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Changes in microbial communities in rhizosphere soil of *Arnebia guttata* at different altitudes in south region of Xinjiang, China

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Abstract [Purpose] To investigate the physicochemical properties, microbial diversity, and community composition of the rhizosphere soil of *Arnebia guttata* at different altitudes, to explore the interaction between the rhizosphere microbial communities and environmental factors, and to provide a basis for the conservation and utilization of its wild resources. [Methods] Using *Arnebia guttata* from Wushi County in the northwest of the Tarim Basin as the study subject, the soil physicochemical properties, rhizosphere microbial diversity, and community composition were observed at five altitudes (1200 meters, 1225 meters, 1506 meters, 2053 meters, and 2492 meters). [Results] At an altitude of 1506 meters, the maximum values of SMC, AVP, and AVK were observed. TN, AVK, and NO₃-N showed a positive correlation with the altitude gradient. The soil pH was acidic, with no significant differences among the altitudes. At 2492 meters, the bacterial and fungal communities were more complex compared to other altitudes, and the microbial diversity was more pronounced. [Conclusion] There are significant differences in the physicochemical properties, microbial community structure, and diversity of the rhizosphere soil of *Arnebia guttata* at different altitudes, indicating that altitude is one of the primary factors affecting the growth of *Arnebia guttata*.

Key words *Arnebia guttata*; altitude; soil physical and chemical properties; rhizosphere microorganisms; diversity; community composition

Arnebia guttata ("Zi Cao" in Chinese), belonging to the genus *Arnebia* of the Boraginaceae family, is widely distributed in the northwest of China^[1]. According to the records in the Pharmacopoeia of the Peoples' Republic of China", *Arnebia guttata* can be used to treat colds, hair problems, vomiting blood, and pulmonary problems^[2]. Other studies have also shown that meroterpenoids^[3], naphthoquinones, phenolic acids, alkaloids, and other compounds can be extracted from the roots of *Arnebia guttata*^[1,4], and they have important biological activities such as anti-inflammatory, anti-tumor, antibacterial, antiviral, liver-protective, immunomodulatory, and anti-cancer activities^[5]. However, in China, due to overexploitation, the wild resources of *Arnebia guttata* are close to exhaustion, and it is listed as a vulnerable (VU) species in the "China Biodiversity Red List - Higher Plants Volume"^[6]. Thus, an important challenge currently faced is how to more effectively protect and develop the existing resources of *Arnebia guttata*.

The rhizosphere, serving as a pivotal junction connecting plant roots and soil, profoundly influences the growth vitality and metabolic efficiency of plant roots^[7]. Within this microscopic world, microbial communities and their metabolic activities play a crucial role, driving the cycling and transformation of soil nutrients, particularly the biogeochemical cycling processes of core elements such as carbon, nitrogen, and phosphorus^[8]. The rhizosphere soil microbial community is stabilized by the establishment of plants^[9], and play an important role in the ecological adaptability of plants to the environment^[10-11]. Beneficial rhizosphere microorganisms directly protect plants from pathogens through antagonism, niche and resource competition, or microbial diversity^[12]. Symbiotic fungi have been proven to strengthen the defense system and effectively reduce the abiotic stress of plants through various mechanisms such as promoting plant nutrient absorption, increasing osmotic regulation, down-regulating stress-related genes, jasmonic acid, high glutathione levels, and increasing the synthesis and expression of stress-resistant genes^[13-15]. On the other hand, root exudates also affect the adjacent soil microbial community by inhibiting or promoting the growth of microorganisms^[16]. For example, the secretion of multiple flavonoids and phenolic acids by the roots of soybeans subjected to long-term continuous cropping has been observed to result in an increase in fungal populations within the rhizosphere soil^[17]. In addition, rhizospheric microbial communities were affected not only by the plant species, but also by some other factors, such as elevation, soil pH, and air humidity, changes in environmental factors may also lead to alterations in the rhizosphere microbial community. In recent years, some studies have revealed the interactions between different plant rhizosphere microbial communities and environmental factors. For instance, In the study of the rhizosphere soil of *Ranunculus glacialis* in the Alps,

significant differences in microbial diversity and community composition were found at different elevations^[9]. HaiYun Zi^[8] also found that altitude gradient affects the rhizosphere bacterial diversity of the ancient wild tea trees in Qianjiazhai, and $\text{NH}_4^+\text{-N}$ is a key soil factor affecting various rhizosphere microbial communities of the ancient wild tea trees. In climate conditions, the reduction in precipitation, changes in wet and dry cycles, and droughts have adverse effects on the diversity of soil microbial communities. Conversely, increased carbon dioxide concentrations, rising temperatures, and increased precipitation show a stimulating effect on these microbial populations^[18]. In summary, soil rhizosphere microbial community is closely related to the changes of environmental factors and has a profound impact on the ecological adaptability of plants. The physicochemical properties, rhizosphere microbial diversity, and community composition of the rhizosphere soil of *Arnebia guttata* were analyzed at five different altitudes (1200 m, 1225 m, 1506 m, 2053 m, and 2492 m) in the southern Xinjiang region of China. Our results not only provide the necessary basic information for a better understanding of the ecological adaptations of *Arnebia guttata*, but also provide theoretical references for the cultivation of *Arnebia guttata* and the conservation of germplasm resources in the context of climate change and anthropogenic activities.

Materials and Methods

Site selection and soil sampling.

The research site of the survey is located in the heart of the southern range of the Tianshan Mountains ($40^\circ 56' 13''\text{N}$ $78^\circ 26' 47''\text{E}$), which falls within the mid-temperate continental arid climate zone. Annual precipitation is sparse, with the summer rainfall accounting for over 80% of the total annual precipitation, and temperatures are significantly influenced by the desert and strong solar radiation^[19]. The distribution of the *Arnebia guttata* is within an altitude range of 1200-2500 meters. As shown in Table 1, We have set up five sampling points to cover the entire altitude range of the: 1200 m, 1373 m, 1506 m, 2053 m, and 2492 m. Three sampling points were randomly select at each altitude. At each sampling point, using Haiyun Zi's method, randomly select three mature plants of *Arnebia guttata* to collect mixed rhizosphere soil samples, ensuring that these plants are at least 5 meters apart. A total of 15 samples were collected, placed in sterile polypropylene bags labeled with tags, and transported to the laboratory in ice boxes for analysis. Upon arrival at the laboratory, some of the soil samples were spread on clean paper and dried in a ventilated room for subsequent elemental analysis. The remaining samples were stored at -80 degrees Celsius for the evaluation of rhizosphere bacterial communities.

