Diversity and assembly processes of microbial eukaryotic communit~~y~~ies 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 investigate the microbial eukaryotic communities of five freshwater lakes on the Fildes Peninsula ~~during~~over three summer seasons. A total of 28 phyla were detected, with ~~the~~ phytoplankton occupying the highest percentage of sequences (accounting for up to 98%). The ~~main~~ dominant taxa ~~were~~consisted of Chrysophyta, Chlorophyta, and Cryptophyta. The species ~~R~~richness (113–268) and Shannon index (1.70~~∼~~–3.50) varied among the lakes, with higher values recorded in ~~lake~~ Lake Chang Hu and ~~lake~~ Lake Kitec~~,~~ and the lowest value obtained for~~in lake~~ Lake Yue Ya. There were significant differences between the ~~in~~ microbial eukaryotic communities of the ~~among~~ lakes, with spatial and temporal heterogenicity in the relative abundance of the dominant taxa (P<0.05). Environmental variables explained about 39% of the variation in community structure, with water temperature and phosphate identified as the driving factors (P<0.05). Network analysis revealed comprehensive co-occurrence relationships (positive correlation 82% vs. negative correlation 18%). The neutral community model revealed~~found~~ that neutral processes explained more than 55% of the community variation. ~~The s~~Stochastic processes (e.g., homogeni~~z~~sing dispersal and undominated processes) predominated in community assembly over~~than~~ the deterministic processes. These findings demonstrate~~revealed~~ the diversity of the microbial eukaryotic communities in the freshwater lakes of the Fildes Peninsula~~community~~ and have important implications for understanding the community assembly in these ecosystems.~~the freshwater lakes of the Fildes Peninsula (Antarctica).~~

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

1 Introduction

The Fildes Peninsula–which makes up the southwestern end of King George Island, South Shetland Islands, Antarctica–is home to a relatively high density of scientific research stations. The peninsula is commonly ice-free throughout the austral summer, making it the largest ice-free area (40 km^2) on King George Island. Located within the maritime Antarctic, the peninsula experiences 400-600 mm of precipitation each year and has an average annual temperature of -3ºC~~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 also occur in the region~~occur~~ (Barsch and Caine, 1984). Lakes on the Fildes Peninsula, along with those found in other ice-free areas of Antarctica, represent the year-round liquid water reservoirs on the continent (Priscu, 2010; Lyons et al., 2007). The availability and quality of water are impacted by sea conditions, macro-fauna usage, and anthropogenic influences, such as solid, volatile, and fluid waste production and disposal ~~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~~ (Priscu, 2010; Lyons et al., 2007)~~.~~ Antarctic lake systems are sentinels for climate change and host globally-relevant microbes and biogeochemical cycles (Marsh et al., 2020; Wilkins et al., 2013), thus making a more complete understanding of the processes shaping microbial communities there a priority. Moreover, the physical stability observed in these lakes makes them a good model system for interrogating biogeochemical processes within water columns (Comeau et al., 2012). ~~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).~~

Microbial eukaryotes (0.2~~∼~~–20 μm, pico-/nanoeukaryotes) are~~constitute~~ important components in microbial food webs and play a significant~~an important~~ role in the biogeochemical cycles (Massana et al., 2015; Unrein et al., 2014; Grob et al., 2007), as well as contributing to plankton biomass and carbon export (Hernandez-Ruiz et al., 2018; Leblanc et al., 2018). ~~-~~However, microbial eukaryotes are generally difficult to study due to their small size and common lack of distinguishing morphological features, especially among pico- and nanoeukaryotes. ~~However, the microbial eukaryotes have been neglected for a long term due to their small cell size and lack of conspicuous morphological features. The~~ A 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 a high taxonomic diversity of microbial eukaryotes in some oligotrophic and extreme regions (Richards et al., 2005; Zhao et al., 2011; Marquardt et al., 2016). Nevertheless, previous research ~~studies focused on~~ exploring the molecular diversity and the population fluctuations in these far cold and oligotrophic lakes of Antarctica ~~lakes are~~is limited.

