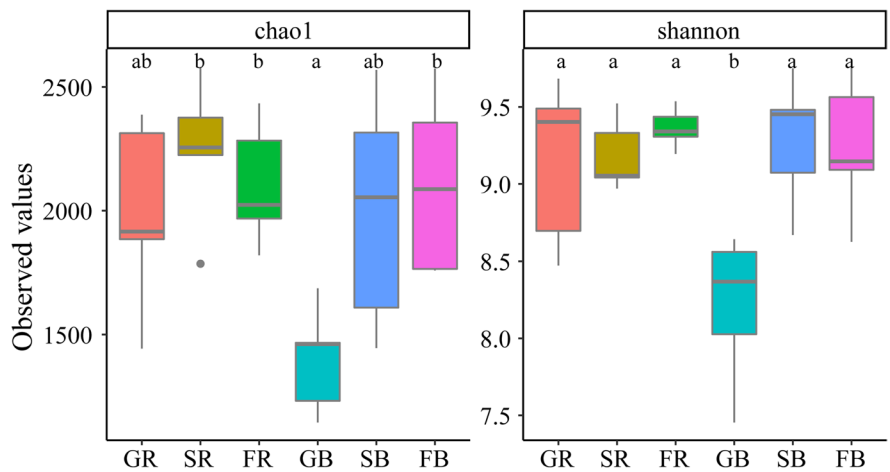


Fig. 2 Box plots of the bacterial α-diversity indexes in the rhizosphere and bulk soils among the three successional stages. (**a**) Chao1 index in the rhizosphere and bulk soils, (**b**) Shannon index in the rhizosphere and bulk soils. Different letters above the boxplots in each row indicate significant differences ($P < 0.05$). GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

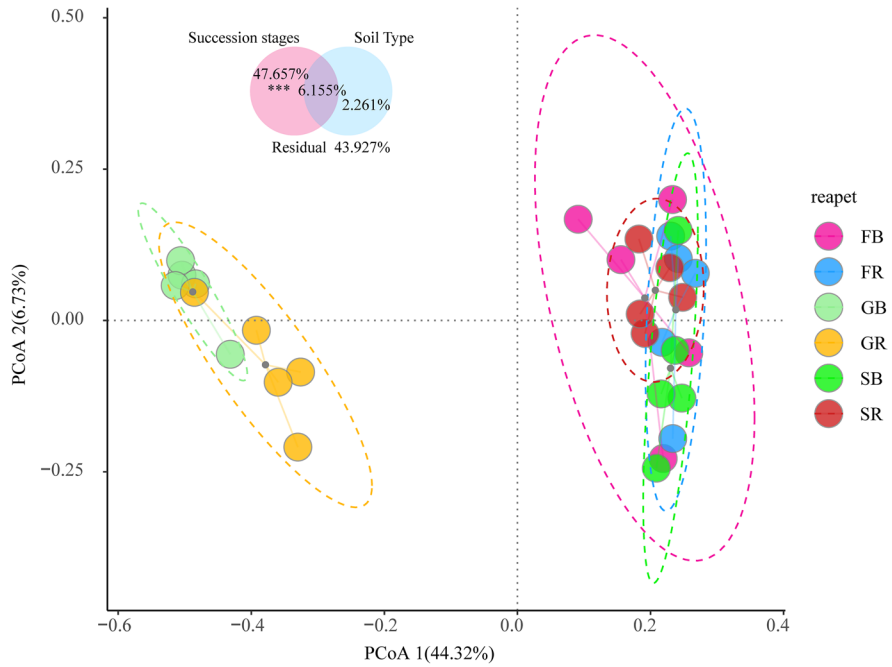


Fig. 3 Principal coordinate analysis (PCoA) plots of the first two principal components of the bacterial community composition in the rhizosphere and bulk soils based on the Bray–Curtis distances during secondary succession. Ellipses represent the 95% confidence intervals for the centroids of bacteria. The Venn diagram represents the variation in the bacterial community that is explained by successional stages (grassland, shrubland and secondary forest) and soil type (rhizosphere and bulk soils) variable and their shared variation. GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

Potential functional groups of soil bacterial communities during secondary forest succession

In this study, 43 and 37 potential bacterial functional taxa were observed in the respective rhizosphere and bulk soils during the three succession stages. The dominant potential bacterial functional groups were chemoheterotrophs (7.42–14.92%), aerobic chemoheterotrophs (7.06–14.19%) and aromatic compound degradation (0.34–3.09%) in both the rhizosphere and bulk soils (Fig. 5). Furthermore, we found that the potential bacterial functional groups involved in the carbon (C), nitrogen (N) and sulfate (S) cycles showed significant changes in the rhizosphere and bulk soils among the three succession stages. For example, the relative abundances of chemoheterotrophs, aerobic chemoheterotrophs, aromatic compound degradation and sulfate_respiration were higher in the shrubland and secondary forest than those in the grassland in both the rhizosphere and bulk soils (Fig. 5). The relative abundances of bacteria involved in nitrification and aerobic ammonia oxidation revealed a contrary trend (Fig. 5). In addition, the relative abundances of bacteria involved in nitrate reduction and fermentation showed no significant difference in the rhizosphere and bulk soils ($F=1.201$, $P=0.339$; $F=2.597$, $P=0.052$) (Fig. 5).

Soil bacterial interactions and keystone bacteria during secondary forest succession

Our results showed that the numbers of edges, average degree and connectedness were higher in the rhizosphere soil of the grassland and secondary forest than those in the shrubland (Fig. S2, Table 1). In the bulk soil, the numbers of nodes, edges, average degree, average path distance, clustering coefficient and connectedness were higher in the shrubland and secondary forest than in the grassland (Fig. S2, Table 1). All bacterial networks at three succession stages were dominated by positive correlations (Fig. S2, Table 1). The average clustering coefficients of the grassland rhizosphere and bulk soils were lower than those of

