Compositional Shifts and Co-occurrence Patterns of Topsoil Bacteria and Micro-Eukaryotes Across a Permafrost Thaw Gradient in Alpine Meadows of the Qilian Mountains, China

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Abstract: Soil microorganisms are pivotal in the biogeochemical cycles of alpine meadow ecosystems affected by permafrost thaw. However, the mechanisms driving microbial community responses to environmental changes, such as variations in permafrost active layer thickness (ALT), are poorly understood. This study utilizes next-generation sequencing to explore the composition and co-occurrence patterns of bacterial and micro-eukaryotic communities in soils along a permafrost thaw gradient. Our findings revealed a decrease in alpha diversity within bacterial communities along the permafrost thaw gradient, while micro-eukaryotic community exhibited an increase. Although shifts were observed in the composition of these communities in both permafrost and seasonally frozen soils, these variations were not statistically significant. Bacterial communities differed more significantly between frozen soil types than within them, a pattern not observed in eukaryotic communities. LEfSe analysis identified more potential biomarkers in bacterial communities than in micro-eukaryotic ones. Furthermore, bacterial co-occurrence networks showed more nodes, edges, and positive linkages compared to those of micro-eukaryotes. Soil texture, ALT, and bulk density significantly influenced bacterial community structures, particularly affecting the abundance of Acidobacteria, Proteobacteria, and Actinobacteria phyla. Conversely, fungal communities (including *Nucletmycea*, *Rhizaria*, *Chloroplastida*, and *Discosea* groups) were more influenced by electrical conductivity, vegetation coverage, and ALT. This study underscores the differential responses of soil bacteria and micro-eukaryotes to permafrost thaw, highlighting implications for microbial community stability under global climate change.

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Importance: This study sheds light on how permafrost thaw affects microbial life in the soil, which has broader implications for our understanding of climate change impacts. As permafrost degrades, it alters the types and numbers of microbes in the soil. These microbes play essential roles in environmental processes, such as nutrient cycling and greenhouse gas emissions. By observing shifts from bacteria-dominated to fungi-dominated communities as permafrost thaws, the study highlights potential changes in these processes. Importantly, this research suggests that the stability of microbial networks decreases with permafrost degradation, potentially disrupting the delicate balance of these ecosystems. The findings not only deepen our understanding of microbial responses to changing climates but also support the development of strategies to monitor and perhaps mitigate the effects of climate change on fragile high-altitude ecosystems.

Key words: Permafrost degradation; Microbial communities; Compositional changes; Co-occurrence networks; Environmental factors; Qilian Mountain area

Introduction

Covering approximately 18% of the Northern Hemisphere**(Zhang et al., 2000; Gruber, 2012)**, permafrost—soil or rock that remains at or below 0°C for at least two consecutive years **(Washburn, 1980)** —is critical in global carbon cycling due to its storage of an estimated 15% of the world's soil carbon**(Post et al., 1982)**. Recent measurements indicate a significant warming of global permafrost by 0.29 ± 0.12 ℃ **(Biskaborn et al., 2019)**, contributing to its rapid degradation characterized by diminishing extent, reduced thickness, and deepening of the seasonally thawed active layer **(Cheng and Wu, 2007; Stendel and Christensen, 2002; Yang et al., 2010)**. These changes disrupt regional thermo-hydrological processes and release stored soil organic carbon, thus affecting ecosystem functions.

Microorganisms within permafrost regions are pivotal to decomposition and mineralization, significantly influencing biogeochemical cycles **(Chu et al., 2011; Männistö et al., 2007)**. The microbial communities swiftly react to permafrost thaw **(Mackelprang et al., 2011)**, transforming the ecosystem from a carbon sinks to a carbon source as they produce greenhouse gases like carbon dioxide and methane through enhanced decomposition activities **(Dutta et al., 2006)**. This transformation is accompanied by an increase in transcriptional activities and methane output **(Schuur and Abbott, 2011)**. As global warming progresses, permafrost degradation modifies

heat and nutrient dynamics within the active layer, affecting the microbial community structure, phylogeny, functional genes, and metabolic pathways **(Wang et al., 2002)**. The thawing process makes previously locked soil organic carbon bioavailable, intensifying microbial metabolism and accelerating organic matter breakdown, thereby augmenting greenhouse gas emissions **(Jansson and Hofmockel, 2020)**. Understanding these microbial dynamics is essential for predicting responses of permafrost ecosystem to climatic changes.

The Qinghai-Tibet Plateau, often dubbed the "Earth's Third Pole" and "Asia's Water Tower", hosts the most extensive distribution of permafrost at low and middle latitudes. This permafrost is notably warmer and thinner than its high-latitude counterparts in North America and Russia, rendering it particularly susceptible to climate change. At the beginning of the 21st century, the Qinghai-Tibet Plateau's permafrost spanned approximately 1.5 million km^2, representing 69.8% of China's total permafrost area **(Zhao et al., 2000; Wang et al., 2008; Wu et al., 2012)**. Recent studies indicate a significant reduction in permafrost coverage, declining by 66,000 km^2 per decade from 1980 to 2010 **(Wang et al., 2019)**. The Qilian Mountains, located along the plateau's northern edge, are acutely responsive to climatic fluctuations, making them a critical area for studying permafrost ecosystem responses to global warming. Research in this region has focused on soil microbial community dynamics, influenced by variables such as elevation gradients **(Duan et al., 2021; Xu et al., 2014)** and vegetation types **(Chen et al., 2019; Zhao et al., 2021)**. Key soil physiochemical properties, including moisture, pH, and organic carbon content, have been linked to shifts in microbial community structure **(Feng et al., 2020; Li et al., 2021)**. Additionally, environmental parameters, such as soil temperature, moisture, vegetation coverage, and carbon storage have experienced significant seasonal and annual fluctuations **(Lawrence et al., 2008; Schuur et al., 2009)**. These changes are found to distinctly influence bacterial and micro-eukaryotic communities, predominantly through variations in the carbon/nitrogen ratio and soil moisture **(Zhang et al., 2014)**, underscoring the complex interplay of environmental factors and microbial responses in the face of climate change.

Despite extensive studies on soil microbial dynamics across environmental gradients, the compositional shifts of microbial communities within permafrost soils, particularly along thaw gradients, remain poorly understood **(Zhang et al., 2007; Zhang et al., 2014)**. This uncertainty restricts our capacity to fully assess the effects

of climate change on the stability of permafrost ecosystem. Previous research has highlighted the distinct microbial phyla associated with different types of frozen soils; however, the extent to which these communities shift due to permafrost degradation is not well-defined **(Wilhelm et al., 2011; Zhang et al., 2017)**.

Addressing this gap, our study focuses on the response of microbial communities to permafrost thaw within the Qilian Mountains, employing a space-for-time substitution approach to simulate future climate scenarios. This method allows us to explore potential relationships between microbial dynamics and the thickness of the active layer (ALT), a critical factor in permafrost studies. The specific objectives of our research are to: (i) Evaluate the alpha diversity and compositional shifts of bacterial and micro-eukaryotic communities in permafrost and seasonally frozen soils; (ii) Analyze co-occurrence patterns and determine the relative importance of various bacterial and micro-eukaryotic taxa in shaping microbial networks within permafrost ecosystems; (iii) Investigate the key environmental factors influencing microbial diversity and community structure across different stages of permafrost thaw. By examining these aspects, our research aims to deepen the understanding of how soil microbial communities adapt to changing permafrost conditions and contribute to biogeochemical cycling in alpine meadow ecosystems. This insight is crucial for predicting and managing the impacts of climate change on permafrost region globally.

1 Materials and methods

1.1 Study sites and soil sample collection

The Heihe River basin, located on the northeastern edge of the Qilian Mountains, is recognized as the second-largest inland river system in China and is currently experiencing significant climatic warming **(Huai et al., 2014; Guo et al., 2015)**. In August 2019, a comprehensive soil sampling campaign was conducted at eight strategic sites along the upper reaches of the Heihe River, spanning geographic coordinates from 98°40′E to 99°20′E and from 38°30′N to 38°60′N (Fig. 1). These sites are situated at altitudes ranging from 3400 to 4700 meters above sea level, with the mean annual precipitation (MAP) varying from 200 to 500 mm **(Wu et al., 2015)** and mean annual temperature (MAT) ranging from -2.5 to -0.3 ℃and **(Li et al., 2018)**. Diverging from the typical high-latitude permafrost, the Qilian Mountains' permafrost exhibits greater variability in environmental factors affecting its stability, with ground temperature being a critical indicator of permafrost degradation in the region **(Cheng**

and Wang, 1982). Based on established classifications, soil samples were delineated as permafrost (sites N1, N2, N3, N4, and N5) and seasonally frozen soils (sites L1, L2, and L3) **(Chen et al., 2019; Smith and Brown, 2009)**. This classification facilitated targeted investigations into the microbial community responses to varying degrees of permafrost degradation, with additional data on active layer thickness sourced from the Chinese National Tibetan Plateau Data Center (available at http://data.tpdc.ac.cn, supported by **Chen et al., 2019**).

Vegetation at these sites includes alpine meadows at the river's origion (N1, N2, and N3) characterized by a grass-felt soil matrix, hosting predominant species such as dwarf *Polygonum viviparum*, *Lagotis brachystachya*, and *Rhodiola algida*. Futher downstream, the vegetation transitions into swamp meadows (N4, N5) and steppe meadow (L1, L2, and L3) dominated by *Kobresia capillifolia* and *Carex moorcroftii*, respectively. Comprehensive details on permafrost parameters and vegetation at each site are summarized in Table S1.

A total of 24 samples were systematically extracted from three distinct soil layers (0-20 cm, 20-40 cm, and 40-50cm) at each site. Samples were collected from five random points within a 5 m × 5 m plot at each site and were pooled to create composite samples representing each depth interval. Soil particle sizes analysis was performed at 10 cm intervals across the 0-50 cm soil profile. Before analysis, all samples were thoroughly mixed and sieved to exclude stones and plant debris. Post-collection, samples were sealed in sterilized plastic bags, kept cool with ice, and transported to the laboratory within 48 hours. Once in the laboratory, each sample was bifurcated: one part was stored at 4 ℃ for immediate physical and chemical analyses, and the other was cryogenically preserved at –80 ℃ in sterilized polyethylene tubes for subsequent DNA extraction and PCR amplification.

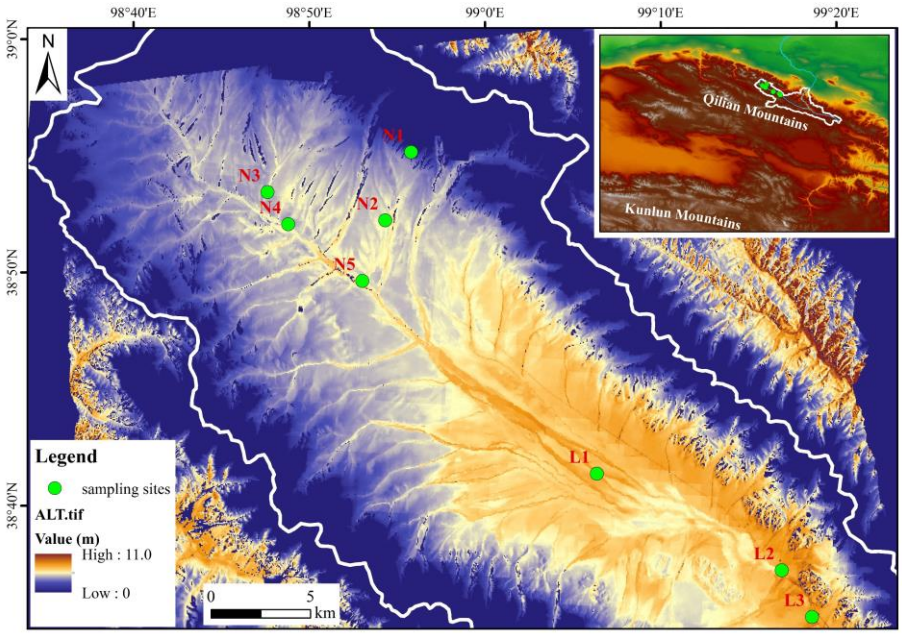

Fig. 1 Sampling sites with locations spanning permafrost soils (N1-N5) and seasonally frozen soils (L1-L3) in the upstream region of the Heihe River.

1.2 Analysis of soil physicochemical properties

A detailed assessment of soil physicochemical properties was performed, including evaluations of both physical (e.g., bulk density, moisture content, temperature, electrical conductivity, particle size distribution) and chemical parameters (e.g., pH, total organic carbon, total nitrogen, NO_3^--N, and NH_4^+-N). For in-situ measurements, soil moisture, temperature, and electrical conductivity were quantified at each sampling depth using a portable W.E.T. Soil Moisture Meter (DELTA Inc., UK). Bulk density was determined using the core cutter method, where soil samples were excavated using a cylindrical core and oven-dried at 90 °C for 24 hours. Particle size composition was assessed through a rapid sieving procedure **(Kettler et al., 2001)** that facilitates rapid classification of soil particles.

Chemical analyses were conducted on soil samples mixed at a weight-to-volume ratio of 1:5 for pH measurement, utilizing a Leici pH electrode (Shanghai, China). Total organic carbon (TOC) and total nitrogen (TN) were quantified using dichromate digestion **(Mebius, 1960)** and Kjeldahl digestion **(Oguntunde et al., 2004)** respectively, which are standard methods for assessing soil nutrient content. Concentrations of soil nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were measured using the KCl extraction followed by indophenol blue colorimetry and phenol disulfonic acid method, respectively **(Wu et al., 2013)**. All soil physicochemical properties were analyzed in triplicate for each soil sample to ensure

reliability and reproducibility of data. The average values from these measurements are systematically presented in Table S1, offering a comprehensive dataset for further interpretation and correlation with microbial community analyses.

1.3 Soil DNA extraction and PCR amplification

Total DNA was extracted from 30-50 ng of fresh soil using the MetaVx™ Library Preparation Kit (GENEWIZ, Inc., South Plainfield, NJ, USA) according to the manufacturer's instructions. DNA concentration was quantified with a Qubit2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and integrity was assessed via electrophoresis on a 1.5% agarose gel using 0.5×TAE buffer.

