Effects of Seasonal Freezing-thawing on the Protistan Communities in a Mountain Lake

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Abstract

Background: Freezing-thawing cycles are common phenomena in temperate regions. Such events may have a significant influence on the composition of the protistan communities in a mountain lake. Protists are single-celled eukaryotic microorganisms that act as links in the aquatic microbial food web, affecting the transfer of substances and energy transformation. Yet little is known about the effects of freezing and thawing on the protistan community in a mountain lake.

Results: The protistan communities in the lake were mainly composed of Ochrophyta, Ciliophora, Choanoflagellida, Cryptophyta, Chlorophyta, Stramenopiles_X, Cercozoa, Dinophyta, and Haptophyta. Seasonal freezing and thawing affected the community composition and diversity of protists. The change in the protistan community structure resulted from a significant change in organic carbon from the ice-covered to ice-free period. During the ice-covered and ice-free periods, temperature and nitrate were the main causes, respectively, for the changes in protistan community structure at different depths. Water depth also affected the structure of the protistan community, but it was not the most important factor.

Conclusions: This study revealed that duration of lake surface icing might affect the function of subalpine lake ecosystems, including the rate of nutrient cycling and energy flow, owing to changes in the structure and biodiversity of the microbial community.

Introduction

Seasonal ice cover is a unique characteristic of lakes in boreal and temperate regions. In these areas, the freeze-thaw process subjects lake ecosystems to considerable seasonal changes. Given the reduction in ice-cover time on lakes and rivers worldwide owing to climate change [1], there is an urgent need to understand the community dynamics in aquatic ecosystems that undergo the freeze-thaw process [2]. According to studies of water temperature trends in lakes [1, 3], ice-cover duration in winter largely determines the characteristics of summer warming trends, which emphasizes the cascading effects between seasons. Cross-seasonal cascades can involve multiple ecosystem processes; for example, ice characteristics influence spring algal growth [4]. Consequently, the ice-cover time and unique environmental conditions of the water under the ice likely drive certain changes in microbial communities [2, 5]. Microbial community composition, activity, and function change considerably under lake ice [6] and sea ice [4] compared with ice-free periods. Changes in microbial composition and activity during the seasonal freezing-thawing periods have important implications for ecosystem functioning and the biogeochemical processes in these ecosystems [7, 8]. Large fluctuations in environmental parameters (e.g., temperature, nutrients, and dissolved oxygen) caused by seasonal freezing and thawing significantly limit microbial activity and thus affect microbial diversity [8–10].

Protists are the main components of lake plankton community and play a key role in lake ecosystem. For example, eukaryotic algae are an important source of primary productivity in lakes, while protozoa transmit materials and energy to the higher trophic levels through the microbial loop [11]. Seasonal ice cover influences the diversity and distribution of organisms in the lake; however, the degree of influence varies with organism type. Previous studies have shown that bacterial communities can rapidly adapt to environmental changes because of their high physiological activities and growth rate, which also enable them to quickly revert to their previous composition when conditions return to normal [2, 10]. Yet few studies have examined the effects of freeze-thaw cycling on freshwater protistan communities in mountain lakes. Moreover, limited information is available on the dynamics of protistan communities at different water depths during the ice-covered to ice-free periods in lakes.

The Ningwu subalpine lake group is located on the northern edge of China's monsoon region [12], and Gonghai Lake is the highest-elevation lake that contains freshwater year round. It begins freezing in early November and remains covered by ice until the end of March of the following year. During the ice-covered period, the lake water is isolated from the external environment. Hardly any exchange of materials or energy occurs between the ice-covered water and the atmosphere. The ice layer gradually melts as the temperature increases in spring, until the end of April (the ice-free period). To explore the effects of freeze-thaw cycling on the structure of protistan communities at different water depths, we compared the physico-chemical parameters of Gonghai Lake water in the winter and spring. We addressed the following questions: (1) Do the structure and diversity of protistan communities change during the ice-covered and ice-free periods at different depths in subalpine lakes? (2) What factors affect the structure and diversity of protistan communities during the freeze-thaw process?

Materials And Methods

Site description and sampling

Gonghai Lake (GH) (38.91°N, 112.23°E) is located at the foot of the Guancen mountains in the southwest of Ningwu County, Shanxi Province, China (Fig. 1). It is located at the edge of the East Asian monsoon region in China, with an altitude of 1854 m, an area of approximately 0.36 km^2, and a maximum water depth of approximately 8 m. The region has a temperate monsoon climate, with cold, long, and snowy winters, cool summers, and abundant precipitation. The samples were collected in December 2018 and April 2019. In the center of the lake, triplicate samples (3 L each) were taken every 2 m from the lake surface to the bottom (0 m, 2 m, 4 m, 6 m, and 8 m). Samples were transported to the laboratory in sterile plastic barrels. Part of the water (2.5 L) was filtered through a 0.2-μm polycarbonate membrane filter (Millipore, Jinteng, Tianjin, China), and the membrane was sealed in a sterile centrifuge tube and stored at -20°C for microbial DNA extraction. The remaining 0.5 L water from each sample was used for analysis of physico-chemical properties.

Physico-chemical analysis

For each sample, water physical parameters including temperature (T), pH, dissolved oxygen (DO), electric conductivity (EC), nitrate (NO_3^-) and ammonium (NH_4^+) content were measured in situ by a portable water multiparameter quality monitor (Aquread AP-2000, England, UK); total nitrogen (TN), nitrite

(NO_2^-), sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) content were measured by an automated discrete analyzer (DeChem-Tech, CleverChem380, Hamburg, Germany); total carbon (TC), total organic carbon (TOC) and inorganic carbon (IC) content were measured by a TOC analyzer (Shimadzu, TOC-V_{CPH}, Shimane, Japan).

