Soil Bacterial Communities and Diversity in Alpine Grasslands on the Tibetan Plateau Based on 16S rRNA Gene Sequencing

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The Tibetan Plateau, widely known as the world’s “Third Pole,” has gained extensive attention due to its susceptibility to climate change. Alpine grasslands are the dominant ecosystem on the Tibetan Plateau, albeit little is known about the microbial community and diversity among different alpine grassland types. Here, soil bacterial composition and diversity in the upper soils of five alpine grassland ecosystems, alpine meadow (AM), alpine steppe (AS), alpine meadow steppe (AMS), alpine desert (AD), and alpine desert steppe (ADS), were investigated based on the 16S rRNA gene sequencing technology. Actinobacteria (46.12%) and Proteobacteria (29.67%) were the two dominant soil bacteria at the phylum level in alpine grasslands. There were significant differences in the relative abundance at the genus level among the five different grassland types, especially for the Rubrobacter, Solirubrobacter, Pseudonocardia, Gaiella, Haliangium, and Geodermatophilus. Six alpha diversity indices were calculated based on the operational taxonomic units (OTUs), including Good's coverage index, phylogenetic diversity (PD) whole tree index, Chao1 index, observed species index, Shannon index, and Simpson index. The Good's coverage index value was around 0.97 for all the grassland types in the study area, meaning the soil bacteria samplings sequenced sufficiently. No statistically significant difference was shown in other diversity indices’ value, indicating the similar richness and evenness of soil bacteria in these alpine grasslands. The beta diversity, represented by Bray–Curtis dissimilarity and the non-metric multidimensional scaling (NMDS), showed that OTUs were clustered within alpine grasslands, indicating a clear separation of soil bacterial communities. In addition, soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), pH, and soil water content (SWC) were closely related to the variations in soil bacterial compositions. These results indicated that soil bacterial taxonomic compositions were similar, while soil bacterial community structures were different among the five alpine grassland types. The environmental conditions, including SOM, TN, TP, pH, and SWC, might influence the soil bacterial communities on the Tibetan Plateau.

Keywords: soil bacteria, soil microorganism, alpine grassland, Tibetan Plateau, 16S rRNA
INTRODUCTION
The Tibetan Plateau, which is considered the world’s “Third Pole,” has attracted extensive attention because of its high altitude and harsh environment (Chen et al., 2013). Environmental factors on the plateau, such as altitude (Cui et al., 2019), precipitation (Zhang K. et al., 2016), and topography (Fridleifsdottir et al., 2019), have a large impact on soils of alpine ecosystems, resulting in marked changes in microbial diversity and function (Donhauser and Frey, 2018). Alpine grasslands are the representative landscape of the Tibetan Plateau, occupying more than 70% of the total area (Lu et al., 2018; Yu et al., 2019). Based on geographical, climatic, and vegetation characteristics, alpine grasslands on the Tibetan Plateau can be classified as alpine meadow (AM), alpine steppe (AS), alpine meadow steppe (AMS), alpine desert (AD), alpine desert steppe (ADS), etc. (Shen et al., 2015; Lu et al., 2018). Extensive studies have been carried out to examine vegetation and soil biogeochemical cycling within these grasslands (Tian et al., 2017; Che et al., 2018; Wu et al., 2019). However, soil microbial communities, which are the key drivers of many below-ground processes, remain poorly understood on the Tibetan Plateau.

