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Title: Distinct microbial communities in the active and permafrost layers on the Tibetan Plateau

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Running title: Microbial communities in Tibetan permafrost

Abstract

Permafrost represents an important understudied genetic resource. Soil microorganisms play important roles in regulating biogeochemical cycles and maintaining ecosystem function. However, our knowledge of patterns and drivers of permafrost microbial communities is limited over broad geographic scales. Using high-throughput Illumina sequencing, this study compared soil bacterial, archaeal and fungal communities between the active and permafrost layers on the Tibetan Plateau. Our results indicated that microbial alpha diversity was significantly higher in the active layer than in the permafrost layer with the exception of fungal Shannon-Wiener index and Simpson's diversity index, and microbial community structures were significantly different between the two layers. Our results also revealed that environmental factors such as soil fertility (soil organic carbon and nitrogen contents) were the primary drivers of the beta diversity of bacterial, archaeal and fungal communities in the active layer. In contrast, environmental variables such as the mean annual precipitation and total phosphorus played dominant roles in driving the microbial beta diversity in the permafrost layer. Spatial distance was important for predicting the bacterial and archaeal beta diversity in both the active and permafrost layers, but not for fungal communities. Collectively, these results demonstrated different driving factors of microbial beta diversity between the active layer and permafrost layer, implying that the drivers of the microbial beta diversity observed in the active layer cannot be used to predict the biogeographic patterns of the microbial beta diversity in the permafrost layer.

1 INTRODUCTION

Permafrost is defined as lithospheric materials that are exposed to temperatures≤ 0°C and frozen for at least two consecutive years (Margesin, 2009). Permafrost covers 25% of the Earth's terrestrial surface and contains a huge amount of buried organic carbon (Zhang, Barry,

Knowles, Heginbottom, & Brown, 2008). In recent decades, climate warming leads to widespread permafrost thawing, which is predicted to promote soil organic matter (SOM) decomposition and greenhouse gas emissions (Schuur et al., 2008; DeConto et al., 2012). The transformation of carbon and nitrogen in permafrost is considered to be largely dependent on microbial activity through processes such as respiration, methanogenesis, methane oxidation, nitrification and denitrification (Mackelprang et al., 2011). Therefore, a better understanding of the factors that shape permafrost microbial communities is important in the elucidation of microbial processes, and can improve our predictions of permafrost ecosystem function in a changing climate.

Despite being assumed to be a harsh environment, permafrost hosts comparatively phylogenetically-diverse microbial assemblages. Using high-throughput sequencing and metagenomics, recent studies shed light on permafrost microbial communities (Steven, Pollard, Greer, & Whyte, 2008; Yergeau, Hogues, Whyte, & Greer, 2010; Wilhelm, Niederberger, Greer, & Whyte, 2011; Frey et al., 2016) and their responses to permafrost thaw (Mackelprang et al., 2011; McCalley et al., 2014). However, our understanding of permafrost microbial communities is still limited in the following three aspects. First, it remains poorly understood whether the controlling factors of microbial communities differ between the active layer and the permafrost layer. The active layer, which is a seasonally unfrozen surface layer, experiences repeated freeze-thaw cycles and suffers frequent environmental disturbances (Steven et al., 2008). Unlike the relatively friendly environment in the active layer, permafrost is considered to be an extreme environment with prolonged subzero temperatures, low water activity and extremely low nutrient transformation rates (Jasson & Tas, 2014; Chen et al., 2016a). Moreover, compared with the microbial community in the active layer which could respond to the annually fluctuating conditions, the permafrost community has the benefit of a relatively stable, though extreme, habitat. These differences in physicochemical and biological properties imply that the factors shaping microbial communities may be different between the two layers. Up to now, a number of studies

revealed that microbial beta diversity in the active layer was mainly related to local environmental conditions (contemporary environment) rather than spatial distance (dispersal-related processes), such as soil organic carbon content (Siciliano et al., 2014; Tytgat et al., 2016), pH (Chu et al., 2010; Ganzert, Bajerski, & Wagner, 2014) and climate (Yergeau, Newsham, Pearce, & Kowalchuk, 2007). However, most available studies about permafrost microbial communities were based on a limited number of soil cores (Steven et al., 2008; Yergeau et al., 2010; Hu et al., 2014, 2015, 2016; Wei et al., 2014; Frey et al., 2016). Consequently, it is still an open question whether the relative importance of environmental variables and spatial distance in determining permafrost microbial community is similar to those reported for the active layer.

Second, it is poorly understood whether there are differences in the relative contributions of environmental variables and spatial distance in structuring microbial communities between bacteria, archaea and fungi in the permafrost layer. It has been reported that the drivers of microbial beta diversity in the active layer were different between bacteria and fungi. To be specific, mounting evidence reveals that plant diversity represents a prevalent determinant of soil surface fungal community structure over broad geographic scales (Prober et al., 2015; Chen et al., 2017), while soil pH is a key regulator in shaping soil surface bacterial community structure (Fierer & Jackson, 2006; Chu et al., 2010). Nevertheless, it is still unclear whether similar patterns observed in the active layer hold in the permafrost layer. Compared with the active layer, permafrost soils usually have lower soil nutrient concentration which may facilitate the growth of fungi over bacteria, as fungi possess a competitive advantage related to nitrogen and phosphorus absorption (Van der Heijden, Bardgett, & Van Straalen, 2008). Permafrost soils also usually have high soil pH (Zhang et al., 2014), which would also have a weaker influence on fungi than bacteria, as individual species of fungi have a broader range of pH for optimal growth when compared to individual species of bacteria (Rousk et al., 2010). Given that these differences in the responses to environmental factors between fungi and bacteria, it is presumed that the driving factors

would be different between bacteria and fungi in the permafrost layer.

Third, it is poorly understood whether microbial communities are different between the high-altitude and high-latitude permafrost regions. Currently, the majority of studies on permafrost affected soil microbial communities is derived from high-latitude permafrost regions, such as Alaska (Mackelprang et al., 2011; Deng et al., 2015), the Canadian Arctic (Steven et al., 2008; Yergeau et al., 2010; Wilhelm et al., 2011), Siberia (Gittel et al., 2014), Greenland (Ganzert et al., 2014) and Antarctica (Yergeau et al., 2007; Tytgat et al., 2016). Unlike the high-latitude permafrost region, permafrost affected soils in the high-altitude regions like the Tibetan Plateau are characterized by a thick active layer more than 2.4 m (Pang, Cheng, Li, & Zhang, 2009; Wu, & Zhang, 2010) and low ice content because of the arid climate, high evaporation rates and solar radiation load (Wang & French, 1995; Yang, Nelson, Shiklomanov, Guo, & Wan, 2010; Chen et al., 2016a). Moreover, compared with the high-latitude permafrost region, the Tibetan permafrost affected soils have lower organic carbon (Ding et al., 2016) and nitrogen (Chen et al., 2016b) content, which were mainly associated with larger outputs driven by higher temperature, good drainage, aerobic conditions and lower inputs from vegetation production in this region. Furthermore, compared with the high-latitude permafrost region, the Tibetan permafrost affected soils have higher carbon lability due to the higher relative abundance of herbaceous litter (Zhang, Wang, Chen, Li, & Zhao, 1988; Walker et al., 2005). Consequently, the unique characteristics in permafrost affected soils on the Tibetan Plateau might harbor different microbial communities from those in the high-latitude permafrost region. However, a comprehensive survey of microbial communities in both the active and permafrost layers covering multiple sites on the Tibetan Plateau is still unavailable, given that emerging attention was mainly paid to microbial community compositions through one permafrost core (Hu et al., 2014, 2015, 2016; Wei et al., 2014) or only in the active layer (Guan et al., 2013; Zhang et al., 2014).

In this study, we compared the distribution patterns and drivers of soil bacterial, archaeal and

fungal communities in the active and permafrost layers on the Tibetan Plateau. A total of 60 samples collected from 30 soil cores at ten sites were used to characterize the microbial communities by targeting 16S rRNA genes and Internal Transcribed Spacer (ITS) regions using high-throughput sequencing. We hypothesized that (i) contemporary environment could be the major driving factor for microbial beta diversity in the active layer, whereas spatial distance (historical event) was predominant in the permafrost layer, (ii) the driving factors of the beta diversity of bacterial and archaeal, and fungal communities in the permafrost layer could be different, and (iii) microbial communities in the high-altitude permafrost region could differ from those in the high-latitude permafrost region.

2 MATERIALS AND METHODS

2.1 Study sites and soil sampling

The Tibetan Plateau, with a mean elevation of ～4000 m, is the largest high-altitude permafrost region around the world, accounting for approximately three quarters of the Northern Hemisphere's mountain permafrost (Yang et al., 2010). The permafrost is distributed in the central Tibetan Plateau (Li & Cheng, 1996; Ran et al., 2012). Within this permafrost region, the Xidatan-Amdo transect along the Tibetan highway and the Gonghe-Qingshuihe transect along the Gonghe-Yushu highway traverse the permafrost areas, and are usually included in long-term permafrost monitoring scheme due to their representativeness and easy access (Jin, Chang, & Wang, 2007). In this study, we sampled ten permafrost sites along the two transects between July and August of 2013 (Figure 1, Table S1). These sites included three alpine steppes (KLSK, BDQ, and WDL), three alpine meadows (XDW, TGLS, and AD) and four swamp meadows (YY, CMH, HSX, and YSP) (Fig. S1, Table S1).