Table.1 Elevation/m and location

Elevation/m	Location
1200	Wushi County
1225	Wushi County
1506	Ahu Township, Artux City
2053	Uqurqat County
2492	Akto County

Physico-chemical characteristics of soil

All rhizospheric soil subsamples were naturally air-dried at indoor and subsequent analysis was performed after screening with a 2 mm sieve. Methods for measuring soil pH with a pH meter. Soil moisture content (SMC) was determined by the drying method, Soil organic matter (SOM) and soluble organic carbon content (SOC) were determined by the potassium dichromate volumetric method, total nitrogen (TN) content by the Kjeldahl method, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ by extraction with 50 mL of 2 mol/L potassium chloride solution followed by determination using the copper-cadmium reduction-diazotization coupling colorimetric method and indophenol blue colorimetric method respectively^[20]. Total phosphorus (TP) and available phosphorus (AVP) content by the molybdenum-antimony anti-colorimetric method, available potassium, total potassium (TK) and available potassium (AVK) was quantified using the flame photometry technique, and microbial biomass by the chloroform fumigation extraction method. ^[8,21].

High-throughput 16S ribosomal RNA gene sequencing

Total genomic DNA was extracted from soil samples using the TGuide S96 Magnetic Soil /Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) according to manufacturer's instructions. The V1-V9 hypervariable regions of the 16S rRNA gene were amplified using primers (27F: AGRGTTTGATYNTGGCTCAG; 1492R: TASGGHTACCTTGTTASGACTT). The amplicons were quantified, after which the normalized equimolar concentrations of amplicons were pooled and sequenced on the PacBio Sequel II platform (Beijing Biomarker Technologies Co., Ltd., Beijing, China).

High-throughput ITS (Internal transcribed spacer) gene sequencing

Total genomic DNA was extracted from soil samples using the TGuide S96 Magnetic Soil /Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) according to manufacturer's instructions. The ITS1-4 hypervariable regions of the ITS (Internal transcribed spacer) gene were amplified using primers (ITS1F: CTTGGTCATTTAGAGGAAGTAA; ITS4: TCCTCCGCTTATTGATATGC). The amplicons were quantified, after which the normalized equimolar concentrations of amplicons were pooled and sequenced on the PacBio Sequel II platform (Beijing Tsingke Biology Co., Ltd., China).

Bioinformatic Analysis

The qualified sequences with more than 97% similarity thresholds were allocated to one operational taxonomic unit (OTU) using USEARCH (version 10.0). Taxonomy annotation of the OTUs/ASVs was performed based on the Naive Bayes classifier in QIIME2^[22] using the SILVA database^[23] (release 138.1) with a confidence threshold of 70%. Alpha was performed to identify the complexity of species diversity of each sample utilizing QIIME2 software. Beta diversity calculations were analyzed by principal coordinate analysis (PCoA) to assess the diversity in samples for species complexity. One-way analysis of variance was used to compare bacterial abundance and diversity. Linear discriminant analysis (LDA) coupled with effect size (LEfSe^[24]) was applied to evaluate the differentially abundant taxa. The online platform BMKCloud (<https://www.biocloud.net>) was used to analyze the sequencing data.

Soil Physico-Chemical characteristics

Table 2 showed that the rhizosphere soil physicochemical properties of *Arnebia guttata* significantly vary among different elevations. Soil moisture content (SMC), available phosphorus (AVP), and available potassium (AVK) reach their maximum values at an elevation of 1506 m, indicating that the site at 1506 m has better moisture conditions and higher available soil nutrients. Soil total nitrogen (TN), total potassium (TK), soil organic matter (SOM), soil organic carbon (SOC), NO₃⁻-N, and NH₄⁺-N content reach their maximum values at an elevation of 2053 m. Soil pH is acidic in the sampled soils, ranging from 6.65 to 6.69, with no significant differences among the samples selected at different elevations.

Soil total nitrogen (TN), available potassium (AVK), and nitrate nitrogen (NO₃⁻-N) showed significant positive correlations with the elevation gradient (Table 3, $P < 0.05$). Additionally, both NO₃⁻-N and ammonium nitrogen (NH₄⁺-N) were significantly positively correlated with soil organic matter (SOM), soil organic carbon (SOC), TN, and AVK ($P < 0.05$). Available phosphorus (AVP) was significantly negatively correlated with soil moisture content (SMC) and significantly positively correlated with the carbon-to-phosphorus ratio (C/P ratio) and total potassium (TK) ($P < 0.05$).

Table 2 Physicochemical properties of rhizosphere soil at different altitudes and *Arnebia guttata* Changes in microbial community composition and diversity in the rhizosphere

Elevation	SMC	Soil pH	TN	TP	TK	SOM	SOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N	AVP	AVK	C/N ratio	C/P ratio	N/P ratio
1200 m	0.06±0.00 ^b	6.76±0.13 ^a	0.24±0.01 ^c	0.09±0.00 ^e	1.86±0.02 ^e	0.14±0.00 ^d	0.09±0.00 ^d	3.66±0.15 ^c	0.83±0.03 ^b	5.35±0.23 ^d	33.23±0.25 ^d	3.62±0.25 ^d	9.60±0.72 ^c	2.66±0.14 ^a
1225 m	0.02±0.00 ^{cd}	6.65±0.06 ^a	0.26±0.00 ^c	0.15±0.00 ^d	3.38±0.01 ^c	0.46±0.02 ^b	0.27±0.01 ^b	2.06±0.09 ^d	0.47±0.02 ^d	6.03±0.19 ^{bc}	19.68±0.21 ^e	10.51±0.33 ^b	17.97±1.01 ^a	1.71±0.04 ^b
1506 m	0.07±0.00 ^a	6.94±0.12 ^a	0.18±0.01 ^d	0.29±0.00 ^a	3.20±0.02 ^d	0.42±0.01 ^b	0.24±0.00 ^{bc}	1.81±0.05 ^d	0.72±0.03 ^c	7.75±0.21 ^a	136.72±1.54 ^a	13.85±0.29 ^a	8.35±0.22 ^c	0.60±0.02 ^c
2053 m	0.01±0.00 ^d	6.99±0.15 ^a	0.46±0.01 ^a	0.26±0.00 ^b	6.84±0.01 ^a	0.76±0.02 ^a	0.44±0.01 ^a	17.12±0.76 ^a	1.40±0.03 ^a	6.51±0.16 ^b	65.50±0.10 ^c	9.62±0.13 ^b	17.30±0.68 ^a	1.80±0.05 ^b
2492 m	0.02±0.00 ^c	6.66±0.05 ^a	0.33±0.01 ^b	0.17±0.00 ^c	5.15±0.03 ^b	0.37±0.01 ^c	0.22±0.01 ^c	6.51±0.26 ^b	0.76±0.02 ^{bc}	5.71±0.17 ^{cd}	86.90±0.29 ^b	6.63±0.39 ^c	12.69±0.70 ^b	1.92±0.07 ^b
SIG	***	ns	***	***	***	***	***	***	***	***	***	***	***	***

Notes: SMC, soil moisture content; SOM, soil organic matter; AVP, available phosphorus; Soil pH, soil pH value; SOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; C/N ratio, soil carbon/nitrogen ratio; C/P ratio, soil carbon/phosphorus ratio; N/P ratio, soil nitrogen/phosphorus ratio; TK, total potassium; AVK, available potassium; NO₃⁻-N, nitrate nitrogen; NH₄⁺-N, ammonium nitro-gen. “*” means $P<0.05$, “**” means $P<0.01$, “***” means $P<0.001$.

