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Soil properties and root traits are important factors driving rhizosphere soil bacterial and fungal community variations in alpine *Rhododendron nitidulum* shrub ecosystems along an altitudinal gradient

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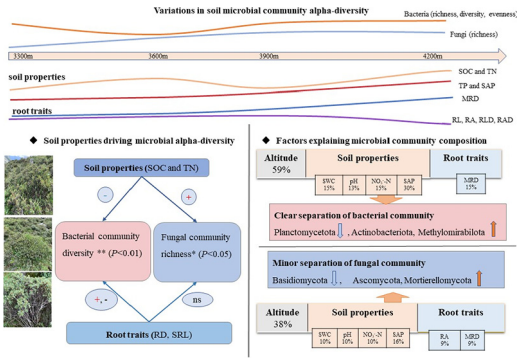
HIGHLIGHTS

- Rhizosphere soil bacterial and fungal alpha-diversity and community structure varied along altitudes.
- Rhizosphere soil microbial alpha-diversity was related closely with soil properties.
- Rhizosphere soil microbial community variations were mainly affected by soil properties and root traits of host plant.
- The responses to altitude of soil microbes varied with microbial taxa.

GRAPHICAL ABSTRACT



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ABSTRACT

Both soil properties and plant root traits are pivotal factors affecting microbial communities. However, there is still limited information about their importance in shaping rhizosphere soil microbial communities, particularly in less-studied alpine shrub ecosystems. To investigate the effects of altitude (3300, 3600, 3900, and 4200 m) on the diversity and composition of rhizosphere soil bacterial and fungal communities, as well as the factors shaping rhizosphere soil microbial communities, we conducted this study in alpine *Rhododendron nitidulum* shrub ecosystems from the Zheduo mountain of the eastern Tibetan Plateau. Results demonstrated that bacterial community diversity and richness decreased to the lowest value at 3600 m and then increased at higher altitudes compared with 3300 m; whereas fungal richness at 3300 m was much lower than at other altitudes, and was closely related to soil properties and root traits. The composition of rhizosphere soil bacterial and fungal communities at the low altitude (3300 m) was different from that at high altitudes. Permutational multivariate analysis of variance and redundancy analysis indicated that soil properties (soil water content, pH, NO_3^--N, and available phosphorus) and root traits (surface area, and maximum depth) were the major factors explaining the variations of rhizosphere soil bacterial and fungal communities. Specific bacterial and fungal taxa along altitudes were identified. The bacterial taxa Planctomycetota was dominant at 3300 and 3600 m with low soil nutrient availability and high root surface area, whereas the fungal taxa Mortierellomycota was abundant at 3900 and 4200 m with high soil nutrient availability and low root surface area. These results suggested that different soil microbes can respond differently to altitude. This study provides a novel insight into factors

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driving rhizosphere soil bacterial and fungal community variations, which could improve our understanding of microbial ecology in alpine *R. nitidulum* shrub ecosystems along altitude.

1. Introduction

Soil microorganisms play a curial role in soil biogeochemical cycles (Saitta et al., 2018). Bacteria and fungi are the most abundant and diverse microorganisms in soil, with a high plasticity and adaptability to environments (Frąc et al., 2018; Gans et al., 2005). They regulate soil nutrient bioavailability to maintain ecosystem functions by mineralizing organic matter, binding of humus compounds in soil mineral layers, and providing nutrients for plants (Dahlberg and Bültmann, 2013; Koranda et al., 2013; Nacke et al., 2011). Soil microbial diversity, composition and fungi-to-bacteria-ratio are dependent on altitude, soil properties, and vegetation composition (Bayranvand et al., 2021; Tedersoo et al., 2014). Alpine ecosystems represent a primary reservoir of global biodiversity to provide various ecological services (Li et al., 2018; Llado et al., 2017). In alpine ecosystems, climate, plant and soil traits differ significantly over a short spatial distance (Bayranvand et al., 2021). Altitude could serve as a natural platform to investigate soil microbial variations and adaptations. However, the altitudinal patterns of microbial community diversity appear to be complex, such as decreasing (Shen et al., 2015), increasing (Wang et al., 2017a), getting peak in the mid-altitude (Singh et al., 2012), and no consistent (Shen et al., 2013) patterns. Moreover, due to the progresses and difficulties of sampling in alpine regions, the altitudinal pattern of soil microbial communities in alpine ecosystems still remains controversial.

In recent years, some studies have investigated the variations and important factors affecting soil bacterial and fungal community diversity, biomass, and composition along an altitudinal gradient (Ren et al., 2018; Siles and Margesin, 2016; Nakayama et al., 2019). Bayranvand et al. (2021) reported that soil bacterial and fungal community diversity and the abundance of some taxa, such as Acidobacteria, monotonically decreased with an increasing altitudinal gradient (0–2500 m), which further suggested that moderate N concentration and pH contribute to high microbial diversity (Bayranvand et al., 2021; Chen et al., 2018). However, Cui et al. (2019) observed different changes in soil bacterial and fungal alpha-diversity along a high altitudinal gradient (2800, 3000, 3200, and 3500 m) in the Tibetan Plateau, showing an initial increase and then a decrease for the bacterial Shannon–Wiener diversity, whereas the fungal diversity was maintained stable. They also revealed that soil bacterial and fungal communities were governed by specific soil factors, e.g., total phosphorus (P) content for bacteria and nitrate-nitrogen (NO_3^--N) content for fungi. Therefore, it is important to understand the patterns and drivers of soil microbial community along an altitudinal gradient of alpine ecosystems for elucidating microbial processes and predicting the functions of alpine ecosystems.

Soil nutrient availability, plant properties, and spatial factors affect soil microbial diversity and community composition along an altitudinal gradient (Bayranvand et al., 2021; Jamil et al., 2020). Soil pH is considered as a decisive factor for the global distribution of soil bacterial community (Shen et al., 2013, 2015; Shi et al., 2014), such as Acidobacteria (Shen et al., 2015). Both soil carbon (C) and nitrogen (N) are also the determining factors for fungal community composition. Excessive soil N is often negatively associated with fungal diversity (Bahram et al., 2012; Yao et al., 2017), whereas a high C content in soils may enhance microbial growth, support a wider range of fungal taxa (e.g., Basidiomycota and Ascomycota), and modify microbial life strategies (Bahram et al., 2012; Siles and Margesin, 2016). Furthermore, soil microbial communities are highly dependent on root traits (Spitzer et al., 2021; Yang et al., 2021). They can influence soil microbial communities through inputs of C by root litters (Yang et al., 2021), or through the symbiotic root organisms (Canini et al., 2019). For example, root morphological traits (e.g., root diameter, length and surface area) that are related to nutrient acquisition strategies and C inputs drive soil microbial communities in the process of C flow and nutrient dynamics (Sweeney et al., 2021; Table S1). Other studies have also suggested that there are no clear relationships between altitude and root traits across plant species (Weemstra et al., 2020; Spitzer et al., 2021). Based on the expected relationships between root traits and altitude (Table S1), it is necessary to explore how root traits affect microbial communities along altitude.

The rhizosphere is the chemical, physical and biological complex zone of soil surrounding plant roots and primarily influenced by the activity of roots (Urrutia et al., 2015), whereas microbial communities are dependent on soil properties and root performance (Wang et al., 2020). Although some studies were conducted on alpine ecosystems (Bayranvand et al., 2021; Tedersoo et al., 2014), the complex impact of altitude on the structural composition of rhizosphere microbial communities associated with root traits and soil properties are still limited. *Rhododendrons*, distributing widely between 2700 and 3700 m a.s.l, are important shrub species in alpine ecosystems and have specific associations with ericoid mycorrhiza that will considerably affect the diversity and composition of rhizosphere microbial communities (Wang et al., 2017c). Here, we collected rhizosphere soil and root samples of *Rhododendron nitidulum* along an altitudinal gradient in alpine shrub ecosystems to investigate the effects of altitude on rhizosphere soil microbial communities, and explore the driving factors in relation to soil properties and root traits. We hypothesized that: (i) the alpha-diversity and composition of soil bacterial and fungal communities would change with altitude and alpha-diversity would be low at high altitudes (3900 and 4200 m) due to low pH and soil nutrient availabilities, (ii) soil properties would be important factors affecting the diversity and composition of bacterial and fungal communities along an altitudinal gradient, and (iii) root morphological traits (e.g., length, surface area, and diameter) could be largely related to soil bacterial and fungal communities, as they can affect soil microbial communities by driving C and nutrient inputs to soil microbes in the process of nutrient flow.