Soil microorganisms are crucial for maintaining soil fertility and sustainability because of their role in regulating organic matter and nutrient cycling (Fierer, 2017). For example, Trichoderma, associated with plant roots, can trigger systemic resistance and improve plant nutrient uptake (Contreras-Cornejo et al., 2016). Additionally, genomically divergent microorganisms were reported as mediators of soil carbon (C) and nitrogen (N) compound turnovers in Mediterranean grasslands (Diamond et al., 2019). Therefore, understanding the differences in microbial communities among multiple ecosystems and their underlying mechanisms is essential for improving ecosystem function predictions and their responses to climate change and human activities (Zhang Y. et al., 2016; Karlowsky et al., 2018). Equally important are the microbial co-occurrence and network analysis to reveal keystone taxa that significantly impact the microbiome structure and functioning regardless of their abundance across space and time (Banerjee et al., 2018). Recent studies have also found that both competition and environmental filtering affect the abundance, composition, and encoded gene functions of bacterial and fungal communities (Bahram et al., 2018). In a tropical mountain ecosystem on Mount Kilimanjaro, interactions between climate and land use explained the soil microbial composition satisfactorily, indicating that climate and land use can modulate soil biodiversity and ecosystem functions (Peters et al., 2019). Steidinger et al. (2019) analyzed the global distribution of the dominant root-associated microbial symbionts and found that climate variables are the primary drivers of the global distribution of major symbionts. Even in other ecosystems, such as deserts (Evans et al., 2019) and farmlands (Jiao et al., 2020), environmental factors also considerably influenced the soil microbial community, but these effects may differ among different ecosystem types.

On the Tibetan Plateau, the spatial distribution of alpine grassland vegetation communities is strongly related to soil properties (Lu et al., 2015), climatic conditions (Wu et al., 2019), and elevation (Ren et al., 2018; Wu et al., 2019). The variations in these factors could also explain soil microbe distribution (Ciccazzo et al., 2016; Ren et al., 2018). For example, Chu et al. (2016) found that the microbial community structure was distinct between the surface and subsurface soil layers, strongly correlating with variation in the total carbon and carbon to nitrogen ratio (C:N) in the western Tibetan Plateau. Chen et al. (2016) found that soil microbial community variations in Tibetan alpine grasslands were mainly explained by edaphic factors, such as soil organic carbon (SOC), C:N ratio, pH, and soil texture, followed by biotic factors, including aboveground biomass and plant species richness, and further by climatic factors, such as mean annual precipitation. Xu et al. (2014) found that soil pH was a major factor affecting microbial communities, and the impact of soil pH was closely correlated with temperature and vegetation changes along the elevational gradient of Mount Segri on the Tibetan Plateau. Therefore, the main controlling factors may vary at different regional scales because the spatial regulators of soil microbes vary at different spatial scales and in different ecosystems (White et al., 2020).
controlled by cold and extreme drought conditions, covering 6.71% of the total grassland area. AMS is a transitional type of alpine grassland from the steppe and ADS is a transitional type of alpine grassland from steppe to desert, covering 7.32 and 10.7%, respectively, of the total grassland area in the Tibetan Plateau (Lu et al., 2018).

Twenty-one sampling sites were set from east to west across the Tibetan Plateau according to the distribution of alpine grassland types (Supplementary Table S1). Five sampling sites were selected for each of the three main natural grassland types, AM, AS, and ADS, and three sampling sites were selected at the relatively smaller natural grassland areas, AMS and AD. At each sample site, three random quadrats (1 m × 1 m) were laid out at intervals of approximately 50 m during July and August 2019. In total, 63 quadrats of alpine grasslands on the Tibetan Plateau were sampled with 45 quadrats (15 sites × 3 quadrats) for AM, AS, and ADS, and 18 quadrats (6 sites × 3 quadrats) for AMS and AD. Five soil samples were collected from each quadrat at the depths of 0–15 cm and then composited into a single soil sample. Rocks and visible roots were removed from the soil, and it was passed through a 2 mm mesh. All samples were shipped in a refrigerator (−21°C) and immediately transported to the laboratory. All the soil samples were divided into two parts: one part was used to analyze the soil physiochemical properties, and the other was used to analyze soil bacteria based on 16S rRNA gene sequencing. The samples for genomic deoxyribonucleic acid (DNA) extraction and molecular analysis were stored at −80°C.

