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Dynamic changes in rhizosphere bacterial communities of *Rhododendron simsii* at different growth stages

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ABSTRACT:
Rhododendron simsii plays important roles in maintaining ecological system stability in the north temperate zone. However, its natural growth is greatly affected by soil microorganisms, particularly rhizosphere microbes. In this study, a comparative analysis was conducted of the bacterial community structure in the rhizosphere of *R. simsii* at the old, adult, juvenile, and seedlings stages. The results showed that Proteobacteria (38.53%–47.63%), Actinobacteria (23.45%–34.03%), and Acidobacteria (10.33%–17.79%) were the dominant phyla in the *R. simsii* rhizosphere. In particular, 3, 5, 42, and 33 OTUs were unique to the soil samples of 'old trees', 'adult trees', 'juvenile trees', and 'seedlings', respectively. Across four sets of *R. simsii* rhizosphere microbes sampled from seedlings to old trees, the OTUs first increased, then decreased, and finally increased. Overall, alpha diversity (Chao, ACE, and Sobs) revealed similar trends with the highest value i–n recorded for the rhizosphere sample of 'adult trees' and the lowest for the 'seedlings' sample. The bacterial genera in the rhizosphere samples from 'old trees' and 'adult trees' exhibited close clustering. Notably, the *R. simsii* population of 'juvenile trees', demonstrating the highest genetic diversity, were rich in *Bradyrhizobium* and *Streptomycetes*. This research serves to benefit the domestication of wild *R. simsii* and other *Rhododendron* resources.

Keywords:
bacterial populations, community structure, high-throughput sequencing, rhizosphere, *Rhododendron* species

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INTRODUCTION

Rhizosphere-associated microbes possess highly diverse metabolic capabilities, some of which are called plant growth-promoting rhizobacteria (PGPR) due to their contribution to increasing nutrient availability, shoot and root development, and phytohormone production, assisting plants to withstand abiotic stresses, and inhibiting the growth of potential pathogens by producing antibiotics, antifungal chemicals, and even insecticides (Zhang *et al.* 2017; Calabrese *et al.* 2022; de Andrade *et al.* 2023; Koprivova *et al.* 2023). In particular, rhizosphere-associated microbes also have the capacity to solubilise phosphate and nitrogen, and facilitate their uptake by roots (Zhao *et al.* 2016; Yang *et al.* 2017; Huang *et al.* 2020). Meanwhile, plants also exert selective pressures on the structural and functional diversity of microbial populations through root exudation, which may vary significantly depending on plant species, plant growth stage, soil properties, as well as other stress factors (Gomes *et al.* 2001; Yang *et al.* 2017; Lombardi *et al.* 2018; Kapagianni *et al.* 2021). Moreover, the change in

Table 1. Information on four *Rhododendron simsii* populations at different growth stages.

Group	Tree ages of *Rhododendron simsii*	Branches	Basal diameter (cm)
'seedlings' population	1–3 years	1	shorter than 2.5
'juvenile trees' population	5–10 years	5–10	2.5–7.5
'adult trees' population	50–100 years	20–25	7.5–12.5
'old trees' population	more than 100 years	more than 40	bigger than 12.5

mineral nutrition caused by root exudates is also essential for rhizosphere microbiota (Nelson & Mele 2007). Therefore, detailed research on soil microbiota serves to facilitate the comprehensive analysis of the taxonomy and functional diversity of microbial communities (Liu *et al.* 2014).

The Ericaceae family (comprising over 1000 species), a specialised plant group widely spread on nutrient-poor acidic soils of heathlands, peatlands, mire complexes, and even in the ground layers of temperate forests, may produce recalcitrant litter low in nitrogen and phosphorus, which may affect the characteristic chemistry of soils (Read *et al.* 2004; Popescu & Kopp 2013). Certain species have a tendency to form dense root systems, dominated by hair roots with inflated rhizodermal cells serving as hosts for microorganisms (Cairney & Ashford 2010). Accordingly, these microorganisms possess effective enzymatic apparatus for scavenging nutrients from proteins, chitin, peptides, mycelium, and plant-mycorrhizal necromass, thus changing the nutrient flows in soils (Kerley & Read 1998; Vohník *et al.* 2012). As integral members of this family, *Rhododendron* species exhibit different ecological types ranging from creeping plants growing a few centimetres tall to trees more than 30 meters high, thus playing vital roles in maintaining the stability of ecological systems (Wang *et al.* 2017a, 2021). For example, *R. ponticum* has the potential to affect soil ecology and reduce the number of a range of earthworm species (Vohník *et al.* 2012). As the dominant vegetation inhabiting the alpine tundra, *R. aureum* is vital in forming soil microbiota and even improving the soil fertility of the Changbai Mountains (Zhao *et al.* 2016).

Rhododendron simsii Planch. (2n = 26), a typical member of the *Rhododendron* genus with beautiful vegetative forms and brightly-coloured flowers, is a perennial deciduous shrub and is distributed at altitudes of 500–2500 m (Wang *et al.* 2019a). *Rhododendron simsii*, predominantly outcrossing with neighbouring individuals, possesses high out-crossing rates and significant biparental inbreeding (Wang *et al.* 2019a). Moreover, the flowering phenology of *R. simsii* is characterised by mass-flowering, gravity/wind seed dispersal, and pollen/pollinator limitation, which can result in 10–20 m gene dispersal distances (Wang *et al.* 2019a). Widely distributed across the Dabie Mountains (central China), *R. simsii* is even a constructive species in several regions, such as the Guifeng woods (Wang *et al.* 2017a). In addition to preventing soil erosion and maintaining the stability of ecological systems, wild *R. simsii* germplasm resources are also vital for developing new cultivars with desired ornamental characteristics (Hahn *et al.* 2017). In our previous study, high genetic variation was observed for the *R. simsii* population at different growth stages (Wang *et al.* 2019a, b).