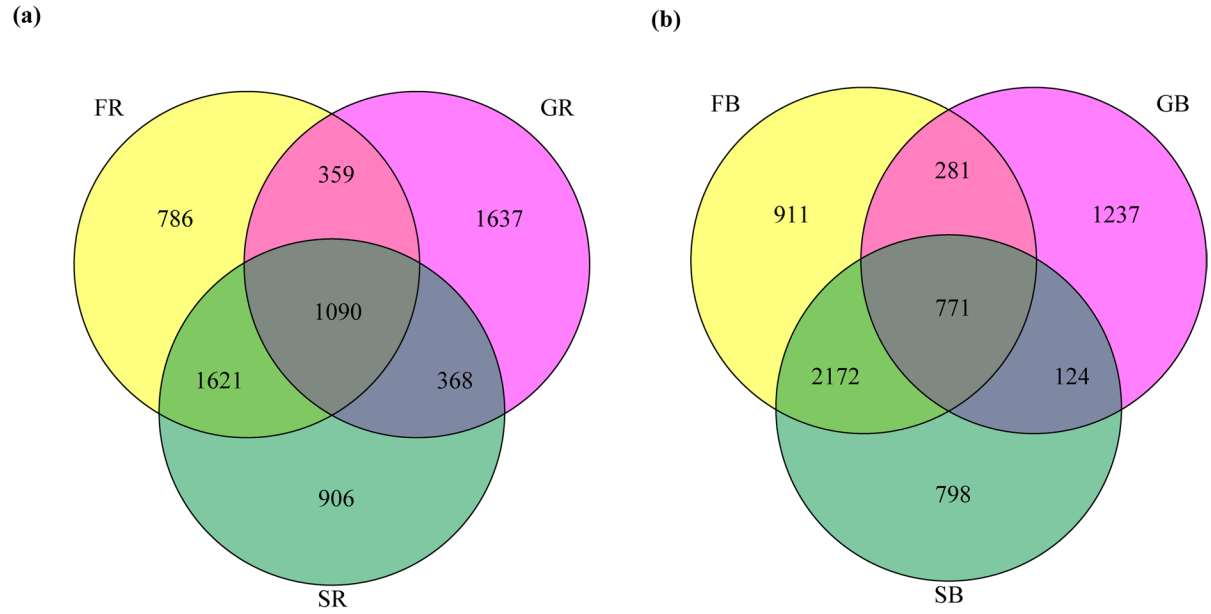


Fig. 4 Venn diagrams of the bacterial community composition. (**a**) OTU numbers in the rhizosphere soil during the three successional stages, (**b**) OTU numbers in the bulk soils during the three successional stages. GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

the shrubland and secondary forest (Fig. S2, Table 1). The modularity values of the bacterial networks in the rhizosphere and bulk soils at the three successional stages were > 0.4, indicating that these networks had a modular structure (Fig. S2, Table 1).

The intermodular connectivity (*Pi*) and intramodular connectivity (*Zi*) of the bacterial molecular ecological network in the rhizosphere and bulk soils of the three successional stages indicate that (1) most nodes of soil bacteria at the three successional stages were peripheral (grassland 96.76–98.03%, shrubland 96.44–98.38%, secondary forest 96.66–97.95%) ($Zi < 2.5$, $Pi < 0.62$) (Fig. 6a, 6b). All OTUs with $Zi \geq 2.5$ and $Pi \geq 0.62$ were identified as keystone species (Deng et al. 2012; Olesen et al. 2007; Yu et al. 2021). The keystone bacteria in the rhizosphere soil of the grassland included Proteobacteria (Caulobacteraceae, Micropepsaceae, Betaproteobacteriales, *Lysobacter*, *Rudaea*), Planctomycetes (Tepidisphaerales), Actinobacteria (Microbacteriaceae, Gaiellales, Solirubrobacterales, Microtrichales, *Iamiaceae*); Gemmatimonadetes (Gemmatimonadaceae), Acidobacteria, and Proteobacteria (Rhizobiaceae, Burkholderiaceae, *Dyella*) were dominant key taxa in the bulk soil of the grassland; Proteobacteria (Betaproteobacteriales), Actinobacteria (Microtrichales, *Pseudonocardia*, *Conexibacter*) and Acidobacteria were dominant in the rhizosphere soil of the shrubland; Proteobacteria (Acetobacteraceae, Nitrosomonadaceae, Sphingomonas, *Acidibacter*, Xanthobacteraceae), Bacteroidetes (Chitinophagaceae, Sphingobacteriales), Actinobacteria (Gaiellales) and Acidobacteria (*Candidatus Solibacter*) were dominant key taxa in the bulk soil of the shrubland; Proteobacteria (Nitrosomonadaceae, *Haliangium*, *Pedomicrobium*), Bacteroidetes (Sphingobacteriales, *Niastella*), Actinobacteria (*Pseudonocardia*, *Actinoplanes*) and Acidobacteria (Acidobacteriales) were dominant key taxa in the rhizosphere soil of the secondary forest; Proteobacteria (*Dokdonella*, Rhizobiales, *Arenimonas*), Bacteroidetes (*Ferruginibacter*) and Actinobacteria (*Cryptosporangium*, *Kineosporia*) and Acidobacteria were dominant key taxa in the bulk soil of the secondary forest.

Effects of soil properties on soil bacterial communities and functional groups during secondary forest succession

Correlation analysis showed that soil factors had no significant effect on the bacterial α-diversity in the