**Analyses of Soil Properties**

The soil water content (SWC) was measured gravimetrically after 10 h of desiccation at 105°C. Soil pH was determined at a fresh soil to water ratio of 1:2.5 using a pH monitor (Mettler-Toledo, Switzerland). Above-ground plant biomass (APB) and underground plant biomass (UPB) were measured gravimetrically after drying at 65°C for 48 h. Soil organic matter (SOM) and soil total nitrogen (TN) concentrations were determined with an element analyzer (Elementar, LiquiTOC, Hanau, Germany). The soil total phosphorus (TP) was determined using the NaHCO3 alkali digestion method, and the molybdenum antimony colorimetric method.

**16S rRNA Gene Sequencing Technology**

Total genomic DNA was extracted using DNeasy PowerSoil Kit # 12888-100 (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were verified using NanoDrop and agarose gel electrophoresis. Extracted DNA was diluted to a concentration of 1 ng/µL and stored at −20°C until further processing. The diluted DNA was used as a template for polymerase chain reaction (PCR) amplification of bacterial 16S rRNA genes with the barcoded primers and Takara Ex Taq (Takara Biochemicals, Beijing, China).

For bacterial diversity analysis, V3–V4 variable regions of 16S rRNA genes were amplified with the universal primers 343F and 798R in PCR amplification (580BR10905, Bio-Rad, CA, United States). The PCR was performed according to previously described PCR methods (Wu et al., 2015; Scarlett et al., 2020). The first thermocycling involved an initial denaturation at 94°C for 5 min followed by 26 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 5 min, which included a combined annealing and extension time. Amplicon quality was visualized using gel electrophoresis, purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, United States), and amplified for another round of PCR (seven cycles); the second thermocycling was the same as in the first round. After purification with the AMPure XP beads again, the final amplicon was quantified using the Qubit dsDNA assay kit (Q328520, Life Technologies, Grand Island, NY, United States). Equal amounts of purified amplicon were pooled for subsequent sequencing on a MiSeq Machine (Illumina, San Diego, CA, United States).

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using Trimmmomatic software (Bolger et al., 2014) to detect and cut off ambiguous bases. Low-quality sequences with an average quality score below 20 were cut off using a sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH software (Reynol et al., 2012). The assembly parameters were: 10 bp of minimal overlapping, 200 bp of maximum overlapping, and 20% of maximum mismatch rate. Sequences were subjected to further denoising as follows: reads with ambiguous, homologous sequences or below 200 bp were removed. Reads with 75% of bases above Q20 were retained. Then, reads with chimera were detected and removed. These two steps were performed using QIME software (Quantitative Insights Into Microbial Ecology, version 1.8.0) (Caporaso et al., 2010). Clean reads were subjected to primer sequence removal and clustering to generate operational taxonomic units (OTUs) using Vsearch software with a 97% similarity cutoff (Edgar et al., 2011). The representative read of each OTU was selected using the QIIME package. All representative reads were annotated and blasted against the SILVA database (Version 132) using the RDP classifier (confidence threshold of 70%) (Wang et al., 2007).

**Diversity Estimates**

Six commonly used alpha diversity indices of the bacterial community were calculated. The Good’s coverage index represents the sample coverage detected. A higher Good’s coverage index means that the result approximates to the actual situation (Yuan et al., 2018). The phylogenetic diversity (PD) whole tree index is defined as the sum of the branch lengths of a phylogenetic tree connecting all species in the target assemblage, which can be regarded as a phylogenetic generalization of species richness (Chao et al., 2016). Chao1 is an index of species richness, which is sensitive to rare species, but does not represent abundance or uniformity (Chao and Bunge, 2002). The observed species index is the number of species in the sample (Yuan et al., 2018). The Shannon index is positively correlated with species richness and evenness, and that gives more weight per individual to rare than common species (Hill et al., 2003). The Simpson index focuses on the uniformity of the community (Zhong et al., 2010). Beta diversity was represented by Bray–Curtis dissimilarity between each type of grassland pair (Chen et al., 2019), and then non-metric multidimensional scaling (NMDS) was used to explain the pairwise dissimilarity between objects in a low-dimensional space. Additionally, the analysis of similarities
FIGURE 1 | Sampling sites of alpine grasslands on the Tibetan Plateau.

