Diversity and assembly processes of microbial eukaryotic community in Fildes Peninsula Lakes (West Antarctica)

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Abstract

The diversity, co-occurrence patterns, and assembly processes of microbial eukaryotes (0.2~20 μm) in Antarctic freshwater lakes are not well understood, despite their wide distribution and ecological importance. This study used Illumina high-throughput sequencing to explore five freshwater lakes' microbial eukaryotic communities on the Fildes Peninsula during three summer seasons. A total of ~~33~~ 28 phyla were detected, with the phytoplankton occupying the highest percentage of sequences (accounting for up to 98%). ~~Meanwhile, t~~The main dominant taxa were Chrysophyta, Chlorophyta, and Cryptophyta. ~~Alpha diversity~~ Richness (113~268) and Shannon index (1.70~3.50) varied among lakes, with higher values in lake Chang Hu and lake Kitec, the lowest value in lake Yue Ya.~~with Changhu (CH), Kitec (KT) lake having higher values, and Yue Ya (YY) lake having the lowest value.~~ There were significant differences in microbial eukaryotic communities ~~between~~among lakes, with spatial and temporal heterogenicity~~variation~~ in the relative abundance of dominant taxa ($P<0.05$). Environmental variables ~~only~~ explained about ~~30~~39% of the variation in community structure, with water temperature and phosphate identified as the driving factors ($P<0.05$). ~~In the co-occurrence network, microeukaryotes tended to be more symbiotic than competitive with each other (positive correlation 82% vs. negative correlation 18%),~~ Network analysis revealed comprehensive co-occurrence relationships (positive correlation 82% vs. negative correlation 18%). The neutral community model found that neutral processes explained more than ~~56~~55% of the community variation. The stochastic processes (e.g., homogenizing dispersal and undominated process) predominated in community assembly than the deterministic processes. These findings revealed the diversity of the microbial eukaryotic community and have important implications for understanding the community assembly in the freshwater lakes of the Fildes Peninsula (Antarctica).

Keywords: Microbial eukaryotic community; Diversity; Co-occurrence network; Stochastic processes; Fildes Peninsula lakes.

1 Introduction

The Fildes Peninsula locates in the southwestern part of King George Island of the West Antarctic region, which has a high concentration of Antarctic scientific research stations worldwide and is commonly free of ice during summer. It is the largest ice-free area (40 km^2) on King George Island, South Shetlands. This area falls within the “maritime Antarctic” with precipitation of 400-600 mm year^{-1} and an average temperature of -3 ºC (Holdgate 1977). Nevertheless, permafrost and periglacial processes occur (Barsch and Caine 1984). ~~The w~~Waters are affected by natural factors such as the sea, animals, and anthropogenic pressures in solid, volatile, and fluid wastes (Kawecka et al., 1998). Lakes in Fildes Peninsula, along with those found in other ice-free areas in Antarctica, represent the year-round liquid water reservoirs on the continent (Lyons et al., 2007; Priscu 2010). Antarctica lake systems are sentinels for climate change and contain chemical elements and microorganisms of global relevance (Marsh et al., 2020; Wilkins et al., 2013). Because of their physical stability, such lakes have been identified as model systems for inferring biogeochemical processes within water columns (Comeau et al., 2012).

~~As an essential component of microorganisms in Antarctic freshwater lake systems, micro~~Microbial eukaryotes (0.2~20 μm, pico-/nanoeukaryotes) constitute important components in microbial food webs and play an important role in the biogeochemical cycles (Grob et al., 2007; Massana et al., 2015; Unrein et al., 2014)~~has shown critical roles in the ecosystem services~~, as well as contributing to plankton biomass and carbon export (Hernandez-Ruiz et al., 2018; Leblanc et al., 2018).~~acting as the main food source and the primary contributors to material circulation(Grob et al., 2007; Mo et al., 2018b; Moreno-Pino et al., 2016; Zeng et al., 2014)~~ ~~.~~ However, the microbial eukaryotes have been neglected for a long term due to their small cell size and lack of conspicuous morphological features. The molecular approach can be used to compensate for the lack of traditional microscopic methods, providing us with a convenient way to study these small-sized eukaryotes. The application of 18S rRNA gene-based molecular tools has revealed high taxonomic diversity of microbial eukaryotes in some oligotrophic and extreme regions (Marquardt et al., 2016; Richards et al., 2005; Zhao et al., 2011). Nevertheless, research studies focused on exploring the molecular diversity and the population fluctuations in these far cold and oligotrophic Antarctica lakes are limited.

The small size, short generation time, rapid growth, sensitivity to environmental conditions, and genetic plasticity render ~~them~~microbial eukaryotes capable of quick reflection to environmental changes (Karimi et al., 2017). Hence, they are excellent bioindicators of the impact of environmental perturbations and ecosystem quality (Bouchez et al., 2016). Alveolate ~~The Ciliophora, Cryptomycota, Chlorophyta,~~ and dinoflagellates~~Bacillariophyta~~ have been detected in the ~~Freshwater Glacier Lake, East~~ size fraction 0.2~5 μm of Antarctica plankton eukaryotes, the biodiversity ~~seem to be~~was affected by ~~the~~ temperature and salinity (Lopez-Garcia et al., 2001). Based on microscopic observation, diatom in the lakes of Fildes Peninsula region accounted for 59.8% of the total number of phytoplankton species, and the water temperature and nutrients resulted in the variation of nano-and microphytoplankton abundance and composition (Zhu et al., 2010). A pretty low number of taxa, the abundance of diatom genera such as *Nitzschia*, *Achnanthes*, and *Navicula*, etc., have been investigated from the oligotrophic and periodically brackish water ponds near the Polish Antarctic Station on King George Island (Kawecka et al., 1998). The ~~microeukaryotic~~microphytoplankton communities of shallow lakes from the Antarctic Peninsula, ~~are~~were influenced by nutrients and surrounding inputs (Mataloni et al., 2000). The composition of pico/nanoeukaryotic community was significantly related to phosphate in the Bohai Sea and North Yellow Sea (Wang et al., 2020b). However, the spatio-temporal variation, co-occurrence pattern, and community assembly of microbial eukaryotes in Antarctic (Fildes Peninsula) freshwater lakes have been rarely reported.

Deterministic and stochastic processes have been considered the two main ecological processes in community assembly (Ofiteru et al., 2010). Deterministic processes are based on ecological niche theory; some deterministic factors (environmental conditions and species interactions) influence and determine community assembly (Powell et al., 2015). Stochastic processes are based on the neutral theory, which believes that random birth or death, drift, and dispersal events also play an essential role in community composition (Bahram et al., 2016). Deterministic processes (selection) are prevalent in building whole ecosystem communities (Liu et al., 2020a), selection leads to species classification, and applying similar habitats results in similar community assemblages. Although other studies supported a role for stochastic processes (drift and dispersal) in community assembly, dispersal was the movement of species in spatial location, and drift was associated with the relative abundance of species (Massana and Logares 2013; Wu et al., 2019). Stochastic processes accounted for up to 95% of the microbial eukaryotic community assembly mechanism in a set of lakes in Eastern Antarctica (Logares et al., 2018). Stochastic processes also were found to prevail in micro-and nanoplankton eukaryotic communities in intertidal zones in Intertidal Zones of Southeast Fujian, China (Kong et al., 2019). Nonetheless, the study of microbial eukaryotic diversity and its community assembly processes in Antarctica still requires further investigations for a more comprehensive view.

Few studies have been conducted on microbial eukaryotic diversity and community assembly processes of the freshwater lakes in the Fildes Peninsula, Antarctica. Our study attempted to analyze the microbial eukaryotic samples of five freshwater lakes from three summers using high-throughput 18S rRNA sequencing. We aimed to (I) understand the diversity and co-occurrence of microbial eukaryotes; ~~and~~ (II) ~~to~~ explore the influencing factors and their community assembly processes.

2 Material and Method

2.1 Sampling collecting

Field samples were collected on the 34th (2017/2018), 35th (2018/2019), and 36th (2018/2019) Chinese Antarctic Research Expedition (CHINARE) in January 2018 (34th), December 2018 (35th), and December 2019 (36th), respectively. The investigations were conducted in the Chinese Great Wall Station area at King George Island, the largest island in southern Shetland Island. Surface water samples were collected from five lakes, Chang_Hu (CH), Kitec (KT), Xi_~~hu~~ Hu (XH), Yan_~~ou~~ Ou (YO), and Yue_Ya (YY) (Fig. 1). Moreover, the physio-chemical parameters were measured synchronously.

~~Lake Chang Hu (~~CH~~)~~ is a narrow strip shape, surrounded by bulges, with major inputs from surrounding glacial melting water. ~~Lake Kitezh (~~KT~~)~~ is the closest to the Corinthian ice cap and is the source of drinking water for the Chilean station, near the airport for access to the Fildes Peninsula in Antarctica. The KT is the largest lake in this investigation area. ~~Lake Xi Hu (~~XH~~)~~ is the drinking water source area for the Great Wall Station scientific expedition station. ~~Lake Yann Ou (~~YO is surrounded by mountains and snow-covered, with moss and lichen growing in the soil. It is the smallest lake ~~of~~in this investigation area and is relatively sensitive to the effects of scientific expeditions. ~~Lake Yue Ya (~~YY, situated on Ardley Island, is far from human activities but influenced by penguins dwelling on the island, which brings massive penguins ~~excrements~~ excrement inputs.

Water temperature (WT), pH, and salinity (Sal) were measured using an~~a~~ ~~YSI Model 30~~RBRconcerto C.T.D (Canada~~Yellow Springs Instruments, Yellow Springs, USA~~). Chlorophyll *a* (Chl *a*) was extracted with acetone and measured spectrophotometrically. Nutrients, including ammonia (NH_4^+), nitrite (NO_2^-), silicate (SiO_3^{2-}), and phosphate (PO_4^{3-}) were measured spectrophotometrically with a continuous flow autoanalyzer Scan++ (Skalar, the Netherlands) after filtering water through 0.45 μm cellulose acetate membrane filters (Whatman) as described by (HP

Hansen and Koroleff 1999).

2.2 PCR and Illumina MiSeq

~~For Illumina MiSeq2000,~~ 1 L surface seawater was collected and prefiltered through a 20 µm mesh sieve to remove most of the mesozooplankton and large particles, then directly filtered through a 0.2 µm pore size nucleopore membrane filter (Whatman). The filters were frozen at –80°C in CTAB buffer until laboratory experiments. DNA extraction was performed as described by (Luo et al., 2015).

PCR was performed using primers with barcode flanking the hypervariable V4 region of the 18S rRNA gene: 3NDf (Charvet et al., 2012) with the reverse primer V4_euk_R2 (Brate et al., 2010). Polymerase chain reactions (PCRs) were conducted in 20 µL reactions with 0.2 µM each primer, 10 ng of template DNA, 1 × PCR buffer, and 2.5 U of Pfu DNA Polymerase (Promega, USA). The amplification program consisted of an initial denaturation step at 95 °C for 2 mins, followed by 30 cycles of 95 °C for 30 s, 55°C for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min. ~~PCR products were pooled and purified using the DNA gel extraction kit (Axygen, Hangzhou, China). The DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Germany) and was quality controlled on a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Finally, amplicons of all samples were pooled in equimolar concentrations.~~ The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer’s instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego,USA) according to the standard protocols by Wefind Biotechnology Co., Ltd. (Wuhan, China).

The raw 18S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0 (Chen et al., 2018) and merged by FLASH version 1.2.7

(Magoc and Salzberg 2011) with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching.

Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE version 7.1 (Edgar 2013), and chimeric sequences were identified and removed. The taxonomy of each representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al., 2007) against the 18S rRNA database (Silva v138) (Quast C et al., 2013) using confidence threshold of 0.7.

The analysis of OTUs obtained using the UPARSE clustering method have still been widely used for high-throughput sequencing analysis (Gad et al., 2022; Reboul et al., 2021; Sun et al., 2022; Xu et al., 2022; Zhang et al., 2022). Some of the diversity indices in our study were more comparable to previous similar research using 97% sequence similarity OTUs (Chen et al., 2022; Wang et al., 2021; Wang et al., 2020b), as comparisons of these statistics using the same bioinformatics tool still seem to remain persuasive (Glassman and Martiny 2018; Li et al., 2019).

A total of 726,700 valid sequences of the 18S rRNA gene in all samples were obtained, and the average length of the acquired reads was 443 base pairs. The OTUs, classified as metazoa, unassigned and unclassified sequences, were filtered based on taxonomic metadata. Finally, the sequences were normalized at the lowest sequences depth and rarefied at 16,717 reads, yielding a total of 520 OTUs, distributed among 28 phyla. The Good's coverage values were above 99.9%, confirming that the libraries could represent most species in these lakes, with rarefaction curves reaching saturation (Fig. S1).