At each site, a 10 m × 10 m plot was established, plant species richness (SR) was counted in five 1 m × 1 m quadrates along a diagonal line of the plot, and aboveground biomass (AGB) within each quadrate was clipped at the ground level. AGB was oven-dried at 65°C for 48 h

and then weighed after being transported to the lab. Three replicate deep soil cores were collected along a diagonal line across the 10 m × 10 m plot, and drilled at depths of 0-10, 10-20, 20-30, 30-50, 50-70, 70-100, 100-150, 150-200, 200-250, and 250-300 cm using motorized coring equipment with a machine driven by diesel (Ding et al., 2016). The core sections (Fig. S2) were homogenized and stored in autoclaved bags, and transported on ice packs to the laboratory as soon as possible.

We assessed the ALT through the location of segregated ice in deep sediment cores. Based on the active layer thickness (ALT) in this study (Table S1) and the reported mean ALT (2.41 m) on the Tibetan Plateau (Pang et al., 2009; Wu et al., 2010), we selected the surface soil (0-10 cm) to represent the active layer and the deepest segment (2.5-3.0 m) of each core to represent the permafrost layer. Notably, the surface soil in the top 10 cm was selected to represent the active layer for easy comparison with previous studies (Steven et al., 2008; Yergeau et al., 2010), while permafrost from the same layer was chosen to eliminate difference from different soil depths (Eilers, Debenport, Anderson, & Fierer, 2012). To explore whether the vertical distance from the upper horizon of the permafrost to the 2.5 m could affect microbial communities in the permafrost layer, we conducted Mantel test and partial Mantel test using this vertical distance as a factor. Our analyses revealed that, although the distance was variable among various sampling sites, its effect on microbial communities was negligible (Table 1, Table S2).

To obtain the mean annual temperature (MAT) and mean annual precipitation (MAP) for each sampling site, we interpolated MAT and MAP with altitude as a covariant to account for the topographic effects through the Cokriging method (Chen et al., 2016c). We then retrieved the interpolated climate data for each sampling site at a spatial resolution of 10 × 10 km^2. The original data, including records from 61 weather stations on the plateau, were obtained from the China Meteorological Data Sharing Service System (http://cdc.nmic.cn/home.do). The interpolation analyses were performed using ArcMap 10.0 (Environmental Systems Research

Institute, Inc., Redlands, CA, USA).

2.2 Soil physical and chemical analyses

In the laboratory, roots and stones were removed from soil samples by hand, and the resulting samples were homogenized and sieved through a 2.0-mm mesh for physical and chemical analyses. Soil moisture was determined by oven drying 10 g of fresh soil at 105°C for 48 h. Soil texture was determined by a particle size analyzer (Malvern Masterizer 2000, UK) after removal of organic matter and calcium carbonates using 30% hydrogen peroxide and 30% hydrochloric acid, respectively. Soil pH was measured by suspending soil in 2M KCl with a soil:water ratio of 1:2.5 and then analyzed by a pH electrode (PB-10, Sartorius, Germany). Total carbon (TC) and total nitrogen (TN) content was determined on a milled sample by combustion at 950°C using an elemental analyzer (Vario EL III, Elementar, Germany). Soil inorganic carbon content was determined with a carbonate content analyzer (Eijkelkamp, Giesbeek, Netherlands). SOC content was calculated as TC minus inorganic carbon content, and soil carbon:nitrogen (C:N) ratios were calculated as the quotient of SOC and TN. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted with 0.5 M K_2SO_4 and measured with a multi-NC-analyzer (Analytik Jena, Germany). Total phosphorous (TP) was determined by acid-dissolved molybdenum, antimony, and scandium colorimetry.

2.3 DNA extraction, amplification and MiSeq sequencing

Soils for DNA extraction were stored at -80°C after being sieved through a 2.0-mm mesh in the lab. We extracted DNA from 0.25 g of each soil using the Fast DNA® SPIN Kit for Soil (Q-BIOgene, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted DNA was examined on a 1% agarose gel and quantified using a NanoDrop® ND-2000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For bacteria and archaea, the V4 region of the 16S rRNA gene was amplified using the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′)

(Bates et al., 2010). The thermal-cycling conditions were as follows: 94°C for 3 min, 35 cycles of 94°C for 45 s, 50°C for 60 s, and 72°C for 60 s, followed by 72°C for 10 min. For fungi, the primer set ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White, Bruns, Lee, & Taylor, 1990; Gardes & Bruns, 1993) was used to amplify the ITS1 region. PCR conditions were 3 min at 95°C, 35 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 30 s, followed by 72°C for 8 min. Each sample was amplified in triplicates and pooled prior to purification with the QIAEX Gel Extraction Kit (Qiagen, Valencia, CA, USA) and quantification with a Quant-iT dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA). The final PCR pools were created by combining equimolar ratios of amplicons from individual samples. The MiSeq V2 kit was used for 2 × 250 bp paired-end sequencing on a MiSeq machine (Illumina, San Diego, CA, USA) at the Chinese National Human Genome Center at Shanghai (http://www.chgc.sh.cn/).

2.4 Bioinformatic analysis

Raw reads without adapter from the MiSeq sequencer were assigned to different samples based on the barcodes (Table S3). Paired-end reads with at least a 50-bp overlap and <5% mismatches were combined using FLASH (version 1.0.0; Magoč & Salzberg, 2011). A threshold of average quality scores > 30 over 5-bp window size was used to trim the unqualified sequences using Btrim (version 1.0.0; Kong, 2011). Any joined sequences with ambiguous bases and lengths < 200 bp were discarded. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using UPARSE (version usearch v7.0.1001_i86linux64; Edgar et al., 2013) with the chimeras and all singletons being discarded meanwhile. We determined taxonomic annotations using the Ribosomal Database Project (RDP) Classifier (version 1.0.0) with a 50% confidence score (Wang, Garrity, Tiedje, & Cole, 2007). The 16S sequences were assigned using the Greengenes database (McDonald et al., 2011), while ITS sequences were assigned using the UNITE database (Abarenkov et al., 2010). To correct for sampling effects, we normalized all samples to an even number of sequences per sample (19,300 and 14,000 for 16S rDNA and ITS, respectively; Table S4) for

further analysis. The above-mentioned steps were performed using an in-house pipeline that was built on the Galaxy platform (http://mem.rcees.ac.cn:8080). The bacterial, archaeal and fungal DNA sequences have been deposited in the SRA of the NCBI database under the accession numbers SRR5527125 and SRR5527119, respectively.

2.5 Statistical analyses

Alpha diversity metrics including the Shannon-Wiener index and Simpson's diversity index were calculated using the "diversity" function in the vegan package (version 2.4-4; Oksanen et al., 2013) in the software R version 3.3.2 (R Development Core Team, 2015). A two-way ANOVA was performed to analyze the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on soil properties, microbial alpha diversity (number of OTUs, Shannon-Wiener index, and Simpson's diversity index) and the relative abundances of different microbial phyla. Pearson correlations were used to assess the associations between the microbial alpha diversity and environmental factors. A value of *P* less than 0.05 was considered to be statistically significant. We tested the heterogeneity of variances, and the original data were normalized using log-transformation or standardization prior to statistical analysis when necessary. These statistical analyses were performed using SPSS 20.0 (IBM Corporation, Armonk, NY, USA).

The bacterial, archaeal and fungal community structure was visualized by non-metric multidimensional scaling (NMDS) ordinations that were based on the Bray-Curtis dissimilarity matrices using the vegan package (Oksanen et al., 2013) in R software (R Development Core Team, 2015). To test the effects of horizon, ecosystem type, and their interactions on microbial Bray-Curtis dissimilarity, a two-way permutational multivariate analysis of variance (PerMANOVA) was performed using the adonis function in the vegan package (Oksanen et al., 2013) in R software (R Development Core Team, 2015). Mantel test was performed to assess the correlations of microbial beta diversity and environmental variables and geographic distance with the ecodist package (version 2.0.1; Goslee & Urban,

2007) in R software (R Development Core Team, 2015). During this analysis, the Bray–Curtis dissimilarity matrix was used for microbial community datasets, and the distances among samples were calculated on the basis of Euclidean dissimilarity for the environmental factors. To control covarying effects of various factors, partial Mantel test were performed using vegan package (Oksanen et al., 2013) in R software (R Development Core Team, 2015). Based on partial Mantel test, the significant environmental variables were selected to construct an environmental matrix for conducting variation partitioning analysis. The principal coordinates of neighbor matrices (PCNM) were calculated to reflect the spatial distance, and the most significant PCNM variables were selected by conducting forward selection procedures with 999 permutations using the pcnm function in the vegan package (Oksanen et al., 2013) in R software (R Development Core Team, 2015). Variation partitioning analysis was then performed to determine the relative importance of environmental variables and spatial distance in explaining the microbial community compositions by a redundancy analysis using the varpart function in the vegan package (Oksanen et al., 2013) in R software (R Development Core Team, 2015).