(ANOSIM) was used to determine the significance of separation along alpine grassland types (Ren et al., 2018).

Statistical Analyses

All statistical analyses were performed in the R environment (v4.0.0). The relative abundance of the top 15 species at the phylum and genus levels was used to compare the distribution of soil bacterial species in the five alpine grassland ecosystems. Before conducting a one-way analysis of variance (ANOVA), the Shapiro function was used to test for the normality of residuals, and the Bartlett function was used to test the homogeneity of variance. The ANOVA was used when the application conditions were satisfied; otherwise, the non-parametric statistics (Kruskal–Wallis test) was chosen. The least significant difference (LSD) comparison of bacteria among different alpine grasslands was performed using the “agricolae” package (Version 1.3-3) (Felipe, 2020); then the indicator bacteria were determined using the random forest model (“randomForest” package, Version 4.6-14) (Liaw and Wiene, 2002).

The alpha diversity indices of the soil bacteria community were calculated based on OTUs using the QIIME software (Version 1.80) (Caporaso et al., 2010). Beta diversity was estimated based on Bray–Curtis dissimilarities between samples, where the distance was computed using OTU tables. The “vegan” package (Version 2.5-6) (Jari Oksanen et al., 2019) was used to compute Bray–Curtis dissimilarity, NMDS, and ANOSIM, which were used to assess the beta diversity of soil bacterial communities. Correlations between the soil bacterial compositions and environmental factors were determined using redundancy analysis (RDA) in the vegan’ package. The selected environmental factors were APB, UPB, SOM, TN, TP, pH, SWC, and altitude. The significance of the RDA results was tested using the Monte Carlo permutation test. Graphs were drawn by the “ggplot2” package (Version 3.3.2) (Wickham, 2016).

RESULTS

Soil Bacterial Community Structure in Alpine Grasslands

A total of 48,366–51,566 clean reads with high-quality 16S rRNA sequences were identified from the 21 samples from the Tibetan Plateau. After chimera detection and removal, about 37,532–47,699 valid tags with an average read length of 412.52–416.3 bp were obtained. Accordingly, an average of 1,799–3,089 OTUs at a 97% similarity cutoff were generated from 16S rRNA sequencing analysis. The number of different OUTs was 855, covering 94 different genera and eight different phyla.

In these five alpine grassland ecosystems, Actinobacteria (46.12%) and Proteobacteria (29.67%) were the two dominant bacteria at the phylum level, followed by Gemmatimonadetes (8.30%), Bacteroidetes (7.25%), Firmicutes (4.23%), and

1http://www.r-project.org/
Acidobacteria (2.35%) (Figure 2A). At the genus level, the dominant bacteria were inhomogeneously distributed in different alpine grasslands, accounting for 20% of all relative abundance of taxonomic, where each dominant genus was between 0.7 and 2.4%. The relative abundance from highest to lowest was *Rubrobacter* (2.38%), *Solirubrobacter* (2.25%), *Blastococcus* (1.65%), *Pseudonocardia* (1.43%), *Crossiella* (1.29%), *Gaiella* (1.17%), *Bacteroides* (1.17%), *Nocardioides* (1.17%), *Sphingomonas* (1.12%), *Gemmatirosa* (1.10%), *Escherichia-Shigella* (1.07%), MND1 (0.90%), *Haliangium* (0.78%), *Geodermatophilus* (0.77%), and *Microvirga* (0.73%) (Figure 2B). The relative abundance of *Rubrobacter* was statistically similar in AD, ADS, and AS; the relative abundance of *Geodermatophilus* in AD was only higher than in AMS. The relative abundance of *Geodermatophilus* in the soils of AD and ADS was 0.65 and 1.45%, respectively, 9.29 and 20.71 times higher than that of the AMS (0.07%), respectively. Moreover, the relative abundance of *Solirubrobacter, Pseudonocardia, Gaiella,*
and *Haliangium* were also the least observed genera in AMS soils. Nevertheless, these bacteria were the most abundant in the AM soils, where their content was two to four times that in AMS soils (Figure 3). Furthermore, the relative abundance of *Gaiella* showed obvious differentiation in alpine grasslands and it was most abundant in the AM soils.

