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WANG Yanying
Tibet University
Junze ZHANG
Tibet University
YIN Yebing
Tibet University
LUO Sicen
Tibet University
Yu LI
Tibet University
ZHAO Wenxiang
Tibet University
YANG Zuopeng
Tibet University
ZHENG Shirui
Tibet University
XU Guoqi
Tibet University
CAO Pengxi
Tibet University
Yixuan LIU
yixuan.liu@utibet.edu.cn
Tibet University

Research Article

Keywords: Northern Tibetan Plateau, alpine grassland, rhizosphere bacteria, diversity characteristics, community construction

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11376682/v1

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Additional Declarations: No competing interests reported.

Plant rhizosphere bacterial community structure in northern Tibetan alpine grassland shows host preference

WANG Yanying[1,2,4†], ZHANG Junze[1], YIN Yebing[1], LUO Sicen[1], LI Yu[1], ZHAO Wenxiang[1], YANG Zuopeng[1], ZHENG Shirui[1], XU Guoqi[1], CAO Pengxi[1,3], LIU Yixuan[1,2,3, †*]

[1]Key Laboratory of Biodiversity and Environment on the Qinghai-Tibetan Plateau, Ministry of Education, Xizang University, Lhasa, 850000, China

[2]Nagqu Mitika, Alpine Wetland Ecosystem, Observation and Research Station of Xizang Autonomous Region, Xizang University, Lhasa, 850000, China

[3]Yani Wetland Ecosystem Positioning Observation and Research Station, Xizang University, Lhasa 850000, China

[4]Lhasa Plateau Ecosystem Research Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

*****Yixuan Liu

Correspondence: yixuan.liu@utibet.edu.cn; Tel.: +86-133-9809-9819

[†]These authors contributed equally to this work.

Running Title: Alpine grassland rhizosphere bacterial community

Abstract

The Qiangtang area in northern Xizang is a typical alpine grassland ecosystem on the Qinghai-Xizang Plateau In this study, using high-throughput sequencing, bioinformatics methods, and analysis of the bacterial diversity characteristics of the non-rhizosphere soil and rhizosphere soil of dominant plants in the alpine grassland in northern Xizang, it was found that, compared to geographical and environmental factors, environmental factors had more effects on non-rhizosphere soil bacterial diversity than on plant rhizosphere bacterial diversity. The construction of soil bacterial communities in the rhizosphere showed stronger host preference than the communities in the non-rhizosphere soil. Additionally, stochastic processes played a more dominant role in soil bacterial community construction than deterministic processes did. The results of this study provide a theoretical basis for further revealing the adaptation mechanisms of rhizosphere bacteria and their symbiotic plants to extreme environments and the response mechanisms of these systems to global change.

Keywords: Northern Tibetan Plateau, alpine grassland, rhizosphere bacteria, diversity characteristics, community construction

1 Introduction

The "plant-soil-microorganism" interface is driven by a combination of geographic, physicochemical, and biological factors, providing a unique niche for plant rhizosphere microorganisms. In recent years, many studies have found that the factors driving plant rhizosphere microbiome construction include two categories: plant species and soil physicochemical properties. Plant rhizosphere microorganisms originate from soil microorganisms, and this is an important factor regulating their

composition and structure (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012) . Rhizosphere microbial communities are constructed through the migration of microorganisms from the non-rhizosphere soil to the plant rhizosphere, where they eventually form a stable community; thus, this community is initially determined by the local soil environment (Erlandson *et al.*, 2018). Eventually, the rhizosphere microbial community forms specific characteristics depending on the combination of soil and plants in the environment. Zuo et al. (Zuo *et al.*, 2020), who studied the rhizosphere microorganisms (*Ammopiptanthus mongolicus*) in desert areas of northwest China, found that community assembly strongly depends on the specific nature of the soil and the plant cover. Owing to the influence of the rhizosphere effect, each plant has unique rhizosphere microorganisms (Li *et al.*, 2019). Plants recruit microorganisms in the surrounding soil through root exudates, attracting life-promoting effects that are conducive to nutrient absorption and responding to external stress (Chaparro *et al.*, 2014). Ultimately, this allows the formation of a stable rhizosphere microbial community structure, which can be rapidly adjusted according to the spatial and temporal requirements needed during plant growth (Chaparro *et al.*, 2014). Studies show that different host plants that recruit rhizosphere microbes may have preferences for certain microbial species. For example, Jaiswal et al. (Jaiswal *et al.*, 2019) found that different varieties of peanuts had obvious selectivity for different rhizosphere-related bacteria, potentially because changes in the soil prompted the plants to adjust the composition of their rhizosphere bacterial community. Recent studies have shown that for crops (Ji, 2019; Tang, 2021), grassland plants (Zhao *et al.*, 2019; Zhou *et al.*, 2019), and desert plants (Kang *et al.*, 2021), the community composition, number, and diversity of the rhizosphere microorganisms significantly differ among different plants in the same ecosystem. Furthermore, the level of plant diversity or presence of different species may have a significant impact on the rhizosphere microbial community (Garbeva *et al.*, 2004; Berg and Smalla, 2009). The diversity promotion hypothesis (i.e., diversity begets diversity) suggests that plant communities with high diversity provide richer food resources for microorganisms and can therefore promote soil microbial diversity; consequently, the large and diverse soil microorganism community can feed back into plant growth through the nutrient cycle process, promoting plant diversity (Leff *et al.*, 2018). Studies have found that "diversity effects" can sufficiently explain the effects of plants on soil bacterial diversity at large spatial scales (Delgado‐Baquerizo *et al.*, 2017). In contrast, at small local scales, plant diversity is more closely related to soil environmental factors (Leff *et al.*, 2018), so it is difficult to determine whether the host plant itself or soil environmental factors affect the construction pattern of plant rhizosphere microbial communities.

The interaction mechanisms between soil bacteria and plants in extreme environments are underpinned by complex coevolutionary processes that facilitate mutual adaptation and survival. Several studies highlight the significance of these interactions in maintaining ecosystem stability and promoting plant health. Shen et al. (Shen *et al.*, 2022) emphasize the importance of understanding soil-vegetation relationships, which provides a foundational context for exploring how soil microbial communities influence plant characteristics, especially in challenging environments. Harnessing coevolutionary mechanisms has practical implications, such as utilizing Streptomyces and other soil bacteria to mitigate drought stress, illustrating how eco-evolutionary strategies can enhance plant resilience in extreme conditions (Liu *et al.*, 2024). At the molecular level, bacterial adaptation to plant environments involves coevolved traits that facilitate mutualism, with genetic and population mechanisms driving these evolutionary processes (Saati-Santamaría *et al.*, 2026). The evolution of symbiotic bacteria within plant-soil systems further exemplifies the interplay of molecular and population dynamics, emphasizing the importance of coevolution in maintaining functional microbial communities in harsh environments (Delaux and Schornack, 2021). Collectively, these studies underscore that soil bacteria and plant coevolution in extreme environments is a multifaceted process driven by mutualistic and antagonistic interactions, genetic adaptations, and ecological dynamics.

Understanding these mechanisms provides a theoretical basis for advancing research into soil-plant-microbe interactions, with implications for ecosystem management and sustainable agriculture in challenging environments. This study provides a theoretical basis for further studying the interaction mechanism between soil bacteria and plant coevolution in extreme environments, and also lays the groundwork for systematic sampling in 2028 to establish temporal comparisons, thereby helping to reveal changes in rhizosphere microbial community structure, host preference, and their environmental drivers across different periods.