For the construction of the bacterial 16S rDNA amplification library, primers targeting the V3-V4 hypervariable regions were used: forward primer "CCTACGGRRBGCASCAGKVRVGAAT" and reverse primer "GGACTACNVGGGTWTCTAATCC". For the micro-eukaryotic 18S rDNA library, the V7-V8 hypervariable regions were amplified using the forward primer "CGWTAACGAACGAG" and the reverse primer "AICCATTCAATCGG". These primers also include adaptor sequences necessary for subsequent high-complexity library amplification and sequencing on the Illumina Miseq platform.

PCR amplifications were performed in triplicate in a 25 μL reaction mixture containing 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of each primer, and 20 ng of template DNA. The thermal profile for amplifying 16S rRNA fragments included an initial denaturation at 94 ℃ for 3 minutes, followed by 24 cycles of denaturation at 94 ℃ for 5 seconds, annealing at 57 ℃ for 90 seconds, and elongation at 72 ℃ for 10 seconds, with a final elongation step at 72 ℃ for 5 minutes. For 18S rDNA, the protocol began with an initial denaturation at 94 ℃ for 5 minutes, followed by 25 cycles of denaturation at 94 ℃ for 30 seconds, annealing at 57 ℃ for 30 seconds, and elongation at 72 ℃ for 30 seconds, ending with a final elongation at 72 ℃ for 5 minutes. Both PCR products (16S rRNA and 18S rRNA genes) were subsequently purified and quantified to prepare composite DNA samples for library construction. The prepared libraries were sequenced on an Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA) at GENEWIZ, Inc. (Suzhou, China), ensuring comprehensive analysis of microbial community dynamics in the sampled soils.

1.4 High-throughput sequencing and data processing

High-throughput sequencing was conducted on an Illumina Miseq platform using

the PE250 paired-end configuration. Image analysis and base calling were managed by the MiSeq Control Software (MCS) installed on the MiSeq system. Raw sequence data were initially processed using the Quantitative Insights into Microbial Ecology (QIIME) software package (version 1.9.1; http://qiime.org/) **(Caporaso et al., 2010)**. Sequences were filtered to retain only those longer than 200bp and devoid of ambiguous bases ("N"). These high-quality sequences were then clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the VSEARCH algorithm (version 1.9.6). Taxonomic assignment of each OTU, for both 16S rDNA and 18S rDNA sequences, was performed using the Ribosomal Database Program (RDP) classifier (version 2.2; http://sourceforge.net/project/rdp-classifier/), with a confidence threshold set at 0.8 **(Wang et al., 2007)**. The RDP classifier utilized the Silva 132 database (Release 132; http://www.arb-silva.de), which allows taxonomic predictions down to the species level, ensuring a comprehensive and reliable annotation of microbial community structure.

1.5 Statistical analysis

The One-way Analysis of Variance (ANOVA) was employed to detect significant differences in soil physicochemical properties and microbial community relative abundances between permafrost soils (group N) and seasonally frozen soils (group L), with significance set at $P < 0.05$. Changes in relative abundances within bacterial and micro-eukaryotic communities across the permafrost thaw gradient were analyzed using generalized linear models implemented in SPSS (version 25.0, IBM Corp., Chicago, IL, USA). Microbial alpha-diversity indices, including Richness, Chao1, and Shannon, were computed from evenly rarefied OTU matrices using the "phyloseq" package in R (v.3.6.2, **R Core Team, 2013**). Boxplots illustrating these indices were generated in R for visual representation of diversity patterns. The structure of bacterial and micro-eukaryotic communities was assessed using ecological distance measures and multivariate statistical methods in R. Community comparisons were performed using the UniFrac distance **(Lozupone and Knight, 2005)**. Beta-diversity between sampling sites was analyzed through nonmetric multidimensional scaling (NMDS) and Analysis of Similarities (ANOSIM) based on Bray-Curtis distances, reflecting variations in OTU composition. Linear Discriminant Analysis Effect Size (LEfSe) was used to identify microbial biomarkers differentially abundant between permafrost and seasonally frozen soils, applying the Kruskal-Wallis test with a significance

threshold of LDA score > 2.0 and $P < 0.05$. The co-occurrence networks for bacterial and micro-eukaryotic communities were constructed using the IDENAP process (http://mem.rcees.ac.cn:8081), which elucidates intricate intra-domain associations **(Feng et al., 2022)**. Network analysis was performed using a correlation coefficient matrix with a threshold cutoff selected at 0.94 (P >0.05) for network validity. Networks were visualized using Gephi (version 0.9.6) **(Bastian et al., 2009)**. The relationship between soil properties and microbial community compositions was examined via Mantel tests, conducted using the "ggcor" and "vegan" packages in R. Redundancy Analysis (RDA) was conducted using CANOCO 4.5 for Windows (Biometris, Wageningen, Netherlands) to discern environmental drivers influencing microbial community differentiation at the phylum level, with the significance of these relationships assessed using a Monte Carlo permutation test (999 permutations).

2 Results

2.1 Variations in the soil physicochemical properties

Soil physical and chemical variables were depicted in Fig. 2, displaying substantial differences between permafrost soils (N1-N5) and seasonally frozen soils (L1-L3). Bulk density across all samples exhibited minor fluctuations but generally increased with soil depth. Soil moisture was higher in the topsoil layers of permafrost sites, whereas it was higher in deeper layers of seasonally frozen soils. Soil temperature and electrical conductivity were significantly higher ($P < 0.05$) in seasonally frozen soils than in permafrost soils. Along the soil profile, while bulk density and electrical conductivity increased, soil temperature showed a decreasing trend.

The pH values ranged from 7.80 to 8.55, indicating alkaline conditions across all samples. Total organic carbon (TOC) and total nitrogen (TN) ranged from 2.0 to 18.5 g/kg and 1.0 to 2.5 g/kg, respectively, with TOC generally decreasing with depth. TN, nitrate, and ammonia levels did not exhibit a consistent pattern across the soil profile. Notably, TOC, TN, and ammonia concentrations were highest at the N1 site. Additionally, permafrost soils exhibited higher average concentrations of TOC, TN, and nitrate compared to seasonally frozen soils, whereas ammonia concentrations were higher in seasonally frozen soils.

Gravel was absent in all sampling sites. Soil particle size analysis (Fig. S1), performed at 10 cm intervals, revealed that clay content was generally low across all

samples, averaging 10.2% in permafrost soils and 6.8% in seasonally frozen soils. The average percentages of silt and sand were 54.2% and 36.5%, respectively, with seasonally frozen soils containing a higher proportion of sand grains. According to the FAO/USDA soil texture classification **(Baillie, 2001)**, samples N1-N4 were categorized as silt loam, N5 as sandy loam and the topsoil of L1, L2, and L3 as sandy loam, while their deeper layers were classified as silt loam.

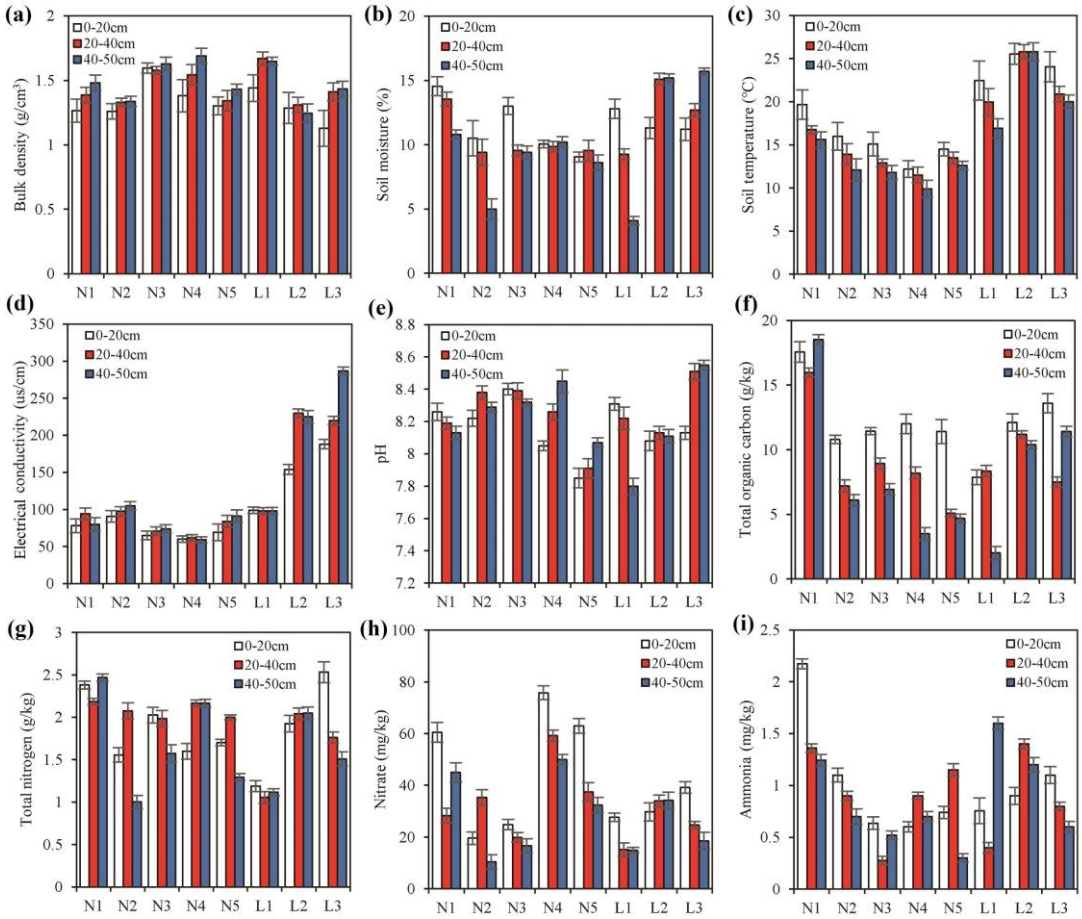

Fig.2. Soil properties of permafrost soils (N1-N5) and seasonally frozen soils (L1-L3) under different layers (white: 0-20 cm, red: 20-40 cm, blue: 40-50 cm) at sampling sites: (a) Bulk density, (b) Soil moisture, (c) Soil temperature, (d) Electrical conductivity, (e) pH, (f) Total organic carbon, (g) Total nitrogen, (h) Nitrate, and (i) Ammonia. All data are presented as "mean ± standard deviation" calculated from triplicated samples.

2.2 Sequence data and diversity of microbial communities

Comprehensive sequencing efforts yielded a total of 444,279 high-quality bacterial sequences and 700,980 micro-eukaryotic sequences across eight study sites. The average sequence lengths were 453 bp for bacteria and 355 bp for micro-eukaryotes. Analysis identified 1,981 bacterial and 1,808 eukaryotic operational taxonomic units (OTUs) at 97% similarity. The Venn diagrams (Fig. 3) revealed that

45.2% (n=896) of bacterial OTUs and 35.0% (n=632) of eukaryotic OTUs were common between permafrost soils and seasonally frozen soils. Upset diagrams (Fig. S2) highlight the distribution of exclusive OTUs across sites, with bacterial unique OTUs ranging from 2 to 18, and eukaryotic unique OTUs ranging from 16 to 79 per site. Notably, the number of endemic OTUs peaked at site N4 for both groups, illustrating a humpbacked pattern correlating with increasing active layer thickness.

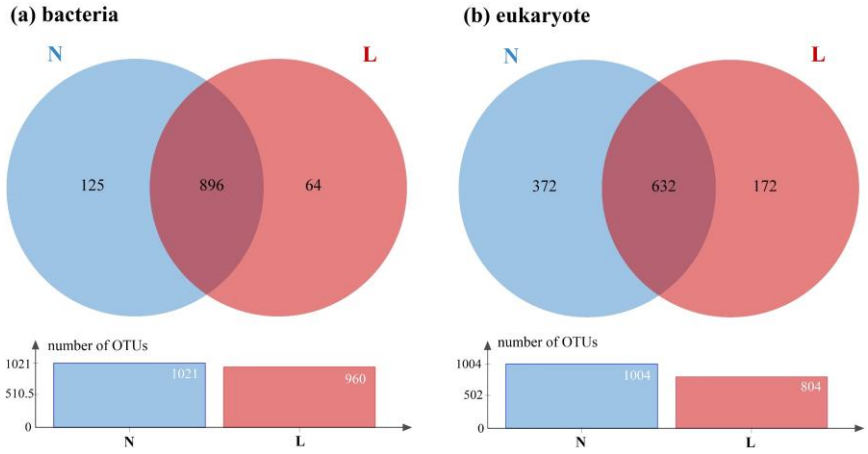

Fig. 3. Venn diagram of OTUs among the permafrost soils (N), and seasonally frozen soils (L) for bacteria (a) and micro-eukaryotes (b), with the total number of OTUs in each group shown on the histogram.

Good's coverage values exceeded 0.99 for all samples (Fig. 4), indicating comprehensive sampling of microbial diversity. Rarefaction curves confirmed that sequencing depth was sufficient (Fig. S3). Alpha diversity analysis showed divergent trends between bacterial and micro-eukaryotic communities in permafrost (N) and seasonally frozen soils (L). Permafrost degradation had an insignificant impact on bacterial alpha diversity ($P > 0.05$) but slightly increased the alpha diversity of micro-eukaryotes. Interestingly, bacterial communities generally exhibited higher alpha diversity indices than their eukaryotic counterparts.

