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# EFFECTS OF PLANT SPECIES ON RHIZOSPHERE BACTERIAL COMMUNITIES IN TWO ALPINE MINES OF THE TIBETAN PLATEAU, CHINA

The maintenance and restoration of ecosystem functions in alpine ecosystems are heavily reliant on the structure and diversity of soil bacterial communities. Unique rhizosphere microenvironments with varying soil properties are shaped by different plant species through their active roots, leading to the formation of distinct rhizosphere bacterial communities. In harsh environments characterized by high altitude, aridity, and nutrient-poor soils, the composition and diversity of rhizosphere soil bacterial communities are primarily influenced by plant species. A field experiment was conducted to investigate the differences and primary drivers of rhizosphere soil bacterial communities among leguminous and non-leguminous plants, as well as herbs and shrubs, in two alpine mines of the Tibetan Plateau, China. Our study indicates that soil properties (such as soil water content, pH, total carbon, and total nitrogen content), as well as the composition and diversity of soil bacterial communities, were significantly influenced by root nodules and varied according to plant species. This work has important ecological implications, suggesting that selecting restoration plants and improving soil nutrient conditions may contribute to the restoration of ecosystem functions in alpine mining areas.

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LIST OF SYMBOLS

TC – total organic carbon concentration
TN – soil total nitrogen concentration
$NH_4^+$-N – ammonia nitrogen concentration
$NO_3^-$-N – nitrate nitrogen concentration
*AP* – available phosphorus concentration
*AK* – available potassium concentration
PS – plant species
RN – root nodules
S1 – S1 alpine mines
S2 – S2 alpine mines
JB – bulk soil of S1
CB – bulk soil of S2
JD – rhizosphere soil of *Artemisia desertorum* at S1
CD – rhizosphere soil of *Artemisia desertorum* at S2
JL – rhizosphere soil of *Astragalus laxmannii* at S1
CL – rhizosphere soil of *Astragalus laxmannii* at S2
JC – rhizosphere soil of *Oxytropis coerulea* at S1
CM – rhizosphere soil of *Sophora moorcroftiana* at S2

## 1. INTRODUCTION

As a vital repository for global biodiversity, the Qinghai-Tibetan Plateau (QTP) has been shown to provide major ecological benefits for preserving ecosystem stability and advancing biodiversity conservation [1]. However, significant challenges and potential threats to this invaluable resource are posed by the rapid expansion of the global mining industry [2]. In alpine mining regions, numerous environmental issues are particularly pronounced, with trends such as biodiversity loss, ecological degradation, and the decline of ecosystem functions becoming increasingly evident [3]. Interestingly, under extreme environmental circumstances, microbes display unique adaptations and functional diversity, which are essential for maintaining the stability of alpine ecosystems.

Alpine mines are characterized by unique environmental conditions, such as extreme cold, aridity, and nutrient-poor soils. The maintenance of ecological functions in these areas primarily depends on the intricate interactions between plants and soil bacterial communities [4]. Soil pH and nutrient content have been proven to be major factors influencing the vertical distribution of bacterial communities in alpine wetland [5]. Yu et al. [6] highlighted that in arid regions, the interaction of climatic factors and human activities (such as intensive agriculture) affects soil bacterial diversity. For instance, climate warming can directly explain part of the changes in bacterial community structure, while soil properties and plant forms can indirectly explain more variations. These studies indicate

that the composition and diversity of soil bacterial communities are influenced by multiple factors. Despite these findings, the specific effects of different plant species on rhizosphere bacterial communities in alpine mines remain underexplored.

The interactions between microorganisms, roots, and soil constitute the rhizosphere microenvironment, which is a key area around the active roots of plants [7]. Highly diverse and dynamic bacterial communities are present in the rhizosphere microenvironment, benefiting plants by inhibiting pathogen invasion and aiding in nutrient acquisition from the soil [8]. The microenvironment can be altered by changes in soil pH, carbon, nitrogen, water content, and oxygen brought about by plant roots [9]. However, there has been controversy about the diversity of rhizosphere bacterial communities in different plants. Woody plants, due to their larger biomass, often exhibit higher rhizosphere bacterial community diversity compared to grasses [10], but some studies have reported that grasses, due to their ability to decompose litter, show greater increases in microbial biomass [11].

It has been suggested that rhizosphere bacterial communities are a subset of soil microbial communities, thus displaying lower diversity [12]. Interestingly, legumes form root nodules by establishing symbiotic relationships with specific rhizobacteria, which in turn fix nitrogen [13]. This not only provides the required nitrogen source for plant growth but also promotes the colonisation and diversity of other bacteria in the rhizosphere soil [14]. Therefore, further research on the effects of different plants on rhizosphere soil bacterial communities in alpine mining areas is essential.

Understanding the effects of plant species on rhizosphere soil bacterial communities in alpine mining areas of the Qinghai-Tibetan Plateau is crucial for developing effective restoration strategies. This study aims to investigate the differences in rhizosphere soil bacterial communities among leguminous and non-leguminous plants, as well as herbs and shrubs, in two alpine mines, and to identify the environmental factors and the main drivers of rhizosphere soil bacterial communities.

Therefore, we tested the following hypotheses in two alpine mines:

*Hypothesis 1.* Pant species significantly influence the physicochemical properties of rhizosphere soil and the composition and diversity of bacterial communities, with shrubs having greater rhizosphere soil nutrient levels and bacterial diversity compared to herbs.