DNA extractions, PCR amplification, and high-throughput sequencing

DNA was extracted with the Fast DNA SPIN extraction kit (MP Biomedicals, Solon, OH, USA), the concentration and purity of DNA were determined, and the DNA was stored at -20°C. PCR amplification was performed using the universal eukaryotic primers TAReuk454WD1F (5'-CCAGCAS CYGCGGTAATTCC-3') and TAReukEV3R (5'-ACTTTCGTTCTTGATYRA-3') to amplify the V4 region of the 18S rRNA gene. The amplification system and conditions were conducted according to Stoeck [13]. The amplified products were sequenced with the Illumina miseq high-throughput sequencing platform (Shanghai Majorbio Biomedical Technology Co., Ltd.). Before analysis, raw sequencing reads were demultiplexed and quality filtered using QIIME (version1.9.1). The low-quality sequences were filtered using the following criteria: sequences that had a length of < 150 bp, sequences that had average Phred scores of < 20, sequences that contained ambiguous bases and sequences that contained mononucleotide repeats of > 8 bp. Paired-end reads were assembled using FLASH and Trimmomatic. After chimera detection, the remaining high-quality sequences were clustered into OTUs at 98% sequence identity by UCLUST. A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by BLAST search of the representative sequences set against the Protist_PR2_v4.5database using the best hit. An OTU table was generated to record the abundance and taxonomy of every OTU in each sample. Before analysis non protist were removed from the OTUs table. To minimize the differences in sequencing depth across samples, all were normalized to the number of sequences in the smallest data set for further analysis. Sequence data analyses were mainly performed using QIIME and R packages (version 4.0.0).

Nucleic acid sequences

The sequence data of protist 18S rDNA genes were submitted to the NCBI GenBank as accession number SRP301277.

Data analysis

The protistan community alpha diversity in each habitat was compared using observed OTUs and the Shannon and Simpson indices. Shapiro-Wilk tests were used to test the normality of the alpha diversity data, and no violations of normality were detected. The one-way ANOVA was used to assess the differential of physico-chemical factors and alpha diversity index in different seasons and depths, and the LSD significance difference test was used for multiple comparisons. The two-way ANOVA was used to assess the effects of physico-chemical factors and sampling depth on alpha diversity. All of the above tests were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). The correlation between environmental factors and the dominant protist taxa (including the dominant phylum, order, and genus) was expressed using the Spearman correlation coefficient, and it was visualized through heat maps via the corrplot package in R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). Beta diversity analysis was performed to investigate the structural variation of protistan communities across two seasons and five depths using the Bray–Curtis distance visualized via principal coordinates analysis (PCoA) (Canoco for Windows version 5.0). The effects of environmental factors and sampling depth on community structure were obtained by permutational multivariate analysis (PERMANOVA). All environmental factors were selected by stepwise regression and the Monte Carlo permutation test (Supplementary Table S1), and finally, the environmental factors with a variance inflation factor (VIF) of less than ten were retained in PERMANOVA. The confidence interval for all statistical analyses was 95% ($P < 0.05$).

Results

Physico-chemical conditions of ice-covered and ice-free lake water

The seasonal freeze-thaw process caused significant changes in the physical and chemical parameters of lake water. Factors such as T, DO, NO_3^-, NO_2^-, TC, TOC, and IC concentration increased significantly after the melting of lake ice, whereas the EC and NH_4^+ concentration were higher under the winter ice cover ($P < 0.05$) (Table 1). The variations in the physico-chemical parameters with sampling depth differed between the ice-covered and ice-free periods. In the ice-covered winter, the maximum values of T, pH, and SO_4^{2-} concentration were recorded at the bottom of the lake (8 m); however, EC and TC concentration were the lowest at 8 m. At 4 m, changes were observed for NO_3^-, TOC, and IC. In the ice-free period, T and NO_3^- concentration were the highest at 8 m, while pH, EC, TOC, and PO_4^{3-} were the highest at the surface. At the same time during this period, the concentrations of TC and IC did not change significantly from the surface to the bottom (Supplementary Fig. S1).

Table 1
Physico-chemical parameters in ice-covered and ice-free periods of Gonghai Lake

Factors	ice-covered	Ice-free
T (°C)	3.60 ± 0.74**b**	6.17 ± 1.26**a**
pH	7.94 ± 0.05**b**	8.69 ± 0.10**a**
DO ($mg·L^{-1}$)	8.90 ± 0.96**b**	11.23 ± 0.75**a**
EC ($μS·cm^{-1}$)	963.27 ± 14.15**a**	826.13 ± 18.97**b**
NO_3^- ($mg·L^{-1}$)	0.01 ± 0.01**b**	0.03 ± 0.01**a**
NO_2^- ($mg·L^{-1}$)	0.02 ± 0.01**b**	0.03 ± 0.02**a**
NH_4^+ ($mg·L^{-1}$)	4.21 ± 0.18**a**	0.11 ± 0.06**b**
TC ($mg·L^{-1}$)	119.53 ± 1.92**b**	131.62 ± 1.09**a**
TOC ($mg·L^{-1}$)	18.94 ± 2.31**b**	29.14 ± 1.34**a**
IC ($mg·L^{-1}$)	100.60 ± 1.56**b**	102.47 ± 0.66**a**
SO_4^{2-} ($mg·L^{-1}$)	19.72 ± 5.08**a**	20.23 ± 2.55**a**
PO_4^{3-} ($mg·L^{-1}$)	0.45 ± 0.38**a**	0.25 ± 0.16**a**
Note: The data were shown as the means ± standard error.		
Abbreviations: T represents temperature; DO represents dissolved oxygen; EC represents electroconductibility; TN represents total nitrogen; NO_3^- represents nitrate; NO_2^- represents nitrite; NH_4^+ represents Ammonium; TC represents total carbon; IC represents inorganic carbon ; TOC represents organic carbon; SO_4^{2-} represents sulfate and PO_4^{3-} represents phosphate. Significant differences between samples were determined using one-way ANOVA at $P < 0.05$ and different letters indicate significant differences		

Changes in protistan community abundance across the seasonal freezing -thawing and lake depths

Taxonomic composition in ice-covered and ice-free periods

The taxa with a relative abundance greater than 1% were defined as dominant, whereas those with relative abundances of less than 1% were combined together as “others”. In the ice-covered winter, protistan communities consisted of eight dominant phyla, which were dominated by Ochrophyta (53.72%), followed by Ciliophora (9.96%), Choanoflagellida (8.26%), Cryptophyta (8.24%), Chlorophyta (7.84%), Stramenopiles_X (6.37%), Cercozoa (2.70%), and Dinophyta (2.24%) (Fig. 2A). In the ice-free spring, protistan communities consisted of seven dominant phyla, which were dominated by Ochrophyta (38.35%), followed by Chlorophyta (22.84%), Cryptophyta (14.59%), Ciliophora (11.99%), Cercozoa (5.07%), Dinophyta (2.57%), and Stramenopiles_X (2.12%) (Fig. 2A). Of the 21 dominant orders, Bacillariophyta_X (47.22%) was the dominant order in winter, while Dictyochophyceae_X (22.49%) was the dominant order in spring (Supplementary Fig. S2A). There were 27 dominant genera, with *Stephanodiscus* (22.49%) being the highest in winter and *Pedinellales_X* (22.32%) being the dominant genus in spring (Supplementary Fig. S2B).