3 RESULTS

3.1 Differences in soil characteristics between the active and permafrost layers

Most soil parameters varied significantly between the two layers and between ecosystem types (Table S5, Table S6). SOC, TN, C:N ratio, sand content, DOC, and TDN in the active layer were significantly higher than those in the permafrost layer (all $P < 0.05$), whereas clay content showed the opposite trend ($P < 0.05$). There were no significant differences in TP, soil moisture, soil pH and silt content between the two layers (all $P > 0.05$). Pearson's correlation analysis showed that there were strong correlations among different environmental variables, and those correlations existed in both layers (Table S7).

3.2 Microbial alpha diversity and taxa in the active and permafrost layers

A total of 3,679,345 high-quality bacterial and archeal and 1,829,329 fungal sequences were

identified across all the samples (Table S4). Bacterial, archaeal and fungal sequences were clustered into 11,479 and 2,043 OTUs, respectively. Microbial alpha diversity including the number of OTUs, the Shannon-Wiener diversity, and Simpson's diversity index differed between horizons, and among alpine steppe, alpine meadow and swamp meadow (Figures 2-3, Table S8). Microbial alpha diversity in the active layer was significantly higher than in the permafrost layer ($P < 0.05$) with the exception of the fungal Shannon-Wiener index, which did not show any significant difference between the two layers ($P = 0.32$). Fungal Simpson's diversity index in the permafrost layer was significantly higher than that in the active layer ($P < 0.05$).

The dominant bacterial phyla in the active and permafrost layers were *Proteobacteria* (25.9% *vs.* 48.5%), *Actinobacteria* (25.0% *vs.* 25.4%), *Acidobacteria* (17.2% *vs.* 2.1%) and *Chloroflexi* (10.1% *vs.* 2.5%) (Figure 4a, Table S9). The *Proteobacteria* populations were dominated by *Alphaproteobacteria* in the active layer (14.1% of all sequences) and by *Gammaproteobacteria* in the permafrost layer (29.8% of all sequences) (Figure 4b). *Verrucomicrobia*, *Planctomycetes*, *Bacteroidetes*, *Nitrospirae*, *Gemmatimonadetes*, *Firmicutes* and *Crenarchaeota* were present at lower abundances in all the samples (Figure 4a, Table S9). Bacterial and archaeal communities showed significant differences at the phylum level between horizons and among ecosystem types (Table S10), including a higher relative abundance of *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Planctomycetes*, *Nitrospirae*, *Gemmatimonadetes* and *Crenarchaeota* in the active layer (all $P < 0.05$) and a higher relative abundance of *Proteobacteria* and *Firmicutes* in the permafrost layer (all $P < 0.05$).

The most abundant fungal phyla were *Ascomycota* (71.7% in the active layer *vs.* 69.0% in the permafrost layer) and *Basidiomycota* (19.0% in the active layer *vs.* 18.3% in the permafrost layer) (Figure 4c). Other phyla including *Chytridiomycota*, *Glomeromycota*, and *Zygomycota* accounted for only a minor fraction of the fungal community composition (Figure 4c, Table

S11). The influence of horizon was apparent on the fungal minor phyla including *Chytridiomycota*, *Zygomycota* and *Glomeromycota* (Table S12). For instance, the relative abundances of *Chytridiomycota* and *Zygomycota* in the active layer were significantly higher than those in the permafrost layer (all $P < 0.05$), whereas the relative abundance of *Glomeromycota* showed the opposite trend ($P < 0.05$). At the class level, fungal communities were dominated by *Sordariomycetes* in the active layer (44.0%) while by *Dothideomycetes* in the permafrost layer (32.0%) (Figure 4d).

3.3 Associations of bacterial, archaeal and fungal beta diversity with environmental variables and spatial distance

The non-metric multidimensional scaling (NMDS) showed that bacterial, archaeal and fungal community structures were significantly different between the active and permafrost layers (Figure 5, Table S13). Significant interactions of horizon and ecosystem type were also found for microbial community structures (all $P < 0.05$; Table S13). Both Mantel test and partial Mantel test indicated that spatial distance, AGB, SR, MAP, SOC, TN, C:N ratio, TP, pH, soil moisture, silt content, sand content, DOC and TDN significantly influenced bacterial and archaeal beta diversity in the active layer (all $P < 0.05$; Table 1, Table S2). By contrast, spatial distance, MAP, MAT, and TP influenced bacterial and archaeal beta diversity in the permafrost layer (all $P < 0.05$; Table 1, Table S2). In addition, soil fungal beta diversity was significantly influenced by environmental factors such as SOC, TN and DOC in the active layer, and by MAP and TP in the permafrost layer (all $P < 0.05$; Table 1, Table S2).

Partial Mantel test revealed that both environmental and geographic distances were positively correlated with bacterial and archaeal community dissimilarity in both layers (all $P < 0.05$; Table 2), indicating a significant distance-decay relationship. Variation partitioning analysis showed that environmental variables were the major contributor to bacterial and archaeal community dissimilarity, explaining 43% and 25.8% of the variation in the active and permafrost layers, respectively (Figure 6). Geographic distance did not exhibit any significant

correlation with fungal community dissimilarity in either the active layer or the permafrost layer (all $P > 0.05$; Table 2). By comparison, environmental variables were important predictors of fungal beta diversity in both layers (all $P < 0.05$; Table 2). These results indicated that contemporary environment plays a more important role than geographic distance in shaping the composition of fungal communities.

4 DISCUSSION

To our knowledge, this study provides a comprehensive comparison of patterns and drivers of microbial communities between the active layer and permafrost layer on the Tibetan Plateau. Our results indicated that the microbial alpha diversity in the active layer was higher than that in the permafrost layer with the exception of fungal Shannon-Wiener index and Simpson's diversity index. Soil bacterial, archaeal and fungal beta diversity was mainly determined by soil fertility (defined as SOC, DOC, and TN) in the active layer but by TP and MAP in the permafrost layer, partly supporting our first hypothesis that microbial beta diversity was mainly predicted by contemporary environment in the active layer whereas by spatial distance in the permafrost layer. Additionally, our results revealed that the beta diversity of bacterial and archaeal communities was explained by a combination of geographic and environmental distances, while fungal beta diversity in the permafrost layer was predicted by contemporary environment, supporting our second hypothesis that the driving factors of the beta diversity of bacterial, archaeal and fungal communities in the permafrost layer are different. At last, the dominant bacterial phylum was *Proteobacteria* in the high-altitude permafrost region, supporting our third hypothesis that bacterial communities are different between the high-altitude and high-latitude permafrost regions.

4.1 Significant difference in microbial alpha diversity and community structures between the active layer and permafrost layer

Our results revealed that microbial alpha diversity was significantly lower in the permafrost layer than in the active layer, indicating that permafrost is a less hospitable habit. Our results

also showed that the bacterial and fungal community structures were significantly different between the two layers. This result was supported by several studies which reported that microbial communities differed significantly between the active layer and permafrost layer on the Tibetan Plateau (Hu et al., 2014, 2015, 2016) and in the Arctic region (Steven et al., 2008; Mackelprang et al., 2011). The observed difference in microbial communities could be ascribed to the different environmental conditions between the two layers. While the active layer experiences larger environmental fluctuations, the permafrost layer is characterized by many restraining factors such as low temperature, limited oxygen content, low nutrient and water availability, low thermal energy, and high viscosity (Jansson & Tas, 2014). Only microbes (psychrotolerant or phsychrophilic) with structural and functional adaptations to the harsh environmental conditions are able to survive in the permafrost layer. For instance, microbes might have evolved multiple strategies such as entering a dormant state or producing specialized proteins to survive at subzero temperatures in permafrost (Jansson & Tas, 2014). Our results suggest that the different environment might cause niche separation, and subsequent differences in microbial communities between the two horizons. However, another study derived from European alpine permafrost reported that microbial alpha diversity (number of OTUs and Shannon-Wiener diversity) in the permafrost layer was higher than that in the active layer (Frey et al., 2016). This inconsistency suggests that differences in microbial alpha diversity between the active layer and permafrost layer are, to some degree, region specific.

4.2 Contrasting drivers of bacterial, archaeal and fungal beta diversity between the active layer and permafrost layer

Our results indicated that soil fertility (defined as SOC, DOC, and TN) was a major driver of bacterial, archaeal and fungal beta diversity in the active layer, suggesting that soil microbial communities in high-altitude permafrost region are limited by carbon. This finding is consistent with an earlier study which revealed that bacterial and fungal community compositions were correlated with carbon and nitrogen concentration in permafrost-affected

soils on the Tibetan Plateau (Zhang et al., 2014). This finding is also supported by previous observations derived from high-latitude permafrost regions (Sililiano et al., 2014). Soil fertility might provide the nutritional properties that allow the more appropriately adapted microbial taxa within a community to grow, dominate and exclude other members of the community (Siciliano et al., 2014). Soil fertility could also affect microbial communities through its effect on plant diversity, as microbes depend on litter and root exudates (Millard & Singh, 2010). Consistent with this assumption, a significant relationship was observed between bacterial and archaeal community compositions in the active layer and plant variables (AGB and SR) (Table 1, Table S2).