Table 3. Pearson correlation coefficients among physiochemical properties and elevations.

	Altitude	SMC	SOM	AVP	Soil pH	SOC	TN	TP	C/N ratio	C/P ratio	N/P ratio	AVK	TK	NO ₃ ⁻ -N	NH ₄ ⁺ -N
SMC	0.423	1.0													
SOM	0.397	-0.593*	1.0												
AVP	-0.25	-0.638*	0.501	1.0											
Soil pH	0.042	-0.361	0.341	-0.114	1.0										
SOC	0.407	-0.583*	1.0	0.497	0.333	1.0									
TN	0.634*	0.073	0.693**	0.097	0.191	0.7**	1.0								
TP	0.319	-0.594*	0.666**	0.104	0.537*	0.661**	0.128	1.0							
C/N ratio	-0.071	-0.818***	0.549*	0.484	0.254	0.542*	-0.208	0.82***	1.0						
C/P ratio	0.15	-0.32	0.677**	0.736**	-0.186	0.681**	0.633*	-0.049	0.118	1.0					
N/P ratio	-0.038	0.618*	-0.363	-0.246	-0.291	-0.355	0.336	-0.85***	-0.932***	0.18	1.0				
AVK	0.789***	-0.106	0.853***	0.17	0.245	0.86***	0.879***	0.51	0.193	0.564*	-0.119	1.0			
TK	0.079	-0.064	0.263	0.733**	-0.444	0.266	0.26	-0.313	0.016	0.799***	0.173	0.236	1.0		
NO ₃ ⁻ -N	0.569*	-0.069	0.742**	0.0	0.377	0.748**	0.944***	0.326	-0.079	0.479	0.181	0.866***	-0.004	1.0	
NH ₄ ⁺ -N	0.435	-0.069	0.59*	-0.244	0.449	0.594*	0.787***	0.361	-0.102	0.203	0.165	0.69**	-0.343	0.922***	1.0

Notes: SMC, soil moisture content; SOM, soil organic matter; AVP, available phosphorus; Soil pH, soil pH value; SOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; C/N ratio, soil carbon/nitrogen ratio; C/P ratio, soil carbon/phosphorus ratio; N/P ratio, soil nitrogen/phosphorus ratio; TK, total potassium; AVK, available potassium; NO₃⁻-N, nitrate nitrogen; NH₄⁺-N, ammonium nitrogen. “*” means P<0.05, “**” means P<0.01, “***” means P<0.001.

Changes in rhizospheric bacterial and Fungi community composition and diversity.

Upon sequencing 15 samples and subsequent identification via Barcode analysis, a total of 383,197 circular consensus sequences (CCS) were generated. Of these, 193,996 sequences were identified as bacterial in origin, while 189,201 sequences were attributed to fungal taxa. The bacterial and fungal sequences were clustered into 2,193 and 762 operational taxonomic units (OTUs), respectively. For both bacterial and fungal communities, the number of OTUs along with Shannon-Wiener and Simpson diversity indices demonstrated significant variations along the altitudinal gradient (Fig. 1).