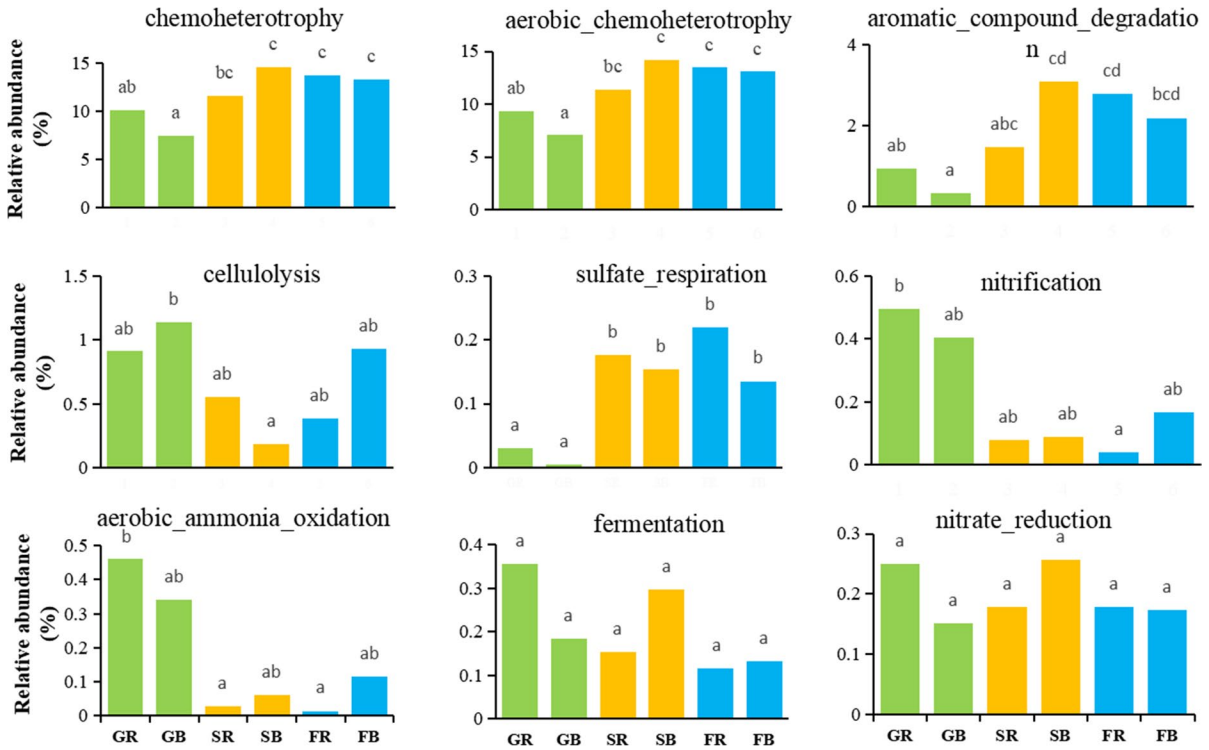
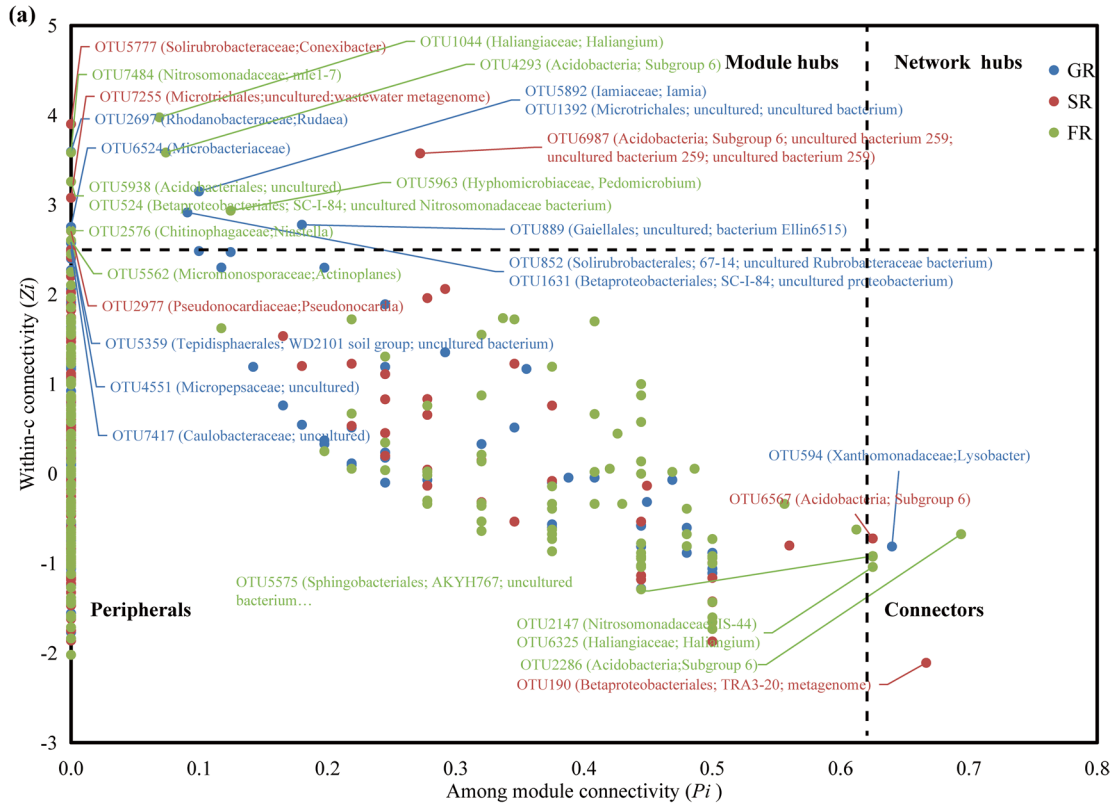


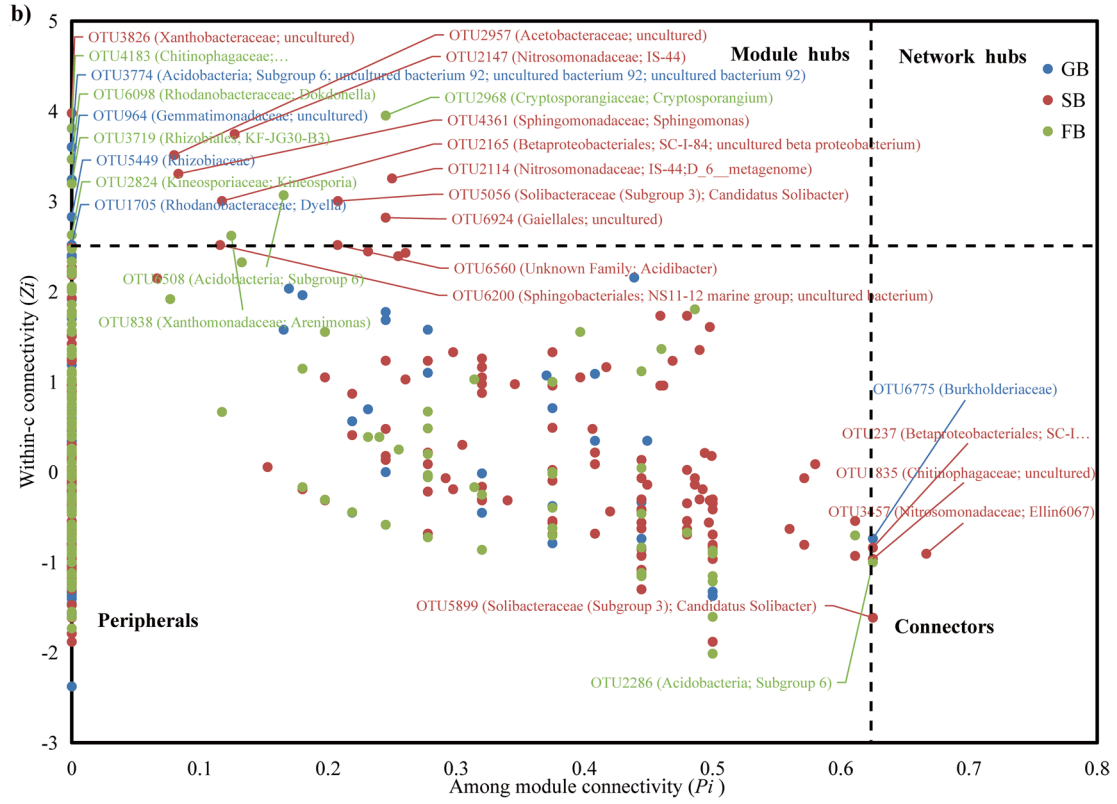
Fig.5 Changes in the soil bacterial functional groups in the rhizosphere and bulk soils at different stages of succession. Different letters indicate significant differences ($P<0.05$) between treatments. GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