*Hypothesis 2.* Leguminous plants showed greater improvement in rhizosphere soil nitrogen content and bacterial diversity compared to non-leguminous plants.

*Hypothesis 3.* The rhizosphere microenvironment (root systems and soil nutrients) is the primary driving factor for microorganisms in alpine mines, as it directly affects the microenvironment and energy sources for bacterial colonization.

By elucidating the relationships between plant species and rhizosphere soil bacterial communities, this study provides insights into the ecological restoration of alpine mining areas, suggesting that selecting appropriate restoration plants and improving soil nutrient conditions may contribute to the restoration of ecosystem functions in these regions.

## 2. MATERIALS AND METHODS

*Site description*. The rhizosphere soil samples of three dominant plants, *Artemisia desertorum*, *Astragalus laxmannii*, and *Sophora moorcroftiana*, were collected from the alpine mine wasteland at two sites (3589 and 3720; S1 and S2, respectively) from Lhasa, Tibet Autonomous Region, Tibetan Plateau, China. These plant forms are non-leguminous herbaceous plants, leguminous herbaceous plants, and leguminous shrubs, respectively (Table 1). The study area is characterized by a plateau climate with cold, dry winters and short, warm summers. The region exhibits significant diurnal temperature variations and distinct vertical climatic zonation. The annual mean temperature and annual mean precipitation are 7.4 °C and 340 mm at the S1 site, and 5.8 °C and 444.8 mm at the S2 site. The soils at both study sites are classified as sandy loam.

Table 1

Basic information on sample sites

| Mine | Altitude | Longitude | Dominant species | Plant growth form | Root nodules |
|---|---|---|---|---|---|
| S1 | 3589 | 90°85′1″E 29°32′82″N | *Artemisia desertorum* (JD) | non-leguminous herbaceous plant | – |
| | | | *Astragalus laxmannii* (JL) | leguminous herbaceous plant | + |
| | | | *Oxytropis coerulea* (JC) | leguminous herbaceous plant | + |
| S2 | 3720 | 90°87′40″E 29°71′13″N | *Artemisia desertorum* (CD) | non-leguminous herbaceous plant | – |
| | | | *Astragalus laxmannii* (CL) | leguminous herbaceous plant | + |
| | | | *Sophora moorcroftiana* (CM) | leguminous shrub | + |

*Field sampling.* Vegetation is commonly sparse in the alpine mines due to anthropogenic disturbances and other factors. Thus, to collect rhizosphere and bulk soil, three dominant plants in each site were chosen in this study. The area of the alpine mine is approximately 8000 $m^2$. Bulk soils and rhizospheres were sampled from the 0–30 cm layer in July 2023. The herbs and shrubs were carefully dug up to collect all of the soil that adhered to the roots. The rhizosphere soil was sampled using sterile brushes and was defined as the soil that remained attached to the root system following mild manual shaking. The soil with no vegetation growth was considered bulk soil. Through these procedures, we obtained rhizosphere and bulk soil samples for different plants at each site.

The soil samples were separated into zip bags, transported in an icebox, and stored in the laboratory at –4 °C for further experiments. The samples were divided into two parts. One part was air-dried and utilized to characterize the soil. The other part was kept in the laboratory at –80 °C for use in soil microbiological analysis after coarse roots and stones (2 mm) were removed, before it was shipped in aseptic bags in refrigerators.

*Determining the properties of the soil.* The soil pH and water content were measured immediately after sampling, followed by the extraction of $NH_4^+$-N and $NO_3^-$-N. Soil water content (SWC) was determined by calculating the mass loss after drying the soil at

105 °C for 24 hours. Soil pH was assessed using a soil-water slurry prepared at a 1:2.5 (w:v) ratio, which was stirred thoroughly and allowed to stand for 30 minutes before measurement. Ammonium nitrogen and nitrate nitrogen were extracted with KCl solution (2 mol/dm$^3$), and their concentrations were measured using a continuous flow analyzer (Seal AA3, Germany). Then the soil available phosphorus (*AP*) was extracted using $NaHCO_3$ solution (0.5 mol/dm$^3$), and its concentration was measured by colorimetry. The soil available potassium (*AK*) was extracted using ammonium acetate solution (1 mol/dm$^3$), and its concentration was measured by flame photometry. The dichromate oxidation method was utilized to quantify the amount of soil organic carbon (TC).

*DNA extraction, amplification, and sequencing.* Total DNA was precisely extracted from 1.0 g of soil for each sample. The DNA extraction kit (LABGENE Biotechnology, Chengdu, China) was used to extract bacterial DNA in accordance with the manufacturer's instructions. The V3–V4 region of the bacterial 16S rRNA gene was amplified with primer pairs 341 F (5′-ACTCCTACGGGGAGGCAGCA-3′) and 805 R (5′-GGACT ACHVGGGTWTCTAAT-3′) using an Applied Biosystems® Gene Amp® PCR System 9700 in a 25-μL reaction volume. The PCR thermal cycling conditions were as follows: initial denaturation at 98 °C for 2 minutes, followed by 30 cycles of denaturation at 98 °C for 15 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes, ending at 4 °C. The PCR products were separated on a 1.2% agarose gel and purified using the QIAquick Gel Extraction Kit (QIAGEN) according to the manufacturer's instructions. The purified products were quantified using a Qubit@2.0 fluorometer (Thermo Scientific). The amplicons were then sequenced using the HiSeq 2500 PE250 platform (Qingke Company, Chengdu, China).

