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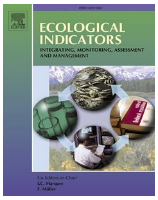

Soil bacterial communities in alpine wetlands in arid Central Asia remain stable during the seasonal freeze–thaw period

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ABSTRACT

Under the background of climate change, freeze–thaw patterns tend to be turbulent: ecosystem function processes and their mutual feedback mechanisms with microorganisms in sensitive areas around the world are currently a hot topic of research. We studied changes of soil properties in alpine wetlands located in arid areas of Central Asia during the seasonal freeze–thaw period (which included an initial freezing period, a deep freezing period, and a thawing period), and analyzed changes in soil bacterial community diversity, structure, network in different stages with the help of high-throughput sequencing technology. The results showed that the α diversity of the soil bacterial community showed a continuous decreasing trend during the seasonal freeze–thaw period. The relative abundance of dominant bacterial groups (Proteobacteria (39.04%–41.28%) and Bacteroidota (14.61%–20.12%)) did not change significantly during the freeze–thaw period. At the genus level, different genera belonging to the same phylum dominated in different stages, or there were clusters of genera belonging to different phylum. For example, *g_Ellin6067*, *g_unclassified_f_Geobacteraceae*, *g_unclassified_f_Gemmatimonadaceae* coexisted in the same cluster, belonging to Proteobacteria, Desulfobacterota and Gemmatimonadota respectively, and their abundance increased significantly during the freezing period. This "adaptive freeze–thaw" phylogenetic model suggests a heterogeneous stress resistance of bacteria during the freeze–thaw period. In addition, network analysis showed that, although the bacterial network was affected to some extent by environmental changes during the initial freezing period and its recovery in the thawing period lagged behind, the network complexity and stability did not change much as a whole. Our results prove that soil bacterial communities in alpine wetlands are highly resistant and adaptive to seasonal freeze–thaw conditions. As far as we know, compared with short-term freeze–thaw cycles research, this is the first study examining the influence of seasonal freeze–thaw on soil bacterial communities in alpine wetlands. Overall, our findings provide a solid base for further investigations of biogeochemical cycle processes under future climate change.

1. Introduction

Being at the transitional zone between land and water, wetland ecosystems play an irreplaceable role in maintaining freshwater resources, protecting biodiversity, regulating regional climate, and sustaining social production (Chen et al., 2021; Hu et al., 2022). Wetlands are the terrestrial ecosystems with the highest carbon storage per unit area: a parameter considered as an indicator of climate change. The arid area of Central Asia is the largest non-zonal arid area in the world with high climate change sensitivity (Wen et al., 2023). The Swan Lake Wetland in Bayinbuluk, an alpine wetland located in this arid region, is of great value in maintaining the stability of regional water resource systems and ecosystems (Yao et al., 2021). In the last 40 years, the area and the interval of time characterized by soil freezing in the northern hemisphere have been significantly reduced; moreover, the freeze–thaw state has shown larger changes in Eurasia than in North America (Wu

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et al., 2022). The rate of climate change in alpine regions is 2–3 times that in plain regions, and the ecological function processes of the pedosphere, hydrosphere, and biosphere are affected by complex changes in the freeze–thaw pattern (Broadbent et al., 2021). Alpine wetlands in the arid region of Central Asia have multiple identities: they are considered as arid regions, alpine regions, and wetlands. Since they have been identified as extremely sensitive to global climate change, their ecosystem stability and biogeochemical cycle processes need to be explored urgently.

Freeze–thaw is a natural phenomenon commonly occurring at middle and high latitudes, in which the surface soil water and the heat conditions change due to temperature fluctuations (Rosinger et al., 2022; Zhao et al., 2022). Compared with permafrost, seasonal frozen soil (which freezes in winter and melts in summer) has a wider distribution range and contains larger amounts of soil nutrients and microbial resources (Wu et al., 2022). The freeze–thaw process, and especially the involved changes of water phase, can directly destroy plant residues and soil aggregates, as well as kill and dissolve some microorganisms. In the meanwhile, a large amount of nutrients is leached and released (Li et al., 2022). Previous studies have proved that the composition of soil microbial communities is driven by temperature and humidity during the freeze–thaw period. Certain microorganisms may enter a state of dormancy or perish due to their limited ability to adapt. Conversely, microorganisms that possess a high level of resistance are capable of sustaining a robust physiological activity during winter (under low temperature and drought conditions) by utilizing the ample organic matter that is released from deceased microbial cells or present in the soil (Schostag et al., 2019; Meisner et al., 2021; Zong et al., 2023). Microbial communities are integral to the various biogeochemical cycling processes observed throughout terrestrial ecosystems. Consequently, the manner in which microbial composition and diversity react to global changes has a direct impact on its function in facilitating elemental cycle and upholding ecosystem stability (De Nijs et al., 2019). There remain ambiguities pertaining to the features and survival strategies employed by microbial communities, as well as the role of nutrient cycles within this framework.

Bacteria are generally expected to show obvious stage characteristics during the freeze–thaw period (Perez-Mon et al., 2020; Sang et al., 2021). A recent *meta*-analysis on the potential impact of global freezing and thawing on soil microbial community showed that the freezing and thawing period did not reduce the diversity of microbial, but significantly changed the structure of soil microbial community (Ji et al., 2022). Based on the Meta-analysis, the team further carried out the research on the influence of freezing and thawing on the microbial diversity of forest soil. The results showed that the diversity, composition and symbiotic network of bacterial communities did not change significantly (Liu et al., 2022). The possible reason of inconsistent results is that litter or snow affects the freezing strength of soil and the variations in soil background characteristics (Ji et al., 2022). Naturally, bacteria could utilize a suite of ecological strategies, such as oligotrophic or copiotrophs, or have different C-utilization strategies. Within the framework of copiotrophic/oligotrophic system development, copiotrophic bacteria respond relatively quickly to the increase of the availability of unstable nutrients, showing a high growth rate, while oligotrophic bacteria usually have a low growth rate and respond slowly and rarely to the increase of the availability of unstable nutrients. Trophic lifestyle may indicate the response of bacterial groups to soil freezing and thawing process and nutrient changes (Fierer et al., 2007; Schostag et al., 2019). Furthermore, it should be considered that bacterial taxa responding to nutrient changes driven by freeze–thaw events may have an “adaptive freeze–thaw” phylogenetic model (Sorensen et al., 2020). Adaptive freeze–thaw refers to the overall stability of community diversity under freeze–thaw stress, but community composition responds to freeze–thaw events at a small scale (such as genus level). Research conducted in alpine grasslands has revealed a significant correlation between the temporal dynamics of soil microbial community composition and variations in soil microbial function and biogeochemical cycling (Broadbent et al., 2021). The bacterial groups contributing to breaking down organic compounds increased rapidly in winter, and the dominant bacteria such as actinomycetes and Bacillus were closely related to cellulose degradation (Broadbent et al., 2021). The distribution of dominating bacteria, which are capable of acquiring resources for survival and rapid growth, is not random but rather exhibits clustering patterns (Sorensen et al., 2020; Broadbent et al., 2021; Isobe et al., 2022). Previous studies have shown comparable findings in many environments, including forests, farmland and polar ecosystems, indicating that the structure of soil microbial communities during winter differs from that observed in other seasons (Perez-Mon et al, 2020; Isobe et al., 2022; Zong et al, 2023). The investigation demonstrates that phylogenetically patterned responses have the potential to enhance our predictive comprehension of microbial community reactions to winter climate change (Isobe et al., 2022). This research contributes to the advancement of knowledge in the fields of microbial ecology and biogeochemistry.