2 Materials and methods

2.1 Sampling area

Samples were taken from six locations in Qiangtang National Nature Reserve in the northern Tibetan Plateau, namely Lungring Township (LR), Wuqiong Lake (WQ), PR, Puruogangri (PR), Yangbajain Town (YB), Shuanghu County (SH), Zharen Township (ZR) (Fig. 1). Prior to the investigation, the study plan was reported to and approved by the Xizang Autonomous Region Forestry Department and the relevant nature reserve departments.

Plant sampling was conducted with permission from the Nagqu Forestry and Grassland Bureau, Tibet, China. Voucher specimens for all plant species have been deposited in the Germplasm Resource Bank of Tibet University (deposition numbers: 1284‑1316). Plant species were identified by Mr. Shengping Ming (Tibet University) with reference to the Flora of Tibet (online version).

2.2 Sampling method

Samples for this study were collected in August 2018. To ensure result accuracy, three sampling points were established at each of six sites, with an inter-point distance exceeding 200 meters. A total of 15 plant root-associated bacterial strains were isolated from diverse locations and ecological types(Table 1). Non-root-associated soil samples from the six sites (LRcon, WQcon, PRcon, SHcon, YBcon, and ZRcon) served as controls. In total, 18 non-root-associated and 99 plant root-associated soil samples were collected, yielding 117 samples.During rhizosphere soil collection, the dominant plant in that habitat was removed from the soil, and the roots were gently shaken to dislodge the soil into a sterile sampling bag. The roots and residual soil were loaded into 50 mL sterile centrifuge tubes and brought in a f20 ℃ vehicle refrigerator back to the laboratory, where they were cryopreserved at 80 ℃ until extraction of total soil bacterial DNA. Soil samples were also brought back to the laboratory and stored at 4 ℃ until soil physical and chemical factor determination. For the non-rhizosphere soil samples, approximately 200 g of the 0–20 cm soil layer without vegetation cover was collected and divided into two samples. The first sample consisted of approximately 5 g of soil loaded into a 50 mL sterile centrifuge tube, which was then transported back to the laboratory in a 20 ℃ vehicle refrigerator and stored at 80 ℃ until extraction of the total DNA of the soil bacteria. The second sample consisted of the remaining soil (approximately 200 g), which was placed in a sterile bag, air dried, transported back to the laboratory, and stored at 4 ℃ until determination of the soil physicochemical factors. Details of the grassland plant species and sample sites can be found in the supplementary materials.

Other environmental factors were determined in the field at the time of sampling. The altitude (ALT) was determined using a handheld GPS (GARMIN, eTrex309X). Using an illuminometer (HANNA, HI97500), the illumination intensity (II), relative humidity (Relative humidity, RH), dew-point temperature (DT), and atmospheric temperature (AT) were determined in the field. Using a carbon dioxide concentration meter (HANNA, HI4005-03), the atmospheric carbon dioxide concentration

(CO_2 concentration, CC) was also measured in the field. Soil temperature (ST) was measured in the field with a soil thermometer (TopYunnon, TRS-II).

2.3 DNA extraction and PCR amplification of the 16-S rRNA fragment

Total DNA was extracted from the soil samples using SDS, and the purity and concentration of the DNA samples were determined by 1% agarose gel electrophoresis. PCR amplification of the bacterial 16S rRNA V3 V4 region was performed using universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Eppendorf, Germany). The PCR system consisted of 5 μL buffer, 5 μL dNTP, 3 μL $MgCl_2$, 2.6 μL of each primer, 2 μL template, and 0.4 μL Taq enzyme, supplemented with ddH2O to 50 μL. The PCR conditions were 95 °C for 5 min; 30 cycles of 95 °C for 1 min, 55 °C for 45 s, and 72 °C for 1 min; terminal extension of 72 °C for 7 min; and termination at 4 °C.

2.4 High-throughput sequencing and bioinformatics analysis

The PCR products were purified using the Illumina MiSeq platform, and small fragment libraries were constructed using paired-end sequencing. The results were obtained using the I-Sanger cloud platform (http://om.i-sanger.cn/).

Using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.17) software for the quality control of the raw sequencing data, double-terminal paired-end reads were assembled into a single sequence. Using FLASH (version 1.2.11), the splicing sequence of the overlapping region allowed a maximum mismatch ratio of 0.2. Quality filtering of reads was conducted using Trimmomatic (version 0.33) (El-Tohamy, 2019), where reads below 50 bp or containing N bases were removed and the sequence direction was corrected. The number of allowed mismatches at the end of the sequence was 0 and the maximum number of primer mismatches was 2. The optimized clean reads were obtained after filtering out singletons.

The uparse algorithm in USEARCH (version 11.0) was used to cluster non-repetitive sequences (operational taxonomic units, OTUs) according to 97% similarity, and the resulting OTUs were annotated at different taxonomic levels based on the Silva (Release132) database and sorted according to the minimum number of sample sequences. Using mothur (version v.1.30.1), the alpha diversity index was calculated under different random sampling of OTUs with 97% similarity. Dilution plots were made using R (version 3.3.1). The Student's t-test was performed on the Sobs index. The Principal Coordinates Analysis (PCoA) distance matrix was calculated based on the Bray-Curtis distance using QIIME software. Permutational multivariate analysis of variance (PERMANOVA) was conducted to interpret the sample differences, and statistical significance of the partition was determined using the permutation test.

Based on the taxonomic analysis results, the common and endemic flora in the soil samples under different grassland types were analyzed at the phylum and genus levels, respectively, using R.

Environmental factors with a variance inflation factor (VIF) < 10 were selected by measuring the multicollinearity relationship between environmental factors. The OTU table for samples with 97% similarity was analyzed using detrended correspondence analysis, and according to the results, the canonical correspondence analysis model (CCA) was selected to analyze the relationship between microflora and environmental factors. The significance of the CCA was judged by ANOVA using the R package “permutest.” According to the results of the genus-level beta-diversity analysis, linear regression analysis was performed with the beta-diversity index of each sample as the y-axis and the

environmental factor corresponding to the sample as the x-axis. The impact of environmental factors on bacterial communities was quantitatively assessed by variance partitioning analysis (VPA) ANOVA decomposition. Through PCoA sorting, procrustes analysis was performed based on the Binary Jaccard distance, and through continuous iteration, the maximum overlap (maximal superimposition) of the standard canonical shape was analyzed. Prokaryotic microbial community randomness and certainty were calculated based on the beta nearest taxon index (β-NTI)/Bray–Curtis-based Raup–Crick (RC Bray). The above analysis was conducted using the Meiji Cloud platform (https://cloud.majorbio.com/page/tools/).

2.5 Soil physicochemical factor determination

Soil pH was determined using a potentiometer. Specifically, 10 g of air-dried soil samples were added to 25 mL of pure water, stirred for 5 min, and allowed to rest for 1–3 h, and then the pH was measured using a corrected pH meter. The soil water content (soil moisture, SM) was determined according to the drying method, where 5 g of fresh soil was placed in a drying oven at a constant temperature of 105 ± 2 ℃ for 48 h. The formula for calculating the SM was SM (%) = [(5 – dry weight) / 5] *100%. Electrical conductivity (EC) was measured using a conductivity instrument (HANNA, HI98304) with a soil and water ratio of 3:1. Using ammonium acetate extraction-atomic absorption and spectrophotometery, the contents of organic matter (OM), potassium, available potassium (AK), available phosphate (AP), and ammonium nitrogen (AN) were determined (Carrillo *et al.*, 2019; Chang *et al.*, 2021; He *et al.*, 2024; Tie *et al.*, 2024; Ye *et al.*, 2024).