*Sequence processing.* All raw 16S rRNA gene sequences were processed using Qiime (version 1.7.0, http: //qiime.org/) to filter sequences, as high-throughput sequencing can introduce PCR amplification errors, point mutations, and chimeric sequences [15]. For instance, chimeric sequences were removed using the uchime method in mothur (version 1.31.2, http: //www.mothur.org/) [16]. Finally, quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the uclust method in Qiime, resulting in high-quality sequences. The diversity index is calculated based on the data after levelling to the lowest sequencing depth.

*Analysis of microbial diversity indices.* To quantify the microbial diversity, three key alpha-diversity indices (Chao1, Shannon, and Simpson) were calculated, and their formulations are detailed as follows:

The Chao1 index was employed to estimate the number of operational taxonomic units (OTUs), serving as a proxy for species richness in the sample. It accounts for rare

taxa (species with low occurrence frequencies) to improve the estimation accuracy. The formulation of the Chao1 index is:

$$S_{\text{Chao}} = S_{\text{obs}} + \frac{n_1(n_1-1)}{2(n_2+1)}$$

where $S_{\text{obs}}$ is the number of species observed, $n_1$ is the number of species occurring only once, $n_2$ is the number of species that occur twice.

The Shannon index was used to characterize the combined effects of microbial species richness (number of unique taxa) and evenness (uniformity of their abundance distribution). Conceptually, a higher Shannon value indicates greater uncertainty in predicting the next-occurring taxon in a sequence, which corresponds to higher community diversity. It is defined as:

$$H' = -\sum_{i=1}^{n} p_i \ln p_i$$

where $p_i$ is the proportion of observations (abundances) belonging to the $i$th species.

The Simpson index was utilized to measure the "concentration" of microbial taxa, reflecting the probability that two randomly selected individuals from the sample belong to the same OTU. It integrates both species richness and evenness, with higher values indicating lower diversity (more dominance by a few taxa). The formula for the Simpson index is:

$$D = 1 - \sum_{i=1}^{n} \frac{n_i(n_i-1)}{N(N-1)}$$

where $D$ is the Simpson index, $S$ represents the total number of species in the sample, $n_i$ denotes the number of individuals of the $i$th species, $N$ denotes the total number of individuals in the sample.

*Statistical analyses*. All statistical analyses were performed using SPSS 21.0. One-way analysis of variance (ANOVA) was used to analyze the differences in the α-diversity index and soil properties ($n \geq 3$), with $p < 0.05$ considered statistically significant. Principal coordinate analysis ( PcoA) was conducted to assess the similarity between bacterial communities in each soil sample. Spearman's rank correlation was utilized to analyze the effects of environmental factors on bacterial communities, while heat maps were generated to depict the relative abundance of soil bacteria. The piecewiseSEM framework enables the development of integrative models to analyze soil bacterial diversity in alpine ecosystems, thereby elucidating the mechanistic drivers of rhizosphere bacterial community dynamics in alpine mining environments. The piecewise SEM was implemented using the piecewiseSEM package within the *R* statistical software v4.3.2. The other graphs were plotted using the *R* package ggplot2.

# 3. RESULTS

## 3.1. EFFECTS OF DIFFERENT PLANTS ON RHIZOSPHERE SOIL PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of rhizosphere and bulk soils of different plants at the S1 and S2 sites were measured. The soil properties varied by plant species and were significantly influenced by living roots (Table 2).

Table 2

Rhizosphere soil properties among various plants at the S1 and S2 sites

| Parameter | S1 | | | |
|---|---|---|---|---|
| | JB | JD | JL | JC |
| SWC, % | 3.86±0.23 c | 5.53±0.12 b | 6.42±0.21 a | 5.39±0.05 b |
| pH | 8.37±0.06 a | 8.24±0.02 b | 8.22±0.04 b | 8.18±0.02 b |
| TC, g·kg$^{-1}$ | 1.03±0.05 c | 1.62±0.06 b | 4.06±0.48 a | 1.45±0.15 b |
| TN, g·kg$^{-1}$ | 0.1±0.00 d | 0.38±0.02 c | 0.81±0.02 a | 0.63±0.02 b |
| $NH_4^+$-N, mg·kg$^{-1}$ | 2.59±0.26 c | 3.31±0.1 b | 4.29±0.25 a | 2.8±0.16 c |
| $NO_3^-$-N, mg·kg$^{-1}$ | 3.11±0.38 c | 5.83±1.00 a | 26.12±1.32 a | 2.79±0.23 c |
| *AP*, mg·kg$^{-1}$ | 17.36±1.49 b | 24.48±1.14 a | 25.25±1.92 a | 17.17±2.23 b |
| *AK*, mg·kg$_{-1}$ | 17.02±1.08 d | 38.14±9.32 c | 138.35±2.72 a | 76.92±2.12 b |
| C/N | 10.42±0.49 a | 4.33±0.22 b | 5.01±0.57 b | 2.33±0.32 c |
| Parameter | S2 | | | |
| | CB | CD | CL | CM |
| SWC, % | 3.24±0.08 c | 3.61±0.25 c | 6.43±0.2 b | 8.62±0.25 a |
| pH | 8.81±0.07 a | 8.73±0.04 a | 8.49±0.06 b | 8.32±0.13 c |
| TC, g·kg$^{-1}$ | 1.18±0.06 c | 5.24±0.46 b | 5.53±0.43 b | 9.27±0.66 a |
| TN, g·kg$^{-1}$ | 0.16±0.02 d | 0.48±0.09 c | 0.64±0.06 b | 1.32±0.08 a |
| $NH_4^+$-N, mg·kg$^{-1}$ | 2.18±0.36 c | 1.31±0.04 d | 3.65±0.18 b | 9.38±0.41 a |
| $NO_3^-$-N, mg·kg$^{-1}$ | 5.1±1.03 d | 13.39±2.7 c | 36.86±1.5 b | 47.62±3.88 a |
| *AP*, mg·kg$^{-1}$ | 8.84±1.5 b | 12.43±2.57 ab | 14.57±2.84 a | 17.75±3.96 a |
| *AK*, mg·kg$_{-1}$ | 31.5±5.53 c | 29.8±10.15 c | 64.01±4.14 b | 169.86±10.57 a |
| C/N | 7.41±1.26 b | 11.05±1.12 a | 8.77±1.61 ab | 7.04±0.79 b |