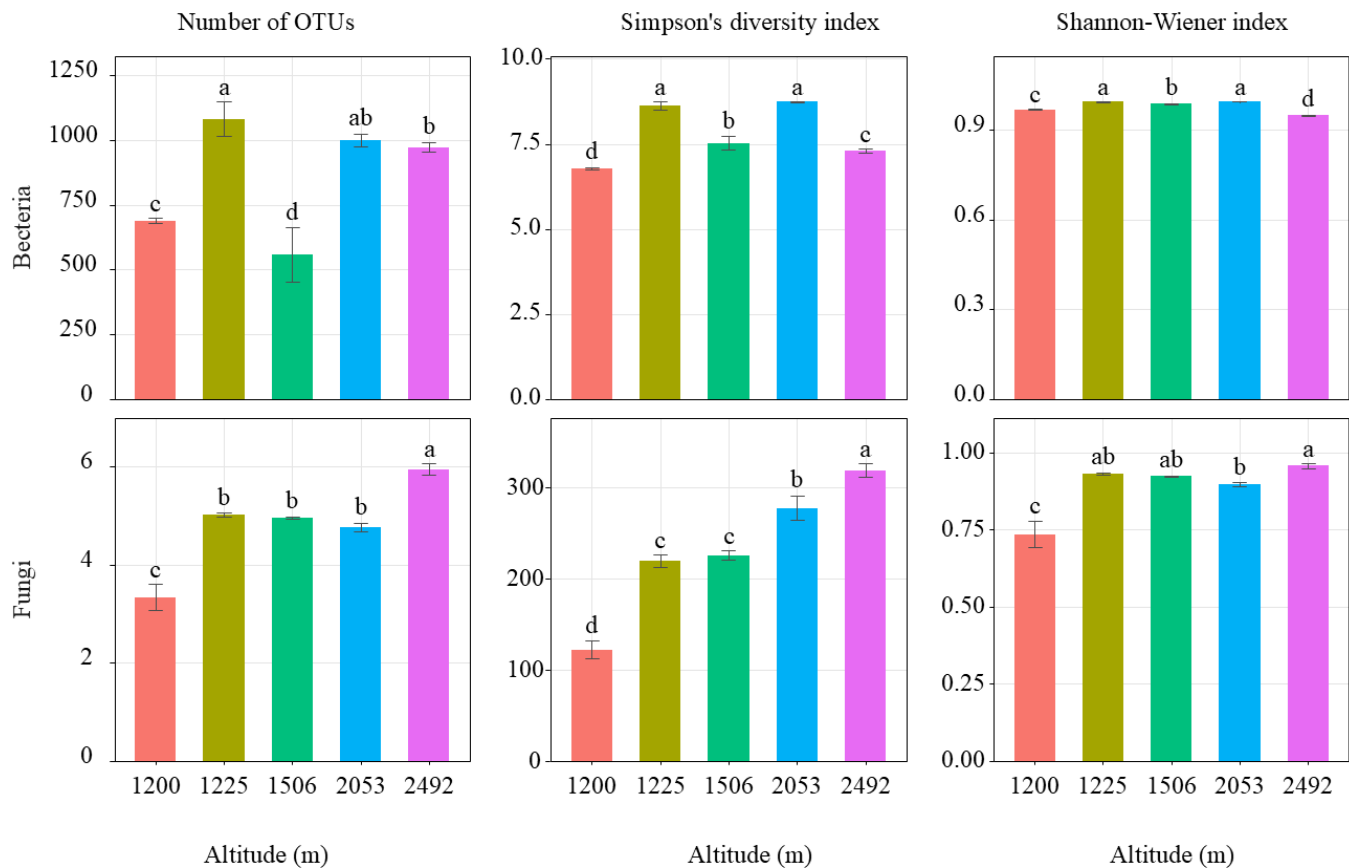


Fig.1 The differences in alpha diversity of bacteria (a, c, e) and fungi (b, d, f) among different altitudes. All data are expressed as mean $\pm$ standard error. Lowercase letters indicate significant differences ($P < 0.05$) in mean values among rhizosphere soils at different altitudes.

Among all sampling sites, the dominant bacterial phyla (relative abundance $>10\%$) were *Proteobacteria* (41.9%), *Acidobacteria* (19.9%), and *Firmicutes* (14.84%). *Proteobacteria* was the most abundant bacterial phylum at elevations of 1,200 m, 1,225 m, 1,506 m, and 2,053 m, with relative abundances of 72.5%, 39%, 36.3%, and 41.7%, respectively. In contrast, *Acidobacteria* dominated at 2,492 m (26.2% relative abundance). Minor bacterial phyla (relative abundance 1–10%) included *Bacteroidota* (8.7%), *Myxococcota* (2.9%), *Patescibacteria* (2.1%), *Acidobacteriota* (1.6%), *Gemmatimonadota* (1.1%), and *Verrucomicrobiota* (1.1%) (Figure 1a,c). The dominant fungal phyla (relative abundance $>10\%$) were *Ascomycota* (75%) and *Basidiomycota* (16.4%), while the minor fungal phylum (relative abundance 1–10%) was *Chytridiomycota* (4.7%) (Figure 1b,d). Except for *Verrucomicrobiota*, all other bacterial and fungal phyla showed significant differences in relative abundance across elevations. The dominant bacterial phylum *Proteobacteria* and the dominant fungal phylum *Ascomycota* both reached their highest relative abundances at 1,200 m. Other major bacterial phyla, such as *Actinobacteriota* and *Firmicutes*, peaked at 2,053 m and 1,506 m, respectively, while the fungal phylum *Basidiomycota* reached its maximum abundance at 1,225 m (Figure 2e,f).

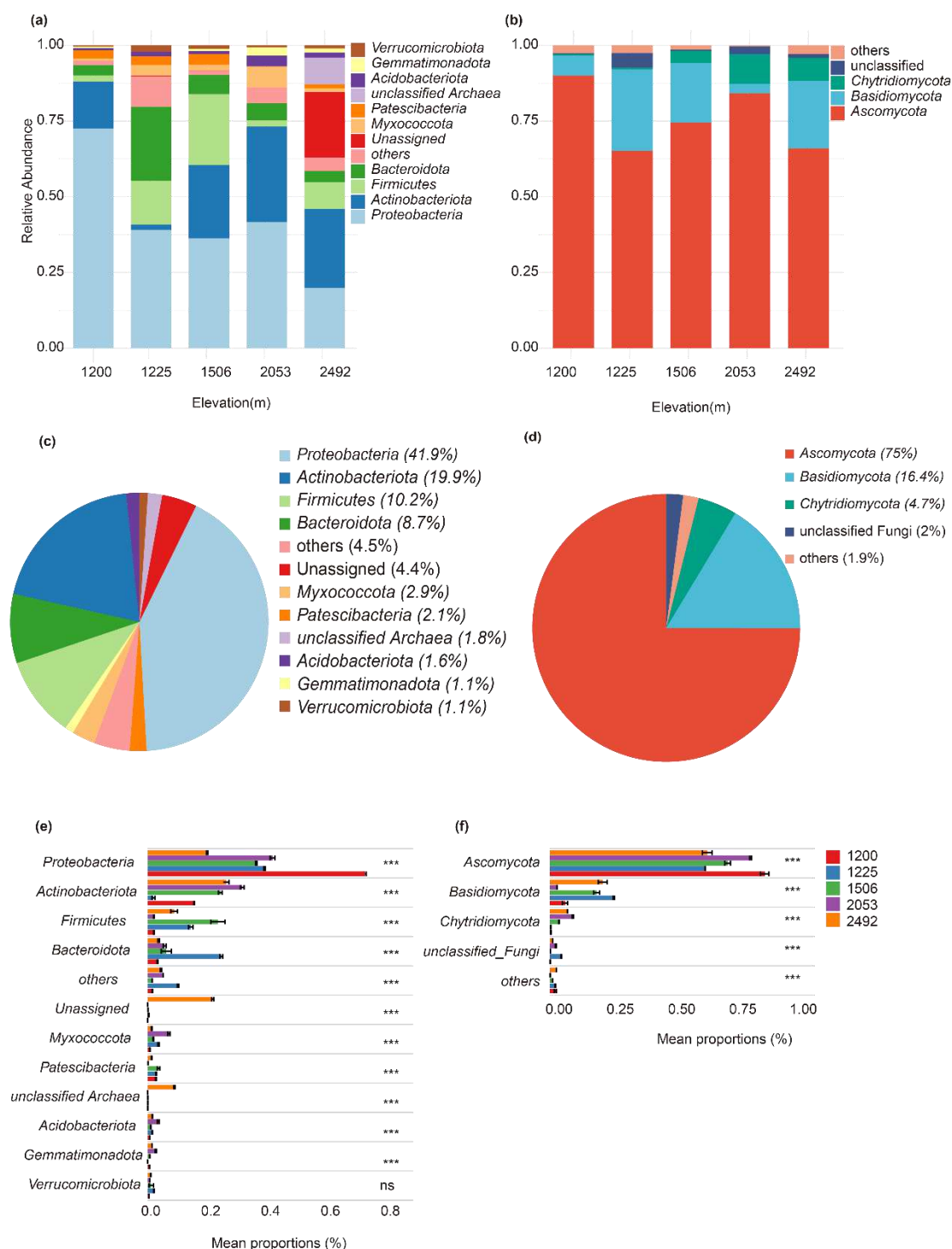


Figure 2. Taxonomic summary of relative abundances for bacterial phyla and genera across elevations.