As mutual environmental factors, vegetation and soil can interact with each other (Zhao *et al.* 2016). Soil characteristics, including chemical properties and microbial populations, may affect the occurrence, development, and even succession speed of vegetation (Zhao *et al.* 2016). Growing the same crop year after year may serve to lower soil pH, alter the ammonium nitrogen and carbon contents in the soil, and further affect the diversity and distribution of bacterial communities (Lauber *et al.* 2009; Zi *et al.* 2020). In the context of *R. simsii* species, the community structure of rhizosphere microbiota remains unclear. Understanding how plants influence microbial communities and their corresponding activities is very important for the transplantation and domestication of *R. simsii*. To investigate the dynamic responses of soil microbial communities to *R. simsii* plants at different growth stages, a comparative analysis of microbial community structure using high-throughput Illumina sequencing technologies was performed. Furthermore, the association between the genetic diversity of *R. simsii* populations revealed by microsatellites and rhizosphere microbial community composition was also investigated with the aim of clarifying the effects of rhizosphere-associated microbes on *R. simsii* populations.

MATERIALS AND METHODS

Materials. The growth stages of each *R. simsii* plant located in Huangshizhai forest park (N 31°10′14″, E 115°31′56″, 700–860 m, Hubei province, central China, in August 2021) were clarified according to the size of the basal diameter and the number of branches (Loubère *et al.* 2004): 'seedlings', 'juvenile trees', 'adult trees', and 'old trees' (Table 1). Three plants from the same group were identified (12 in total) and rhizosphere samples were taken as described below. Additionally, each plant was randomly framed within a 20 × 20 m quadrat and

the leaves from 30 plants per square plot were taken for assessing the diversity of the plants with SSR markers within the plot. The young leaves of *R. simsii* were directly frozen in liquid nitrogen, and then stored at -80°C for further use.

The minimum distance between two adjacent plots was set as 20 m (Cong *et al.* 2015). The rhizosphere was sampled as follows: firstly, excess bulk soils were flaked away, and those attached to roots were selected as rhizosphere; secondly, the obtained rhizospheres were washed off with sterile NaCl solution (0.85%). The samples were labelled as: 'seedlings' rhizosphere sample (Stage I-1, Stage I-2, and Stage I-3), 'juvenile trees' rhizosphere sample (Stage II-1, Stage II-2, and Stage II-3), 'adult trees' rhizosphere sample (Stage III-1, Stage III-2, and Stage III-3), and 'old trees' rhizosphere sample (Stage IV-1, Stage IV-2, and Stage IV-3). After removing plant and animal residues, these soil samples were sieved through a 2 mm mesh sieve. The rhizospheres were directly frozen in liquid nitrogen, and then stored at -80°C for further use.

Soil DNA extraction, purification, and quantification. Soil DNA extraction from each of the 1.0 g soil samples was performed using E.Z.N.A.® soil kit (Omega Bio-tek, Norcross, GA, U.S.A.) according to the manufacturer's instructions, and the quality and quantity of the DNA was verified using a Nanodrop spectrophotometer (Thermo Fisher Scientific/Nanodrop Products, Wilmington, Delaware, U.S.A.) and 1% agarose gel electrophoresis. The DNA was stored at -20°C until further analysis. Primers targeting the V3-V4 variable region (about 468 bp) of 16S rRNA were used: 338F: 5'-ACTCCTACGGGAGGCAGCAG-3'; 806R: 5'-GGACTACHVGGGTWTCTAAT-3' (Caporaso *et al.* 2011). PCR amplification was carried out on a GeneAmp 9700 thermocycler (Applied Biosystems) in a volume of 20 μL containing 4 μL 5 × Fast Pfu buffer, 0.8 μL primer pairs (5 μM), 20 ng genomic DNA, as well as 0.4 μL *Pfu* DNA polymerase (TransGen Biotech). PCR amplification conditions were set as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles (95°C for 30 s, annealing at 53°C for 30 s, and 72°C for 30 s), as well as a 10 min elongation at 72°C. Barcodes were attached to each amplified sample. The PCR products were then examined on 3% agarose gels, and purified by a DNA gel extraction kit (Axygen, Union City, CA, U.S.A.). Based on gel electrophoresis of the 16s rRNA gene amplification, precise quantitation was further performed using the QuantiFluor™dsDNA System (Promega). Specifically, the PCR products from all the samples were pooled in equimolar ratios.

Illumina Sequencing and data processing. Library construction was carried out using a TruSeq™ DNA Sample Prep Kit. The 2 × 250bp paired-end sequencing of the 16S rRNA PCR amplicons was performed by means of the Illumina MiSeq platform (Illumina, San Diego, CA, U.S.A.). Raw sequences were dereplicated into separate files by barcodes with the Galaxy Illumina sequencing pipeline (http://rccc.ou.edu), and quality trimming was performed using Btrim (Kong *et al.* 2011; Derakhshani *et al.* 2016). Short sequences (shorter than 50 bp) and those containing ambiguous bases were removed. Both forward and reverse raw reads were incorporated into full-length sequences with FLASH, and were clustered *de novo* into operational taxonomic units (OTUs) at a 97% similarity threshold (Edgar 2010; Magoč & Salzberg 2011; Devine *et al.* 2013). The taxonomy of different clusters was assigned at 97% sequence similarity against the SILVA database (version 132). Rarefaction analysis was performed with the original detected OTUs, and the taxonomic assignment was carried out using the RDP classifier with minimal 50% confidence estimates (Wang *et al.* 2007).