Table 1 Topological features of bacterial networks in the rhizosphere and bulk soils across the three successional stages

	GR	SR	FR	GB	SB	FB
No. of nodes	340	371	389	254	393	391
No. of edges	823	645	979	578	1232	1023
Positive edges	509 (61.85%)	372 (57.67%)	550 (56.18%)	322 (55.71%)	840 (68.18%)	605 (59.14%)
Negative edges	314 (38.15%)	273 (42.33%)	429 (43.82%)	256 (44.29%)	392 (31.82%)	418 (40.86%)
R2 of power-law	0.811	0.803	0.768	0.799	0.831	0.783
Average degree	4.841	3.477	5.033	4.551	6.270	5.233
Average path distance	6.817	9.109	7.486	6.090	6.202	6.549
Connectedness	0.629	0.485	0.886	0.491	0.887	0.650
Clustering coefficient	0.354	0.359	0.356	0.327	0.350	0.335
Modularity	0.765	0.888	0.761	0.819	0.670	0.745

rhizosphere soil ($P>0.05$) (Fig. S3a). In contrast, in the bulk soil, SOC ($\rho=0.936$, $P<0.001$), TN ($\rho=0.817$, $P<0.001$), NH_4^+-N ($\rho=0.814$, $P<0.001$), NO_3^--N ($\rho=0.825$, $P<0.001$), pH ($\rho=0.689$, $P=0.008$), EC ($\rho=0.796$, $P=0.001$), SM ($\rho=0.850$, $P<0.001$), C:P ($\rho=0.929$, $P<0.001$), N:P ($\rho=0.779$, $P=0.002$) and C:N ($\rho=0.893$, $P<0.001$) were significantly and positively correlated with the bacterial Shannon index, whereas soil texture ($P>0.05$) and soil TP ($\rho=-0.554$, $P=0.050$; $\rho=-0.543$, $P=0.054$) had no significant


(a)
Module hubs
Network hubs
GR
SR
FR
OTU5777 (Solirubrobacteraceae;Conexibacter)
OTU1044 (Haliangiaceae; Haliangium)
OTU7484 (Nitrosomonadaceae; nitc1-7)
OTU4293 (Acidobacteria; Subgroup 6)
OTU7255 (Microtrichales;uncultured;wastewater metagenome)
OTU5892 (Iamiaceae; Iamia)
OTU2697 (Rhodanobacteraceae;Rudaea)
OTU1392 (Microtrichales; uncultured; uncultured bacterium)
OTU6524 (Microbacteriaceae)
OTU6987 (Acidobacteria; Subgroup 6; uncultured bacterium 259; uncultured bacterium 259; uncultured bacterium 259)
OTU5938 (Acidobacteriales; uncultured)
OTU5963 (Hyphomicrobiaceae, Pedomicrobium)
OTU524 (Betaproteobacteriales; SC-I-84; uncultured Nitrosomonadaceae bacterium)
OTU2576 (Chitinophagaceae;Niastella)
OTU889 (Gaiellales; uncultured; bacterium Ellin6515)
OTU852 (Solirubrobacterales; 67-14; uncultured Rubrobacteraceae bacterium)
OTU1631 (Betaproteobacteriales; SC-I-84; uncultured proteobacterium)
OTU5562 (Micromonosporaceae;Actinoplanes)
OTU2977 (Pseudonocardiaceae;Pseudonocardia)
OTU5359 (Tepidisphaerales; WD2101 soil group; uncultured bacterium)
OTU4551 (Micropepsaceae; uncultured)
OTU7417 (Caulobacteraceae; uncultured)
OTU594 (Xanthomonadaceae;Lysobacter)
OTU6567 (Acidobacteria; Subgroup 6)
OTU5575 (Sphingobacteriales; AKYH767; uncultured bacterium…
Peripherals
Connectors
OTU2147 (Nitrosomonadaceae; IS-44)
OTU6325 (Haliangiaceae; Haliangium)
OTU2286 (Acidobacteria;Subgroup 6)
OTU190 (Betaproteobacteriales; TRA3-20; metagenome)
Within-c connectivity (Zi)
Among module connectivity (Pi)
5
4
3
2
1
0
-1
-2
-3
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8


(b)
Module hubs
Network hubs
GB
SB
FB
OTU3826 (Xanthobacteraceae; uncultured)
OTU2957 (Acetobacteraceae; uncultured)
OTU4183 (Chitinophagaceae;…
OTU2147 (Nitrosomonadaceae; IS-44)
OTU3774 (Acidobacteria; Subgroup 6; uncultured bacterium 92; uncultured bacterium 92; uncultured bacterium 92)
OTU6098 (Rhodanobacteraceae; Dokdonella)
OTU2968 (Cryptosporangiaceae; Cryptosporangium)
OTU964 (Gemmatimonadaceae; uncultured)
OTU4361 (Sphingomonadaceae; Sphingomonas)
OTU3719 (Rhizobiales; KF-JG30-B3)
OTU2165 (Betaproteobacteriales; SC-I-84; uncultured beta proteobacterium)
OTU5449 (Rhizobiaceae)
OTU2114 (Nitrosomonadaceae; IS-44;D_6__metagenome)
OTU2824 (Kineosporiaceae; Kineosporia)
OTU5056 (Solibacteraceae (Subgroup 3); Candidatus Solibacter)
OTU1705 (Rhodanobacteraceae; Dyella)
OTU6924 (Gaiellales; uncultured)
OTU6560 (Unknown Family; Acidibacter)
OTU508 (Acidobacteria; Subgroup 6)
OTU6200 (Sphingobacteriales; NS11-12 marine group; uncultured bacterium)
OTU838 (Xanthomonadaceae; Arenimonas)
OTU6775 (Burkholderiaceae)
OTU237 (Betaproteobacteriales; SC-I…
OTU1835 (Chitinophagaceae; uncultured)
OTU3457 (Nitrosomonadaceae; Ellin6067)
OTU5899 (Solibacteraceae (Subgroup 3); Candidatus Solibacter)
Peripherals
Connectors
OTU2286 (Acidobacteria; Subgroup 6)
Within-c connectivity (Zi)
Among module connectivity (Pi)
5
4
3
2
1
0
-1
-2
-3
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

◂**Fig. 6** Putative keystone species in the rhizosphere and bulk soils during the three successional stages. GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

effect on the bacterial Chao 1 and Shannon indexes, respectively (Fig. S3b).