The small size, short generation time, ~~rapid growth,~~ quick reflection of environmental conditions~~sensitivity to environmental conditions,~~ and genetic plasticity ~~render~~ of microbial eukaryotes make them excellent bioindicators reflecting environmental disturbances and the quality of ecosystems~~capable of quick reflection to environmental changes~~ (Karimi et al., 2017; Bouchez et al., 2016)~~. Hence, they are excellent bioindicators of the impact of environmental perturbations and ecosystem quality~~. A previous study revealed the presence of Alveolates and ~~dinoflagellates~~ Dinoflagellates among small deep-sea Antarctic plankton eukaryotes (0.2–5 μm)~~have~~

~~been detected in the size fraction 0.2~5 µm of Antarctica plankton eukaryotes, the biodiversity~~ and demonstrated that their diversity was affected by temperature and salinity (Lopez-Garcia et al., 2001). ~~Based on m~~Microscopic observations have also been used to show that~~,~~ diatoms in the lakes of Fildes Peninsula region accounted for 59.8% of the total number of phytoplankton species, and that water temperature and nutrients resulted in the variations ~~of~~in nano-and microphytoplankton abundance and composition (Zhu et al., 2010). A relatively~~pretty~~ low number of taxa, ~~the abundance of diatom genera~~ such as *Nitzschia*, *Achnanthes*, and *Navicula*, etc., have also been investigated from ~~the oligotrophic and~~ periodically brackish water ponds near the Polish Antarctic Station on King George Island, whose abundance were influenced by the nutrients in the water (Kawecka et al., 1998). Indeed, ~~T~~the microphytoplankton communities of the shallow lakes ~~from~~of the Antarctic Peninsula, ~~were~~are 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).~~ Although several studies on other regions have explored the fluctuations of microbial eukaryotes and their relationship with environmental factors,~~However,~~ the spatio-temporal variation, co-occurrence pattern, and community assembly of microbial eukaryotes in Antarctic (Fildes Peninsula) freshwater lakes have been rarely investigated~~reported~~.

Deterministic and stochastic processes are~~have been~~ considered the two main ecological processes in community assembly (Ofiteru et al., 2010). Deterministic processes are based on the 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 states~~believes~~ that random birth or death, drift, and dispersal events also play an essential role in community assembly~~composition~~ (Bahram et al., 2016). Deterministic processes~~ (selection)~~ are prevalent in building whole ecosystem communities (Liu et al., 2020)~~,~~ where selection leads to species classification, and ~~and~~

applying similar habitats thus results in similar community assemblages. ~~Although~~ However, other studies have supported an important role ~~for~~of stochastic processes (e.g., drift and dispersal) in community assembly, where dispersal ~~was~~ is the movement of species over~~in~~ spatial locations and drift ~~was~~ is associated with the relative abundance of the species (Massana and Logares, 2013; Wu et al., 2019). Stochastic processes have been shown to account~~ed~~ 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 have also been~~also were~~ found to prevail in micro-and nanoplankton eukaryotic communities in the intertidal zones ~~in Intertidal Zones~~ of ~~S~~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 the microbial eukaryotic diversity and community assembly processes of the freshwater lakes ~~in~~ on the Fildes Peninsula, Antarctica. Our study attempted to analyze the microbial eukaryotic samples of five freshwater lakes collected over~~from~~ three summers using high-throughput 18S rRNA sequencing. We aimed to (I) understand the diversity and co-occurrence of microbial eukaryotes~~;~~ and (II) explore the influencing factors and their community assembly processes.

2 Material and Method

2.1 Study area, sample collection and measurement of environmental variables ~~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~~ on King George Island, the largest of the South~~island in southern~~ Shetland Islands. Surface water samples were collected from five lakes, namely Chang Hu (CH), Kitec (KT), Xi

Hu (XH), Yan Ou (YO), and Yue Ya (YY) (Fig. 1). Moreover, the physio-chemical parameters of water samples were measured synchronously.

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

Water temperature (WT), pH, and salinity (Sal) were measured using an RBRconcerto C.T.D (Canada). In addition to the in-situ measurements, a water sample obtained 0.5 m below the surface was collected at each monitoring site using a 5 L plexiglass sampler. For measurements of chlorophyll a (Chl *a*) contents, 1 L water samples were filtered using GF/F filters (0.70 μm, Whatman), and Chl *a* was then extracted with 90% acetone over 24 h and measured spectrophotometrically. ~~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 San^{++} continuous flow ~~autoanalyzer~~ auto-analyser (Skalar, the Netherlands) after filtering following the procedure~~as~~ described by ~~(~~Hansen and Koroleff~~,~~ (1999).