~~We used QIIME default parameters for quality filtering (reads truncated at first low-quality base and excluded if: (1) overlap ≤10bp while the coupled reads were assembled into one single sequence, (2) less than 80% of reading length was consecutive high-quality base calls, (3) more than 1 errors were present in the bar code, (4) the length was less than 50 bases . We picked operational taxonomic units (OTUs) with a 97% similarity cut-off using available reference UPARSE version7.1 (http://drive5.com/uparse/). Reads that did not match any sequences in the reference database at ≥ 97% identity were clustered de novo. The taxonomic identity of eukaryotic representative sequences was performed using RDP classifier against the SILVA database (version 132 NR) at a bootstrap cutoff of 80%.~~

2.3 Community composition and diversity

The ~~OTUs~~richness and Shannon index (H) were measured using the “vegan” R package based on the OTUs table, respectively. The nearest-taxon index (NTI) was used to measure the degree of phylogenetic clustering of taxa on a within-community scale for communities. High or positive values indicated clustering taxa across the overall phylogeny, while lower negative values indicated overdispersion of taxa across the phylogeny (Horner-Devine and Bohannan 2006). The nearest taxon index (NTI) quantifies the number of standard deviations that the observed ~~MNTD~~mean nearest taxon distance (MNTD) is from the mean of the null distribution with 999 randomizations in the “Picante” R package.

Non-metric multidimensional scaling (NMDS) of microbial eukaryotic communities was performed with the relative abundance of OTUs (Roberts 2013). Analysis of similarity (ANOSIM) investigated differences in the microbial eukaryotic communities between groups. The unweighted pair-group method with arithmetic means (UPGMA) was used to determine the similarity between samples by clustering analysis according to community composition similarity. These analyses were performed in the R package “Vegan” and “Phangorn”. All calculations were based on

~~similarity matrices calculated with the Bray-Curtis similarity index.~~ similarity matrices (1-dissimilarity of the Bray–Curtis distance metric).

2.4 Influencing factors of the community structure

Canoco 4.5 software (Braak and Smilauer 2002) was used to rank species and environmental factor data, and the ranking model was determined by de-trending correspondence analysis (DCA) of OTUs data. All environmental factors, except pH, were log (x+1) transformed before analysis to improve normality and homoscedasticity. To reduce multicollinearity among environmental factors, all variance inflation factors (VIFs) were kept below 10. The environmental factors influencing the composition of the microbial eukaryotic community were selected by 999 Monte Carlo permutation tests at the significant level (P<0.05). In addition, the relative importance of water temperature, physicochemical factors, and nutrients was assessed using the variation partitioning analysis (VPA).

2.5 Co-occurrence Network Analysis

We constructed one co-occurrence network based on samples from the whole study period. ~~The samples collected were performed by co-occurrence network analysis.~~ To reduce the complexity of the data sets, OTUs ~~represented Occurred~~ occurred in at least 5 samples were retained to construct networks. Only robust (|r| > 0.6) and statistically significant (P<0.05) correlations were incorporated into network analyses. Finally, network visualization was conducted using Gephi software (Bastian M et al., 2009). ~~Previous studies identified potential keystone taxa as nodes with degree > 30 and betweenness centrality < 5000 (Ma et al., 2020; Zhang et al., 2021a).~~

2.6 Ecological community assembly analysis

The Neutral community model (NCM) was used to measure the potential role of stochastic processes in the assembly of microbial eukaryotic communities based on the relationship between OTUs frequency and relative abundance (Chen et al., 2019). The model ~~is~~was derived from neutral theory (Zhou et al., 2014). The parameter Nm represents the metacommunity size, and R^2 represents the degree of fit to a neutral model.

To further evaluate the contributions of deterministic and stochastic processes to community assembly, the Stegen null model was used (Stegen et al., 2012). The β-nearest taxon index (βNTI) was calculated using phylogenetic distance and OTUs abundance (Stegen et al., 2013; Webb et al., 2002). The relative contribution of variable selection and homogeneous selection was estimated from the percentage of pairwise comparisons whose βNTI were > 2 and < −2, respectively. We further calculated the Bray-Curtis-based Raup-Crick index (RC_{bray}) to investigate pairwise comparisons that deviated from selection (Evans et al., 2017; Stegen et al., 2013). Integrated with the value of $|RC_{bray}|$, the underlying community assembly processes could be homogenizing dispersal (| βNTI| < 2 and $RC_{bray} < -0.95$), dispersal limitation (| βNTI| < 2 and $RC_{bray} > +0.95$) and undominated processes (i.e. weak selection, weak dispersal, diversification, and drift processes) with | βNTI| < 2 and $|RC_{bray}| < 0.95$. The null community of all the samples was randomized 999 times to obtain average null expectations.

3 Result

3.1 Physico-chemical properties

The ~~water temperatures~~ WT varied from 0.90 °C to 7.14 °C~~of all five lakes had similar values as 0.90°C to 7.14°C~~ (Table S1), while the YO ~~lake~~ was significantly higher than other lakes (*P*<0.05). Nutrients were low with ~~nitrite (~~NO_2-N~~)~~, ~~ammonium nitrogen (~~NH_4^+~~)~~, and~~ phosphate~~ PO_4^{3-}~~ (PO_4-P)~~ concentrations with 0.00~0.15 µM~~ L^{-1}~~, 0.05~0.74 µM~~ L^{-1}~~, and 0.02~2.29 µM~~ L^{-1}~~, respectively. YY ~~lake~~ had higher concentrations of PO_4^{3-}~~phosphate~~ , NH_4^+~~ammonium nitrogen~~, and NO_2^-~~nitrite~~, while XH had lower NO_2^-~~nitrite~~ and~~ phosphate~~ PO_4^{3-} .~~.~~ ~~Silicate (~~SiO_3^{2-}~~)~~ varied from 1.43 to 51.5 µM~~ L^{-1}~~, with the highest value in CH and lowest value in YY. The range of Chl *a* was 0.25~2.11 µg L^{-1}, with the YY highest and the CH lowest. pH ranged from 7.65 to 8.27. ~~Salinity~~Sal was 0.00-0.14 PSU, which in YO ~~lake~~ exhibited a significantly lower value compared to other lakes (*P*<0.05).

3.2 Diversity and composition of microbial eukaryotic community

~~A total of 726,700 valid sequences of the 18S rRNA gene in all samples was obtained, and the average length of the acquired reads was 443 base pairs. These sequences clustered into 547 OTUs at 97% similarity level, distributed among 33 phyla. The Good's coverage values were above 99.9%, confirming that the libraries could represent most species in these lakes.~~

A total of ~~10~~ 9 dominant phyla were identified, accounting for over 98% of the sequences.~~96.02% sequences in CH, 97.01% in KT, 98.30% in XH, 94.19% YO, and 98.27% YY.~~ These dominant phyla were mainly composed of microbial eukaryotic phytoplankton, with various relative abundances between different lakes (Fig. 2a). The Chrysophyta (~~35.04~~34.91% in CH~~~76.69~~ 78.88% in XH), Chlorophyta (~~13.94~~15.17% in KT~~~35.37~~35.88% in YY), and Cryptophyta (0.01% in YO~23.~~73~~71% in CH) were most abundant in lakes. The Cryptophyta in KT was significantly more abundant than in XH and YO, and Pyrrophyta~~Alveolate~~ in ~~KT~~ XH was significantly more abundant than in ~~YO~~ YY (*P*<0.05) (Fig. 2b). ~~Meanwhile, it was noticed that the relative abundances of some phyla varied between lakes but not significantly, with Chytridiomycota, Cercozoa, and Cryptophyta in XH being higher than those in YO.~~ The ~~Arthropoda~~ Haptophyta represented ~~0.10~~0.09% in ~~CH~~KT~~~4.11~~4.93% in YO; Alveolate represented ~~0.03~~0.02% in XH~~~1.01~~1.00% in CH, and unclassified Stramenopiles-Alveolates-Rhizaria (SAR) represented ~~1.07~~ 1.12 % in XH~~~5.27~~8.05 % in YO.

The relative abundance of the dominant taxa in the same lake had some interannual variation. The Chrysophyta in CH_19, YO_19, and YY_19 samples were lower than the other samples, while the Cryptophyta in CH_19 and YY_19 were lower than the other samples in CH, and YY, respectively~~. The proportion of Arthropoda in YO_19 reached 70.09%, which was remarkably higher than the different samples~~ (Fig. 2a).

A total of ~~24~~ 21 dominant genera were identified (Fig. 2c), accounting for over 87% of the sequences.~~81.22% sequences in CH, 79.43% in KT, 61.22% in XH, 65.95% in YO, and 59.06% in YY.~~ The dominant genera were mainly *Hydrurus*, *Paraphysomonas*, *Ochromonas*, *Synura*, and *Monochrysis* belonging to Chrysophyta, *Komma* in Cryptophyta, *Monomastix*, *Chlamydomonas*, and *Mantoniella*~~*Raphidonema*~~ in Chlorophyta.

As shown in Fig. 2c, the abundance of the dominant genera differed among the lakes investigated interannually. The relative abundance of *Komma* varied from 0 to 48.~~49~~52%, which showed an increasing trend over the year in CH and YY. The ranges of *Paraphysomonas* and *Ochromonas* were ~~0.28~~3.06~~~41.98~~56.88% and ~~0.22~~0.06~~~15.82~~18.86%, showing an increase followed by a decrease in XH ~~and YO~~ over the year. *The Hydrurus* in XH_18 and YO_18 was higher than in the other samples. *Mantoniella*~~*Raphidonema*~~ was significantly more abundant in CH than in other lakes. *Ochromonas*~~*Chrysosphaerell and Synura*~~ in ~~KT~~CH~~, except for compared with CH, were~~ was significantly ~~higher~~ lower than ~~other lakes~~in YO and XH ($P<0.05$, Table S2).

The ~~indices (OTUs~~richness, Shannon index, and NTI~~)~~ had interannual variation but showed no significance ($P>0.05$) (Fig. 3a, c, e). The order of NTI and Shannon all showed 2018>2017>2019; ~~OTUs~~ Richness were highest in the expedition season 2017 and lowest in ~~2019~~2018. The ranges of ~~OTUs~~richness and Shannon index were ~~151~~113~~~244~~ 268 and ~~2.06~~1.70~~~3.26~~3.50, respectively, with YY having the lowest value and was significantly lower than ~~CH and~~ KT (Fig. 3b, d, $P<0.05$). The range of NTI was ~~0.80~~0.62~~~1.42~~1.56, with the lowest value in YO and significantly lower than KT (Fig. 3f, $P<0.05$). KT had the highest Shannon index, richness, and NTI, ~~while~~and CH also had the ~~highest~~ higher ~~number of OTUs~~richness (Fig. 3b, d, f).

The total number of OTUs shared in 2017-2019 was ~~276~~239, and the unique OTUs were ~~31~~ 32 (2017), ~~34~~ 49 (2018), ~~70~~ 66 (2019) (Fig. 3g). The Venn diagram showed that the total number of OTUs shared by the five lakes was ~~129~~103, and the unique OTUs were ~~62~~ 59 (CH),~~37~~ 43 (KT),~~5~~ 9 (XH),14 (YO), and ~~14~~ 15 (YY) (Fig. 3h).

The NMDS results divided the samples into five clusters according to their similarity of microbial eukaryotic community (stress value = 0.~~14~~12) (Fig. 4a). In addition, the analysis of similarity (ANOSIM) based on Bray-Curtis distance indicated that the differences between lakes were significant (Global R = 0.~~613~~70, *P*<0.01). Meanwhile, no significant differences were detected by ANOSIM among interannual variations (R=0.013, *P*=0.393).

UPGMA clustering analysis (Fig.4b) showed the same lakes in a different year, such as CH_17 and CH_18, YY_17 and YY_18, YO_17 and YO_18 clustered together~~clustered into one clade~~, respectively. For other lakes, ~~KT_18 and XH_18 clustered as one clade,~~ CH_19, and YY_19 clustered together~~as one clade, CH_17, and XH_19 clustered as one clade~~. YO lake was distant from other lakes and clustered into a separate one.

3.3 Driving factors and co-occurrence patterns

Canonical correspondence analysis (CCA) demonstrated that the first two sequencing axes explained ~~16.7~~21.84% and ~~15.5~~17.11% of community variation (Fig. 5a). The samples from the same lake were closer, with a more similar community structure. More importantly, the Monte Carlo analysis confirmed that the ~~water temperature~~WT and PO_4^{3-} significantly affected the microbial eukaryotic community (*P*<0.~~01~~05). The variation partition analysis (VPA) indicated that environmental factors monitored explained ~~14.19~~9.34% of microbial eukaryotic community variability among lakes and still had a large amount of unexplained community variation (~~85.8~~90.66%, Fig. 5b).

