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# Composition and diversity of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau

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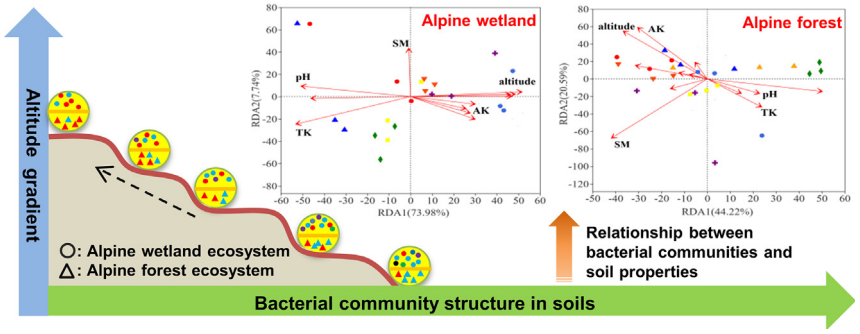
## HIGHLIGHTS

- Bacterial α-diversity decreased with altitude in the Tibetan alpine wetland soils.
- Bacterial β-diversity was significantly different in alpine wetland and forest soils.
- Wetland and forest ecosystems showed little difference in soil microbial composition.
- Total/available K and moisture influenced the structure of soil microbial communities.
- Potential functional pathways involved in human disease were identified in the soils.

## GRAPHICAL ABSTRACT

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## ABSTRACT

While the composition and diversity of soil microbial communities play a central and essential role in biogeochemical cycling of nutrients, they are known to be shaped by the physical and chemical properties of soils and various environmental factors. This study investigated the composition and diversity of microbial communities in 48 samples of seasonally frozen soils collected from 16 sites in an alpine wetland region (Lhasa River basin) and an alpine forest region (Nyang River basin) on the Tibetan Plateau using high-throughput sequencing that targeted the V3-V4 region of 16S rRNA gene. The dominant soil microbial phyla included *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* in the alpine wetland and alpine forest ecosystems, and no significant difference was observed for their microbial composition. Linear discriminant analysis Effect Size (LEfSe) analysis showed that significant enrichment of *Hymenobacteraceae* and *Cytophagales* (belonging to *Bacteroidetes*) existed in the alpine wetland soils, while the alpine forest soils were enriched with *Alphaproteobacteria* (belonging to *Proteobacteria*), suggesting that these species could be potential biomarkers for alpine wetland and alpine forest ecosystems. Results of redundancy analysis (RDA) suggest that the microbial community diversity and abundance in the seasonally frozen soils on the Tibetan Plateau were mainly related to the total potassium in the alpine wetland ecosystem, and available potassium and soil moisture in the alpine forest ecosystem, respectively. In addition, function prediction analysis by Tax4Fun revealed the existence of potential functional pathways involved in human diseases in all soil samples. These results provide insights on the structure and function of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau, while the potential risk to human health from the pathogenic microbes in the seasonally frozen soils deserves attention.



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## 1. Introduction

Soil microorganisms play crucial roles in the biogeochemical cycling of carbon, nitrogen, sulfur, phosphorus, and metals, as well as biodegradation or stabilization of environmental contaminants in the terrestrial ecosystems (Fierer and Jackson, 2006; Green et al., 2008). For example, soil organic matter mainly comes from the decomposition and transformation of plant and animal materials brought by soil microbes (Prescott, 2010), and microbial mineralization of soil organic matter is an important pathway for the transfer of greenhouse gases from the terrestrial ecosystem to the atmosphere (Chauvin et al., 2015; Zhang et al., 2006). In addition, different microorganisms exhibit distinct ecological functions in biogeochemical cycling of elements, and microbial community structure can influence a variety of ecosystem processes (Allison et al., 2010; Allison et al., 2013). The physical and chemical properties of soils are known to affect the soil microbial community structure and diversity. For examples, soil pH has been shown to be a key factor driving the diversity and abundance of soil bacterial communities, as the pH conditions affect the adaptation and selection of particular phylogenic groups (Feng et al., 2014; Kim et al., 2014; Wu et al., 2017). Soil moisture is another important determinant of the microbial community structure (Brockett et al., 2012; Sheik et al., 2011; Singh et al., 2010), because it directly and indirectly affects the physiological activities of microorganisms, as well as the diffusion of soil gases and oxygen availability (Singh et al., 2010). It has also been reported that nitrogen content (Stark et al., 2012), carbon content (Zhang et al., 2014a), and C/N ratio (Zhang et al., 2006) constitute the major abiotic controls over soil microbial community structure. In addition, Wu et al. (2017) observed that the soil depth was significantly correlated with the dominance of specific microbial species, such as *Acidobacteria*, *Proteobacteria*, *Nitrospirae*, and *Gemmatimonadetes*. Yang et al. (2014) observed that the microbial gene diversity exhibited a change along the altitude gradient in the grassland soils on the Tibetan Plateau. Guo et al. (2015) found that the structure of soil autotrophic microbial communities dramatically shifted along the altitude gradient in grassland soils on the Tibetan Plateau, which was jointly driven by soil temperature, nutrients, soil moisture, and plant types.

It has been demonstrated that soil microbial communities are more sensitive to environmental changes compared to plants and animals (Qian and Ricklefs, 2004; Willig et al., 2003; Zhang et al., 2013; Zhou et al., 2008). There has been growing interest in understanding the structure, diversity, and potential functional pathways of soil microbial communities and their relationship with the physicochemical properties of soils (van Meeteren et al., 2008; Zhang et al., 2013). However, studying the microbial community structure is quite challenging because soil microbial communities are diverse and have high spatial variations (Ettema and Wardle, 2002; O'Brien et al., 2016). Furthermore, it is well recognized that most soil microorganisms (80–90%) are not characterizable using the classical cultivation techniques, which poses a major barrier for soil microbial analysis (Amann et al., 1995; Ellis et al., 2003).

The Tibetan Plateau, which is also known as "third pole of the world" and "water tower of the Asia", is the source and upper reaches of major rivers of Central and South Asia (Dong et al., 2010; Harris, 2010; Wang et al., 2015; Zhao et al., 2018). With complex interactions of cryospheric, atmospheric, geographic, hydrological, and environmental processes, the Tibetan Plateau plays a key role in the Earth's climate, biodiversity, and water cycle (Yang et al., 2019). The total area of natural wetlands on the Qinghai-Tibetan Plateau is estimated to be up to $13.3 \times 10^4$ km$^2$ (Deng et al., 2014). Meanwhile, the southeast part of the Tibetan Plateau has rich forestry resources. These alpine wetlands and forests play important roles in maintaining carbon sequestration, water balance, ecological security, and biodiversity on the Tibetan Plateau (Siles et al., 2016; Zhang et al., 2011). The vegetation inputs and vegetation types are obviously different between these two types of ecosystems, which can induce difference in the soil nutrients (Zhang and Zhang, 2008). Soil microbes, which are crucial in maintaining the functions of ecosystems (e.g., decomposition of organic matter), serve as a key link between soils and plants (Chen et al., 2019). Soil conditions, such as pH, moisture content, and nutrients, are well known to have significant impact on the diversity and composition of soil microbial communities (Brockett et al., 2012; Kim et al., 2014; Stark et al., 2012). Therefore, comparing the structures of microbial communities and the key factors driving the assemblages of soil microbiota between the alpine wetland and alpine forest ecosystems could greatly enhance the understanding of soil microbial diversity and ecosystem function on the Tibetan Plateau. Meanwhile, the Tibetan ecosystems are particularly vulnerable to climate warming (Yang et al., 2014). With melting of glaciers and frozen soils brought by global and regional climate change, it is important to understand the structure and function of microbial communities in the seasonally frozen soils and the potential risk to human health.

Several studies have investigated the soil microbial communities in the alpine wetland ecosystem (An et al., 2019; Gu et al., 2018; Lei et al., 2017) and the alpine forest ecosystem on the Tibetan Plateau (Siles and Margesin, 2016; Sun et al., 2019; Xu et al., 2017). Nonetheless, the difference in soil microbial community structure between the alpine wetland and alpine forest ecosystems has not been studied. In this study, the Lhasa River basin and the Nyang River basin on the Tibetan Plateau were selected to investigate the soil microbial communities in the alpine wetland and alpine forest ecosystems, respectively. The main objective of this study was to explore the variations in soil microbial community diversity and composition in the alpine wetland and alpine forest ecosystems based on 16S rRNA gene sequencing analysis, and understand the key driving factors of soil microbial diversity on the Tibetan Plateau. The following three hypotheses were examined in this study: (1) soil microbial diversity in the alpine forest and wetland ecosystems on the Tibetan Plateau is largely determined by abiotic factors (i.e., altitude, climate, and soil properties), while plant factors (i.e., vegetation) also play non-negligible roles; (2) driven by the difference in climate conditions and soil properties, soil microbial community diversity and abundance have significant spatial variations, especially along the altitude gradient; (3) the significantly different plant types and vegetation input between the alpine wetland and alpine forest ecosystems have impact on their soil microbial communities.

## 2. Materials and methods

### 2.1. Study sites and soil sample collection

The sampling sites of alpine wetland ecosystem (90°54′-92°10′E, 29°24′-29°48′N) and alpine forest ecosystem (92°47′-94°46′E, 29°13′-29°58′N) are located in the Lhasa River and Nyang River basins, respectively, which are within the Mira Mountain (92°21′E, 29°49′N) watershed, and their waters discharge into the Yarlung Zangbo River. The higher mountains on the Tibetan Plateau are in permafrost region, while the lower places are in seasonally frozen soil region (Sato, 2001). All the study sites in the Lhasa River and Nyang River basins are located in the latter region. During the thawing period (May to October), the seasonally frozen soils melt from the surface, while freezing of the soil layer occurs from November to April of the next year (Chen et al., 2013). The mean annual temperature (MAT) and mean annual precipitation (MAP) of the study sites in the Lhasa River basin are −7.1 to 9.2 °C and 340 to 700 mm (Han et al., 2018b), while the MAT and MAP of the study sites in the Nyang River basin are 7.6 to 10.3 °C and 452.4 to 1295.1 mm, respectively (Jin et al., 2019). More details on the climatic parameters of the study regions can be found in the Supplementary data (Table S1). The study sites in the Lhasa River basin, which is one of the typical distribution regions of alpine wetlands, are predominantly covered by Kobresia (*Kobresia humilis* and *Kobresia littledalei*). The land type of the study sites in the Nyang River basin belongs to alpine forest, and the region is mainly covered with cedar and alpine pine, such as *Abies forrestii*, *Picea asperata Mast*, and *Pinus densata*

*Mast*. The main vegetation for the sampling site on the Mira Mountain is shrubs and grasses, including *R. kongboense*, *S. gyamdaensis*, and *Carex Linn*.