The relative abundances of Ochrophyta, Chlorophyta, Choanoflagellida, and Stramenopiles_X differed significantly between the two seasons. The relative abundances of Ochrophyta, Choanoflagellida, and Stramenopiles were significantly higher in the ice-covered period, but the relative abundance of Chlorophyta was significantly higher in the ice-free period (Fig. 3A). Among the top 15 dominant orders, eight differed significantly between the two seasons (Supplementary Fig. S3A). Among the top 15 dominant genera, ten differed significantly between the two seasons (Supplementary Fig. S3B).

Taxonomic composition at different depths

In the ice-covered period, there were nine dominant phyla at five sampling points. Ochrophyta (45.91–61.61%) was the dominant phylum at all five sampling sites and gradually increased from the surface to the bottom. Variations in the relative abundances of the eight other dominant phyla (Ciliophora, Choanoflagellida, Cryptophyta, Chlorophyta, Stramenopiles_X, Cercozoa, Dinophyta, and Haptophyta) were different from that of Ochrophyta (Fig. 2B). Among the 20 dominant orders and 22 dominant genera, the relative abundances of Bacillariophyta_X and *Stephanodiscus* were the largest and gradually increased from the surface to the bottom (Supplementary Fig. S2C and S2D). During the ice-free period, there were eight dominant phyla at five sampling points. At the 4 m depth, Ochrophyta (19.44–52.68%) was the dominant phylum; Chlorophyta (11.27–50.45%) and Cercozoa (3.12–11.56%) were the dominant phyla at the 8 m depth; and Cryptophyta (2.52–34.45%) and Ciliophora (7.75–17.11%) were the dominant phyla in the surface layer (Fig. 2C). The relative abundances of the 18 dominant orders and 27 dominant genera varied with sampling depth (Supplementary Fig. S2E and S2F).

The relative abundance of Cercozoa in the surface layer (0 m) was significantly higher than that in the bottom layer (8 m) during the ice-covered period. There was no significant difference among the other eight dominant phyla at different sampling depths (Fig. 3B). The relative abundances of Ochrophyta, Dinophyta, and Choanoflagellida were the lowest at the 8 m depth in the ice-free period (Fig. 3C). Among the top 15 dominant orders and 15 dominant genera, six orders and eight genera differed significantly with depth in the ice-covered period (Supplementary Fig. S3C and S3D). Among the top 15 dominant orders and 15 dominant genera, eight orders and ten genera differed significantly with depth in the ice-free period (Supplementary Fig. S3E and S3F).

The relationship between dominant taxa and environmental factors

There was a significant correlation between the abundance of dominant taxa and physico-chemical variables (Fig. 4, Supplementary Fig. S4). Changes in the abundances of dominant taxa (dominant phyla, orders, and genera) from ice-covered waters to ice-free waters were significantly correlated with changes in T, pH, DO, EC, and nutrient (carbon and nitrogen) concentrations (Fig. 4A, Supplementary Fig. S4A-B). During the ice-covered period, the abundance of dominant taxa was significantly correlated with T, pH, DO, and lake depth (Fig. 4B, Supplementary Fig. S4C-D). During the ice-free period, NO_3^- was the most important factor affecting the abundances of the dominant taxa (Fig. 4C, Supplementary Fig. S4E-F).

Protistan community diversity

Alpha diversity and driving factors

From the ice-covered to the ice-free period, OTU numbers and Shannon indices increased significantly ($P < 0.05$), from 135.13 ± 2.46 to 157.81 ± 7.04 and from 2.70 ± 0.07 to 3.06 ± 0.05, respectively. Meanwhile, the Simpson index decreased significantly between these two periods, from 0.20 ± 0.01 to 0.11 ± 0.00 (Fig. 5). Among the five sampling depths from the surface to the bottom, there were also differences in alpha diversity. In the ice-covered period, the OTU numbers did not change significantly among different sampling depths, the Shannon index decreased gradually from the water surface (0 m) to the lake bottom (8 m), and the Simpson index and Shannon index exhibited opposite trends (Fig. 5). In the ice-free period, the OTU numbers at 0 m and 8 m were larger than those at the middle layers (2 m, 4 m, 6 m), the Shannon index did not change significantly among different sampling depths, and the Simpson index was the highest at 4 m (Fig. 5).

The seasonal effects of lake freezing-thawing and sampling depths affected the alpha diversity of protistan communities; however, the interactive effect of these factors only had a significant effect on the OTUs (Table 2). The effect of seasonal variation on alpha diversity was greater than that of sampling depth. The environmental gradients caused by freezing-thawing, including those of pH, EC, NH_4^+, TC, TOC, and SO_4^{2-}, had significant effects on the Shannon and Simpson indices (Table S2).

Table 2
Two-way ANOVA of the effects of freezing and thawing and sampling depth on alpha diversity on protistan communities

	Factors	df	F	*P*
OTUs	Seasons	1	13.564	**0.001**
	Depth	4	6.056	**0.002**
	Seasons×Depth	4	4.466	**0.01**
Shannon index	Seasons	1	21.646	**0.000**
	Depth	4	2.927	**0.047**
	Seasons×Depth	4	0.846	0.513
Simpson index	Seasons	1	31.189	**0.000**
	Depth	4	3.916	**0.017**
	Seasons×Depth	4	0.915	0.474

Beta diversity and driving factors

Variations in the community structure of the protistan communities across two seasons and five sampling depths were statistically analyzed using PCoA based on Bray–Curtis distance (Fig. 6). ANOSIM verification indicated that the community compositions of the ice-covered and ice-free periods, as well as those among the different depths of these two periods, were significantly different (all $P < 0.05$) (Fig. 6).