Different letters within a row indicate significant differences between the rhizosphere soils of the three plant species and between these soils and the bulk soil ($p < 0.05$).

The study sites generally have high evaporation and low precipitation, resulting in low soil water content. The presence of plants significantly increased the soil water content in the rhizosphere ($p < 0.05$), with an increase of 39.66–166.09% compared to bulk soil (Table 3).

Table 3

The contribution of living roots from different plants to soil properties [%]

| | | SWC | pH | TC | TN | $NH_4^+$-N | $NO_3^-$-N | *AP* | *AK* | C/N |
|---|---|---|---|---|---|---|---|---|---|---|
| S1 | JD | 43.14 b | –1.19 a | 57.28 b | 274.80 c | 27.96 b | 87.49 b | 40.99 a | 124.10 c | –58.45 a |
| | JL | 66.24 a | –1.39 a | 294.39 a | 711.80 a | 65.67 a | 739.79 a | 45.46 a | 712.85 a | –51.99 a |
| | JC | 39.66 b | –1.51 a | 40.58 b | 523.17 b | 8.16 c | –10.42 c | –1.11 b | 351.92 b | –77.64 b |
| S2 | CD | 11.57 c | –0.95 a | 344.21 b | 200.27 c | –39.85 c | 162.61 c | 40.76 b | –5.40 c | 49.15 a |
| | CL | 98.35 b | –3.63 b | 368.73 b | 299.06 b | 67.35 b | 622.67 b | 64.96 b | 103.22 b | 18.35 b |
| | CM | 166.09 a | –5.60 c | 685.50 a | 726.33 a | 330.20 a | 833.75 a | 101.07 a | 439.22 a | –5.03 c |

Different letters within a row indicate significant differences between the rhizosphere soils of the three plant species and between these soils and the bulk soil ( $p < 0.05$).

## 3.2. EFFECTS OF DIFFERENT PLANTS ON THE STRUCTURE AND DIVERSITY OF BACTERIAL COMMUNITIES IN RHIZOSPHERE SOILS

Using 16S rRNA sequencing technology, the composition and diversity of bacterial communities in the rhizosphere soils of different plants at the S1 and S2 sites were analyzed. In this soil sequencing analysis, a total of 930,392 raw reads were obtained. 422,181 high-quality sequences remained after chimeras were cut, denoising, and removed. Furthermore, all sample sparsity curves were saturated (Fig. 1), indicating adequate sequencing depth and offering a more complete view of the bacterial community composition of the sequenced samples.

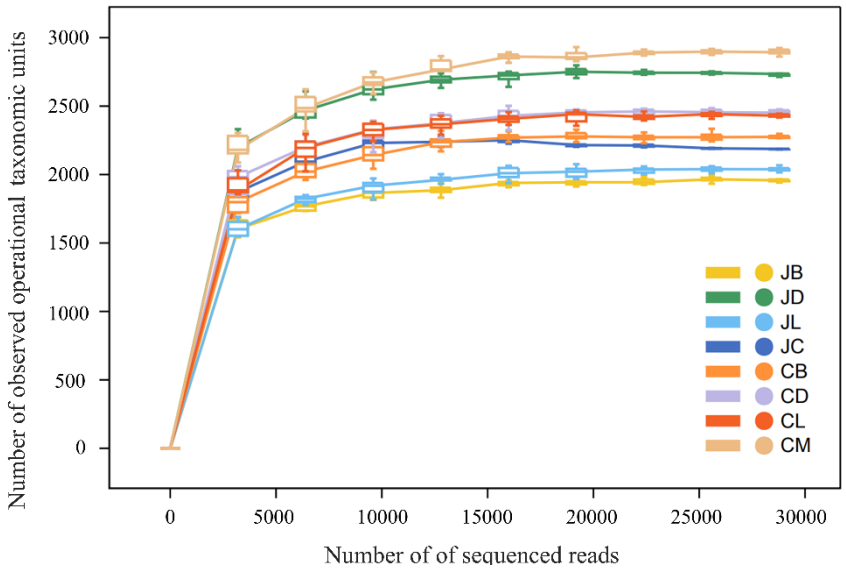


Fig. 1. OTU sparsity curve of rhizosphere soil bacteria of different plants at two alpine mines. Different colors denote bacterial communities from distinct sample groups

Heatmap analysis was performed to determine the abundance of dominant bacteria at the phylum and genus levels in the rhizosphere soils of three dominant plants at each site. The relative abundance patterns of the major bacterial phyla in the rhizosphere soils of different plants at both sites were Actinobacteria > Proteobacteria > Acidobacteria (Fig. 2a, b). However, the soil bacterial communities at the genus level differed between the two sites.