A total of 48 samples of surface soil (0–10 cm) were taken from the alpine wetlands (L), Mira Mountain (M), and alpine forests (N) in July 2019 (Fig. 1). At each sampling site, three 5 m × 5 m quadrats with relatively similar microrelief conditions (i.e., flat lands, without patchy distribution of vegetation) were selected. The distance between each quadrat was greater than 20 m, thus the quadrats were considered independent from each other. At each quadrat, five sub-samples of soil were randomly collected and pooled into a composite sample to represent the site. These samples were mixed, homogenized, and sieved (<2 mm) to remove the stones, roots, and other plant materials, respectively. The samples from the Lhasa River basin and Nyang River basin were designated as alpine wetland soils (L1-L7) and alpine forest soils (N1-N8), respectively. The geographic information (latitude, longitude, and altitude) of each site was recorded with a GPS device. All soil samples were sealed in sterilized plastic bags, stored in a cooler containing ice before being transported back to the laboratory within 3 days. Once in the laboratory, a portion of each soil sample was taken for characterization of physical and chemical properties, while the rest was stored at −20 °C and processed for DNA extraction and PCR amplification within 10 days.

### 2.2. Analysis of soil properties

The soil pH was measured in a suspension of soil and deionized water (at a weight-to-volume ratio of 1:5) by a pH meter. Total contents of nitrogen (TN), hydrogen (TH), and carbon (TC) of the soil samples were measured using an elemental analyzer (Elementar vario EL cube). The content of soil moisture (SM) was measured as the water loss after oven drying at 105 °C for 8 h (Wu et al., 2017). Total phosphorus (TP) and total potassium (TK) contents were determined using the sodium hydroxide extraction-molybdenum-antimony anti-spectrophotometric method and the sodium hydroxide fusion-flame photometric method, respectively (Ade et al., 2018). Available phosphorus (AP) content was determined based on the sodium bicarbonate extraction-molybdenum antimony anti-colorimetric method (Ade et al., 2018). Available potassium (AK) content was analyzed by the ammonium acetate extraction-flame photometric method (Bao, 2000).

### 2.3. DNA extraction and PCR amplification

The total microbial DNA was extracted from 0.5 g of soil with a FastDNA SPIN Kit for soil and the FastPrep Instrument (MP Biomedicals), following the standard operating protocol. Extractions were performed in triplicates for each of the 48 samples, and the extracts were then pooled for further analysis. The extracted DNA was purified with a silica-based GENECLEAN SPIN filter, then the DNA sample was loaded on 1.2% agarose gel (0.5× TAE).

The V3-V4 region of the bacterial 16S rRNA gene was amplified by PCR (ABI GeneAmp 9700) with the primers 338F (5′-ACTCCTACGGG AGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Chu et al., 2016). The conditions for amplification were 93 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and then a final extension step at 72 °C for 10 min. The PCR amplification was performed in triplicates with a 20 μL mixture containing 0.2 μM of both forward primer and reverse primer, which was prepared from 10 ng template DNA, 4 μL FastpPfu buffer, 2 μL dNTPs (2.5 mM), 0.8 μL Forward Primer (5 μM) and Reverse Primer (5 μM), 0.4 μL FastPfu polymerase, 0.2 μL bovine serum albumin (BSA), and milli-Q water. The samples with a bright strip between 220 and 520 bp were chosen for

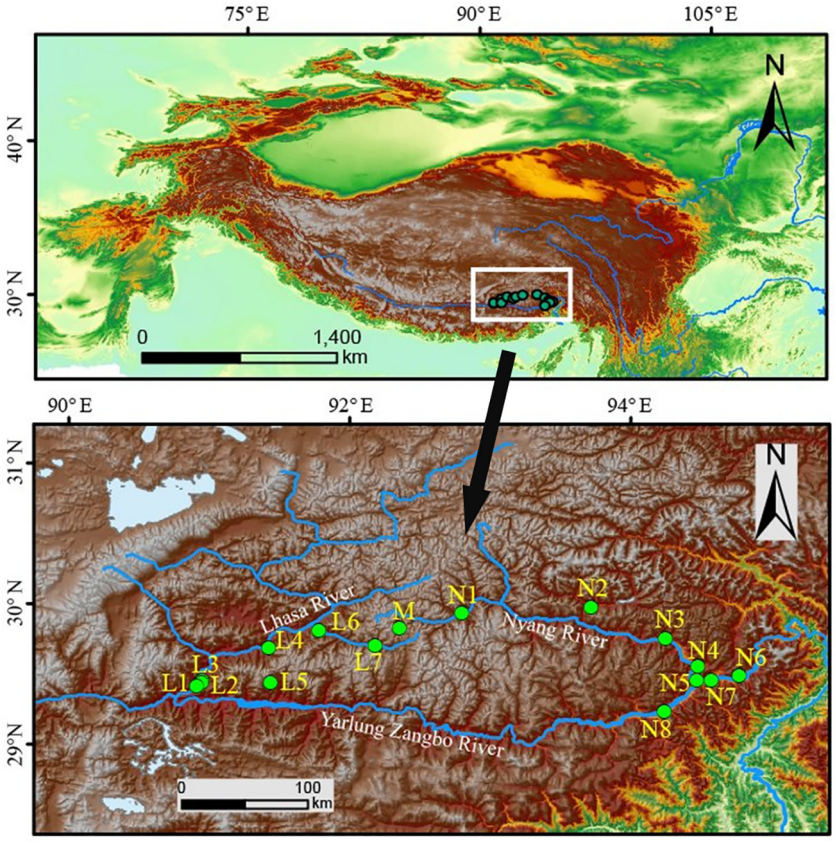


**Fig. 1.** Sampling sites of the alpine wetland soils (L1-L7) in the Lhasa River basin, Mira Mountain soil (M), and alpine forest soils (N1-N8) in the Nyang River basin on the Tibetan Plateau.

further analysis. The 3 μL loading buffer was mixed with the PCR products and visualized with 2% agarose gel electrophoresis. Then the PCR products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) and quantified using QuantiFluor™-ST (Promega), according to the manufacturer's protocol.

### 2.4. Processing of the sequencing data

The purified barcode tagged amplicons were pooled equally and paired-end sequenced (2 × 300) on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology (Shanghai, China), following the standard protocol (Li et al., 2016; Zhou et al., 2019). The fastp (https://github.com/OpenGene/fastp, version 0.20.0) and FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.7) were used to quality-filter and merge the raw fastQ files of 48 soil samples, following the detailed procedures reported by Li et al. (2016). Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE OTU clustering, while chimeric sequences were identified and removed using Usearch (ver. 7.0, http://www.drive5.com/usearch/) (Caporaso et al., 2012). The taxonomic assignment of each 16S rRNA gene sequence was performed using the Ribosomal Database Project (RDP) classifier (version 2.2, http://sourceforge.net/projects/rdp-classifier/). Each 16S rRNA gene sequence was aligned against the Silva database (Release 132, http://www.arb-silva.de) with a confidence threshold of 70% (Gu et al., 2018; Zhou et al., 2019).

### 2.5. Statistical analysis

The sequencing data were analyzed on the Majorbio Cloud Platform (www.majorbio.com). The rarefaction curves were plotted for each sample based on the OTU information. The α-diversity indices, including community diversity indices (Shannon index), community richness parameters (Chao index), as well as a sequencing depth index (Good's coverage), were calculated by the Mothur software (version 1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). The β-diversity was presented through the principal co-ordinate analysis (PCoA) and the analysis of similarities (ANOSIM), which were conducted according to the Bray-Curtis distance matrix algorithm using the OTU information from each sample in R. Biomarker analysis was performed by the linear discriminant analysis (LDA) for effect size (LEfSe) using the Kruskal-Wallis test to determine the significant difference in soil microbial species between the alpine wetland and alpine forest ecosystems. LDA was performed to evaluate the difference of each microbial taxon with a threshold value of 4.0. A redundancy analysis (RDA) was performed using R to identify the relationship between microbial community structure and physicochemical properties of the soils. In addition, 16S rRNA gene sequences were also used to calculate and predict the functional pathways in KEGG databases by the Tax4Fun package in R.

## 3. Results and discussion

### 3.1. Physicochemical properties and gene sequences of the soil samples

As shown in Table 1, the altitudes of the soil sampling sites ranged from 2859 to 4925 m, while there was no obvious fluctuation in the values of pH and contents of TP, TN, and TH across most soil samples. Nevertheless, the C/N ratio and contents of TH, TN, and TC of L7 were significantly higher than the others ($p < 0.05$). The other physical and chemical properties of these soil samples, including SM, H/C ratio, AP, and AK, differed significantly ($p < 0.05$). Specifically, the SM values were significantly higher in N4-N8 than the other soils ($p < 0.05$). L7 had the lowest pH and H/C ratio, while M had the highest values. In addition, L4, L7, N1, and N7 exhibited significantly higher AP and AK contents than the other soil samples ($p < 0.05$). The contents of TC and TN were rather low in all the surface soil samples, which is consistent with the findings of other investigations conducted on Tibetan soils (Luo et al., 2020; Shang et al., 2016). The poor soil nutrition is the combined results of many factors, including parent material, topography, climate, and vegetation (Post et al., 1982). In addition, soil erosion is another important factor that causes reduction in the contents of organic carbon and other nutrients in surface soils on the Tibetan Plateau (Zhang et al., 2019).