To explore the key environmental drivers shaping protistan communities, environmental variables were analyzed by PERMANOVA. Among the seven selected environmental variables (TOC, NO_3^-, DO, T, IC, NO_2^-, and PO_4^{3-}), six factors, excluding PO_4^{3-}, significantly affected the seasonal variation of protistan communities, and TOC ($R^2 = 0.561$, $P = 0.001$) had the largest explanation while NO_2^- ($R^2 = 0.161$, $P = 0.007$) had the smallest (Table 3). Temperature ($R^2 = 0.346$, $P = 0.001$), sampling depth ($R^2 = 0.330$, $P = 0.001$) and NH_4^+ concentration ($R^2 = 0.199$, $P = 0.011$) were the main factors affecting the structure of protistan communities at different depths during the ice-covered period. NO_3^- ($R^2 = 0.374$, $P = 0.005$), sampling depth ($R^2 = 0.340$, $P = 0.002$), T ($R^2 = 0.271$, $P = 0.016$), SO_4^{2-} ($R^2 = 0.263$, $P = 0.018$), and IC concentration ($R^2 = 0.244$, $P = 0.026$) were the main factors affecting the protistan community structures at different depths during the ice-free period (Table 3).

Table 3
Permutational multivariate analysis of variance (PERMANOVA) of the effect of environmental parameters with variance inflation factor(VIF)less than 10 on protistan community structures

	Parameters	**S S**	**M S**	**F**	R^2	*P*
Between two periods	TOC	3.903	3.903	35.726	0.561	**0.001**
	NO_3^-	2.720	2.720	17.958	0.391	**0.001**
	DO	2.509	2.509	15.781	0.360	**0.001**
	T	2.398	2.398	14.716	0.345	**0.001**
	IC	1.562	1.562	8.098	0.224	**0.001**
	NO_2^-	1.123	1.123	5.387	0.161	**0.007**
	PO_4^{3-}	0.534	0.534	2.328	0.077	0.084
	Total	4.158	4.158	41.529	0.597	**0.001**
Ice-covered period	T	0.169	0.169	6.874	0.346	**0.001**
	Depth	0.161	0.161	6.398	0.330	**0.001**
	NH_4^+	0.097	0.097	3.237	0.199	**0.008**
	NO_3^-	0.070	0.070	2.180	0.144	0.065
	TC	0.050	0.050	1.489	0.103	0.195
	IC	0.049	0.049	1.436	0.099	0.216
	PO_4^{3-}	0.044	0.044	1.297	0.091	0.269
	SO_4^{2-}	0.025	0.025	0.707	0.052	0.662
	Total	0.313	0.078	4.453	0.640	**0.001**
Ice-free period	NO_3^-	0.866	0.866	7.773	0.374	**0.005**
	Depth	0.787	0.787	6.695	0.340	**0.002**
	T	0.627	0.627	4.825	0.271	**0.016**
	SO_4^{2-}	0.608	0.608	4.627	0.263	**0.018**
	IC	0.564	0.564	4.190	0.244	**0.026**

Abbreviations: MS, mean sum of squares; SS, sum of squares. The larger R^2, the higher explanation degree of the environmental variables to the community differences.

	Parameters	S S	M S	F	R^2	*P*
	TC	0.457	0.457	3.196	0.197	0.053
	pH	0.221	0.221	1.372	0.095	0.235
	NH_4^+	0.201	0.201	1.233	0.087	0.241
	NO_2^-	0.027	0.027	0.154	0.012	0.970
	Total	1.914	0.478	11.923	0.827	**0.001**

Abbreviations: MS, mean sum of squares; SS, sum of squares. The larger R^2, the higher explanation degree of the environmental variables to the community differences.

Discussion

Protists are a major component of freshwater lakes communities, forming the base of the food web on which all other aquatic organisms depend [14]. Here, we present a detailed analysis of changes in protistan communities from the ice-covered period to ice-free period in a freshwater mountain lake with seasonal freezing and thawing. From ice cover to ice melting, the lake water environment undergoes dramatic seasonal fluctuations (Table 1). Changes in composition and abundance are often regarded as a response of protistan communities to climate fluctuations and changes in nutrient availability [4, 10], especially in mountainous lakes [15].

Effects of seasonal freezing-thawing on protistan communities

We found that the abundances of the protistan communities during the two periods showed clear differences from the phylum to genus levels, driven by multiple environmental factors, especially T, pH, DO, and nutrients (NO_3^- and TOC). Temperature change was the main factor directly influencing the freeze-thaw process. In general, water temperature clearly rises after ice melting, and this was seen in our study (Table 1). The influence of temperature on microorganisms includes many aspects. First, temperature changes directly affect the metabolic activity and abundance of microorganisms [2, 16, 17]; Second, temperature also affects microorganisms by mediating the release of nutrients in sediments [3]. Moreover, temperature is closely related to other environmental factors [18], and our results also confirm that temperature is collinear with other environmental factors (Table S1). The relative abundances of Ochrophyta, Choanoflagellida, and Stramenopiles X were higher in the ice-covered period, while the relative abundance of Chlorophyta increased significantly during the ice-free period (Fig. 3A). This was because the abundances of the genera *Stephanodiscus* and *unclassified_F_Polar-centric-Mesophyceae* (belonging to the Ochrophyta phylum) and genus *unclassified_C_Choanoflagellatea* (belonging to the Choanoflagellida phylum) were higher during the ice-covered winter. The physical stability of the lake under the ice was identified as the key factor behind the initiation of these particular groups, which are well adapted to winter temperature and low light conditions [5]. *Stephanodiscus* readily adapts to low temperature, with its membrane lipid composition changing in cold environments [19], to ensure that the activity of its cells can be sustained. The abundances of the genera *unclassified_f__Cryptomonadales*, *Pedinellales X*, *Cryptomonas*, and *Chlamydomonas* increased significantly after ice melting. With the melting of the ice cover, the abundances of these groups with higher demands for temperature and light increased in spring [20]. Meanwhile, other physical and chemical parameters of the water were changing. The increase in nutrients and DO promoted the increase of aerobic and heterotrophic protozoa (Ciliophora, Cercozoa), which further indicates that the environmental changes caused by freezing and thawing had an important impact on protistan community composition.