The genetic diversity of four *Rhododendron simsii* populations. The genomic DNA of the *R. simsii* leaves was extracted using the cetyltrimethylammonium bromide method, and 12 microsatellite markers were used according to our previous study (Wang *et al.* 2018). PCR amplification was carried out in 15 μL reaction volumes: 1.5 μL 10 × *Taq* buffer, 50 ng genomic DNA, 0.25 μM each primer, 250 μM dNTPs (2.5 mM each), and as 0.5 U *Taq* DNA polymerase (Tiangen). The PCR amplification conditions consisted of initial denaturation at 95°C for 8 min, 35 cycles (94°C for 40 s, annealing for 40 s, and 72°C for 50 s), and 5 minutes of longation at 72°C. Subequently, the PCR products were visualised on 6% (w/v) silver-stained denaturing polyacrylamide sequencing gels. The size of each DNA amplicon was determined by a comparison with the 20 bp DNA ladder (20–600 bp, Takara, China). The genetic parameters per locus, including the number of alleles (*Na*), Shannon's diversity index (*I*), observed heterozygosity (H_O), expected heterozygosity (H_E), and Nei's gene diversity (*h*), were calculated using Popgene 32 software (Wang *et al.* 2018).

Statistical analysis. The richness of microbial communities was calculated by counting all the samples and making comparisons between different groups. The overall alpha diversity indices, including Chao (richness estimator), ACE (abundance-based coverage estimator), Sobs (the observed number of species), as well as Shannon's and Simpson's diversity indices, were used to evaluate microbial community diversity. In addition, the Bray-Curtis distance was used to measure dissimilarities between any two types and ANOSIM was used to test the presence of any statistical differences between the microbial communities (Anderson & Walsh 2013; Vasquez *et al.* 2022). Mantel tests were used to evaluate the links between the rhizosphere microbial structure and the age of the *R. simsii* trees. Positive/negative cor-

Table 2. The sequencing information of the 16S rRNA gene in four rhizosphere samples.

Soil samples	Raw reads	Base number	Mean length	Minimum length	Maximum length	Clean reads
'seedlings' rhizosphere sample	69,621	30,156,889	433.16	279	530	25,349
'juvenile trees' rhizosphere sample	50,906	22,089,602	433.93	267	490	19,018
'adult trees' rhizosphere sample	61,844	26,833,522	433.89	260	484	18,024
'old trees' rhizosphere sample	58,784	25,498,167	433.76	279	546	22,395

Table 3. The diversity indices of the 16S rRNA gene in four rhizosphere soil samples, including Shannon's index, alpha diversity indexes (Chao, ACE, and Sobs), Simpson's index, and operational taxonomic units (OTUs).

Soil samples	Shannon	Chao	ACE	Sobs	Simpson	OTUs
'seedlings' rhizosphere sample	5.43	1036	1012	932	0.0120	1040
'juvenile trees' rhizosphere sample	5.47	1071	1059	958	0.0150	1133
'adult trees' rhizosphere sample	5.59	1080	1064	972	0.0145	1083
'old trees' rhizosphere sample	5.36	1062	1054	944	0.0156	1107

relations between the abundance of rhizosphere bacteria and the genetic diversity of the *R. simsii* populations were assessed using SPSS software. All the other statistical tests were performed with the Vegan package (Oksanen *et al.* 2020).

RESULTS

The structural variance in the bacterial communities of *Rhododendron simsii* rhizosphere. A total of 50,906–69,621 raw reads (22,089,602–30,156,889 bp) were obtained from four types of rhizosphere samples after sequencing the V3–V4 region of the 16S rRNA gene (Table 2). The minimum lengths were 279bp (the 'seedlings' rhizosphere sample and the 'old trees' rhizosphere sample), 267bp (the 'juvenile trees' rhizosphere sample), and 260bp (the 'adult trees' rhizosphere sample); while the maximum lengths were 530bp (the 'seedlings' rhizosphere sample), 490bp (the 'juvenile trees' rhizosphere sample), 484bp (the 'adult trees' rhizosphere sample), and 546bp (the 'old trees' rhizosphere sample). An average of 25,349, 19,018, 18,024, and 22,395 high quality clean reads were obtained for the 'seedlings' rhizosphere sample, the 'juvenile trees' rhizosphere sample, the 'adult trees' rhizosphere sample, and the 'old trees' rhizosphere sample, respectively.

At the cut off levels of 3%, 1040–1133 OTUs were identified in the four groups of rhizosphere samples. Across four sets of *R. simsii* rhizosphere microbes sampled from the seedlings to old trees, the OTUs first increased in the 'juvenile trees' rhizosphere sample, then decreased in the 'adult trees' rhizosphere sample, and finally increased in the 'old trees' rhizosphere sample. In particular, 897 OTUs were shared by all four groups. Meanwhile, 33, 42, 5 and 3 OTUs appeared only in the 'seedlings' rhizosphere sample, the 'juvenile trees' rhizosphere sample, the 'adult trees' rhizosphere sample, and the 'old trees' rhizosphere sample, respectively (Fig. 1). The Shannon and Chao indexes yielded values ranging from 5.36 to 5.59 and from 1036 to 1080, suggesting a relatively high diversity of bacterial sequences in the rhizosphere of *R. simsii* (Table 3). Moreover, the values of ACE were all above 1012. Overall, the indexes of Chao, ACE, and Sobs revealed similar trends, with the highest value observed for the 'adult trees' rhizosphere sample, and the lowest for the 'seedlings' rhizosphere sample, inferring that more microbial species were discovered in the 'adult trees' rhizosphere sample.

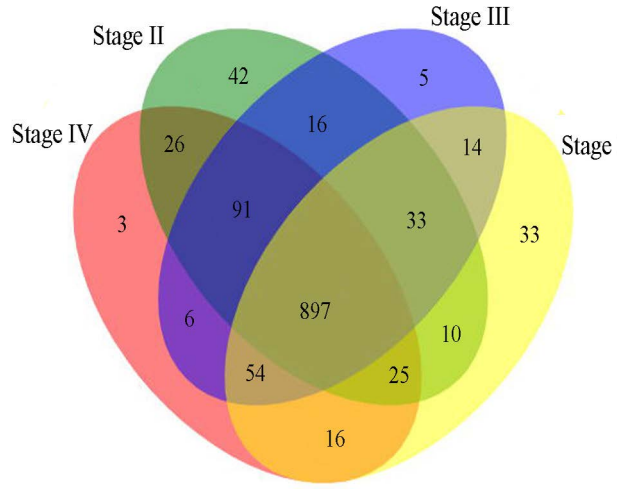

Fig. 1. Venn diagram of the number of OTUs shared by four types of rhizosphere bacteria. "Stage I", "Stage II", "Stage III", and "Stage IV" referred to the 'seedlings' rhizosphere sample, the 'juvenile trees' rhizosphere sample, the 'adult trees' rhizosphere sample, and the 'old trees' rhizosphere sample, respectively.