2. Materials and methods

2.1. Study site

The study site was located in the Zheduo mountain (30°05′ N, 101°82′ E), Kangding county, in western Sichuan province, which is characterized by rainy summer and snowy winter. The annual mean temperature was 7.09 °C, and the monthly mean temperature ranged from −2.2 °C in January to 15.5 °C in July. The annual precipitation was 800–950 mm, and 77 % of the rainfall occurred from May to September (Chen et al., 2016). The dominant shrub was *Rhododendron nitidulum* Rehd. et Wils., which ranged from 3300 to 4200 m with altitudinal gradient (Table 1, Fig. S1).

2.2. Experimental design and sampling

In the study area, 20 plots (4 altitudes × 5 plots each altitude) were selected over 300 m intervals with an altitudinal gradient (3300, 3600, 3900 and 4200 m a.s.l.). At each altitude, we collected samples from five plots with 10 × 10 m being separated by a distance of at least 20 m from each other. The field study was conducted in August 2019. Plant height and basal diameter of *R. nitidulum* in each plot were measured. From 3300 m to 4200 m, the average height was 63.19, 59.39, 20.74, and 16.42 cm, respectively (Table 1).

After removing the upper litters, we excavated five clusters of *R. nitidulum* containing the whole roots using a spade from the center and the four sides of each plot (Adamo et al., 2021). Each cluster had a soil cube (50 × 50 × 50 cm). The maximum root depth of each cluster was

Table 1
Basic information of *Rhododendron nitidulum* shrubs, environmental data and dominant species along altitude (mean ± SE, n = 5).

Altitude	Height (cm)	BD (mm)	MAT (°C)	MAP (mm)	Dominant species
3300 m	63.19 ± 8.79	5.89 ± 0.97	5.74	756.27	Shrub: *Rhododendron nitidulum*, *Lonicera tangutica* Herb: *Thalictrum uncatum*, *Geranium wilfordii*, *Rodgersia sambucifolia*
3600 m	59.39 ± 9.37	7.33 ± 1.88	3.82	758.17	Shrub: *Rhododendron nitidulum*, *Salix cupularis* Herb: *Carex capilliformis*, *Deyeuxia scabrescens*, *Festuca ovina*
3900 m	20.74 ± 4.03	4.679 ± 1.02	1.91	757.36	Shrub: *Rhododendron nitidulum* Herb: *Polygonum viviparum*, *Deyeuxia scabrescens*, *Festuca ovina*
4200 m	16.42 ± 6.18	5.03 ± 2.05	0.79	758.17	Shrub: *Rhododendron nitidulum* Herb: *Polygonum viviparum*, *Bistorta macrophylla*, *Deyeuxia scabrescens*

BD, basal diameter; MAT, mean annual temperature; MAP, mean annual precipitation. The environmental data were extracted by WorldClim (http://www.worldclim.org/).

measured using a tape. Rhizosphere soil was obtained using the method of De Feudis et al. (2017). Specifically, the soil firmly adhering to the fine roots was considered as rhizosphere soil and was collected by shaking and soft brushing. Soil samples were sieved below 2-mm mesh, transported to the laboratory and then divided into two subsamples, of which one subsample was stored at −80 °C for DNA analysis, and the other was stored at 4 °C to measure soil properties. After collecting the rhizosphere soil, we collected root samples. Root samples were cleaned up using water and then transported to the laboratory at 4 °C to measure root traits.

2.3. Determination of soil properties and root traits

Soil water content (SWC) was determined with the gravimetric method by drying fresh soil at 105 °C for 24 h. Soil pH was measured using a pH meter in a 1:2.5 (m:v) soil-to-water extract. The concentrations of NO_3^--N and ammonium (NH_4^+-N) were measured using a continuous flow analyzer (Auto Analyzer III, SEAL Analytical GmbH, Norderstedt, Germany) according to a previously described method (Wang et al., 2020). Concentrations of soil organic carbon (SOC) and total N (TN) were determined using an Elementary Analyzer (Vario MACRO cube, Elementar, Hanau, Germany) (Wang et al., 2020). Total phosphorus (TP) was extracted from the soil samples using the digestion of $HClO_4$ + H_2SO_4 acids (Parkinson and Allen, 1975). The concentration of soil available phosphorus (SAP) (Olsen-P) was determined by ultraviolet visible spectrophotometer (Olsen and Sommers, 1982).

In the laboratory, after collecting and dissecting roots samples, the identified living fine roots (<2 mm) were washed gently in distilled water, and then scanned at 600 dpi using a scanner (Epson expression 10000XL, Seiko Epson Corporation, Nagano, Japan). The obtained images were processed using the WinRHIZO software (WinRHIZO Pro 2007, Régent Instruments, Quebec, Canada) to get its mean diameter, length, and surface area. After scanning, root samples were oven dried at 60 °C to reach a constant weight. Specific root length (m g^{-1}) and surface area (m^2 g^{-1}) were calculated as the total length and surface area divided by the corresponding dry mass, respectively. Root tissue density (g cm^{-3}) was calculated as the root dry mass divided by the total volume. Root surface area density (m^2 m^{-3}) was calculated as the total surface area per soil volume, and root length density (km m^{-3}) was calculated as the total length per soil volume (Li et al., 2018).

2.4. DNA extraction, amplification, and sequencing

Microbial DNA was extracted from 0.5 g rhizosphere soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) following the manufacturer's instructions. The V4-V5 region of the bacterial 16S rRNA gene was amplified with primer pairs 515F (5′-GTGCCAGCMGCCG CGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) using an ABI GeneAmp® 9700 polymerase chain reaction (PCR) thermocycler (ABI, CA, USA) (Jiang et al., 2018, 2019). PCRs were carried out in triplicates in a 20 μL reaction mixture containing 4 μL of 5 × *TransStart* FastPfu buffer, 2 μL (2.5 mM) dNTPs, 0.8 μL (5 μM) of each primer, 0.4 μL *TransStart* FastPfu DNA Polymerase, 10 ng template DNA, and 10 μL double-distilled water. The PCR thermal cycling program consisted of initial denaturation at 95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and then a final extension at 72 °C for 10 min, and end at 4 °C. PCR product was extracted from 2 % agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using Quantus™ Fluorometer (Promega, Madison, WI, USA). Raw amplicon sequences were deposited in the NCBI short read archive under Bioproject ID (PRJNA765434).

The fungal ITS1 region was amplified using the primers ITS1F (5′-CTTG GTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCATCGATGC-3′) (Adams et al., 2013). PCR steps were performed as described for the bacterial 16S rRNA gene amplification. Purified amplicons pooled in equal amounts, with paired-end sequenced (2 × 300 bp), were performed using the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai) via the Illumina MiSeq platform (Illumina). Raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA765449).

2.5. Bioinformatic analysis

The Illumina sequencing data were analyzed using Flash with the option max-overlap 200 (Magoc and Salzberg, 2011), and the unassembled sequences were removed (572,640 and 1,090,576 sequences for bacterial and fungal community, respectively). FASTQ files were processed and implemented in QIIME 1.9.1 (Caporaso et al., 2010). Bacterial sequences were searched against the Ribosomal Database Project (RDP) Classifier to identify and discard chimeric sequences using Mothur operated under default setting (Schloss et al., 2009). Operational taxonomic units (OTUs) with 97 % similarity cutoff were clustered using UPARSE (version 7.1) (5366 and 3959 OTUs remaining for bacterial and fungal communities, respectively), and the taxonomy of each OTU representative sequence was analyzed against the 16S rRNA database (Silva) using confidence threshold of 0.7. Fungal ITS sequences were assigned to taxa using the naive Bayesian classifier, and the UNITE-curated International Nucleotide Sequence Database (Abarenkov et al., 2010). OTUs with 97 % similarity cutoff (Caporaso et al., 2010) were clustered using UPARSE version 7.1 (Edgar, 2013).