The impact of global change on microbial diversity and structure has significant implications for their function in driving element cycling and upholding ecosystem stability (De Nijs et al., 2019; Ji et al., 2022). The cyclical fluctuations in soil temperature and water phase in the process of freezing and thawing cause direct physical damage to microorganisms, as well as indirect consequences such as alterations in soil structure and the redistribution of nutrients and mineral elements (Ren et al., 2018). The alterations in the soil microbial community caused by freezing and thawing can potentially impact its capacity to sustain soil nutrients (Ji et al., 2022). A particular study underscored the significance of seasonal variations in soil microbial communities their impact on biogeochemical cycles. The study discovered that the process of thawing induced a sudden change in the composition of soil microbial communities in alpine grassland. This shift was found to be strongly linked to alterations in soil microbial functioning, as well as changes in biogeochemical pools and fluxes. The impact of precipitation and temperature on the freeze–thaw cycle of alpine wetlands, and their subsequent influence on ecosystem function processes, has been identified as a crucial factor (Broadbent et al., 2021). This phenomenon gives rise to interconnected inquiries: What are the implications of freeze–thaw cycles on biogeochemical cycling? To what extent are bacterial communities that have undergone significant changes in their composition susceptible to climate variations and environmental shifts? Is it possible that the seasonal freeze–thaw cycles have a minimal impact on the microbial community? Can the soil microbial communities that have adapted to freezing and thawing in alpine wetlands be utilized as indicators of stress tolerance in the face of the constantly shifting freezing and thawing conditions? Will there be a succession of microbial communities that adapt to the intensifying freeze–thaw pattern of climate change? The aforementioned inquiries are of pressing nature and necessitate resolution (Bahram et al., 2018; Garcia et al., 2020; Ji et al., 2022).

Past research on the evolution of soil microbial communities during the freeze–thaw period has mostly focused on permafrost regions and forest ecosystems; meanwhile, wetland ecosystems, which are characterized by particularly complex changes in hydrothermal conditions, have not been considered much (Chen et al., 2021). Additionally, most previous studies have been based on laboratory simulation experiments, which cannot provide accurate information about the survival strategy of microorganisms under real natural conditions (Sang et al., 2021; Ji et al., 2022). Most previous research has focused on short-term freeze–thaw cycles (i.e., day-and-night cycles), but not on longer-term, periodic seasonal freeze–thaw processes (Song et al., 2017; Ren et al., 2018). Due to the above limitations, it is currently difficult to disentangle biogeochemical cycle processes under the background of intensified climate changes and unstable freeze–thaw patterns. Therefore, in order to study the stage change characteristics of bacterial community diversity and structure (symbiotic network) in wetland soil during seasonal freezing and thawing, and to investigate the survival potential and strategy of soil bacterial communities (such as "adaptive freeze–thaw" soil microbial groups as the stress tolerance indicators) in alpine wetlands during the seasonal freeze–thaw period, we carried out field experiments in an alpine wetland in northwest China. Based on previous research results, we assumed that: (1) during seasonal freezing and thawing, the bacterial community diversity would change in synchronicity with soil temperature and soil moisture; (2) the low temperature and drought conditions experienced in winter would reduce the complexity and stability of the bacterial community, and the community structure would show obvious stage characteristics. Overall, the results of this study can enrich the research on the functional process of alpine wetland ecosystem of Central Asia, provide scientific basis for the research on microbial mechanism of biogeochemical cycle of alpine wetland, and have practical guiding significance for the formulation of management measures for alpine wetland ecosystem to adapt to future climate change.

2. Materials and methods

2.1. Study design and sample collection

The Swan Lake Wetland in Bayinbuluk, northwest China (N 42°40′–43° 00′, E 83°40′–84° 35′), has an important ecological barrier function in the arid region of Central Asia. It covers a total area of 770 km^2 and is located at an altitude of 2300–3042 m, where the annual winter minimum temperature remains below − 35 °C. The main soil type is swamp soil (Chen et al., 2021; Abulaizi et al., 2023). According to historical meteorological data and real-time monitoring data, four batches of topsoil (0–10 cm depth) samples (i.e., A10 (October 2021, topsoil temperature was lower than 0°C for the first time), B12 (December 2021, the average daily temperature of topsoil was below −5°C), C01 (January 2022, the average daily temperature of topsoil was below −10°C)), and D05 (May 2022, the average daily temperature of topsoil was higher than 10°C)) were collected during the seasonal freeze–thaw period (from October 2021 to May 2022). Meanwhile, the three consecutive sampling intervals were defined as the initial freezing period (frost appeared on the soil surface, the topsoil temperature gradually dropped below 0°C, and the soil moisture plummeted), the deep freezing period (topsoil temperature continued to drop, the soil was deeply frozen and mixed with ice, and the soil moisture gradually decreased slowly), and the thawing period (topsoil temperature continued to increase, the ice in the soil completely melted, and the soil moisture rose rapidly) (Fig. 1). Four repeated plots (3 m × 3 m, at a respective distance > 30 m) were selected in the field. Four composite samples were hence collected from each plot diagonally in each batch (Fig. 2). A total of 16 composite samples were obtained in this way and stored in liquid nitrogen for high-throughput sequencing. In addition, 48 composite samples were collected by the same sampling method for analyzing the soil characteristics and as backup (Hu et al., 2021).

2.2. Soil properties analysis

Data of soil temperature (ST) and soil moisture (SM) were recorded and transmitted by a weather station. Meanwhile, soil pH was measured by a pH meter, the soil organic carbon (SOC) content was measured with the external heating method (involving sample dissolution in H_2SO_4–$K_2Cr_2O_7$), and the total nitrogen (TN) content was measured with the Kjeldahl digestion–distillation method (Lu, 2000).