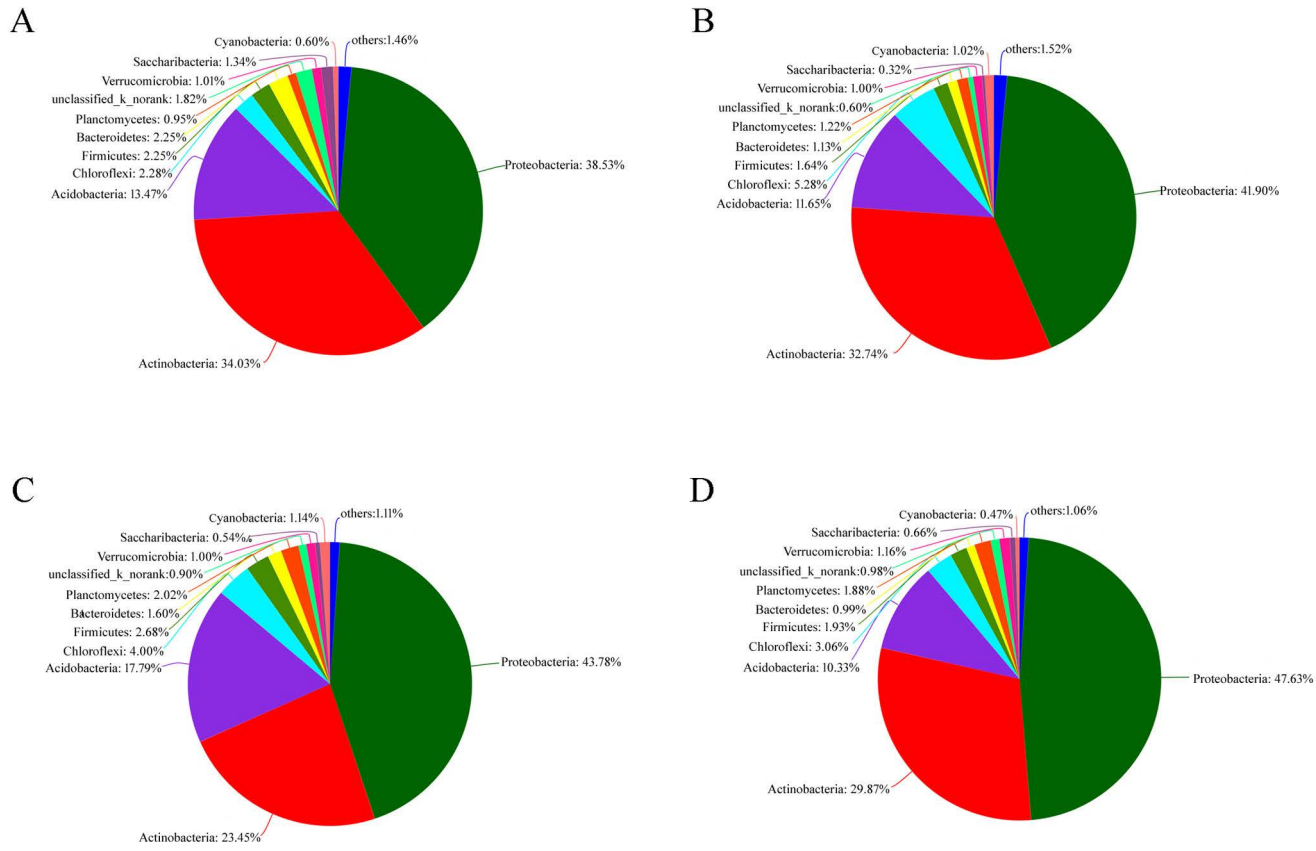

Fig. 2. The relative abundance of the rhizosphere bacteria at phylum level: (A) the 'seedlings' rhizosphere sample, (B) the 'juvenile trees' rhizosphere sample, (C) the 'adult trees' rhizosphere sample, (D) the 'old trees' rhizosphere sample.

The bacterial community in the rhizosphere of *Rhododendron simsii*. More than 98.5% of the microbial sequences in 12 rhizosphere samples were classified, including phylum Proteobacteria (38.53%–47.63%), Actinobacteria (23.45%–34.03%), Acidobacteria (10.33%–17.79%), Chloroflexi (2.28%–5.28%), Firmicutes (1.64%–2.68%), Planctomycetes (0.95%–2.02%), Verrucomicrobia (1%–1.16%), Bacteroidetes (0.99%–2.25%), Saccharibacteria (0.32%–1.34%), and Cyanobacteria (0.47%–1.14%) (Fig. 2). The largest proportion was the phylum Proteobacteria, followed by Actinobacteria in all four types of rhizosphere samples. As *R. simsii* grew, the percentage of Proteobacteria increased from 38.53% (the 'seedlings' rhizosphere sample) to 47.63% (the 'old trees' rhizosphere sample). Compared with the 'old trees' rhizosphere sample, the percentages of Bacteroidetes and Cyanobacteria were higher than Verrucomicrobia in the 'juvenile trees' and the 'adult trees' rhizosphere samples. Moreover, the phyla Verrucomicrobia, Bacteroidetes, and Saccharibacteria constituted a larger proportion than the phylum Planctomycetes in the 'seedlings' rhizosphere sample. The bacteria in the 'adult trees' and 'old trees' rhizosphere samples were clustered together, and then formed a further cluster with the bacteria of the 'juvenile trees' rhizosphere sample (Fig. 3). Notably, the bacteria of the 'seedlings' rhizosphere sample were clustered independently due to the low degree of similarity.