A total of 2,407,531 quality sequences were obtained from the 48 soil samples, and the fragment lengths ranged from 200 to 536 bp. The 48 samples contained 19,235 to 923,280 reads, and the smallest number (19,235) was chosen to compare all of them at the same sequencing level. After clustering and alignment, a total of 10,676 OTUs were observed based on a 97% threshold with a range of 1263 to 2721 OTUs, indicating remarkable variations of the microbial OTU number across the samples. The lowest OTU number was observed in L7 while the highest one was observed in N4. Among these OTUs, 0.55% ($n = 59$), 0.35% ($n = 37$), 0.24% ($n = 26$), 0.31% ($n = 33$), 0.12% ($n = 13$), 0.09% ($n = 10$), 0.13% ($n = 14$), 0.33% ($n = 35$), 0.07% ($n = 8$), 0.28% ($n = 30$), 0.27% ($n = 29$), 0.27% ($n = 29$), 0.65% ($n = 69$), 0.25% ($n = 27$), 0.11% ($n = 12$), and 0.23% ($n = 25$) were exclusively detected in L1, L2, L3, L4, L5, L6, L7, M, N1, N2, N3, N4, N5, N6, N7, and N8, respectively. However, most OTUs ($n = 6448$, 60.4%) were shared by the alpine wetland soils (L1-L7) and alpine forest soils (N1-N8) (Fig. S1), indicating the alpine wetland and alpine forest ecosystems had little difference in soil microbial composition at the OTU level. The average percentage of unclassified sequences increased with the depth of classification, ranging from 0.04% (phylum level) to 5.82% (genus level) in the alpine wetland soils, and 0.05% (phylum level) to 6.97% (genus level) in the alpine forest soils. These results suggest that there were more original sequences in the alpine forest soils than the alpine wetland soils.

### 3.2. Diversity of soil microbial communities

The observed OTUs and rarefaction curves of Good's coverage, Chao richness, and Shannon index in all soil samples were saturated, and the Good's coverage values were over 0.96 and showed no significant difference among all soil samples (Figs. 2a–b and S2). Thus, the sequencing depth was adequate to cover most soil microorganisms, and some rare species were also possibly included (Zhou et al., 2019). There was significant ($p < 0.05$) difference in the microbial richness, as reflected by the Chao index, among the alpine wetland soils and alpine forest soils (Fig. 2c). Generally, the soil microbial richness decreased with increases in the altitude of the sampling sites (Fig. 2c). For example, the site with the highest altitude in the Lhasa River basin, L7, had the lowest Chao index value, indicating this site had the least number of microbial species in the soil compared to the other sites in the alpine wetland ecosystem.

As demonstrated by the Shannon index, the α-diversity of soil microbial communities in the alpine wetland ecosystem decreased with increases in altitude of the sampling sites (Fig. 2d). This is consistent with the findings of a previous study, which showed that the α-diversity of soil microbial communities differed significantly ($p < 0.05$) among the grasslands at 3200, 3400, and 3600 m on the Tibetan Plateau (Yang et al., 2014). However, the α-diversity of soil microbial communities in the alpine forest ecosystem did not exhibit such a pattern. PCoA results show that the β-diversity of the soil microbial communities at L1, L3, L4, N4, N6, and N7 clustered together and were relatively isolated from the others (Fig. 3a). The first two principal components (PC 1 and PC 2) could explain 26.6 and 11.5% of the variation, respectively. The results of ANOSIM ($r^2 = 0.9976$, $p = 0.001$) show the grouping was meaningful (Fig. 3b). Thus, there was significant difference among all groups with different sampling sites and no difference within each group.

The varying altitude and climate conditions result in soils with different physical and chemical properties, which influence the local soil microbial communities. Various patterns (such as humpbacked,

**Table 1**
Physical and chemical properties of the alpine wetland soils (L1-L7), Mira Mountain soil (M), and alpine forest soils (N1-N8) on the Tibetan Plateau.

| Soil sample | Altitude (m) | pH | SM (%) | TC (%) | TH (%) | TN (%) | C/N | H/C | TP (g/kg) | TK (g/kg) | AP (mg/kg) | AK (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L1 | 3580 | 8.1 ± 0.2a | 10.2 ± 1.0cd | 0.74 ± 0.03fgh | 0.34 ± 0.01hi | 0.12 ± 0.03efgh | 7.6 ± 1.5d | 5.59 ± 0.31cde | 0.64 ± 0.04de | 23.6 ± 1.5abc | 7.9 ± 0.8d | 52.8 ± 5.2jk |
| L2 | 3603 | 7.9 ± 0.2ab | 8.4 ± 1.2d | 0.69 ± 0.01gh | 0.33 ± 0.01hi | 0.08 ± 0.03hi | 10.8 ± 4.8abcd | 5.74 ± 0.05bcd | 0.61 ± 0.03e | 24.2 ± 1.9ab | 7.6 ± 0.5d | 65.5 ± 3.5hijk |
| L3 | 3635 | 7.7 ± 0.1ab | 4.1 ± 1.0f | 0.61 ± 0.01hi | 0.31 ± 0.01hi | 0.10 ± 0.01gh | 7.4 ± 0.6d | 6.16 ± 0.06bc | 0.79 ± 0.03bc | 25.2 ± 2.1a | 7.5 ± 0.4d | 75.7 ± 4.1fghi |
| L4 | 3690 | 7.5 ± 0.2abcd | 4.1 ± 0.7f | 1.80 ± 0.01b | 0.49 ± 0.01cd | 0.21 ± 0.01b | 10.2 ± 0.3abcd | 3.29 ± 0.04f | 0.68 ± 0.01cde | 22.6 ± 1.6abc | 17.8 ± 1.9b | 309.3 ± 8.0a |
| L5 | 3806 | 7.5 ± 0.2 abcd | 9.5 ± 1.8d | 1.15 ± 0.04d | 0.45 ± 0.01e | 0.15 ± 0.01 cde | 8.7 ± 0.4bcd | 4.61 ± 0.07e | 0.68 ± 0.01bcde | 21.3 ± 0.5abcd | 8.3 ± 0.5d | 58.3 ± 5.0ijk |
| L6 | 3912 | 7.7 ± 0.2 abc | 11.8 ± 1.2c | 1.05 ± 0.02de | 0.46 ± 0.01e | 0.10 ± 0.01gh | 12.7 ± 0.8abc | 5.25 ± 0.03de | 0.39 ± 0.04f | 22.5 ± 3.1abc | 12.1 ± 1.2c | 83.5 ± 4.9efgh |
| L7 | 4246 | 6.3 ± 0.1e | 6.3 ± 1.2e | 7.59 ± 0.11a | 1.46 ± 0.02a | 0.63 ± 0.01a | 14.0 ± 0.5a | 2.30 ± 0.02 g | 0.98 ± 0.01a | 20.8 ± 2.1bcd | 14.9 ± 1.2c | 279.9 ± 10.8b |
| M | 4925 | 7.2 ± 0.2abcde | 10.5 ± 1.3cd | 0.42 ± 0.02i | 0.41 ± 0.02f | 0.06 ± 0.01i | 8.6 ± 1.0 cd | 12.90 ± 0.89a | 0.58 ± 0.01e | 22.1 ± 1.2abcd | 7.4 ± 0.5d | 67.6 ± 7.4ghij |
| N1 | 3836 | 6.7 ± 0.4de | 10.0 ± 1.7cd | 1.17 ± 0.01d | 0.34 ± 0.01hi | 0.13 ± 0.01efg | 10.6 ± 0.7abcd | 3.46 ± 0.03f | 0.70 ± 0.01bcde | 18.3 ± 0.9d | 22.7 ± 0.8a | 138.3 ± 6.1c |
| N2 | 3324 | 6.8 ± 0.2cde | 10.2 ± 0.5cd | 1.15 ± 0.02d | 0.35 ± 0.02gh | 0.13 ± 0.01efg | 10.1 ± 1.0abcd | 3.86 ± 0.14f | 0.58 ± 0.03e | 21.9 ± 1.3abcd | 2.4 ± 0.5f | 90.5 ± 8.7ef |
| N3 | 3045 | 7.0 ± 0.2bcde | 8.8 ± 0.9d | 1.51 ± 0.01c | 0.47 ± 0.01de | 0.14 ± 0.01def | 12.6 ± 0.5ab | 3.77 ± 0.04f | 0.56 ± 0.02e | 23.8 ± 1.1ab | 4.1 ± 0.5ef | 83.8 ± 5.5efgh |
| N4 | 2930 | 7.3 ± 0.2abcd | 20.7 ± 1.4a | 0.92 ± 0.02ef | 0.41 ± 0.01f | 0.12 ± 0.01efgh | 9.0 ± 0.5bcd | 5.29 ± 0.16cde | 0.83 ± 0.03b | 25.4 ± 1.8a | 6.9 ± 1.0de | 85.3 ± 1.7efg |
| N5 | 2885 | 7.5 ± 0.3abcd | 22.2 ± 1.3a | 1.21 ± 0.08d | 0.38 ± 0.01g | 0.12 ± 0.01efg | 11.7 ± 0.3abcd | 3.90 ± 0.28f | 0.58 ± 0.04e | 22.0 ± 1.6abc | 4.0 ± 0.3ef | 49.0 ± 2.7k |
| N6 | 2859 | 7.4 ± 0.2abcd | 22.3 ± 2.4a | 1.52 ± 0.01c | 0.73 ± 0.01b | 0.17 ± 0.01bcd | 9.8 ± 0.7abcd | 5.80 ± 0.04bcd | 0.77 ± 0.01bcd | 22.8 ± 1.7abcd | 8.6 ± 0.7d | 90.2 ± 5.0de |
| N7 | 2870 | 7.6 ± 0.3 abcd | 18.4 ± 2.5b | 1.59 ± 0.02c | 0.51 ± 0.01c | 0.19 ± 0.01bc | 9.9 ± 0.4abcd | 3.74 ± 0.05f | 0.77 ± 0.01bcd | 21.2 ± 3.2abc | 19.4 ± 1.4b | 111.4 ± 9.4d |
| N8 | 2924 | 8.0 ± 0.1 a | 17.0 ± 1.5b | 0.82 ± 0.02fg | 0.45 ± 0.01e | 0.10 ± 0.01fgh | 8.9 ± 0.3bcd | 6.64 ± 0.18b | 1.00 ± 0.03a | 19.1 ± 0.6cd | 12.9 ± 1.0c | 86.4 ± 3.6efg |