(a, e) Relative abundances of bacterial phyla at different elevations; (b, f) Relative abundances of fungal phyla at different elevations; (c) Mean relative abundances of bacterial phyla across all samples; (d) Mean relative abundances of fungal phyla across all samples. Taxa are ordered by total relative abundance across all samples, with only bacterial phyla (genera) $\geq 1\%$ relative abundance displayed; those $< 1\%$ are grouped as "Others." The most abundant phyla are positioned at the bottom of the y-axis, while the least abundant phyla (genera) are placed at the top. Correspondingly, phylum names in the legend are ordered from the most abundant (bottom) to the least abundant (top).

Principal Component Analysis (PCA) revealed significant differences in the bacterial and fungal community structures across different altitudes (Figure 3). Based on the topological properties of co-occurrence networks, the complexity of bacterial and fungal communities reached its maximum at 2492 m, as evidenced by the highest number of nodes and edges (Figure 3; Table 4). This altitude (2492 m) may harbor more organic

matter and ecological niches, thereby providing increased opportunities for interactions among different species. In the bacterial network, nodes primarily belonged to nine phylas: *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Bacteroidota*, *Myxococcota*, *Patescibacteria*, *Acidobacteriota*, *Gemmatimonadota*, and *Verrucomicrobiota*. In contrast, the fungal network nodes were mainly distributed across five phyla: *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytridiomycota*, and *Glomeromycota*. The dominant phylas in the root soil community of *Arnebia guttata* may play crucial ecological roles in microbial community assembly.

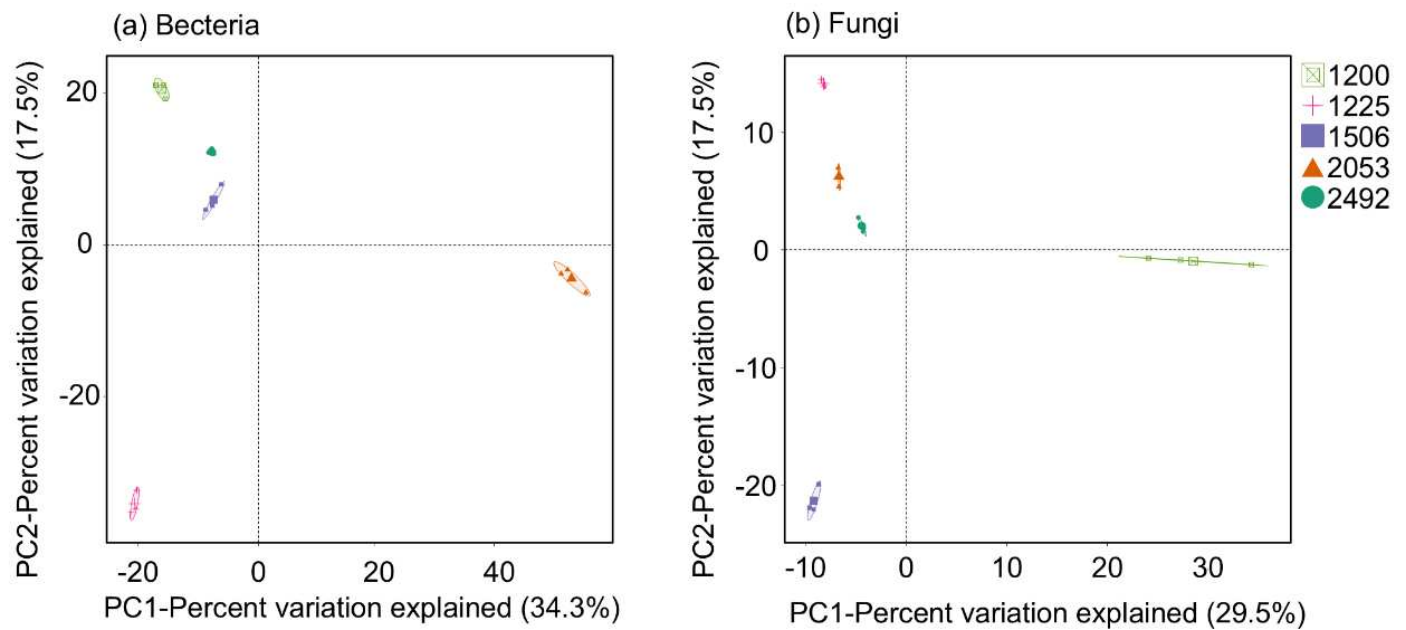


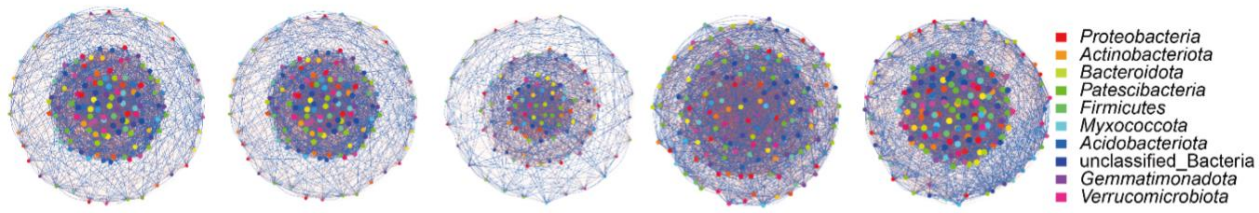
Figure 3. Principal component analysis of bacterial (a) and fungal (b) community structures in rhizosphere soil and bulk soil at different altitudes.

Table 4 Comparative analysis of the topological characteristics of bacterial and fungal phyla networks at different altitudes

Index	Bacteria					Fungi				
	1200	1225	1506	2053	2492	1200	1225	1506	2053	2492
Nodes	129	145	108	153	178	36	59	64	59	74
Edges	2773	3616	1990	3838	5352	241	581	657	556	926
Pos/Neg edges	1403/1370	1833/1783	1022/968	2005/1833	2852/2500	141/100	286/295	319/338	278/278	448/478
Average degree	42.99	49.88	36.85	50.17	60.13	13.39	19.69	20.53	18.85	25.03

Cluster coefficient	1	1	1	1	1	1	1	1	1	1
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(a) Bacteria



(b) Fungi

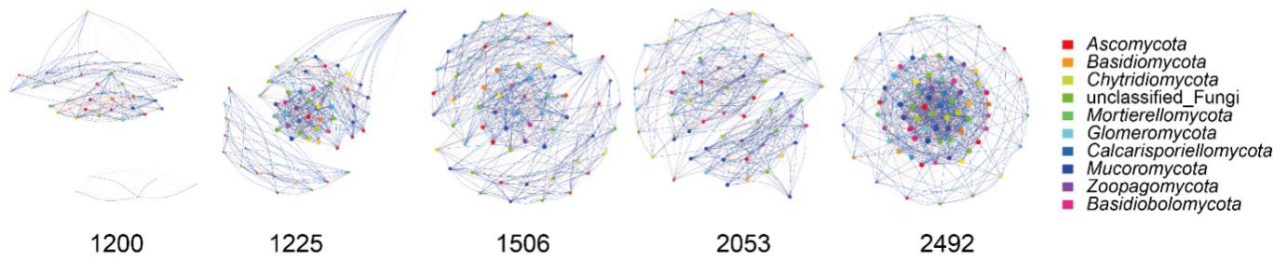


Figure.4 The correlation between bacterial communities and soil physicochemical properties.