At the family level, the rhizosphere bacteria of *R. simsii* were clustered into 26 families. The top three main families varied in different rhizosphere samples: Burkholderiaceae, Acidothermaceae, and Bradyrhizobiaceae were dominant in the 'adult trees' and 'old trees' rhizosphere samples; Xanthobacteraceae, Bradyrhizobiaceae, and Acidothermaceae were the first three families detected in the 'juvenile trees' rhizosphere sample; Bradyrhizobiaceae, Solibacteraceae, and Xanthobacteraceae predominated in the 'seedlings' rhizosphere sample (Fig. 4). The Bradyrhizobiaceae family was dominant in all the rhizosphere samples. The Acetobacteraceae family was found in the 'adult trees', 'old trees', and 'seedlings' rhizosphere samples at percentages of 2.13%, 2.30%, and 2.71%, but absent in the 'juvenile trees' rhizosphere sample. Micrococcaceae (2.23%), Hyphomicrobiaceae (1.5%), and Streptomycetaceae (3.25%) were unique to the 'juvenile trees' rhizosphere sample. Caulobacteraceae (1.52%) was only found in the 'seedlings' rhizosphere sample.

At the genus level, *Burkholderia*, *Acidothermus*, and *Bradyrhizobium* were the main genera detected in

Table 4. The genetic diversity of four *Rhododendron simsii* populations at different life stages, including the number of alleles (*Na*), Shannon's diversity index (*I*), observed heterozygosity (H_O), expected heterozygosity (H_E), and Nei's gene diversity (*h*).

Populations	Growth stage	Numbers	*Na*	*I*	*Ho*	H_E	*h*
'seedlings' population	seeding	30	43	1.021	0.675	0.612	0.598
'juvenile trees' population	juvenile	30	52	1.198	0.889	0.693	0.655
'adult trees' population	adult	30	48	1.352	0.835	0.659	0.632
'old trees' population	old	30	47	1.143	0.847	0.674	0.643

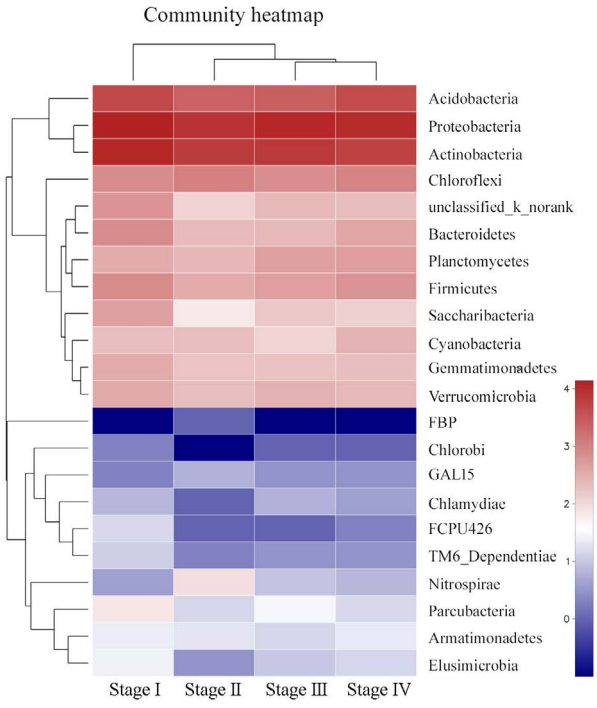

Fig. 3. Community heatmap of four types of rhizosphere bacteria. "Stage I", "Stage II", "Stage III", and "Stage IV" referred to the 'seedlings' rhizosphere sample, the 'juvenile trees' rhizosphere sample, the 'adult trees' rhizosphere sample, and the 'old trees' rhizosphere sample, respectively.

the community (Fig. 5). The species of the genus *Burkholderia* were the most abundant in the 'old trees' rhizosphere sample (13.97%); *Bradyrhizobium* was dominant in both the 'adult trees' and 'juvenile trees' rhizosphere samples, with percentages of 9.8% and 8%, respectively; and *Acidothermus* was the main genus in the 'seedlings' rhizosphere sample (14.58%). Moreover, *Mycobacterium*, *Variibacter*, *Candidatus_Solibacter*, and *Bryobacter* were also detected in the rhizosphere of the *R. simsii* populations at all ages. *Roseiarcus* was found only in the 'old trees' rhizosphere at a percentage of 1.45%. *Rhizomicrobium* was only found in the 'adult trees' rhizosphere at a percentage of 1.68%. *Streptomyces* (2.48%) and bacteria belonging to the family Micrococcaceae (2.23%) were unique to the 'juvenile trees' rhizosphere. Moreover, *Crossiella* (2.69%) was only observed in the 'seedlings' rhizosphere.

The genetic diversity of different *Rhododendron* populations and related analysis. The observed number of alleles (*Na*) in each *R. simsii* population ranged between 43 (the 'seedlings' *R. simsii* population) and 52 (the 'juvenile trees' *R. simsii* population). For each locus, the *Na* values varied from 2 to 7. Shannon's diversity index (*I*) was in the range of 1.021 (the 'seedlings' *R. simsii* population) to 1.352 (the 'adult trees' *R. simsii* population). The observed heterozygosity (H_O) and expected heterozygosity (H_E) varied in the ranges of 0.675–0.889 and 0.612–0.693, respectively. Nei's gene diversity (*h*) reached its highest level in the 'juvenile trees' *R. simsii* population ($h = 0.655$), but its lowest in the 'seedlings' *R. simsii* population ($h = 0.598$). Overall, the lowest genetic diversity was observed in the 'seedlings' population, followed by the 'old trees' population, and was at its highest in the 'juvenile trees' population (Table 4).