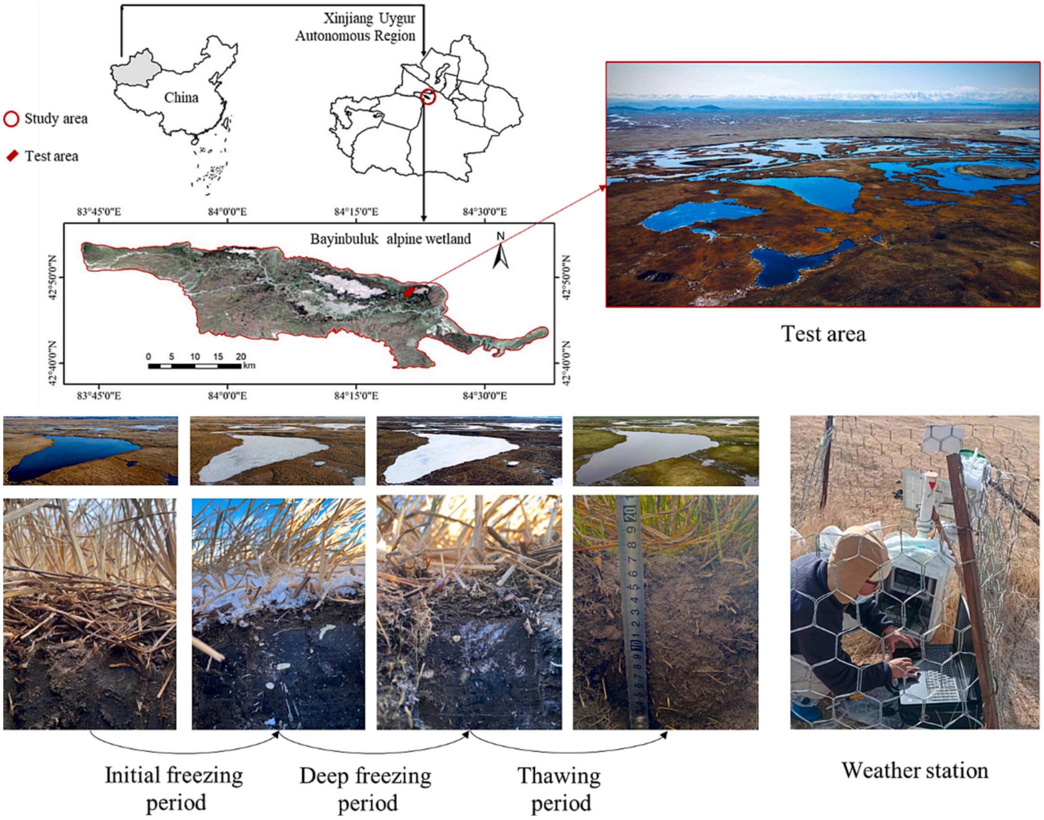

Fig. 1. The sampling location overview map and the landscape pictures. The location of the study area and the test area in Bayinbuluk alpine wetland. Gray area: Xinjiang Uygur Autonomous Region, China. The profiles of the surface soil (depth > 10 cm) in four batches of sampling, and the three intervals of seasonal freeze–thaw period (initial freezing period, deep freezing period and thawing period). Weather station was used to measure and record soil temperature and moisture data.

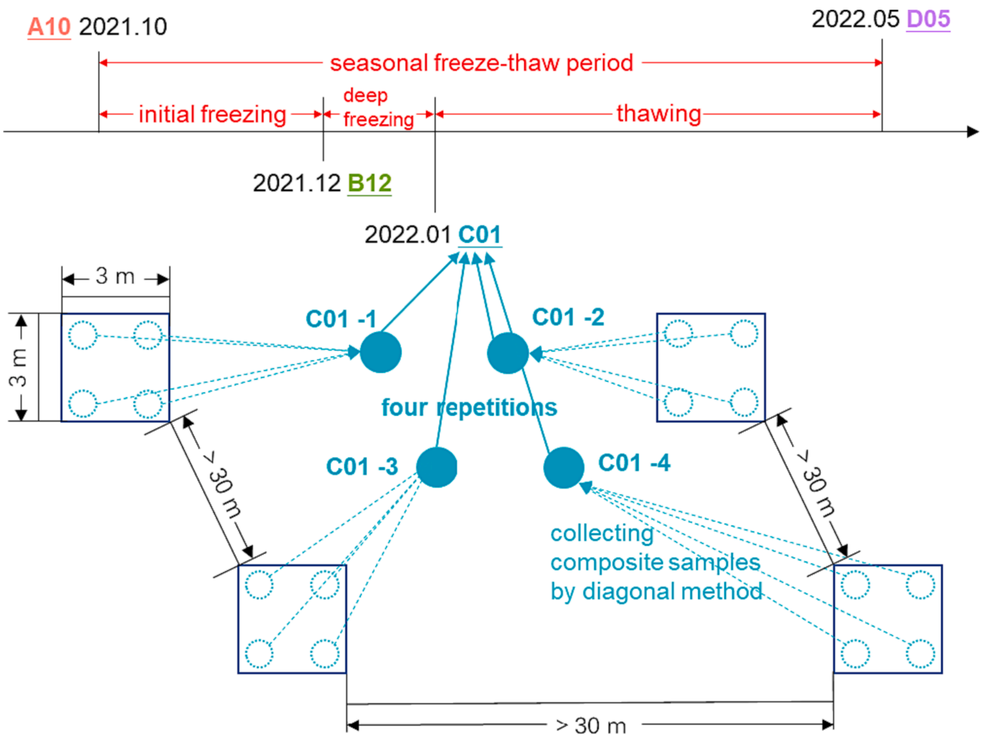

Fig. 2. Schematic diagram showing the stages of the seasonal freeze–thaw period and the sampling times. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period. The consecutive sampling intervals were defined as the initial freezing period, the deep freezing period and the thawing period. The sampling method is illustrated with C01 as an example.

2.3. DNA extraction, sequencing, and bioinformatics analysis

Soil microbial DNA was extracted by using a Power Soil DNA Isolation kit (MoBio Laboratories Inc., CA, USA). The DNA was determined by spectrophotometry with a NanoDrop-2000 (Thermo Scientific, MA, USA). The bacterial V3–V4 region was amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). All bioinformatics services were performed using an Illumina HiSeq 2500 (Chen et al., 2021). Trimmomatic (v. 0.33) was used to filter reads (raw reads counts: 79683–80243) obtained by sequencing. Cutadapt (v. 1.9.1) was used to identify and remove primer sequences. Usearch (v. 10.0) was used for splicing and length filtering of clean reads. Low-quality sequences were removed, and sequences with ambiguous base calls were removed. DADA2 method in QIIME2 (v. 2020.6) was applied to denoise sequences and remove chimera sequence, obtain valid data (non-chimeric reads counts: 52205–63938) and generate ASVs. Naive Bayesian classifier was used to annotate the feature sequence in taxonomy with Silva (Release138, http://www.arb-silva.de) as the reference database. The sequence read archive (SRA) records are accessible at the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA928857.

2.4. Statistical analyses

R (v. 4.1.1) and ggplot2 package (v. 3.3.6) were used for data analysis and drawing. Moreover, the EasyStat (v. 0.1.0) package was used to analyze the differences between groups, the vegan (v. 2.5–7) package for non-metric multidimensional scaling (NMDS) analysis, the microeco (v. 0.12.1) package and the pheatmap (v. 1.0.12) package for stacking column charts and heat maps, the ggvegan (v. 0.1.0) package for redundancy analysis (db-RDA), the linkET (v. 0.0.2.9) package for correlation analysis and Mantel testing, and the phyloseq (v. 1.39.1), ggClusternet (v. 0.1.0), igraph (v. 1.2.11), and Gephi (v. 0.9.7) packages for network analysis (Yuan et al., 2021; Wen et al., 2022).

3. Results

3.1. Changes in soil properties during the seasonal freeze–thaw period

During the freeze–thaw period, we observed a significant change of the soil properties (Fig. 3). Soil temperature and moisture decreased significantly in groups B12 and C01 (from 10.34 ℃ and 99.78 % in group A10 to − 5.96 ℃ and 21.13 % in group B12, and to − 14.44 ℃ and 12.09 % in group C01, respectively), while they increased significantly in group D05 (to 12.30 ℃ and 64.68 %, respectively). Meanwhile, soil pH, SOC, and TN increased significantly from A10 to groups B12 and C01, but decreased significantly ($P < 0.05$) in group D05. Soil SOC and TN decreased from 107.14 g kg^{-1} and 7.66 g kg^{-1} in group C01 to 82.05 g kg^{-1} and 7.13 g kg^{-1} in group D05.