Pearson correlation analysis revealed that, except for Soil pH, TN, TP, N/P ratio, NO_3^- -N, and NH_4^+ -N, all other environmental factors were significantly correlated with bacterial α diversity (Table 5). In contrast, fungal α -diversity was significantly correlated with SOM, SOC, TP, N/P ratio, and AVK. The number of bacterial OTUs was significantly positively correlated with AVP, TN, C/P ratio, AVK, and TK. The number of fungal OTUs was significantly positively correlated with SOM, SOC, TN, and AVK. Mantel tests indicated that bacterial community structure was extremely significantly correlated with Altitude ($r=0.66$, $P=0.001$), SMC ($r=0.52$, $P=0.01$), AVP ($r=0.46$, $P=0.001$), AVK($r=0.40$, $P=0.002$), TK($r=0.51$, $P=0.002$), and C/N ratio ($r=0.36$, $P=0.007$). Fungal community structure was extremely significantly correlated with SOM ($r=0.48$, $P=0.001$), SOC ($r=0.48$, $P=0.001$), AVK($r=0.43$, $P=0.004$), TK($r=0.41$, $P=0.004$), NO_3^- -N($r=0.37$, $P=0.04$), and NH_4^+ -N ($r=0.55$, $P=0.001$).

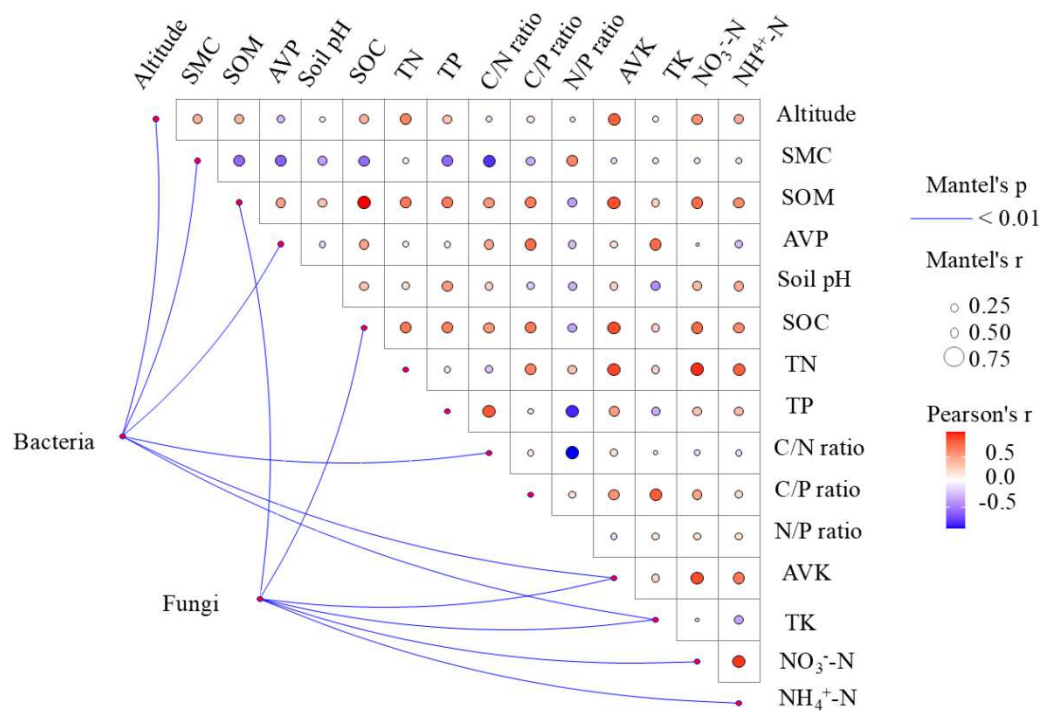


Figure 5. Mantel test for the correlation between rhizosphere soil bacterial community, fungal community structure, and physicochemical properties of rhizosphere soil.

Table 5 Pearson correlation coefficients between bacterial and fungal alpha diversity and environmental variables.

	Bacteria			Fungi		
	Number of OTUs	Simpson's diversity index	Shannon-Wiener index	Number of OTUs	Simpson's diversity index	Shannon-Wiener index
SMC	0.013	-0.9581***	-0.7056**	0.0597	-0.2479	0.0425
SOM	0.476	0.5854*	0.8634***	0.6224*	0.5436*	0.412
AVP	0.5471*	0.6444**	0.8021***	0.163	0.4614	0.2734
Soil pH	-0.3489	0.3321	0.1103	-0.0252	-0.0265	-0.1279
SOC	0.4857	0.5766*	0.8627***	0.6282*	0.5433*	0.4157
TN	0.6231*	0.0472	0.5041	0.5475*	0.153	0.2143
TP	-0.2076	0.441	0.3925	0.5045	0.5630*	0.4262
C/N ratio	-0.0969	0.6439**	0.5351*	0.3156	0.6358*	0.4168
C/P ratio	0.8847***	0.4096	0.8462***	0.3837	0.3461	0.2768
N/P ratio	0.296	-0.4021	-0.2662	-0.3427	-0.6484**	-0.4784
AVK	0.5573*	0.1028	0.5982*	0.8275***	0.5379*	0.5597*
TK	0.8785***	0.0928	0.5882*	0.3466	0.4683	0.4531
NO ₃ ⁻ -N	0.4054	0.178	0.4745	0.4637	0.0746	0.0929
NH ₄ ⁺ -N	0.0773	0.179	0.2523	0.2464	-0.1678	-0.1523

In Redundancy Analysis, the explanatory power of soil physical and chemical properties on bacterial diversity reached 64.09%, with the first and second axes explaining 36.32% and 27.87% of the variance, respectively. For fungi, the explanatory power was 81.21%, with the first and second axes accounting for 50.65% and 30.57% of the variance, respectively (Figure 6). Among the soil factors, AVP had the highest explanatory power for bacteria (32.4%), followed by NO_3^- -N (27.4%) and AVK (22.5%). For fungi, the factors with the highest explanatory power were NH_4^+ -N (46.4%), NO_3^- -N (31.3%), and C/P ratio (14.1%).