The random forest model revealed the indicator bacteria at the genus level, the genus with high essential values was also the genus with high relative abundance. According to the ordering of essential values (Figure 4), *Rubrobacter* and *Solirubrobacter* were extremely sensitive indicator bacteria in the alpine grasslands (importance > 1); *Blastococcus*, *Pseudonocardia*, *Crossiella*, and *Gaiella* also had high values of importance.

### Alpha Diversity of Soil Bacterial Community in Alpine Grasslands

Six alpha diversity indices, the PD whole tree index, Chao1 index, Good’s coverage index, observed species index, Shannon index, and Simpson index, were calculated to compare soil microbial community differences among the five different alpine grassland types (Table 1). The Good’s coverage indices in all alpine grasslands were higher than 0.97, and the Good’s coverage index of the AD was significantly higher than that of other alpine grasslands (*P* < 0.05), indicating that that our sequenced sample was sufficient to reveal the true diversity. Significance in the other five alpha diversity indices among alpine grassland types demonstrated a similarity in alpha diversity among the five investigated alpine grasslands.

### Beta Diversity of Soil Bacterial Community in Alpine Grasslands

The NMDS based on Bray–Curtis dissimilarities was conducted to reflect the soil bacterial beta diversity of the alpine grasslands on the Tibetan Plateau. The results showed that OTUs were clustered within alpine grasslands (Figure 5), indicating a clear separation of soil bacterial communities among five alpine grassland ecosystems (stress = 0.0284). For instance, the Bray–Curtis dissimilarity from the AM to the AD was relatively far, highlighting the significant difference of soil bacterial communities between them. In addition, the Bray–Curtis dissimilarity of AS, ADS, and AMS was close, implying the similar soil bacteria community structures, especially the relative

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**FIGURE 3** Differences in soil bacteria among alpine grasslands at the genus level on the Tibetan Plateau. AD, alpine desert; ADS, alpine desert steppe; AM, alpine meadow; AMS, alpine meadow steppe; AS, alpine steppe. Different lowercase letters marking the same genus represent significant differences between two alpine grasslands (*P* < 0.05).
abundance of genus. Furthermore, the ANOSIM highlighted the differences in soil bacterial communities in alpine grasslands (Table 2). The soil bacteria community of AM was substantially different from those of AMS, ADS, and AD (P < 0.05). Moreover, the soil bacterial community at AS was different from that at AD, and there were also even differences between the two transition states alpine grasslands (AMS and ADS).

**Relationships Between Soil Bacterial Community and the Environmental Factors**

The RDA showed that the variations in the soil bacterial community were determined by the environmental factors (Figure 6). The permutation test indicated significance in the
Non-metric multidimensional scaling (NMDS) analysis of the soil bacteria in alpine grasslands on the Tibetan Plateau. Different shaped dots represent the samples from different alpine grassland types, and the bumps of the samples bound the faces of different colors. AD, alpine desert; ADS, alpine desert steppe; AM, alpine meadow; AMS, alpine meadow steppe; AS, alpine steppe.

Environmental variables had an explanation rate of 41.69% for the soil bacterial community, with the first and second axes accounting for 24.49 and 17.2% of the variation, respectively. The results also revealed correlations between each genus and environmental variations; the dominant factors for different soil bacteria genera were different. For example, soil pH was positively correlated to Nocardioides, Escherichia-Shigella, MND1, and Haliangium, but negatively correlated to Rubrobacter, Solirubrobacter, Gemmatirosa, and Geodermatophilus. In addition, the differences in sampling points can also be reflected by environmental variables, and the soil bacterial abundance at different points also showed variability. For instance, the projection point of the corresponding sample points in AM on the corresponding vector of Pseudonocardia was in front of that of AD, implying that the Pseudonocardia has greater abundance potential in the AM than in AD. Environmental factors, especially SOM, TN, TP, pH, and SWC, were closely related to the soil bacterial community ($P < 0.05$) (Table 3). The difference in environmental factors in alpine grasslands can explain the differences in soil bacterial communities among different alpine grassland types.