3.2. Changes in soil bacterial community diversity during the seasonal freeze–thaw period

During the freeze–thaw period, the α-diversity of the soil bacterial community changed (Fig. 4a,b). The Shannon diversity and Chao1 indexes of group D05 were the lowest, but there was no significant difference with B12 and C01 group. The Shannon diversity and Chao1 indexes of groups B12, C01, and D05 were significantly lower than those of group A10 ($P < 0.05$) (Fig. 4a,b). Our NMDS analysis revealed a pressure function value of 0.1231, and that the ranking model had a certain explanatory significance to the distance between samples (Fig. 4c). There was an obvious overlap between the samples of each group, which indicated that the β-diversity of soil bacterial communities during the freezing and thawing period was different but not significant (Fig. 4c).

3.3. Changes in soil bacterial community structure during the seasonal freeze–thaw period

During the freeze–thaw period, the dominant phyla of the soil

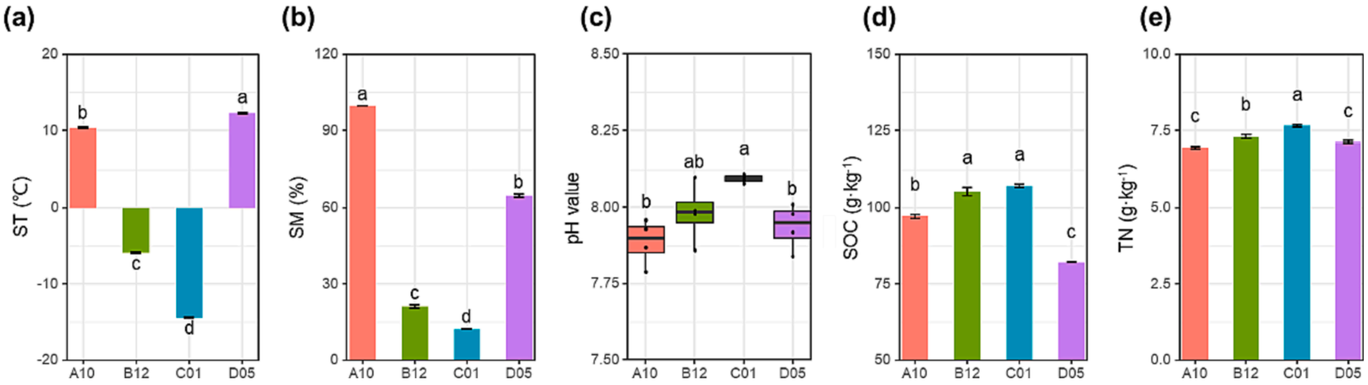

Fig. 3. Changes in soil properties during the seasonal freeze–thaw period. Results are shown as the mean ± se. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period. The different letters at the top of the bar chart (or box chart) indicate significant differences between groups, and the bar heights marked by a, ab, b, c and d decrease significantly in turn ($p < 0.05$). ST, soil temperature; SM, soil moisture; SOC, soil organic carbon; TN, total nitrogen.

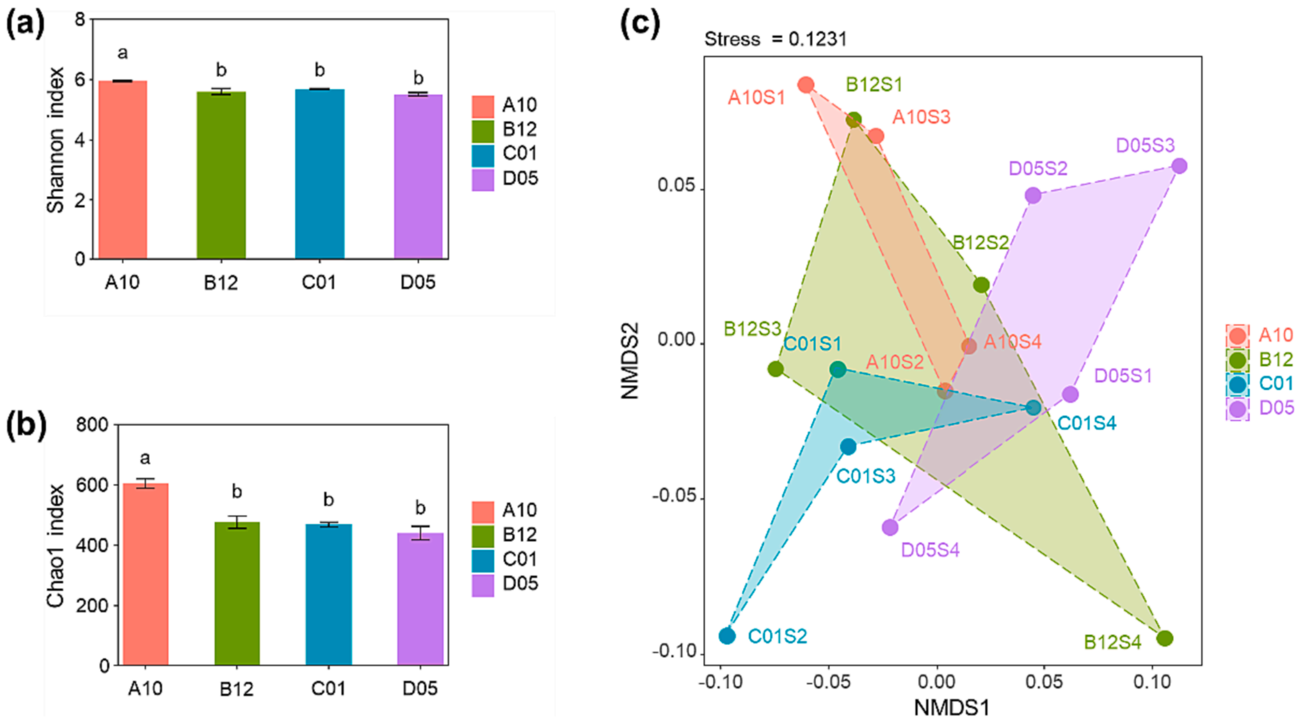

Fig. 4. Changes in soil bacterial community diversity during the seasonal freeze–thaw period. (a) Changes in the Shannon diversity index during the seasonal freeze–thaw period. The different letters at the top of the bar chart indicate significant differences between groups, and the bar marked with a is significantly higher than that marked with b ($p < 0.05$). (b) Changes in the Chao1 diversity index during the seasonal freeze–thaw period. The different letters at the top of the bar chart indicate significant differences between groups, and the bar marked with a is significantly higher than that marked with b ($p < 0.05$). (c) Changes in β-diversity during the seasonal freeze–thaw period. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period.