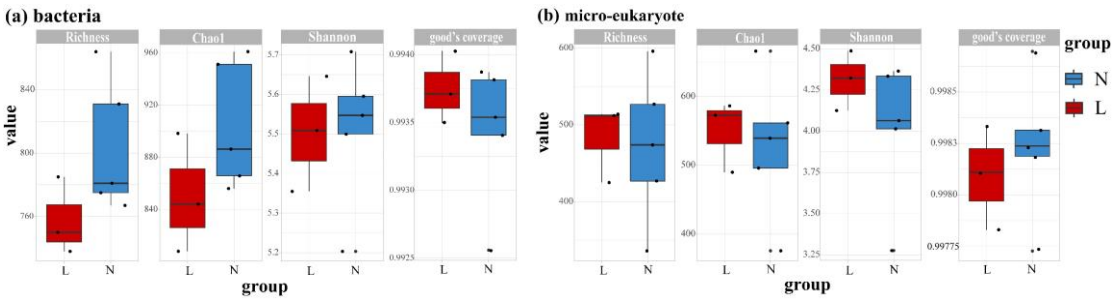

Fig. 4. Alpha diversity of permafrost soils (N) and seasonally frozen soils (L) for bacteria (a) and micro-eukaryotes (b).

Non-metric multidimensional scaling (NMDS) plots demonstrated distinct

community structures between bacterial and eukaryotic communities in permafrost versus seasonally frozen soils. However, analysis of similarities (ANOSIM) indicated that permafrost degradation significantly influenced bacterial community structure (R = 0.61, $P < 0.05$), unlike the eukaryotic community where no significant effect was observed (R = –0.128, $P > 0.05$) (Fig. 5). These findings underscore the complex and differential responses of soil bacterial and micro-eukaryotic communities to permafrost thaw, suggesting that microbial dynamics are closely tied to changes in the permafrost environment.

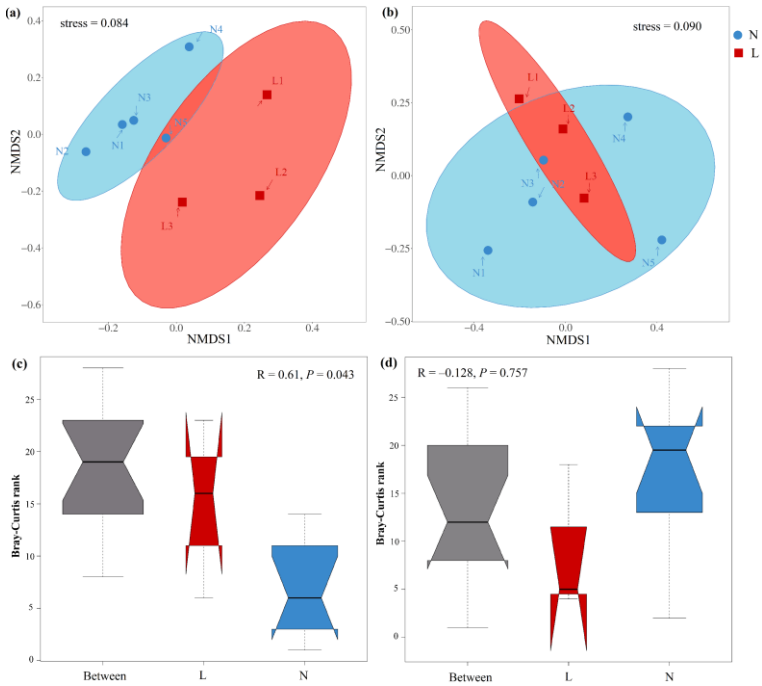

Fig. 5. Nonmetric multidimensional scaling (NMDS) plots of bacterial community (a) and eukaryote (b) across the permafrost degradation gradient; Analysis of similarity (ANOSIM) plots of bacterial community (c) and eukaryote (d) across the permafrost degradation gradient. Stress > 0 means

2.3 Composition of soil microbial communities

The bacterial component of the soil microbiome was highly diverse, with sequences assignable to 20 different phyla, 61 classes, 120 orders, 151 families, and 198 genera. Dominant phyla included Acidobacteria (32.9%), Proteobacteria (32.0%), Actinobacteria (10.0%), Bacteroidetes (9.0%), which together accounted for 84.0% of all bacterial sequences (Fig. 6(a)). Among these, the phylum Nitrospirae exhibited a significant decrease ($P < 0.01$) along the degradation gradient. At the class level, the most prevalent were Acidobacteria_Blastocatellia_[Subgroup_4] (17.8%), Alphaproteobacteria (17.5%), Gammaproteobacteria (11.5%), and Acidobacteria_Subgroup_6 (10.5%) (Fig. 6(b)). Lesser-known phyla constituted 75.9%

of the total diversity (Table S2). The top 19 bacterial genera were led by RB41 (13.0%), *Sphingomonas* (5.3%), *Nitrospira* (4.1%), uncultivated soil bacterium clone C112 (2.3%), and *Terrimonas* (1.1%). Notably, differences in the relative abundance of these genera between permafrost and seasonally frozen soils were statistically significant (Fig. 6(c)), illustrating spatial variations in bacterial community compositions that suggest a strong influence of permafrost degradation.

Eukaryotic diversity spanned six lineages, with Opisthokonta, SAR, and Archaeplastida being the most dominant groups (Fig. 7(a)). Among the 12 eukaryotic phyla identified, Nucletmycea and Rhizaria were predominant, comprising 86.4% of the total eukaryotic sequences. The remaining phyla, including Chloroplastida (8.4%) and Discosea (2.5%), represented a minor fraction of the community (2.7%) (Fig. 7(b)). Two Phyla, Holozoa and Rhizaria, exhibited a significant increase in relative abundance with increasing ALT ($P < 0.05$) (Table S4). Heatmap analysis highlighted several dominant fungal families such as *Mortierellaceae*, *Agaricales*, *Leptosphaeriaceae*, etc. which accounted for 98.1% of the Nucletmycea phylum (Fig. 7(c)). Among the top 20 fungal groups within the micro-eukaryotic community, *Hygrophoraceae* and *Microascaceae* displayed significant differences between the two soil types ($P < 0.05$) as determined by generalized linear models. Overall, the fungal component of the micro-eukaryotic community showed less variability along the degradation gradient than the bacterial community, suggesting a relative stability of fungal populations in response to permafrost changes.

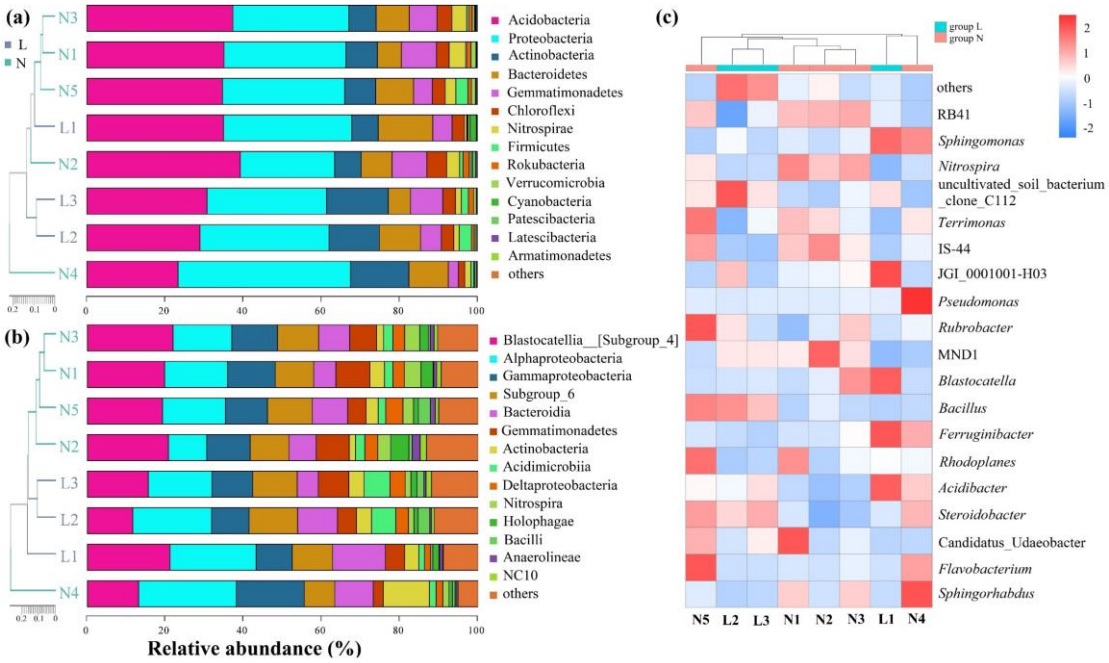

Fig.6. Relative abundance of the soil bacterial community under the permafrost soils (N1-N5) and seasonally frozen soils (L1-L3): (a) Phylum, (b) Class, and (c) Classified Genus. "Others" represents sequences that were unclassified and sequences that were less than 1% of the total.

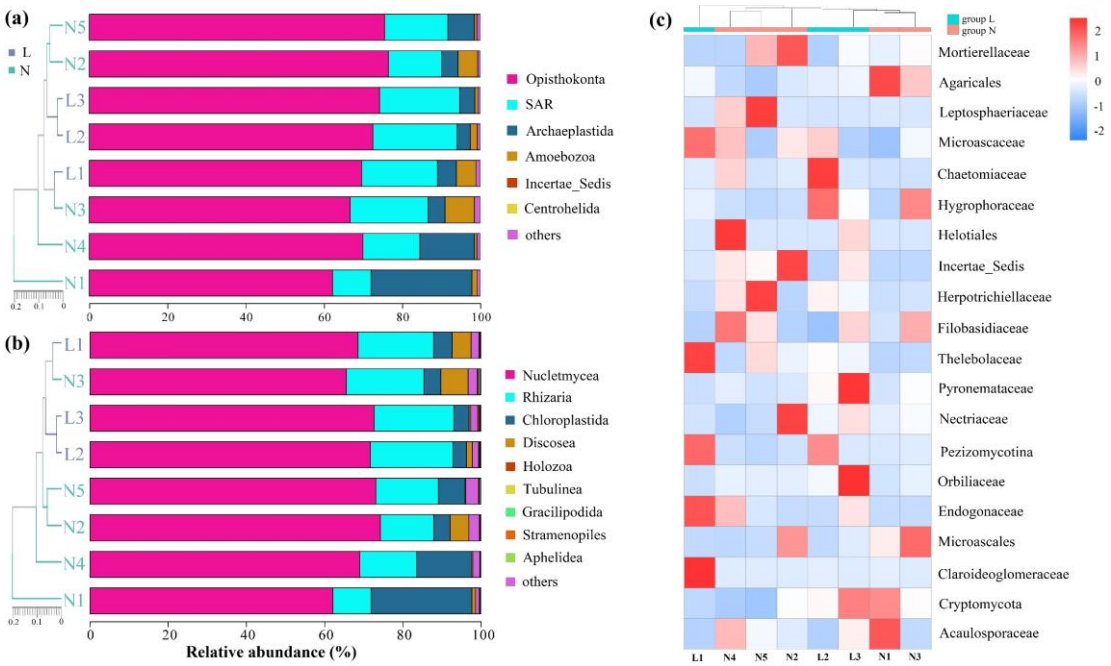

Fig.7. Relative abundance of the eukaryotic community under the permafrost soils (N1–N5) and seasonally frozen soils (L1–L3): (a) Kingdom, (b) Phylum, and (c) Fungal groups of Nucletmycea. "Others" represents sequences that were unclassified and sequences that were less than 1% of the total.

As depicted in Fig. 8(a), LEfSe analysis identified 37 bacterial clades that demonstrated significant differences across all soil samples, with a LDA threshold of 2.0. In the permafrost soils (N1–N5), 22 bacterial clades were notably enriched, whereas in the seasonally frozen soils, 15 clades were more abundant. Notably, the seasonally frozen soils (gourp L) showed a higher prevalence of members from the Cytophagales order and uncultivated soil bacterium clone C112 at multiple taxonomic levels (order, family, and genus). Conversely, the permafrost soils (group N) displayed enrichment in Gammaproteobacteria (class), Nitrosomonadaceae (family), and Nitrospiraceae (family). Previous research on the Qinghai-Tibet Plateau highlighted Cytophagales as potential biomarkers for alpine wetland environments **(Wang et al., 2020)**.

The LEfSe analysis for the micro-eukaryotic community, as shown in Fig. 8(b), indicated that six eukaryotic clades—belonging to the Nuclemycea, Rhizaria, Holozoa, and Chloroplastida groups—showed significant susceptibilities to soil degradation. Among these, Acaulosporaceae emerged as a potential biomarker in the permafrost soils, with its highest abundance in sample N1 (2.24%). Conversely, Nectriaceae was

suggested as a potential biomarker for seasonally frozen soils, most notably in sample L3 (2.68%). These findings provide insights into the differential responses of bacterial and micro-eukaryotic communities to environmental changes in permafrost regions, highlighting specific taxa that may serve as indicators of ecological shifts due to permafrost thaw.