The alpha diversity of the protistan communities increased significantly in the ice-free period compared with that in the ice-covered period (Fig. 5). Our results are consistent with previous studies, and microbial diversity in the water was lower during the ice-covered period [5, 21]. Ice cover acts as a shield over the lake surface, which reduces light intensity and the input of terrestrial nutrients and organic matter, and hinders gas exchange with the atmosphere [5, 10]. During the ice-free period, material exchange can occur between water and the surrounding environment. The increase in nutrients (mainly from rain erosion) in water promoted the photosynthesis of phytoplankton, thus changing the balance of free carbon dioxide and carbonate in the water, and finally increased the pH of the water. Precipitation in the ice-covered period was 9.4 mm, whereas precipitation during the ice-free period was three times that amount (Supplementary Table S3), which indicates that rainfall is also an important factor causing physico-chemical changes in the aquatic environment. With Gonghai Lake being a basin lake, nutrients from the surrounding land enter the lake with rainfall, which increases the concentration of nutrients in the lake water. Thus, the diversity of microbial communities increased in an environment with abundant nutrients.

Seasonal freezing and thawing resulted in significant changes in the distribution pattern of protistan communities ($P < 0.01$, Fig. 6A), and their distribution was affected by multiple factors, especially TOC and NO_3^- (Table 3). Within lakes, some organic carbon originates from terrestrially derived dissolved organic matter from surrounding soils. Many studies have confirmed that microbial community composition, structure, and metabolic strategies are driven by soluble organic matter [22–24]. In this study, the concentration of organic carbon increased significantly during the ice-free period. This suggests that terrestrial carbon subsidies make an important contribution to seasonal trends in microbial communities in the mountain lakes. TOC can be utilized by aquatic heterotrophic microorganisms [25, 26]. Because organic carbon is an important energy material for heterogeneous microorganisms, changes in organic carbon lead to changes in protistan community structure. Eukaryotic algae are the main group of protists in water, and changes in nutrient concentrations have the greatest effect on their communities [27]. It is generally accepted that nitrate will accumulate in water bodies in the winter [2, 10]. In this study, however, the increase in terrestrial nitrate nitrogen may have led to the change in the bacterial community structure involved in the nitrogen cycle. Bacteria and protists are closely linked through the food web, and changes in the bacterial community will inevitably lead to changes in the protistan community [24].

Effects of water depth on protistan communities

Water depth greatly influences the composition and diversity of biological communities [4]. Solar radiation penetrates the ice layer, and the surface photosynthetic autotrophs increase, leading to changes in nutrient concentrations at different depths. Coupled with the release of heat from lake-bottom sediments, a vertical temperature gradient is formed, resulting in the downward mixing of water bodies [5]. In these ways, convective mixing can make microorganisms circulate under the ice and improve their access to nutrients [28]. Therefore, it is necessary to study different depth gradients to further understand the changes in microbial communities caused by freezing and thawing. Our results also confirm that water depth affected the composition and diversity of the protistan communities. Stratification of the lake will hinder the vertical material exchange at different water depths, and then affect various biochemical processes of the lake water, biological metabolism, and material decomposition [29]. The relative abundances of the dominant groups from phylum to genus varied with sampling depth, and the variation trend of the same group differed by season. The heterogeneity of the physico-chemical environment at different depths directly caused this change (Fig. 4). Light availability, along with water mass characteristics, strongly impacts microbial communities in the vertical gradients [29]. There will also be stable stratification of water below the ice, with the main driving factors being the heat flux of sediments and the solar radiation infiltrating the ice [3]. Winter climatic fluctuations proved to be a key element in a linked chain of causal factors including the cooling of hypolimnetic waters, deep vertical mixing, and epilimnetic nutrient replenishment.

In contrast to the ice-covered period, the OTUs of protists were highest in the surface water; however, the Shannon index did not differ significantly at different depths during the ice-free period (Fig. 5). During the ice-free period, the nutrient concentrations in the surface layer and the vertical mixing intensity of the water body increased significantly with the aggravation of external disturbances (such as rainfall and gales), which resulted in the vertical difference in alpha diversity for the protistan communities. The OTU number did not differ significantly at different depths during the ice-covered period. This was mainly due to the following reasons. First, different protist groups have different requirements for light intensity and nutrient concentration [2]. Second, in winter, external disturbances are much smaller, and the water body is relatively stable.

The results of PERMANOVA analysis showed that temperature was the main reason for the differences in protistan communities at different depths during the ice-covered period (Table 3). The temperature gradient is the main factor leading to water stratification at different depths, and it forms nutrient gradients. Althoug Bertilsson [5] showed that temperature usually does not limit the growth of phytoplankton in winter in shallow lakes, we found that the vertical difference in temperature and sampling depth together affect the spatial distribution of protistan communities. Under the ice, photoautotrophic protist activities are often limited by the availability of photosynthetically active radiation [2, 5, 30]. Limited light penetration reduces the photodegradation of organic matter under ice and changes the quality and biodegradability of organic carbon available to microorganisms. Some phototrophic phytoplankton can perform aerobic photosynthesis in the upper waters, yet the light in the deeper waters is more limited, and the distribution of photoautotrophs at the bottom is lower. Owing to the reduced availability of light and the reduction of bacterial biomass during the ice-covered period, the life history strategies of protists became more diverse. During the ice-free period, the bottom layer (8 m) and other depths (0–6 m) differed significantly (Fig. 6). The water at the depth of 8 m is directly connected with the sediment, which is rich in organic and inorganic substances. The release of nutrients in sediments can promote the growth and diversity of microorganisms in water. For example, the release of nitrate and organic carbon in sediments is an important source of microbial nutrients in deep water [28]. Because the concentration of heat and mixed energy near the sediment–water interface is conducive to the activity of microorganisms in the bottom boundary layer, the characteristics of the bottom-water environment led to the obvious division of its community from those of other layers.

Conclusions

This study aimed to understand how the composition and structure of protistan communities change over extreme seasonal transitions and how they are affected by sampling depth. Our results suggest that ice melting caused shifts in the composition of protistan communities. The increase in diversity during the ice-free period suggests that the input of terrestrial nutrients may have caused these changes. Additionally, sampling depth affects the composition and structure of the protozoan community, but the effect of depth is less than that of environmental fluctuations caused by seasonal changes. The main factors influencing the community structure of protists change with the seasons. The concentration of organic carbon was the most important factor causing changes in the structure of protistan communities from freezing to thawing. The vertical gradients of water temperature during the ice-covered period led to the changes in protistan community structures, while the vertical gradients of nitrate concentration during the ice-free period were the most important factors. Attaining a better understanding of the impact of freezing and thawing on the distribution of protists will be important for characterizing the changes in exogenous substances and the duration of lake ice cover on biogeochemical fluxes. To better understand how seasonal variations and ice cover impact ecosystem function over the long-term, the temporal changes in microbial community composition and biogeochemical cycling must be characterized.