A total of ~~223~~ 220 nodes linked by ~~1941~~ 1521 edges ~~was~~ were made up microbial eukaryotic network. The majority of nodes in the network had many connections. Notably, the positive associations among species were predominant in the network (Fig. 5c), with ~~82~~81.82~~.25~~%, whereas the portion of negative association was only

~~17.75~~18.18%. In addition, the positive interactions were mainly within the same taxonomic affiliations, such as Chrysophyta, or between a few different taxonomic affiliations, such as Chrysophyta and Chlorophyta. While the negative correlations mainly were reflected between Chrysophyta and Chlorophyta. We found that only about 8% of OTUs directly correlated with environmental factors ($P<0.05$). Meanwhile, only ~~four~~ two of the top 20 OTUs with the highest degree centrality were directly associated with environmental factors (WT~~,~~ and PO_4^{3-}~~PO_4-P~~), and ~~three~~ one belonged to Chrysophyta and one to Chytridiomycota~~Cercozoa~~.

~~24 nodes were identified as potential keystone species (Table S3), which contained *Heteromita* belonging to Cercozoa, seven genera belonging to Chrysophyta, such as *Spumella*, *Ochromonas*, and *Chromulina*. The Chlorophyta keystone genera included *Chloromonas* and *Chlamydomonas*, and other genera were from Bacillariophyta and Alveolata.~~

3.4 Community assembly processes

The Sloan neutral community model (NCM) showed the importance of stochastic processes for microbial eukaryotic communities (Fig. 6a), with the neutral processes explaining ~~56.8~~55.5% community variation. In addition, the ~~Sloan neutral model~~NCM classified microbial eukaryotic taxa into three groups (above prediction, below prediction, and neutral prediction). We found that the neutral group (within 95% confidence interval), with richness and abundance ratios of ~~79.7~~80.0% and ~~90.4~~86.4%, respectively, were both much higher than the above and below prediction groups, which was dominated by Chrysophyta, Chlorophyta, and Cryptophyta (Fig. 6b, c). The above prediction group accounted for ~~8.8~~10.58% of the microbial eukaryotic richness but corresponded to only ~~0.75~~0.96% of the abundance, dominated by Chrysophyta, Chlorophyta, and ~~Chytridiomycota~~Cercozoa. Cryptophyta accounted for ~~13.3~~16.58% of the abundance in the neutral group but was almost absent in the ~~other two groups~~above group. In contrast, Cercozoa~~Chytridiomycota~~ was present in ~~10.4~~9.38% abundance in the ~~two groups mentioned~~ above group, but only ~~0.1~~0.32%

in the neutral group.

~~The variation of βNTI ranged from -1.65~1.31 with a mean value of -0.48 (Fig. 6d), which~~ The βNTI was mainly distributed in the region of stochastic processes with a mean value of -0.48 and supported the results of the neutral model. The community assembly process analysis showed that stochastic rather than deterministic processes controlled the community assembly. Among them, homogenizing dispersal dominated, with a proportion of ~~64.76~~59.05%, followed by undominated process and dispersal limitation (Fig. 6d).

4 Discussion

4.1 Diversity and dominant taxa

The environmental conditions (e.g., low light and low nutrient, etc.) in Antarctic freshwater lakes differed from temperate lakes. These special features and relative isolation resulted in unique communities and the survival strategies of the species adapted to such conditions (Pearce 2008). In our study, the survival of taxa (Chrysophyta, Chlorophyta, and Cryptophyta) might depend on their survival strategies to withstand harsh conditions, which made them as the predominant species.

Chrysophyta dominated in five lakes examined in our study, including *Hydrurus*, *Paraphysomonas*, *Ochromonas*, and *Monochrysis*. Firstly, the dominance ~~may~~might be due to the adaptation to low nutrient availability~~;~~.~~ -~~ Chrysophyta have been well represented mostly in oligo and mesotrophic lakes from both the Maritime and Continental regions (Allende 2009; Allende and Izaguirre 2003; Izaguirre et al., 2020; Richards et al., 2005).~~the relatively high surface to volume ratio contributes to the uptake of nutrients at low concentrations, Which have been reported in high latitude polar lakes~~ (Charvet et al., 2012)~~ .~~. Secondly, Chrysophyta still retained high cell density~~keeps a high proportion~~ under low light conditions, as they ~~can~~could adapt to changing light conditions (Yubuki et al., 2008). Furthermore, Chrysophyta ~~is~~was mixotrophic and even ~~can~~could swim, which allow~~s~~ed them to get available nutrients from other microorganisms, reducing the need for dissolved nutrients in the water

(Katechakis and Stibor 2006; Pick and Lean 1984). In addition, when the environmental conditions changed dramatically, such as freezing and nutrient changes, Chrysophyta ~~can~~could form cysts (Nicholls 1995), protecting cells from resisting an unsuitable environment. All these aspects ~~make~~made Chrysophyta ~~has~~have the advantage to be the predominant species in the five Antarctic lakes.

Chlorophyta was the second most dominant taxon in our study (~~13.94~~15.17%~~~35.37~~35.88%), containing mainly *Monomastix*, *Chlamydomonas*, and *Mantoniella*~~*Raphidonema*~~. Chlorophyta ~~is~~was typically represented by flagellated species such as *Chlamydomonas* spp., which dominate ~~the~~ phytoplankton communities in different trophic statuses and respond to adverse environmental conditions by forming temporary, non-swimming cell populations encased in a gelatinous mother cell membrane~~by forming temporary groups~~ (Allende and Mataloni 2013; Izaguirre et al., 2003; Toro et al., 2007). Several unicellular algae ~~can~~could mix acid fermentation, and some obligate photoautotrophic species responded by photo-acclimation processes involving the accumulation of chlorophyll to increase the light capture efficiency (Atteia et al., 2013; Morgan-Kiss et al., 2016). These characteristics might partially be explained how Chlorophyta survived and occupied a specific advantage of the important reason in lakes we studied.

Cryptophyta was the third dominant taxon observed. Indeed, their dominance has been interpreted as evidence of heterotrophic growth in winter and mixotrophic throughout the year (Unrein et al., 2014). Cryptophyta dominate~~s~~d under perennially ice-covered and coastal saline lakes in continental Antarctica. The ingestion of bacteria by mixotrophic Cryptophyta has been observed in two perennially ice-covered lakes (Fryxell and Hoare) in the McMurdo Dry Valleys (Roberts and Laybourn-Parry 1999).

Compared with other aquatic ecosystems (Hernandez-Ruiz et al., 2018; Wang et al., 2021; Wang et al., 2020b), the diversity of microbial eukaryotes in Antarctic lakes was significantly lower (Shannon ~~2.06~~1.70~~~3.26~~3.50, ~~OTUs~~richness ~~151~~113~~~244~~268).

The diversity of microorganisms reported decreases from mid-latitude to the poles (Santos et al., 2020). The isolation and harsh conditions, especially the lower temperatures and nutrients, prevailing in Antarctic lakes accounted for a low microbial eukaryotic diversity. In addition, the species-area relationships model (SAR) states that increased species number with increasing habitat area within a specific area (Ma 2018). An increase in the ice-free area drastically modifie~~s~~d biodiversity (Duffy et al., 2017; Lee et al., 2017; Pertierra et al., 2017). Our results supported the SAR model, observing more alpha diversity ~~and richness~~ in CH and KT, where habitat areas were much larger than the YY and YO.

4.2 Influence of environmental factors on the community

Previous great efforts have demonstrated that abiotic factors affect microbial diversity and community composition (Quiroga et al., 2013; Sun et al., 2017). Our study found that ~~only water temperature~~ WT and PO_4^{3-} ~~was~~were ~~a~~ significant driving factors for community change among the abiotic factors analyzed. Some microorganisms have evolved to grow under a defined temperature, allowing differences in temperature adaptation of different species (Wilkins et al., 2013). ~~Water temperature~~WT has become a major driving factor for ~~changes in~~ microbial eukaryotic communit~~ies~~y variations by regulating cellular activity and metabolic rates (Margesin and Miteva 2011). The retreat of glaciers due to global warming had the risk of reducing the abundance and diversity of microorganisms, and more attention should be paid to the impact of ~~water~~ temperature changes on community structure (Garcia-Rodriguez et al., 2021). The ~~water temperature~~WT in the YO ~~lakes~~ was significantly higher than in the other lakes, and YO clustered into a separate clade (Fig. 5b). Microorganisms have been highly sensitive to nutrients, and nutrients were important factors of community differences in microbial eukaryotes in different areas (Zouari et al., 2018). PO_4^{3-} has been demonstrated to be one of the environmental variables that “best” explained the picoeukaryotic distributions in the Western Pacific Ocean (Liu et al., 2021).

Nevertheless, a small amount of community variation could be explained by measured environmental variables in our analysis. This indicated that these environmental factors played a minor role in shaping microbial eukaryotic community structure. There were many unexplained variations (Fig. 5), and some possible causes have been indicated. Firstly, it was not easy to obtain all environmental factors, and some important factors might exist that were not fully obtained or taken into account in the current study (Wang et al., 2020a).~~Firstly, the nonconsecutive of environmental factors among different expedition seasons was deficient in our study.~~ There ~~are~~were also many vital abiotic factors in Antarctic freshwater lakes, including solar cycle, light availability, ice cover (thickness and duration), physical changes as snow melts and mixes, and hydrological changes (Allende and Izaguirre 2003; Lizotte 2008). Secondly, the relationship between microorganisms~~ (symbiotic or competitive)~~ ~~cannot~~ couldn't be quantified, which ~~is~~was an essential factor influencing community structure. Predation pressure manifested itself in lakes as a top-down control of microbial eukaryotes (Blomqvist 1997). Thirdly, stochastic processes such as ecological drift (birth, death) ~~may~~might cause unexplained community variation (Zhang et al., 2018).

4.3 Co-occurrence patterns ~~and keystone taxa~~

Network analysis could help us understand complex biological interactions and ecological rules for community assembly within a specific ecological niche (Li and Hu 2021; Lupatini et al., 2014). Microorganisms form various ecological relationships, ranging from mutualism to competition, ultimately shaping microbial abundances (Faust and Raes 2012). Positive associations in a network often indicate common preferred environmental conditions or niche-overlapping, whereas negative associations mean competition or niche division (Faust and Raes 2012). By analyzing the network, we found that the positive correlations were much more than the negative correlations in the co-occurrence network (~~87~~82% vs. ~~13~~18%), revealing that assumed positive relationships (e.g. due to cross-feeding, niche overlap, mutualism and/or commensalism) might exhibit a more important role than negative relationships (e.g. predator-prey, host-parasite and/or competition) (Chen and Wen 2021) in studied Antarctic lake ecosystem. Similar result has been found in small planktonic eukaryotes (0.2~20 μm) inhabiting surface waters of a coastal upwelling system (Hernandez-Ruiz et al., 2018). Notwithstanding, further studies are necessary to corroborate the biological interactions and other nonrandom processes (for example, cross-feeding versus niche overlap) between species pairs detected by network analyses.~~indicating that species coexistence was achieved mainly by symbiotic relationships between species.~~ In addition, only 8% OTUs were significantly correlated with environmental factors, suggesting that microbial eukaryotes had a relatively lower response to environmental factors and these ~~could~~ might weaken the role of environment selection in community assembly. Previous studies have shown the high response of microbial eukaryotic communities to mid-and late-stage diatom blooms promotes deterministic processes (Hou et al., 2020).

~~In co-occurrence networks, keystone species play a critical role in maintaining the structure and function of the microbial community, and the loss of essential species may lead to the fracturing of networks (Zhang et al., 2022). (Xue et al., 2018)The keystone species in this study belonged mainly to Chlorophyta, Chrysophyta, Bacillariphyta, and Cercozoa. *Heteromita* has significant genetic variation and promotes bacterial degradation of alkylbenzenes through predation (Ekelund et al., 2004). *Spumella* is a heterotrophic microorganism commonly found in freshwater and soil (Boenigk et al., 2005). As mixed trophic organisms, *Ochromonas* prey on bacteria and are, therefore, a critical link between bacteria and higher trophic levels (Andersson et al., 1989). *Chloromonas* has motile trophic cells that can grow in the snow to give it a green color and, together with Chlamydomonas, are thought to have a strong carbon concentration mechanism (Hu 1998).(Belevich et al., 2020; Tragin and Vaulot 2019).~~

4.4 Community assembly processes

In general, deterministic and stochastic processes existed simultaneously in the community assembly (Chase 2010; He et al., 2021). Several factors such as habitat connectivity and size (Orrock and Watling 2010), productivity (Chase 2010), disturbance (Liang et al., 2020), predation (Chase et al., 2009), and resource availability (Kardol et al., 2013) influenced the relative importance of stochastic and deterministic processes in the community assembly. The importance of stochastic processes has been previously illustrated for other microbial eukaryotic communities from the aquatic ecosystem (Chen et al., 2019; Wang et al., 2020a). The picoeukaryotic communities in the lower oxygen layer of Bengal Bay were dominated by stochastic processes (Chen et al., 2022). In our study, the results supported the prominent role of stochastic processes in shaping the microbial eukaryotic community assembly than deterministic processes. Hence, environmental variables explained only a small number of variations in our study's microbial eukaryotic community, and a small number of taxa were significantly correlated with environmental factors.