3 Results

3.1 Sequencing results and diversity analysis

After optimizing the sequencing results of the 117 samples, 11,352 OTUs with 97% similarity were obtained. The average sequencing coverage was 98.38%. The rarefaction curve shows that as the amount of sequencing data increased, the diversity of the flora in the sample also increased (Fig. 2A). Although a small number of bacteria were not identified, the existing sequences met the requirements of subsequent analysis. The OTU number of the non-rhizosphere soil bacteria was much higher than that of the plant rhizosphere bacteria (Fig. 2A). The group difference tests based on the ACE diversity index and Shannon diversity index (Fig. 2B, C) showed that there were significant differences in the richness and diversity of non-rhizosphere soil bacteria.

PERMANOVA based on the Binary Jaccard distance revealed that plant species, soil type, and the geographical location of the sampling point all had significant effects on the bacterial community composition ($P = 0.001$; Table 2). The structural variation in the microflora according to the plant species, soil type, and sampling location was 61.82%, 32.17%, and 18.11%, respectively (Table 2. The PCoA analysis of the OTU level showed that the PC1 axis explained 10.04% of the changes between the non-rhizosphere and soil bacterial communities among different sites, and the PC2 axis explained 4.64% of the changes. These results indicated that there were significant differences between the non-rhizosphere and rhizosphere soil bacterial communities in terms of species composition and abundance at different sites (Fig. 2D).

3.2 Structure of bacterial communities in non-rhizosphere and rhizosphere soils

By working with the Silva database (https://www.arb-silva.de/), we obtained 11,352 OTUs of non-rhizosphere and rhizosphere bacteria in Longren Township, belonging to 46 phyla, 152 classes, 376 orders, 674 families, and 1440 genera. At the phylum level, Actinobacteriota, Chloroflexi,

Acidobacteriota, Bacteroidota, and Gemmatimonadota had higher abundances in the non-rhizosphere soil than in the rhizosphere soil, whereas Proteobacteria and Firmicutes had much higher abundances in the rhizosphere soil than in the non-rhizosphere soil (Fig. 3A). At the genus level, the non-rhizosphere soil bacteria had similar structures in the same ecological types, while the rhizosphere soil bacteria differed significantly in the same ecological types (Fig. 3B). The phyla with a relative abundance greater than 1% were defined as the core flora. The core flora in the non-rhizosphere soil included Proteobacteria, Actinobacteriota, Chloroflexi, Acidobacteriota, and Bacteroidota. The core flora of the rhizosphere soils included Proteobacteria, Firmicutes, and Actinobacteriota.

3.3 Effects of soil environmental factors on the bacterial community

The environmental factors of the root soil at different sampling sites are shown in Table 3. According to the VIF analysis, EC, OM, AK, AN, ALT, LAT, and II were found to be significantly related to the bacterial community structure, reflecting their substantial impact on the bacterial community.

The relevant results of the CCA showed that the first- and second-ranking axes explained 4.96% of the total variation in the species data (Fig. 4A). AK, AN, EC, and OM were the environmental factors with the strongest effects on the bacterial communities in the non-rhizosphere soil, while LAT and ALT had the strongest effects on the rhizosphere soil communities. The coefficients (R^2 values) for AK, AN, EC, OM, LAT, and ALT were 0.57, 0.39, 0,31, 0.19, 0.16, and 0.06, respectively, and the order of their influence on the flora was AK > EC > II > AN > OM > LAT. The results of the linear regression analysis showed that although OM, AN, and II had significant effects on the beta diversity ($P < 0.05$), there were few differences in the community composition (Fig. 4B–D).

The environmental factors were classified into three different types: geographic (ALT, LON, and LAT), climate (II, RH, DP, CC, and AT), and soil (pH, SM, EC, OM, AK, AP, AN, and ST). Variation partitioning analysis (VPA) showed that, together, the environmental factors explained 46.14% of the differences in the non-rhizosphere soil bacterial communities. Among the environmental factors, the combined effect of soil and climate factors as well as the combined effect of soil, climate, and geographic factors were the most explanatory. The interpretation degree of the geographic factors alone was 0. The community difference that was not explained by the above environmental factors was 54% (Fig. 5A). The combination of all the environmental factors explained 4.09% of the differences for the rhizosphere bacteria community. Accordingly, 95.91% of the community differences were not explained by the above environmental factors (Fig. 5B). The influence of environmental factors on the composition of non-rhizosphere soil communities (Mantel test, r = 0.554, P = 0.001) was greater than that on the composition of rhizosphere bacteria (Mantel test, r = 0.018, P = 0.701). According to the results of the procrustes analysis comparing the fit between the spatial ranking of the bacterial community structure in non-rhizosphere soil and environmental factors, the correlation between non-rhizosphere soil and environmental factors (M^2 = 0.574, P = 0) was greater than the correlation between rhizosphere soil and environmental factors (M^2 = 0.959, P = 0.027) (Fig. 5C, D).

The β-NTI/RC Bray analysis of the construction of the non-rhizosphere and rhizosphere soil bacterial communities in northern Tibetan alpine grasslands showed that the construction mechanisms for the two types of bacterial communities were differently influenced by stochasticity and deterministic processes at different sampling sites. In general, the construction of the soil bacterial community in the alpine grassland of northern Xizang is more affected by random processes than by deterministic processes, diffusion limitation and the non-dominant process are the main factors affecting

stochasticity, and heterogeneity selection is the dominant factor in the deterministic processes (Fig. 6).

4 Discussion

In the alpine grassland of northern Xizang, the richness and diversity of non-rhizosphere soil bacteria is much higher than that of rhizosphere soil bacteria, and there are significant differences between non-rhizosphere bacteria. These results confirm that the composition of rhizosphere bacterial communities comes from soil (Bai *et al.*, 2022), and that the bacteria are specifically recruited by plants in the non-rhizosphere soil.

Regarding the differences in the composition and abundance between rhizosphere and non-rhizosphere bacteria, the composition of plant rhizosphere bacteria was dominated by Proteobacteria, Firmicutes, and Actinobacteriota, whereas that of non-rhizosphere bacteria was dominated by Proteobacteria, Actinobacteriota, Chloroflexi, Acidobacteriota, and Bacteroidota. Compared with the Chloroflexi in the non-rhizosphere soil, the Proteobacteria in the rhizosphere soil grows more rapidly and, like Actinobacteriota and Firmicutes, it plays an important role in the absorption of nutrients and trace elements in the plant rhizosphere (Tian *et al.*, 2019). At the genus level, the structural composition of the non-rhizosphere bacteria was significantly different than that of the rhizosphere bacteria. The structural composition of plant rhizosphere bacteria varied greatly between different grassland types, or even within the same grassland type, and it was simpler than that of non-rhizosphere bacteria, with a relative abundance of less than 1%. The relative abundance of *Acinetobacter* in alpine meadow was relatively high (21.21%), as was that of *Bacillus* in the same sampling location (31.51%). The relative abundance of *Lachnoclostridium* in the alpine grassland and Shuanghu County was relatively high (45.72% and 68.91%, respectively). Additionally, the relative abundances of *Lelliottia* (64.59%) and *Hafnia-Obesumbacterium* in the high desert were high (46.73%). Most of the above bacteria have the functions of nitrogen fixation, phosphorus lysis, potassium solution, and resistance to extreme environmental conditions (Wei, 2017; Wang, 2021). They are therefore bacteria that can potentially promote plant life, which can subsequently improve the nutrient cycle in the plant rhizosphere microecological environment in alpine grasslands.