Abbreviations

LSD: Least significant difference; PCoA: Principal coordinates analysis; PERMANOVA: Permutational multivariate analysis; VIF: Variance inflation factor T: Temperature; DO: Dissolved oxygen; EC: Electroconductibility; TN: Total nitrogen; NO_3^- : Nitrate; NO_2^- : Nitrite; NH_4^+ : Ammonium; TC: Total carbon; IC: Inorganic carbon ; TOC: Organic carbon; SO_4^{2-}: Sulfate; PO_4^{3-}: Phosphate.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors consent for publication.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BC and JL designed the study. JL and XL collected water samples. BC, JL, XL performed the experiments. JL and XL performed statistical analyses and prepared the draft of the manuscript. JL advised on the Figures and Tables. All the authors revised the manuscript and approved the final version.

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Figures

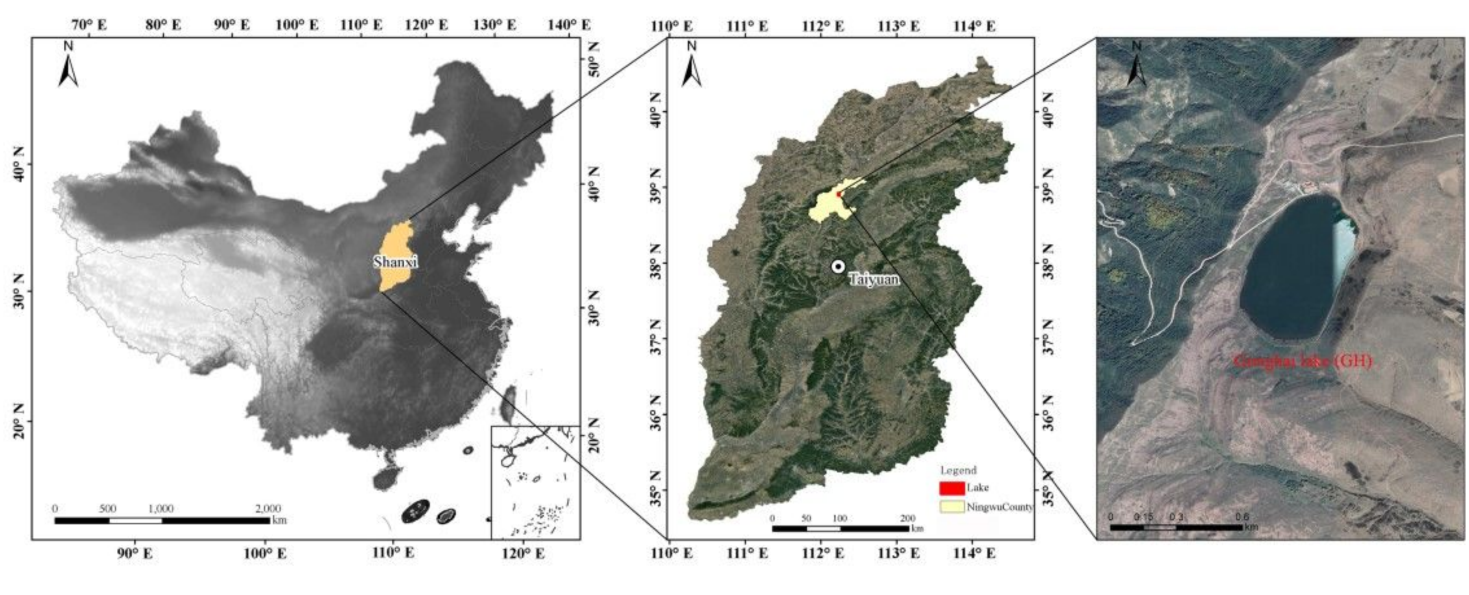

Figure 1

Map showing the location of sampling sites and spatial distribution of Gonghai Lake (GH) in the Ningwu County of Shanxi, China Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

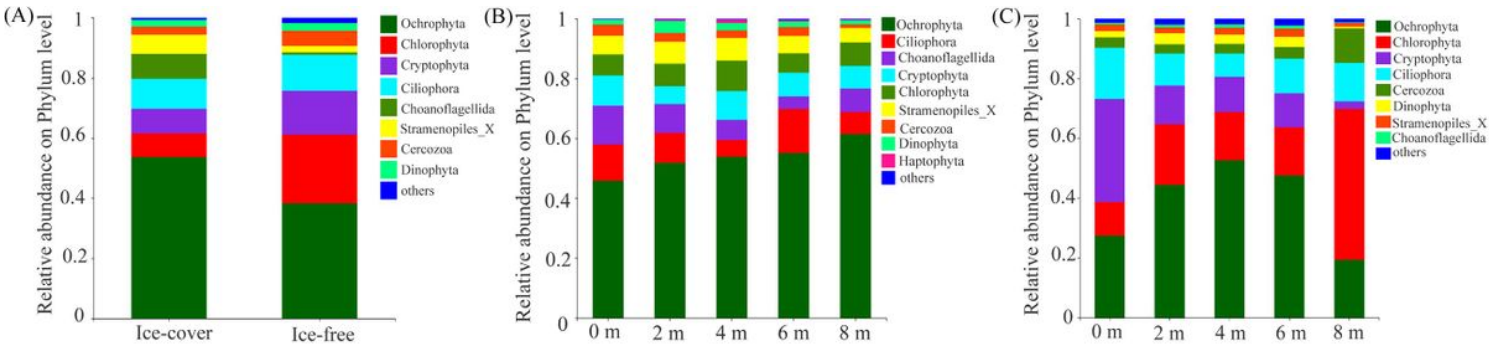

Figure 2

Relative abundance of the dominant protist phyla (with average relative abundance > 1%) during the (A) two periods, the (B) ice-covered period and the (C) ice-free period.