OTUs of bacterial and fungal datasets with low abundance (≤10 sequences across all 20 samples) were filtered and discarded from the OTU table (Brown et al., 2015; Oliver et al., 2015). Rarefaction curves for soil bacterial and fungal community were obtained (Fig. S2). Alpha-diversity of bacterial and fungal communities, including the OTU richness (Sobs), Shannon's diversity index (Shannon), and Shannon's evenness index (Shannon-even), were calculated using the Mothur (version 1.30.2) program (Schloss et al., 2009).

2.6. Data analysis

To test the effects of altitude on the rhizosphere soil properties, root traits and microbial community diversity, we performed a one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests ($P < 0.05$). All data were first tested for the homogeneity of variance and normality of

distribution before statistical analyses, and the log-transformation analysis was used when necessary. Correlations between microbial alpha-diversity and soil properties, and root traits were determined using the Spearman correlation analysis.

Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity matrix ("vegan" package) were conducted to visualize differences in microbial community composition along altitudes (dimensions = 2, initial configurations = 100, model = global, maximum number of iterations = 200, convergence ratio for stress = 0.999999) (Oksanen et al., 2015). Before further analysis, the "vif. cca" command was conducted to find the values of variance inflation factors (VIF) of all variables to resolve the collinearity of the explanatory variables (VIF <10, Oksanen et al., 2015). Thus, eight factors (pH, SWC, SAP, NH_4^+-N, NO_3^--N, root surface area, mean diameter, and maximum root depth) were used as explanatory variables.

Permutational multivariate analysis of variance (PERMANOVA) was run based on Bray–Curtis distance matrices obtained of Hellinger-transformed OTU tables with 9999 permutations (with the Adonis function) (Anderson, 2001) to test the respective effect of root traits and soil properties, as well as altitude, on the rhizosphere soil microbial communities. The relationship between root traits, soil properties and rhizosphere microbial community (at phylum level) was determined by redundancy analysis (RDA) using the vegan package (Ji et al., 2021). Heatmap (Spearman's rank correlation) was presented by the ggplots, RColorBrewer, and vegan packages in R (Chen et al., 2021) to explore the relationship between root and soil variables and relative abundances of dominant phylum in rhizosphere soil bacterial and fungal communities. All of above analyses were performed using R version 3.6.2 (R Development Core Team, 2019).

3. Results

3.1. Rhizosphere soil properties and root traits varied with altitudes

Rhizosphere soil properties varied with altitudes (Fig. 1). Soil water content was the lowest at 3300 m and then increased at high altitudes (Fig. 1A). Conversely, soil pH was the highest at 3300 m and decreased at high altitudes (Fig. 1B). NH_4^+-N concentration increased from 3300 to 3600 m and then decreased at high altitudes (3900 and 4200 m), with the highest value observed at 3600 m (Fig. 1D). While NO_3^--N concentration at 4200 m was significantly higher than other altitudes, and no significant differences were found among 3300, 3600 and 3900 m (Fig. 1E). Concentrations of SOC and TN showed a similar trend of 4200 > 3600 > 3900 > 3300 m (Fig. 1C, F). Both concentrations of TP and SAP showed a trend of 4200 > 3900 > 3600 and 3300 m, and there was no significant difference between 3300 and 3600 m (Fig. 1G, H).

Root length, surface area, specific root length, specific root surface area, root length density and root surface area density were significantly higher at low altitudes (3300, 3600 and 3900 m) than at 4200 m, and there were no significant differences among these three altitudes (Fig. 2B, C, E, F, H, I). Root mean diameter, root tissue density and maximum root depth had the highest values in 4200 m, and no significant differences were observed among 3300, 3600 and 3900 m (Fig. 2A, D, G).

3.2. Alpha-diversity of rhizosphere soil microbial communities

Alpha-diversity of rhizosphere soil bacterial communities varied with altitudes. The richness, diversity, and evenness had the lowest values at

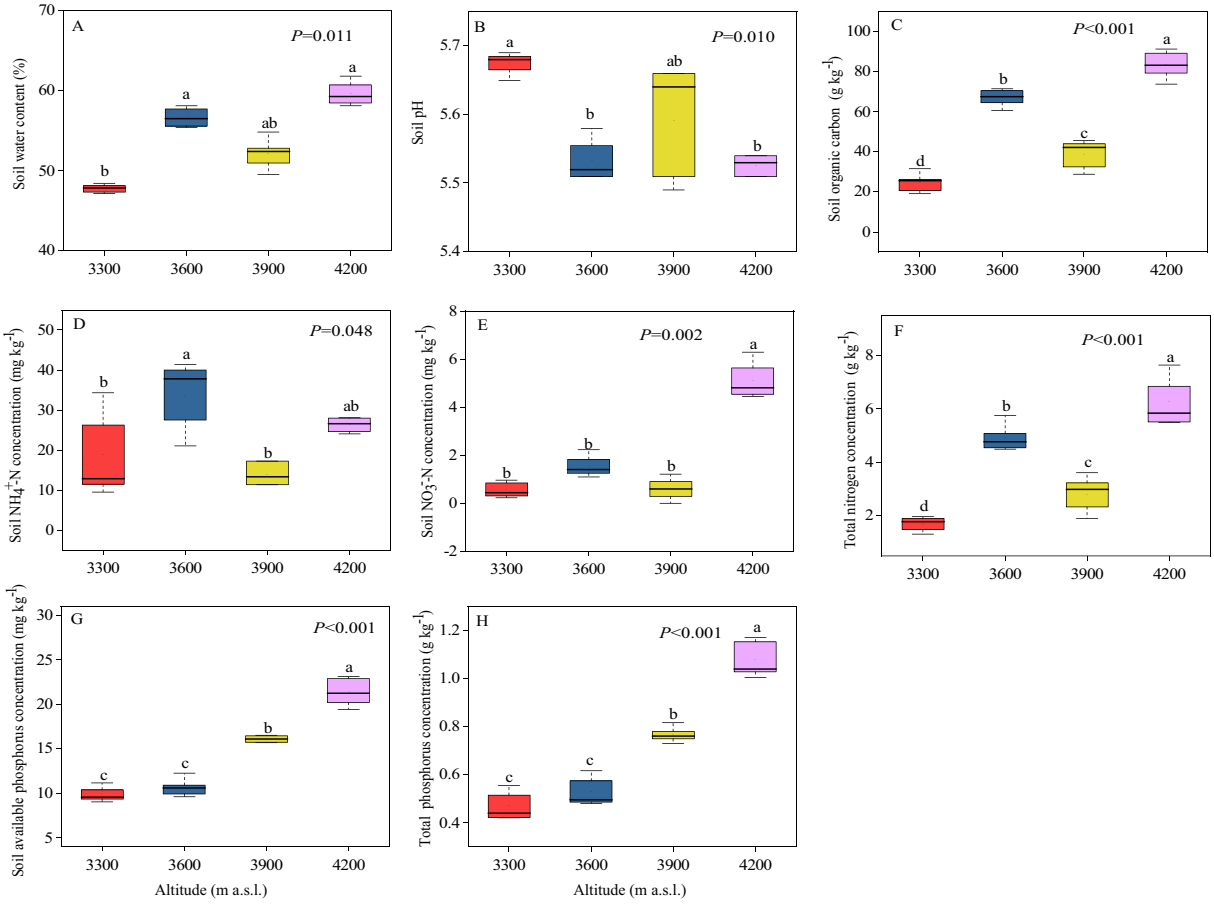

Fig. 1. Variations of rhizosphere soil water content (A), pH (B), organic carbon concentration (C), NH_4^+-N concentration (D), NO_3^--N concentration (E), total nitrogen concentration (F), available phosphorus concentration (G) and total phosphorus concentration (H) (mean ± SE, n = 5) along altitude. Letters indicate significant differences (P < 0.05; post hoc Tukey HSD test).