In contrast with the significant role of soil fertility in driving microbial beta diversity in the active layer, soil bacterial, archaeal and fungal beta diversity in the permafrost layer was mainly determined by TP and MAP. This finding indicates that the observed drivers of microbial beta diversity in the active layer did not hold true for microbial communities in the permafrost layer. Then, an interesting question arises why soil P regulates microbial communities in the permafrost layer. Such a pattern could be due to the potential P limitation for microbial growth (Liu, Gundersen, Zhang, & Mo, 2012). In support of this deduction, our results indicated that the C:P and N:P ratios in the permafrost layer were significantly higher than those in the active layer (all $P < 0.05$), implying that P is much less abundant than C and N in the permafrost layer. Moreover, previous studies reported that P addition exerted significant effects on microbial beta diversity in Tibetan alpine grasslands (He et al., 2016), indicating that P is an important soil nutrient regulating microbial communities in these ecosystems. Although soils store a large quantity of total P, the available P is rather limited on the Tibetan Plateau (He et al., 2016). The low P availability might alter the growth of certain microbial populations (Siciliano et al., 2014) and thus affect the microbial communities. In support of this deduction, further analysis showed significant correlations among TP and the relative abundance of different phyla such as *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes*, *Zygomycota* and *Chytridiomycota* (all $P < 0.05$;

Tables S14-15) and microbial alpha diversity (Tables S16-17) in the permafrost layer. Collectively, our study illustrates that P functions as a growth-limiting nutrient that is important in microbial community development. In addition to TP, MAP was another important factor in predicting microbial beta diversity in the permafrost layer. Precipitation may alleviate or aggravate substrate diffusion for permafrost microorganisms, as microbes possess semi-permeable membranes (Schimel, Balser, & Wallenstein, 2007). Precipitation may also indirectly affect permafrost microorganisms through P because there was significant correlation between these two factors in this layer ($P < 0.01$; Table S7). Notably, our study appears to be contradictory to several previous studies reporting that bacterial and fungal beta diversity in the permafrost layer was correlated with soil carbon and nitrogen content, and pH on the Tibetan Plateau (Hu et al., 2014, 2015, 2016). Such a difference may be due to the scale differences across studies: this study focused on the permafrost layer across multiple sampling sites over broad geographic scales, while Hu et al. (2014, 2015, 2016) were based on a 10 m deep soil core at one site.

Our results also illustrated that driving factors of microbial beta diversity in the permafrost layer differed between bacteria and fungi. To be specific, an obvious difference was that spatial distance significantly influenced bacterial but not fungal beta diversity. It has been reported that bacteria have limited capacity for long distance dispersal over broad geographic scales (Bowers, McLetchie, Knight, & Fierer, 2011; Wang et al., 2015; except Smith et al., 2013), partly due to burial and freezing in the permafrost layer. However, there was no significant effect of spatial distance on fungi, possibly due to unexpectedly efficient dispersal via a number of known agents, including wind (Egan, Li, & Klironomos, 2014), birds and human activities (Davison et al., 2015). Given that many fungal species can form spores (Smith & Read, 2008), they might form a propagule bank to efficiently exploit even harsh environmental conditions (Davison et al., 2015). Consistent with this assumption, we observed that the relative abundance of *Glomeromycota*, which generally form large spores, was significant higher in the permafrost layer than in the active layer (Figure 4).

Our results further demonstrated that the active layer thickness (ALT) was another driving factor that differed between bacteria, archaea and fungi in the permafrost layer. ALT, which is equivalent to the distance between the soil surface and the permafrost table, was a significant factor determining bacterial and archaeal but not fungal beta diversity (Tables S2), indicating that effect of ALT cannot be neglectable in the permafrost layer. The permafrost table, acting as a physical and biogeochemical barrier, can limit the infiltration of external environmental variables, surface water and the vertical movement of microbial species (Steven et al., 2008). ALT may provide a good reflection of MAT across the sampling sites, as there were strong correlations between ALT and MAT (Table S7), which was an important predictor of bacterial and archaeal but not fungal beta diversity in the permafrost layer (Table S2). MAT plays a predominant role in shaping variations in microbial diversity over broad geographic scales mainly through altering the rates of metabolism and growth, ecosystem productivity (Zhou et al., 2016).

4.3 Comparisons of microbial communities between the high-altitude and high-latitude permafrost regions

Our results showed that bacterial communities on the Tibetan Plateau were dominated by *Proteobacteria*, which was contradictory to previous findings that permafrost bacterial communities were dominated by *Actinobacteria* (Yergeau et al., 2010; Wilhelm et al., 2011), Bacteroidetes (Deng et al., 2015) in high-latitude permafrost region with relatively high carbon. Our results were also partly inconsistent with several studies which reported that bacterial communities were dominated by *Actinobacteria* (Goordial et al., 2017) or by *Proteobacteria* (Blanco et al., 2012; Rivkina et al., 2016) in high-latitude region with relatively low carbon. These discrepancies may be ascribed to the differences in water content, carbon and nitrogen content, and carbon lability between the high-altitude and high-latitude permafrost regions. For instance, compared with the high-latitude permafrost region, the high-altitude permafrost region is characterized by lower ice content, carbon and

nitrogen content, because of the higher evaporation rates and temperature but lower vegetation productivity (Ding et al., 2016). Moreover, it is well known that the vegetation is dominated by graminoids and sedges in our study area (Chinese Academy of Sciences, 2001) while by dwarf shrubs, mosses and coniferous trees in high-latitude areas (Walker et al., 2005), leading to higher carbon lability of herbaceous litter than shrub and moss litter (Chen et al., 2016a). These differences could then contribute to the observed differences in bacterial community compositions. Nevertheless, these discrepancies could also be partly induced by methodological differences. DNA extraction (Vishnivetskaya et al., 2014), primer specificity, PCR amplification, Illumnia sequencing and downstream analysis could produce unequal read counts and stochastic differences in the relative abundances of different microbial phyla (Frey et al., 2016). Consequently, methodological differences can also lead to potential discrepancies in microbial community composition among different studies.

In summary, this study provides insights into the distribution patterns and drivers of microbial communities over broad geographic scales, and improves our understanding of microbial ecology in permafrost regions. Our results revealed significant differences in microbial alpha diversity and microbial community structures between the active layer and permafrost layer. These findings suggest that the distinctness in environmental conditions between the two horizons have profound influences on microbial niche differentiation, and further imply that the microbe-driven ecosystem processes would be different between the two horizons in changing environments. Our results also demonstrated that the driving factors differed between the active layer and the permafrost layer as well as between bacteria, archaea and fungi in the permafrost layer. These findings imply that different variables should be used to predict microbial distributions in different layers, and in different microbial groups using species distribution models, and further illustrate the importance of considering soil layers and microbial groups when predicting microbial responses to environmental changes in permafrost-affected regions. Finally, our results demonstrated that the high-altitude permafrost region harbored different dominant bacterial phylum compared with

the high-latitude permafrost region, highlighting the necessity of studying both the high-altitude and high-latitude permafrost regions to gain a complete understanding on permafrost microbial ecology.

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DATA ACCESSIBILITY

The bacterial and archaeal, and fungal DNA sequences have been deposited in the SRA of the NCBI database under the accession numbers SRR5527125 and SRR5527119, respectively. The OTU tables are uploaded in Dryad with doi:10.5061/dryad.63474.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Y.Y. and Y.L. designed the study; J.Z. F.L. and Y.Y. performed samplings; Y.L., Y.D., H.W., T.L., G.B. and Y.Y. performed the experiment, analyzed data and wrote the manuscript.

TABLE 1 Relationships of bacterial, archaeal and fungal community compositions with environmental variables in the active layer and permafrost layer as revealed by Mantel test. Values in bold indicate significant correlations ($P < 0.05$).

Factors	Bacteria and archaea				Fungi			
	Active layer		Permafrost layer		Active layer		Permafrost layer	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Spatial distance (km)	0.49	**0.001**	0.39	**0.001**	0.022	0.630	0.14	**0.008**
AGB (g m^{-2})	0.33	**0.001**	0.016	0.847	0.11	0.485	0.069	0.497

SR	0.47	**0.001**	0.12	0.113	0.093	0.518	-0.025	0.792
MAT (^{o}C)	0.029	0.623	0.25	**0.001**	-0.096	0.319	0.028	0.703
MAP (mm)	0.42	**0.001**	0.34	**0.001**	0.015	0.863	0.29	**0.001**
SOC (%)	0.72	**0.001**	0.14	0.071	0.33	**0.006**	0.21	**0.037**
TN (%)	0.72	**0.001**	0.15	0.062	0.37	**0.003**	0.071	0.483
C:N ratio	0.43	**0.001**	0.025	0.738	0.25	**0.013**	0.086	0.360
TP (mg g^{-1})	0.55	**0.001**	0.25	**0.002**	0.17	0.095	0.27	**0.004**
pH	0.68	**0.001**	-0.050	0.507	0.20	0.069	0.0020	0.975
Soil moisture (%)	0.66	**0.001**	0.12	0.81	0.23	**0.025**	-0.0010	0.990
Clay content (%)	0.27	**0.001**	-0.038	0.703	0.047	0.654	-0.017	0.862
Silt content (%)	0.38	**0.001**	0.11	0.126	0.13	0.101	0.096	0.296
Sand content (%)	0.38	**0.001**	0.11	0.144	0.13	0.102	0.089	0.354
DOC (mg kg^{-1})	0.37	**0.001**	0.20	**0.010**	0.30	**0.024**	0.14	0.122
TDN (mg kg^{-1})	0.42	**0.001**	0.16	0.057	0.13	0.412	0.099	0.391
ALT (m)	-	-	0.27	**0.001**	-	-	0.072	0.241
Vertical distance (m)	-	-	0.27	**0.001**	0.072	0.243	-	-

AGB, aboveground biomass; SR, plant species richness; MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen; C:N ratio, the ratio of SOC to TN; TP, total phosphorus; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; ALT, active layer thickness; Vertical distance, distance from the upper horizon of the permafrost to the 2.5 m.