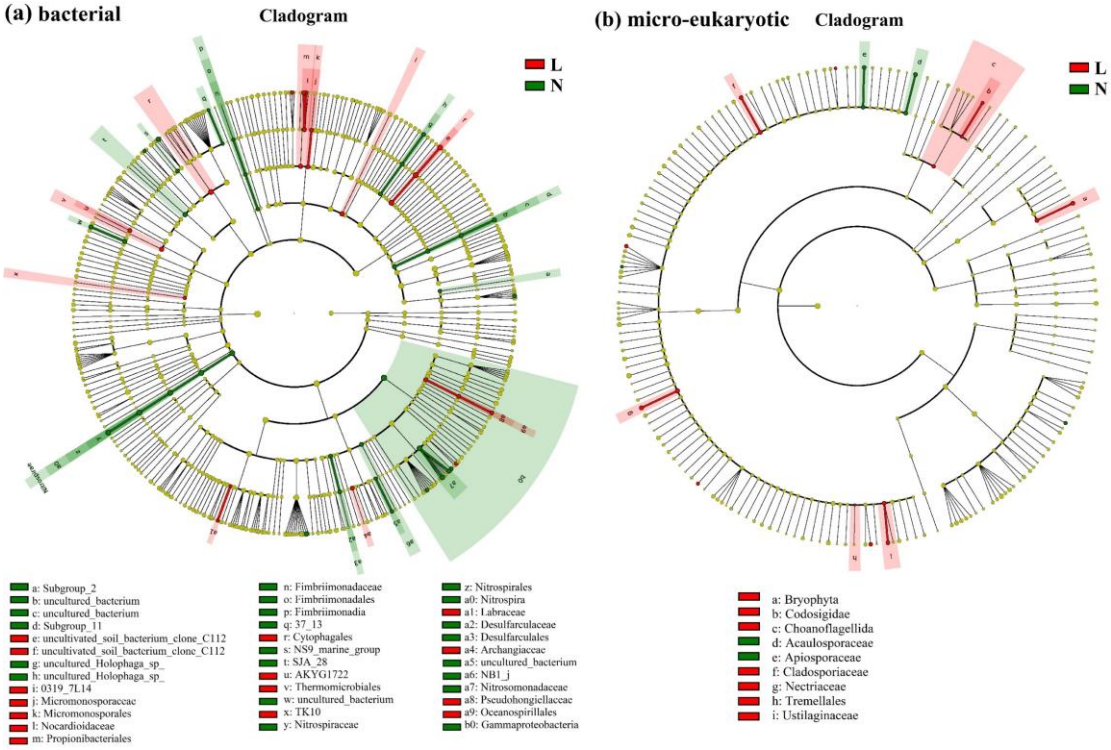

Fig. 8. LEfSe analysis and Histogram of LDA scores with a threshold value of 2.0 of soil microbial abundance in permafrost soils (group N) and seasonally frozen soils (group L): soil bacteria (a) and eukaryote (b). Small circles and shading with different colors illustrate the taxa that are enriched in group N and group L.

2.4 Co-occurrence networks response to permafrost thaw gradient

The co-occurrence networks were constructed for bacterial and micro-eukaryotic communities to elucidate their interaction patterns in response to the permafrost thaw gradient. These networks were established from robust (R = 0.94) and statistically significant ($P < 0.05$) correlations among OTUs, highlighting the intricate relationships within these microbial communities. The bacterial network was extensive, comprising 633 nodes interconnected by 1127 edges, demonstrating a balance of 54.75% positive and 45.25% negative interactions (Fig. 9(a)). This network's dominant taxa included Proteobacteria, Acidobacteria, and Bacteroidetes, indicating their pivotal roles in microbial interactions within permafrost soils. The node-level topological features of the bacterial and eukaryotic networks were

examined (Table 2). The network's modularity index exceeded 0.4, suggesting a pronounced modular structure, which is indicative of highly interconnected clusters or communities within the network **(Newman, 2006)**. These modules, encompassing 59.11% of the total network, varied in their representation, ranging from 4.11% to 8.06% of nodes (Fig. 9(b)).

Contrastingly, the micro-eukaryotic network was characterized by fewer nodes (255) and edges (258), with a significant predominance of negative interactions (65.89%) over positive interactions (34.11%), reflecting potentially competitive or antagonistic relationships (Fig. 9(c)). This network, primarily comprising Opisthokonta and SAR, was also divided into nine major modules, similar to the bacterial network, suggesting a modular organization that may facilitate specialized functional responses to environmental changes (Fig. 9(d)). This distinct patterns of positive and negative correlations within the bacterial and eukaryotic networks underscore how these communities adapt differently to environmental stresses associated with permafrost thaw.

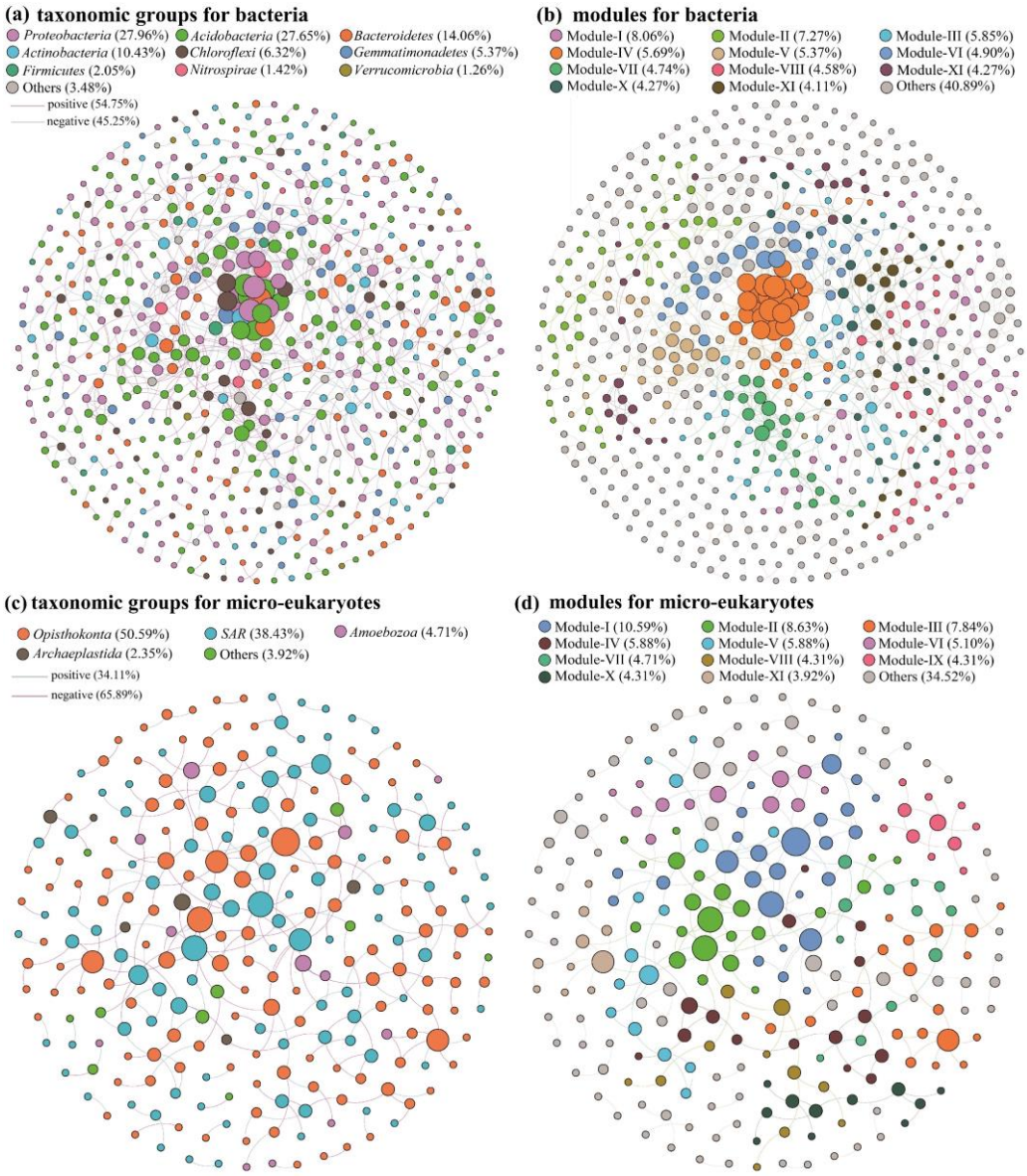

Fig. 9. The co-occurrence networks among the OTUs of soil bacterial and micro-eukaryotic communities colored by taxonomic groups ((a), (c)) and modules ((b), (d)) based on Spearman correlation analysis.

Table 2. The key topological features of soil microbial networks.

Topological Parameters	Bacteria	Eukaryota
Number of edges	1127	258
Number of nodes	633	255
Network density	0.006	0.008
Characteristic degree	3.561	2.024
Characteristic path length	8.299	7.275
Clustering coefficient	0.369	0.125
Modularity	0.822	0.863

2.5 Effects of environmental factors on soil microbial communities

The analyses of correlations between environmental factors and microbial communities underscored distinct influences on soil bacteria and micro-eukaryotes. Soil chemical properties exhibited mutual positive correlations, whereas elevation,

ALT, soil temperature, and electrical conductivity showed negative relationships with one another. The Mantel test revealed that only a select few environmental factors significantly impacted the soil microbial communities ($P < 0.05$). For the bacterial community, soil temperature ($r \geq 0.4$, $P < 0.05$) and electrical conductivity ($r \geq 0.4$, $P \geq 0.05$) emerged as significant influencers, both demonstrating a robust correlation. Elevation and vegetation cover ($0.2 < r < 0.4$, $P < 0.05$), although less influential, still showed moderate correlations. In contrast, the micro-eukaryotic communities were primarily influenced by the carbon-to-nitrogen ratio ($r \geq 0.4$, $P < 0.05$) and the silt content ($0.2 < r < 0.4$, $P < 0.05$), suggesting a dependency on soil nutrient dynamics. Interestingly, ALT presented as a considerable factor for micro-eukaryotes ($0.2 < r < 0.4$, $P \geq 0.05$) but was not significant for bacterial communities (Fig. 10(a)).

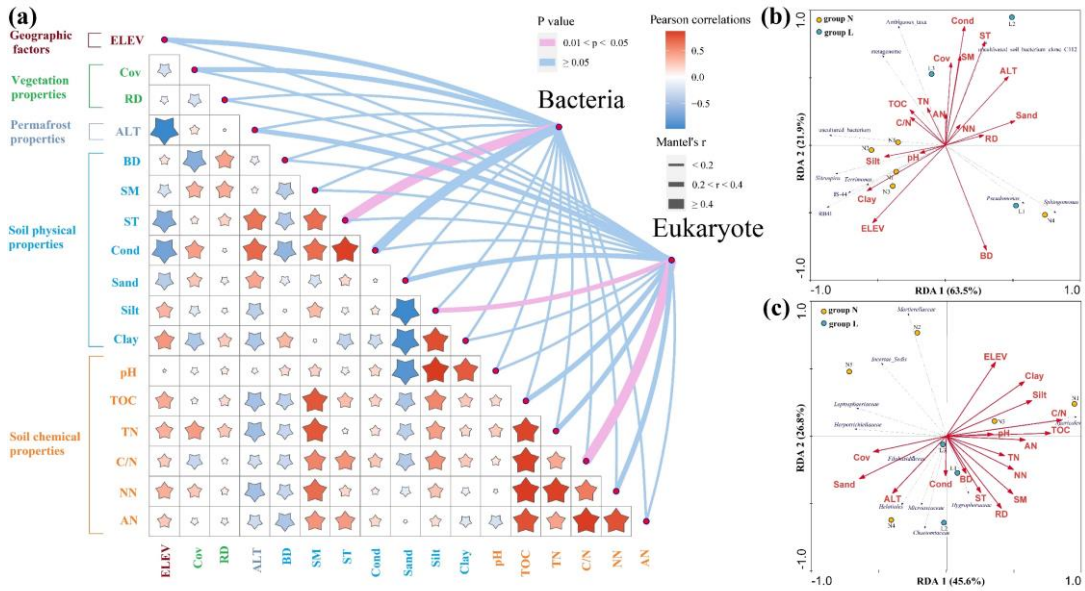

Fig. 10. Relationship between environmental factors and soil microbial communities at phylum level revealed by (a) Mantel test and at genus level revealed by RDA analysis for (b) bacterial community and (c) micro-eukaryotic community. ELEV, RD, Cov, ALT, BD, SM, ST, and Cond indicate elevation, root depth, coverage, active layer thickness, bulk density, soil moisture, soil temperature, and electrical conductivity, respectively. TOC, TN, C/N, NN, and AN indicate total organic carbon, total nitrogen, the ratio of C and N, nitrate, and ammonia, respectively. The size of the star is in proportion to the correlation among physicochemical properties. The arrow length corresponds to the variation of microbial community structure that can be explained by the environmental factors and the direction indicates an increasing magnitude of the soil property. Group N and L represent permafrost soils (N1-N5) and seasonally frozen soils (L1-L3), respectively.

The redundancy analysis helped further delineate the relationships between microbial community structure at the genus level and environmental parameters. In the bacterial community, the electrical conductivity, soil temperature, and bulk density

were the major driving factors (Fig. 10(b)). Notably, the genus RB41, a member of Acidobacteria, showed a significant correlation with clay content ($P < 0.05$), highlighting the potential regulation of this bacterial genus by factors affecting soil aeration and moisture retention. Soil texture components, particularly in permafrost soils (group N), were significant drivers of bacterial community composition, with electrical conductivity being especially influential in seasonally frozen soils (group L).

For micro-eukaryotic community, the carbon-to-nitrogen ratio, root depth, and sand content were key environmental drivers influencing community structure (Fig. 10(c)). This finding underscores the greater impact of soil physical properties over chemical properties on microbial community dynamics within our study sites. This is perhaps attributable to the more pronounced changes in soil physical properties along the permafrost thaw gradient compared to chemical properties.

In summary, our study illustrates that bacterial communities are significantly affected by soil physical properties, whereas eukaryotic communities are more responsive to variations in soil nutrient content. These differences highlight the distinct ecological niches and adaptive strategies employed by soil bacterial and micro-eukaryotic communities in permafrost-affected regions.

3 Discussion

3.1 Impact of permafrost degradation on microbial diversity

Results of our study illuminates the significant impact of permafrost degradation on the diversity of bacterial and eukaryotic communities within the alpine ecosystems of the Qilian Mountains. Although numerous studies have examined microbial diversity across different types of frozen soil, findings have been inconsistent **(Wu et al., 2017; Yang et al., 2015)**. For instance, while research in northern Sweden identified a monotonous trend in alpha diversity across three distinct permafrost thaw stages **(Mondav et al., 2017)**, a contrasting humpback pattern was observed in the Qinghai-Tibet plateau **(Zhang et al., 2017)**. Our results align with the letter, showing an initial increase and subsequent decrease in bacterial diversity along the active layer thickness gradient, whereas micro-eukaryotic diversity displayed an opposing trend (Fig. 4). This pattern can be explained by the mid-dominant effect, where moderate environmental changes along the permafrost thaw gradient benefit bacterial communities but only up to a certain limit, beyond

which the effect reverses **(Singh et al., 2012; Miyamoto et al., 2014)**.