bacterial community (Proteobacteria (39.04 %–41.28 %), Bacteroidota (14.61 %–20.12 %), and Acidobacteriota (11.98 %–13.27 %)) showed higher relative abundance followed by Chloroflexi (7.46 %–12.14 %), Desulfobacterota (5.80 %–8.37 %), Actinobacteriota (2.91 %–5.29 %), Myxococcota (1.41 %–5.53 %), Gemmatimonadota (0.72 %–2.17 %), Nitrospirota (0.73 %–2.11 %) and Firmicutes (0.42 %–1.73 %) (Fig. 5a). There was no significant difference in the dominant phyla between the groups. The relative abundance of Chloroflexi and Firmicutes was significantly higher in group D05 than that in other groups. The relative abundance of Desulfobacterota, Actinobacteriota and Nitrospirota was significantly higher in group C01 than that in other groups. Interestingly, the relative abundance of Myxococcus and Gemmatimonadota was relatively low, but they were the only phyla in group A10 and B12, respectively, which were significantly higher than those in other groups ($P < 0.05$). At the class level (TOP10), similarly to the phylum level, there were no significant differences in terms of dominant bacteria (Gammaproteobacteria (26.40 %–34.01 %) and Bacteroidia (11.39 %–16.71 %)) among groups of samples (Fig. 5b). The relative abundances of Alphaproteobacteria, Polyangia, Acidobacteriae, and Acidimicrobiia were the lowest in group D05, while the relative abundance of Anaerolineae was the highest in group D05. The relative abundance of Alphaproteobacteria decreased significantly from 12.71 % in group C01 to 5.97 % in group D05, while the relative abundance of Anaerolineae increased significantly from 6.91 % in group C01 to 11.93 % in group D05 ($P < 0.05$). At genus level (TOP30), the changes in bacterial community structure were evident (Fig. 5c). The relative abundances of 6 of the 7 genera included in Sub1 were higher in A10 compared to other groups, and extremely low in group D05. On the contrary, the relative

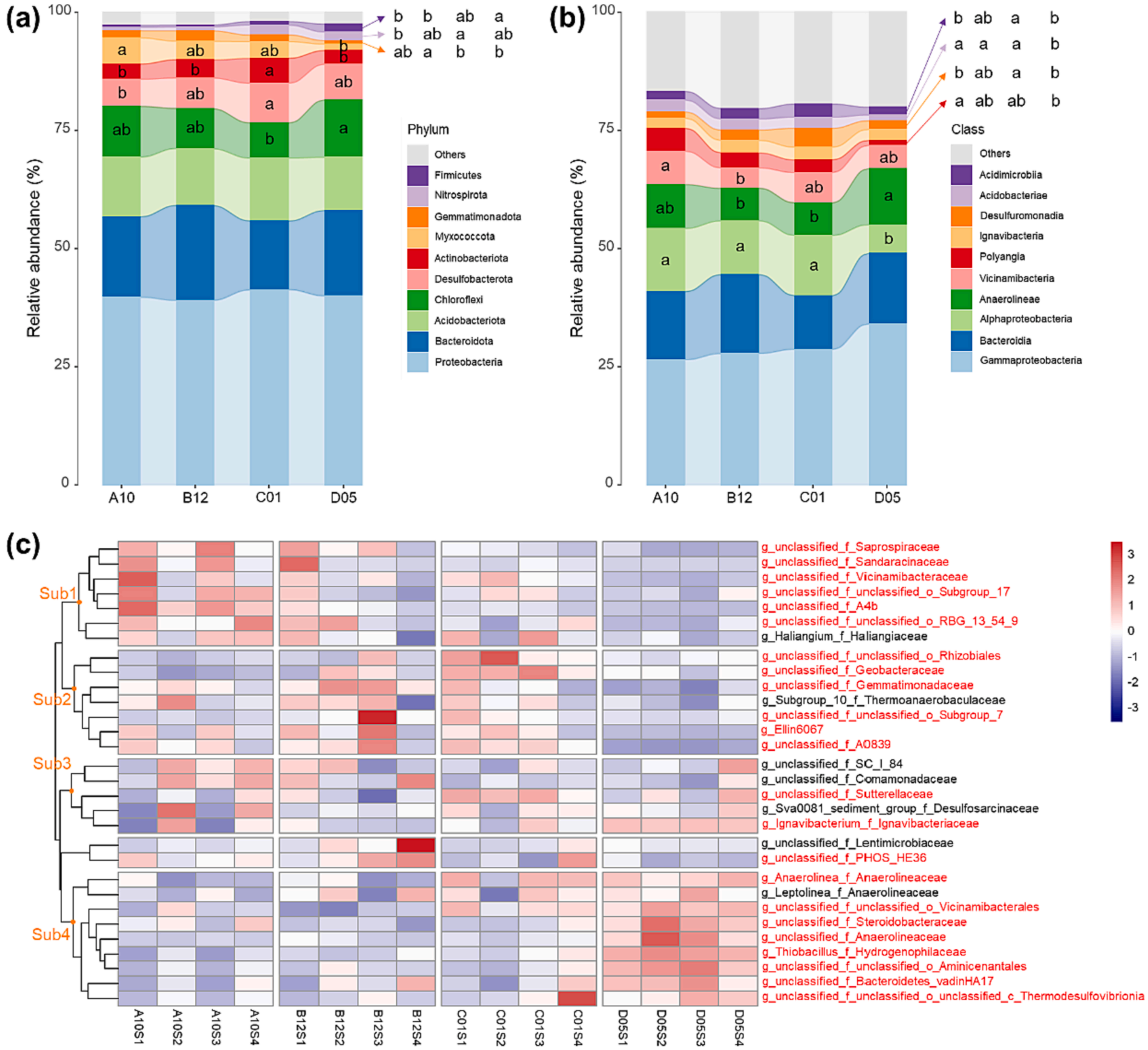

Fig. 5. Changes in the soil bacterial community structure during the seasonal freeze–thaw period. (a) Changes in the soil bacterial community structure (phylum level, top 10) during the seasonal freeze–thaw period. The different letters above the bars indicate significant differences between groups, and the bar heights marked by a, ab and b decrease significantly in turn ($p < 0.05$). (b) Changes in the soil bacterial community structure (class level, top 10) during the seasonal freeze–thaw period. The different letters above the bars indicate significant differences between groups, and the bar heights marked by a, ab and b decrease significantly in turn ($p < 0.05$). (c) Changes in the soil bacterial community structure (genus level, top 30) during the seasonal freeze–thaw period. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period. Bacterial genera with significant differences between groups are highlighted in red ($p < 0.05$). Sub1-4 represents four subsets of bacterial communities by cluster analysis at the genus level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

abundances of 8 of the 9 genera included in Sub4 were higher in D05 compared to other groups. The genera contained in Sub2 has a high relative abundance in groups B12 and C01, but an extremely low relative abundance in group D05. The genera contained in Sub3 occurred in all groups.

3.4. Relationship between the soil bacterial community and the soil properties during the seasonal freeze–thaw period

Based on our ASV level data, a db-RDA was conducted on the soil bacterial community and on the soil properties (Fig. 6a). Its results showed that the samples of each group were clustered, and that the distance between groups A10 and D05 was larger than that between groups B12 and C01. A permutation test showed that ST, SM, and pH were all significantly correlated with changes in the soil bacterial community ($P < 0.05$), although SOC was the soil property contributing the most to such changes ($P < 0.001$). We also analyzed the correlation between the soil bacterial community and soil properties at the phylum level (Fig. 6b). The results showed no significant correlations between changes in the relative abundance of dominant bacteria (e.g., Proteobacteria, Bacteroidota, and Acidobacteriota) and the soil properties, while Chloroflexi and Actinobacteriota were significantly affected by