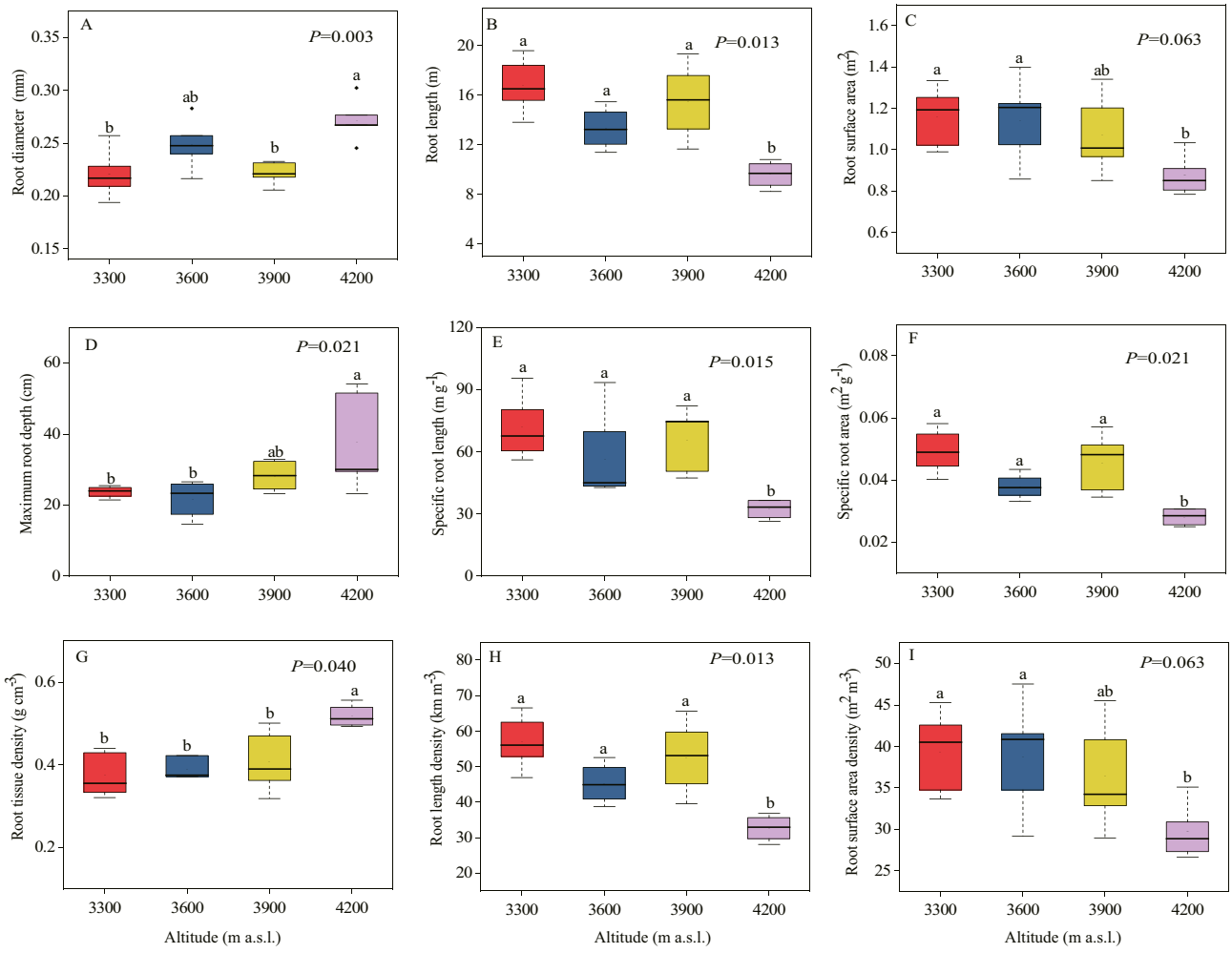

Fig. 2. Variations of root diameter (A), length (B), surface area (C), maximum root depth (D), specific root length (E), specific root area (F), and root tissue density (G), length density (H), area density (I) (mean ± SE, $n = 5$) along altitude. Letters indicate significant differences ($P < 0.05$; post hoc Tukey HSD test).

3600 m, decreasing from 3300 m to 3600 m, and then increased from 3600 to 3900 and 4200 m (Fig. 3A, B, C). Richness of soil fungal communities was significantly affected by altitude, showing higher values at 3600, 3900 and 4200 m than at 3300 m (Fig. 3D). No significant differences in diversity and evenness of soil fungal communities were found among altitudes (Fig. 3E, F).

3.3. Composition of rhizosphere soil microbial communities

NMDS ordination revealed a clear separation for rhizosphere soil bacterial community composition among altitudes (Fig. 4A). The results of PERMANOVA also demonstrated that altitude was a determinant factor for separation (59.00 %, Table 2). Averagely, the top ten phyla of soil bacterial dataset across the altitudinal gradient were: Proteobacteria (35.10 %), Acidobacteriota (19.71 %), Actinobacteriota (16.07 %), Planctomycetota (7.15 %), Chloroflexi (5.55 %), Bacteroidota (5.11 %), Myxococcota (2.40 %), Gemmatimonadota (1.88 %), Methylomirabilota (0.89 %), and Firmicutes (0.88 %) (Fig. 4C, Table S2). At phylum and class levels, their relative abundances differed along altitudes (Table S2). Proteobacteria and Acidobacteriota were more abundant at 3600 m, and their relative abundance declined from 3600 to 4200 m, whereas Actinobacteriota was most abundant at 4200 m (Fig. 4C, Table S2). The relative abundance of Planctomycetota, Chloroflexi, Myxococcota and Gemmatimonadota decreased with altitude. The relative abundances of Bacteroidota, Methylomirabilota and Firmicutes increased with altitude, and their highest relative abundances were detected at 4200 m (Fig. 4C, Table S2). Alphaproteobacteria was more highly represented at 3300 and 3600 m, and Gammaproteobacteria was more highly rich at 3900 and 4200 m (Table S2). The relative abundance of Acidobacteriota decreased from 3300 to 3600 m and then increased at 4200 m. Thermoleophilia, Acidimicrobiia and MB-A2–108 were most abundant at 4200 m, whereas Actinobacteria were more highly represented at 3300 m. Planctomycetes and Phycisphaera were more abundant at 3300 m. Members of the class Ktedonobacteria and KD4-96 were more abundant at 3900 and 4200 m, while Anaerolineae and Chloroflexiaand were more abundant at 3300 m. Relative abundance of Bacteroidia and Methylomirabilia increased with altitude, and reached the highest value at 4200 m, whereas Gemmatimonadetes showed an opposite trend (Fig. S3A, Table S2).

Soil fungal communities showed a distinct separation at 3300 m from other altitudes (Fig. 4B). At phylum and class levels, their relative abundances altered with altitude (Table S3). The dominant soil fungal phyla were Basidiomycota (40.36 %), Ascomycota (40.07 %) and Mortierellomycota (16.64 %) (Fig. 4D, Table S3). The relative abundances of Basidiomycota and three classes (Agaricomycetes, Tremellomycetes and Geminibasidiomycetes) were high at 3300 and 3600 m, and decreased with altitude. While the relative abundances of Ascomycota and four classes (Archaeorhizomycetes, Leotiomycetes, Dothideomycetes, Sordariomycetes), and Mortierellomycota (Mortierellomycetes) were high at 3900 and 4200 m (Fig. S3B, Table S3).