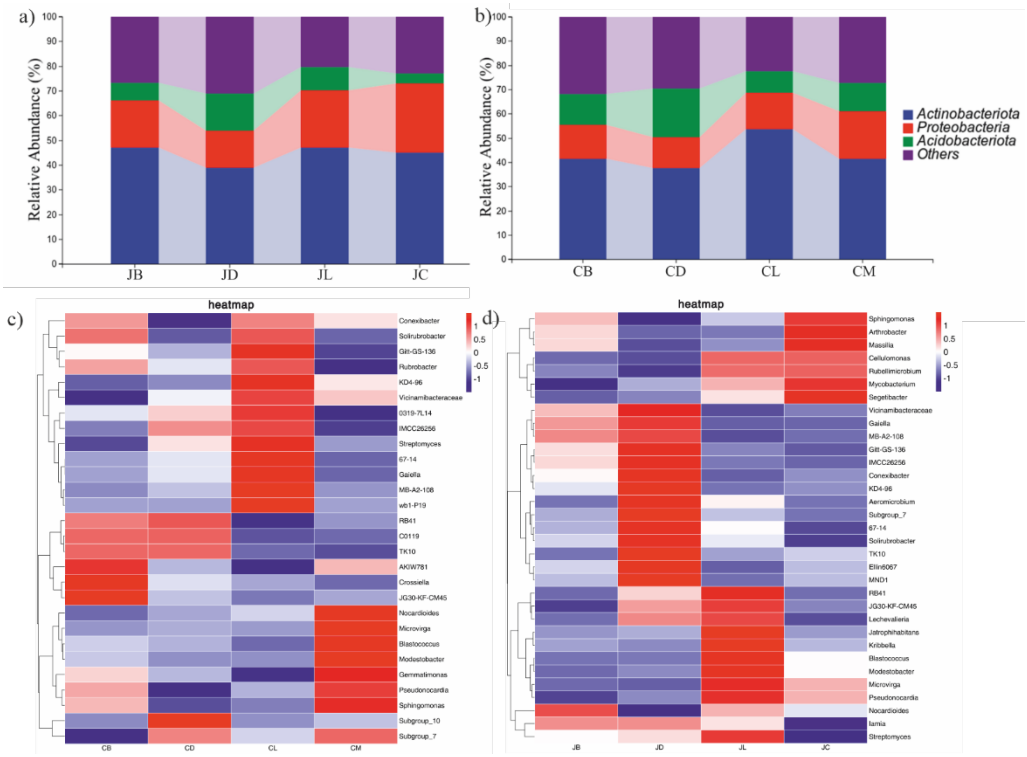


Fig. 2. The relative abundance of major bacterial phyla (abundance > 1%) in the rhizosphere soil of different plants at S1 (a) and S2 (b). Heatmaps of the top 10 bacterial genera in bulk and rhizosphere soil samples of different plants at S1 (c) and S2 (d)

To identify the dominant bacterial genera in bulk soil and rhizosphere soils of different plants, the top ten most abundant genera in each soil sample were selected. In total, 33 genera were identified at the S1 site (Fig. 2c), and 28 genera at the S2 site (Fig. 2d). The results showed that the dominant bacterial genera varied among the rhizosphere soils of different plants at both sites. At the S1 site, genera such as *Gitt-GS-136*, *IMCC26256*, and *Conexibacter* were more abundant in the rhizosphere soil of JD, while *Jatrophihabitans*, *Kribbella*, and *Blastococcus* were more abundant in the rhizosphere soil of JL. Gen-

era such as *Arthrobacter*, *Massilia*, and *Segetibacter* were more abundant in the rhizosphere soil of JC. The abundance of *Cellulomonas* and *Rubellimicrobium* was similar in the rhizosphere soils of JL and JC. At the S2 site, *Subgroup_10* had the highest abundance in the rhizosphere soil of CD, while *Streptomyces*, *67-14*, and *Gaiella* were more abundant in the rhizosphere soil of CL. Genera such as *Nocardioides*, *Microvirga*, and *Blastococcus* were more abundant in the rhizosphere soil of CM. Additionally, *Vicinamibacteraceae* showed similar abundance in the rhizosphere soils of CL and CM.