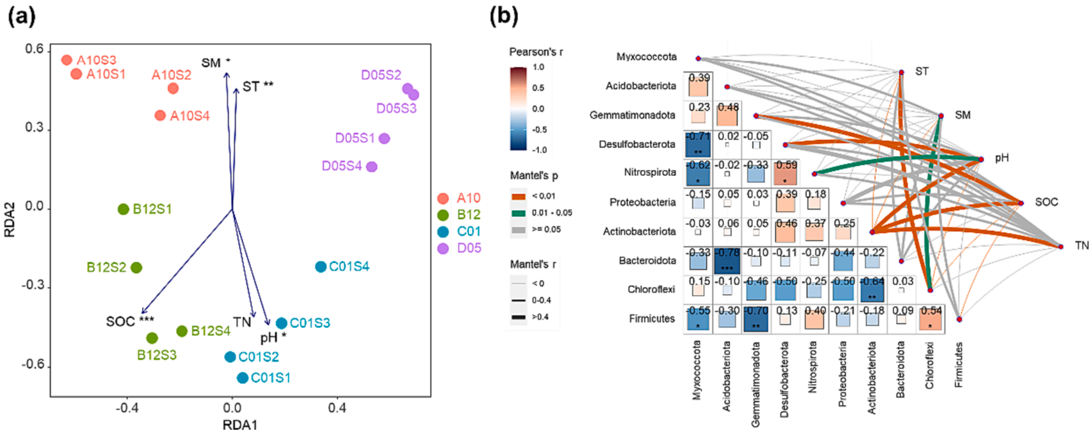

Fig. 6. Relationships between the soil properties and the bacterial community during the seasonal freeze–thaw period. (a) Distance-based redundancy analysis (db-RDA) of the relationship between the soil properties and the bacterial community during the seasonal freeze–thaw period. (b) Correlation analysis between the soil properties and the bacterial community during the seasonal freeze–thaw period. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period. The "*", "* *", and "* * *" indicate significant differences at the levels of 0.05, 0.01, and 0.001.

the soil properties. Notably, changes in ST, SM, pH, SOC, and TN were all significantly correlated with the relative abundance of 2, 2, 4, 4, and 1 phyla, respectively ($P < 0.05$). Overall, SOC and pH showed the greatest influence on the bacterial community structure at the phylum level.

3.5. Network analysis of the soil bacterial community in different stages of the seasonal freeze–thaw period

The variation range of the soil bacterial network composition in different stages of the freeze–thaw period was relatively small (Fig. 7). The largest variations in the proportion of Proteobacteria (36.84 %–39.26 %), Bacteroidota (14.59 %–15.51 %), Acidobacteriota (13.08 %–13.90 %), and Chloroflexi (7.29 %–9.76 %) (i.e., the dominant phyla) were 2.42 %, 0.92 %, 0.82 %, and 2.47 %, respectively. During the freeze–thaw period, the proportion of Desulfobacterota (5.77 %, 6.37 %, 7.56 % and 7.62 %, respectively) in groups A10, B12, C01 and D05 showed an overall increasing trend, while the proportions of Myxococcota (5.77 %, 5.84 %, 5.26 % and 4.12 %, respectively) and Others (5.00 %, 4.77 %, 4.46 % and 3.80 %, respectively) showed opposite trends. We compared the topological properties of the network in different stages of the freeze–thaw period and analyzed its complexity. The results showed that the number of nodes (780, 754, and 741, respectively) and edges (15,358, 14,017, and 14,348, respectively) in groups A10, B12, and C01 was significantly higher than in group D05 (characterized by 656 points and 12,353 edges). Furthermore, the proportion of negative edges in group A10 (25.95 %) was higher than in other groups (Fig. 7a). At the same time, the average degree, the relative modularity, and the modularity of groups B12 and D05 were relatively low, proving a low network complexity for these two groups. We also analyzed the similarity of the bacterial network modules in each stage and the stability of the network. From the point of view of network robustness, no obvious differences were noted among groups (Fig. 7e). Interestingly, the networks in each group could be divided into 28 modules. Between groups A10 and B12, A10 and C01, and A10 and D05 there were 18, 21, and 14 pairs of similarity modules, respectively. Between groups B12 and C01, and B12 and D05 there were 16 and 18 pairs of similarity modules, respectively. Between groups C01 and D05 there were 25 pairs of similarity modules (Fig. 7f). The results of our network composition stability analysis show that, compared with the differences between groups A10 and D05, the changes in network composition within each stage were relatively small (Fig. 7g).

4. Discussion

During the seasonal freeze–thaw period, the nutrient cycling process in surface soil is directly affected by drastic changes in the hydrothermal conditions and in microbial activity (Ji et al., 2022). In this study, the temperature and humidity of surface soil in an alpine wetland during the freeze–thaw period decreased to − 11.44 ℃ and 12.09 %, respectively, in the initial freezing period, and increased to 12.30 ℃ and 64.68 %, respectively, in the successive thawing period (Fig. 3). Changes in soil moisture and, especially, water phase changes can accelerate the breaking of soil surface litter, promoting the leaching and release of litter nutrients (Isobe et al., 2022; Zong et al., 2023). Previous studies have proved that the structure of soil aggregates tends to change during freezing and thawing; in particular, the melting of ice crystals would greatly accelerate the release of unstable nutrients (Sang et al., 2021). Drastic changes in soil temperature would not only affect soil moisture, but also lead to a decrease or to the disappearance of some microorganisms, further affecting the process of nutrient migration and release (Miura et al., 2020). Our research results confirm this view. In the initial freezing and deep freezing periods, the SOC and TN of group C01 significantly grew (by 10.27 % and 10.06 %, respectively) compared to those of the group A10. A significant decrease in SOC and TN was observed instead during the freeze–thaw period, which lasted for 4 months in this study. In this occasion, soil water phase changes increased the availability and transport rate of nutrients; meanwhile, plant growth and the utilization of microorganisms by plants improved the soil living environment and subsequently led to the consumption of great amounts of nutrients (Chen et al., 2021; Zhao et al., 2021). Small variations of pH were registered in this study, in accord with the results of Sang et al. (2021). The processes behind the observed changes in soil properties during the freeze–thaw period, as well as the complex relationships between soil properties and microbial communities need to be urgently explained in view of future climate change (Hicks et al., 2022).

Our research showed that the soil bacterial community diversity declined with soil temperature during the initial freezing and deep freezing periods, which is consistent with hypothesis (1). Previous studies on the freeze–thaw cycle have shown that soil bacteria adopting an r-strategy are more sensitive to environmental pressure (Sang et al., 2021). During the freeze–thaw period, low temperatures and water shortages significantly affect the physiological activities of the bacterial community and even cause the death of part of the population (especially of bacteria having poor resistance to environmental stress and a high dependency on nutrients) (Mazur, 1984; Robroek et al., 2013;