TABLE 2 Relationships among bacterial, archaeal and fungal community dissimilarity and environmental variables and geographic distance as revealed by partial Mantel test. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Factors	Control for	r_M in the active layer	r_M in the permafrost layer
Bacteria and archaea			
Geographic distance	Environmental distance	0.26**	0.26**
Environmental distance	Geographic distance	0.57***	0.25**
Fungi			
Geographic distance	Environmental distance	-0.12	0.02
Environmental distance	Geographic distance	0.24*	0.23**

FIGURE LEGENDS

FIGURE 1 Map of the sampling sites along two transects on the Tibetan Plateau. The background map shows the distribution of permafrost on the Tibetan Plateau, which was derived from Li & Cheng (1996). AS, alpine steppe; AM, alpine meadow; SM, swamp meadow; YY, youyun; CMH, changmahe; HSX, huashixia; XDW, xiadawu; KLSK, kunlunshankou; BDQ, budongquan; WDL, wudaoliang; TGLS, tanggulashan; YSP, yanshiping; AD, anduo.

FIGURE 2 Differences in bacterial and archaeal alpha diversity between the active and permafrost layers. (a) Number of OTUs, (b) Shannon-Wiener index, and (c) Simpson's diversity index. All data are presented as the mean ± standard error. Asterisks indicate that a horizon has significant influence (*** indicate $P < 0.001$; ** indicate $P < 0.01$; * indicate $P < 0.05$; ns, not significant). AS, alpine steppe; AM,

alpine meadow; SM, swamp meadow.

FIGURE 3 Differences in fungal alpha diversity between the active and permafrost layers. (a) Number of OTUs, (b) Shannon-Wiener index, and (c) Simpson's diversity index. All data are presented as the mean ± standard error. Asterisks indicate that a horizon has significant influence (*** indicate $P < 0.001$; ** indicate $P < 0.01$; * indicate $P < 0.05$; ns, not significant). AS, alpine steppe; AM, alpine meadow; SM, swamp meadow.

FIGURE 4 The relative abundance of dominant bacterial and archaeal (left panel) and fungal (right panel) taxa in the active and permafrost layers. (a) Relative abundance of bacterial and archaeal phyla (%), (b) Relative abundance of *Proteobacteria* (%), (c) Relative abundance of fungal phyla (%), and (d) Relative abundance of fungal classes. "Others" include phyla with a relative abundance < 2.0% on the phylum level for bacteria and archaea, and < 1% on the class level for fungi.

FIGURE 5 Non-metric multidimensional scaling (NMDS) analysis of bacterial and archaeal (a) and fungal (b) community structures in the active and permafrost layers.

FIGURE 6 Variation partitioning analysis showing the percentages of variance in bacterial and archaeal communities explained by the principal coordinates of neighbor matrices (PCNM) and environmental variables in the active layer (a) and permafrost layer (b).