At present, most of the studies on the rhizosphere microbial composition of oats (*Avena sativa* L.), bromeliads, and agave in different geographical locations have found significant differences in the composition and diversity of the rhizosphere microbial communities (Coleman‐Derr *et al.*, 2015; Liu, 2018; Ren *et al.*, 2025). In our study, the effect of biogeographic factors on the variation in beta diversity of the microflora showed that plant species had a greater impact than soil type (rhizosphere soil vs. non-rhizosphere soil) and the sampling location, in that order. This result implies that the bacterial communities in the rhizosphere soil had a stronger host preference compared with their preference for the geographical location. Several existing studies support our conclusions. Niu et al. (Niu *et al.*, 2017) studied the microbial composition of different cities in the northeast and midwest of China, and found similar community composition in different cities in the former but significant differences in the latter, indicating that geographical location cannot fully explain the formation of the rhizosphere microbiome. Bonito et al. (Bonito *et al.*, 2014) investigated the soil properties in poplar, oak, and pine trees in three soil environments, and they found that the host type had a strong influence on the rhizosphere bacterial composition. Edwards et al. (Edwards *et al.*, 2015) found that the soil type and host plant genotype jointly affected the construction of the rice rhizosphere microbiome.

Abiotic and biological factors in the soil, such as the complexity of the soil ecosystem and crops, soil type, legacy effects, climate, and pH, can affect the assembly of the rhizosphere microbial community and cause changes in the plant rhizosphere microecological environment. This has led to many studies being conducted on the rhizosphere microbial community construction process, though the conclusions substantially differ. For example, some research has found that pH is a key factor affecting the assembly of nitrogen fixation in the microbial communities in the wheat rhizosphere (Fan *et al.*, 2018), and other research has found that the developmental stage of Arabidopsis greatly affects its rhizosphere microbial community assembly (Chaparro *et al.*, 2014). Several studies related to alpine grasslands on the Tibetan Plateau have shown that the stability of plant rhizosphere microbial communities is closely related to environmental factors such as soil pH, available phosphorus, available nitrogen, organic carbon content, and altitude (Ma, 2019; Liu *et al.*, 2021; Fu, 2022; Zhang *et al.*, 2025). In contrast to the results of previous studies, this study suggests that the construction of the rhizosphere soil bacterial community in alpine grassland is more affected by plants than by spatial and environmental factors.

The assembly of microbial communities is shaped by both deterministic and stochastic processes (Dini-Andreote *et al.*, 2015). Deterministic processes are based on niche theory, assuming that differences in community structure and metabolic function result from microbial preferences and adaptability to different habitats, and that community construction is determined by biological and abiotic factors such as species characteristics, interspecies interactions, and environmental conditions. Stochastic processes are based on neutral process theory, assuming that stochastic processes such as birth, death, migration, speciation, and dispersal restriction lead to a balance between the loss and gain of microbial taxa, ultimately shaping the community structure of microorganisms. This study found that the construction of soil bacterial communities in different habitats of the alpine grassland in northern Xizang varied greatly in response to random and non-random processes. Overall, the construction of bacterial communities is dominated by stochastic processes, and diffusion limitation and non-dominant processes are the main influencing factors, indicating that the influence of distress termination processes, such as drift, is greater than the expected turnover rate. Regarding deterministic processes, heterogenous selection was the main influencing factor, while the effect of homogenized selection did not exceed 10% in each habitat, indicating that the effect of biological interactions is much greater than that of environmental selection.

5 Conclusion

This study demonstrates that rhizosphere bacterial communities in the alpine grasslands of northern Xizang are strongly shaped by host preference, with plants selectively recruiting specific taxa from the surrounding soil pool. Although these findings provide valuable insights into plant–soil–microbe interactions in extreme environments, several limitations should be acknowledged. The samples were collected in 2018; therefore, the results represent a snapshot of rhizosphere and non-rhizosphere bacterial communities at that time and cannot fully capture interannual variation, seasonal dynamics, or long-term successional trends. Furthermore, this study relied primarily on 16S rRNA gene amplicon sequencing, which limits direct inference of microbial functional potential, activity, and mechanistic interactions between plants and microbes. Additionally, plant root traits, root exudates, soil enzyme activities, and functional gene profiles were not systematically integrated, thereby precluding identification of the physiological and molecular mechanisms underlying host preference.

Future research should therefore incorporate systematic resampling in 2028 to establish a temporal comparison with the 2018 samples, thereby enabling examination of changes in rhizosphere bacterial community structure, host preference, and environmental drivers over time. The integration of

metagenomics, metatranscriptomics, metabolomics, and stable isotope probing would facilitate linking community composition to microbial function and activity. The isolation and functional validation of key rhizosphere taxa identified in this study could further elucidate their roles in nitrogen fixation, phosphorus and potassium solubilization, and stress tolerance. Comparative studies across plant functional types, elevation gradients, and grassland degradation stages would further enhance understanding of how host preference and environmental selection jointly shape rhizosphere microbiomes in extreme environments. Collectively, this study establishes a baseline for long-term monitoring and mechanistic exploration of rhizosphere microbial assembly in alpine grasslands, and highlights promising directions for future research on plant–microbe co-adaptation on the Tibetan Plateau.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Ethics approval and consent to participate

Permission for plant sample collection was obtained from the Nagqu Forestry and Grassland Bureau, Tibet, China. Sampling was conducted on public land with the approval of the appropriate governing body.

8 Consent for publication

Not applicable.

9 Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI Sequence Read Archive repository, PRJNA1063442 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1063442).

10 Competing Interests

The authors declare that they have no competing interests.

11 Author Contributions

Conceptualization, Y.W.; data curation, Y.W. and Y.L. (Yu Li); investigation, Y.W., S.L., and Y.L. (Yi-xuan Liu); validation, Y.W., S.Z., and G.X.; writing—original draft, J.Z.; writing—review and editing, P.C., S.L., Y.L. (Yi-xuan Liu), Y.W., and Y.Y.; visualization, J.Z., S.L., Y.L. (Yi-xuan Liu), Y.W., Y.Y., Y.L. (Yu Li), W.Z., S.Z., G.X., H.M., and P.C. All authors have read and agreed to the published version of the manuscript.

12 Funding

This research was funded by the Central Special Fund for Supporting Local Universities' Reform and Development in 2026 (Zang Cai Yu Zhi [2026] No. 1), the Ecological High-level Team Building Project (grant number XMK25-000842), and the Science and Technology Projects of Xizang Autonomous Region, China (grant number XZ202501JD0016).

13 Acknowledgments

We would like to thank Guoqi Xu of Xizang University and Xiaoyan Li of Wuhan University for their assistance in sample collection.

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11 Data Availability Statement

Raw sequences are available at the NCBI Sequence Read Archive (PRJNA1063442).