Successional stages explained most of the variations (47.657%) in the soil bacterial community composition, while soil type only explained 2.261% of the variation in the soil bacterial communities (Fig. 3). The RDA results showed that soil properties explained 31.85% (*PERMANOVA*, F = 2.018, *df* = 11, *P* = 0.001) and 27.83% (*PERMANOVA*, F = 1.933, *df* = 11, *P* = 0.001) of the variations in the bacterial *β*-diversity in the rhizosphere and bulk soils during secondary forest succession, respectively (Fig. 7a, b; Table S4). Soil SOC, TN, NH_4^+-N, TP, pH, EC, SM, C:N, C:P and N:P significantly affected the bacterial communities in both the rhizosphere and bulk soils at the three successional stages ($P < 0.05$) (Fig. 7a, b; Table S4). Among them, soil TP ($R^2 = 0.915$, $P < 0.001$) and NH_4^+-N ($R^2 = 0.937$, $P < 0.001$) were the most significant factors determining the bacterial *β*-diversity in the rhizosphere and bulk soils, respectively (Fig. 7a, b; Table S4). However, changes in soil NO_3^--N ($R^2 = 0.028$, $P = 0.841$) had no significant influence on the bacterial *β*-diversity in the rhizosphere soil (Fig. 7a). Soil texture had no significant influence on the bacterial *β*-diversity in the bulk soil ($P > 0.05$) (Fig. 7b).

The RDA results showed that soil properties explained 78.23% (*PERMANOVA*, F = 5.947, *df* = 11, *P* = 0.001) and 75.30% (*PERMANOVA*, F = 7.155, *df* = 11, *P* = 0.001) of the variations in the bacterial functional groups in the rhizosphere and bulk soils during secondary forest succession, respectively (Fig. 7c, d; Table S5). SOC ($R^2 = 0.514$, $P = 0.012$), NH_4^+-N ($R^2 = 0.512$, $P = 0.014$), TP ($R^2 = 0.623$, $P = 0.002$), C:P ($R^2 = 0.709$, $P < 0.001$), N:P ($R^2 = 0.694$, $P = 0.001$), pH ($R^2 = 0.660$, $P = 0.003$) and SM ($R^2 = 0.434$, $P = 0.035$) were the important factors that explained the changes in the bacterial functional groups in the rhizosphere soil (Fig. 7c; Table S5). Among them, C:P was the most important soil factor ($R^2 = 0.709$, $P < 0.001$). Furthermore, soil SOC ($R^2 = 0.425$, $P = 0.041$), NH_4^+-N ($R^2 = 0.730$, $P = 0.001$), TP ($R^2 = 0.896$, $P < 0.001$), pH ($R^2 = 0.797$, $P < 0.001$), SM ($R^2 = 0.631$, $P = 0.004$), EC ($R^2 = 0.589$, $P = 0.004$), C:P ($R^2 = 0.618$, $P = 0.002$), C:N ($R^2 = 0.588$, $P = 0.006$) and N:P ($R^2 = 0.707$, $P = 0.001$) had significant influences on the bacterial functional groups in the bulk soil (Fig. 7d; Table S5). Among them, TP ($R^2 = 0.896$, $P < 0.001$) and pH ($R^2 = 0.797$, $P < 0.001$) were the key driving factors.

Discussion

Differential responses of bacterial diversities in the rhizosphere and bulk soils to secondary forest succession

In this study, bacterial *α*-diversity in the rhizosphere soil revealed no significant changes during secondary forest succession ($P > 0.05$). This result is consistent with a previous study on karst mountainous secondary forest succession (Li et al. 2021). This result may be mainly related to the influence of dominant plants. In this study, the dominant specie in the grassland successional stage was *Poa annua*, which is arbuscular mycorrhizal (AM) specie (Montiel-Rozas et al. 2017). While the dominant species *Quercus aquifolioides* and *Picea asperata* in the shrubland and forest, respectively. *Quercus aquifolioides* and *Picea asperata* are ECM tree species and form symbiotes with ECM fungi (Zhang et al. 2022b). Previous studies have reported that AM fungi community assembly (Gruyter et al. 2022) and ECM fungi (Cumming et al. 2015; Wang et al. 2021a) affect the rhizosphere bacterial community and builds a unique rhizosphere bacterial community. Previous study in the subalpine region have found that the relative abundance of AM fungi in grassland soils was significantly higher than that in shrubland and secondary forest soils, while the relative abundance of ECM fungi revealed a contrary result (Zhang et al. 2022b). Thus, the specific changes in AM fungi communities in the grassland and ECM fungi communities in the shrubland and secondary forest during succession influenced rhizosphere bacterial community structure to lead to a stable bacterial *α*-diversity. The result of the correlation analysis also revealed no significant influences of soil physicochemical properties on bacterial *α*-diversity in the rhizosphere soil (Fig. S3a). These results indicated