Notes: All values are reported as "mean ± standard deviation" based on measurement results for triplicated samples; SM - soil moisture; TN - total nitrogen; TC - total carbon; TH - total hydrogen; TP - total phosphorus; TK - total potassium; AP - available phosphorus; AK - available potassium; values in the same line without shared lowercases letters mean significant difference at $p < 0.05$ among the samples.

monotonic decreasing, hollow, or no change) in the variations of soil bacterial community diversity along the altitude gradient have been reported in previous studies. Han et al. (2018a) observed a hump-backed curve for the bacterial α-diversity in the primitive pine forest soils along the altitude gradient in the Changbai Mountain of Northeast China. Cong (2013) found that the bacterial diversity decreased monotonically with increases in altitude in the soils of four major types of forest in the Shennongjia Natural Reserve of Hunan province in China. Wang et al. (2012) reported that the biodiversity patterns for biofilm bacterial communities on stream stones exhibited a hollow pattern along an elevation gradient (1820 to 4050 m) in the Laojun Mountain, Yunnan province of China. Meanwhile, for alpine forest soils, no apparent change in soil bacterial diversity with increases in altitude has also been observed in previous investigations (Fierer et al., 2011; Shen et al., 2013). The highly variable patterns observed in different studies could be attributed to the difference in the primary factors controlling the bacterial diversity in soils from different regions. The negative correlation between bacterial diversity and altitude observed for the alpine wetland soils could actually result from the impact of soil pH, not altitude (Fierer et al., 2011). The results of this study indicate that soil pH was a key driver affecting bacterial diversity in the alpine wetland soils on the Tibetan Plateau, but not in the alpine forest soils (to be discussed later). In addition, vegetation type, which determines litter composition and soil conditions, is also an important factor shaping soil microbial community structure (Han et al., 2018a). The changes in vegetation and soil properties along the altitude gradient were more significant in the alpine wetland ecosystem compared to the alpine forest ecosystem, which resulted in an apparent correlation between soil bacterial communities and elevation for the former. On the other hand, it has been suggested the difference in soil microbial communities observed along an elevation gradient of the Tibetan grasslands could be partially explained by the relationship between soil heterogeneity and geographic distance (Yang et al., 2014). It has also been reported that the dramatic shift in the structure of soil microbial communities along the altitude gradient in the grassland soils on the Tibetan Plateau was jointly influenced by soil moisture, temperature, nutrient contents, and plant types (Guo et al., 2015). The Lhasa River basin and Nyang River basin, where the alpine wetland soils and alpine forest soils were collected, belong to the zones of warm semi-arid climate and warm semi-humid climate, respectively. Changes in soil moisture and temperature not only have direct impact on soil microbial habitats (e.g., soil nitrogen and carbon), but can also alter the diversity of soil microbial communities (Yang et al., 2019). In this study, clear spatial variations of soil microbial diversity in the alpine wetland and alpine forest ecosystems was observed, which could be attributed primarily to the significant difference in physical and chemical properties (e.g., soil moisture and soil nutrients) of the soils.

### *3.3. Composition of soil microbial communities in the alpine wetland and alpine forest ecosystems*

The soil microbial gene sequences were analyzed by the RDP classifier algorithm against the Silva 16S rRNA database at a confidence threshold of 70% and clustered into different taxa, including 1 domain, 41 phyla, 116 classes, 317 orders, 554 families, and 1161 genera. Fig. 4a shows the microbial composition at phylum level (relative abundance >1%) in the 48 soil samples. The classified sequences were primarily assigned to *Proteobacteria* in both the alpine wetland and alpine forest soils, and its average abundance was 30.47, 21.51, 28.15, 32.02, 42.71, 39.03, 44.48, 22.19, 42.80, 38.52, 22.49, 36.21, 35.62, 47.02, 33.89, and 34.59% in L1, L2, L3, L4, L5, L6, L7, M, N1, N2, N3, N4, N5, N6, N7, and N8, respectively. These results are consistent with the findings that soil bacterial communities at phylum level were mainly composed of *Proteobacteria* in alpine wetland ecosystem (An et al., 2019; Gu et al., 2018) and alpine forest ecosystem (Siles and

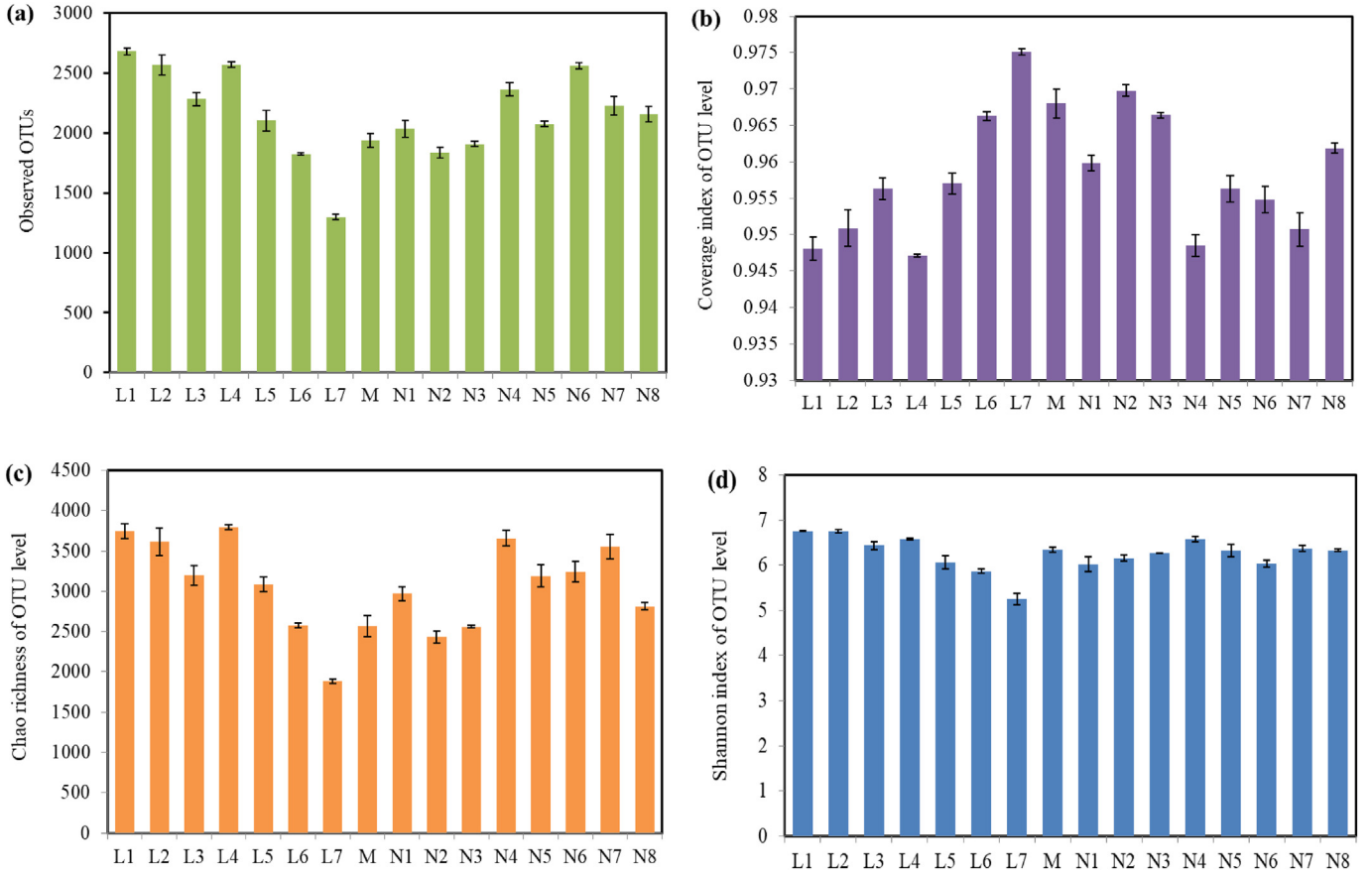


**Fig. 2.** α-diversity of microbial communities in the alpine wetland soils (L1-L7), Mira Mountain soil (M), and alpine forest soils (N1-N8): (a) Observed OTUs, (b) Good's coverage, (c) Chao richness, and (d) Shannon index. All data are presented as "mean ± standard deviation" calculated from biological triplicates.

Margesin, 2016). It has also been reported that *Proteobacteria* were the most abundant bacterial phylum in the permafrost soil layer and the soils from alpine meadows on the Tibetan Plateau (Chen et al., 2017; Li et al., 2016; Wu et al., 2017). Nonetheless, a recent study found that *Actinobacteria* were the predominant soil bacterial phylum in the alpine steppes with different degrees of degradation on the Tibetan Plateau (Zhou et al., 2019). Mainly composed of *Deltaproteobacteria* and *Betaproteobacteria*, which are classified as copiotrophs stimulated by increased nutrients in the environment (Fierer et al., 2007; Röske et al., 2011), *Proteobacteria* have phylogenetic, ecological, and pathogenic significance, and participate in energy metabolism, e.g., oxidation of organic and inorganic compounds and obtaining energy from light (Bryant and Frigaard, 2006; Mukhopadhya et al., 2012). The important role played by *Proteobacteria* in the decomposition of organic matter could partially explain its greater abundance in the alpine wetland and alpine forest ecosystems, which have higher organic matter contents than the soils of degraded alpine steppes.