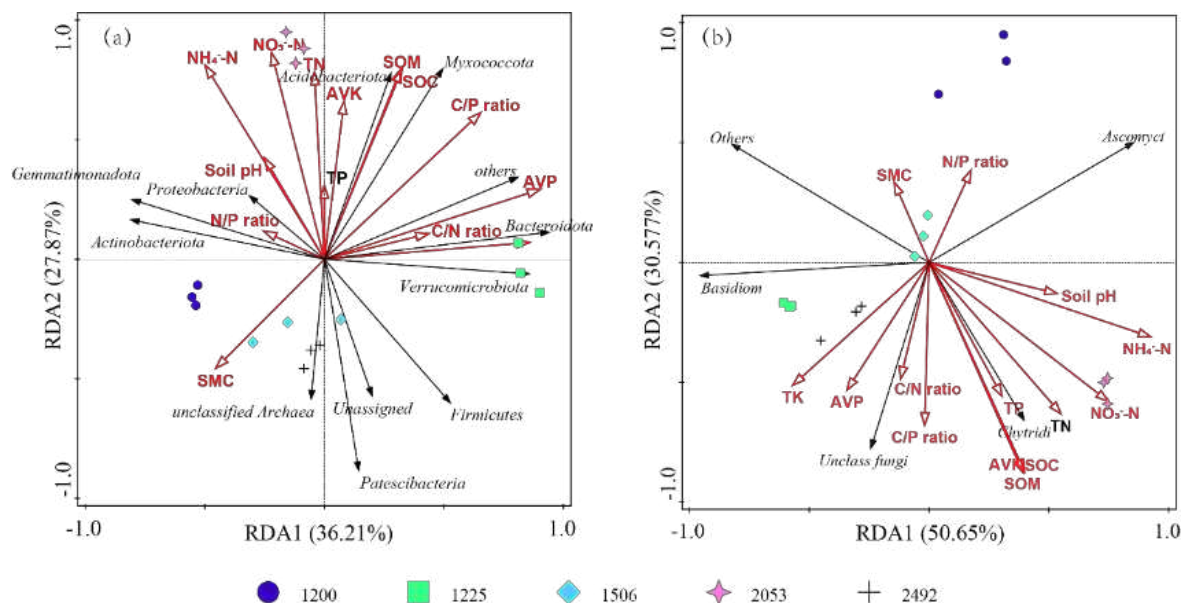


Figure 6. Redundancy analysis of rhizosphere soil properties and rhizosphere bacteria (a) bacterial phyla (b) fungal phyla. SMC, soil moisture content; SOM, soil organic matter; AVP, available phosphorus; Soil pH, soil pH value; SOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; C/N ratio, soil carbon/nitrogen ratio; C/P ratio, soil carbon/phosphorus ratio; N/P ratio, soil nitrogen/phosphorus ratio; TK, total potassium; AVK, available potassium; NO_3^- -N, nitrate nitrogen; NH_4^+ -N, ammonium nitrogen.

Discussion

In the process of plant community succession, plants and their communities are constantly adapting to and changing the soil. As a valuable medicinal resource, *Arnebia guttata* is seriously mined and stolen. Ding Wenhuan and his team found, during their investigation and analysis of the current status of three species of soft purple grass in Xinjiang, that this plant is facing severe over-harvesting. For instance, at the Bayinbuluke Delbilige Ranch in Hejing (latitude and longitude: N42° 38' 613" E84° 6' 486", 2137m above sea level), which was densely populated with high-quality soft purple grass and far from residential areas during a 2004 survey, the plant has been almost entirely depleted in recent years [25]. Long-term harvesting has severely disrupted the growth of *Arnebia guttata*, leading to a gradual decrease in plant litter production. Plant litter plays an important role in the formation of soil organic matter [26]. The decrease of litter will lead to the decrease of nutrient return, thus reducing the material source of soil nutrient reservoir and causes changes in the nutrient content of the soil [27].

In this study, the majority of soil physicochemical properties in the rhizosphere of *Arnebia guttata* reached their peak at 1506m, with favorable water and nutrient conditions. The highest levels of TN, NO_3^- -N, and NH_4^+ -N were observed at 2053m, while the lowest levels were found at 1056m. According to Ma Yuan et al., rhizosphere secretions can accelerate nitrogen cycling by promoting rhizosphere microorganisms and enhance the mineralization of soil organic matter through rhizosphere carbon deposition, thereby improving the of carbon and nitrogen nutrient cycling [28]. Rhizosphere secretions at different altitudes may be one of the factors influencing the changes in TN, NO_3^- -N, and NH_4^+ -N content. The pH value of the sampled soil is acidic, ranging from 6.65 to 6.69. Soil acidification may be caused by the litter and secretions of *Arnebia guttata*, including organic acids, carbonic acid, and polyphenols. Chen Leiyi et al. also noted that vegetation types influence the chemical properties of litter and root exudates (organic acids) [29][30], which provides a theoretical basis for our hypothesis that soil acidity is due to the litter and secretions of *Arnebia guttata*.

The diversity and community structure of soil microorganisms influence the degree of material metabolism in soil and are key indicators for

assessing soil fertility and nutrient levels. Bacteria are the most abundant group in soil microorganisms^[31]. This study, using Illumina sequencing analysis, found that the dominant bacteria in the soil of *Arnebia guttata* are *Proteobacteria* (41.9%), *Acidobacteria* (19.9%), and *Firmicutes* (14.84%). These findings are similar to those reported by Zihai Yun et al. in their research on tea trees^[8]. The *Proteobacteria* dominates soil bacteria and plays a crucial role in the global carbon, nitrogen, and sulfur cycles. It is also the predominant bacterial group in the rhizosphere of many plants. As shown in Figure 2, the *Proteobacteria* is the most abundant bacterial phylum at altitudes of 1200m, 1225m, 1506m, and 2053m, with relative abundances of 72.5%, 39%, 36.3%, and 41.7%, respectively. By comparing the bacterial phylum types at different altitudes, it is evident that the *Proteobacteria* dominates at almost every altitude. This finding aligns with the research conclusions by Cui Wantong et al., who studied the effects of different varieties of grasses on soil enzyme activity and microbial diversity^[31]. At an altitude of 2,492m, the *Acidobacteria* is dominant (with a relative abundance of 26.2%). *Acidobacteria* are typically oligotrophic microorganisms, with higher concentrations found in nutrient-poor soils at high altitudes, which is consistent with the findings of Ma Fulong et al., who reported that *Proteobacteria* and *Acidobacteria* are the most abundant phyla in high-altitude regions^[32]. As shown in Figure 2, the relative abundance of fungal groups changes with altitude. For instance, Chytridiomycota reaches its peak at an altitude of 2053m, showing a trend of initial increase followed by a decline. This finding aligns with the research of Pragardine et al., who noted that the composition of fungal communities varies with altitude, with certain groups increasing in abundance at mid-altitudes and decreasing at high or low altitudes^[9].