Table 4

Richness and diversity indices of bacterial communities in bulk and rhizosphere soils of different plants

| Parameter | S1 | | | |
|---|---|---|---|---|
| | JB | JD | JL | JC |
| Shannon index | 9.39±0.12 b | 10.2±0.17 a | 10.03±0.09 a | 10.28±0.1 a |
| Simpson index | 0.98±0.001 b | 0.99±0.02 a | 0.99±00.001 a | 0.99±0.001 a |
| Chao1 index | 2,202.83±54.49 c | 2,615.25±55.98 a | 2,291.95±57.55 c | 2,486.8±67.06 b |
| Parameter | S2 | | | |
| | C B | CD | CL | CM |
| Shannon index | 10.05±0.03 b | 10.05±0.08 b | 10.11±0.08 b | 10.45±0.07 a |
| Simpson index | 1.00±0.001 a | 0.99±00.003 a | 1.00±00.002 a | 1.00±0.002 a |
| Chao1 index | 2,344.77±114.19 c | 2,571.41±107.12 b | 2,558.65±129.86 b | 2,782.58±44.73 a |

Different letters in a row indicate significant differences among four types of soil samples ( $p < 0.05$).

The bacterial diversity in rhizosphere soils was significantly higher than in bulk soils (Table 4). At S1, the Chao1, Shannon, and Simpson indices in the rhizosphere soils of the three plants were significantly higher than those in the bulk soils ( $p < 0.05$). The rhizosphere soil of JL had the highest Chao1 index, significantly higher than the other two plants ( $p < 0.05$), while the Shannon index did not show significant variation among the rhizosphere soils of different plants. Similarly, at S2, the Chao1 indices in the rhizosphere soils of the three plants were significantly higher than those in the bulk soils ( $p < 0.05$). The rhizosphere soil of CM had the highest Chao1 and Shannon indices, significantly higher than the other two plants ( $p < 0.05$).

### 3.3. RELATIONSHIPS BETWEEN THE PHYSICOCHEMICAL PROPERTIES AND BACTERIAL COMMUNITIES IN RHIZOSPHERE SOILS

To investigate the combined effects of different plant species on soil nutrients and bacterial community structure, redundancy analysis (RDA) at two alpine mines was conducted. The RDA plots showed a close relationship between the soil properties and the top ten dominant bacterial phyla in the rhizosphere soils of different plants. The results indicated that different plant species had varying impacts on rhizosphere soil properties and bacterial community structures. At S1, JL showed a positive correlation with almost all soil nutrients compared to the other two plants (Fig. 3a), and was positively correlated

with the abundances of Actinobacteriota, Cyanobacteria, and Acidobacteriota. Similarly, at S2, CM showed a negative correlation with pH and a positive correlation with almost all other soil physicochemical properties, and was positively correlated with the abundances of Proteobacteria, Myxococcota, and Cyanobacteria (Fig. 3b). Thus, the presence of vegetation in alpine mines can significantly influence soil properties.

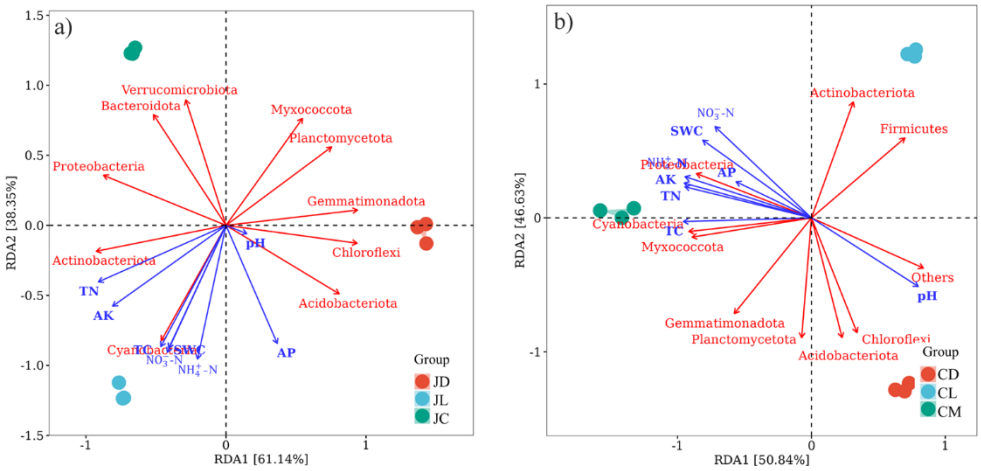


Fig. 3. Results of redundancy analysis (RDA) showing the correlation between soil properties in the rhizosphere of different plants and the top 10 dominant bacterial phyla at S1 (a) and S2 (b). The ordination plots display the species scores of dominant bacterial phyla (in red) and the scores of environmental factors (in blue) from the RDA

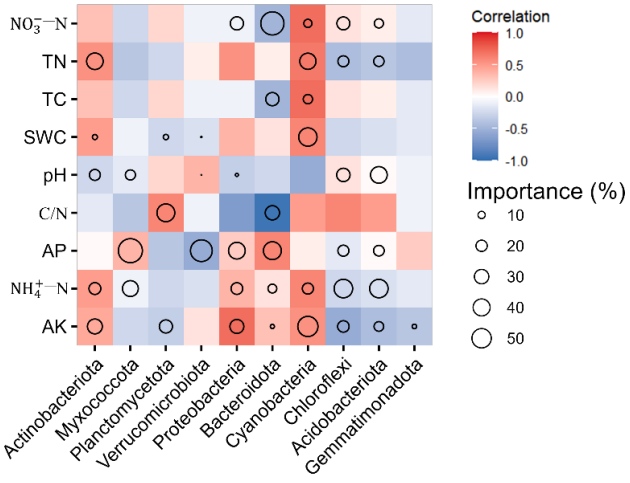


Fig. 4. Correlation heatmap showing relationships between rhizosphere soil properties, bacterial community structure, and the relative abundance of bacterial phyla. Circle size indicates variable importance (% explained variance from multiple regression and variance partitioning), and color indicates Spearman correlation coefficients