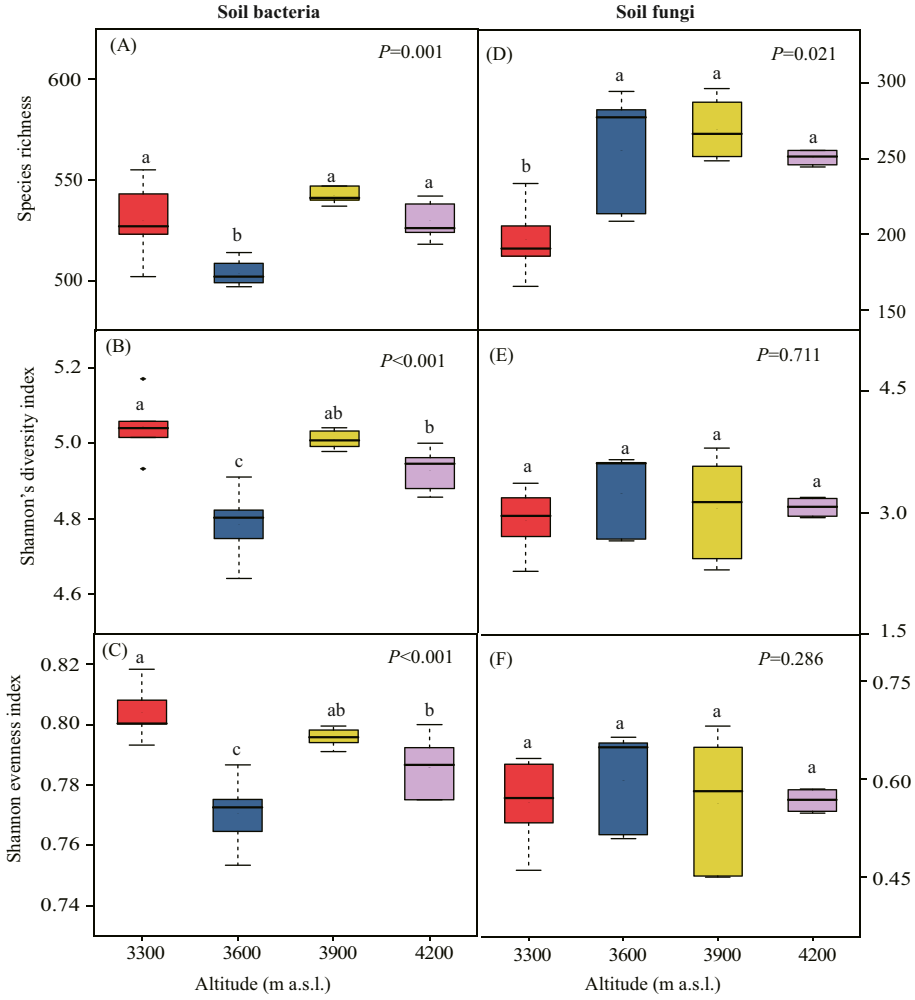

Fig. 3. Richness, diversity and evenness of rhizosphere soil bacterial community (A, B, C) and fungal community (D, E, F) (mean ± SE, n = 5) along altitude. Letters indicate significant differences ($P < 0.05$; post hoc Tukey HSD test).

3.4. Relationship between soil properties, root traits and rhizosphere soil microbial communities

The PERMANOVA analysis was conducted to analyze the proportion of variations explained by each factor of soil properties and root traits in rhizosphere soil microbial communities. Soil properties, including SWC (15.09 %), pH (12.96 %), NO_3^--N (10.43 %), SAP (30.68 %), and root traits (maximum root depth (15.05 %)) were the major factors explaining the variance in bacterial communities (Table 2). While SWC (10.37 %), pH (10.69 %), NO_3^--N (9.98 %), SAP (15.83 %), and root traits, including surface area (8.86 %) and maximum root depth (8.97 %), were important factors explaining fungal community variations (Table 2).

RDA and correlation heatmap analysis revealed that rhizosphere bacterial and fungal communities correlated differently with specific soil properties and root traits (Figs. 5, 6). Acidobacteriota, Actinobacteriota, and Methylomirabilota strongly correlated with soil properties (available P, NO_3^--N, NH_4^+-N and soil water content) and root traits (mean diameter and maximum root depth) at high altitudes, whereas Planctomycetota was highly related with soil pH and root surface area at low altitude (3300 m), and its relative abundance correlated positively with root surface area, but negatively related with soil water content, NO_3^--N and root mean diameter (Figs. 5A, 6A). The dominant soil fungal phyla were Basidiomycota, Ascomycota and Mortierellomycota. Basidiomycota was dominant at low altitude (3300 m) and its relative abundance correlated positively with soil pH, but negatively with available P and maximum root depth (Figs. 5B, 6B). Ascomycota was dominant at high altitudes (3900 m and 4200 m) with high availability of nutrients (available P and N) (Fig. 5B). Similarly, Mortierellomycota was dominant at high altitudes (3900 m and 4200 m), and its relative abundance was correlated positively with soil water content, root mean diameter, and available P, but negatively with soil pH (Figs. 5B, 6B).

4. Discussion

4.1. Alpha-diversity of rhizosphere soil bacterial and fungal communities

The finding that the alpha-diversity of rhizosphere soil bacterial communities varied with altitude (Fig. 3A–C) was consistent with previous studies (Shen et al., 2015; Singh et al., 2012). However, differed with our hypothesis 1, bacterial alpha-diversity reached the lowest value at 3600 m, but had no significant differences among other altitudes (Fig. 3A–C). These results may be attributed to variations in soil nutrients (Table 3). Bacterial community diversity inversely correlated with soil nutrients (SOC, NH_4^+-N, NO_3^--N and TN) (Table 2), suggesting that soil nutrients are vital to explaining the bacterial diversity variations with altitude (Bayranvand et al., 2021; Cui et al., 2019). Previous studies also demonstrated that higher nutrient supplies were generally associated with a low diversity of soil microbes (Cui et al., 2019; Bahram et al., 2012), and higher bacterial species diversity was also found at altitudes with low nutrient availability (Singh et al., 2012). We speculated that there may be intense competition