In the 'juvenile trees' *R. simsii* rhizosphere, Xanthobacteraceae (8.85%), Bradyrhizobiaceae (8.32%), and Acidothermaceae (5.97%) were the main rhizosphere bacteria. However, Acidothermaceae (14.58%), Bradyrhizobiaceae (7.09%), Solibacteraceae (6.36%), and Xanthobacteraceae (5.39%) were predominant in the 'seedlings' *R. simsii* rhizosphere. Moreover, *Streptomyces* and bacteria belonging to the family Micrococcaceae were only found in the 'juvenile trees' rhizosphere sample at percentages of 2.48% and 2.23%, respectively. At the significance level ($p < 0.05$), there was a strong significant positive correlation between the genetic diversity of the *R. simsii* population revealed by Shannon's diversity index (*I*) and the diversity of the *R. simsii* rhizosphere microbiome revealed by Sobs index ($r = 0.985$).

DISCUSSION

Rhododendron species, important horticultural plants with deciduous and evergreen species, are widely used in landscape greening and ecotourism (Christiaens *et al.* 2014). In greenhouse production, the most important cultivars are derived from *R. simsii* (Christiaens *et al.*

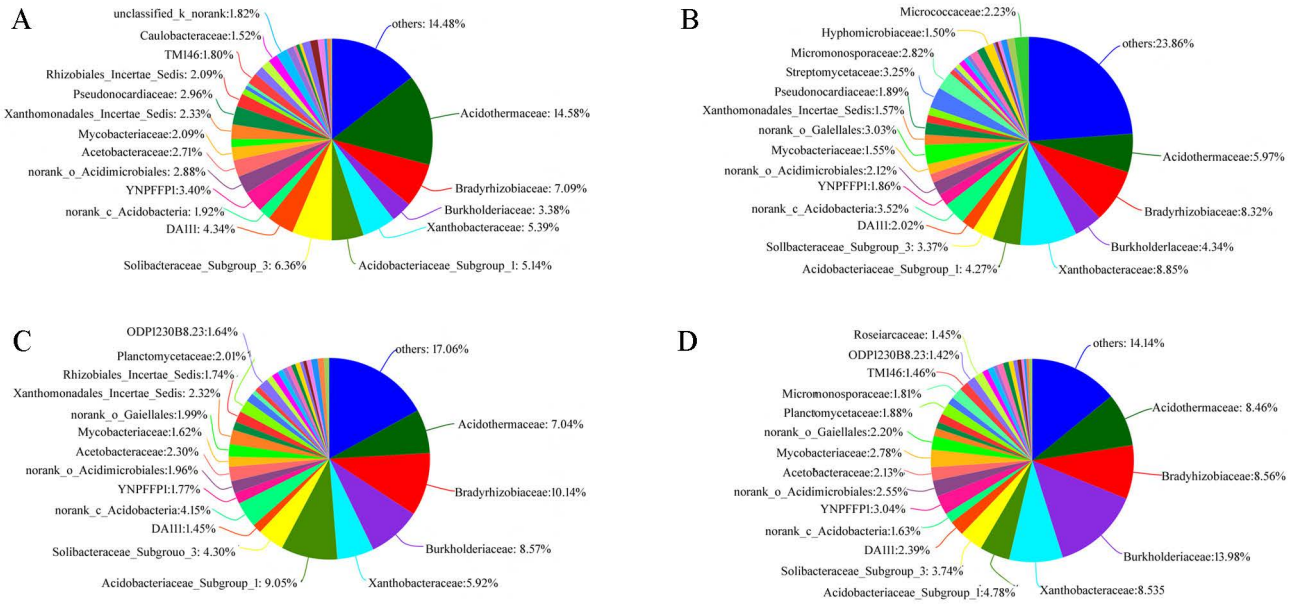

Fig. 4. The relative abundance of the rhizosphere bacteria at family level: (A) the 'seedlings' rhizosphere sample, (B) the 'juvenile trees' rhizosphere sample, (C) the 'adult trees' rhizosphere sample, (D) the 'old trees' rhizosphere sample.