The impact of soil properties on bacterial community variation and the relative abundance of bacterial phyla in alpine mines was further assessed. The Spearman's correlation analysis was used to establish the relationships between the rhizosphere soil samples at S1 and S2. The results indicated that rhizosphere soil characteristics had a significant influence on soil bacterial communities (Fig. 4). Rhizosphere soil properties were strong positive predictors of differences in bacterial community composition and the relative abundance of most phyla. For example, the level of ammonium niytogen was significantly correlated with differences in bacterial communities and the abundances of Actinobacteriota, Myxococcota, and Acidobacteriota. Similarly, *AP* was significantly correlated with the abundances of Myxococcota, Proteobacteria, and Bacteroidota (Fig. 4).

### 3.4. DRIVERS OF RHIZOSPHERE SOIL BACTERIAL COMMUNITIES

To better understand the drivers behind changes in rhizosphere bacterial communities, the Piecewise SEM was employed to establish comprehensive pathways affecting soil bacterial diversity in alpine ecosystems such as alpine mines (AIC = 347.85, Fisher C = 20.25, $p = 0.132$, Fig. 5a).

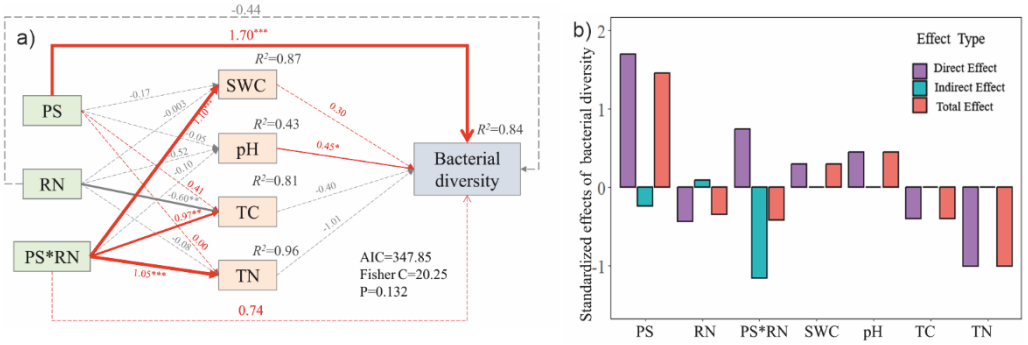


Fig. 5. Results of piecewise structural equation modeling ( piecewise SEM) showing the direct and indirect effects of plant species, root traits (the presence or absence of root nodules), and soil properties on soil bacterial diversity. Positive and negative effects are represented by red and gray arrows, respectively. Solid and dashed lines indicate significant ( $p < 0.05$) and non-significant ( $p > 0.05$) coefficients, respectively. Arrows between composite variables with numbers represent direct paths and standardized path coefficients, with arrow width proportional to the strength of the path coefficient

Given that plant species, soil properties, and root characteristics of legumes (the presence or absence of root nodules in this study) may directly or indirectly affect the community structure and diversity of rhizosphere bacteria, these possibilities were explicitly tested in our analysis. During the construction of the SEM, variables with high collinearity were excluded, and paths with $p > 0.05$ were selected to construct the final SEM. The results indicate that bacterial diversity can be directly affected by plant species (1.70). Soil

properties such as SWC (1.10), TC (0.97), and TN (1.05) are significantly influenced by plant species and root nodules. Additionally, soil bacterial diversity is significantly impacted by pH (0.45). In terms of total standardized effect coefficients, plant species has the greatest impact on soil bacterial diversity, followed by pH and SWC (Fig. 5b).

## 4. DISCUSSION

In the same location, the changes in soil physicochemical properties, microbial activity, and biogeochemical cycling induced by the rhizosphere, compared to bulk soil (the area away from roots), are referred to as the rhizosphere effect. This effect is recognized as a significant influence of plants on underground ecological processes [7]. Unique rhizosphere effects are exhibited by different plant species, leading to variations in the structure of rhizosphere soil microbial communities [9]. In this study, it was observed that the physicochemical properties of rhizosphere soil were greater than those of bulk soil, and these properties varied among different plant species, resulting in differences in the structure and diversity of bacterial communities. Specifically, it was demonstrated that, in alpine mining areas, soil nutrient content of rhizosphere soil was significantly improved, and bacterial diversity of rhizosphere soil was enhanced by leguminous shrubs compared to non-leguminous herbaceous plants and leguminous herbaceous plants.

### 4.1. DIFFERENCES IN SOIL PROPERTIES OF RHIZOSPHERE SOILS OF DIFFERENT PLANTS IN ALPINE MINES

The physicochemical properties of rhizosphere soil differ significantly among plant species. The root nodules of leguminous plants can effectively fix nitrogen from the air and convert it into organic nitrogen, thereby increasing the total nitrogen content in the soil [17]. Similarly, our study indicated that compared to *Artemisia desertorum* (a non-leguminous plant), *Astragalus laxmannii* and *Oxytropis coerulea* (leguminous herbaceous plants) showed more significant improvements in soil properties. Furthermore, compared to *Astragalus laxmannii*, *Sophora moorcroftiana* (a leguminous shrub) showed the most significant improvement in all soil properties in its rhizosphere soil, supporting our hypotheses 1 and 2.