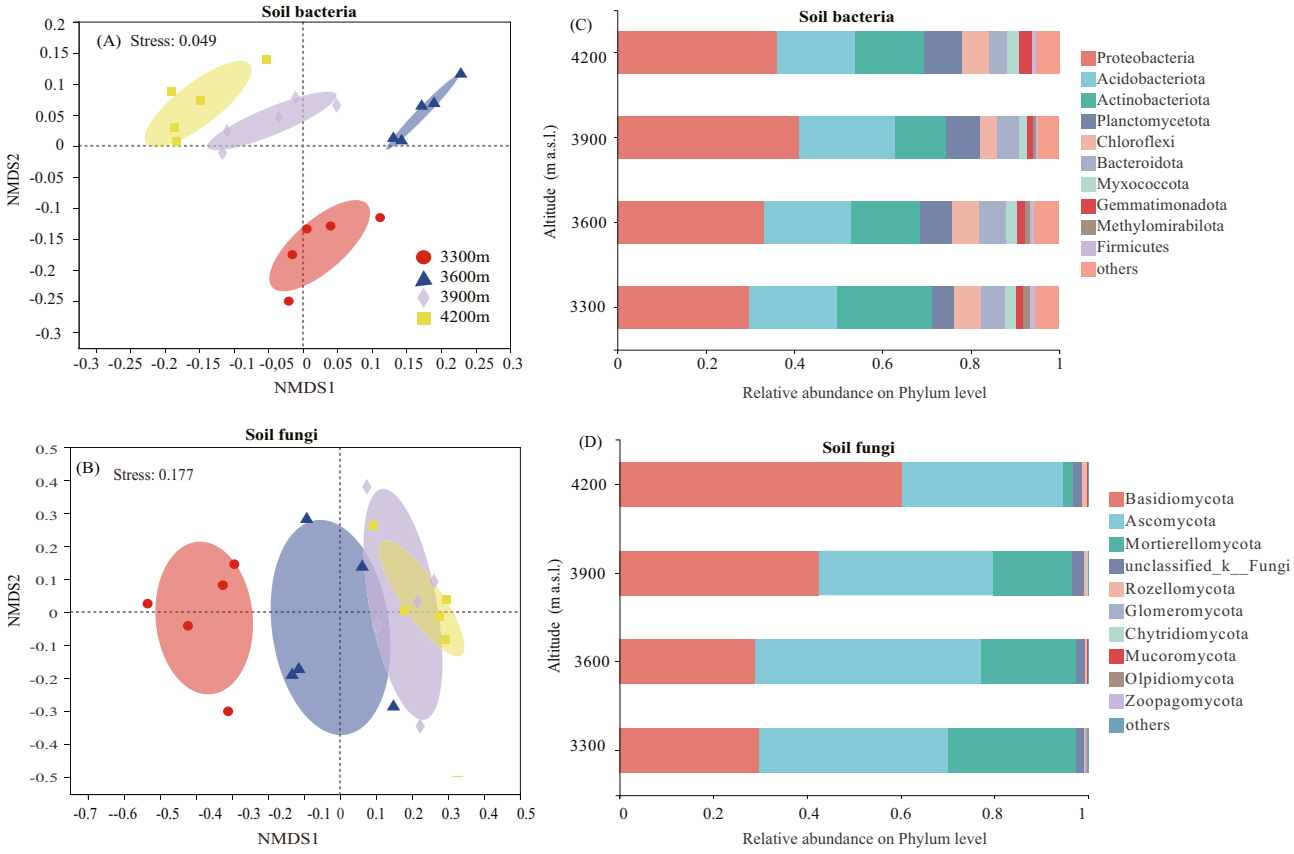

Fig. 4. Non-metric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in the composition of rhizosphere soil bacterial and fungal communities (A, B) along altitude. Bar graph displayed the relative abundances of soil bacterial and fungal communities on phylum level along altitudes (C, D).

for nutrients at 3600 m with high soil nutrient availability, thus leading to the reduction or even disappearance of some microbial taxa. This was also partly supported by the lowest abundances of Actinobacteriota, Chloroflexi, Myxococcota, Gemmatimonadota and Firmicutes at 3600 m, where some microbial taxa such as MB-A2-108 disappeared (Table S2).

For rhizosphere fungal communities, the richness at higher altitudes (3600, 3900 and 4200 m) was significantly higher than that at 3300 m (Fig. 3D), possibly due to the variations in soil nutrients along altitudes (Fig. 1). Soil C content and nutrient availabilities were confirmed to be the major drivers of soil fungal community composition (Bahram et al., 2012; Wang et al., 2015). We found positive relationships between soil fungal richness and SOC and TN (Table 3), because the high C and moderate N concentrations could enhance both microbial growth and the relative abundance of some fungal taxa (e.g., Ascomycota and Mortierellomycota), whereas low energy support could reduce the competitive ability of species and have a negative effect on the richness of soil fungi (Bahram et al., 2012; Siles and Margesin, 2016). Hence, the increased contents of SOC and TN with altitude (Fig. 1C, F) promote fungi growth and improve competitive ability, resulting in the marked improvements in soil fungal richness at higher altitudes, as reported by Siles and Margesin (2016). Moreover, altitude exerted a stronger effect on rhizosphere bacterial community alpha-diversity than on fungal alpha-diversity (Table 2), which is consistent with previous studies (Cui et al., 2019; Ren et al., 2018). Possible explanations were (1) a relatively stable habitat (low nutrient fluctuation and microbial metabolic process) created by low soil temperature in the alpine ecosystem (Cui et al., 2019; Zhou et al., 2016), and (2) compared with fungi, bacteria could adapt to more complex ecological habitats for their wider range of metabolic and nutritional strategies (Li et al., 2021).

Table 2
Proportion of variations in rhizosphere soil microbial communities, explained by soil and root variables calculated independently with permutational multivariate analysis of variance.

Variable	Soil bacteria		Soil fungi	
	Variance (%)	*P*	Variance (%)	*P*
Altitude	59.00	**0.001**	38.19	**0.001**
Soil properties				
SWC	15.09	**0.013**	10.37	**0.004**
pH	12.96	**0.022**	10.69	**0.002**
NH_4^+-N	10.43	0.054	7.02	0.096
NO_3^-N	14.69	**0.008**	9.98	**0.003**
SAP	30.68	**0.001**	15.83	**0.001**
Root traits				
Diameter	9.95	0.096	7.62	0.069
Surface area	9.87	0.079	8.86	**0.018**
MRD	15.05	**0.006**	8.97	**0.018**

SWC, soil water content; pH, soil pH; NH_4^+-N, soil NH_4^+-N concentration; NO_3^--N, soil NO_3^--N concentration; SAP, soil available phosphorus concentration; MRD: maximum root depth.
Bold values indicate significant effects at $P < 0.05$.

4.2. Variations in rhizosphere soil microbial community composition

There was an obvious variation in rhizosphere bacterial community composition among altitudes (Fig. 4A), which was primarily related to the differential abundances of ten phyla-induced by soil properties and root traits (Figs. 5, 6). Specifically, at low altitudes (3300 and 3600 m) with low nutrient availabilities (SOC, TN, SAP and TP) (Fig. 1), rhizosphere bacterial community was dominated by Planctomycetota, Proteobacteria, and Gemmatimonadota. Although Planctomycetota was more abundant in nutrient-poor soils (Fig. 5A), its relative abundance was correlated positively with root surface area (Fig. 6A). Thereby, there may be a cooperative

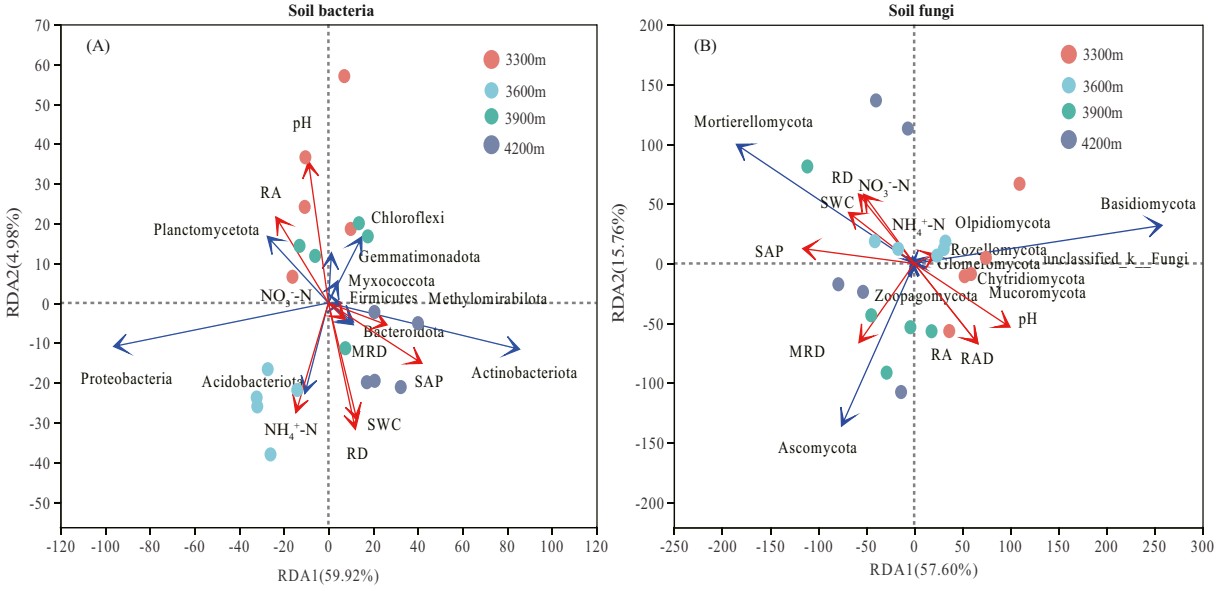

Fig. 5. Result of redundancy analysis of rhizosphere soil bacterial and fungal communities (at phylum level) in relation to soil properties and root traits. Ordination diagrams presented species scores (blue) and environmental factor scores (red) in the redundancy analysis. SWC, soil water content; pH, soil pH; NH_4^+-N, soil NH_4^+-N concentration; NO_3^--N, soil NO_3^--N concentration; SAP, soil available phosphorus concentration; RA, root surface area; RD, root diameter; MRD, maximum root depth.