FIGURE 1

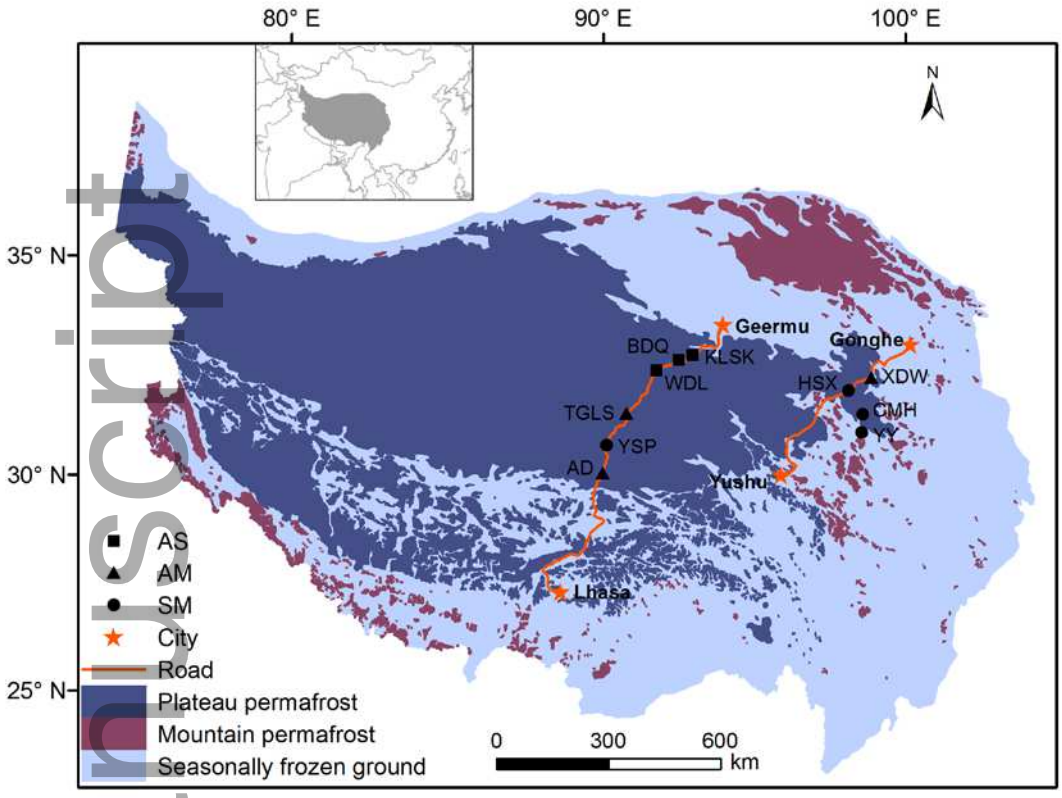


FIGURE 2

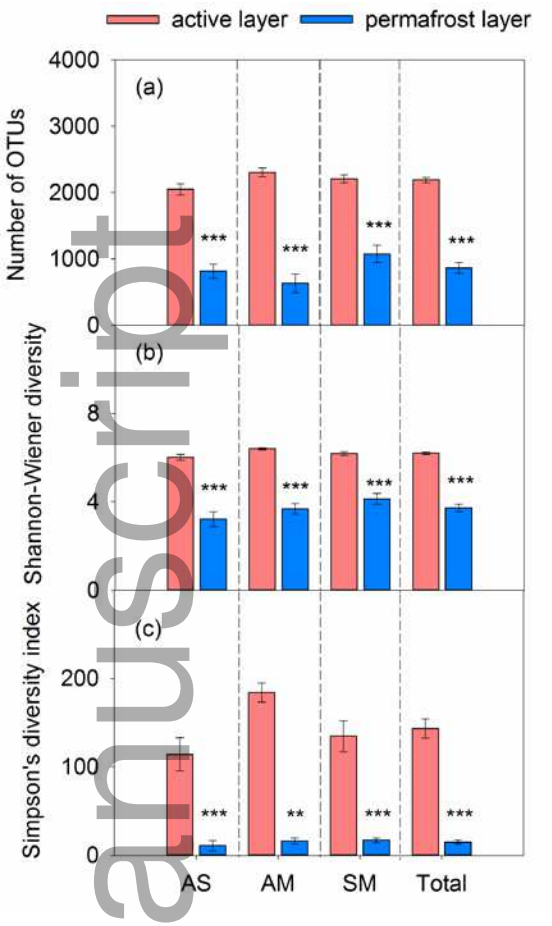


FIGURE 3

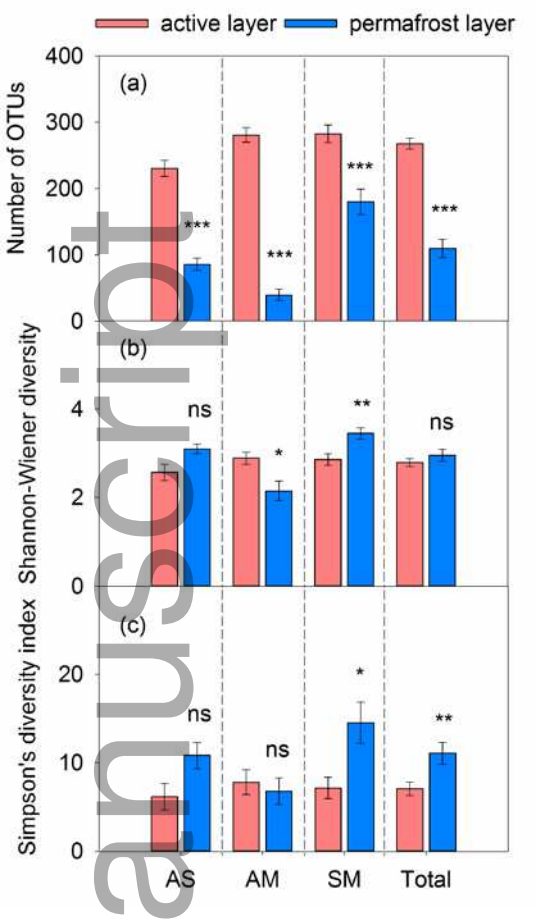


FIGURE 4

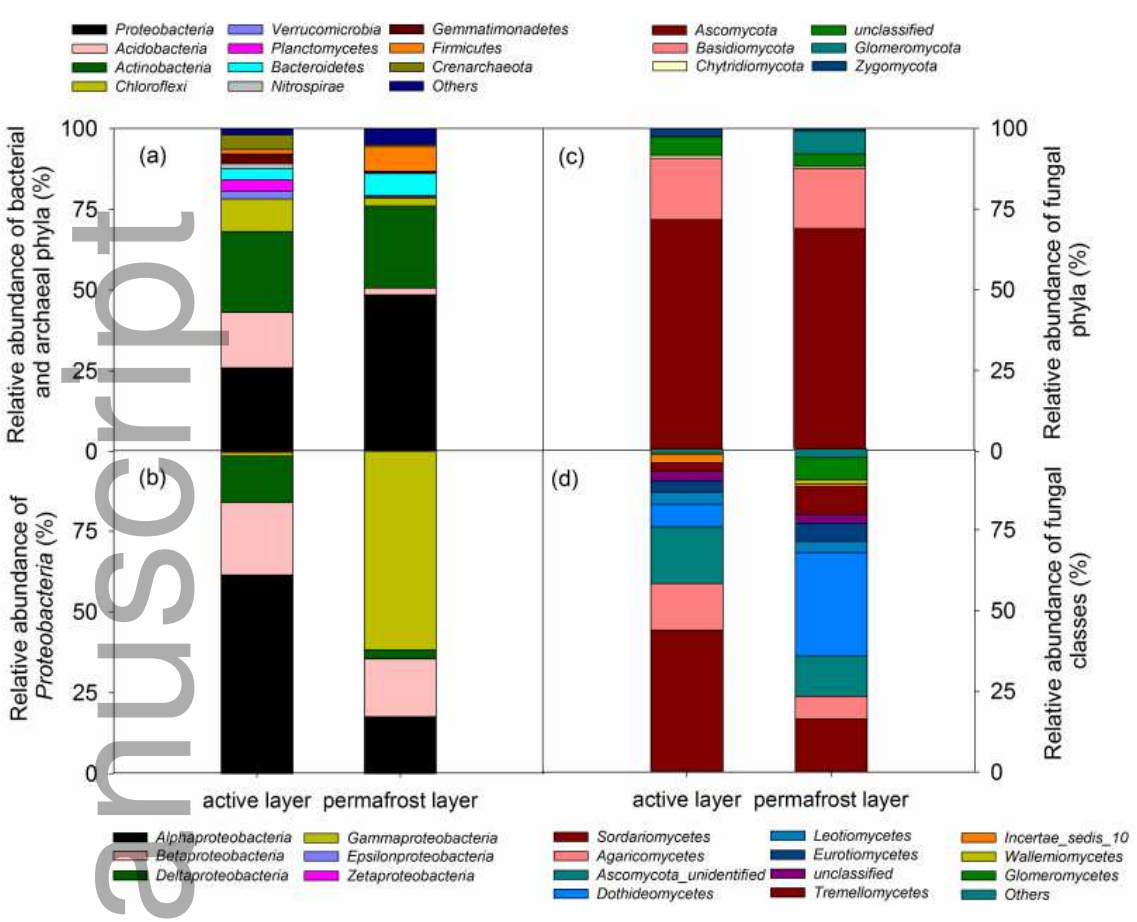


FIGURE 5

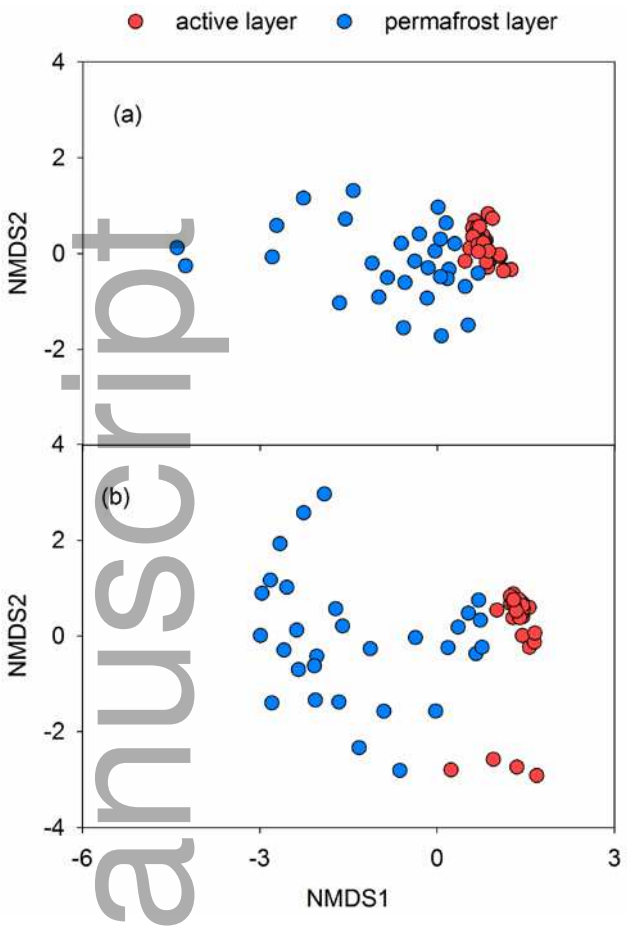
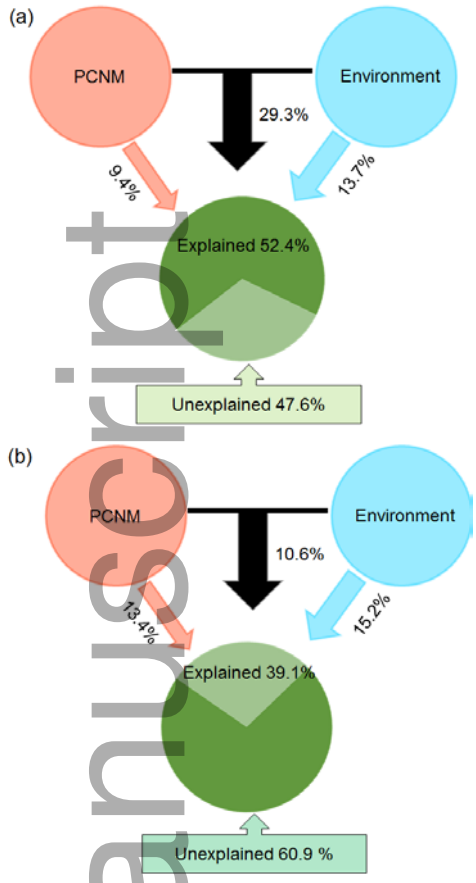


FIGURE 6



SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1 Locations and characteristics of the ten permafrost sites on the Tibetan Plateau.

Table S2 Relationships of bacterial, archaeal and fungal beta diversity with environmental variables for the active and permafrost layers, respectively. These analyses are based on partial Mantel test. Values in bold indicate significant correlations ($P < 0.05$).

Table S3 The barcodes used to identify samples within the pooled sequencing run.

Table S4 The number of microbial sequences for each sample, which were obtained by FLASH and UPARSE. More details could be found in the Materials and methods section.

Table S5 Soil parameters in the active and permafrost layers. Values for horizons at individual sites are the mean values of the three replicated soil cores (mean ± standard error). Values for overall means are averages of the 30 replicated soil cores

(mean ± standard error).

Table S6 Results of two-way ANOVAs showing the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on soil parameters for the active and permafrost layers, respectively. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S7 Pearson correlation coefficient (r) among different environmental variables for the active and permafrost layers, respectively. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S8 Results of two-way ANOVAs showing the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on microbial alpha diversity. Asterisks indicate significant influence (*** indicates $P < 0.001$, ** indicates $P < 0.01$; * indicates $P < 0.05$).

Table S9 The relative abundances (%) of bacterial and archaeal phyla in the active and permafrost layers. Values at individual sites are mean values of three replicated soil cores (mean ± standard error). Values for overall means are the averages of the 30 replicated soil cores (mean ± standard error).

Table S10 Results of two-way ANOVAs showing the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on the relative abundances of bacterial and archaeal phyla. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S11 The relative abundances (%) of fungal phyla in the active and permafrost layers. Values at individual sites are mean values of three replicated soil cores (mean ± standard error). Values for overall means are averages of the 30 replicated soil cores (mean ± standard error).

Table S12 Results of two-way ANOVAs showing the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on the relative abundances of fungal phyla. Asterisks

indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S13 Two-way permutational multivariate analysis of variance (PerMANOVA) showing the effects of horizon (active layer and permafrost layer), ecosystem type (alpine steppe, alpine meadow and swamp meadow), and their interactions on microbial community structures. Asterisks indicate significant influence (*** indicates $P < 0.001$, ** indicates $P < 0.01$; * indicates $P < 0.05$).