The harsh conditions prevalent in permafrost regions, such as low temperatures, freeze-thaw cycles, and limited water availability, harbor a complex and largely unknown microbial life. A substantial proportion of soil bacterial and micro-eukaryotic communities in our study were unclassified OTUs, constituting 59.1% and 55.0% of total OTUs, respectively. The soil microbial compositions in seasonally frozen and permafrost soils exhibit distinctive characteristics **(Doherty et al., 2019)**, with dominant bacterial phyla including Acidobacteria, Proteobacteria, and Actinobacteria (Fig. 6), consistent with other permafrost studies **(Shi et al., 2015; Duan et al., 2021)**. The Actinobacteria/Proteobacteria ratio, considered a marker of permafrost degradation, increased with the ALT, aligning with findings from the Shule River basin **(Zhang et al., 2017)**. Nitrospira, part of the nitrifying bacterial group, decreased significantly along the permafrost thaw gradient, suggesting a stagnation in nitrogen cycling in soil ecosystems (Fig. 6 (b)).

For micro-eukaryotes, the *Mortierellacea* genus, a significant decomposer of chitin, decreased with increasing ALT (Fig. 7(c)), indicating a reduced capacity for organic matter decomposition and nutrient formation for plant uptake **(Kim et al., 2008)**. The LEfSe analysis identified GammaProteobateria (Class) and *Nitrospira* (Genus) as bacterial biomarkers in permafrost soils, and Cytophagales (order) in seasonally frozen soils. Eukaryotic biomarkers included Acaulosporaceae in permafrost soils and Nectriaceae in seasonally frozen soils (Fig. 8), though biomarker detection can vary by region **(Tripathi et al., 2018)**.

Our microbial network analysis suggested that bacterial communities possess a balanced mix of positive and negative interactions, whereas eukaryotic communities exhibit more mutual inhibition, indicating differing strategies within these microbial domains (Fig. 9) **(Hu et al., 2020; Yuan et al., 2021)**. This analysis suggests that bacterial communities may better adapt and respond to environmental disturbances than eukaryotic communities, a notion supported by the stability of fungal communities despite temperature changes observed in our studies **(Feng et al., 2020)**.

Our research found that permafrost degradation may intensify the instability of bacterial and eukaryotic networks because with permafrost degradation, decreased bacterial community diversity leads to decreased positive feedback between networks, while increased fungal diversity leads to increased negative feedback in networks. Another study conducted in Qilian Mountains also showed that compared with the

slightly degraded permafrost, the node connectivity, centrality, and complexity all increased significantly in the severely degraded permafrost **(Wu et al., 2021)**. Such an increase in the network properties suggested a lower network stability with permafrost degraded. These findings collectively suggest that the degradation of frozen soil diminishes the stability of the soil microbial community.

3.2 Responses of microbial communities to environmental changes

The alteration of soil microbial communities in response to permafrost thaw is well established, yet the intricate mechanisms driving these changes under permafrost degradation remain incompletely understood. Prior research has indicated that warming trends might shift microbial communities from predominantly bacterial to fungal in the alpine meadow of the Qinghai-Tibet Plateau, underscoring distinct responses between these community types **(Wang et al., 2017)**. Our findings reinforced that soil temperature and carbon-to-nitrogen ratio were critical determinants of microbial community structures at the phylum level (Fig. 10 (a)). Specifically, soil texture and electrical conductivity significantly influenced bacterial communities in permafrost and seasonally frozen soils respectively, while the carbon-to-nitrogen ratio and sand content were pivotal for micro-eukaryotic communities. To investigate the effects of rising temperature on soil microorganisms in the permafrost regions, a long-term soil experiment conducted in the Alaska tundra found that while short-term (1.5 years) temperature increases did not alter microbial structures significantly, longer-term warming (4.5-years) did have substantial impacts **(Johnston et al., 2019)**. In our study, the distribution of soil temperature did not align neatly with ALT gradients, reflecting a complex interplay of elevation and soil conditions. The soil temperature decreased with a reduction in altitude in permafrost regions but increased in areas with seasonally frozen soils. During the summer sampling period, the melting of seasonally frozen soils was greater than that of permafrost soils, enhancing soil moisture and electrical conductivity, thereby fostering microbial metabolism and enzyme activity. Changes in soil water content influence soil permeability and oxygen availability, potentially decreasing anaerobic microorganisms while boosting aerobic populations, thus altering the microbial community composition and structure **(Liebner et al., 2008**; **Geng et al., 2012; Feng et al., 2020)**. Interestingly, while nutrient content typically has a profound impact on microbial communities, our study highlighted a more pronounced influence of soil

physical properties. Previous studies often selected sampling points on a basin scale, and our sample points have a distribution area of less than 10 km^2, which may lead to relatively small changes in the nutrient content in this area, thus magnifying the impact of soil physical characteristics. This suggested that microbial responses to environmental factors may vary depending on the geographic scale of the study area.

Although ALT was not identified as a predominant factor affecting microbial communities (Fig. 10), changes in soil temperature and carbon-to-nitrogen ratio were directly influenced by variations in ALT. Previous researches highlighted the significant role of ALT in storing soil organic matter (SOM), which is crucial for maintaining soil fertility and ecosystem function **(Rodionov et al., 2007; Schuur et al., 2015)**. Degradation of permafrost likely leads to enhanced groundwater flow, which can increase the mineralization of SOM by improving soil drainage and oxygen availability **(Goulden et al., 1998; Hobbie et al., 2000)**. Consequently, as permafrost degrades, the reduced availability of nutrients such as soil organic carbon (SOC) and total nitrogen (TN) may trigger shifts from highland to drier communities, lowlands, and finally even wetter community structures, making a significant ecological transformation **(Jin et al., 2021; Xu et al., 2018)**.

These shifts in microbial community composition and diversity, driven by changes in water and nutrient availability due to permafrost degradation, will inevitably trigger broader ecological changes. Such transformations are often irreversible under ongoing climatic status, underscoring the urgency of developing robust ecological security and early warming systems. This study provides a foundational scientific basis for such initiatives by elucidating the interactions between soil microbial communities and environmental factors within permafrost ecosystems.

4 Conclusions

Permafrost significantly influences climatic feedback mechanisms due to its integral role in water and nutrient cycles. Our study underscored the profound impact of soil properties on the structure and diversity of soil microbial communities within the alpine meadow ecosystem. Notably, soil temperature predominantly shaped the bacterial community, whereas the carbon-to-nitrogen ratio predominantly influenced the eukaryotic community. The responses of soil bacterial and micro-eukaryotes to permafrost degradation exhibited distinct trends and mechanisms, leading to

decreased bacterial diversity and increased fungal diversity as permafrost degrades. The co-occurrence networks revealed a dominance of positive correlations within bacterial communities and negative correlations within fungal communities, suggesting different interaction dynamics. Bacterial networks, characterized by more nodes and connections, indicated greater stability compared to the less stable micro-eukaryotic communities. Environmental disturbances were more likely to induce substantial changes in the micro-eukaryotic community, while bacterial diversity trends to remain more constant.

Under scenarios of future global warming, a potential shift in microbial community dynamics from a bacterial-dominated to a eukaryotic-dominated system is anticipated. This shift is likely to coincide with a reduction in the stability of the microbial network as soil conditions continue to change. The implications of these shifts are critical for understanding and predicting changes in alpine meadow ecosystems in response to climate change.

CRediT authorship contribution statement

Zhu Wang: Conceptualization, Methodology, Writing - original draft. Yang Liu: Supervision, Writing - review & editing. Fang Wang: Supervision, Funding acquisition.

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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Supplementary materials

Supplementary material associated with this article can be found online at: .

References

Baillie, I.C., 2001. Soil survey staff 1999, soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, agricultural handbook 436, Natural Resources Conservation Service, USDA, Washington DC, USA, pp. 869.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks, in: Proceedings of the International AAAI Conference on Web and Social Media. pp. 361–362.

Biskaborn, B.K., Smith, S.L., Noetzli, J., Matthes, H., Vieira, G., Streletskiy, D.A., Schoeneich, P., Romanovsky, V.E., Lewkowicz, A.G., Abramov, A., 2019. Permafrost is warming at a global scale. Nat. Commun. 10, 264.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Chen, Q.Y., Lei, T.Z., Wu, Y.Q., Si, G.C., Xi, C.W., Zhang, G.X., 2019. Comparison of soil organic matter transformation processes in different alpine ecosystems in the Qinghai-Tibet Plateau. J. Geophys. Res. Biogeosciences 124, 33–45.

Chen, Y.X., Jiang, L.M., Liang, L.L., Zhou, Z.W., 2019. Monitoring permafrost deformation in the upstream Heihe River, Qilian Mountain by using multi-temporal Sentinel-1 InSAR dataset. Chin. J. Geophys. 62, 2441–2454.

Cheng, G.D., Wu, T.H., 2007. Responses of permafrost to climate change and their environmental significance, Qinghai-Tibet Plateau. J. Geophys. Res. Earth Surf. 112.

Cheng, G.D., Wang, S.L., 1982. On the zonation of high-altitude permafrost in China. J. Glaciol. Geocryol. 4, 1–17.

Chu, H., Neufeld, J.D., Walker, V.K., Grogan, P., 2011. The influence of vegetation type on the dominant soil bacteria, archaea, and fungi in a low Arctic tundra landscape. Soil Sci. Soc. Am. J. 75, 1756–1765.

Doherty, S.J., Barbato, R., Dorrepaal, E., Johansson, M., Monteux, S., Bennett, K.A., Ernakovich, A., Mackay, J., Ernakovich, J.G., 2019. Assembly of microbial communities in thawed permafrost and implications for carbon processing, in: AGU Fall Meeting Abstracts. pp. B23J-2555.

Duan, Y.L., Lian, J., Wang, L.L., Wang, X.Y., Luo, Y.Q., Wang, W.F., Wu, F.S., Zhao, J.H., Ding, Y., Ma, J., 2021. Variation in soil microbial communities along an elevational gradient in alpine meadows of the Qilian Mountains, China. Front. Microbiol. 12, 684386.

Dutta, K., Schuur, E.A.G., Neff, J.C., Zimov, S.A., 2006. Potential carbon release from permafrost soils of Northeastern Siberia. Glob. Change Biol. 12, 2336–2351.

Feng, J.J., Wang, C., Lei, J.S., Yang, Y.F., Yan, Q.Y., Zhou, X.S., Tao, X.Y., Ning, D.L., Yuan, M.T., Qin, Y.J., 2020. Warming-induced permafrost thaw exacerbates tundra soil carbon decomposition mediated by microbial community. Microbiome 8, 1–12.

Feng, K., Peng, X., Zhang, Z., Gu, S.S., He, Q., Shen, W.L., Wang, Z.J., Wang, D.R., Hu, Q.L., Li, Y., 2022. iNAP: an integrated network analysis pipeline for microbiome studies. iMeta 1, e13.

Frey, K.E., McClelland, J.W., 2009. Impacts of permafrost degradation on arctic river biogeochemistry. Hydrol. Process. Int. J. 23, 169–182.

Geng, Y., Wang, Y.H., Yang, K., Wang, S.P., Zeng, H., Baumann, F., Kuehn, P., Scholten, T., He, J.S., 2012. Soil respiration in Tibetan alpine grasslands: belowground biomass and soil moisture, but not soil temperature, best explain the large-scale patterns. PloS One 7, e34968.

Goulden, M.L., Wofsy, S.C., Harden, J.W., Trumbore, S.E., Crill, P.M., Gower, S.T., Fries, T., Daube, B.C., Fan, S.-M., Sutton, D.J., 1998. Sensitivity of boreal forest carbon balance to soil thaw. Science 279, 214–217.

Gruber, S., 2012. Derivation and analysis of a high-resolution estimate of global permafrost zonation. The Cryosphere 6, 221–233.

Guo, W.Q., Liu, S.Y., Xu, J.L., Wu, L.Z., Shangguan, D.H., Yao, X.J., Wei, J.F., Bao, W.J., Yu, P.C., Liu, Q., 2015. The second Chinese glacier inventory: data, methods and results. J. Glaciol. 61, 357–372.

Hobbie, S.E., Schimel, J.P., Trumbore, S.E., Randerson, J.R., 2000. Controls over carbon storage and turnover in high-latitude soils. Glob. Change Biol. 6, 196–210.

Hu, Q.L., Tan, L., Gu, S.S., Xiao, Y.S., Xiong, X.Y., Zeng, W.A., Feng, K., Wei, Z., Deng, Y., 2020. Network analysis infers the wilt pathogen invasion associated with non-detrimental bacteria. Npj Biofilms Microbiomes 6, 8.

Huai, B., Li, Z., Wang, S., Sun, M., Zhou, P., Xiao, Y., 2014. RS analysis of glaciers change in the Heihe River Basin, Northwest China, during the recent decades. J. Geogr. Sci. 24, 993–1008.

Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nat. Rev. Microbiol. 18, 35–46.

Jin, X.-Y., Jin, H.-J., Iwahana, G., Marchenko, S.S., Luo, D.-L., Li, X.-Y., Liang, S.-H., 2021. Impacts of climate-induced permafrost degradation on vegetation: A review. Adv. Clim. Change Res. 12, 29–47.

Johnston, E.R., Hatt, J.K., He, Z., Wu, L., Guo, X., Luo, Y., Schuur, E.A., Tiedje, J.M., Zhou, J., Konstantinidis, K.T., 2019. Responses of tundra soil microbial communities to half a decade of experimental warming at two critical depths. Proc. Natl. Acad. Sci. 116, 15096–15105.

Jorgenson, M.T., Shur, Y.L., Pullman, E.R., 2006. Abrupt increase in permafrost degradation in Arctic Alaska. Geophys. Res. Lett. 33.

Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci. Soc. Am. J. 65, 849–852.

Kim, Y.-J., Zhao, Y., Oh, K.-T., Nguyen, V.-N., Park, R.-D., 2008. Enzymatic deacetylation of chitin by extracellular chitin deacetylase from a newly screened Mortierella sp. DY-52. J. Microbiol. Biotechnol. 18, 759–766.