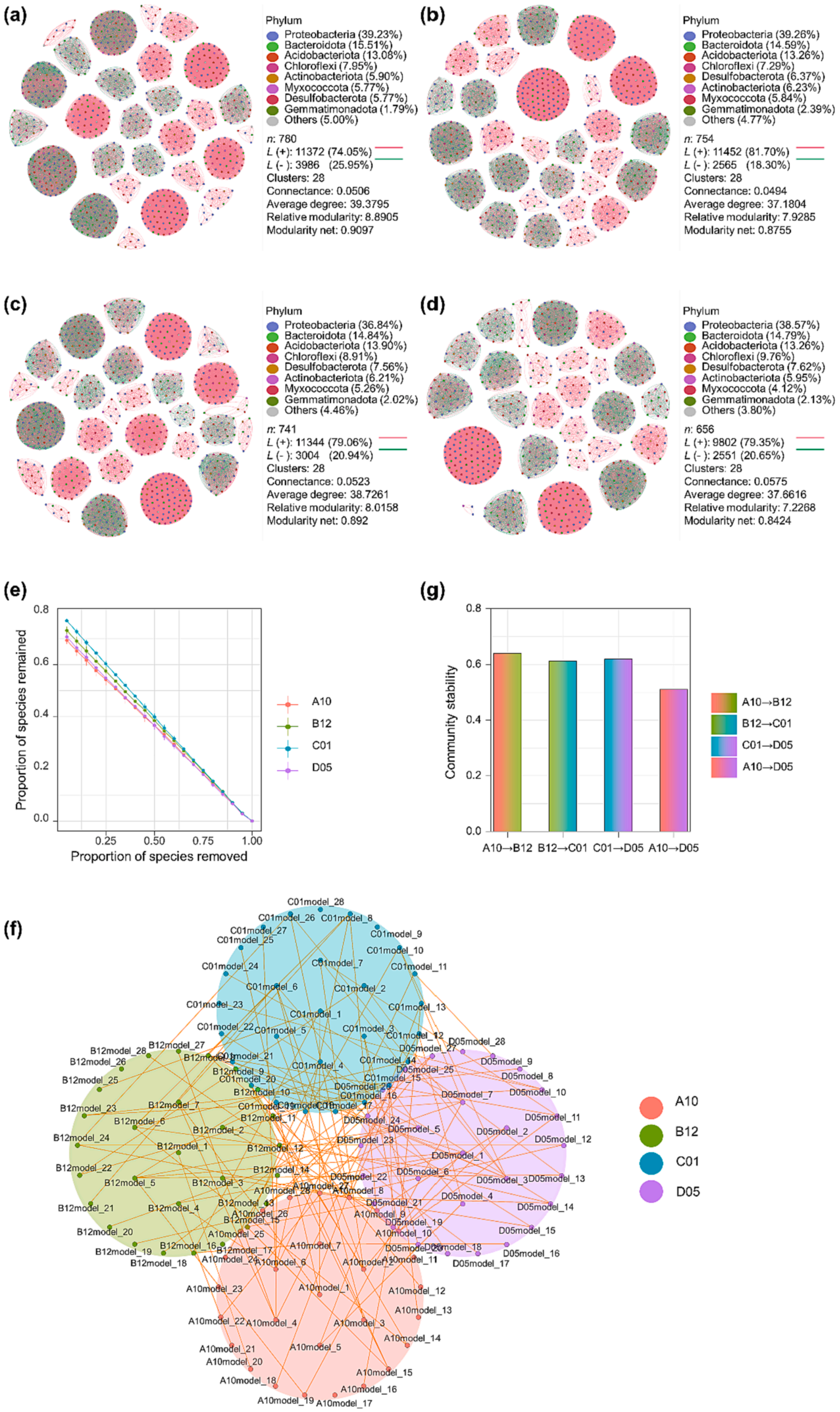

Fig. 7. Network analysis of the soil the bacterial community in different stages of the seasonal freeze–thaw period. (a) Network of the soil bacterial community (A10). (b) Network of the soil bacterial community (B12). (c) Network of the soil bacterial community (C01). (d) Network of the soil bacterial community (D05). (e) Robustness of the soil bacterial community network in different stages of the seasonal freeze–thaw period. (f) Similarity analysis of the soil bacterial community network model in different stages of the seasonal freeze–thaw period. (g) Stability of the soil bacterial community network in different stages of the seasonal freeze–thaw period. A10, B12, C01 and D05 represent four batches of sampling in seasonal freeze–thaw period.

Wubs et al., 2018; Perez-Mon et al., 2020). In this study, although soil nutrients (including biological residues) were more abundant in the initial freezing and deep freezing period, the harsh low temperatures and water shortage that occurred at the same time would have caused the abundance of some bacterial groups to decrease sharply or even disappear under multiple stresses. This, in turn, would have led to a continuous decrease of the bacterial community diversity (Song et al., 2017; Sang et al., 2021). Contrary to hypothesis (1), during the thawing period, soil temperature and humidity increased, while the soil environment improved; in the meanwhile, the soil bacterial community diversity decreased further, although this variation was not statistically significant. This actually may support the "r-strategist" idea if competitive genera are the ones growing at the expense of others at this initial burst of nutrients. Some previous studies have found that an increase of the soil water content during the thawing period would lead to a decrease (or even death) of aerobic bacteria, while the remaining (high-activity) bacteria would strive for nutrients (Supramaniam et al., 2016; Yang et al., 2019). Additionally, the growth of the soil bacterial community after freezing and thawing tends to be slow, and the recovery of its physiological activity lags behind (Koponen and Bååth, 2016; Ji et al., 2022). Ren et al. (2018) analyzed in detail the short-term multi-day freeze–thaw cycle in spring and autumn and the inter-seasonal freeze–thaw cycle. They demonstrated that the bacterial community diversity increased after the short-term multi-day freeze–thaw cycle (although not significantly), which is basically consistent with the mainstream view on the matter (Ren et al. 2018; Hentschel et al., 2008; Hu et al., 2018). In the seasonal freeze–thaw period, the bacterial diversity decreased, although not significantly, which is consistent with our results. Liu et al. (2022) recently carried out a study on the diversity and versatility of soil bacterial communities in forest ecosystems during the freeze–thaw period. They found that the α-diversity of bacterial communities did not change significantly during freeze–thaw events and supported it with a *meta*-analysis. The above evidence indicates that the effects of short-term multi-day freeze–thaw cycles and longer-term seasonal freeze–thaw events on soil bacterial communities can vary and be even opposite (Männistö et al., 2018; Perez-Mon et al., 2020; Liu et al., 2022). This is mainly due to the complexity of natural environmental conditions and to differences in the experimental conditions during the freeze–thaw period. In addition, some issues need to be considered, such as the time required for cell death under frozen conditions, and more detailed division of freeze–thaw cycles in order to more accurately explore freeze–thaw events. This also proves the importance and necessity of our research anyway.