12 Figure captions

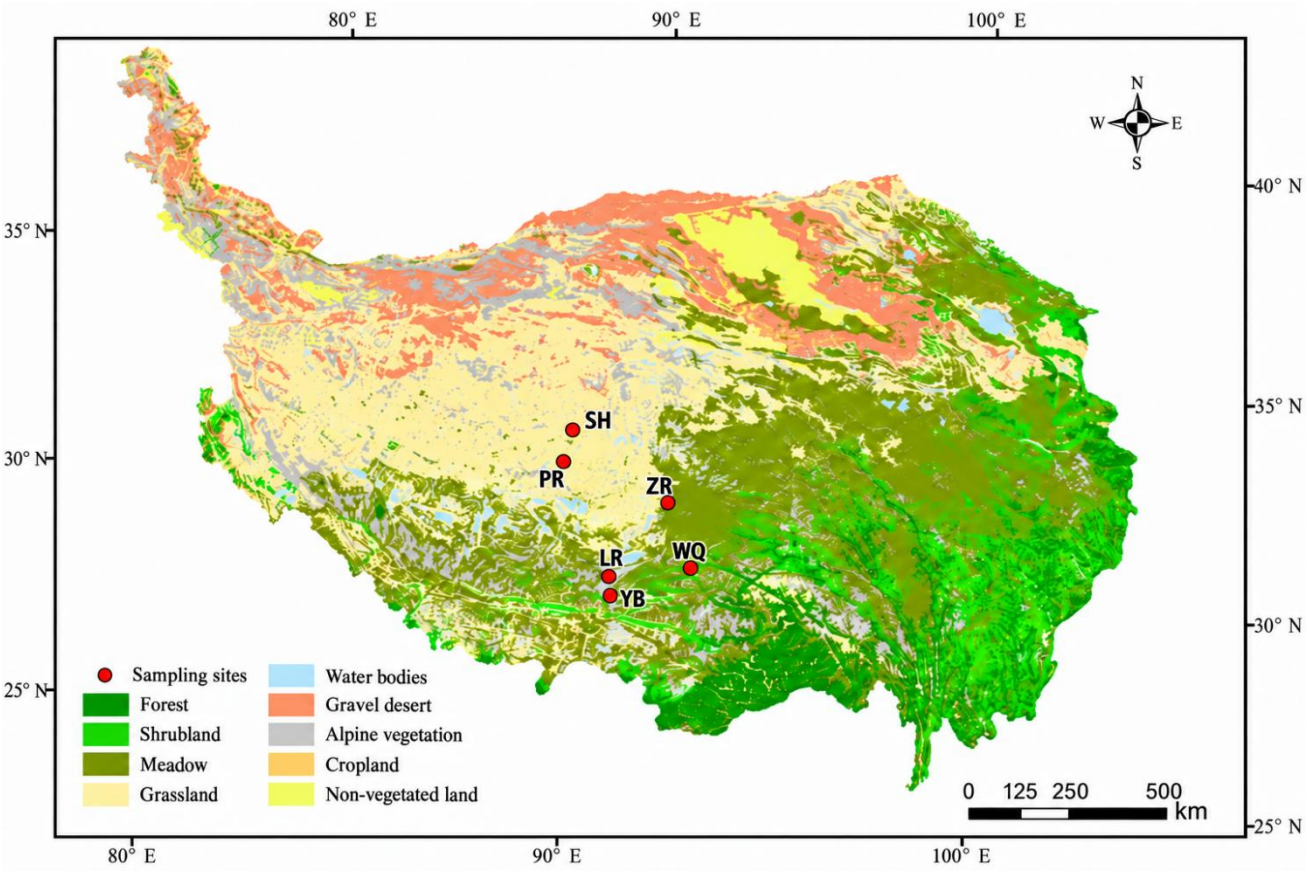
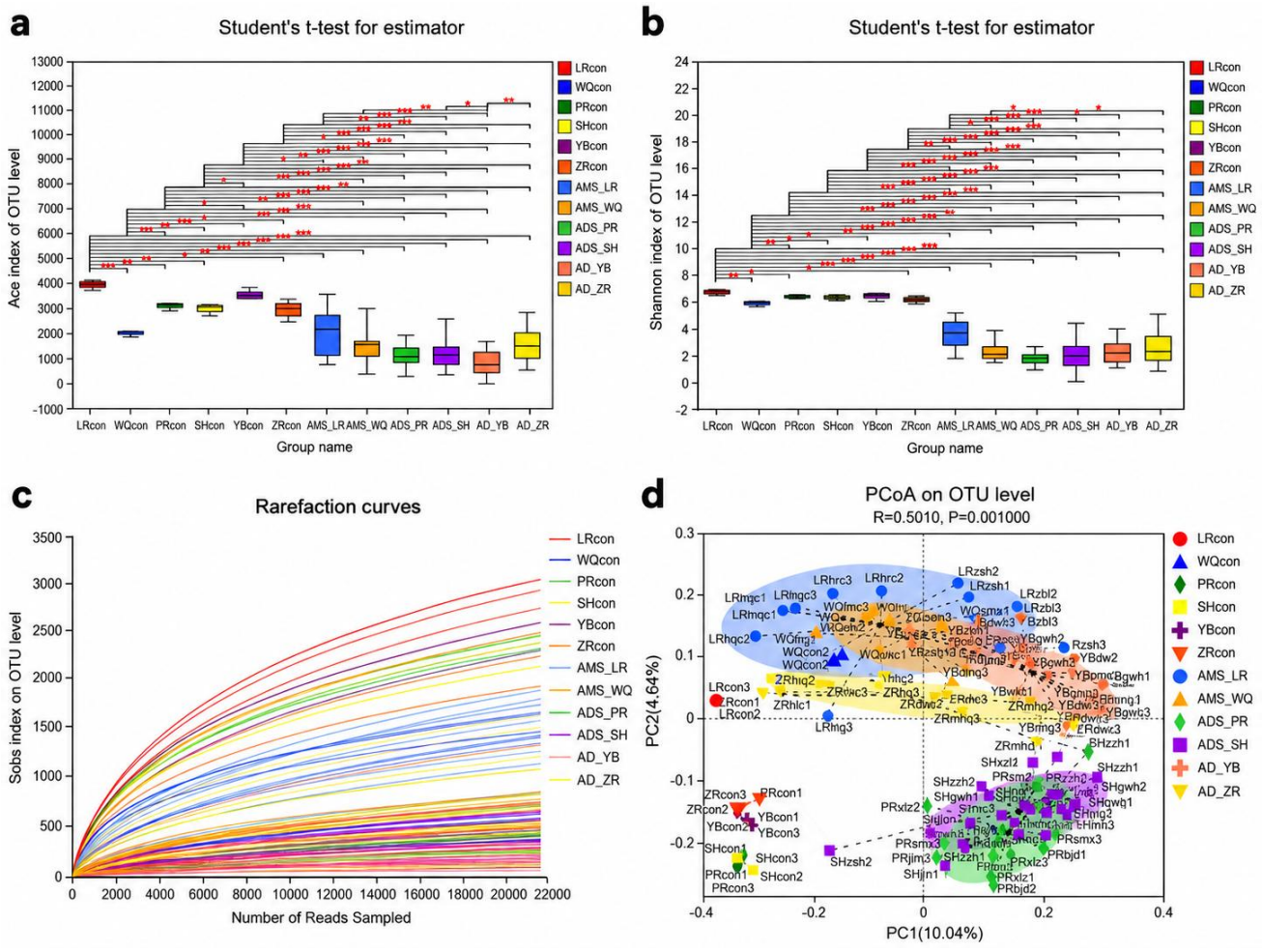
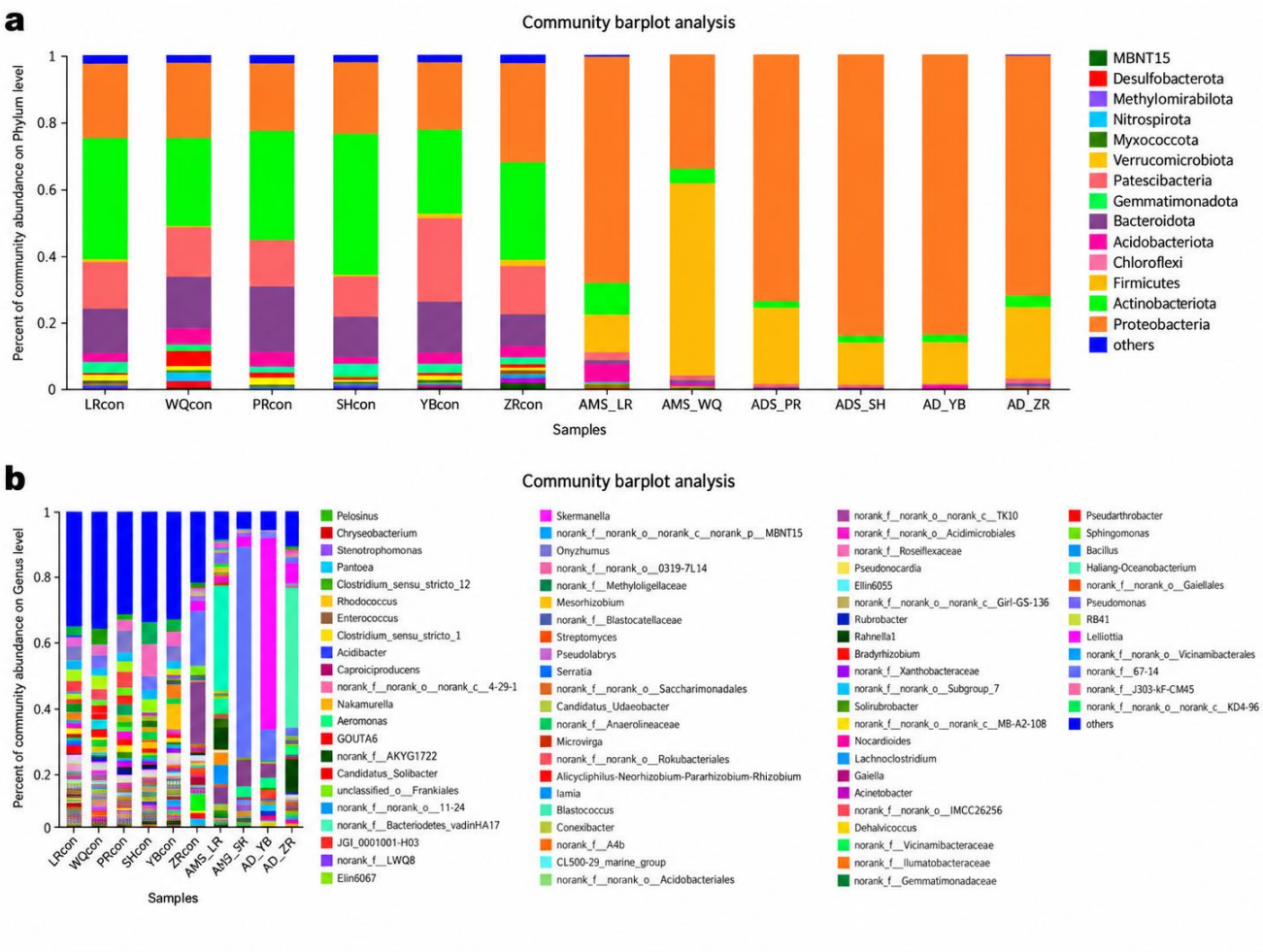