2.2 Polymerase chain reaction (PCR) and Illumina MiSeq

1 L surface water 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 prior to the~~until~~ laboratory experiments. The DNA extraction was performed as described by (Luo et al., 2015).

PCR was performed using primers by 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 (~~The PCRs~~)~~ were conducted in 20 µL reactions with 0.2 µM of 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. 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 a 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 described 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 a 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 et al., 2013) using a confidence threshold of 0.7.

The analysis of the OTUs obtained using the UPARSE clustering method have ~~still~~ been widely used for high-throughput sequencing analysis (Gad et al., 2022; Xu et al., 2022; Zhang et al., 2022; Reboul et al., 2021; Sun 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 (Li et al., 2019; Glassman and Martiny, 2018).

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~~ normalised 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).

2.3 Community composition and diversity

The 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 the taxa on a within-community scale for communities. High or positive values indicated clustering taxa across the overall phylogeny, while lower negative values indicated an 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 mean nearest taxon distance (MNTD) is from the mean of the null distribution with 999 ~~randomizations~~ randomisations determined in the “Picante” R package.

The ~~Non~~non-metric multidimensional scaling (NMDS) of the microbial eukaryotic communities was performed ~~with~~ using the relative abundance of OTUs (Roberts, 2013). An Analysis of similarity (ANOSIM) investigated differences in the microbial eukaryotic communities between the groups. The unweighted pair-group method with arithmetic means (UPGMA) was used to determine the similarity between the samples ~~by~~ using clustering analysis according to community composition similarity. These analyses were performed in the ~~R package~~ “Vegan” and “Phangorn” R packages. All calculations were based on similarity matrices (1-dissimilarity of the Bray–Curtis distance metric).

2.4 Influencing factors of the community structure

The Canoco 4.5 software (Ter Braak and Smilauer, 2002) was used to rank species and environmental factor data, and the ranking model was determined by the de-trending correspondence analysis (DCA) of OTUs data. All environmental factors, except pH, were log (x+1) transformed prior to~~before~~ analysis to improve normality and homoscedasticity. To reduce multicollinearity among the 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~~ network ~~Analysis~~analysis

We constructed one co-occurrence network based on the samples acquired over~~from~~ the whole study period. To reduce the complexity of the data sets, OTUs occurring~~ed~~ in at least 5 samples were retained to construct the network~~networks~~. Only robust (|r| > 0.6) and statistically significant (*P*<0.05) correlations were incorporated into the network analyses. Finally, the network was visualised by Gephi software~~visualization was conducted using Gephi software~~ (Bastian et al., 2009).

2.6 Ecological community assembly analysis

The ~~Neutral~~ 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 the OTUs frequency and their relative abundance (Chen et al., 2019), which ~~The model~~ 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 community model.

The Stegen null model was used ~~To~~ 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 the phylogenetic distance and OTU~~s~~ abundance (Stegen et al., 2013; Webb et al., 2002). The relative contributions of variable selection and homogeneous selection ~~was~~ were 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 the pairwise comparisons that deviated from the selection (Evans et al., 2017; Stegen et al., 2013). When ~~Integrated~~ integrated with the value of |RC_{bray}|, the underlying community assembly processes could represent a~~ 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~~ randomised 999 times to obtain the average null expectations.