The second most dominant phylum was *Acidobacteria* (3.88–32.60%), which are known to degrade organic matter and participate in nutrient cycling (Eichorst et al., 2018; Fang et al., 2017). After *Acidobacteria*, the dominant phyla were *Actinobacteria* (8.90–22.83%), *Chloroflexi* (1.92–23.57%), and *Bacteroidetes* (1.33–17.74%). With an active DNA repair mechanism, *Actinobacteria* have a strong metabolic capacity at low temperatures (Johnson et al., 2007; Yergeau et al., 2010). *Bacteroidetes*, which are involved in C and N metabolism (Wu et al., 2017) and occur widely across various ecological niches (Garrity and Holt, 2001), are expected to be well adapted in the seasonally frozen soils. The other less abundant phyla found in the alpine wetland and alpine forest soils included *Gemmatimonadetes* (0.69–7.62%), *Patescibacteria* (0.18–7.30%), *Firmicutes* (0.19–3.59%), *Verrucomicrobia* (0.21–5.41%), *Rokubacteria* (0.05–3.52%), *Planctomycetes* (0.08–1.15%), *Cyanobacteria* (0.03–2.68%), *unclassified_k_norank_d_Bacteria* (0.05–1.57%), and *Latescibacteria* (0.00%–1.01%). The rest had relative abundance below 1% in all soil samples, and were thus designated as "other phyla" (1.06–2.03%).

Fig. 4b displays the relative abundance of the 28 shared species (relative abundance >1%) at class level in the soil samples. The most abundant classes were *Alphaproteobacteria* and *Actinobacteria*, followed by *Gammaproteobacteria*, *Blastocatellia_subgroup_4*, *subgroup_6*, and *Bacteroidia*. As shown on Fig. 4a and b, the relative abundance of dominant phyla and classes in the alpine wetland and alpine forest soils differed along the altitude gradient. The change in soil microbial community abundance with altitude is not surprising, as rises in altitude result in increases in environmental harshness (Margesin et al., 2008). Further analysis of the top 50 genera reveals that members of *Acidobacteria*, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Chloroflexi*, *Rokubacteria*, *Actinobacteria*, *Nitrospirae*, *Gemmatimonadetes*, *Patescibacteria*, and *Firmicutes* were shared by all the soil samples (Fig. 4c). The most representative bacterial genera were *RB41*, *Sphingomonas*, and *norank_c_Subgroup_6*. In addition, 36 phyla (87.8%), 101 classes (87.07%), and 875 genera (75.37%) were shared by the alpine wetland and alpine forest ecosystems, respectively (Fig. S3). Overall, the composition of bacterial phylum, class, and genus was similar across all the soil samples, although the relative abundance exhibited spatial variations, suggesting that stable microbial community composition existed in the soils of alpine wetland and alpine forest ecosystems (Zhang et al., 2014b). Bacterial phyla, including *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes*, also existed as the dominant species in the soils of alpine steppes (Zhou et al., 2019), alpine meadows (Li et al., 2016), alpine wetlands (Gu et al.,

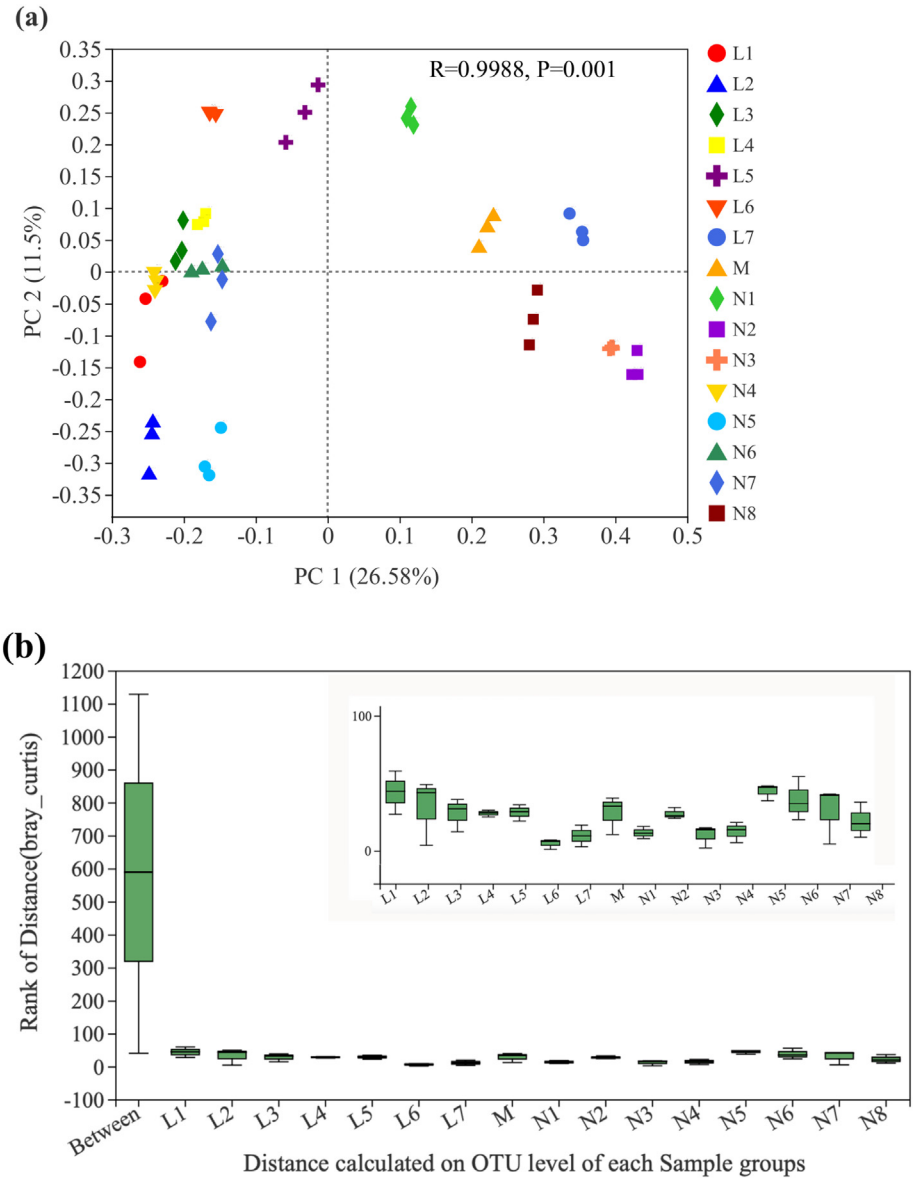


**Fig. 3.** β-diversity of microbial communities in the alpine wetland soils (L1-L7), Mira Mountain soil (M), and alpine forest soils (N1-N8): (a) Principal co-ordinate analysis, and (b) Analysis of similarities. On (b), the box corresponding to the "between" represents the distance value of the difference among groups, while the other boxes represent the distance value of the difference within each group.

2018), and some permafrost regions on the Tibetan Plateau (Wu et al., 2017). As shown in Table 1, the soil pH and TN values exhibited little fluctuation between the alpine wetland soils and alpine forest soils. The results of RDA analysis also showed soil pH and TN were the key drivers of soil microbial communities across all the soil samples (Fig. S4). Previous studies have shown that soil pH (Feng et al., 2014) and nitrogen content (Stark et al., 2012) could serve as the key abiotic controls over microbial community structure. It has also been reported that the dramatic shift in the structure of soil microbial communities on the Tibetan Plateau was influenced by soil temperature (Guo et al., 2015). On the other hand, the MAT values of the alpine wetland and alpine forest ecosystems investigated in this study are comparable (Table S1). Together, the above abiotic factors could probably explain the lack of significant difference in the soil microbial composition in the two types of ecosystems on the Tibetan Plateau.

Difference in the soil microbial structure was found when the LEfSe algorithm was used to determine the taxon that best characterizes each biological class. As shown on Fig. 5, 21 bacteria clades exhibited significant difference in all soil samples with an LDA threshold of 4.0. The soil sample from Mira Mountain (M) was enriched with bacteria that were different, while the alpine wetland soils and alpine forest soils only had 2 and 3 abundant bacteria clades, respectively. Specifically, the alpine wetland soils were rich in *Cytophagales* (order) and *Hymenobacteraceae* (family), while the alpine forest soils were enriched with *Alphaproteobacteria* (class), *Rhizobiales* (order), and *Xanthobacteraceae* (family). These results suggest that *Hymenobacteraceae* and *Cytophagales* may be potential biomarkers for the alpine wetland soils, while *Alphaproteobacteria* could serve as a potential biomarker for the alpine forest soils. Besides, the most enriched bacteria at the phylum level were *Bacteroidetes* (from order to family) and *Proteobacteria* (from class to genus) in the alpine wetland soils and alpine forest soils, respectively. *Bacteroidete* is involved in C and N metabolism, such as degradation of organic matter and nitrite oxidation (Eichorst et al., 2018; Fang et al., 2017), while *Alphaproteobacteria* shows a significant positive correlation with the organic carbon content of the soils, suggesting that increases in root secretions or plant litter decomposition products stimulate their growth (He et al., 2017). These results indicate that the microbes related to organic matter degradation can survive

better in the alpine wetland soils compared to the alpine forest soils. Nonetheless, there is difference in the degradation of soil organic matter between wetland and forest soils. For instance, the labile to moderate fractions of organic matter in alpine wetland soils degrade faster than those in alpine forest soils, while the moderate to recalcitrant fractions of organic matter in alpine wetland soils degraded more slowly than those in alpine forest soils (Chen et al., 2019). Such difference in the rate of organic matter degradation could potentially impact the soil microbial community structure in the alpine wetland and alpine forest soils, such as the fungi/bacteria ratio (Malik et al., 2016). Meanwhile, the enrichment of *Alphaproteobacteria* in the alpine forest soils could be attributed to the good vegetation coverage and higher plant biomass. In the alpine forest ecosystem, *Abies forrestii*, *Picea asperata Mast*, and *Pinus densata Mast* are the dominant vegetation types, and abundant shrubs and weeds are also distributed on the ground. Plant types and vegetation input are significantly different between the alpine wetland and alpine forest ecosystems, which can lead to difference in the soil carbon fractions (Zhang and Zhang, 2008), and subsequently impact