**DISCUSSION**

**Soil Microbial Community Structures in Alpine Grasslands**

Alpine ecosystems are susceptible to climate change and human disturbance, causing variability in soil bacterial composition and diversity (Donhauser and Frey, 2018). Soil bacteria play an essential role in the cycling organic matter in nature, soil formation, and soil fertility. Simultaneously, various secondary metabolites produced by soil bacteria during their life processes are also crucial to the soil ecological environment (Chen et al., 2020). In our study, the dominant bacteria at the phylum level were Actinobacteria, Proteobacteria, Gemmatimonadetes, Bacteroidetes, Firmicutes, and Acidobacteria, accounting for approximately 94% of the total abundance of the bacterial community. Despite differences in relative abundance, Actinobacteria, Proteobacterial, Acidobacteria, and Chloroflexi were also the dominant bacteria found by Chu et al. (2016) and Zhou et al. (2019).

Actinobacteria constitute one of the largest bacterial phyla in many types of soil ecosystems (Barka et al., 2015). They often
TABLE 2 | Analysis of similarities (ANOSIM) of soil bacteria in alpine grasslands on the Tibetan Plateau.

| Different groups | R   | P-value | Sig |
|------------------|-----|---------|-----|
| AM/AS            | 0.144 | 0.094 |     |
| AM/AMS           | 0.651 | 0.015 | *   |
| AM/ADS           | 0.616 | 0.011 | *   |
| AM/AD            | 0.846 | 0.024 | *   |
| AS/AMS           | 0.426 | 0.066 |     |
| AS/ADS           | 0.076 | 0.215 |     |
| AS/AD            | 0.569 | 0.025 | *   |
| AMS/ADS          | 0.569 | 0.031 | *   |
| AMS/AD           | 0.296 | 0.200 |     |

Asterisks denote significant differences between two alpine grasslands (P < 0.05). AD, alpine desert; ADS, alpine desert steppe; AM, alpine meadow; AMS, alpine meadow steppe; AS, alpine steppe.

live as plant commensals (Fitzpatrick et al., 2018), nitrogen-fixing symbionts in soil, involved in the SOM formation and biogeochemical cycles (Yu et al., 2020), and are also a significant source of extracellular enzymes and secondary metabolites (Alvarez et al., 2017). In the present study, the relative abundance of Acidobacteria was higher in all five alpine grasslands. Proteobacteria, one of the main phyla of ammonifying microorganisms, were also abundant in the five alpine grasslands, especially in AM, AMS, and AS, which might be due to the different vegetation types and their litters, resulting in the structural difference of the dominant species of Proteobacteria (Chen et al., 2020). Gemmatimonadetes, Entotheonellaeota, and Fusobacteria were also found in alpine grassland soils. Gemmatimonadetes and Entotheonellaeota are also adapted to low soil moisture or arid environments (DeBruyn et al., 2011; Wilson et al., 2014; Zethof et al., 2020) that are similar to our sampling area conditions. Fusobacteria are often found in fecal microbiota, which were probably introduced by ruminants (Wu et al., 2016).

At the genus level, the average relative abundance of the dominant species was all lower than 2.5%, and the diverse community composition might be related specific functions of soil bacteria. For example, Rubrobacter was abundant in AD, ADS, and AS; a previous study showed that Rubrobacter contains many highly specific proteins that play a vital role in the evolution of bacteriochlorophyll-based photosynthesis (Gupta and Khadka, 2016). Geodermatophilus is abundant in ADS and AS, as it often thrived in nutrient-poor habitats, such as the surface of calcareous stones or sandy desert soils (Sghair et al., 2016). Pseudonocardia has received extensive attention for degrading recalcitrant cyclic ether pollutants, mutualism with soil organisms, as well as producing antibiotics (Tanvir et al., 2016; Li et al., 2018; Ma et al., 2018); it is abundant in AM and AS. The Gaiella, as a good indicator of C:N in the soil (Hermans et al., 2017), and the Haliangium, involving in soil denitrification and playing an essential role in the biochemical cycling of N and fermentation of C sources (Lévesque et al., 2020), were both found with the higher relative abundance in AM.