Figure 1. Sampling sites.LR, Lungring Township;WQ, Wuqiong Lake;PR, Puruogangri;YB, Yangbajain Town;SH, Shuanghu County;ZR, Zharen Township.



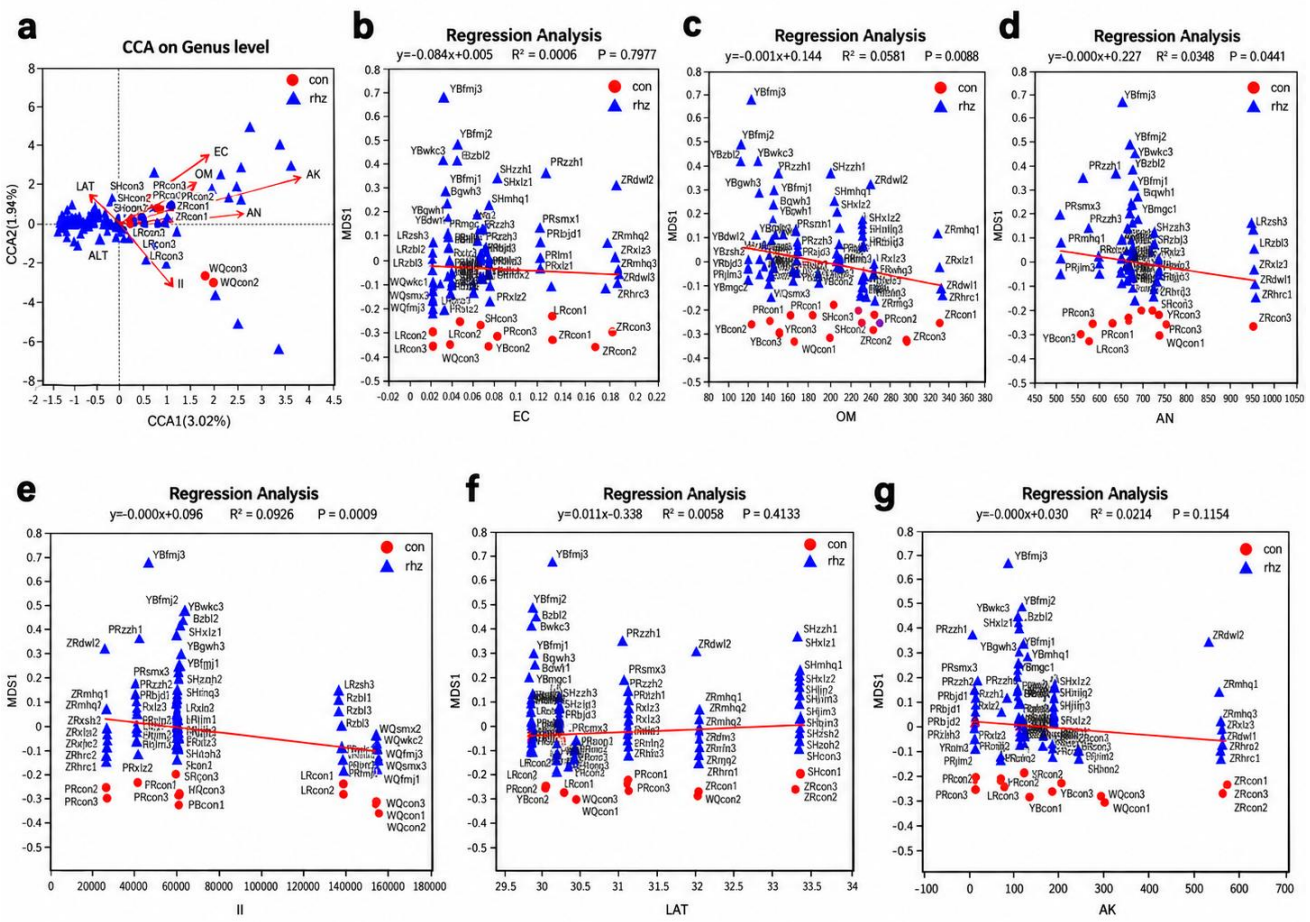
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513 **Figure 2.** Diversity analysis between rhizosphere and non-rhizosphere bacteria. A. Rarefaction
514 curves. B. Intergroup difference examination of the ACE index. C. Intergroup difference examination
515 of the Shannon index. D. Beta diversity analysis.