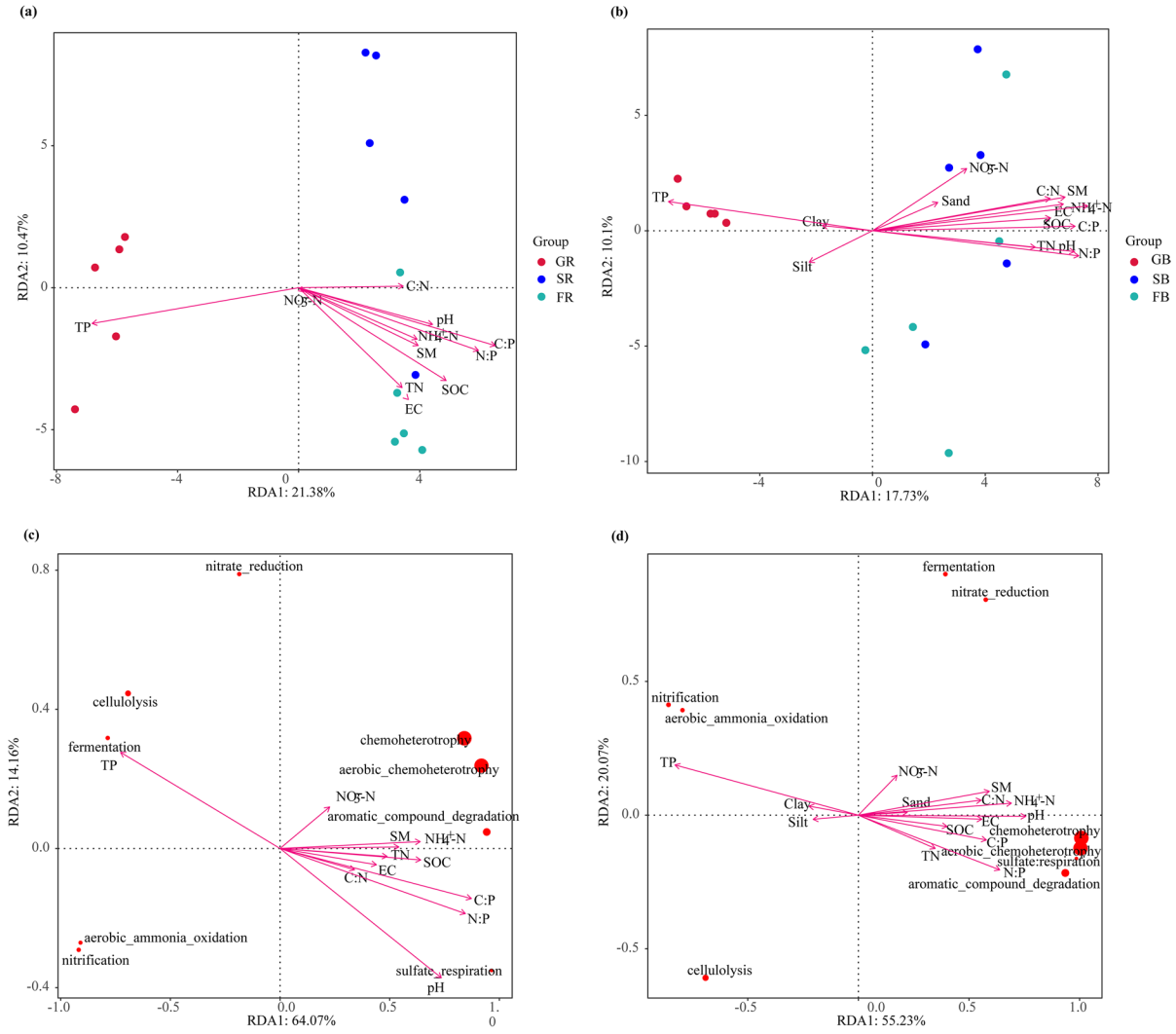


Fig. 7 Ordination plots of the redundancy analysis (RDA) results to identify the relationships among the (a) bacterial community (coloured circles) in the rhizosphere soil, (b) bacterial community (coloured circles) in the bulk soil, (c) bacterial functional groups (coloured circles) in the rhizosphere soil, (d) bacterial functional groups (coloured circles) in the bulk soil and the soil physicochemical properties (red arrows). The results of the significance tests for all parameters are provided in the supplementary materials. GR and GB represent the rhizosphere and bulk soils of the grassland, respectively; SR and SB represent the rhizosphere and bulk soils of the shrubland, respectively; FR and FB represent the rhizosphere and bulk soils of the secondary forest, respectively

that soil properties were not the major determining factors of bacterial α-diversity in the rhizosphere soil. By comparison, the bacterial α-diversity in the bulk soil in the shrubland and secondary forest was higher than that in the grassland ($P < 0.05$). This is consistent with previous studies on the succession of boreal forests (Jiang et al. 2021) and secondary forest succession on the Loess Plateau (Chai et al. 2019). This could be explained by the variations in plant richness and diversity (Chai et al. 2019), plant C and N concentrations (Liu et al. 2015) increased significantly during forest succession, causing resource diversity and microhabitat heterogeneity for microbial decomposers (Santonja et al. 2018), thereby enhancing bacterial α-diversity in the mid- and later succession stages. Moreover, bacterial α-diversity was also influenced by soil SOC, TN and NH_4^+-N during secondary forest succession, because the increase in soil

nutrients could provide more nutrients and energy sources for bacterial growth. The result of correlation analysis also revealed that SOC, TN, NH_4^+-N and NO_3^--N were significantly and positively correlated with bacterial α-diversity in the bulk soil ($P < 0.001$) (Fig. S3b). This result suggested that bacteria α-diversity in the rhizosphere soil was mainly affected by the dominant plant but was mainly affected by soil factors in the bulk soil.

The bacterial β-diversity in the rhizosphere soil showed significant changes during secondary forest succession ($P < 0.05$), which is consistent with the findings of Song et al. (2019) for grassland succession in a semiarid region. Indeed, previous studies pointed out that different plant species in a high mountain ecosystem selected specific rhizobacterial communities (Ciccazzo et al. 2014). These results are probably because plants secrete different root exudates (i.e., organic carbon, amino acids, polysaccharides and luteolin) during their growth, which could specifically promote or restrict the bacterial communities in the rhizosphere soil (Qu et al. 2020; Vives-Peris et al. 2020; Zhalnina et al. 2018). For example, grass secretes polysaccharides, which could recruit the specific bacterium *Azospirillum*, and coniferous forests secrete organic carbon and recruit Betaproteobacteria, Gammaproteobacteria and Actinobacteria (Qu et al. 2020). This indicated that the changes in dominant plant species have important influences on the bacterial community composition. In addition to plant species, other factors, such as soil factors, also have important influences on rhizobacterial communities (Song et al. 2019; Xu et al. 2021b). However, in the bulk soil, the bacterial β-diversity in the grassland was significantly different from shrubland and secondary forest, while the bacterial β-diversity was similar between the shrubland and secondary forest ($R = 0.04$, $P = 0.296$). This phenomenon is likely related to significant changes from grassland to shrubland and secondary forest, while small changes in soil properties (i.e., SOC, TN, NH_4^+-N, NO_3^--N, TP) at the mid- and later succession stages ($P > 0.05$) (Table S6). In summary, our results showed that secondary succession caused changes in soil bacterial diversity and community changes. However, due to the influence of the dominant plants, the bacterial diversity and community composition in the rhizosphere and bulk soil response inconsistently to forest succession. In addition, our result indicated that the effect of succession stage is stronger than the soil type (rhizosphere and bulk soil) (Fig. 3).