Based on the soil bacterial communities in alpine grasslands on the Tibetan Plateau, soil bacterial taxonomic compositions and alpha diversity were similar among the five different alpine grassland types. There were significant differences in the relative abundance at the genus level among the alpine grassland types, especially for the Rubrobacter, Solirubrobacter, Pseudonocardia, Gaiella, Haliangium, and Geodermatophilus. The parallel composition of soil bacteria in different alpine grasslands reflected their strong adaptability to the environment. In contrast, the relative abundance of some soil bacteria varied among the five alpine grasslands at the genus levels, suggesting a filtering effect of environmental factors. The relative abundance of soil bacteria changed significantly in other alpine soils (Rime et al., 2016; Malard and Pearce, 2018). For example, the relative abundance of soil bacteria in different alpine edaphic conditions showed significant differences, especially for Chloroflexi, whose relative abundance significantly increased with elevation and indicated a high colonization potential in the Swiss Alps (Adamczyk et al., 2019).

Soil Bacterial Diversity in Alpine Grasslands

The vegetation and soil nutrients were heterogeneous among the five alpine grasslands on the Tibetan Plateau, which may lead to variations in soil bacterial diversity. However, we did not detect significant differences in the alpha diversity of soil bacteria among the alpine grassland types, although supported by other studies on the Tibetan Plateau and other regions (French et al., 2017; Zhou et al., 2019; Su et al., 2020). For instance, there were no differences in the alpha diversity of microbes in five degraded AS in Qinghai Province, China (Zhou et al., 2019). Furthermore, a study in southeast England found that bacterial diversity and specific beneficial taxa did not change when the land-use changed (French et al., 2017). Ramirez et al. (2014) reported that soil bacterial diversity in Central Park in New York City was very similar to the multiple locations globally.

It seems that two possible reasons can account for the similar alpha diversity of soil bacteria in alpine grasslands. First, the alpha diversity may be influenced by soil bacterial colonization strategies, and some bacteria have large population sizes with greater dispersal ability. For instance, Actinobacteria OTUs were found to contribute up to 52% of the soil bacterial communities in Australian and northern Antarctica soils, indicating the remarkable dispersion ability of Actinobacteria (Araujo et al., 2020). Second, nutrient restriction may also affect soil bacterial alpha diversity in alpine grasslands, with C, N, phosphorus (P), and especially SOC, acting as limiting factors. Ma et al. (2019) reported that total bacteria slightly increased with P addition only when plants remained N limited, and most bacterial taxa were limited by resources other than N and P. Hence, similar species richness and evenness usually occurred due to constraints of the same resource (Ho et al., 2017).

Bray–Curtis dissimilarities represent beta diversity, and the results showed the differentiation of soil microbial communities among the five types of alpine grasslands. The significant difference in soil bacterial communities between AM and AD was demonstrated because the Bray–Curtis dissimilarity from AM
FIGURE 6 | Redundancy analysis (RDA) of soil bacterial communities and environmental factors in alpine grasslands on the Tibetan Plateau. Points of the same color with a symbol denote sampling sites of an alpine grassland type. The blue arrows represent environmental factors, the red arrows represent soil bacteria (genus), and the cosine of the angle within two vector arrows represents the correlation between vectors. AD, alpine desert; ADS, alpine desert steppe; AM, alpine meadow; AMS, alpine meadow steppe; AS, alpine steppe; APB, aboveground plant biomass; UPB, underground plant biomass; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; SWC, soil water content.