In our study, the microbial eukaryotic community showed a good fit~~ (57%)~~ to the neutral model (Fig. 6a), which suggested community variation could~~can~~ be explained by stochastic processes such as birth, death, and migration to a large extent. The NCM also attributed the observed patterns of community assembly to different population behavior (Zhang et al., 2021b). The NCM separated taxa into three groups, and these groups were different in community structure (Fig. 6), which was similar to the result of microbial eukaryotic community in the Middle Route Project of the South-to-North Water Diversion Project ~~channel~~ (Zhang et al., 2021b), indicating these taxa might differ in their adaptability to the environment or dispersal rate (Chen et al., 2019). In addition, the neutral group contributed a high proportion to both abundance (~~90.4~~86.4%) and richness (~~79.7~~80.0%) in our study. In a subtropical river, the neutral group also dominated the microbial eukaryotic community in terms of richness and abundance (Chen et al., 2019). Similar results in this study suggested that microbial eukaryotes~~ in this study~~ were more susceptible to stochastic processes.

~~Furthermore, the null model results showed that stochastic processes (mainly homogenizing dispersal and undominated process) dominated the community assembly (Fig. 6c). The importance of stochastic processes has been previously illustrated by the microeukaryotic communities of lakes in East Antarctica (Logares et al., 2018). Abrupt changes in environmental conditions can affect the relative contribution of community assembly processes. For example, increasing the nutrients and regulating ecological scheduling (Chan et al., 2002; Jiang and Patel 2008; Liu et al., 2019), perennial fertilization in the soil (Liang et al., 2020), and the activities of long-term cultivation of rice fields (Liu et al., 2020b) all have cause changes in the relative contribution of stochastic and deterministic processes. It has been believed that if changing environmental factors are not significant or do not force selection on species, stochastic processes still dominate (Zhou et al., 2014). (Lei et al., 2021)The extreme environmental conditions over a long period might lessen the ecological selection pressure on microeukaryotes. Furthermore, the explanation for the dominance of the stochastic process might also be due to the long-term adaptation of species to the environment, which leads to a low response, as also confirmed by the fungal community assembly (Powell et al., 2015).~~

~~Our study's microeukaryotic community tended to homogenize during dispersal, and the community compositions were relatively stable.~~ The importance of stochastic processes has been previously illustrated by the microbial eukaryotic communities of lakes in East Antarctica (Logares et al., 2018). Antarctic freshwater lakes can receive external microbial colonies by the input of microorganisms from the surrounding ice melt, atmospheric transport, human activities, or bird migration (Unrein et al., 2005). ~~Water bodies have been reported occupied with a high proportion of homogenizing~~ Homogenizing dispersal occupied a high proportion in water bodies (Zeng et al., 2019), and the similar result was found in our study. Most microorganisms detected in the sea also have been found present in lakes in East Antarctica, pointing to that some marine taxa in the lake ~~may~~could be the product of homogenizing dispersal from the ocean to the lake (Logares et al., 2018). In addition, the lakes were covered in ice for most of the year and were limited by geographical distance, resulting in the dispersal limitation of microorganisms (~~2.86~~0.95%). Undominant processes accounted for ~~32.38~~40.00% of community assembly in our study, including ecological drift and other complex processes that have not been fully quantified, such as weak selection and diffusion (Mo et al., 2018a), suggesting that microbial eukaryotic communities might be formed by some highly complex assembly mechanisms in Antarctic freshwater lakes.

5 Conclusion

In conclusion, the unique microbial eukaryotic community structure and low alpha diversity (richness and Shannon index) were demonstrated in~~the microeukaryotic community was dominated by phytoplankton, mainly Chrysophyta, Chlorophyta, and Cryptophyta, with spatial and temporal variation in the relative abundance of dominant taxa from~~ five freshwater lakes on the Fildes Peninsula, Antarctica. ~~This study highlighted the first time the i~~Importance of stochastic processes and co-occurrence patterns in shaping the microbial eukaryotic community of this area were proved. WT and PO_4^{3-} were identified as important driving factors for variation of community structure ($P<0.05$). ~~The environmental variables explained only about 30% of the community variation. Microbial interactions were predominantly symbiotic, indicating common preferred environmental conditions or niche overlapping.~~ Stochastic processes played a very prominent role in microbial eukaryotic community assembly~~, and the low response to environmental factors might enhance the proportion of stochastic processes~~. Our study provides a better understanding of the dynamic patterns and ecological processes of microbial eukaryotic community structure in Antarctic oligotrophic lakes (Fildes Peninsula).

Data Availability Statement

The raw 18S reads have been deposited into the NCBI Sequence Read Archive database with the accession numbers of PRJNA805287~~SRP359325~~.

Author Contribution Statement

Conceptualization: Chunmei Zhang and Yonghong Bi. Methodology: Chunmei Zhang and Wei Luo. Molecular technique：Huirong Li. Physico-chemical properties: Bin Wang, Yangjie Li, and Zhongqiang Ji. Sample collection： Yinxin Zeng and Haitao Ding. Funding acquisition: Yonghong Bi and Wei Luo. Supervision: Yonghong Bi and Wei Luo. Writing - original draft: Chunmei Zhang. Writing - review & editing: Yonghong Bi and Wei Luo.

Competing interests

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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Reference

Allende, L. (2009) Combined effects of nutrients and grazers on bacterioplankton and phytoplankton abundance in an Antarctic lake with even food-chain links. Polar Biology 32(3), 493-501. https://doi.org/10.1007/s00300-008-0545-6.

Allende, L. and Izaguirre, I. (2003) The role of physical stability on the establishment of steady states in the phytoplankton community of two Maritime Antarctic lakes. Hydrobiologia 502(1-3), 211-224.

https://doi.org/10.1023/B:HYDR.0000004283.11230.4a.

Allende, L. and Mataloni, G. (2013) Short-term analysis of the phytoplankton structure and dynamics in two ponds with distinct trophic states from Cierva Point (maritime Antarctica). Polar Biology 36(5), 629-644. https://doi.org/10.1007/s00300-013-1290-z.

Andersson, A., Falk, S., Samuelsson, G. and Hagstrom, A. (1989) Nutritional Characteristics of a Mixotrophic Nanoflagellate, Ochromonas Sp. Microbial Ecology 17(3), 251-262. https://doi.org/10.1007/Bf02012838.

Atteia, A., van Lis, R., Tielens, A.G.M. and Martin, W.F. (2013) Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochimica Et Biophysica Acta-Bioenergetics 1827(2), 210-223. https://doi.org/10.1016/j.bbabio.2012.08.002.

Bahram, M., Kohout, P., Anslan, S., Harend, H., Abarenkov, K. and Tedersoo, L. (2016) Stochastic distribution of small soil eukaryotes resulting from high dispersal and drift in a local environment. Isme Journal 10(4), 885-896. https://doi.org/10.1038/ismej.2015.164.

Barsch, D. and Caine, N. (1984) The Nature of Mountain Geomorphology. Mountain Research and Development 4(4), 287-298. https://doi.org/10.2307/3673231.

Bastian M, Heymann S. and Jacomy M. (2009) Gephi: An open source software for exploring and manipulating networks. Int Conf Weblogs Soc media. https://doi.org/10.13140/2.1.1341.1520.

Belevich, T.A., Ilyash, L.V., Milyutina, I.A., Logacheva, M.D. and Troitsky, A.V. (2020) Photosynthetic Picoeukaryotes Diversity in the Underlying Ice Waters of the White Sea, Russia. Diversity 12(3). https://doi.org/10.3390/d12030093.

Blomqvist, P. (1997) Early summer phytoplankton responses to experimental manipulations of grazing and nutrients in unlimed and limed Lake Njupfatet, central Sweden. Archiv Fur Hydrobiologie 140(3), 321-346. https://doi.org/10.1127/archiv-hydrobiol/137/1996/425.

Boenigk, J., Pfandl, K., Stadler, P. and Chatzinotas, A. (2005) High diversity of the 'Spumella-like' flagellates: an investigation based on the SSU rRNA gene sequences of isolates from habitats located in six different geographic regions. Environ Microbiol 7(5), 685-697. https://doi.org/10.1111/j.1462-2920.2005.00743.x.

Bouchez, T., Blieux, A.L., Dequiedt, S., Domaizon, I., Dufresne, A., Ferreira, S., Godon, J.J., Hellal, J., Joulian, C., Quaiser, A., Martin-Laurent, F., Mauffret, A., Monier, J.M., Peyret, P., Schmitt-Koplin, P., Sibourg, O., D'oiron, E., Bispo, A., Deportes, I., Grand, C., Cuny, P., Maron, P.A. and Ranjard, L. (2016) Molecular microbiology methods for environmental diagnosis. Environmental Chemistry Letters 14(4), 423-441. https://doi.org/10.1007/s10311-016-0581-3.

Braak, C.J.F.t. and Smilauer, P. (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). https://doi.org/https://edepot.wur.nl/405659.

Brate, J., Logares, R., Berney, C., Ree, D.K., Klaveness, D., Jakobsen, K.S. and Shalchian-Tabrizi, K. (2010) Freshwater Perkinsea and marine-freshwater colonizations revealed by pyrosequencing and phylogeny of environmental rDNA. Isme Journal 4(9), 1144-1153. https://doi.org/10.1038/ismej.2010.39.

Chan, T.U., Hamilton, D.P., Robson, B.J., Hodges, B.R. and Dallimore, C. (2002) Impacts of hydrological changes on phytoplankton succession in the Swan River, Western Australia. Estuaries Coasts 25, 1406–11421. https://doi.org/10.1007/BF02692234.

Charvet, S., F., W., Vincent， and Lovejoy., C. (2012) Chrysophytes and other protists in High Arctic lakes: molecular gene surveys, pigment signatures and microscopy. Polar Biology 35(5), 733-748. https://doi.org/10.1007/s00300-011-1118-7.

Chase, J.M. (2010) Stochastic Community Assembly Causes Higher Biodiversity in More Productive Environments. science 328(5984), 1388-1391.

https://doi.org/10.1126/science.1187820.

Chase, J.M., Biro, E.G., Ryberg, W.A. and Smith, K.G. (2009) Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. Ecology Letters 12(11), 1210-1218. https://doi.org/10.1111/j.1461-0248.2009.01362.x.

Chen, S.F., Zhou, Y.Q., Chen, Y.R. and Gu, J. (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34(17), 884-890. https://doi.org/10.1093/bioinformatics/bty560.

Chen, W., Ren, K., Isabwe, A., Chen, H., Liu, M. and Yang, J. (2019) Stochastic processes shape microeukaryotic community assembly in a subtropical river across wet and dry seasons. Microbiome 7(1), 138. https://doi.org/10.1186/s40168-019-0749-8.

Chen, W. and Wen, D. (2021) Archaeal and bacterial communities assembly and co-occurrence networks in subtropical mangrove sediments under Spartina alterniflora invasion. Environ Microbiome 16(1), 10. https://doi.org/10.1186/s40793-021-00377-y.

Chen, Z., Gu, T., Wang, X., Wu, X. and Sun, J. (2022) Oxygen gradients shape the unique structure of picoeukaryotic communities in the Bay of Bengal. Science of the Total Environment 814, 152862. https://doi.org/10.1016/j.scitotenv.2021.152862.

Comeau, A.M., Harding, T., Galand, P.E., Vincent, W.F. and Lovejoy, C. (2012) Vertical distribution of microbial communities in a perennially stratified Arctic lake with saline, anoxic bottom waters. Sci Rep 2, 604. https://doi.org/10.1038/srep00604.

Duffy, G.A., Coetzee, B.W.T., Latombe, G., Akerman, A.H., McGeoch, M.A. and Chown, S.L. (2017) Barriers to globally invasive species are weakening across the Antarctic. Diversity and Distributions 23(9), 982-996. https://doi.org/10.1111/ddi.12593.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10(10), 996-+. https://doi.org/10.1038/Nmeth.2604.

Ekelund, F., Daugbjerg, N. and Fredslund, L. (2004) Phylogeny of Heteromita, Cercomonas and Thaumatomonas based on SSU rDNA sequences, including the description of Neocercomonas jutlandica sp nov. gen. nov. European Journal of Protistology 40(2), 119-135. https://doi.org/10.1016/j.ejop.2003.12.002.

Evans, S., Martiny, J.B. and Allison, S.D. (2017) Effects of dispersal and selection on stochastic assembly in microbial communities. Isme Journal 11(1), 176-185. https://doi.org/10.1038/ismej.2016.96.

Faust, K. and Raes, J. (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10(8), 538-550. https://doi.org/10.1038/nrmicro2832.

Gad, M., Hou, L., Cao, M., Adyari, B., Zhang, L., Qin, D., Yu, C.P., Sun, Q. and Hu, A. (2022) Tracking microeukaryotic footprint in a peri-urban watershed, China through machine-learning approaches. Science of the Total Environment 806(Pt 1), 150401. https://doi.org/10.1016/j.scitotenv.2021.150401.