Potential bacterial functional groups reveal a consistent trend in the rhizosphere and bulk soils during secondary forest succession

Potential bacterial functional groups changed dramatically in the rhizosphere and bulk soils during secondary forest succession and revealed a consistent trend. In general, chemoheterotrophs and aerobic chemoheterotrophs were the dominant potential functional groups in the rhizosphere and bulk soils during the three succession stages, indicating that most bacteria cannot fix carbon and need to acquire carbon sources and energy sources by the oxidation of organic compounds (Zhang et al. 2018). The increase in chemoheterotrophs and aerobic chemoheterotrophs during secondary forest succession is beneficial to promote organic compound degradation and increase the soil nutrient content to meet the higher nutrient requirements during the conversion from grasslands to forests (Pellegrini 2016). In addition, bacteria involved in aromatic compound degradation were more abundant in shrubland and secondary forest than in grassland, which could promote the degradation of recalcitrant aromatic heteropolymer lignin in shrubland and secondary forest (Nacke et al. 2014). This result is consistent with that reported by Nacke et al. (2014), who revealed that the relative abundance of aromatic compound- degrading bacteria (*Phenylobacterium* and *Burkholderia*) was significantly higher in forest soil than that in grassland soil. Moreover, the relative abundance of sulfate-respiration bacteria increased significantly in the rhizosphere and bulk soils during secondary forest succession. This result might be associated with a significant increase in soil pH from grassland to the secondary forest (Table S6). Previous studies have also reported a strong positive correlation between sulphate-reducing bacteria and pH (George et al. 2020). In contrast, the relative abundance of bacteria involved in nitrification and aerobic ammonia oxidation (N cycle) in the shrubland and secondary forest was lower than that in the grassland in the rhizosphere and bulk soils during secondary forest succession. This might be due to nitrifying bacteria being more competitive in an oligotrophic environment (Che et al. 2018; Kits et al. 2017). Thus, the higher soil C and N contents in the shrubland and secondary forest resulted in a decrease in nitrifying bacteria from grassland to the secondary forest. The consistent change of bacterial functional groups in

the rhizosphere and bulk soil to secondary succession might be related to bacterial functional redundancy. That is, functional similarities exist between different taxonomic microorganisms to better adapt to environmental changes (Yang. 2021). For example, a wide variety of microorganisms can carry out aerobic respiration, thus the removal of one or several microbes might not affect microbial core functions (Yang. 2021). Previous studies have reported that the variations in microbial functional abundance are less than taxonomic variations in the soil and ocean, which is important for functional stability in different environments (Balmonte et al. 2018; Goss-Souza et al. 2019; Yang. 2021). In addition, many microorganisms reveal various functions, for example, *Bradyrhizobium*, *Streptomyces* and *Rhizobium* are not only the key C cycle bacteria, but also participate in N fixation (Stone et al. 2021; Thilakarathna and Raizada. 2017; Zhao et al. 2020; Terpolilli et al. 2016), which could also cause a similar change in bacterial function in the rhizosphere and bulk soil. Notably, although our results provide information on bacterial potential functional groups during succession, while less than 30% of bacterial taxa can be assigned to a potential function due to the limitation of the sequence of individual microbial and traditional pure-culture measures, which means that this result for bacterial functions is based on only some specific taxa rather than the whole bacterial community.

Bacterial co-occurrence network and keystone species are different in the rhizosphere and bulk soils during secondary forest succession

The co-occurrence networks of bacteria showed different patterns in the rhizosphere and bulk soils among the three successional stages; this result is in line with hypothesis 2. In the rhizosphere soil, the numbers of nodes, edges, average degree and connectedness were the highest in the secondary forest (Table 1), indicating the highest ecological network complexity in the rhizosphere soil of the secondary forest. Additionally, positive interactions were dominant (accounting for 56.18%—61.85%) among bacterial species at the three succession stages, indicating a higher mutualism rather than competitiveness of the rhizobacteria during secondary succession. In contrast, in the bulk soil, the bacterial network complexity was higher in shrubland and secondary forest in the bulk soil than those in grassland, with a higher numbers of nodes, edges, average degree, average path distance, clustering coefficient and connectedness in the shrubland and secondary forest than in the grassland. This suggests that bacterial networks in the grassland are more sensitive to the disturbance of environmental factors, since lower microbial network complexity is easily stressed by the environment (Banerjee et al. 2019). Moreover, we found that positive interactions were higher in shrubland and secondary forest than in grassland, indicating that the connected bacterial taxa were more interdependent in the mid- and later succession stages. This is because positive interactions of the microbial network indicated that the microorganisms were interdependent and showed similar preferences for the environment (Yuan et al. 2021).

Keystone species play critical roles in maintaining ecosystem function and can be detected as network hubs, module hubs and connectors using network analysis. The removal of keystone species will affect microbial community structure and the ecosystem function (Deng et al. 2012; Olesen et al. 2007; Yu et al. 2021). In this study, we identified the keystone species in the bacterial networks in the rhizosphere and bulk soil during the three successional stages (Fig. 6). Interestingly, the majority of the putative keystone species were taxonomically affiliated with Proteobacteria, Acidobacteria and Actinomycetes. This is consistent with previous studies in karst soil under different land uses (Cheng et al. 2021), in forests in ecosystem types from boreal temperate to tropical forests (Tu et al. 2020) and in the cold-temperate montane forests of China (Ji et al. 2022). This might be a result of more abundant bacterial taxa having a higher chance of coexisting with other taxa. Thus, the rich diversity contributes to influencing the ecosystem function. However, at higher taxonomic levels (i.e., at the family and genus levels), keystone taxa were almost completely different in both the rhizosphere and bulk soil among the three successional stages, indicating that secondary succession and dominant plant species had a great influence on keystone bacteria. This is also supported by previous studies, which revealed that different environmental conditions cause different keystone bacteria (Cheng et al. 2021; Li et al. 2022; Tu et al. 2020; Wang et al. 2021b). In this study, Chitinophagaceae,