relationship between such microbial taxa and plant roots to adapt to low-altitudes with low-nutrient supply, and roots possess more effective nutrient acquisition strategies using high root area to obtain nutrients, however, the role of microbes needs further exploration. This may be attributed to their ecological strategies (Ivanova et al., 2016). In fact, Planctomycetes are typically slow-growing oligotrophic bacteria with a low ability to decompose nutrients, so that they can adapt to low temperatures and dry environments, but their abundance substantially declines in stands with more nutrients (Ding et al., 2015; Fierer et al., 2007; Ivanova et al., 2016). Proteobacteria is also typically present in soils with low-nutrient availability (Wang et al., 2017b). Gemmatimonadota is the main component in terrestrial ecosystems and can adapt in soil environments with a high pH (Bayranvand et al., 2021) and low water content (DeBruyn et al., 2011). Conversely, Actinobacteriota, Bacteroidota, Methylomirabilota, and Firmicutes were abundant at higher altitudes with high C, N, and P contents (Fig. 5A). Siles et al. (2016) mentioned that the high nutrient availability at high altitudes could enhance microbial growth leading to high microbial abundance. Some Actinobacteriota are highly presented in nutrient-rich soils due to their copiotrophic lifestyle (Bayranvand et al., 2021), and Bacteroidota was confirmed to be abundant in nutrient-rich environments and positively related to soil organic matter and available P (Wu et al., 2021). Besides, Methylomirabilota was found enriched in soils with high

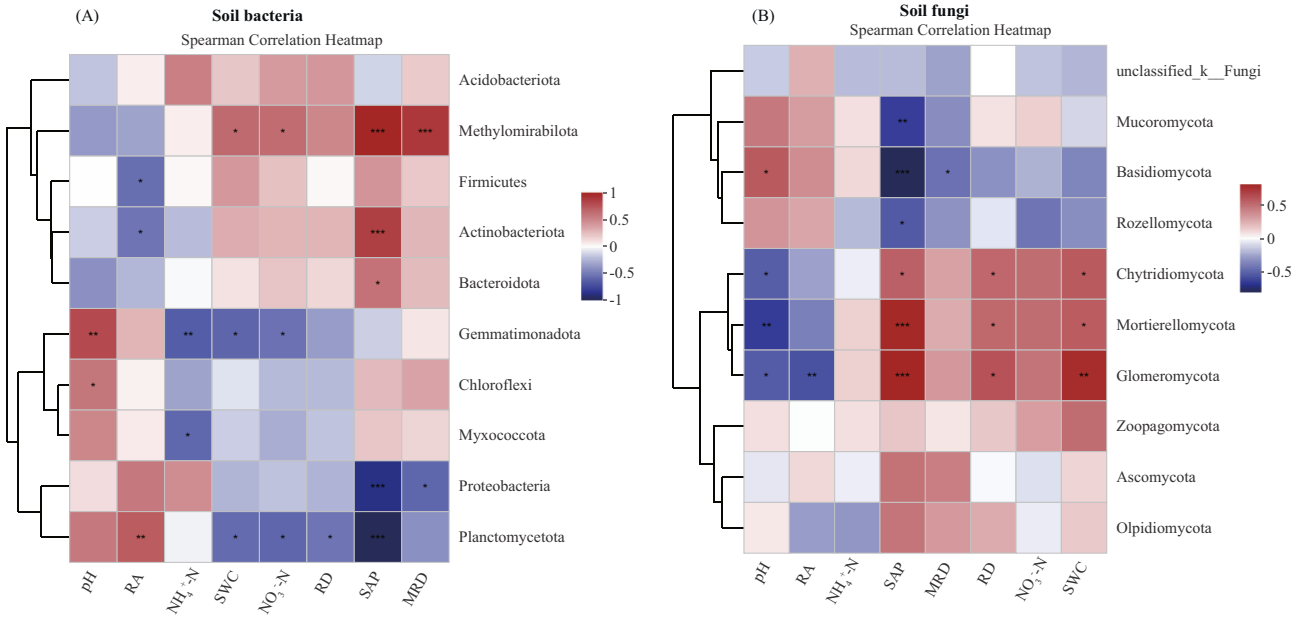

Fig. 6. Correlation heatmap showed the relationship between the relative abundance of dominant phyla in rhizosphere soil bacterial (A) and fungal communities (B) and soil properties and root traits. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. SWC, soil water content; pH, soil pH; NH_4^+-N, soil NH_4^+-N concentration; NO_3^--N, soil NO_3^--N concentration; SAP, soil available phosphorus concentration; RA, root surface area; RD, root diameter; MRD, maximum root depth.

Table 3
Spearman correlation (two-tailed) coefficients between the alpha-diversity of rhizosphere soil microbial communities and soil properties and root traits.

Variable	Soil bacteria			Soil fungi		
	Richness	Diversity	Evenness	Richness	Diversity	Evenness
Soil properties						
SWC	0.031	**−0.464***	**−0.575****	0.375	−0.055	0.410
pH	0.229	**0.445***	**0.502***	**−0.605****	−0.215	0.011
SOC	−0.290	**−0.612****	**−0.666****	**0.507***	0.093	0.308
NH_4^+-N	**−0.479***	**−0.525***	−0.424	0.156	0.063	0.275
NO_3^--N	−0.310	**−0.600****	**−0.602****	**0.463***	0.175	0.414
TN	−0.299	**−0.638****	**−0.687****	**0.493***	0.081	0.325
SAP	0.223	−0.069	−0.185	0.378	0.000	−0.066
TP	0.327	−0.054	−0.205	**0.493***	0.078	0.143
Root traits						
Diameter	−0.166	**−0.464***	**−0.502***	0.169	−0.177	0.308
Length	0.105	0.392	**0.472***	−0.159	0.305	−0.038
Surface area	−0.132	0.119	0.244	−0.133	0.262	0.108
MRD	0.324	−0.047	−0.123	0.098	−0.242	0.073
SRL	0.197	**0.492***	**0.555***	−0.219	0.221	−0.119
SRA	0.093	0.388	**0.480***	−0.168	0.299	−0.023
RTD	−0.006	−0.236	−0.337	0.103	−0.318	−0.107
RLD	0.105	0.392	0.472*	−0.159	0.305	−0.038
RAD	−0.132	0.119	0.244	−0.133	0.262	0.108

SWC, soil water content; pH, soil pH; SOC: soil organic carbon concentration; NH_4^+-N, soil NH_4^+-N concentration; NO_3^--N, soil NO_3^--N concentration; TN, soil total nitrogen concentration; SAP, soil available phosphorus concentration; TP, soil total phosphorus concentration. MRD: maximum root depth; SRL: specific root length; SRA: specific root surface area; RTD: root tissue density; RLD: root length density; RAD: root area density.
Bold values indicate significant effects at $P < 0.05$.
* $P < 0.05$.
** $P < 0.01$.

P availability (Zhang et al., 2021). To summarize, these results suggested that rhizosphere bacterial taxa responded differently to altitude and adapted to the changing environment by virtue of their ecological strategies.

Regarding the rhizosphere soil fungal community composition, the relative abundances of three dominant phyla (Basidiomycota, Ascomycota, and Mortierellomycota) varied with altitude (Table S3). *Rhododendrons* are a group of plants forming specific symbiotic associations with ericoid mycorrhiza from Ascomycota and Basidiomycota (Wang et al., 2017c), and would undoubtedly influence the composition of rhizosphere soil microbial communities. It has been demonstrated that the primary role of mycorrhiza is nutrient acquisition (Smith and Read, 2010), so changes in fungal community composition with altitude are mainly related to nutrient acquisition. In this study, the relative abundance of Basidiomycota decreased with increasing altitude, because high nutrient availability could inhibit their growth (Xiang et al., 2020). However, Ascomycota preferred high altitudes and high P conditions, suggesting that they were key drivers of residue degradation in nutrient-rich soils, regulating nutrient cycling (Zeng et al., 2020). In addition, the relative abundance of Mortierellomycota correlated positively and significantly with soil available P ($P < 0.01$), but negatively and significantly with root surface area ($P < 0.05$) (Fig. 6B). As a type of fast-growing fungi, Mortierellomycota are adapted to copious soluble substrates, such as pectin, and need more nutrients to meet their fast-growing demand (van der Wal et al., 2006; Siles and Margesin, 2016), so they may compete for nutrients with plant roots.