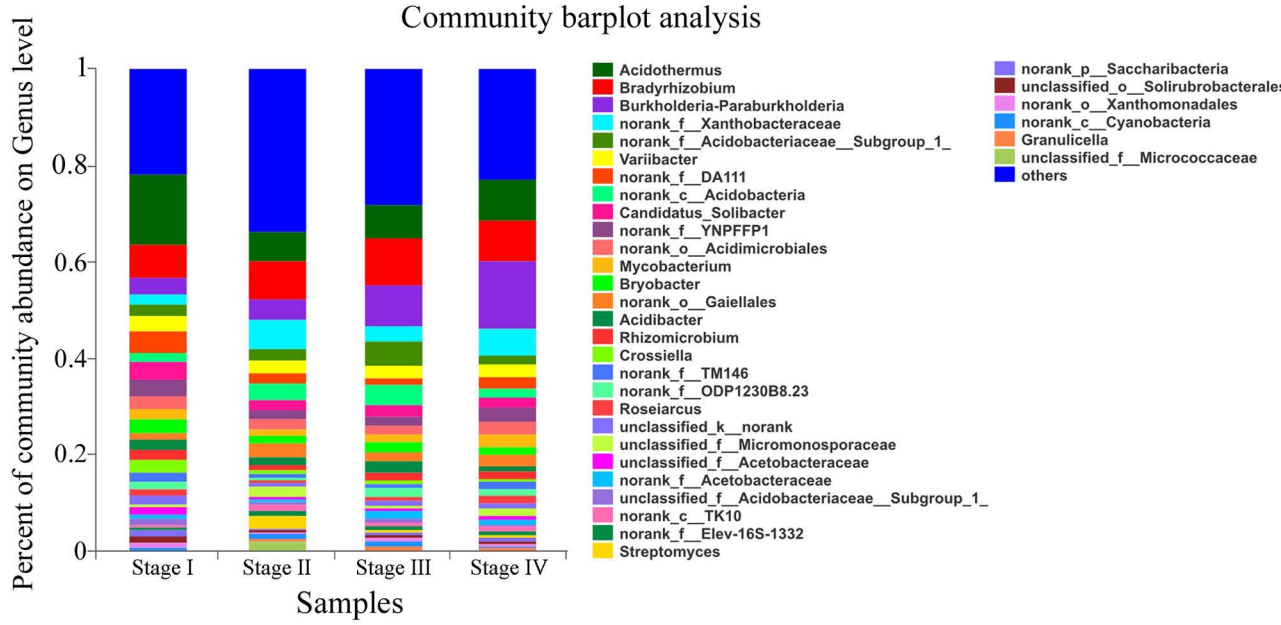

Fig. 5. The relative abundance of the rhizosphere bacteria at genus level: (A) the 'seedlings' rhizosphere sample, (B) the 'juvenile trees' rhizosphere sample, (C) the 'adult trees' rhizosphere sample, (D) the 'old trees' rhizosphere sample.

2014). However, the transplantation and domestication of *R. simsii* from mountains to low elevation areas is inefficient. Specifically, no flowering or delayed flowering, frequently occur after transplanting *R. simsii*. Rhizosphere microorganisms may greatly affect plant growth, and play an important role in the adaptability of plants to adverse environmental stresses (Yue *et al.* 2021). Therefore, in this study, the composition, diversity, and relative abundance of the microbial community in the rhizosphere of *R. simsii* at different life stages were analysed through Illumina high-throughput sequencing.

Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Firmicutes, and Gemmatimonadetes were the main phyla detected in the *R. simsii* rhizosphere, while similar results were also observed in the rhizosphere of maize (Yang *et al.* 2017). Likewise, Pro-

teobacteria (the main Deltaproteo bacteria and Gammaproteo bacteria), Chloroflexi, Bacteroidetes, Planctomycetes and Acidobacteria were found to be dominant in mangrove species *Bruguiera gymnorrhiza*, *Kandelia candel* and *Aegiceras corniculatum* (Wu *et al.* 2016). Proteobacteria is responsible for nitrogen fixation and polycyclic aromatic hydrocarbons (Johnston-Monje *et al.* 2016). Actinobacteria, widely distributed in soil and water ecosystems, has been claimed to play a critical role in humus formation and decomposition processes (Buée *et al.* 2009). Actinobacteria, producing numerous types of bioactive secondary metabolites, serve as biocontrol agents of economically important plant pathogens, including *Rhizotonia*, *Alternaria*, *Colletotrichum*, and *Fusarium* (García-Salamanca *et al.* 2013). Therefore, Actinobacteria (23.45%–34.03%) have the potential to effectively induce the resistance of *R. simsii* against biotic and abiotic stresses, exhibit antagonistic activity toward soil-borne pathogens, as well as promote plant growth. Acidobacteria, dominant irrespective of crop variety and land management, also emerged as an important phylum in the rhizosphere of *R. simsii* (García-Salamanca *et al.* 2013). Moreover, representatives of Acidobacteria may degrade polysaccharides and play essential roles in the carbon cycle of ecosystem (García-Salamanca *et al.* 2013; Noushahi *et al.* 2021).

The species of endophytic bacteria in roots are often similar to those found in soil (Lin *et al.* 2022). *Rhododendron simsii* plants have been observed to promote the formation of a unique soil microbial community, and a similar phenomenon was also detected in *R. aureum* (Wang *et al.* 2017b). In total, 26 families were classified in the *R. simsii* rhizosphere bacterial community, with Bradyrhizobiaceae being a consistent family in all four groups of *R. simsii* rhizospheres. However, the composition and relative abundance of the microbial community in the rhizosphere of *R. simsii* varied largely between different groups, which might be attributed to factors such as soil structure, root exudates, and even nutrients (Anna *et al.* 2021). For the 'juvenile trees' *R. simsii* population exhibiting the highest genetic diversity, the Acetobacteraceae family was not detected, whereas Micrococcaceae, Hyphomicrobiaceae, and Streptomycetaceae were unique in the rhizosphere.

The genera *Burkholderia*, *Acidothermus*, and *Bradyrhizobium* were the main bacteria in the *R. simsii* rhizosphere. As endophytic microbes, the members of the genera *Acidothermus*, *Bradyrhizobium*, and *Burkholderia* were significantly enriched in *R. simsii* roots under heat stress (day/night: 14/10h, 40/35°C), which may serve to assist *R. simsii* plants in resisting stressful environments (Lin *et al.* 2022; Liu *et al.* 2022). In the rhizosphere of eroded areas in the Loess Plateau, mulching was found to promote the relative abundances of *Bradyrhizobium*, which further played a major role in nitrogen fixation (Hao *et al.* 2021). In the rhizosphere of *Calotropis procera*, Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, and Firmicutes were the main bacteria (Ramadan *et al.* 2020). However, the genera *Bacillus*, *Enterobacter*, *Pseudomonas*, *Arthrobacter*, *Burkholderia*, *Acinetobacter*, and *Paenibacillus*, are all common PGPRs in the rhizosphere of numerous species (Zhang *et al.* 2017). *Bacillus* is a typical facultative anaerobic autogenous nitrogen-fixing bacterium (McSpadden Gardener 2004). The added nitrogen could be further exploited by Anaerobacteria, which degrade carbohydrates and simultaneously participate in many element cycles (C, N, Fe, and S) under anaerobic conditions. The interaction between Anaerobacteria and nitrogen-fixing *Bacillus* has the capacity to make soil more fertile (Zhang *et al.* 2019). As a member of the Ericaceae family, *R. simsii* is widely spread on nutrient-poor acidic soils with limited requirements for N elements, which is consistent with the absence of both *Bacillus* and Anaerobacteria in the *R. simsii* rhizosphere.