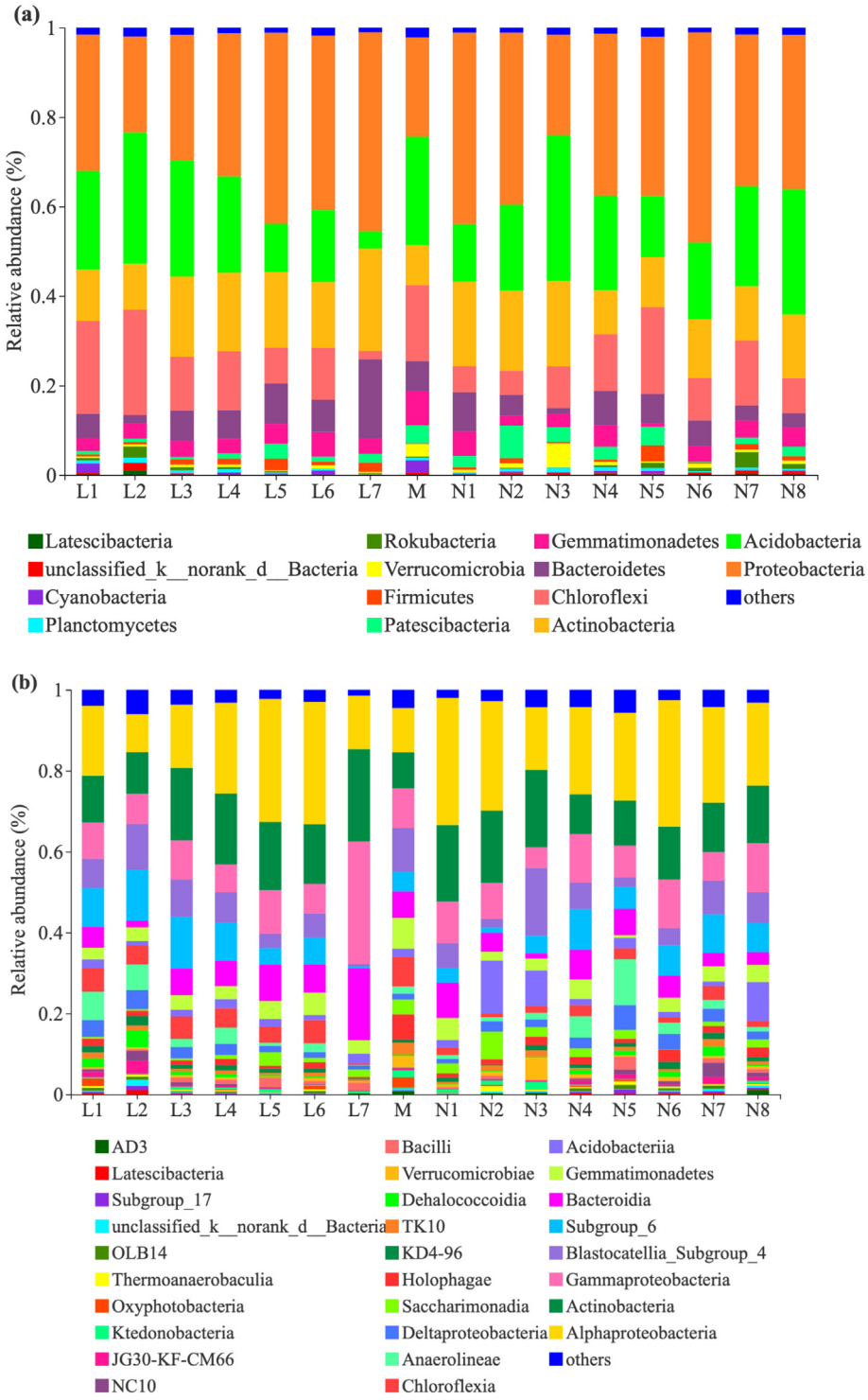


**Fig. 4.** Microbial community composition of the alpine wetland soils (L1-L7), Mira Mountain soil (M), and alpine forest soils (N1-N8) at different levels: (a) Phylum, (b) Class, and (c) Classified genus (top 50).

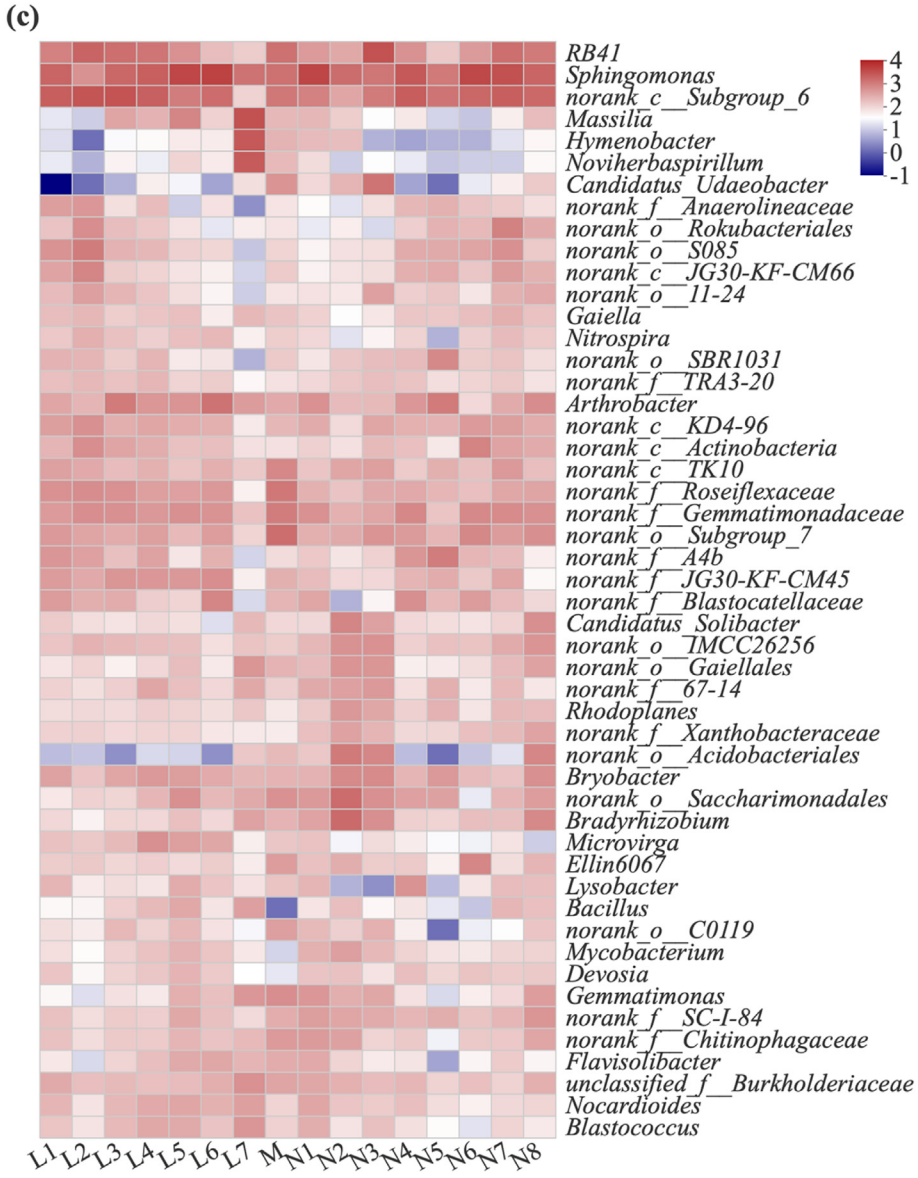


**Fig. 4** (*continued*).

the soil microbial communities. Taken together, the abundance of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau exhibited spatial variations, with climate and soil properties being the primary drivers of the formation and evolution of soil microbial communities, whereas little difference in soil microbial composition existed between these two types of ecosystems.

### *3.4. Relationship between soil properties and microbial communities*

RDA was applied to identify the effect of soil properties on microbial community structure in the alpine wetland and alpine forest ecosystems. Fig. 6a shows that the first and second ordination axes of RDA explained 73.98 and 7.74% of the variance of total phyla in the alpine wetland soils, respectively. TK was the most important factor affecting the soil bacterial community structure, while altitude and pH also played important roles. In the alpine forest soils, the first and second ordination axes of RDA explained 44.22 and 20.59% of the variance of total phyla, respectively (Fig. 6b). Soil moisture was the most important factor affecting the soil bacterial communities, while AK, altitude, and C/N ratio also played important roles. Overall, soil pH, TC, TH, and TN appeared to be the important drivers of soil microbial communities, while altitude exhibited little influence across all the soil samples (Fig. S4).

Understanding the relationship between environmental variables and soil microbial community structure is one of the vital goals of microbial ecology (Gu et al., 2018). The results of this study clearly indicate that the physicochemical properties of soils could play an important role in bacterial community structure, although the impact was variable depending on the microbial species and sampling sites. Previous studies observed that soil pH was the most important driver shaping soil bacterial communities on regional scales (Chu et al., 2016; Fierer and Jackson, 2006; Tripathi et al., 2012). Nonetheless, pH was not detected as a major driver of the soil bacterial community structure in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. This could be explained by the fact that the soil pH gradient was not enough to exert substantial effect on the structure of microbial communities in the regions covered in this study. A recent study also observed that soil pH was not obviously correlated with the structure of soil bacterial communities in a permafrost thaw subsidence area on the southern Tibetan Plateau (Wu et al., 2018).