4.3. Major drivers of rhizosphere soil bacterial and fungal communities

Altitude is an important factor affecting microbial community by associating with soil properties and root traits (Ren et al., 2018). For example, high altitudes with low temperatures often decrease the ability of nutrient decomposition (Berger et al., 2015; Zeng et al., 2020) and change plant root traits (e.g., decrease root length; Prakash et al., 2011; Table S1), thus affecting soil microbial growth and diversity. In this study, soil properties and root traits were confirmed as important factors shaping the variations of rhizosphere soil microbial diversity and community composition along altitudes (Tables 2 and 3). Bayranvand et al. (2021) also demonstrated that the combined effects of plant traits and edaphic variables account for the largest variations in microbial communities along altitudes.

In the present study, our findings suggested that soil properties are key factors determining rhizosphere soil microbial distribution along altitudes (Table 2), supporting our hypothesis 2. Specifically, soil water content and pH can significantly explain the variation of soil bacterial and fungal communities (Table 2), as they can affect soil microbial diversity and composition by directly or indirectly altering soil properties (e.g., enzymes activities, C content, and nutrient availability) (Lauber et al., 2009; Li et al., 2018; Wang et al., 2015; Hirao et al., 2021). Microbial communities were highly explained by NO_3^--N, and SAP contents (Table 2), because their survival and growth depend on soil nutrient availabilities (Bayranvand et al., 2021; Siles and Margesin, 2016; Zhang et al., 2016). N is a vital resource for microbes and exclusively participates in protein synthesis (Bottomley et al., 2012), and a high N availability promotes the activity of soil fungal community and their diversity (Kerfeld et al., 2018; Põlme et al., 2018). Similarly, soil P could affect the growth of microbe, and limit the maximum potential of soil fungal diversity (Cox et al., 2010; Siles and Margesin, 2016; Verbruggen et al., 2012). Importantly, high energy and nutrient availability could support more microbes and enhance their competitive ability in changing environments (Bahram et al., 2012). Hence, shifts in the quality and functionality of soil properties (i.e., C, N and P availabilities) can suppress or surge microbial community due to their ecological niches, as different microorganisms can alter the ability to compete with various nutrients (Bayranvand et al., 2021; Beauregard et al., 2010).

In this study, among all the examined root traits (Fig. 2), root surface area and maximum root depth exerted a considerable impact on soil bacterial and fungal community composition (Table 2), which is consistent with our hypothesis 3. Some studies have reported that root traits play an essential role in microbial communities, as they can be associated with plant C allocation, water acquisition, nutrient utilization, and microbial community assembly (de la Riva et al., 2016; Ma et al., 2018; Spitzer et al., 2021; Yang et al., 2021). However, the relationship between plant roots and microorganisms is complex, and different microbial species had different responses to root traits (Spitzer et al., 2021). In the present study, the relative abundance of the dominant bacterial phyla Planctomycetota and Methylomirabilia positively correlated with root surface area and maximum root depth, respectively (Fig. 6A), suggesting that these species may help plants to optimize resource acquisition by increasing root surface area and depth. However, the relative abundances of Mortierellomycota, Glomeromycota, and Chytridiomycota showed negative relationships with root surface area (Fig. 6B). These results revealed that there may be a competition between soil fungi and plant roots. In fact, previous studies have found that there was a competition between roots and soil fungi in terms of nutrient uptake, such as the competition for photosynthetic C (Hunter et al., 2014; Xie et al., 2021). This implies that plants maintain their growth by enhancing C investment to either fine roots or soil microbes across environmental changes to achieve a higher efficiency in nutrient capture per unit C invested, benefiting the C balance of host plants (Ostonen et al., 2013; Weemstra et al., 2016; Zadworny et al., 2016). In general, plants consume more energy when they increase root growth (such as large surface area, length or depth) to unexplored soil volumes to forage nutrients (Robinson, 2001; Yang et al., 2021), which in turn reduces energy consumption to microorganisms, especially in harsh conditions (Kuzyakov and Xu, 2013; Swaty et al., 2004). However, whether the relationship with plant roots and soil microbes is mutual or competitive may need to be studied on certain microbial taxa, as different taxa exhibit different functions and responses to root traits.

4.4. Further consideration

In this study, we sampled rhizosphere soil only in August of one unique year, which had some limitations to the study for just one collection. According to the field survey, summer season is the growth peak of

Rhododendrons vegetation with vitality (Francon et al., 2017; Li et al., 2016), and we sampled during the same plant physiology and phenology period. Although previous studies have shown that soil microbial communities and plant roots are most active and vigorous as well as have a closer connection during this period than other periods (Xie and Yin, 2022; Ji et al., 2021), it is also necessary to carefully consider other periods such as nongrowing seasons. Future research should involve more multiple samplings than one collection.

Furthermore, given that the abundance of plant species in the plant community plays a role in determining soil and microbial community properties, and root properties along the altitudinal gradient, and species identity can be the main explanatory factor for how root traits varied with altitude (Weemstra et al., 2020), the abundance of *R. nitidulum* and the species in the surrounding community would be an essential contributor to the results on the relative roles of soil properties versus root traits of this one species for microbial communities along the gradient. Therefore, future studies need consider the abundance of target plants and other species. From the field survey, we observed that the dominant shrub in the region was *R. nitidulum* (Table 1), hence, the abundance of plant species was not considered in this study. Weemstra et al. (2020) also suggested that adjustments of plant root traits (resource acquisition strategies) across species in a similar environment may be equally adaptive to overcome specific environmental constraints. Therefore, we believed that it was feasible to investigate the changes in *R. nitidulum* root traits and their effect on soil microbial communities with altitude in this region, as these plant species may exhibit similar root trait performance. Certainly, to explore the effects of root traits with species identity on soil microbial communities along altitude, there is a need for studies at larger scale and using more integrative approaches to integrate the multiple dimensions of root traits. Besides, other factors (including for instance macro and microclimate, grazing pressure) may affect rhizosphere soil microbial communities, more comprehensive and in-depth studies are needed further. In the present study, we focused on how soil properties and root traits are associate with soil microbial communities.

5. Conclusion

This study provides insights into the distributional patterns and drivers of microbial communities in rhizosphere soils along an altitudinal gradient and improves our understanding of microbial ecology in alpine ecosystems. We observed significant differences in rhizosphere soil bacterial and fungal alpha-diversity and community composition along the altitudinal gradient. Altitude exerted an impact on rhizosphere soil microbial communities by associating with soil properties and root traits. Soil properties (soil water content, pH, NO_3^--N and available phosphorus) and root traits (surface area and maximum root depth) were the main drivers of soil bacterial and fungal community variations.

We also identified specific taxa of rhizosphere soil bacterial and fungal communities along the altitudinal gradient. Low altitudes (3300 and 3600 m) with low soil nutrient availability and high root surface area were dominated by the bacterial taxa Planctomycetota, whereas, the fungal taxa Mortierellomycota was abundant at high altitudes (3900 and 4200 m) with high soil nutrient availability and low root surface area. Our study demonstrated that rhizosphere soil microbes in alpine ecosystems possess diverse microbial adaptive strategies and exert a strong relationship with soil nutrients. This study clarified how soil properties and root traits relate to rhizosphere soil microbial communities, and could improve our understanding of microbial ecology in alpine *R. nitidulum* shrub ecosystems along altitude.

CRediT authorship contribution statement

Chunying Yin, Xueyong Pang and Wanting Li conceived the ideas and designed methodology; Xueyong Pang, Qinghua Liu and Wanting Li conducted the field investigation and sample collection; Wanting Li and Lulu Xie carried out the measurements; Lulu Xie analyzed the data; Lulu Xie and Chunying Yin led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability

The authors do not have permission to share data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.161048.

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