Root physiology may be influenced by plant growth stage, further affecting the quality and quantity of root exudates (Houlden *et al.* 2008). These difference in the root exudates of *R. simsii* at different tree ages might exert various selective pressures on root-associated bacteria. Specifically, a relatively high diversity of bacteria sequences existed in the rhizosphere microbial communities of *R. simsii*. The Shannon indexes (5.36–5.59) observed in the rhizosphere of *R. simsii* were similar to those found in maize (Yang *et al.* 2017). Moreover, the Chao index (1036–1080) was lower than that of the maize rhizosphere microbial communities (1276.304–1490.337) (Yang *et al.* 2017). In terms of the diversity indexes of the 16S rRNA gene in four rhizosphere samples, Shannon, Chao, and ACE were all at their highest in the 'adult trees' rhizosphere sample, but lowest in the 'seedlings' rhizosphere sample, which might be partially attributed to the variation in the root exudates of *R. simsii* plants at different growth stages.

These differences in the genetic diversity of *R. simsii* populations might be partially caused by carbon sources, as older *R. simsii* plants release more sugars and amino acids into the rhizosphere, which could act as carbon sources for soil bacteria (Victoria *et al.* 2021). The growth rates of *R. simsii* rhizospheric bacteria were assumed to be controlled by the soluble organic substrate concentrations. Differences in the composition of the microbial community and the proportion of dominant bacteria might also exert a certain influence on the genetic differentiation of *R. simsii* population. Some rhizospheric microbes, also known as plant beneficial rhizospheric microorganisms, may colonise the rhizosphere and thus improve plant growth, development and nutrient use efficiency (Meena *et al.* 2017). Host-plant diversity could drive genetic divergence and even the host-plant-mediated sympatric speciation in European corn borer (Martel *et al.* 2003). Intraspecific genetic

diversity might leave significant imprints on the surrounding community and ecosystem, and the impacts of genetic diversity are system, scale, and context dependent (Tack & Roslin 2011). According to correlation analysis, *Bradyrhizobium* might have a negative effect on the genetic diversity of *R. simsii* populations, while *Streptomyces* and certain bacteria belonging to Micrococcaceae may also impact on the genetic differentiation of *R. simsii* populations. Accordingly, *R. simsii* plants also affect the composition of the bacterial community structure.

In terms of *R. simsii* plants at different growth stages, different dominant bacteria were detected, which certainly holds implications for the transplantation and domestication of *R. simsii* plants. However, the diversity, function, and application of rhizosphere bacteria in relation to *R. simsii* plants require great attention, and corresponding research will provide valuable insights into the roles of microbes in promoting plant growth.

CONCLUSION

In this study, the rhizosphere bacteria of *R. simsii* at different growth stages were investigated with high throughput DNA pyrosequencing of the 16S rRNA gene. Comparative analysis of the bacterial community structure in the rhizosphere of *R. simsii* at seedling, juvenile, adult, and old stages showed that Proteobacteria (38.53%–47.63%), Actinobacteria (23.45%–34.03%), and Acidobacteria (10.33%–17.79%) were the dominant phyla in the *R. simsii* rhizosphere. Across four sets of *R. simsii* rhizosphere microbes sampled from seedlings to old trees, the OTUs first increased, then decreased, and finally increased. The alpha diversity (Chao, ACE, and Sobs) revealed similar trends: the highest value was recorded in the 'adult trees' rhizosphere sample with the lowest value in the 'seedlings' rhizosphere sample. The bacterial genera in the 'old trees' and 'adult trees' rhizosphere samples were clustered together. In particular, the 'juvenile trees' *R. simsii* population, with the highest genetic diversity, were rich in *Bradyrhizobium* and *Streptomycetes*. This research could also provide insights into the correlation between *R. simsii* populations and microbes. Moreover, the dominant soil bacteria identified in this research might be beneficial for the further domestication and genetic improvement of *R. simsii* and other *Rhododendron* species.

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REZIME

Botanica SERBICA

Dinamika promena u bakterijskim zajednicama rizosfere *Rhododendron simsii* u različitim fazama rasta

Jun Fu, Yirong Sun, Yuqing Hu, Lan Lu, Zhiwei Huang, Chunyu Zhang i Shuzhen Wang

Rhododendron simsii igra važnu ulogu u održavanju stabilnosti ekološkog sistema severne umerene zone. Međutim, na njegov prirodni rast u velikoj meri su uticali mikroorganizmi u zemljištu, posebno mikrobi iz rizosfere. U ovoj studiji urađena je komparativna analiza strukture bakterijske zajednice u rizosferi *R. simsii* u starim, odraslim, juvenilnim i fazama klijanaca. Rezultati su pokazali da Proteobacteria (38,53%-47,63%), Actinobacteria (23,45%-34,03%), i Acidobacteria (10,33%-17,79%) su dominantni tip u rizosferi *R. simsii*. Konkretno, 3, 5, 42, i 33 OTUs su unikatni za uzorke zemljišta „starih stabala", „odraslih stabala", „mladih stabala" i „klijanaca". U četiri seta mikroba *R. simsii* rizosfere uzorkovanih od sadnica do starih stabala, OTU su se prvo povećale, zatim smanjile i na kraju povećale. Sve u svemu, alfa raznovrsnost (Chao, ACE i Sobs) otkrila je slične trendove: najveću vrednost u uzorku rizosfere „odraslih stabala", a najnižu u „uzorku klijanaca". Rodovi bakterija u uzorcima rizosfere „starih stabala" i „odraslih stabala" su grupisani zajedno. Zajednice „mladih stabala" *R. simsii* koje poseduju najveću genetičku raznovrsnost, pokazale su bogatstvo sa *Bradyrhizobium* i *Streptomycetes*. Rezultati ovih istraživanja se mogu koristiti u pripitomljavanju divljeg *R. simsii* i drugih resursa *Rhododendron*-a.

Ključne reči: bakterijske populacije, struktura zajednice, sekvenciranje visoke propusnosti, rizosfera, *Rhododendron* vrste