Table S14 Pearson correlation coefficient (*r*) between dominant bacterial and archaeal phyla and environmental variables for the active and permafrost layers, respectively. Asterisks indicate significant correlation (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S15 Pearson correlation coefficient (*r*) between dominant fungal phyla and environmental variables for the active and permafrost layers, respectively. Asterisks indicate significant correlation (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S16 Pearson correlation coefficient (*r*) between bacterial and archaeal alpha diversity and environmental variables for the active and permafrost layers, respectively. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Table S17 Pearson correlation coefficient (*r*) between fungal alpha diversity and environmental variables for the active and permafrost layers, respectively. Asterisks indicate significant influence (*** indicate $P < 0.001$, ** indicate $P < 0.01$; * indicate $P < 0.05$).

Fig. S1 The three different ecosystem types in permafrost-affected region on the Tibetan Plateau. (a) alpine steppe, (b) alpine meadow, and (c) swamp meadow.

Fig. S2 The divided cores during soil sampling in permafrost-affected region on the Tibetan Plateau.

TABLE 1 Relationships of bacterial, archaeal and fungal community compositions with environmental variables in the active layer and permafrost layer as revealed by Mantel test. Values in bold indicate significant correlations ($P < 0.05$).

Factors	Bacteria and archaea				Fungi			
	Active layer		Permafrost layer		Active layer		Permafrost layer	
	r	P	r	P	r	P	r	P
Spatial distance (km)	0.49	**0.001**	0.39	**0.001**	0.022	0.630	0.14	**0.008**
AGB (g m^{-2})	0.33	**0.001**	0.016	0.847	0.11	0.485	0.069	0.497
SR	0.47	**0.001**	0.12	0.113	0.093	0.518	-0.025	0.792
MAT (^{o}C)	0.029	0.623	0.25	**0.001**	-0.096	0.319	0.028	0.703
MAP (mm)	0.42	**0.001**	0.34	**0.001**	0.015	0.863	0.29	**0.001**
SOC (%)	0.72	**0.001**	0.14	0.071	0.33	**0.006**	0.21	**0.037**
TN (%)	0.72	**0.001**	0.15	0.062	0.37	**0.003**	0.071	0.483
C:N ratio	0.43	**0.001**	0.025	0.738	0.25	**0.013**	0.086	0.360
TP (mg g^{-1})	0.55	**0.001**	0.25	**0.002**	0.17	0.095	0.27	**0.004**
pH	0.68	**0.001**	-0.050	0.507	0.20	0.069	0.0020	0.975
Soil moisture (%)	0.66	**0.001**	0.12	0.81	0.23	**0.025**	-0.0010	0.990
Clay content (%)	0.27	**0.001**	-0.038	0.703	0.047	0.654	-0.017	0.862
Silt content (%)	0.38	**0.001**	0.11	0.126	0.13	0.101	0.096	0.296
Sand content (%)	0.38	**0.001**	0.11	0.144	0.13	0.102	0.089	0.354
DOC (mg kg^{-1})	0.37	**0.001**	0.20	**0.010**	0.30	**0.024**	0.14	0.122
TDN (mg kg^{-1})	0.42	**0.001**	0.16	0.057	0.13	0.412	0.099	0.391
ALT (m)	-	-	0.27	**0.001**	-	-	0.072	0.241
Vertical distance (m)	-	-	0.27	**0.001**	0.072	0.243	-	-

AGB, aboveground biomass; SR, plant species richness; MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen; C:N ratio, the ratio of SOC to TN; TP, total phosphorus; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; ALT, active layer thickness; Vertical distance, distance from the upper horizon of the permafrost to the 2.5 m.

TABLE 2 Relationships among bacterial, archaeal and fungal community dissimilarity and environmental variables and geographic distance as revealed by partial Mantel test. Asterisks indicate significant influence (*** indicate P < 0.001, ** indicate P < 0.01; * indicate P < 0.05).

Factors	Control for	r_M in the active layer	r_M in the permafrost layer
Bacteria and archaea			
Geographic distance	Environmental distance	0.26**	0.26**
Environmental distance	Geographic distance	0.57***	0.25**
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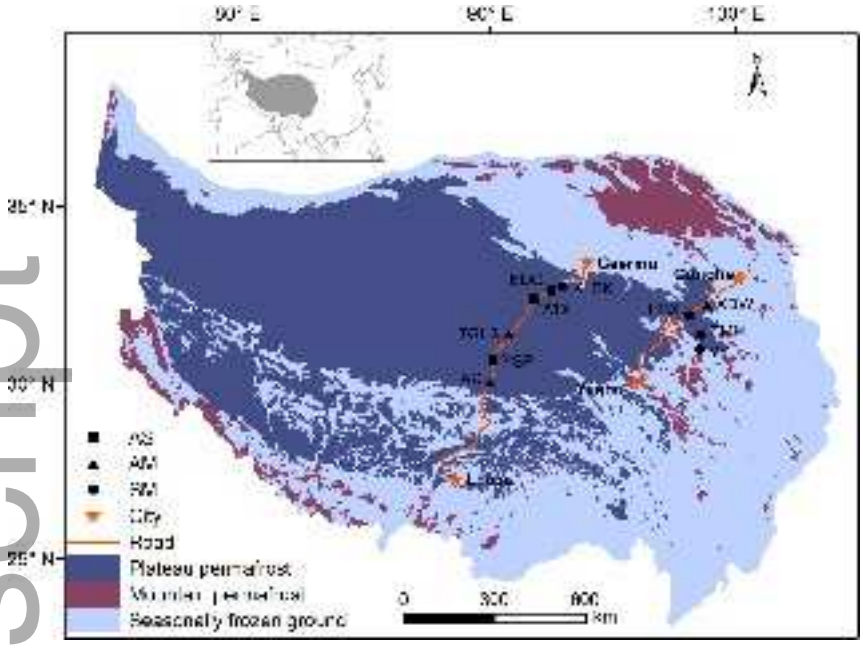
Geographic distance	Environmental distance	-0.12	0.02
Environmental distance	Geographic distance	0.24*	0.23**



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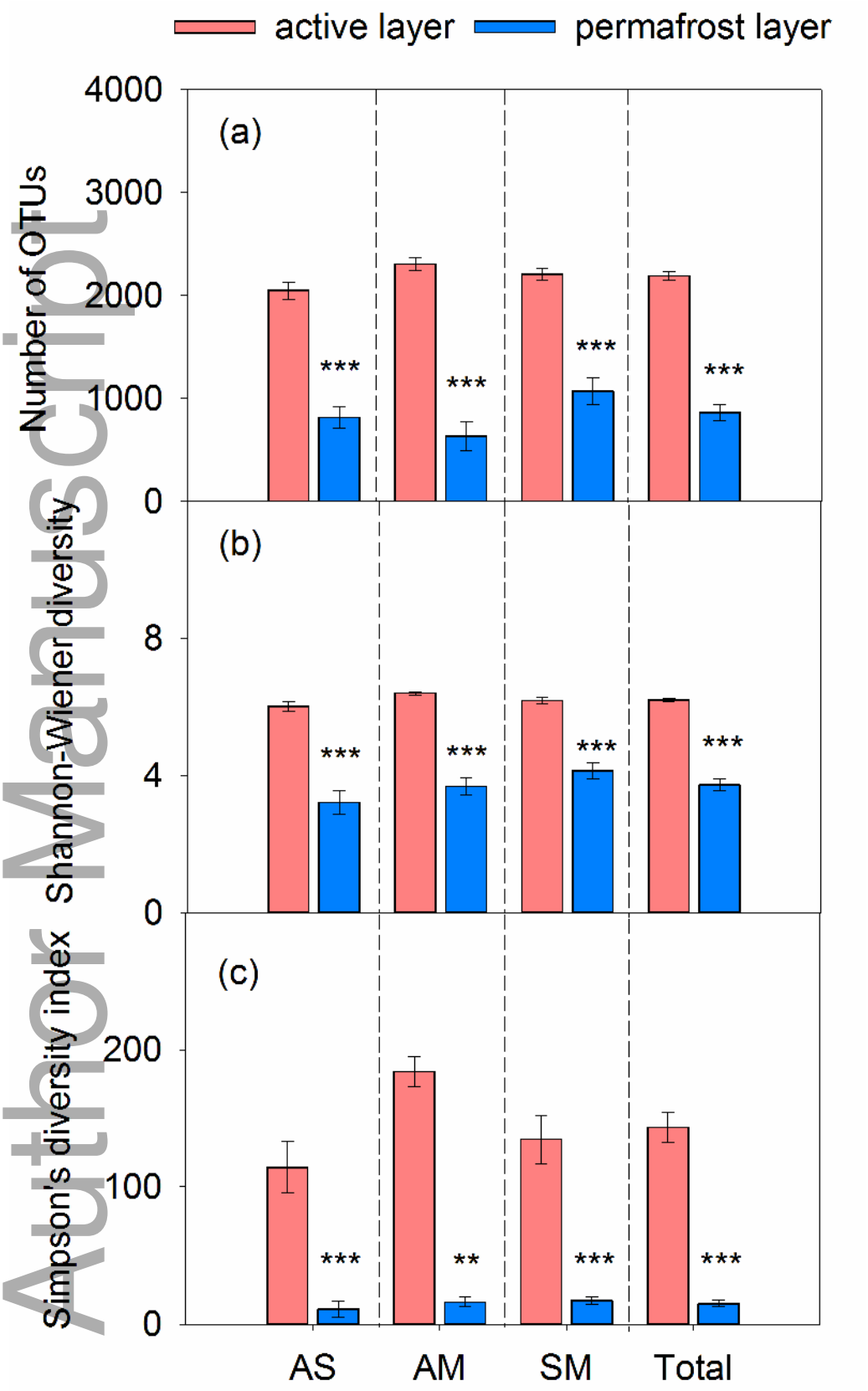