The *Ascomycota* (75%) and *Basidiomycota* (16.4%) are the most abundant phyla in the fungal kingdom. Most *Ascomycota* and *Basidiomycota* can break down plant residues and organic matter, promoting nutrient cycling and nitrogen-phosphorus cycles. They also form stable aggregates by symbiotically with plant roots, improving soil aeration and water retention, which is beneficial for root growth. In this study, the relative abundance of *Ascomycota* generally increases with altitude but then decreases^[33], reaching its peak at 1200m. This trend may be related to the pH levels at different altitudes. Nie Jiaming et al. also found that *Ascomycota* are highly sensitive to changes in soil pH levels, with higher abundance in soils with higher pH levels^[34]. Furthermore, the *Actinobacteriota* plays a crucial role in the decomposition of organic matter, which can enhance the quality of agricultural soil. These bacteria are likely to prefer acidic soils and are heterotrophic bacteria with a medium acidity level. Their relative abundance peaks at 2053m, aligning with the previous findings that the content of NO_3^- -N and NH_4^+ -N is highest at this depth. This also supports the conclusion by Wu Haokun et al., who found that soil nitrogen significantly influences the composition of soil fungal communities^[35].

Based on the topological characteristics of the co-occurrence network, the complexity of bacterial and fungal communities reaches its peak at an altitude of 2492 meters, as evidenced by the highest number of nodes and edges (Figure 3; Table 4). This elevation (2492 meters) may harbor more organic matter and ecological niches, thus offering more opportunities for interactions among different species^[36], which aligns with the findings of Ziyun et al., who observed lower complexity in bacterial communities at lower altitudes, with fewer nodes and edges^[8]. These nodes are primarily composed of relatively abundant phyla, such as *Proteobacteria*, *Acidobacteria*, *Ascomycota*, and *Basidiomycota*, suggesting that the dominant phylum of *Arnebia guttata* may play a significant ecological role in microbial community aggregation.

Through Pearson correlation analysis, we observe that as altitude increases, the number of nodes and edges in the network topology also shows an increasing trend (Table 4). In conjunction with Figure 4 and Table 5, it is found that, except for soil pH, TN, TP, N/P ratio, NO_3^- -N, and NH_4^+ -N, all other environmental factors are significantly correlated with bacterial diversity. Additionally, altitude is a variable that influences the ecosystem, playing a crucial role in affecting microbial diversity^[8].

In this study, we found that the microbial bacterial diversity at a depth of 2492m is significantly the highest. The Mantel test (Figure 5) also indicates a highly significant correlation between bacterial community structure and altitude ($r=0.66$, $P=0.001$). Huang Zhengyi et al. also noted that the soil microbial community structure varies significantly between low, medium, and high altitudes^[37]; and Huang Manshun et al., in their analysis of the soil bacterial community diversity and PICRUST functional predictions of All-leaf green velvet at different altitudes, observed that as altitude increases, the diversity and abundance of All-leaf green velvet soil bacteria also gradually increase^[38]. The research by Ma Fulong and colleagues also confirmed that bacterial diversity increases with altitude. In their study, "The Impact of Altitude Gradient on the Structure and

Diversity of Grassland Plants and Soil Microbial Communities in the Central Slope of the Kunlun Mountains," they noted that soil bacterial OTUs, microbial abundance (ACE, Chao1), and lineage diversity (PD) indices all show a monotonically increasing trend with altitude ^[32]. In this study, bacterial diversity increases with altitude, which may be related to environmental factors such as soil organic matter content, pH value, and microclimate. The differences in soil nutrients and the carbon-to-nitrogen-to-phosphorus ratio at different altitudes are significant factors influencing the diversity of rhizosphere microbial communities.

The redundancy analysis revealed that ammonium nitrogen has the greatest impact on the rhizosphere microorganisms of *Arnebia guttata* at high altitudes (Figure 6). Therefore, we concluded that ammonium nitrogen in the rhizosphere soil is the primary abiotic soil factor influencing the bacterial community structure of *Arnebia guttata* across different altitudes. We hypothesize that the dominant role of ammonium nitrogen in bacterial diversity is closely linked to its central role in the nitrogen cycle. As a key component of the nitrogen cycle, fluctuations in ammonium nitrogen concentration can directly influence microbial metabolic activity, thereby reshaping the community structure. This regulatory mechanism may affect microbial nutrient acquisition pathways, energy metabolism efficiency, and interspecific interactions, ultimately making ammonium nitrogen the leading factor driving changes in bacterial community diversity ^[39].

In conclusion, the diversity and community structure of soil microorganisms vary significantly with the altitude gradient, and the altitude-related soil characteristics and spatial characteristics also have a significant impact on the composition of underground communities. Different altitudes also shape the unique characteristics of different microbial communities, and these characteristics still have potential for further exploration.

Conclusion

The maximum values of SMC, AVP, and AVK were reached at 1506m for the *Arnebia guttata*, indicating that the soil nutrients are sufficient at 1056m, suitable for the growth of *Arnebia guttata*. TN, AVK, NO₃--N show a positive correlation with altitude gradient, and there is a significant correlation among various soil nutrient indicators. The soil pH is acidic and shows no significant difference between different altitudes, suggesting that the cause of soil acidification may be due to the growth and physiological metabolism of the *Arnebia guttata* itself. Through Pearson's test, Mantel test, and other analyses, we found that the bacterial and fungal communities at 2492m are more complex than those at other altitudes, and microbial diversity is also more significant. This also indicates that altitude is one of the main factors affecting ecosystems and plays an important role in microbial diversity. In summary, this experiment has shown that there are significant differences in the physicochemical properties of rhizosphere soil, microbial community structure, and diversity of *Arnebia guttata* at different altitudes, and different altitudes are one of the main factors affecting the growth of yellow soft purple grass.

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Research materials: The plant *Arnebia guttata* was identified by the corresponding author according to the characteristics recorded in Flora of China. The voucher specimens of the material under study are not in the public herbarium.

Data availability: This article presents all data generated or analyzed during this study. Other data may be obtained from the corresponding author upon reasonable request.