Garcia-Rodriguez, F., Piccini, C., Carrizo, D., Sanchez-Garcia, L., Perez, L., Crisci, C., Oaquim, A.B.J., Evangelista, H., Soutullo, A., Azcune, G. and Luning, S. (2021) Centennial glacier retreat increases sedimentation and eutrophication in Subantarctic periglacial lakes: A study case of Lake Uruguay. Science of the Total Environment 754, 142066. https://doi.org/10.1016/j.scitotenv.2020.142066.

Glassman, S.I. and Martiny, J.B.H. (2018) Broadscale Ecological Patterns Are Robust to Use of Exact Sequence Variants versus Operational Taxonomic Units. Ecological and Evolutionary Science 3(4), e00148-00118. https://doi.org/10.1128/mSphere.

Grob, C., Ulloa, O., Li, W.K.W., Alarcon, G., Fukasawa, M. and Watanabe, S. (2007) Picoplankton abundance and biomass across the eastern South Pacific Ocean along latitude 32.5 degrees S. Marine Ecology Progress Series 332, 53-62.

https://doi.org/10.3354/meps332053.

He, Q., Wang, S., Hou, W., Feng, K., Li, F., Hai, W., Zhang, Y., Sun, Y. and Deng, Y. (2021) Temperature and microbial interactions drive the deterministic assembly processes in sediments of hot springs. Science of the Total Environment 772, 145465. https://doi.org/10.1016/j.scitotenv.2021.145465.

Hernandez-Ruiz, M., Barber-Lluch, E., Prieto, A., Alvarez-Salgado, X.A., Logares, R. and Teira, E. (2018) Seasonal succession of small planktonic eukaryotes inhabiting surface waters of a coastal upwelling system. Environ Microbiol 20(8), 2955-2973. https://doi.org/10.1111/1462-2920.14313.

Holdgate, M.W. (1977) Terrestrial Ecosystems in Antarctic. Philosophical Transactions of the Royal Society B-Biological Sciences 279(963), 5-25. https://doi.org/10.1098/rstb.1977.0068.

Horner-Devine, M.C. and Bohannan, B.J.M. (2006) Phylogenetic clustering and overdispersion in bacterial communities. Ecology 87(7), S100-S108. https://doi.org/10.1890/0012-9658(2006)87[100:Pcaoib]2.0.Co;2.

Hou, F., Zhang, H., Xie, W., Zhou, X., Zhu, X. and Zhang, D. (2020) Co-occurrence patterns and assembly processes of microeukaryotic communities in an early-spring diatom bloom. Science of the Total Environment 711, 134624. https://doi.org/10.1016/j.scitotenv.2019.134624.

HP Hansen and Koroleff, F.F. (1999) Determination of nutrients. Method of Seawater Analysis 23(3), 223-229. https://doi.org/10.1111/j.1540-8183.2010.00546.x.

Hu, L. (1998) Snow algae of the Windmill Islands, continental Antarctica3. Chloromonas polyptera (Volvocales, Chlorophyta). Polar Biology 20(5), 320-324. https://doi.org/10.1007/s003000050309.

Izaguirre, I., Allende, L. and Romina Schiaffino, M. (2020) Phytoplankton in Antarctic lakes: biodiversity and main ecological features. Hydrobiologia. https://doi.org/10.1007/s10750-020-04306-x.

Izaguirre, I., L, C, A.M. and Marinone (2003) Comparative study of the planktonic communities from lakes of contrasting trophic status at Hope Bay (Antarctic Peninsula). Journal of Plankton Research 25, 1079–1097. https://doi.org/10.1093/plankt/25.9.1079.

Jiang, L. and Patel, S.N. (2008) Community assembly in the presence of disturbance: A microcosm experiment. Ecology 89(7), 1931-1940. https://doi.org/10.1890/07-1263.1.

Kardol, P., Souza, L. and Classen, A.T. (2013) Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. Oikos 122(1), 84-94. https://doi.org/10.1111/j.1600-0706.2012.20546.x.

Karimi, B., Maron, P.A., Chemidlin-Prevost Boure, N., Bernard, N., Gilbert, D. and Ranjard, L. (2017) Microbial diversity and ecological networks as indicators of environmental quality. Environmental Chemistry Letters 15(2), 265-281. https://doi.org/10.1007/s10311-017-0614-6.

Katechakis, A. and Stibor, H. (2006) The mixotroph Ochromonas tuberculata may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. Oecologia 148(4), 692-701. https://doi.org/10.1007/s00442-006-0413-4.

Kawecka, B., Olech, M., Nowogrodzka-Zagorska, M. and Wojtun, B. (1998) Diatom communities in small water bodies at H. Arctowski Polish Antarctic Station (King George Island, South Shetland Islands, Antarctica). Polar Biology 19(3), 183-192. https://doi.org/10.1007/s003000050233.

Kong, J., Wang, Y., Warren, A., Huang, B. and Sun, P. (2019) Diversity Distribution and Assembly Mechanisms of Planktonic and Benthic Microeukaryote Communities in Intertidal Zones of Southeast Fujian, China. Front Microbiol 10, 2640. https://doi.org/10.3389/fmicb.2019.02640.

Leblanc, K., Queguiner, B., Diaz, F., Cornet, V., Michel-Rodriguez, M., Durrieu de Madron, X., Bowler, C., Malviya, S., Thyssen, M., Gregori, G., Rembauville, M., Grosso, O., Poulain, J., de Vargas, C., Pujo-Pay, M. and Conan, P. (2018)

Nanoplanktonic diatoms are globally overlooked but play a role in spring blooms and carbon export. Nat Commun 9(1), 953. https://doi.org/10.1038/s41467-018-03376-9.

Lee, J.R., Raymond, B., Bracegirdle, T.J., Chades, I., Fuller, R.A., Shaw, J.D. and Terauds, A. (2017) Climate change drives expansion of Antarctic ice-free habitat. Nature 547(7661), 49-+. https://doi.org/10.1038/nature22996.

Lei, J., Liu, C., Zhang, M., Yang, J., Wu, F., Ren, M., Wu, Q. and Shi, X. (2021) The daily effect is more important than the diurnal effect when shaping photosynthetic picoeukaryotes (PPEs) communities in Lake Taihu at a small temporal scale. FEMS Microbiol Ecol 97(7). https://doi.org/10.1093/femsec/fiab090.

Li, Y., Gao, Y., Zhang, W., Wang, C., Wang, P., Niu, L. and Wu, H. (2019) Homogeneous selection dominates the microbial community assembly in the sediment of the Three Gorges Reservoir. Science of the Total Environment 690, 50-60. https://doi.org/10.1016/j.scitotenv.2019.07.014.

Li, Y. and Hu, C. (2021) Biogeographical patterns and mechanisms of microbial community assembly that underlie successional biocrusts across northern China. NPJ Biofilms Microbiomes 7(1), 15. https://doi.org/10.1038/s41522-021-00188-6.

Liang, Y., Ning, D., Lu, Z., Zhang, N., Hale, L., Wu, L., Clark, I.M., McGrath, S.P., Storkey, J., Hirsch, P.R., Sun, B. and Zhou, J. (2020) Century long fertilization reduces stochasticity controlling grassland microbial community succession. Soil Biology and Biochemistry 151. https://doi.org/10.1016/j.soilbio.2020.108023.

Liu, K., Liu, Y., Hu, A., Wang, F., Chen, Y., Gu, Z., Anslan, S. and Hou, J. (2020a) Different community assembly mechanisms underlie similar biogeography of bacteria and microeukaryotes in Tibetan lakes. FEMS Microbiol Ecol 96(6). https://doi.org/10.1093/femsec/fiaa071.

Liu, L., Chen, H., Liu, M., Yang, J.R., Xiao, P., Wilkinson, D.M. and Yang, J. (2019)

Response of the eukaryotic plankton community to the cyanobacterial biomass cycle over 6 years in two subtropical reservoirs. Isme Journal 13(9), 2196-2208. https://doi.org/10.1038/s41396-019-0417-9.

Liu, Q., Zhao, Q., Jiang, Y., Li, Y., Zhang, C., Li, X., Yu, X., Huang, L., Wang, M., Yang, G., Chen, H. and Tian, J. (2021) Diversity and co-occurrence networks of picoeukaryotes as a tool for indicating underlying environmental heterogeneity in the Western Pacific Ocean. Mar Environ Res 170, 105376. https://doi.org/10.1016/j.marenvres.2021.105376.

Liu, W., Graham, E.B., Zhong, L., Zhang, J., Li, W., Li, Z., Lin, X., Feng, Y. and Wang, J. (2020b) Dynamic microbial assembly processes correspond to soil fertility in sustainable paddy agroecosystems. Functional Ecology 34(6), 1244-1256. https://doi.org/10.1111/1365-2435.13550.

Lizotte, M.P. (2008) Phytoplankton and primary production. W. F. & J. Laybourn-Parry (eds), Polar Lakes and Rivers Limnology of Arctic and Antarctic Aquatic Ecosystems. Oxford University Press, Oxford., 157-179.

Logares, R., Tesson, S.V.M., Canback, B., Pontarp, M., Hedlund, K. and Rengefors, K. (2018) Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. Environ Microbiol 20(6), 2231-2240. https://doi.org/10.1111/1462-2920.14265.

Lopez-Garcia, P., Rodriguez-Valera, F., Pedros-Alio, C. and Moreira, D. (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409(6820), 603-607. https://doi.org/10.1038/35054537.

Luo, W., Li, H., Gao, S., Yu, Y., Lin, L. and Zeng, Y. (2015) Molecular diversity of microbial eukaryotes in sea water from Fildes Peninsula, King George Island, Antarctica. Polar Biology 39(4), 605-616. https://doi.org/10.1007/s00300-015-1815-8.

Lupatini, M., Suleiman, A.K.A., Jacques, R.J.S., Antoniolli, Z.I., de Siqueira Ferreira, A.o., Kuramae, E.E. and Roesch, L.F.W. (2014) Network topology reveals high connectance levels and few key microbial genera within soils. Frontiers in Environmental Science 2. https://doi.org/10.3389/fenvs.2014.00010.

Lyons, W.B., Laybourn-Parry J and Welch K A (2007) Antarctic Lake Systems and Climate Change.Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer Netherlands.

Ma, B., Wang, Y., Ye, S., Liu, S., Stirling, E., Gilbert, J.A., Faust, K., Knight, R., Jansson, J.K., Cardona, C., Rottjers, L. and Xu, J. (2020) Earth microbial co-occurrence network reveals interconnection pattern across microbiomes. Microbiome 8(1), 82. https://doi.org/10.1186/s40168-020-00857-2.

Ma, Z.S. (2018) DAR (diversity-area relationship): Extending classic SAR (species-area relationship) for biodiversity and biogeography analyses. Ecology and Evolution 8(20), 10023-10038. https://doi.org/10.1002/ece3.4425.

Magoc, T. and Salzberg, S.L. (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27(21), 2957-2963. https://doi.org/10.1093/bioinformatics/btr507.

Margesin, R. and Miteva, V. (2011) Diversity and ecology of psychrophilic microorganisms. Research in Microbiology 162(3), 346-361. https://doi.org/10.1016/j.resmic.2010.12.004.

Marquardt, M., Vader, A., Stubner, E.I., Reigstad, M. and Gabrielsen, T.M. (2016) Strong Seasonality of Marine Microbial Eukaryotes in a High-Arctic Fjord (Isfjorden, in West Spitsbergen, Norway). Appl Environ Microbiol 82(6), 1868-1880. https://doi.org/10.1128/AEM.03208-15.

Marsh, N.B., Lacelle, D., Faucher, B., Cotroneo, S., Jasperse, L., Clark, I.D. and Andersen, D.T. (2020) Sources of solutes and carbon cycling in perennially ice-covered Lake Untersee, Antarctica. Sci Rep 10(1), 12290. https://doi.org/10.1038/s41598-020-69116-6.

Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., Chambouvet, A., Christen, R., Claverie, J.M., Decelle, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Forn, I., Forster, D., Guillou, L., Jaillon, O., Kooistra, W.H.C.F., Logares, R., Mahe, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M.C., Probert, I., Romac, S., Richards, T., Santini, S., Shalchian-Tabrizi, K., Siano, R., Simon, N., Stoeck, T., Vaulot, D., Zingone, A. and de Vargas, C. (2015) Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. Environmental Microbiology 17(10), 4035-4049. https://doi.org/10.1111/1462-2920.12955.

Massana, R. and Logares, R. (2013) Eukaryotic versus prokaryotic marine picoplankton ecology. Environmental Microbiology 15(5), 1254-1261. https://doi.org/10.1111/1462-2920.12043.

Mataloni, G., Tesolin, G., Sacullo, F. and Tell, G. (2000) Factors regulating summer phytoplankton in a highly eutrophic Antarctic lake. Hydrobiologia 432(1-3), 65-72. https://doi.org/10.1023/A:1004045219437.

Mo, Y., Zhang, W., Yang, J., Lin, Y., Yu, Z. and Lin, S. (2018a) Biogeographic patterns of abundant and rare bacterioplankton in three subtropical bays resulting from selective and neutral processes. Isme Journal 12(9), 2198-2210. https://doi.org/10.1038/s41396-018-0153-6.