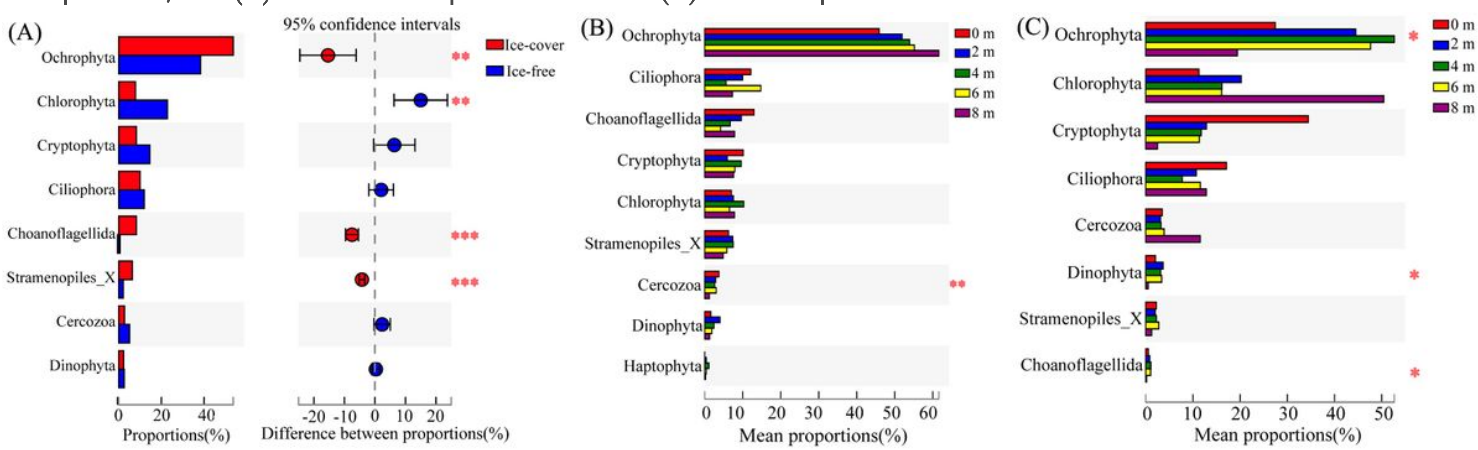

Figure 3

Difference among dominant phyla. during the (A) two periods, the (B) ice-covered period and the (C) ice-free period. *p < 0.05, **p < 0.01 and ***p < 0.001.

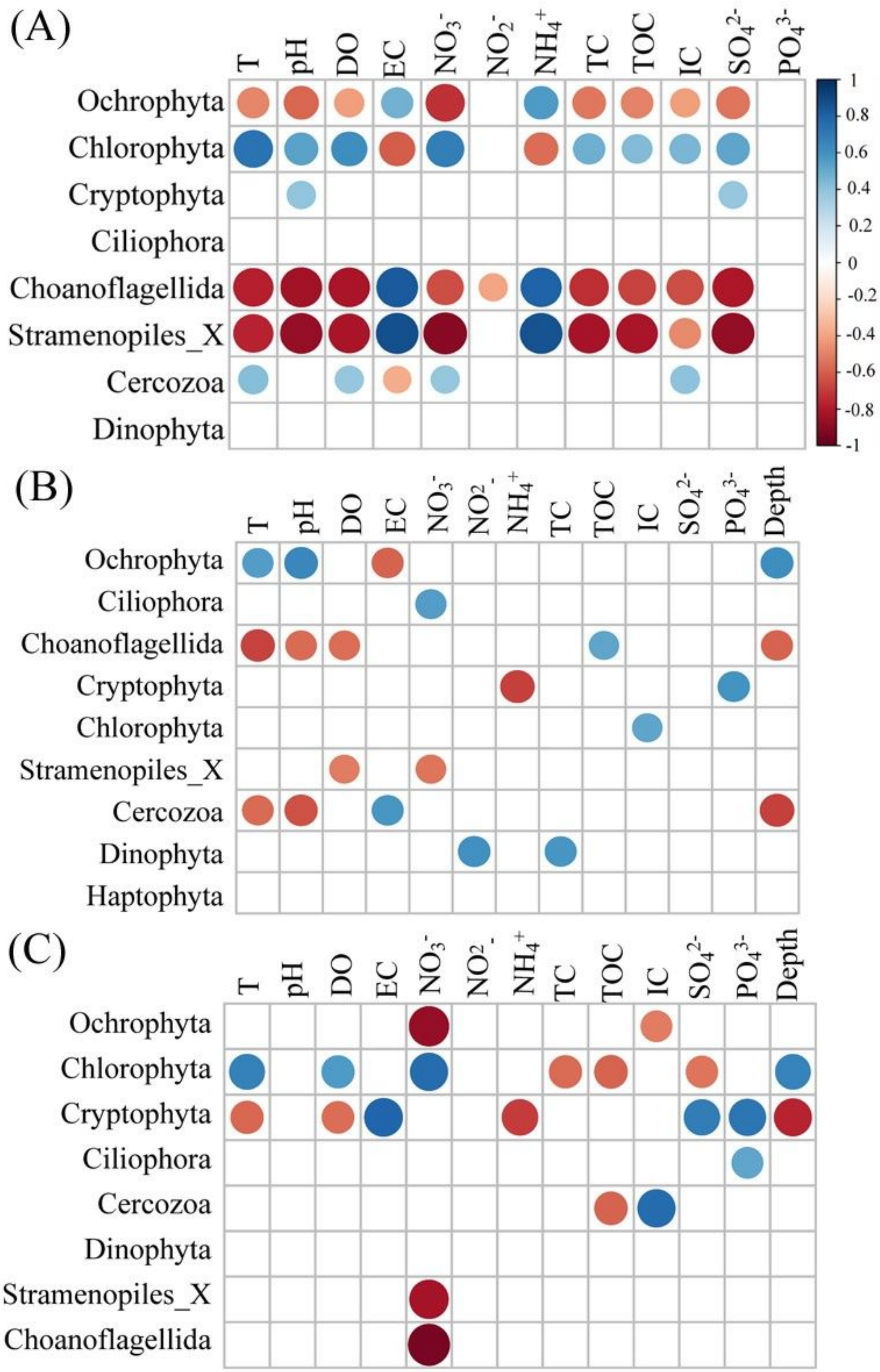

Figure 4

Heatmap showing significant Spearman correlations ($P < 0.05$) between dominant protist phyla and physico-chemical variables for (A) two periods, (B) different depths during the ice-covered period and (C) different depths during the ice-free period. Pairwise correlations without assigned color represent correlations that are not significant.