Lawrence, D.M., Slater, A.G., Tomas, R.A., Holland, M.M., Deser, C., 2008. Accelerated Arctic land warming and permafrost degradation during rapid sea ice loss. Geophys. Res. Lett. 35.

Li, H.Y., Qiu, Y.Z., Yao, T., Han, D.R., Gao, Y.M., Zhang, J.G., Ma, Y.C., Zhang, H.R., Yang, X.L., 2021. Nutrients available in the soil regulate the changes of soil microbial community alongside degradation of alpine meadows in the northeast of the Qinghai-Tibet Plateau. Sci. Total Environ. 792, 148363.

Li, X., Cheng, G.D., Ge, Y.C., Li, H.Y., Han, F., Hu, X.L., Tian, W., Tian, Y., Pan, X.D., Nian, Y.Y., 2018. Hydrological cycle in the Heihe River Basin and its implication for water resource management in endorheic basins. J. Geophys. Res. Atmospheres 123, 890–914.

Liebner, S., Harder, J., Wagner, D., 2008. Bacterial diversity and community structure in polygonal tundra soils from Samoylov Island, Lena Delta, Siberia. Int. Microbiol. 11, 195–202.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Mackelprang, R., Waldrop, M.P., DeAngelis, K.M., David, M.M., Chavarria, K.L., Blazewicz, S.J., Rubin, E.M., Jansson, J.K., 2011. Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. Nature 480, 368–371.

Männistö, M.K., Tiirola, M., Häggblom, M.M., 2007. Bacterial communities in Arctic fjelds of Finnish Lapland are stable but highly pH-dependent. FEMS Microbiol. Ecol. 59, 452–465.

Mebius, L., 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22, 120–124.

Miyamoto, Y., Nakano, T., Hattori, M., Nara, K., 2014. The mid-domain effect in ectomycorrhizal fungi: range overlap along an elevation gradient on Mount Fuji, Japan. ISME J. 8, 1739–1746.

Mondav, R., McCalley, C.K., Hodgkins, S.B., Frolking, S., Saleska, S.R., Rich, V.I., Chanton, J.P., Crill, P.M., 2017. Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. Environ. Microbiol. 19, 3201–3218.

Newman, M.E., 2006. Modularity and community structure in networks. Proc. Natl. Acad. Sci. 103, 8577–8582.

Oguntunde, P.G., Fosu, M., Ajayi, A.E., Van De Giesen, N., 2004. Effects of charcoal production on maize yield, chemical properties and texture of soil. Biol. Fertil. Soils 39, 295–299.

Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G., 1982. Soil carbon pools and world life zones. Nature 298, 156–159.

R Core Team, R., 2013. R: A language and environment for statistical computing.

Rodionov, A., Flessa, H., Grabe, M., Kazansky, O.A., Shibistova, O., Guggenberger, G., 2007. Organic carbon and total nitrogen variability in permafrost-affected soils in a forest tundra ecotone. Eur. J. Soil Sci. 58, 1260–1272.

Schuur, E.A., Abbott, B., 2011. High risk of permafrost thaw. Nature 480, 32–33.

Schuur, E.A., McGuire, A.D., Schädel, C., Grosse, G., Harden, J.W., Hayes, D.J., Hugelius, G., Koven, C.D., Kuhry, P., Lawrence, D.M., 2015. Climate change and the permafrost carbon feedback. Nature 520, 171–179.

Schuur, E.A., Vogel, J.G., Crummer, K.G., Lee, H., Sickman, J.O., Osterkamp, T.E.,

2009. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature 459, 556–559.

Shi, Y., Xiang, X.J., Shen, C.C., Chu, H.Y., Neufeld, J.D., Walker, V.K., Grogan, P., 2015. Vegetation-associated impacts on arctic tundra bacterial and microeukaryotic communities. Appl. Environ. Microbiol. 81, 492–501.

Singh, D., Takahashi, K., Kim, M., Chun, J., Adams, J.M., 2012. A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. Microb. Ecol. 63, 429–437.

Smith, S., Brown, J., 2009. Assessment of the status of the development of the standards for the Terrestrial Essential Climate Variables-T7-Permafrost and seasonally frozen ground.

Stendel, M., Christensen, J.H., 2002. Impact of global warming on permafrost conditions in a coupled GCM. Geophys. Res. Lett. 29, 10–1.

Tripathi, B.M., Kim, M., Kim, Y., Byun, E., Yang, J.-W., Ahn, J., Lee, Y.K., 2018. Variations in bacterial and archaeal communities along depth profiles of Alaskan soil cores. Sci. Rep. 8, 504.

Wang, C.T., Zhao, X.Q., Zi, H.B., Hu, L., Ade, L., Wang, G.X., Lerdau, M., 2017. The effect of simulated warming on root dynamics and soil microbial community in an alpine meadow of the Qinghai-Tibet Plateau. Appl. Soil Ecol. 116, 30–41.

Wang, G.X., Li, Y.S., Wang, Y.B., Wu, Q.B., 2008. Effects of permafrost thawing on vegetation and soil carbon pool losses on the Qinghai–Tibet Plateau, China. Geoderma 143, 143–152.

Wang, G.X., Qian, J., Cheng, G.D., Lai, Y.M., 2002. Soil organic carbon pool of grassland soils on the Qinghai-Tibetan Plateau and its global implication. Sci. Total Environ. 291, 207–217.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Wang, T.Y., Wu, T.H., Wang, P., Li, R., Xie, C.W., Zou, D.F., 2019. Spatial distribution and changes of permafrost on the Qinghai-Tibet Plateau revealed by statistical models during the period of 1980 to 2010. Sci. Total Environ. 650, 661–670.

Wang, X.J., Zhang, Z.C., Yu, Z.Q., Shen, G.F., Cheng, H.F., Tao, S., 2020.

Composition and diversity of soil microbial communities in the alpine wetland and alpine forest ecosystems on the Tibetan Plateau. Sci. Total Environ. 747, 141358.

Washburn, A.L., 1980. Permafrost features as evidence of climatic change. Earth-Sci. Rev. 15, 327–402.

Wilhelm, R.C., Niederberger, T.D., Greer, C., Whyte, L.G., 2011. Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic. Can. J. Microbiol. 57, 303–315.

Wu, F., Zhan, J.Y., Wang, Z., Zhang, Q., 2015. Streamflow variation due to glacier melting and climate change in upstream Heihe River Basin, Northwest China. Phys. Chem. Earth Parts ABC 79, 11–19.

Wu, M.H., Chen, S.Y., Chen, J.W., Xue, K., Chen, S.L., Wang, X.M., Chen, T., Kang, S.C., Rui, J.P., Thies, J.E., 2021. Reduced microbial stability in the active layer is associated with carbon loss under alpine permafrost degradation. Proc. Natl. Acad. Sci. 118, e2025321118.

Wu, W., Tang, X.P., Yang, C., Liu, H.B., Guo, N.J., 2013. Investigation of ecological factors controlling quality of flue-cured tobacco (Nicotiana tabacum L.) using classification methods. Ecol. Inform. 16, 53–61.

Wu, X.D., Xu, H.Y., Liu, G.M., Ma, X.L., Mu, C.C., Zhao, L., 2017. Bacterial communities in the upper soil layers in the permafrost regions on the Qinghai-Tibetan plateau. Appl. Soil Ecol. 120, 81–88.

Wu, X.D., Zhao, L., Chen, M.J., Fang, H.B., Yue, G.Y., Chen, J., Pang, Q.Q., Wang, Z.W., Ding, Y.J., 2012. Soil organic carbon and its relationship to vegetation communities and soil properties in permafrost areas of the Central Western Qinghai-Tibet Plateau, China. Permafr. Periglac. Process. 23, 162–169.

Xu, L.J., Xu, X.L., Tang, X.J., Xin, X.P., Ye, L.M., Yang, G.X., Tang, H.J., Lv, S.J., Xu, D.W., Zhang, Z., 2018. Managed grassland alters soil N dynamics and N2O emissions in temperate steppe. J. Environ. Sci. 66, 20–30.

Xu, L., Zhang, Y., Wang, L., Chen, W.M., Wei, G.H., 2014. Diversity of endophytic bacteria associated with nodules of two indigenous legumes at different altitudes of the Qilian Mountains in China. Syst. Appl. Microbiol. 37, 457–465.

Yang, R.H., Wang, X.L., Su, J.H., Li, Y., Jiang, S.P., Gu, F., Yao, Y.J., 2015. Bacterial diversity in native habitats of the medicinal fungus Ophiocordyceps sinensis

on Tibetan Plateau as determined using Illumina sequencing data. FEMS Microbiol. Lett. 362, fnu044.

Yang, Z.P., Ouyang, H., Xu, X.L., Zhao, L., Song, M.H., Zhou, C.P., 2010. Effects of permafrost degradation on ecosystems. Acta Ecol. Sin. 30, 33–39.

Yuan, M.M., Guo, X., Wu, L.W., Zhang, Y.A., Xiao, N.J., Ning, D.L., Shi, Z., Zhou, X.S., Wu, L.Y., Yang, Y.F., 2021. Climate warming enhances microbial network complexity and stability. Nat. Clim. Change 11, 343–348.

Zhang, B.G., Wu, X.K., Zhang, G.S., Zhang, W., Liu, G.X., Chen, T., Qin, Y., Zhang, B.L., Sun, L.K., 2017. Response of Soil Bacterial Community Structure to Permafrost Degradation in the Upstream Regions of the Shule River Basin, Qinghai–Tibet Plateau. Geomicrobiol. J. 34, 300–308.

Zhang, G.S., Ma, X.J., Niu, F.J., Dong, M.X., Feng, H.Y., An, L.Z., Cheng, G.D., 2007. Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai–Tibet Plateau permafrost region. Extremophiles 11, 415–424.

Zhang, T., Heginbottom, J.A., Barry, R.G., Brown, J., 2000. Further statistics on the distribution of permafrost and ground ice in the Northern Hemisphere. Polar Geogr. 24, 126–131.

Zhang, X.F., Xu, S.J., Li, C.M., Zhao, L., Feng, H.Y., Yue, G.Y., Ren, Z.W., Cheng, G.D., 2014. The soil carbon/nitrogen ratio and moisture affect microbial community structures in alkaline permafrost-affected soils with different vegetation types on the Tibetan plateau. Res. Microbiol. 165, 128–139.

Zhao, L., Cheng, G.D., Li, S.X., Zhao, X.M., Wang, S.L., 2000. The freezing and melting process of the permafrost active layer near Wu Dao Liang region on Tibetan Plateau. Chin. Sci. Bull. 45, 1205–1211.

Zhao, W., Yin, Y.L., Li, S.X., Liu, Y., Liu, J.J., Dong, Y.L., Su, S.F., Ji, L.H., 2021. The characteristics of bacterial communities in different vegetation types in the Qilian Mountains. Acta Prataculturae Sin. 30, 161.

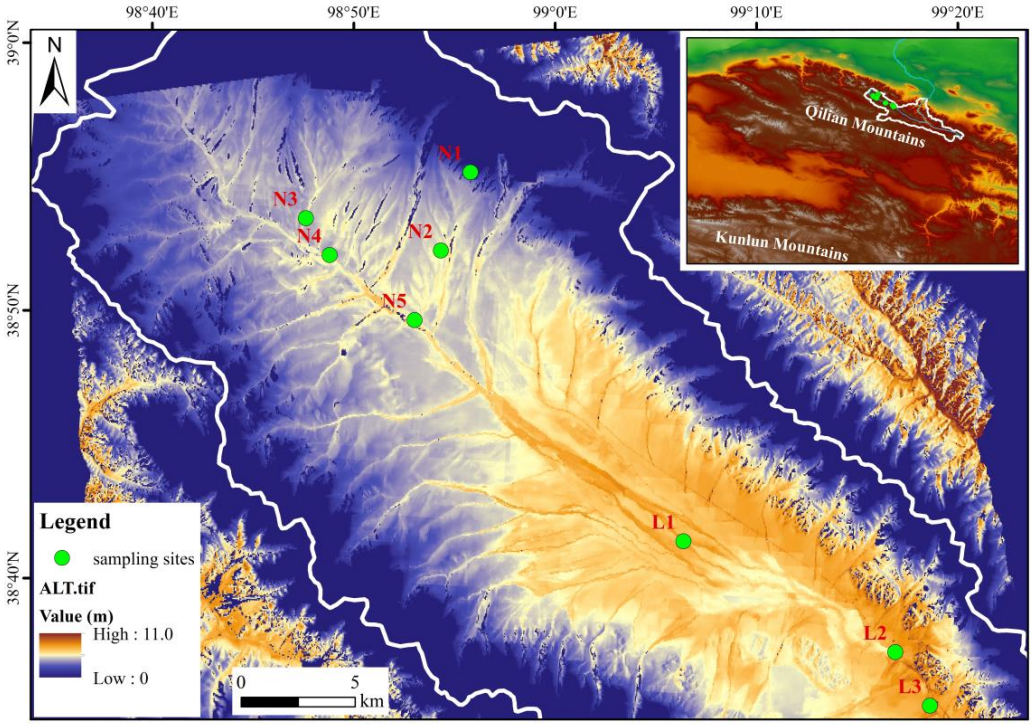

Fig. 1 Sampling sites with locations spanning permafrost soils (N1-N5) and seasonally frozen soils (L1-L3) in the upstream region of the Heihe River.