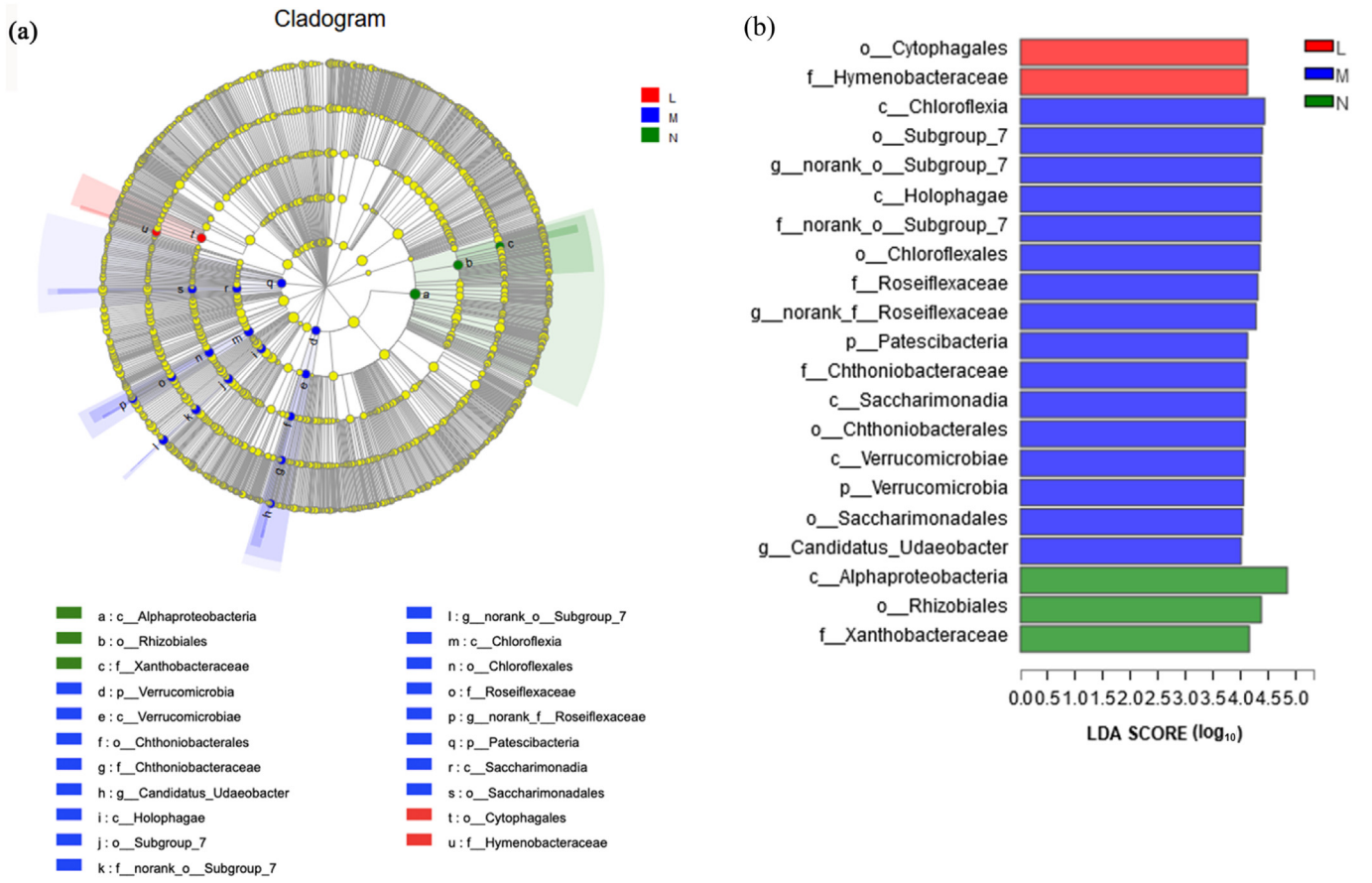


**Fig. 5.** LEfSe analysis of soil microbial abundance in the alpine wetland soils (L), Mira Mountain soil (M), and alpine forest soil (N): (a) LEfSe analysis results of soil bacteria, and (b) Histogram of LDA scores calculated for the differentially abundant microbes with a threshold value of 4.0.

In this work, TK and AK were found as the most important drivers of soil bacterial community structure in the alpine wetland and alpine forest ecosystems, respectively. This finding is consistent with those of a previous study, which showed that TK content was the most important factor influencing the bacterial communities in the alpine wetland soils on the Tibetan Plateau (Gu et al., 2018). Pereira et al. (2014) also observed that the diversity and composition of soil bacterial communities were strongly associated with the TK content. As a plant macronutrient, potassium is easily leached from soils (Gu et al., 2018). Soil bacteria can affect the solubility and availability of potassium through a range of processes, including chelation, redox, acidolysis, and production of different products, which conversely affects the selection of specific bacteria related with potassium (Miransari, 2013; Pereira et al., 2014). Although RDA reveals that soil pH and TN were the key driving factors of the microbial community structure across all the soil samples (Fig. S4), they were not identified as the most important drivers in either the alpine wetland soils or the alpine forest soils. A recent study found that the variations of microbial communities in hummock and hollow soils from three wetlands on the Qinghai-Tibetan Plateau were mainly contributed by soil N (Deng et al., 2014). As vegetation cover can be an important factor affecting the soil microbes (Gu et al., 2018; Han et al., 2018a), it is likely that soil nutrients, including K and N, could influence soil microbial structure indirectly through affecting the plants growing in the soils. The results also indicate that altitude is a strong driver of soil microbial community structure in the alpine wetland and alpine forest ecosystems. With increases in the altitude, soil physical and chemical conditions would change, leading to corresponding variations in microbial communities (Ade et al., 2018; Guo et al., 2015; Yang et al., 2014).

The different climatic conditions (MAT and MAP) could also play an important role in shaping the soil microbial community structure in the alpine wetland and alpine forest ecosystems. The highest MAT values of the alpine wetland sites are similar to those of the alpine forest sites. Nevertheless, the warm and humid air is transported to the Nyang River basin due to influence of the southwest monsoon from May to September (Jin et al., 2019). As a result, the alpine forest ecosystem in the Nyang River basin has overall higher MAP and soil moisture than the alpine wetland ecosystem in the Lhasa River basin because of the topographical influence. Moisture is well known to be an important factor affecting soil microbial communities on the Tibetan Plateau (Wu et al., 2018; Zhang et al., 2012; Zhang et al., 2014a). It has been demonstrated that the soil microbial communities under enhanced rainfall treatment could be strongly affected by the available nutrients and moisture content of soils (Zhang et al., 2016). The process of obtaining nutrients for microbes depends on the flow of water film in soils (Evans and Wallenstein, 2012). Thus, moisture content can impact soil microbial community structure through affecting the availability, transport, and diffusion of nutrients, and the physicochemical properties of soil as well. Previous investigations of the permafrost regions on the Tibetan Plateau have shown that SM content was correlated with the vegetation cover (Wang et al., 2008; Wu et al., 2012). Thus, moisture content of the alpine forest soils plays a more important role in affecting microbial community structure compared to other soil properties. Nonetheless, the composition of microbial communities in the alpine wetland and alpine forest soils was mostly similar, despite of their significant difference in moisture contents, which is indicative of stable soil microbial ecosystems on the Tibetan Plateau.

Many studies have shown that the soil nutrients, mainly organic carbon, nitrogen, total/available phosphorus, and total/available potassium, play key roles in controlling the diversity and abundance of soil microbial community structure. For examples, Gu et al. (2018) observed

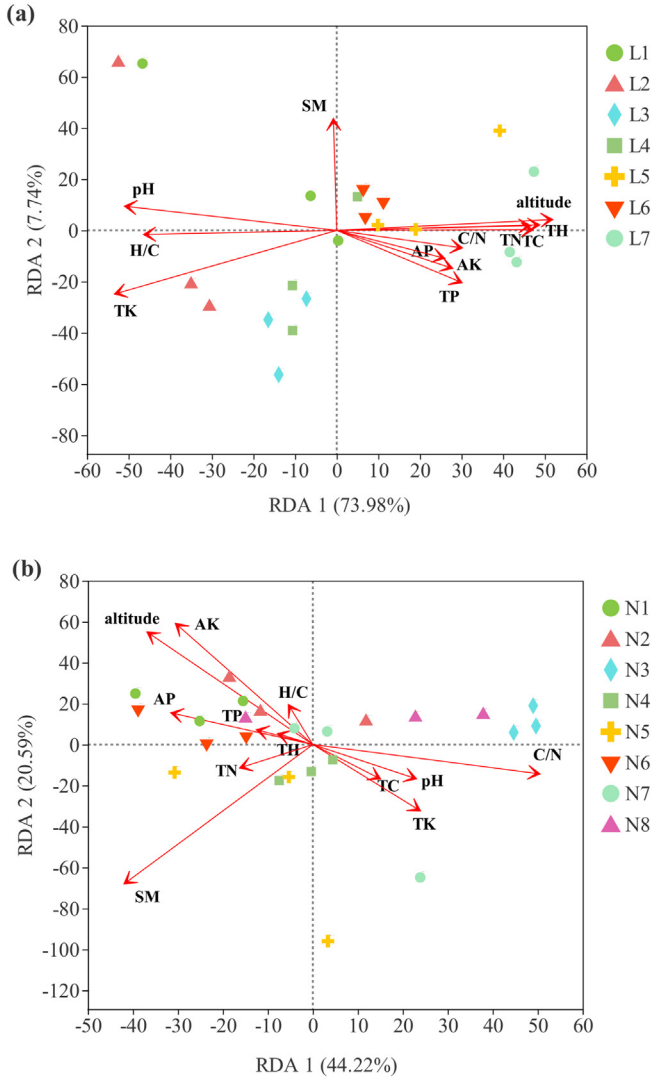


**Fig. 6.** Relationship between soil microbial communities and physicochemical properties of the soils revealed by RDA: (a) The alpine wetland soils, and (b) The alpine forest soils. The arrow length corresponds to the variance of microbial community structure that can be explained by the soil property, and its direction indicates an increasing magnitude of the soil property.

that TK content was the most important factor influencing the bacterial communities in the alpine wetland soils on the Tibetan Plateau. Other researchers also observed that soil nutrients (e.g., TC, TN, TP, AP, and organic carbon) have great influence on bacterial abundance and could explain most variations of the soil microbial communities in the alpine grassland soils on the Tibetan Plateau (Zeng et al., 2018; Zhang et al., 2016; Zhou et al., 2019). In addition, other metal nutrients (e.g., Fe, Cu, Ca, and Mg) could also have significant impact on bacterial diversity and composition in soils. Corneo et al. (2013) evaluated the variations of microbial community structure in vineyard soils across the altitude gradient and in different seasons, and found that soil Cu and Mg could affect the structures of bacterial and fungal communities, while soil Fe, Al, Mn, and Ni were also important determinants of bacteria community structure. Pereira et al. (2014) also observed that the contents of Na, Ni, and Zn were important parameters that influenced the bacterial diversity and composition in the soils from a neutral mine drainage channel. Hamidović et al. (2020) found that the contents of metal nutrients (Ca, K, and Fe) had positive correlation with the microbial community structure in the soils affected by coal mine exploitation. Due to lack of measurement data, the relationship between metal nutrients and the diversity and structure of soil microbial communities in the alpine forest and alpine wetland soils on the Tibetan Plateau could not be evaluated here, but this issue deserves investigation in future research.