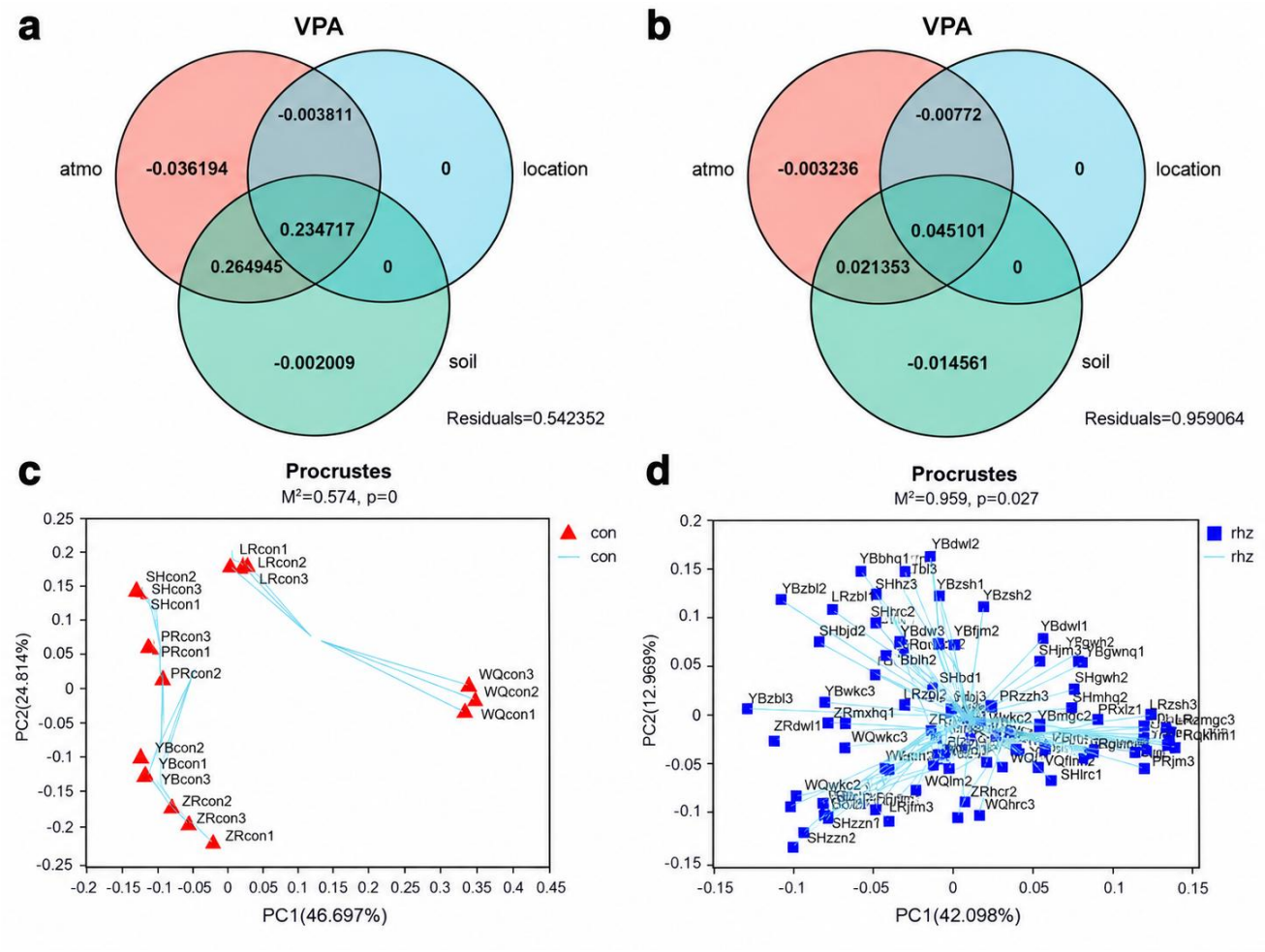
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517 **Figure 3.** Relative abundance of soil bacteria and rhizosphere bacteria in the samples.(A) Phylum
518 level. (B) Genus level.



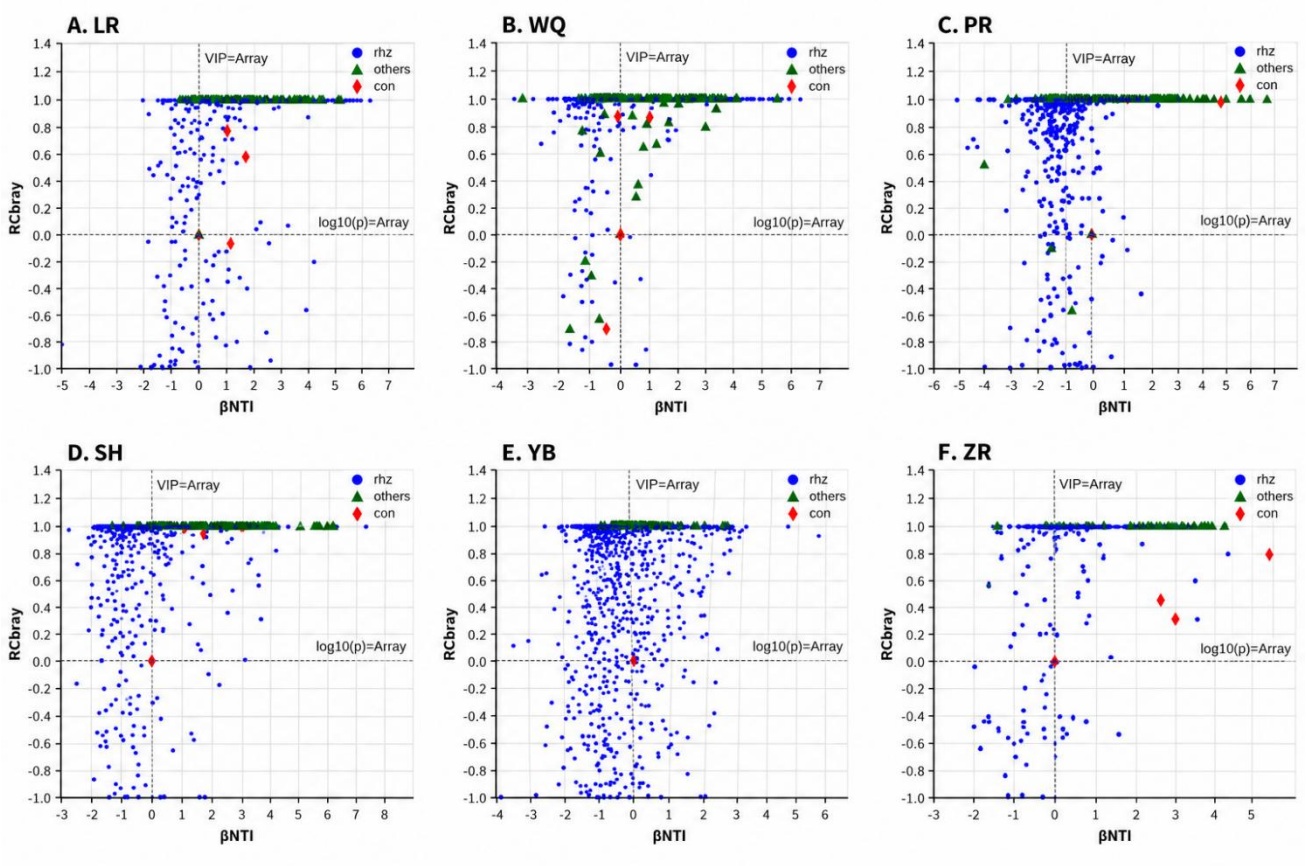
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520 **Figure 4.** Correlation analysis and linear regression analysis of the bacterial community against
521 environmental factors. (A) Canonical correspondence analysis (CCA). (B) Linear regression analysis
522 of illumination intensity (II). (C) Linear regression analysis of organic matter (OM). (D) Linear
523 regression analysis of ammonium nitrogen (AN).