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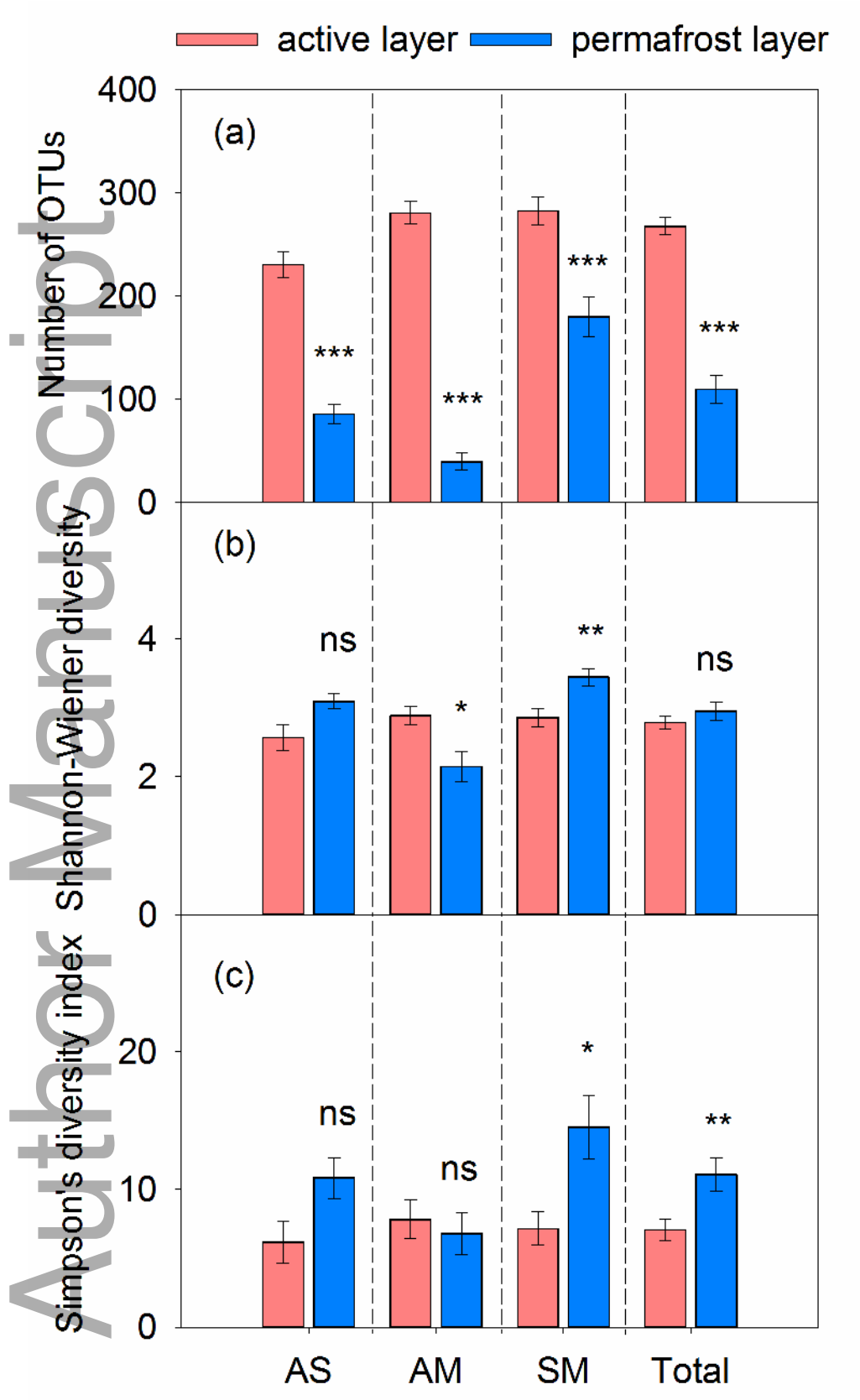

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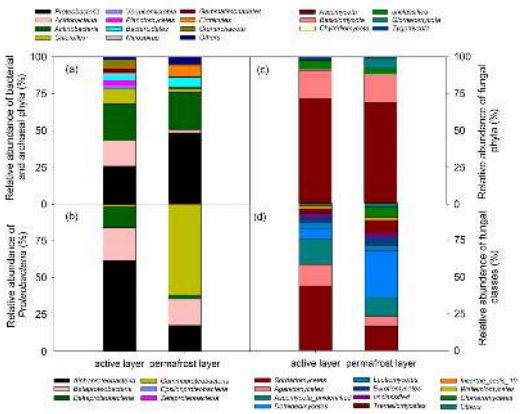

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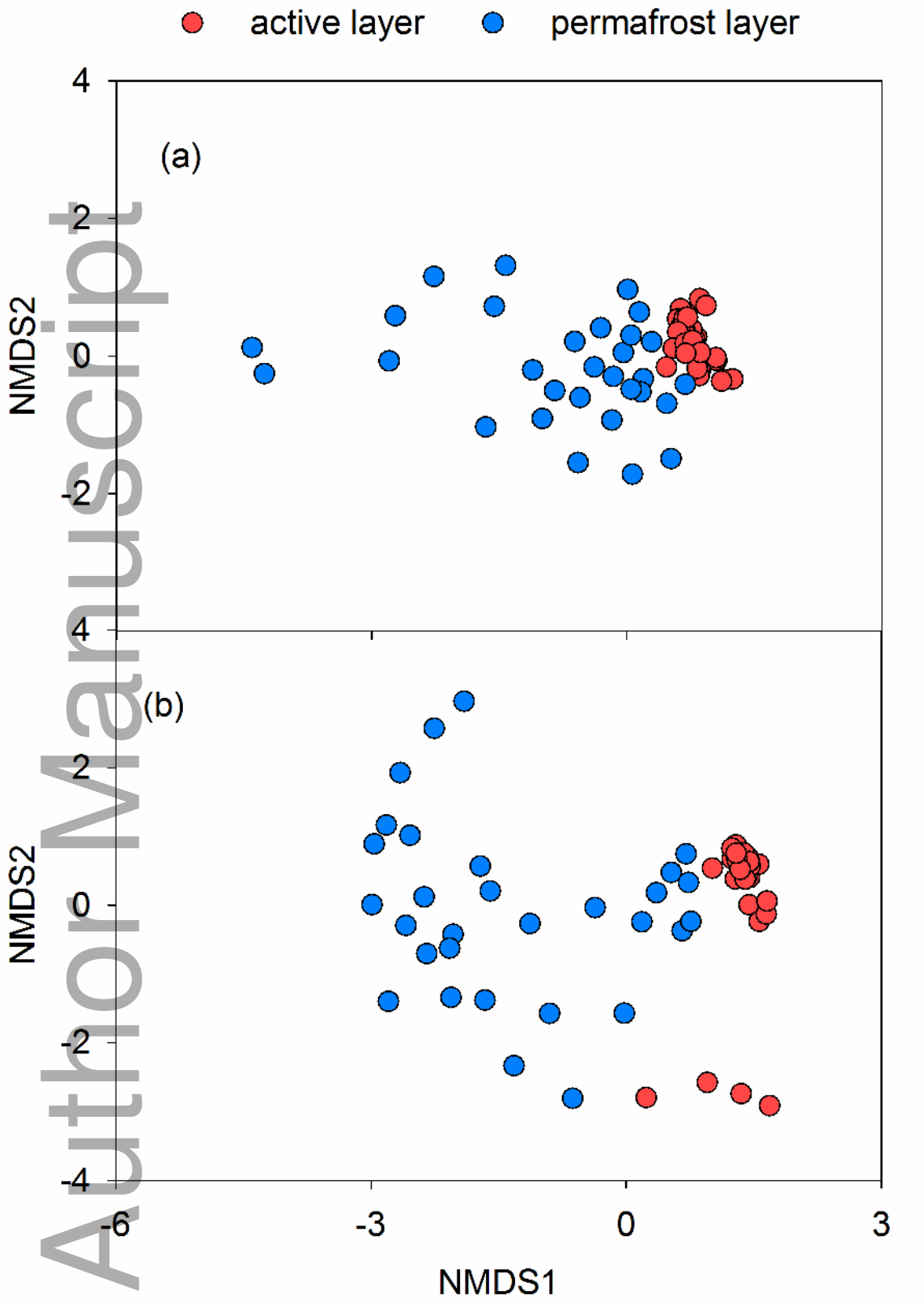

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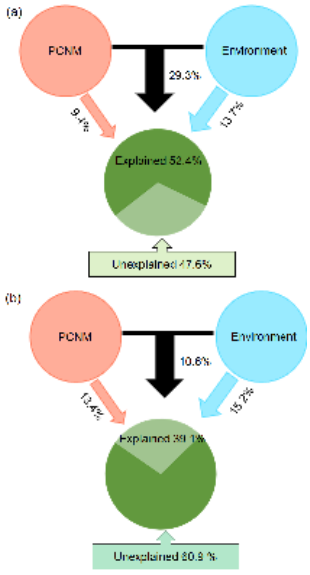

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