Together, the findings of this study suggest that soil physicochemical properties could explain the variations of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. It is well known that altitude can be a major factor that has confounded effects on both biodiversity and soil physiochemical properties, as high altitude ecosystems are often impacted by low temperature, variable precipitation, and soil nutrient stress (Kumar et al., 2019). Therefore, more detailed investigations are still necessary to fully understand the driving factors and mechanism responsible for the structures and diversity of soil bacterial communities observed in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. In addition, the β-diversity of the soil microbial communities and the relationship between soil properties and structure of microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau were evaluated with PCoA and RDA analyses based on a relatively small sample size. Even though the results obtained are statistically significant, further investigation involving a larger size of soil samples are necessary to extend the validity of the findings of this study.

### 3.5. Potential metabolic pathways in soils

As an inexpensive and suitable alternative without metagenomic data, Tax4Fun analysis of 16S rRNA data could be applied for predicting the functional capabilities of microbial communities (Aßhauer et al., 2015; Zhou et al., 2019). A total of 6386 KEGG orthologues, which belong to metabolism, organismal system, genetic information processing, cellular processes, environmental information processing, and even human diseases, were found in the soil samples. Table 2 lists the top abundant functional pathways (relative abundance >1%) at level 2. Among the diverse functional pathways identified for the microbes in the alpine wetland and alpine forest soils, most of them were associated with metabolic pathways, which are similar to those found in the soils of degraded alpine steppes on the Tibetan Plateau (Zhou et al., 2019). Compared to the other pathways, energy metabolism, amino acid metabolism, carbohydrate metabolism, membrane transport, metabolism of cofactors and vitamins, and signal transduction were over-represented. In addition, the bacteria involved in human diseases, including cancers, immune, metabolic, neurodegenerative, cardiovascular, endocrine, and infectious diseases, were also found in the alpine wetland and alpine forest soil samples, with the functional class of infectious diseases being the most abundant. No significant difference in the 18 functional pathways (at level 2) was observed among all the soil samples.

It is worth noting that some bacteria involved in human diseases, especially infectious diseases, were found in the seasonally frozen soils from the Mira Mountain, alpine wetland and alpine forest ecosystems in the Lhasa River and Nyang River basins. It has been well recognized that environmental bacterial profiles are associated with human diseases (Sandifer et al., 2015). Therefore, the occurrence of disease-causing bacteria in the seasonally frozen soils of the Lhasa River and Nyang River basins could negatively affect the health of the residents, particularly those engaging in agricultural and pastoral activities. The elevated abundance of functional pathways involved in human diseases in soil microbes might be partially brought by human activities. A previous study found that the abundance of bacteria involved in human diseases increased with increasing degradation of alpine steppes on the Tibetan Plateau (Zhou et al., 2019). The Lhasa River basin and Nyang River basin have relatively large populations and are economically more developed compared to the rest parts of the Tibetan Plateau, which have negatively impact on the local environment and ecosystem. As the waters of the Lhasa River and Nyang River both discharge into the Yarlung Zangbo River, which is a major international river, shared by China, India, and Bangladesh, the microbes involved in human diseases could potentially enter the major rivers and pose negative impact on the

**Table 2**
Relative abundance (>1%) of potential functional pathways at level 2 in the alpine wetland soils (L1-L7), Mira Mountain soil (M), and alpine forest soils (N1-N8).

| Pathway (level 1) | Pathway (level 2) | L1 | L2 | L3 | L4 | L5 | L6 | L7 | M | N1 | N2 | N3 | N4 | N5 | N6 | N7 | N8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Metabolism | Carbohydrate metabolism | 13.0 | 12.4 | 13.1 | 13.2 | 12.9 | 13.1 | 12.9 | 11.5 | 12.7 | 12.7 | 12.6 | 12.8 | 13.3 | 12.7 | 12.8 | 12.3 |
| | Lipid metabolism | 3.9 | 3.7 | 4.0 | 4.1 | 3.9 | 3.9 | 4.1 | 3.6 | 3.9 | 4.1 | 4.0 | 3.8 | 3.8 | 3.7 | 3.8 | 3.7 |
| | Metabolism of cofactors and vitamins | 7.3 | 7.2 | 7.2 | 7.2 | 7.3 | 7.3 | 7.2 | 7.2 | 7.3 | 7.2 | 7.2 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 |
| | Energy metabolism | 7.3 | 7.5 | 6.9 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.1 | 7.1 | 7.0 | 7.3 | 7.2 | 7.1 | 7.1 |
| | Nucleotide metabolism | 5.1 | 5.1 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.4 | 5.2 | 5.2 | 5.2 | 5.2 | 5.3 | 5.1 | 5.2 | 5.3 |
| | Amino acid metabolism | 12.7 | 12.5 | 12.8 | 12.8 | 13.1 | 13.0 | 13.0 | 13.5 | 13.1 | 12.9 | 13.0 | 12.8 | 12.4 | 13.0 | 13.0 | 13.1 |
| | Metabolism of terpenoids and polyketides | 3.6 | 3.6 | 4.1 | 4.1 | 3.5 | 3.4 | 4.2 | 3.0 | 3.5 | 4.2 | 4.2 | 3.1 | 3.6 | 2.8 | 3.1 | 3.2 |
| | Xenobiotics biodegradation and metabolism | 4.8 | 4.4 | 4.9 | 5.2 | 4.9 | 5.0 | 5.0 | 3.9 | 4.8 | 5.0 | 5.0 | 4.6 | 4.6 | 4.7 | 4.8 | 4.4 |
| | Metabolism of other amino acids | 2.8 | 2.5 | 2.8 | 2.8 | 2.8 | 2.9 | 2.7 | 2.4 | 2.8 | 2.7 | 2.7 | 2.8 | 2.7 | 2.8 | 2.8 | 2.6 |
| | Glycan biosynthesis and metabolism | 2.2 | 2.4 | 2.2 | 2.1 | 2.2 | 2.1 | 2.3 | 2.7 | 2.3 | 2.2 | 2.3 | 2.4 | 2.3 | 2.2 | 2.2 | 2.4 |
| Genetic information processing | Translation | 4.6 | 4.5 | 4.5 | 4.4 | 4.5 | 4.6 | 4.5 | 4.8 | 4.6 | 4.4 | 4.5 | 4.7 | 4.8 | 4.6 | 4.5 | 4.7 |
| | Folding, sorting and degradation | 2.3 | 2.4 | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 | 2.4 | 2.3 | 2.3 | 2.3 | 2.3 | 2.4 | 2.3 | 2.3 | 2.3 |
| | Replication and repair | 4.4 | 4.5 | 4.4 | 4.3 | 4.3 | 4.3 | 4.6 | 5.0 | 4.4 | 4.5 | 4.6 | 4.5 | 4.5 | 4.2 | 4.3 | 4.6 |
| Cellular processes | Cell motility | 1.8 | 2.2 | 1.6 | 1.6 | 1.8 | 1.7 | 1.6 | 2.4 | 1.9 | 1.8 | 1.8 | 2.0 | 1.8 | 2.1 | 2.0 | 2.1 |
| | Cell growth and death | 1.7 | 1.7 | 1.6 | 1.6 | 1.7 | 1.7 | 1.5 | 1.8 | 1.7 | 1.7 | 1.6 | 1.7 | 1.7 | 1.9 | 1.8 | 1.8 |
| Environmental information processing | Membrane transport | 10.9 | 10.3 | 10.9 | 11.0 | 11.2 | 11.4 | 10.2 | 9.4 | 10.8 | 10.3 | 10.0 | 10.7 | 10.7 | 11.4 | 11.3 | 10.5 |
| | Signal transduction | 6.7 | 8.0 | 6.3 | 6.1 | 6.5 | 6.2 | 6.5 | 8.5 | 6.7 | 6.7 | 6.9 | 7.0 | 6.6 | 7.1 | 6.9 | 7.5 |
| Human diseases | Infectious disease: bacterial | 1.2 | 1.2 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.3 | 1.1 | 1.1 | 1.0 | 1.3 | 1.1 | 1.2 | 1.1 | 1.2 |

health of the people living in the lower reaches. It should be noted that the functional capabilities of soil microbial communities in the Lhasa River basin and Nyang River basin were predicted by Tax4Fun analysis based on 16S rRNA sequencing, while further confirmation of the functional pathways of soil microbial communities involved in human diseases by metagenomic shotgun sequencing is necessary for more accurate assessment of the potential human health impact.

## 4. Conclusions

The diversity and composition of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau were investigated based on 16S rRNA sequencing of the DNA extracted from 48 seasonally frozen soil samples, which were collected from 16 sites. The diversity of soil microbial communities exhibited significant spatial variations, with *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* being the dominant phyla in both alpine wetland and alpine forest soils. No significant difference in the composition of soil microbial communities at OTU, phylum, class, and genus levels was observed. LEfSe analysis reveals five biomarkers in the alpine wetland and alpine forest soils, and suggests that various microbes with the special function of organic matter degradation adapted well in the alpine wetland and alpine forest ecosystems. Results of RDA indicate that the diversity and abundance of soil microbial communities were mainly related to the total K in the alpine wetland soils, and available K and moisture content in the alpine forest soils. Tax4Fun analysis indicates the presence of potential functional pathways involved in human diseases in the alpine wetland and alpine forest soils, which could have negative human health implications. These results provide insights on the variations in the composition, structure, and function of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. Nonetheless, more detailed investigation is needed to quantify the contribution of various natural and anthropogenic drivers to the diversity and composition of soil microbes observed.

## CRediT authorship contribution statement

**Xiaojie Wang:** Conceptualization, Data curation, Methodology, Writing - original draft, Writing - review & editing. **Zhichao Zhang:** Data curation, Visualization. **Zhiqiang Yu:** Project administration, Supervision. **Guofeng Shen:** Project administration, Funding acquisition, Resources. **Hefa Cheng:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing. **Shu Tao:** Project administration, Funding acquisition, Resources.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141358.

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