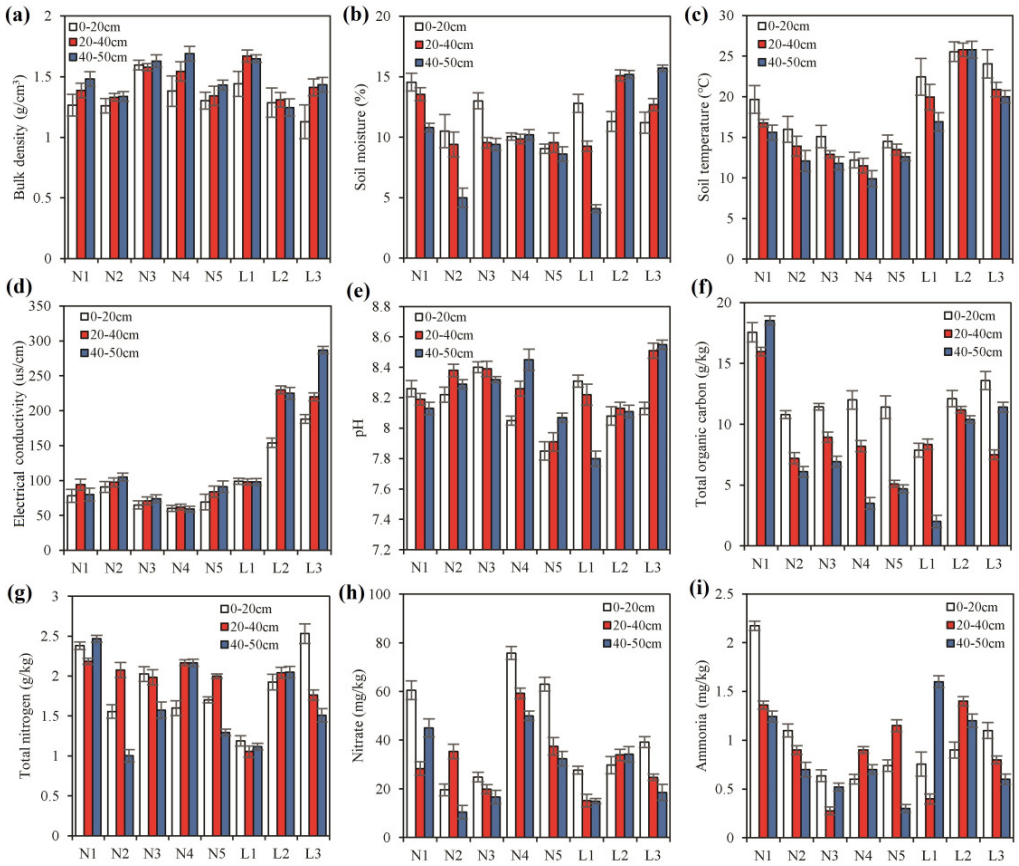

Fig.2. Soil properties of permafrost soils (N1-N5) and seasonally frozen soils (L1-L3) under different layers (white: 0-20 cm, red: 20-40 cm, blue: 40-50 cm) at sampling sites: (a) Bulk density, (b) Soil moisture, (c) Soil temperature, (d) Electrical conductivity, (e) pH, (f) Total organic carbon, (g) Total nitrogen, (h) Nitrate, and (i) Ammonia. All data are presented as "mean ± standard deviation" calculated from triplicated samples.

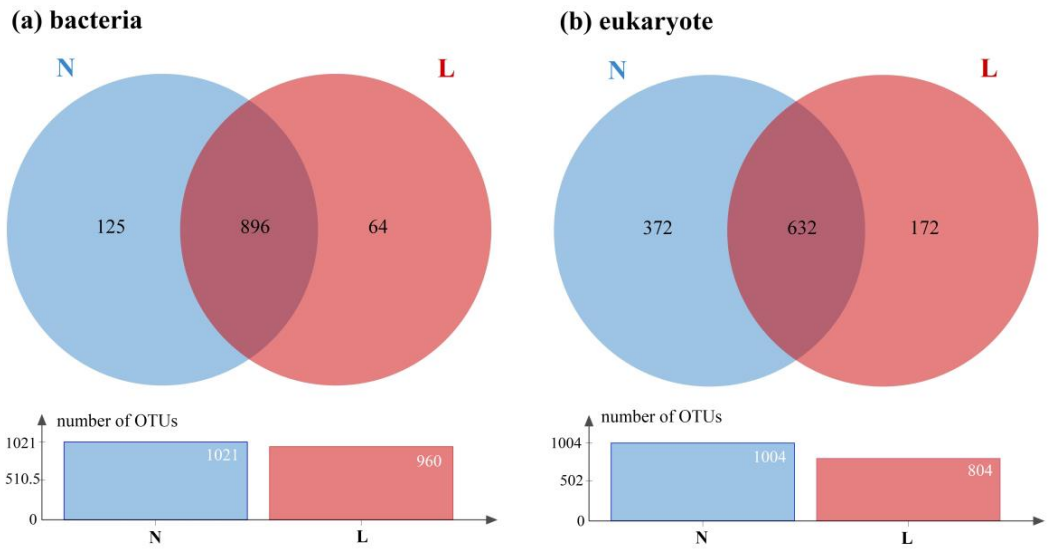

Fig. 3. Venn diagram of OTUs among the permafrost soils (N), and seasonally frozen soils (L) for bacteria (a) and micro-eukaryotes (b), with the total number of OTUs in each group shown on the histogram.

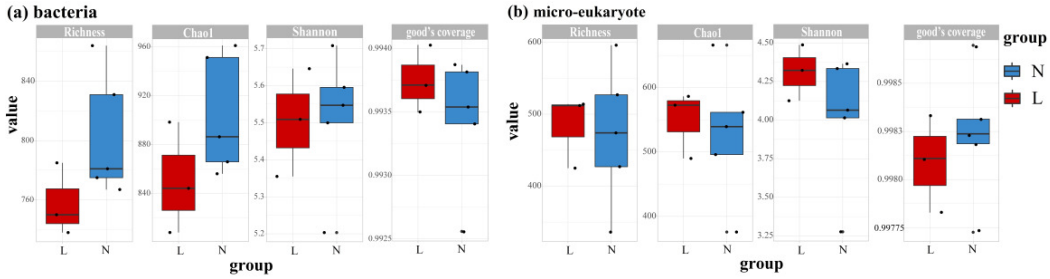

Fig. 4. Alpha diversity of permafrost soils (N) and seasonally frozen soils (L) for bacteria (a) and micro-eukaryotes (b).

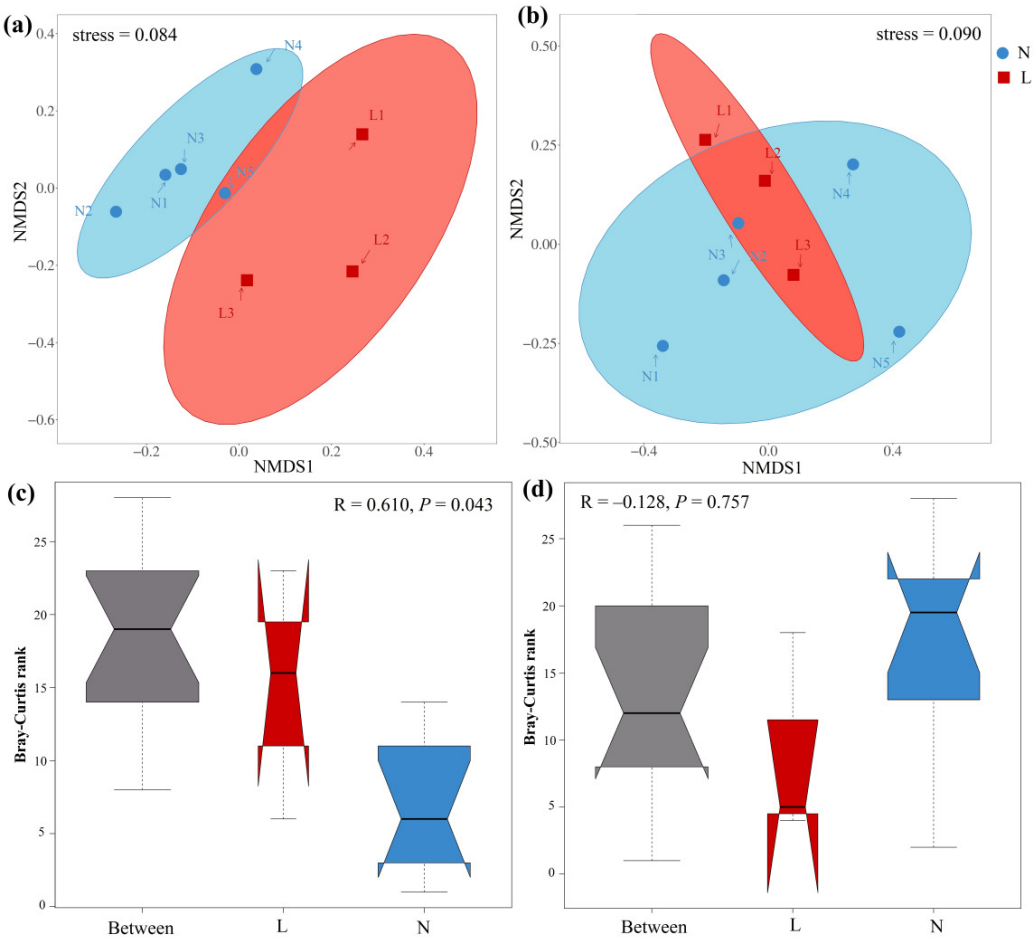

Fig. 5. Nonmetric multidimensional scaling (NMDS) plots of bacterial community (a) and eukaryote (b) across the permafrost degradation gradient; Analysis of similarity (ANOSIM) plots of bacterial community (c) and eukaryote (d) across the permafrost degradation gradient. Stress > 0 means

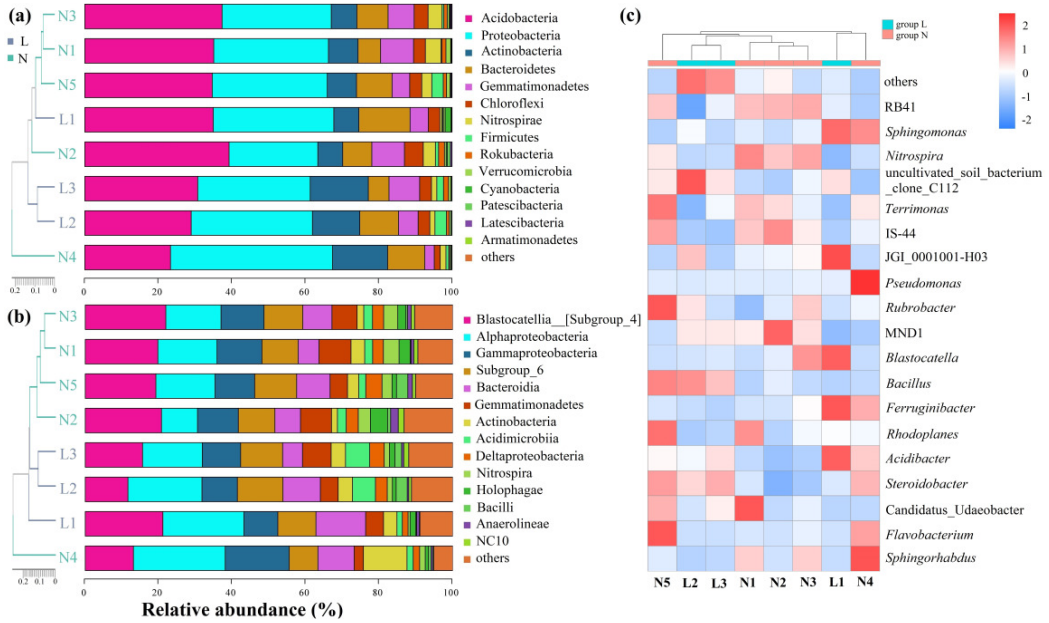

Fig.6. Relative abundance of the soil bacterial community under the permafrost soils (N1-N5) and seasonally frozen soils (L1-L3): (a) Phylum, (b) Class, and (c) Classified Genus. “Others” represents sequences that were unclassified and sequences that were less than 1% of the total.

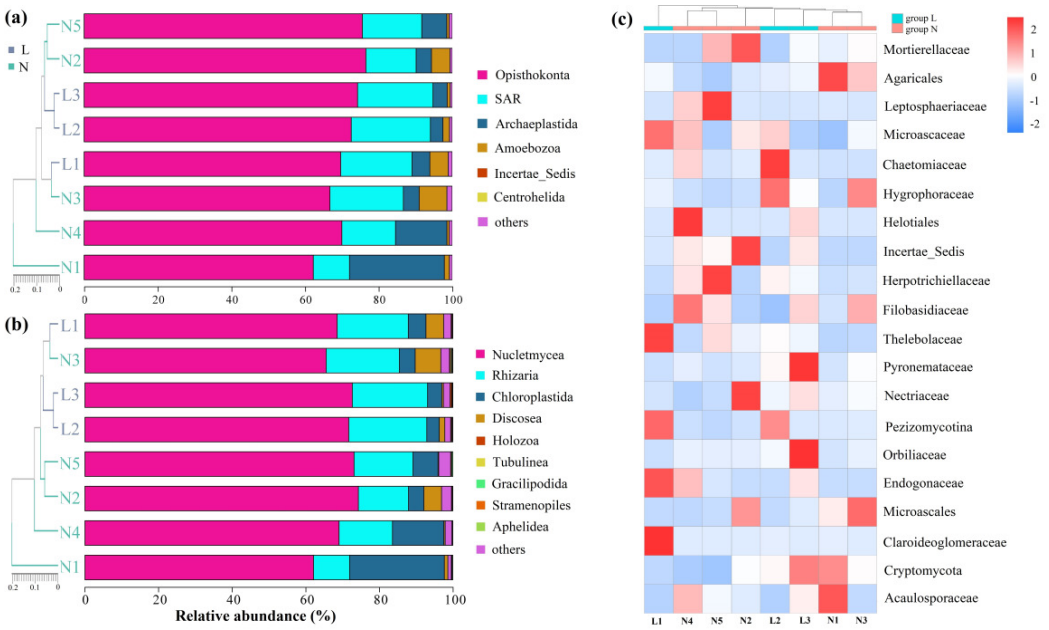

Fig.7. Relative abundance of the eukaryotic community under the permafrost soils (N1–N5) and seasonally frozen soils (L1–L3): (a) Kingdom, (b) Phylum, and (c) Fungal groups of Nucletmycea. “Others” represents sequences that were unclassified and sequences that were less than 1% of the total.