Although some relatively short-term freeze–thaw cycle studies have shown that the bacterial community structure does not change significantly during short-term freeze–thaw cycles (Ji et al., 2022), we hypothesized that soil bacterial communities would change considerably from one stage to another during longer seasonal freeze–thaw periods (under greater temperature changes) (hypothesis (2)). At the phylum level, only bacterial groups with relatively small abundance (e.g., Chloroflexi and Desulfobacterota), changed significantly (Fig. 5a). A similar phenomenon was observed at the class level (Fig. 5b). Overall, the bacterial community structure did not have any obvious stage change characteristics at the phylum and class levels. This fact can explain the β diversity results: significant differences and overlaps were noted between groups of samples (Fig. 4c). Notably, changes between A10 to B12 and between B12 to D05 in the initial freezing and thawing periods were larger than those in the deep freezing period. The relative abundances of typical eutrophic bacteria groups (e.g., Bacteroidota and Gammaproteobacteria) increased in the initial freezing period and in the thawing period, but not significantly. This might have been partly due to the sampling period, but especially by drastic changes in the soil living environment. Our db-RDA analysis showed that the bacterial community as a whole was affected by changes in the soil environment (especially by the amount of nutrients) during the freeze–thaw period (Fig. 6a). The correlation analysis at the class level clearly showed that this effect was particularly pronounced for bacterial groups with a relatively low abundance (e.g., Actinobacteriota and Chloroflexi) (Fig. 6b). Oligotrophic conditions, combined with the soil environment, nutrient changes, and strong competition for living space might further explain the observed changes in bacterial community structure (Fierer et al., 2007; Bouskill et al., 2013; Leff et al., 2015; Sang et al., 2021). More interesting phenomena were noticed at the genus level. For example, *g_Thiobacillus_f_Hydrogenophilaceae* and *g_unclassified_f_A0839*, both belonging to Proteobacteria. The former had highest relative abundance in group D05, while the latter was abundant in groups A10, B12, and C01, but almost absent in group D05. Meanwhile, *g_unclassified_f_Bacteroidetes_vadinHA17* and *g_unclassified_f_saprospiraceae*, both belonging to Bacteroidota. The former had highest relative abundance in group D05, while the latter had its highest relative abundance in group A10 (Fig. 5c). The above results prove that the bacterial community structure was relatively stable at the phylum level, and the relative abundance of some bacteria has changed at the lower taxonomic level (genus level). The clustering results showed that different bacterial groups had heterogeneous resistances to freeze–thaw stress. These findings are supported by some previous studies, which found that taxa with higher resistances and nutrient utilization rates were not randomly distributed, but clustered (Morrissey et al., 2019; Garcia et al., 2020; Sorensen et al., 2020). Cold-tolerant bacteria native of alpine regions, acquire a unique survival mechanism (i.e., cold adaptation mechanism) after long-term exposure to cold conditions. This survival mechanism reduces the degree of tRNA modification after transcription and diminishes cell structural stability, adjusts the cell membrane lipid composition, ensures a good flexibility and fluidity of the membrane, and induces the expression of various cold-shock proteins, with the effect of maintaining transcription, translation, protein folding, and cell membrane permeability regulation under low temperature stress (Tribelli and López, 2018; Collins and Margesin, 2019; Chen et al., 2021). By studying this phylogenetic pattern in bacterial communities, adaptive freeze–thaw microbial communities can be screened, and it would be possible to clarify how microbial communities respond to changes in freeze–thaw patterns (Luláková et al., 2019).

Positive–negative and direct–indirect associations among species play an important role in the process of ecosystem function, creating a closely related whole (Ovaskainen et al., 2017; Wagg et al., 2019). Under climate change, the complex interactions between microbial groups and variations in network complexity and stability caused by dynamic changes have received attention in recent studies (Yuan et al., 2021; Liu et al., 2022). The bacterial community composition of the network analyzed in this study did not show any significant changes (Fig. 7), confirming a relative stability of the bacterial community structure during seasonal freezing and thawing. Based on the topological properties of the network, we found that the number of points and edges, the average degree, the relative modularity, and the net modularity of the bacterial network decreased slightly after the initial freezing period and the thawing period. Only group A10 showed a high negative correlation (25.95 %), while in groups B12, C01, and D05 it was ~20 %. This proves that, in the initial freezing period, hydrothermal conditions gradually become unfavorable for bacterial survival, and there was an increasingly strong competitive relationship between bacteria sharing relatively limited resources (Shi et al., 2016; Yuan et al., 2021). After the initial freezing period, the complexity of the bacterial network decreased. In the deep freezing period, although bacteria with strong cold tolerance survived, nutrients were relatively high and their competition obviously weakened. Some studies have proposed that changes of soil water phase and porosity, linked to a scarcity of water and nutrients, during the freeze–thaw period lead to a better occupation of soil spaces by fungal hyphae, while bacteria would not have this ability (Yuste et al., 2011; Liu et al., 2022). In the thawing period, although the soil environment and nutrients change greatly, the bacterial network does not react quickly, and there can be a lag in recovery (Koponen and Bååth, 2016; Ji et al., 2022). We also analyzed the changes in bacterial community stability. From the perspective of robustness and community composition stability, there were no obvious differences among stages (Fig. 7e,g). The relative stability of the bacterial community in the seasonal freeze–thaw period was reinforced by the similarity modules between networks in each stage (Fig. 7f). Under a soil temperature difference of 26.74 ℃ and a soil moisture difference of 87.69 % (Fig. 3), the complexity and stability of the bacterial community network remained relatively stable during the freeze–thaw period, differently from what we expected (hypothesis (2)).

Our research findings contribute to the existing body of knowledge on the functional processes of alpine wetland on the functional process of alpine wetland ecosystem. Specifically, our study addresses the research gap concerning the microbial aspects of the biogeochemical cycle in alpine wetlands in arid areas of Central Asia during the freeze–thaw period, and have practical guiding significance for the prediction and management of alpine wetland ecosystem ("adaptive freeze–thaw" microorganisms) adapting to future climate change. Naturally, the study also exhibited several limitations. The potential influence of dormancy or environmental DNA on bacterial community stability should be taken into account as potential complicating factors. The primary focus of previous studies on dormant DNA has been the investigation of permafrost (Burkert et al., 2019). However, there is limited mention of dormant DNA in research pertaining to the seasonal freeze–thaw process (Ren et al., 2018; Schostag et al., 2019; Zong et al., 2023). This lack of attention may be attributed to the challenges associated with interpreting results due to the rapid environmental changes experienced by dormant DNA. The assessment of microbial activity is beneficial for comprehending the structure of microbial communities and the mechanisms of transmission (Wang et al., 2023). Despite some methodological explorations, there are still many limitations (Bowsher et al., 2019; Wang et al., 2023). The efficacy of the 16S-RNA sequencing method has yet to be comprehensively assessed, particularly in the context of in-situ experimental settings inside intricate complex ecosystems (Wang et al., 2023). Part of dormant microorganisms may actively participate in the biogeochemical cycle (Barbato et al., 2022; Ernakovich et al., 2022). Hence, it is imperative that future research endeavors encompass the concurrent investigation of microbial activity research and function prediction. It is recommended to integrate multiple omics technologies in order to examine the precise mechanisms of information transmission and resource allocation within networks, hence enhancing the investigation of functional processes (Liu et al., 2022).

5. Conclusion

Compared with short–term indoor simulation of freeze–thaw cycle, it is of great significance to study the ecological process of seasonal freeze–thaw period with intense environmental changes. Under the influence of complex environmental factors during the seasonal freeze–thaw period, the diversity and structure (phylum level) of soil bacterial community and the stability and complexity of bacterial network in an alpine wetland of arid Central Asia remained relatively unchanged. Only the microbial groups with low abundance changed significantly due to the environmental factors during the freeze–thaw period. A phylogenetic model of "adaptive freeze–thaw" appeared at the genus level. Overall, the bacterial communities in alpine wetlands have a good resistance and adaptability to changeable freeze–thaw patterns under climate change.

CRediT authorship contribution statement

Mo Chen: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Zailei Yang:** Software, Methodology, Project administration, Funding acquisition. **Maidinuer Abulaizi:** Software, Visualization, Methodology, Data curation. **Yang Hu:** Investigation, Methodology, Data curation. **Yuxin Tian:** Investigation, Data curation, Visualization. **Yunpeng Hu:** Investigation, Data curation. **Guangling Yu:** Investigation, Data curation. **Xinping Zhu:** Writing – review & editing, Supervision. **Pujia Yu:** Writing – review & editing, Supervision. **Hongtao Jia:** Supervision, Project administration, 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.

Data availability

Data will be made available on request.

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