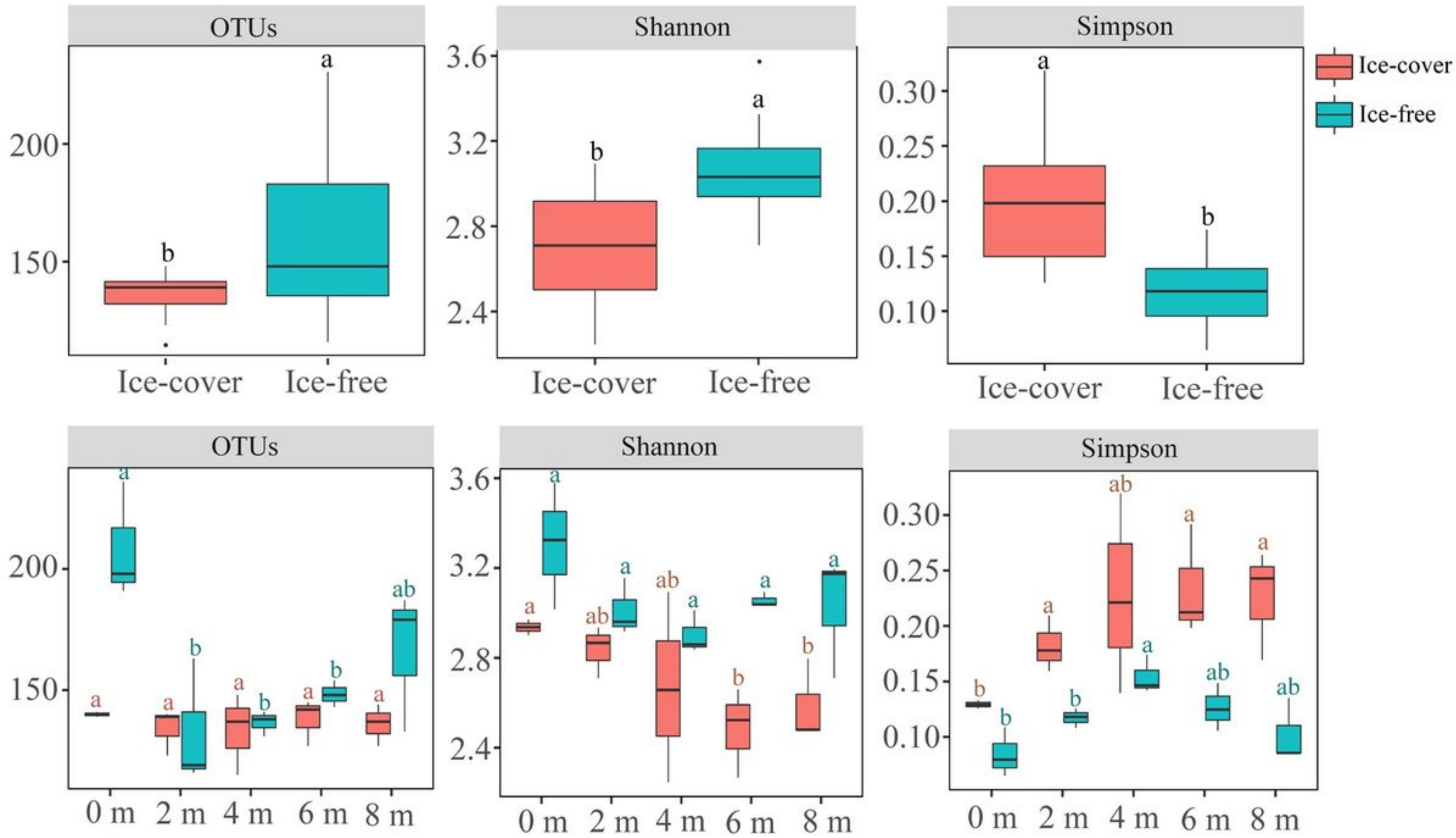

Figure 5

Variation of protistan community alpha diversity between two periods and among water depth for ice-covered and ice-free periods respectively. Significant differences between samples were determined using one-way ANOVA at $P < 0.05$ and different letters indicate significant differences.

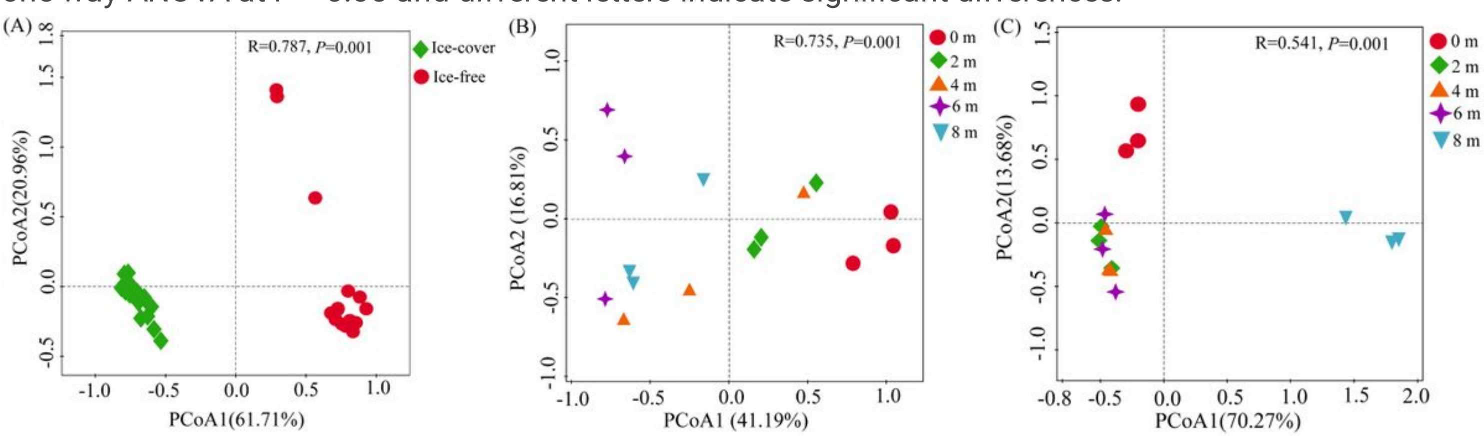

Figure 6

Principal coordinate analysis based on Bray-Curtis distance indicating the distribution patterns of protistan communities (A) two periods, (B) among five different depths of ice-covered period, and (C) among five different depths of ice-free period.

Supplementary Files

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- SupplementalResult.docx