Table 3: Significance of explanatory variables and the variance values in redundancy analysis (RDA).

| Environmental factors | RDA1   | RDA2   | R²    | Pr (>r) |
|-----------------------|--------|--------|-------|---------|
| APB                   | 0.999  | −0.029 | 0.051 | 0.60    |
| UPB                   | 0.886  | 0.464  | 0.037 | 0.73    |
| SOM                   | −0.882 | −0.470 | 0.580 | 0.002   |
| TN                    | −0.934 | −0.357 | 0.342 | 0.025   |
| TP                    | 0.827  | 0.562  | 0.389 | 0.018   |
| pH                    | −0.487 | 0.874  | 0.362 | 0.021   |
| Altitude              | −0.399 | −0.917 | 0.155 | 0.213   |
| SWC                   | −0.833 | −0.552 | 0.355 | 0.025   |

$R^2$, coefficient of determination; Pr, probability, indicating significance; APB, aboveground plant biomass; UPB, under-ground plant biomass; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; SWC, soil water content.

Response of Soil Bacteria to Environmental Factors

Soil bacterial community structures differed among the five different alpine grassland types, despite the similarities in the soil species composition. High consistency of soil bacterial alpha diversity and differentiation of beta diversity were found in the alpine grasslands. These results may indirectly reveal the extensive adaptability of soil bacteria to the environment. In order to explore the reasons for the differences in soil
bacterial communities, correlations between the soil bacterial compositions and environmental factors were determined using RDA. We found that environmental factors, including SOM, TN, TP, SWC, and soil pH, were closely related to the soil bacterial community. Environmental factors were also found to have both positive and negative effects on different alpine soil bacteria. For instance, the result reflected that the soil pH was positively correlated with *Rubrobacter*, while it was negatively correlated with *Nocardioides*.

Soil organic matter provides important sustenance to soil bacteria; the vegetation compositions in alpine grasslands implies multiple litters, which might contribute to the different structures of soil bacterial communities (Leff et al., 2018; Hicks et al., 2019; Yang et al., 2019). Meanwhile, soil N content can affect the microbial mineralization of SOM (Carrillo et al., 2017; Hicks et al., 2019). In the present study, TN, TP, and SWC had a high explanatory power for soil bacteria. Similar results were also found in an AM and a poplar plantation, where RDA revealed that soil pH, extractable organic carbon, and extractable organic nitrogen accounted for most of the variability in the bacterial communities (Zhang et al., 2017; Sun et al., 2020). A study on Mt. Kilimanjaro in East Africa found that bacterial diversity had a U-shaped pattern across the mountain gradient, and pH could explain about 12% of the soil bacteria (Shen et al., 2020). Soil pH may affect soil microorganisms by indirectly influencing the form of available nutrients, and is closely correlated with temperature and vegetation changes along the elevational gradient (Xu et al., 2014). Finally, in alpine grasslands, SWC may affect nutrient solubility and indirectly affect soil microbial communities.

**CONCLUSION**

Based on 16S rRNA gene sequencing, we compared soil bacterial composition and diversity among the five types of alpine grasslands on the Tibetan Plateau. We found that the bacterial composition and diversity among the five types of alpine grasslands were similar; however, the community structure varied. The alpha diversity index showed no significant differences in the alpha diversity of soil bacterial communities, implying that there were similarities in the bacterial richness and evenness among different alpine grasslands. The results from NMDS and ANOSIM assessing the beta diversity among alpine grasslands revealed that the soil bacterial communities in AM diverged significantly from the rest of the alpine grassland types, except AS. Moreover, the soil bacterial communities in AS and AD were dissimilar, and soil bacterial communities varied in two alpine grassland transition types, AMS and ADS. The soil environmental factors were examined to explain the soil bacterial community variations by RDA, highlighting that the distribution pattern of soil bacteria was influenced by some environmental factors, including SOM, TN, TP, pH, and SWC.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, SRR13370619.

**AUTHOR CONTRIBUTIONS**

XL planned and designed the study. HJ, YH, and ZW performed the experiments and conducted fieldwork. HM and YC analyzed the data and wrote the manuscript. All authors contributed critically to the drafts and gave the final approval for publication.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.630722/full#supplementary-material

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**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.

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