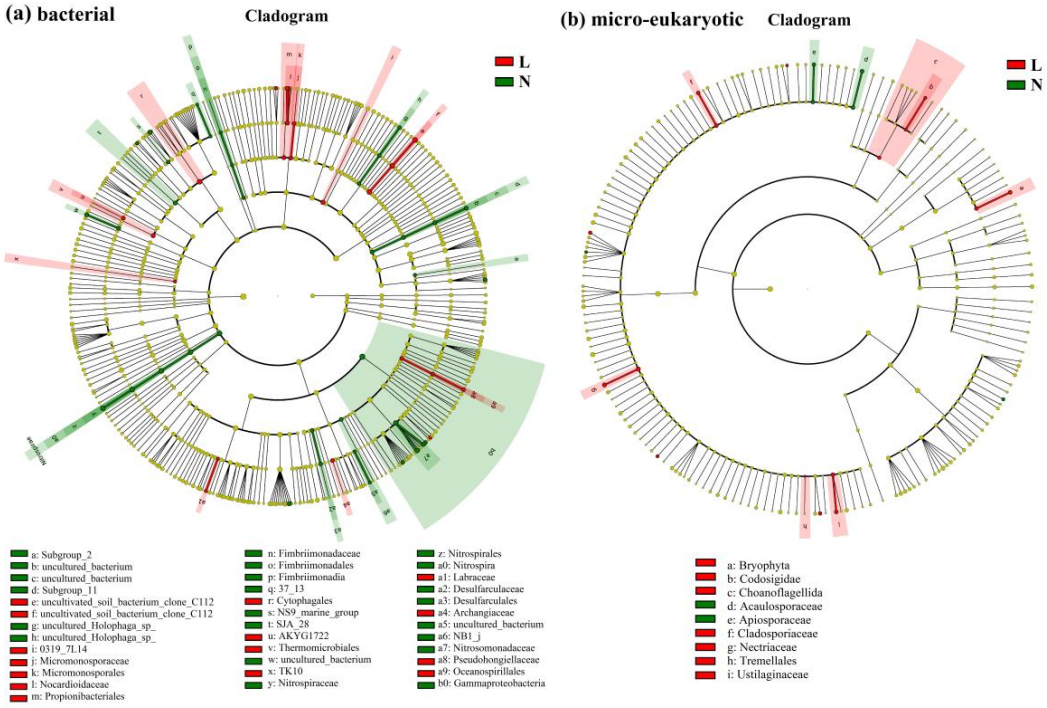

Fig. 8. LEfSe analysis and Histogram of LDA scores with a threshold value of 2.0 of soil microbial abundance in permafrost soils (group N) and seasonally frozen soils (group L): soil bacteria (a) and eukaryote (b). Small circles and shading with different colors illustrate the taxa that are enriched in group N and group L.

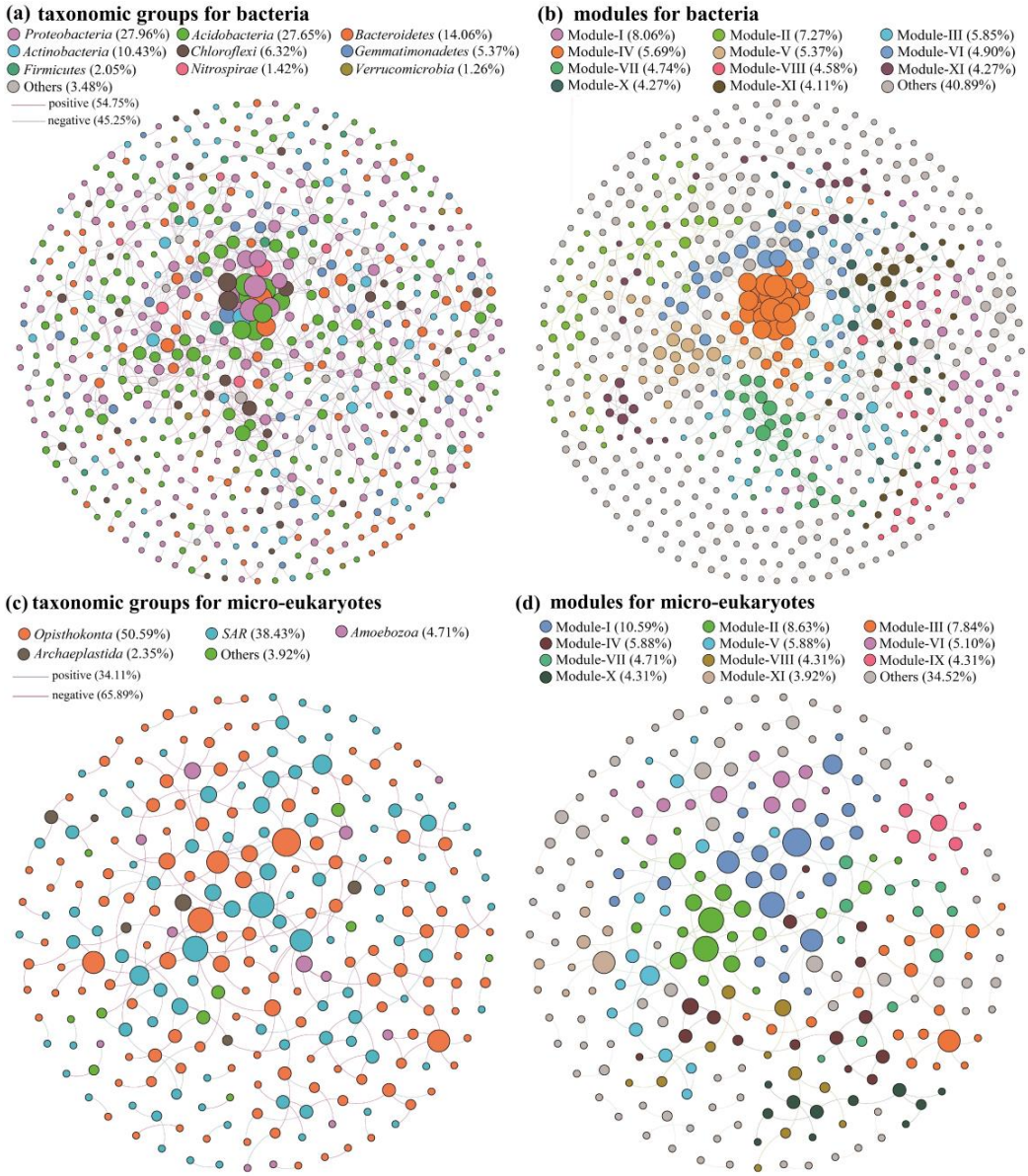

Fig. 9. The co-occurrence networks among the OTUs of soil bacterial and micro-eukaryotic communities colored by taxonomic groups ((a), (c)) and modules ((b), (d)) based on Spearman correlation analysis.

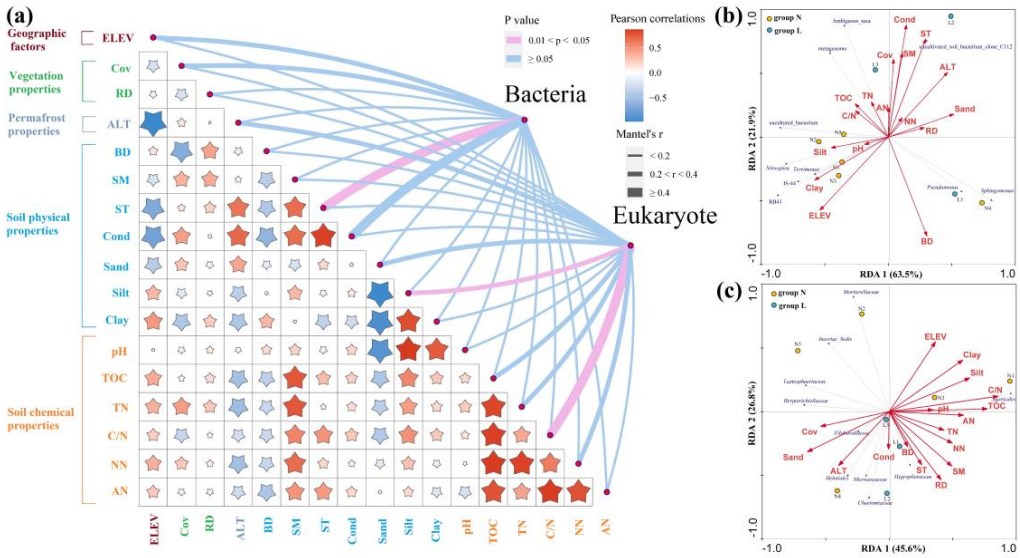

Fig. 10. Relationship between environmental factors and soil microbial communities at phylum level revealed by (a) Mantel test and at genus level revealed by RDA analysis for (b) bacterial community and (c) micro-eukaryotic community. ELEV, RD, Cov, ALT, BD, SM, ST, and Cond indicate elevation, root depth, coverage, active layer thickness, bulk density, soil moisture, soil temperature, and electrical conductivity, respectively. TOC, TN, C/N, NN, and AN indicate total organic carbon, total nitrogen, the ratio of C and N, nitrate, and ammonia, respectively. The size of the star is in proportion to the correlation among physicochemical properties. The arrow length corresponds to the variation of microbial community structure that can be explained by the environmental factors and the direction indicates an increasing magnitude of the soil property. Group N and L represent permafrost soils (N1–N5) and seasonally frozen soils (L1-L3), respectively.

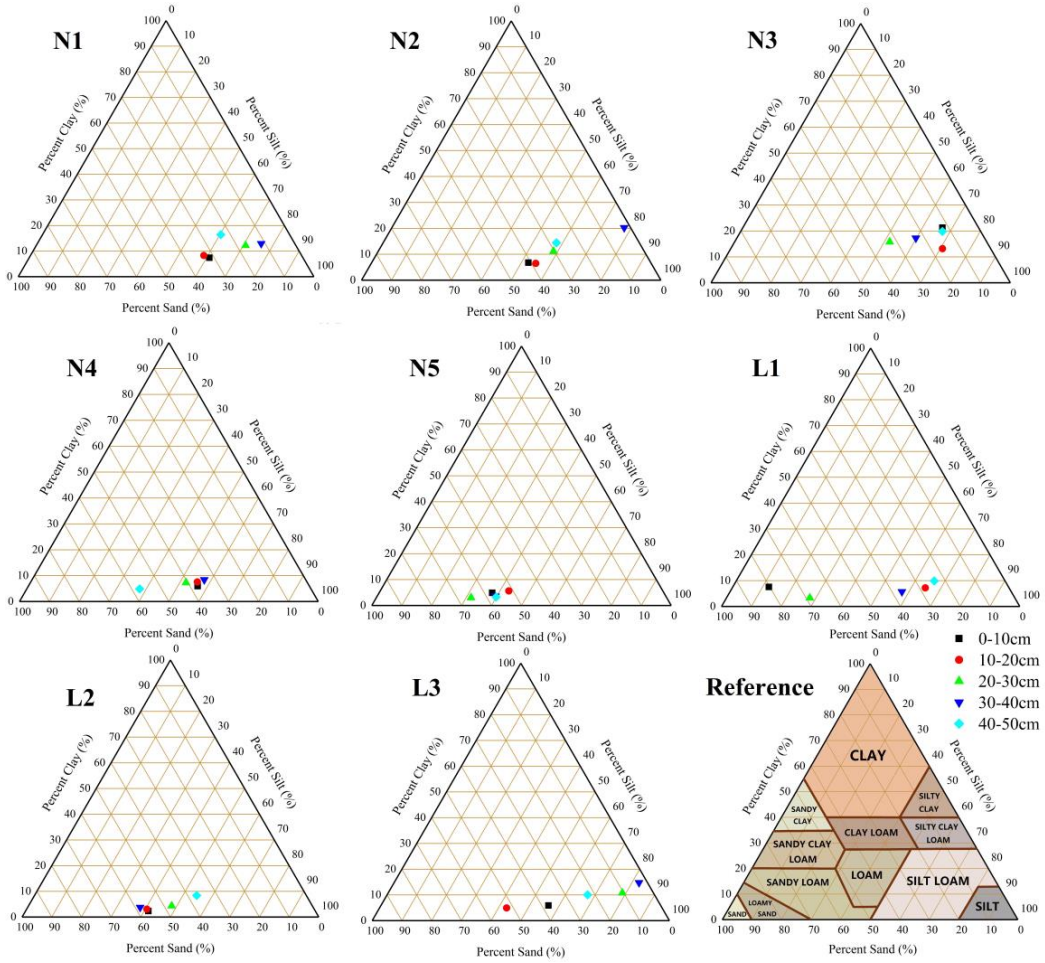

Fig. S1. Soil particle size of sampling sites from permafrost soils (N1-N5) and seasonally frozen soils (L1-L3) under different layers (black: 0-10 cm, red: 10-20 cm, green: 20-30 cm, dark blue: 30-40 cm, light blue: 40-50 cm).

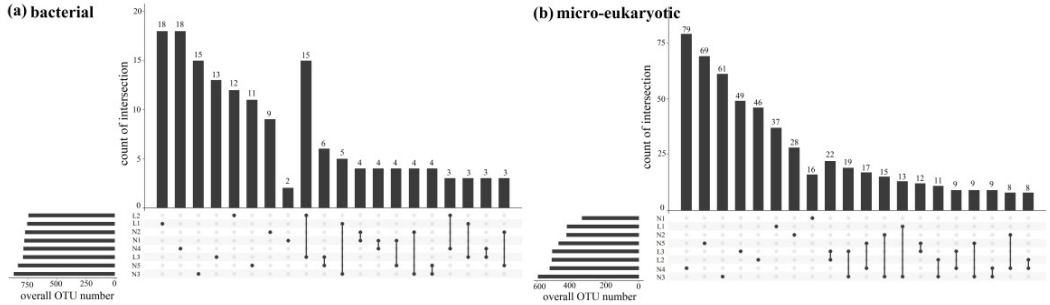

Fig. S2. Upset diagrams of OTUs among the permafrost soils (N) and seasonally frozen soils (L) for bacteria (a) and micro-eukaryotes (b).