Mo, Y.y., Zhang, W.j., Yang, J. and Lin, Y.s. (2018b) Biogeographic patterns of abundant and rare bacterioplankton in three subtropical bays resulting from selective and neutral processes. The ISME Journal 12(9), 2198-2210. https://doi.org/10.1038/s41396-018-0153-6.

Moreno-Pino, M., De la Iglesia, R., Valdivia, N., Henriquez-Castilo, C., Galan, A., Diez, B. and Trefault, N. (2016) Variation in coastal Antarctic microbial community composition at sub-mesoscale: spatial distance or environmental filtering? FEMS Microbiol Ecol 92(7). https://doi.org/10.1093/femsec/fiw088.

Morgan-Kiss, R.M., Lizotte, M.P., Kong, W. and Priscu, J.C. (2016) Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake. Limnology and Oceanography 61(1), 3-13. https://doi.org/10.1002/lno.10107.

Nicholls, K.H. (1995) Chrysophyte Blooms in the Plankton and Neuston of Marine and Freshwater Systems. Chrysophyte Algae, 181-213.

Ofiteru, I.D., Lunn, M., Curtis, T.P., Wells, G.F., Criddle, C.S., Francis, C.A. and Sloan, W.T. (2010) Combined niche and neutral effects in a microbial wastewater treatment community. Proceedings of the National Academy of Sciences of the United States of America 107(35), 15345-15350. https://doi.org/10.1073/pnas.1000604107.

Orrock, J.L. and Watling, J.I. (2010) Local community size mediates ecological drift and competition in metacommunities. Proceedings of the Royal Society B-Biological Sciences 277(1691), 2185-2191. https://doi.org/10.1098/rspb.2009.2344.

Pearce, D.A.G., Pierre E. (2008) Microbial biodiversity and biogeography. In Vincent, W. F. & J. Laybourn-Parry (eds), Polar lakes and rivers. Oxford University Press, Oxford, 213–230.

Pertierra, L.R., Aragón, P., Shaw, J.D., Bergstrom, D.M., Terauds, A. and Olalla-Tárraga, M.Á. (2017) Global therma niche models of two European grasses show high invasion risks in Antarctica. Glob. Chang. Biol 23(7), 2863–2873. https://doi.org/10.1111/gcb.13596.

Pick, F.R. and Lean, D. (1984) Diurnal movements of metalimnetic phytoplankton. Journal of Phycology 20, 430–436. https://doi.org/10.1111/j.0022-3646.1984.00430.x.

Powell, J.R., Karunaratne, S., Campbell, C.D., Yao, H., Robinson, L. and Singh, B.K. (2015) Deterministic processes vary during community assembly for ecologically dissimilar taxa. Nat Commun 6, 8444. https://doi.org/10.1038/ncomms9444.

Priscu, J.C. (2010) The biogeochemistry of nitrous oxide in permanently ice‐covered lakes of the McMurdo Dry Valleys, Antarctica. Global Change Biology 3(4), 301-315. https://doi.org/10.1046/j.1365-2486.1997.00147.x.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, PepliesJ and FO, G. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41, 590-596. https://doi.org/10.1093/nar/gks1219.

Quiroga, M.V., Unrein, F., González Garraza, G., Küppers, G., Lombardo, R., Marinone, M.C., Menu Marque, S., Vinocur, A. and Mataloni, G. (2013) The plankton communities from peat bog pools: structure, temporal variation and environmental factors. Journal of Plankton Research 35(6), 1234-1253. https://doi.org/10.1093/plankt/fbt082.

Reboul, G., Moreira, D., Annenkova, N.V., Bertolino, P., Vershinin, K.E. and Lopez-Garcia, P. (2021) Marine signature taxa and core microbial community stability along latitudinal and vertical gradients in sediments of the deepest freshwater lake. Isme Journal 15(11), 3412-3417. https://doi.org/10.1038/s41396-021-01011-y.

Richards, T.A., Vepritskiy, A.A., Gouliamova, D.E. and Nierzwicki-Bauer, S.A. (2005) The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages. Environ Microbiol 7(9), 1413-1425. https://doi.org/10.1111/j.1462-2920.2005.00828.x.

Roberts, D. (2013) labdsv: Ordination and Multivariate Analysis for Ecology.

Roberts, E.C. and Laybourn-Parry, J. (1999) Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. Freshwater Biology 41, 737–746. https://doi.org/10.1046/j.1365-2427.1999.00401.x.

Santos, J.A.D., Meyer, E. and Sette, L.D. (2020) Fungal Community in Antarctic Soil Along the Retreating Collins Glacier (Fildes Peninsula, King George Island). Microorganisms 8(8). https://doi.org/10.3390/microorganisms8081145.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L. and Konopka, A. (2013) Quantifying community assembly processes and identifying features that impose them. Isme Journal 7(11), 2069-

2079. https://doi.org/10.1038/ismej.2013.93.

Stegen, J.C., Lin, X., Konopka, A.E. and Fredrickson, J.K. (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. Isme Journal 6(9), 1653-1664. https://doi.org/10.1038/ismej.2012.22.

Sun, D., Bi, Q., Li, K., Dai, P., Yu, Y., Zhou, W., Lv, T., Liu, X., Zhu, J., Zhang, Q., Jin, C., Lu, L. and Lin, X. (2017) Significance of temperature and water availability for soil phosphorus transformation and microbial community composition as affected by fertilizer sources. Biology and Fertility of Soils 54(2), 229-241. https://doi.org/10.1007/s00374-017-1252-7.

Sun, P., Wang, Y., Huang, X., Huang, B.Q. and Wang, L. (2022) Water masses and their associated temperature and cross-domain biotic factors co-shape upwelling microbial communities. Water research 215. https://doi.org/ARTN 118274

10.1016/j.watres.2022.118274.

Toro, M., Camacho, A., Rochera, C., Rico, E., Banon, M., Fernandez-Valiente, E., Marco, E., Justel, A., Avendano, M.C., Ariosa, Y., Vincent, W.F. and Quesada, A. (2007) Limnological characteristics of the freshwater ecosystems of Byers Peninsula, Livingston Island, in maritime Antarctica. Polar Biology 30(5), 635-649. https://doi.org/10.1007/s00300-006-0223-5.

Tragin, M. and Vaulot, D. (2019) Novel diversity within marine Mamiellophyceae (Chlorophyta) unveiled by metabarcoding. Sci Rep 9(1), 5190. https://doi.org/10.1038/s41598-019-41680-6.

Unrein, F., Gasol, J.M., Not, F., Forn, I. and Massana, R. (2014) Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. Isme Journal 8(1), 164-176. https://doi.org/10.1038/ismej.2013.132.

Unrein, F., Izaguirre, I., Massana, R., Balague, V. and Gasol, J.M. (2005) Nanoplankton assemblages in maritime Antarctic lakes: characterisation and molecular fingerprinting comparison. Aquatic Microbial Ecology 40(3), 269-282. https://doi.org/10.3354/ame040269.

Wang, F., Huang, B., Xie, Y., Cai, S., Wang, X. and Mu, J. (2021) Diversity, Composition, and Activities of Nano- and Pico-Eukaryotes in the Northern South China Sea With Influences of Kuroshio Intrusion. Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.658233.

Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73(16), 5261-5267. https://doi.org/10.1128/Aem.00062-07.

Wang, W., Ren, K., Chen, H., Gao, X., Ronn, R. and Yang, J. (2020a) Seven-year dynamics of testate amoeba communities driven more by stochastic than deterministic processes in two subtropical reservoirs. Water Res 185, 116232. https://doi.org/10.1016/j.watres.2020.116232.

Wang, Y., Li, G., Shi, F., Dong, J., Gentekaki, E., Zou, S., Zhu, P., Zhang, X. and Gong, J. (2020b) Taxonomic Diversity of Pico-/Nanoeukaryotes Is Related to Dissolved Oxygen and Productivity, but Functional Composition Is Shaped by Limiting Nutrients in Eutrophic Coastal Oceans. Front Microbiol 11, 601037. https://doi.org/10.3389/fmicb.2020.601037.

Webb, C.O., Ackerly, D.D., McPeek, M.A. and Donoghue, M.J. (2002) Phylogenies and Community Ecology. Annual Review of Ecology and Systematics 33(1), 475-505. https://doi.org/10.1146/annurev.ecolsys.33.010802.150448.

Wilkins, D., Yau, S., Williams, T.J., Allen, M.A., Brown, M.V., DeMaere, M.Z., Lauro, F.M. and Cavicchioli, R. (2013) Key microbial drivers in Antarctic aquatic environments. FEMS Microbiol Rev 37(3), 303-335. https://doi.org/10.1111/1574-6976.12007.

Wu, L.W., Ning, D.L., Zhang, B., Li, Y., Zhang, P., Shan, X.Y., Zhang, Q.T., Brown, M.R., Li, Z.X., Van Nostrand, J.D., Ling, F.Q., Xiao, N.J., Zhang, Y., Vierheilig, J., Wells, G.F., Yang, Y.F., Deng, Y., Tu, Q.C., Wang, A.J., Zhang, T., He, Z.L., Keller, J., Nielsen, P.H., Alvarez, P.J.J., Criddle, C.S., Wagner, M., Tiedje, J.M., He, Q., Curtis, T.P., Stahl, D.A., Alvarez-Cohen, L., Rittmann, B.E., Wen, X.H., Zhou, J.Z. and Consortium, G.W.M. (2019) Global diversity and biogeography of bacterial communities in wastewater treatment plants (vol 4, pg 1183, 2019). Nature Microbiology 4(12), 2579-2579. https://doi.org/10.1038/s41564-019-0617-0.

Xu, D., Kong, H., Yang, E.J., Wang, Y., Li, X., Sun, P., Jiao, N., Lee, Y., Jung, J. and Cho, K.H. (2022) Spatial dynamics of active microeukaryotes along a latitudinal gradient: Diversity, assembly process, and co-occurrence relationships. Environ Res 212(Pt A), 113234. https://doi.org/10.1016/j.envres.2022.113234.

Xue, Y.Y., Chen, H.H., Yang, J.R., Liu, M., Huang, B.Q. and Yang, J. (2018) Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. Isme Journal 12(9), 2263-2277. https://doi.org/10.1038/s41396-018-0159-0.

Yubuki, N., Nakayama, T. and Inouye, I. (2008) A unique life cycle and perennation in a colorless chrysophyte Spumella sp. Journal of Phycology 44(1), 164-172. https://doi.org/10.1111/j.1529-8817.2007.00441.x.

Zeng, J., Jiao, C., Zhao, D., Xu, H., Huang, R., Cao, X., Yu, Z. and Wu, Q.L. (2019) Patterns and assembly processes of planktonic and sedimentary bacterial community differ along a trophic gradient in freshwater lakes. Ecological Indicators 106. https://doi.org/10.1016/j.ecolind.2019.105491.

Zeng, Y.X., Yu, Y., Qiao, Z.Y., Jin, H.Y. and Li, H.R. (2014) Diversity of bacterioplankton in coastal seawaters of Fildes Peninsula, King George Island, Antarctica. Arch Microbiol 196(2), 137-147. https://doi.org/10.1007/s00203-013-0950-2.

Zhang, G., Wei, G., Wei, F., Chen, Z., He, M., Jiao, S., Wang, Y., Yu, Y., Dong, L. and Chen, S. (2021a) Homogeneous selection shapes rare biosphere in rhizosphere of medicinal plant. Ecological Indicators 129. https://doi.org/10.1016/j.ecolind.2021.107981.

Zhang, L., Yin, W., Wang, C., Zhang, A., Zhang, H., Zhang, T. and Ju, F. (2021b) Untangling Microbiota Diversity and Assembly Patterns in the World's Largest Water Diversion Canal. Water Res 204, 117617. https://doi.org/10.1016/j.watres.2021.117617.

Zhang, W., Wan, W., Lin, H., Pan, X., Lin, L. and Yang, Y. (2022) Nitrogen rather than phosphorus driving the biogeographic patterns of abundant bacterial taxa in a eutrophic plateau lake. Science of the Total Environment 806(Pt 4), 150947. https://doi.org/10.1016/j.scitotenv.2021.150947.

Zhang, W.J., Pan, Y.B., Yang, J., Chen, H.H., Holohan, B., Vaudrey, J., Lin, S.J. and McManus, G.B. (2018) The diversity and biogeography of abundant and rare intertidal marine microeukaryotes explained by environment and dispersal limitation. Environmental Microbiology 20(2), 462-476. https://doi.org/10.1111/1462-2920.13916.

Zhao, B., Chen, M., Sun, Y., Yang, J. and Chen, F. (2011) Genetic diversity of picoeukaryotes in eight lakes differing in trophic status. Can J Microbiol 57(2), 115-126. https://doi.org/10.1139/w10-107.

Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z., Wu, L., Stahl, D.A., Hazen, T.C., Tiedje, J.M. and Arkin, A.P. (2014) Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proc Natl Acad Sci U S A 111(9), E836-845. https://doi.org/10.1073/pnas.1324044111.