In arid and nutrient-poor environments, nutrient-rich “fertile islands” are created around plant roots through vegetative cover, interception, and root exudates. These microenvironments are typically characterized by higher levels of nutrients and moisture compared to bulk soil. Previous studies have demonstrated that organic substances in root exudates (such as carbohydrates and amino acids) are important sources of rhizosphere carbon [9, 18]. In this study, the higher total carbon content in the rhizosphere soil of *Astragalus laxmannii* may be associated with carbon sources provided by its root exu-

dates. It has been demonstrated that an increase in soil carbon content is positively correlated with soil bacterial diversity and microbial biomass [18]. The lower concentrations of readily available nutrients (such as $NH_4^+$-N, $NO_3^-$-N, and available phosphorus) in the rhizosphere of *Oxytropis coerulea* are likely due to their uptake and depletion during root and bacterial metabolism. Additionally, a decrease in the pH of rhizosphere soil has been observed, likely caused by organic acids and oxalic acid secreted by plant roots, which can directly or indirectly lower soil pH [19].

### 4.2. DIFFERENCES IN RHIZOSPHERE SOIL BACTERIAL COMMUNITIES OF DIFFERENT PLANT SPECIES IN ALPINE MINES

Plant species significantly affected the diversity indices and structure of rhizosphere soil bacterial communities, varying among different plant species (Fig. 2, Table 4). Through sequencing analysis of bacterial communities in the rhizosphere soils of different plants, Actinobacteria were revealed to have the highest relative abundance in the rhizosphere soils of alpine mining areas. However, a study on alpine meadows at an average altitude of 3,800 m indicated that Proteobacteria had the highest relative abundance in rhizosphere bacterial communities [7]. Actinobacteria, with their excellent adaptation to extreme environments, ability to decompose organic matter, phosphate solubilization, and plant growth promotion functions [20], were found to dominate in the harsh and nutrient-poor soils of alpine mining areas.

It was also shown that there were significant differences in the dominant bacterial genera in the rhizosphere soils of different plants. For example, *Gitt-GS-136*, *IMCC26256*, and *Conexibacter* were found to have higher abundances in the rhizosphere soil of *Astragalus laxmannii*, while *Jatrophihabitans*, *Kribbella*, and *Blastococcus* were more abundant in the rhizosphere soil of *Sophora moorcroftiana*, likely due to the variation in root exudates among different plants, as plants select bacteria from the existing microbial community to construct their unique rhizosphere bacterial communities through root exudates [21]. The Chao1 and Shannon indices of bacterial diversity in the rhizosphere soils of *Astragalus laxmannii* (leguminous herb) and *Sophora moorcroftiana* (leguminous shrub) were significantly higher than those of other plants, validating hypothesis 2. This may be driven by significant differences in soil physicochemical properties that lead to niche differentiation [22] and by vegetative cover that provides more carbon sources for bacteria. Soil properties were found to have a broad impact on the bacterial community structure in the region's soils, while the plant species determined which bacteria could enrich in the rhizosphere.

### 4.3. DRIVERS OF RHIZOSPHERE SOIL BACTERIAL COMMUNITY STRUCTURE AND DIVERSITY

In global mountain ecosystems, temperature, vegetation characteristics, and soil pH are considered the primary drivers of microbial community composition, further influencing ecosystem functions [23]. Our study also indicated that plant species, root nodules of

leguminous plants, and soil physicochemical properties play critical roles in the composition and diversity of rhizosphere soil bacterial communities in alpine mines of the Qinghai-Tibetan Plateau, validating hypothesis 3. Redundancy analysis (RDA, Fig. 3) and structural equation modeling (SEM), Fig. 5) demonstrated that plant species play a critical role in shaping the composition and diversity of rhizosphere soil bacterial communities in alpine mines of the Qinghai-Tibetan Plateau. Meanwhile, plant species and root nodules jointly influence the soil physicochemical properties such as pH, soil water content (SWC), total carbon (TC), and total nitrogen (TN). Additionally, a negative correlation between pH and the majority of dominant bacterial phyla was observed in this study. However, this contrasts with the significant positive correlation between the relative abundance of Actinobacteria and Bacteroidetes and soil pH that was reported in previous studies [24]. Such a discrepancy may have occurred due to the unique, strongly alkaline environment of alpine mine soils. Elevated soil pH promotes bacterial community aggregation via deterministic assembly processes, serving as the primary driver of phylogenetic clustering patterns [25]. Therefore, increasing soil bacterial community diversity by improving soil nutrients may have a positive effect on the restoration of degraded ecosystems.

According to the conclusions of this study, the rhizosphere of leguminous shrubs has the best soil nutrient conditions and the highest diversity of soil bacterial communities. However, this study also has some limitations. For example, only two alpine mines were considered, and only the rhizosphere soil bacterial communities were studied, without involving other microbial communities, such as soil fungal communities, and without measuring root exudates. Based on the evidence from this study, the selection of restoration plants such as *Astragalus laxmannii* and *Sophora moorcroftiana* (leguminous plants) and the improvement of soil nutrient conditions in harsh, arid, and nutrient-poor environments are likely to contribute to the restoration of ecological functions in mining areas.

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