524

525 **Figure 5.** Variation partitioning analysis (VPA) and procrustes analysis of the bacteria community
526 against environmental factors. (A) VPA of non-rhizosphere soil. (B) VPA of rhizosphere soil. (C)
527 Procrustes analysis of the non-rhizosphere soil. (D) Procrustes analysis of the rhizosphere soil.



528

529 **Figure 6.** β-NTI/RC Bray analysis of the bacterial community structure. (A) LR. (B) WQ. (C) PR.
530 (D) SH. (E) YB. (F) ZR.

13 Tables

Table 1. Plant species collected from different sampling sites

Grassland Type	Location	Plants Collected
Alpine meadow (AMS)	Lungring Township (LR)	*Astragalus strictus* (LRbhq), *Incarvillea Younghusbandii* (LRzbl), *Poa pratensis* (LRzsh), *Lasiocaryum densiflorum* (LRmgc), *Leontopodium pusillum* (LRhrc)
	Wuqiong Lake (WQ)	*Pedicularis cheilanthifolia* (WQsmx), *Microula sikkimensis* (WQwkc), *Saussurea graminea* (WQfmj)
Altitude desert (AD)	Puruogangri (PR)	*Dracocephalum heterophyllum* (PRzzh), *Oxytropis glacialis* (PRbjd), *Potentilla fruticosa* (PRjlm), *Pedicularis cheilanthifolia* (PRsmx), *Arenaria kansuensis* (PRxlz)
	Yangbajain Town (YB)	*Astragalus strictus* (YBbhq), *Incarvillea younghusbandii* (YBzbl), *Potentilla saundersiana* (YBdwl), *Saussurea graminea* (YBfmj), *Aster bowerii* (YBgwh), *Lasiocaryum densiflorum* (YBmgc), *Microula sikkimensis* (YBwkc), *Poa annua* (YBzsh)
	Zharen Township (ZR)	*Potentilla saundersiana* (ZRdwl), *Astragalus heydei* (ZRmhq), *Leontopodium pusillum* (ZRhrc), *Arenaria kansuensis* (ZRxlz)
Alpine desert steppe (ADS)	Shuanghu County (SH)	*Dracocephalum heterophyllum* (SHzzh), *Oxytropis glacialis* (SHbjd), *Saussurea stoliczkai*, *Kobresia robusta*, *Potentilla fruticosa* (SHjlm), *Astragalus heydei* (SHmhq), *Aster bowerii* (SHgwh), *Leontopodium pusillum* (SHhrc), *Arenaria kansuensis* (SHxlz), *Poa annua* (SHzsh)

Note: AMS, alpine meadow; AD, alpine desert; ADS, alpine desert steppe. LR, WQ, PR, YB, ZR, and SH denote the six sampling sites: Lungring Township, Wuqiong Lake, Puruogangri, Yangbajain Town, Zharen Township, and Shuanghu County, respectively. Codes in parentheses after the species names represent plant sample IDs. Plant species were identified by **Shengping Ming** (Xizang University) according to the *Flora of Xizang* (web version: http://www.cfh.ac.cn/subsite/default.aspx?siteid=tibetplants).

Table 2.PERMANOVA results for bacterial community composition in non-rhizosphere and rhizosphere soils.

Characteristics	Sum of squares	F.Model	R^2	*P* value
Plant species	7.44825	3.32448	0.61826	0.001
Soil type (non-rhizosphere vs. rhizosphere)	3.87593	4.52785	0.32173	0.001
Sampling location	2.16974	25.26207	0.18011	0.001

Note: PERMANOVA was performed to assess the effects of plant species, soil type (non-rhizosphere vs. rhizosphere), and sampling location on bacterial community composition. F.Model denotes the pseudo-F statistic, and R^2 indicates the proportion of total variation explained by each factor. *P* values were obtained by permutation tests, with $P < 0.05$ considered statistically significant. All factors showed significant effects on bacterial community composition ($P = 0.001$).

545 **Table 3.** Environmental properties in the soil at all studied sampling sites.

Sampling site	pH	SM(%)	EC(ms/cm)	OM(mg/kg)	AK(mg/kg)	AP(mg/kg)	AN(mg/kg)	II(lx)	RH(%)	CC(ppm)	DT(°C)	AT (°C)
LR	5.64 ± 0.1	0.14 ± 0.01	0.01 ± 0.01	238.17 ± 19.89	73.25 ± 6.69	5.03 ± 4.11	915.03 ± 33.96	141344	28.9	817	12.3	31.7
WQ	4.24 ± 0.05	0.31 ± 0.02	0.02 ± 0.01	160.67 ± 21.97	273.03 ± 36.98	12.04 ± 0.26	894.8 ± 54.77	155000	43.8	783	6.1	17.9
PR	8.18 ± 0.13	0.12 ± 0.01	0.08 ± 0.02	150 ± 17.06	8.22 ± 3.63	2.44 ± 1.39	590.07 ± 44.51	42976	64.9	846	5.3	12
SH	8.12 ± 0.04	0.12 ± 0	0.05 ± 0.01	211.3 ± 14.25	148.3 ± 38.9	6.2 ± 1.85	768.63 ± 76.08	59296	66.4	932	6.6	12.8
YB	6.27 ± 0.14	0.14 ± 0	0.03 ± 0.01	125.23 ± 15.51	138.8 ± 28.14	12.22 ± 0.1	859.87 ± 45.61	60896	31.3	806	9.1	26.3
ZR	7.29 ± 0.02	0.47 ± 0.04	0.18 ± 0.03	277.03 ± 57.85	539.6 ± 7.31	35.28 ± 6.81	923.5 ± 79.1	28037	62.9	850	13.4	13.5
VIF	471.30	163.98	48.76	25.27	82.62	52.74	11.37	23217.99	2620.01	29890.75	4727.57	94419.87
Selected VIF	/	/	8.10	2.83	5.94	/	7.49	5.12	/	3.17	4.46	/

Note:VIF represents the Variance Inflation Factor value of the environmental factors before selection. "/" represents the environmental factors with insignificant related effects. SM, soil moisture; EC, electrical conductivity; OM, organic matter; AK, available K; AP, available P, AN, ammonium N; II, illumination intensity; RH, relative humidity, CC, CO2 concentration; DT, dewpoint temperature; AT, atmospheric temperature.