Zhu, G.H., OHTANI Shuji, HU Chuan-yu, HE Jian-feng, JIN Mao, YU Pei-song and Jian-ming, P. (2010) Impact of global climate change on antarctic freshwater algae. China Environmental Science 30(3), 400-404.

Zouari, A.B., Hassen, M.B., Balague, V., Sahli, E., Ben Kacem, M.Y., Akrout, F., Hamza, A. and Massana, R. (2018) Picoeukaryotic diversity in the Gulf of Gabes: variability patterns and relationships to nutrients and water masses. Aquatic Microbial Ecology 81(1), 37-53. https://doi.org/10.3354/ame01857.

Figure Captions

Fig. 1 (a) Location of the five studied lakes in Fildes Peninsula, King George Island, Antarctica, (b) Lake Xi Hu (XH), (c) Lake Yan Ou (YO), (d) Lake Chang Hu (CH), (e) Lake Yue Ya (YY), (f) Lake Kitec (KT).

Fig.2 Comparison of microbial eukaryotic community composition. (a)Temporal and spatial dynamics of relative abundance on Phylum level in five lakes from 2017 to 2019. 17, 18, and 19 expedition season of 2017/2018, 2018/2019, and 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different lakes. Lakes that showed no significant differences were not shown (*P*>0.05). (c)Temporal and spatial dynamics of relative abundance on Genus level in five lakes. (Note: **P < 0.01, *P < 0.05). (Chlorophyta: ~~*Aphelida,*~~ *Atractomorpha*, *Chlamydomonas*, ~~*Chloromonas,*~~ *Mantoniella, Chlorella,* ~~*Chlorothrix,*~~ *Choricystis*, *Crustomastix*, *Microglena*, *Monomastix*, *Nannochloris*~~*, Raphidonema*~~; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*, *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*; Cryptophyta: *Komma*; Haptophyta: *Diacronema*; ~~Pyrrophyta: *Heterocapsa*; Glissomonadida~~Cercozoa: *Heteromita*. The relative abundance at any lake was less than 1% was defined as others).

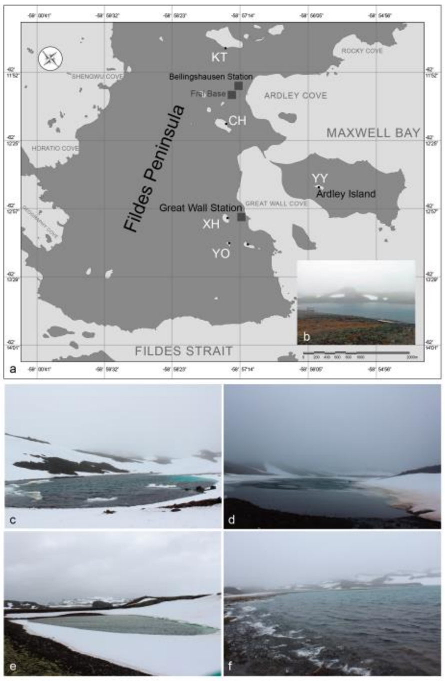
Fig.3 Microbial diversity and Venn diagram in different years and lakes. (a, b) variations in the number of microbial OTUs; (c, d) variations in microbial Shannon index; (e, f) variations in within-community nearest-taxon index (NTI); (g, h) Venn diagram showing the unique and shared operational taxonomic units (OTUs). Homogeneity and one-way ANOVA analysis of variance was used to test the indices' significance. “ns” represents no significant differences (*P*>0.05). The significant differences (*P*<0.05) were indicated by different alphabet letters between lakes, and lakes contained the same alphabet letters showed no significant difference (*P*>0.05)

Fig. 4 Temporal variability analysis of Non-metric multidimensional scaling (NMDS) ordination of microbial eukaryotic communities (a) and clustering of five lakes based on similarity (b).

Fig.5 The effect of environmental variables on microbial eukaryotic communities, and co-occurrence pattern. Canonical correlation analysis plots(a) and variance partitioning analysis (b), respectively. Sal: salinity; WT: water temperature; NO_2 -N: nitrite nitrogen; PO_4^{3-}~~ -P~~: ~~phosphate~~phosphate~~ phosphorus~~. ~~** P < 0.01.~~ (c) Network~~s~~ analysis revealing the interspecies associations between microbial eukaryotic OTUs, and the correlation between environmental factors and OTUs in lakes integrated networks. The size of each OTUs or environmental factor (node) is proportional to the degree centrality. Others: other phyla and unclassified taxa.

Fig.6 Relative influences of deterministic and stochastic processes on microbial eukaryotic community assembly based on the neutral community model (NCM) and the null model. (a) Fit of the neutral community model (NCM) of community assembly. Nm indicates the metacommunity size, and R^2 indicates the fit to the neutral model. Neutral prediction is within 95% confidence interval (black), while non-neutral processes include above and below prediction (dark green and red). (b) Proportions in richness and abundance of the three groups (above prediction, below prediction, and neutral prediction) based on the NCM. (c) Composition of the three groups in abundance for microbial eukaryotic community. ~~(d) β-nearest-taxon index (βNTI) range of community.~~ (d) Null model analysis revealing the fraction of ecological processes. The percent of community assembly is governed primarily by various deterministic processes, including homogenous and heterogeneous selections and stochastic processes, including dispersal limitations and homogenizing dispersal and undominated processes (mainly ecological drift).

Fig. 1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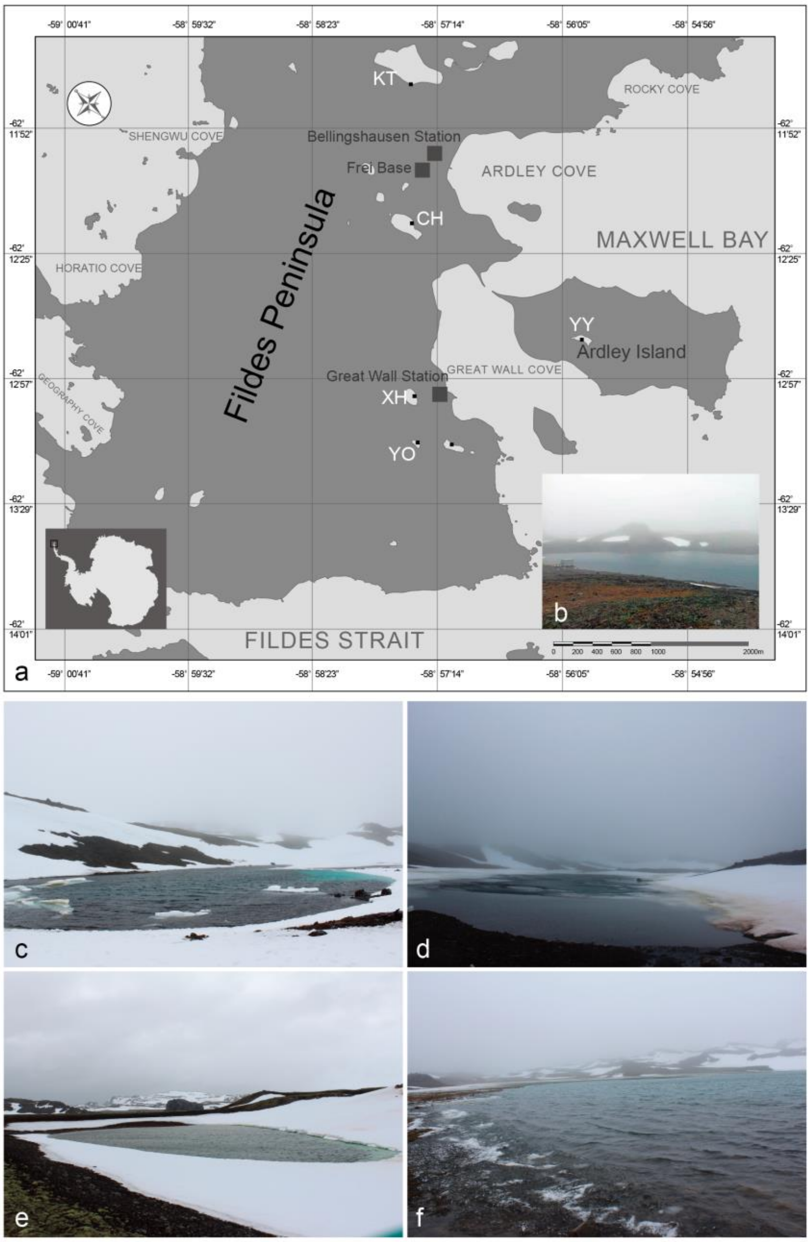
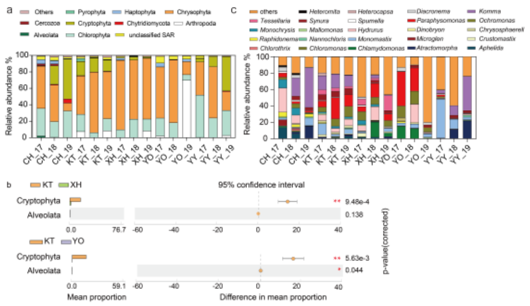


Fig. 1 (a) Location of the five studied lakes in Fildes Peninsula, King George Island, Antarctica, (b) Lake Xi Hu (XH), (c) Lake Yan Ou (YO), (d) Lake Chang Hu (CH), (e) Lake Yue Ya (YY), (f) Lake Kitec (KT).

Fig. 2



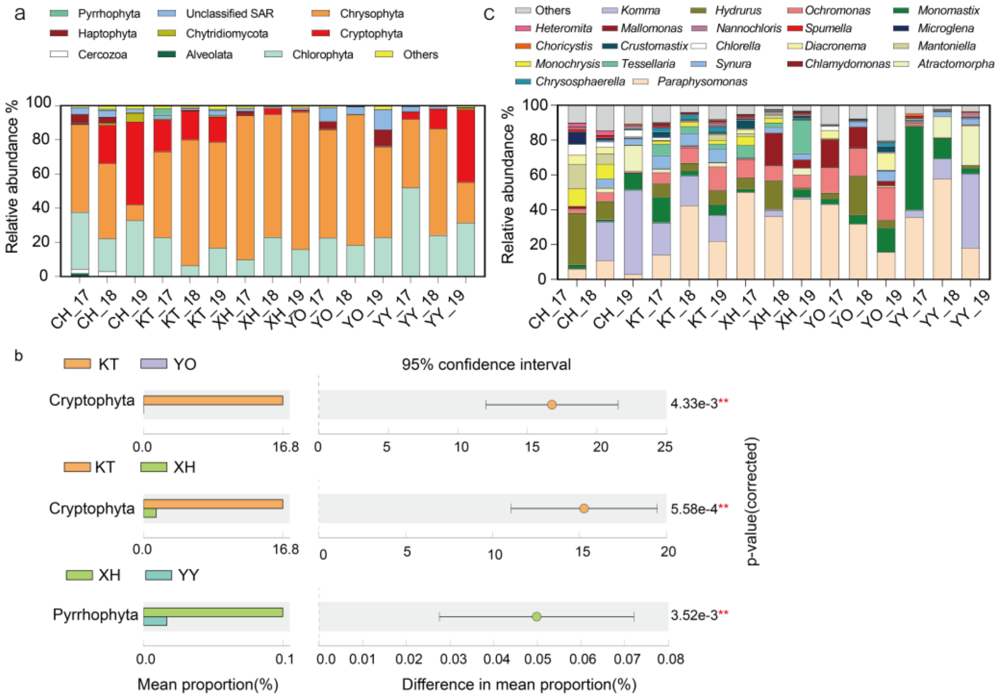
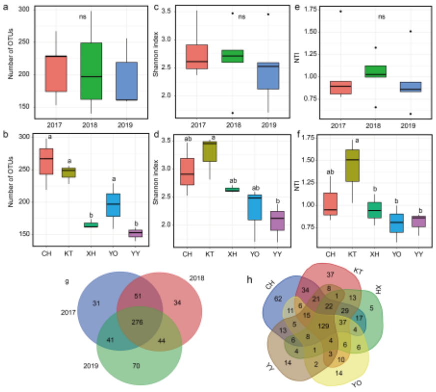


Fig.2 Comparison of microbial eukaryotic community composition. (a)Temporal and spatial dynamics of relative abundance on phylum level in five lakes from 2017 to 2019. 17, 18, and 19 expedition season of 2017/2018, 2018/2019, and 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different lakes. Lakes that showed no significant differences were not shown(P>0.05). (c)Temporal and spatial dynamics of relative abundance on genus level in five lakes. (Note: **$P < 0.01$, *$P < 0.05$). (Chlorophyta: ~~*Aphelida,*~~ *Atractomorpha*,~~ *Chlamydomonas,*~~ *Chloromonas*, ~~*Chlorothrix,*~~ *Monomastix, Chlorella,* *Choricystis*, *Crustomastix*, *Microglen*, *Monomastix*, *Nannochloris*~~, *Raphidonema*~~; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*, *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*,

Tessellaria; Cryptophyta: *Komma*; Haptophyta: *Diacronema*; ~~Pyrrophyta: *Heterocapsa*; Glissomonadida~~Cercozoa: *Heteromita*. The relative abundance at any lake was less than 1% was defined as others).