Solibacteraceae, Burkholderiaceae, Xanthomonadaceae, Sphingobacteriales, Nitrosomonadaceae, *Pseudonocardia* and *Haliangium* were identified as "connectors" in the rhizosphere soil at the three successional stages and might have important influences on the interactions of other bacteria in the network during the forest succession. Specifically, Sphingomonadaceae, Xanthomonadaceae and Burkholderiaceae could participate in the decomposition of chemically recalcitrant C (lignin), and the ability to utilize refractory C sources makes these bacteria more competitive in environments with limited carbon sources (Diaz-Garcia et al. 2020; Goldfarb et al. 2011; Kern and Kirk 1987). Thus, refractory C-utilizing bacteria are likely to promote the soil C cycle during forest succession. *Pseudonocardia* is able to produce cellulase and may play an important role in cellulose degradation during litter decomposition (Ji et al. 2022; Malfait et al. 1984). Nitrosomonadaceae species (primarily *Nitrosovibrio* sp.) were the most essential bacteria participating in ammonia oxidation in the soil of a pine barren forest, which is important for the growth of pine (Shah et al. 2011). Furthermore, many studies have found that Chitinophagaceae, Xanthobacteraceae, Sphingomonadaceae and *Pseudonocardia* are associated with plant disease suppressiveness caused by *Fusarium graminearum* or *Rhizoctonia solani* or *Pythium ultimum*, contributing to plant disease resistance (Campos et al. 2016; Chapelle et al. 2016; Fang et al. 2022; Won et al. 2017). Thus, the presence of such keystone species might directly or indirectly regulate the soil nutrient cycle and plant health and productivity during the forest succession process, thereby further affecting the structure and function of the forest ecosystem during succession. Notably, we found that some rare species were identified as potential keystone species, such as *Tepidisphaerales*, Gaiellales, *Iamia*, *Niastella* and *Kineosporia*. This result suggests that in addition to abundant taxa, rare species also play an important role in maintaining the structure and function of bacterial networks during forest succession. This is supported by Jousset et al. (2017) and Wan et al. (2020), who indicated that rare species may play an important role in regulating ecosystem processes, ecological functional diversity and ecosystem stability against environmental disturbance. In summary, network analysis is important in revealing information on interactions of bacterial communities, keystone species and their responses to changing environments (He et al. 2017), providing more information on the importance of microbial species in ecology, thereby contributing to our understanding of ecosystem function and community structure (Tao et al. 2018).

Contrasting driving factors shaping bacterial communities and functional groups in the rhizosphere and bulk soils during secondary forest succession

Soil properties have been demonstrated to be important in determining the soil bacterial community structure (Chai et al. 2019; Jiang et al. 2021; Wang et al. 2019). In this study, soil properties (i.e., SOC, TN, NH_4^+-N, TP, C:P, C:N, N:P, pH, EC and SM) had significant influences on the bacterial community in the rhizosphere and bulk soils during secondary forest succession ($P < 0.05$) (Fig. 7a, 7b). However, the key driving factor explaining the variation in soil bacterial community dynamics was different in the rhizosphere and bulk soils. Specifically, soil TP was the key influential factor in the bacterial community in the rhizosphere soil ($R^2 = 0.915$, $P < 0.001$), which is in accordance with previous studies on fir in subtropical China (Wang et al. 2021c; Yan et al. 2021). These results were probably due to a significant decrease in the TP content in the rhizosphere soil during secondary forest succession, which could cause P limitation in the middle and later succession stages (Table S6). Previous studies have also reported extensive P limitation in the western Sichuan subalpine region (Ji et al. 2019; Yuan et al. 2019). Moreover, Duan et al. (2020) found that the soil P content can affect the composition of plant root exudates, thus causing changes in the composition of the rhizosphere bacterial community (Qu et al. 2020). In contrast, soil NH_4^+-N was the most important influential factor in the bacterial community in the bulk soil ($R^2 = 0.937$, $P < 0.001$). Previous studies found that the soil NH_4^+-N content exerts important effects on the abundance of Proteobacteria (Wang et al. 2019), Acidobacteria and Firmicutes (Ramirez et al. 2012), which is consistent with our results (Fig. S4b). Notably, previous investigations at different ecosystem and geographic scales have found

that soil texture is an important driver of soil bacterial communities, and the effects of soil texture are highly taxon-dependent (Dini-Andreote et al. 2014; Karimi et al. 2018; Xia et al. 2020). However, soil texture had no significant influence on the bacterial community in the current study ($P > 0.05$). This may be because the soil texture (sand and silt) did not change significantly during the secondary forest succession (Table S6).

For the bacterial functional groups, the soil properties explained 78.23% and 75.30% of the variations in the rhizosphere and bulk soils, respectively, indicating that the shift in bacterial functional groups was largely affected by soil properties during secondary forest succession. As expected in hypothesis 3, the soil bacterial functional groups in the rhizosphere and bulk soils were driven by different soil factors. Specifically, the soil C:P ratio was the most important soil factor that affected the rhizosphere bacterial functional groups ($R^2 = 0.709$, $P < 0.001$). This result implies that changes in the bacterial functional groups in the rhizosphere soil were mainly driven by soil C:P. In contrast, soil TP ($R^2 = 0.896$, $P < 0.001$) and pH ($R^2 = 0.797$, $P < 0.001$) were the key factors that influenced the bacterial functional groups in the bulk soil. The effect of TP may be related to local soil phosphorus limitation. In addition, soil pH increased significantly during secondary forest succession (Table S6), which may lead to changes in bacterial functional groups. Previous studies also found that soil pH significantly affected bacterial functional groups during the succession of boreal forests (Jiang et al. 2021).

Conclusion

Our results showed that bacterial communities revealed inconsistent responses in the rhizosphere and bulk soils to secondary forest succession. Soil properties explained 31.85% and 27.83% of the variations in the bacterial β-diversity in the rhizosphere and bulk soils, respectively, indicating that other factors (i.e., plant species) might also have important influences on bacterial communities. In contrast, bacterial functional groups in the rhizosphere and bulk soils responded consistently to secondary forest succession. Soil properties explained 78.23% and 75.30% of the variations in the bacterial functional groups in the rhizosphere and bulk soils, indicating that soil properties are the key factors contributing to the shift in bacterial functional groups in both the rhizosphere and bulk soils during secondary succession. In addition, the bacterial networks were most complex in the secondary forest in the rhizosphere soil, while in the bulk soil, shrubland revealed higher bacterial network complexity, indicating that rhizosphere and bulk soil bacterial interactions respond differently to secondary forest succession. Notably, distinct keystone bacteria were observed in the rhizosphere and bulk soils among the three succession stages, which are important for maintaining ecosystem function and community structure. In conclusion, our results enhance the understanding of the bacterial communities, functional groups, interactions and driving factors during secondary forest succession.

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Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals Not applicable.

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