Fig. 3



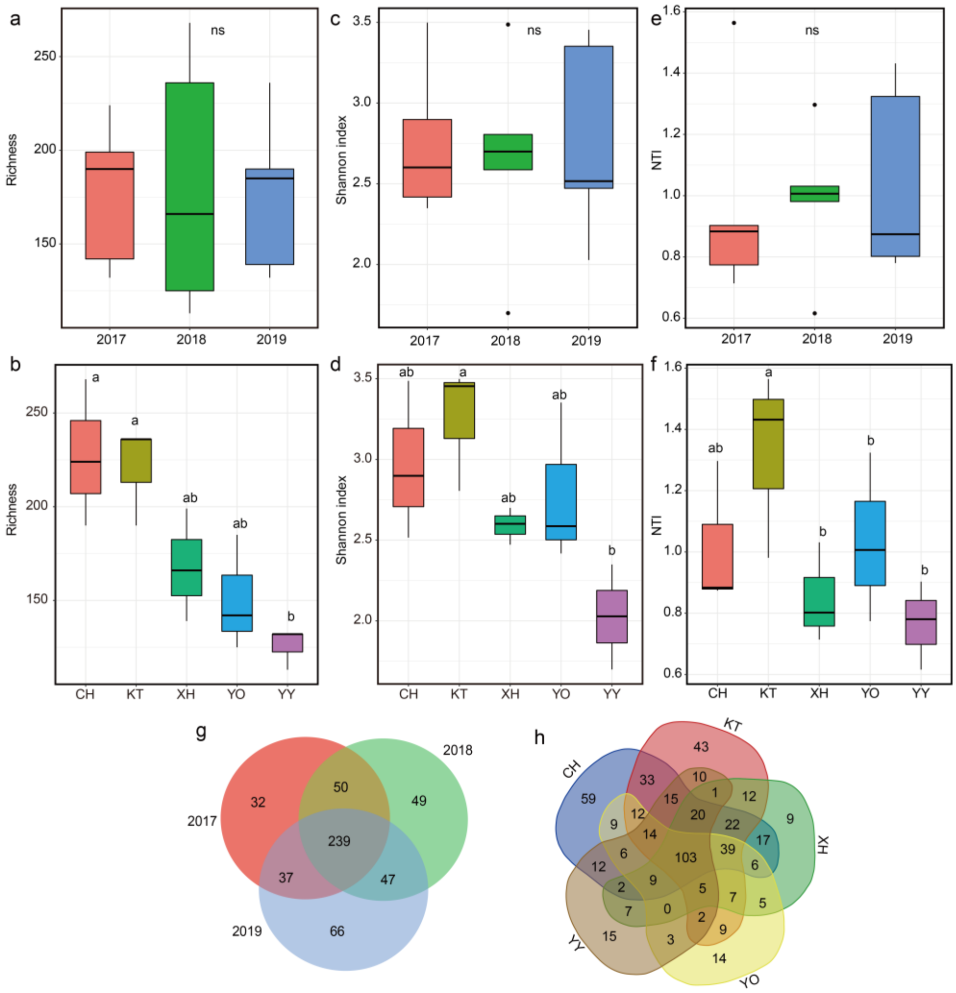
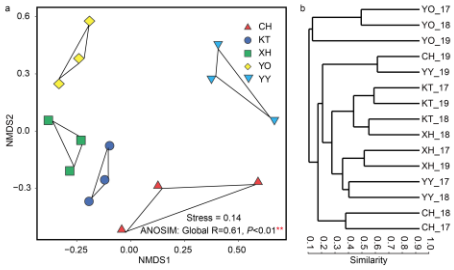


Fig.3 Microbial diversity and Venn diagram in different years and lakes. (a, b) variations in microbial ~~OTUs~~richness. (c, d) variations in microbial Shannon index. (e, f) variations in within-community nearest-taxon index (NTI). (g, h) Venn diagram showing the unique and shared operational taxonomic units (OTUs). Homogeneity and one-way ANOVA analysis of variance was used to test the indices' significance.

“ns” represents no significant differences ($P>0.05$). The significant differences ($\mathrm{P}<0.05$) were indicated by different alphabet letters between lakes, and lakes contained the same alphabet letters showed no significant difference ($P>0.05$) ~~Significant differences ($\mathrm{P}<0.05$) are indicated by different alphabet letters.~~

Fig. 4



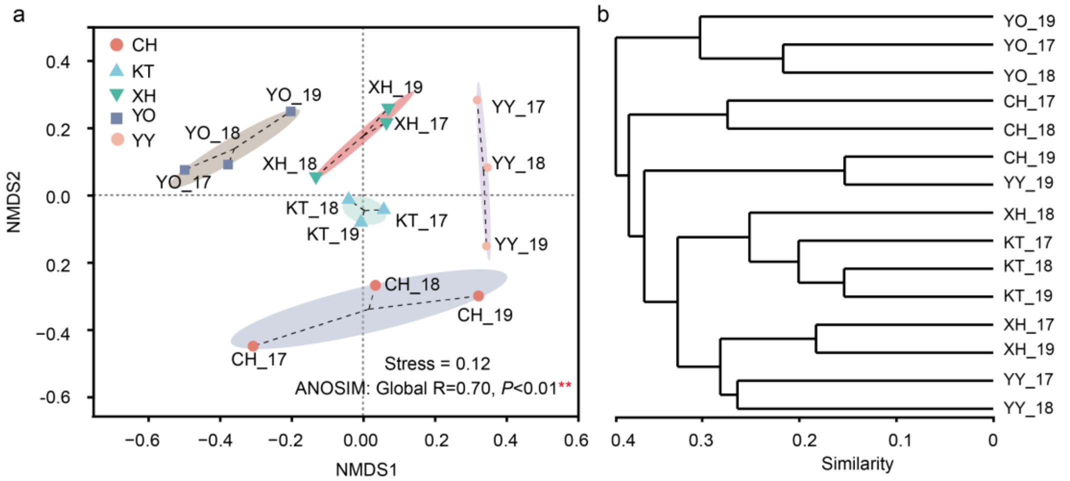
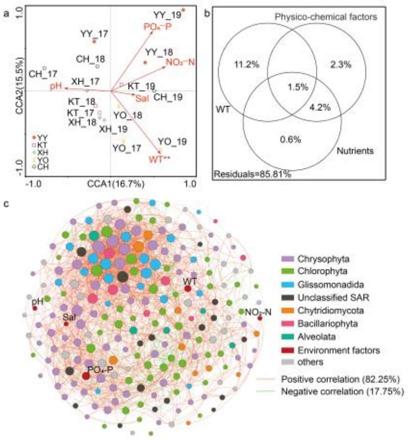


Fig. 4 Temporal variability analysis of Non-metric multidimensional scaling (NMDS) ordination of microbial eukaryotic communities (a) and clustering of five lakes based on similarity (b).

Fig. 5



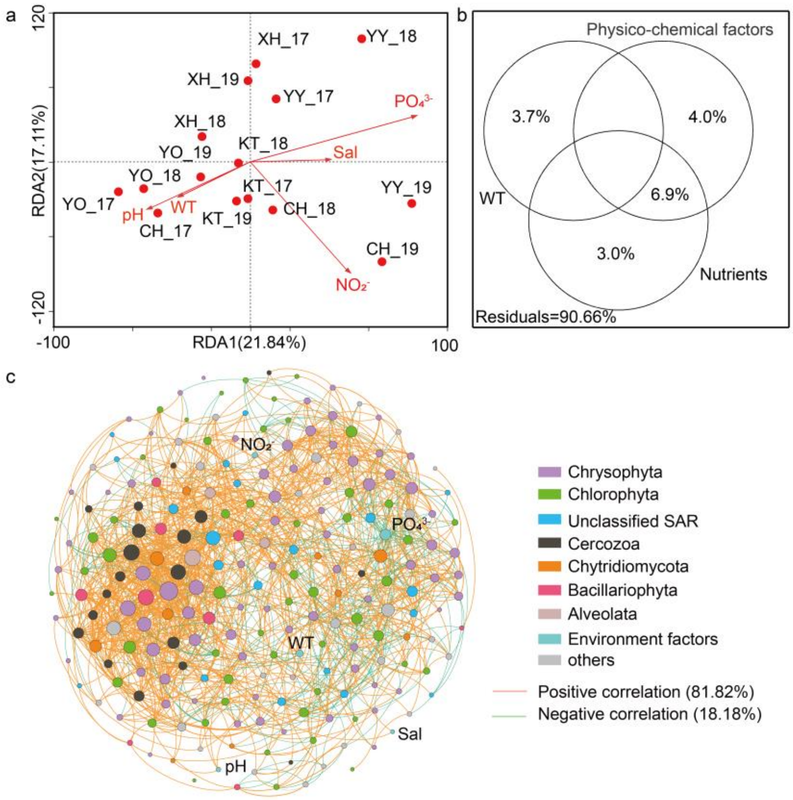
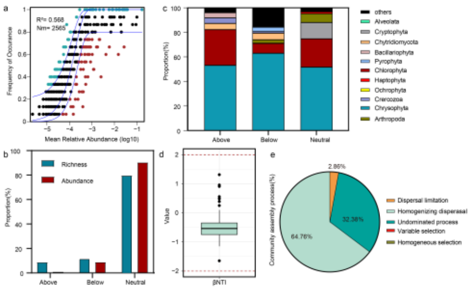


Fig.5 The effect of environmental variables on microbial eukaryotic communities, and co-occurrence pattern. Canonical correlation analysis plots(a) and variance partitioning analysis (b), respectively. ~~** $P < 0.01$.~~ (c) Networks analysis revealing the interspecies associations between microbial eukaryotic OTUs, and the correlation between environmental factors and OTUs in lakes integrated networks. The size of

each OTUs or environmental factor (node) is proportional to the degree centrality. Others: other phyla and unclassified taxa.

Fig. 6



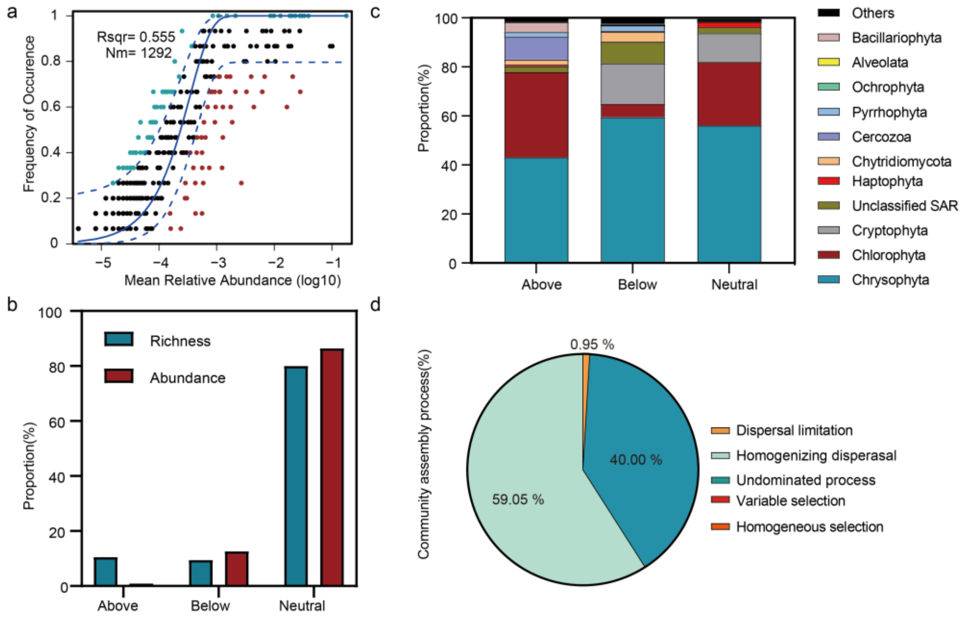


Fig.6 Relative influences of deterministic and stochastic processes on microbial eukaryotic community assembly based on the neutral community model (NCM) and the null model. (a) Fit of the neutral community model (NCM) of community assembly. Nm indicates the metacommunity size, and R^2 indicates the fit to the neutral model. Neutral prediction is within 95% confidence interval (black), while non-neutral processes include above and below prediction (dark green and red). (b) Proportions in richness and abundance of the three groups (above prediction, below prediction, and neutral prediction) based on the NCM. (c) Composition of the three groups in abundance for microbial eukaryotic community. ~~(d) β-nearest-taxon index (βNTI) range of community.~~ (ed) Null model analysis revealing the fraction of

ecological processes. The percent of community assembly is governed primarily by various deterministic processes, including homogenous and heterogeneous selections and stochastic processes, including dispersal limitations and homogenizing dispersal and undominated processes (mainly ecological drift).