3 Results

3.1 Physico-chemical properties

Water temperature ranged from 0.90°C to 7.14°C, and the water temperature in YO was significantly higher than in the other lakes (Table S1, P<0.05). Nutrients in five lakes showed lower values of 0.00-0.15 μM (NO_2^-), 0.05-0.74 μM (NH_4^+) and 0.02-2.29 μM (PO_4^{3-}). Relatively higher and lower levels of nutrients were identified in YY and XH, respectively. The lowest value of SiO_3^{2-} was recorded in YY (1.43 μM) and the highest in CH (51.5 μM). The highest and lowest Chl *a* were reached in YY and CH (2.11 and 0.25 μg L^{-1}, respectively). pH showed minimum and maximum values in YY (7.65) and CH (8.27). Sal values ranged from 0.00-0.14 PSU and were significantly lower in YO than in the other lakes (*P*<0.05).~~The WT varied from 0.90 °C to 7.14 °C (Table S1), while the YO was significantly higher than other lakes (P<0.05). Nutrients were low with NO2-N, NH4+, and PO43− concentrations with 0.00~0.15 μM, 0.05~0.74 μM, and 0.02~2.29 μM, respectively. YY had higher concentrations of PO43−, NH4+, and NO2−, while XH had lower NO2− and PO43−. SiO32- varied from 1.43 to 51.5 μM, 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. Sal was 0.00-0.14 PSU, which in YO exhibited a significantly lower value compared to other lakes (P<0.05).~~

3.2 Diversity and composition of microbial eukaryotic ~~community~~communities

A total of 9 dominant phyla were identified, accounting for over 98% of the sequences. These dominant phyla ~~were~~ mainly consisted~~composed~~ of microbial eukaryotic phytoplankton, with various relative abundances between different lakes (Fig. 2a). ~~The~~ Chrysophyta (34.91% in CH~~~~~–78.88% in XH), Chlorophyta (15.17% in KT~~~~~–35.88% in YY), and Cryptophyta (0.01% in YO~~~~~–23.71% in CH) were most abundant in the studied lakes. ~~The~~ Cryptophyta in KT was significantly more abundant than in XH and YO, and Pyrrophyta in XH was significantly more abundant than in YY ($P<0.05$) (Fig. 2b). ~~The~~ Haptophyta represented 0.09% of the sequence in KT ~~∼~~and up to 4.93% in YO; Alveolates represented 0.02% of the sequence in XH~~∼~~ and up to 1.00% in CH, ~~and~~ while unclassified Stramenopiles-Alveolates-Rhizaria (SAR) represented 1.12 % in XH~~∼~~ and 8.05 % in YO.

The relative abundances of the dominant taxa in the same lake exhibited~~had~~ some interannual variations. ~~The~~ The relative abundances of Chrysophyta in CH_19, YO_19, and YY_19 samples were lower than in the other samples, while ~~the~~ the relative abundances of Cryptophyta in CH_19 and YY_19 ~~were~~ were lower than in the other samples ~~in~~ of CH~~,~~ and YY, respectively (Fig. 2a).

A total of 21 dominant genera were identified (Fig. 2c), accounting for over 87% of the sequences. The dominant genera included~~were mainly~~ *Hydrurus*, *Paraphysomonas*, *Ochromonas*, *Synura*, and *Monochrysis* belonging to Chrysophyta, *Komma* in Cryptophyta, *Monomastix*, *Chlamydomonas*, and *Mantoniella* belonging to~~in~~ Chlorophyta.

As shown in Fig. 2c, the abundance of the dominant genera differed interannually among the investigated lakes~~ investigated interannually~~. The relative abundance of *Komma* varied from 0 to 48.52%, which showed an increasing trend over the years in CH and YY. The ranges of *Paraphysomonas* and *Ochromonas* were 3.06~~∼~~–56.88% and 0.06~~∼~~–18.86%, showing an increase followed by a decrease in XH over the years. The highest relative abundances of *Hydrurus* were in samples XH_18 and YO_18 than in the other samples~~was higher than in the other samples~~. ~~*Mantoniella* was significantly more abundant in CH than in other lakes.~~ *Ochromonas* in CH was significantly lower than in YO and XH ($P<0.05$, Table S2).

The richness, Shannon index, and NTI exhibited~~had~~ interannual variations but showed no significance ($P>0.05$) (Fig. 3a, c, e). The order of NTI and Shannon index values all showed 2018>2017>2019; the species ~~Richness~~ richness were highest ~~in~~ during the expedition season 2017 and lowest in 2018. The ranges of richness and Shannon index were 113~~∼~~–268 and 1.70~~∼~~–3.50, respectively, with YY having the lowest values ~~and~~ which ~~was~~ were significantly lower than in KT (Fig. 3b, d, $P<0.05$). The range of NTI was 0.62~~∼~~–1.56, with the lowest value recorded in YO which was ~~and~~ significantly lower than in KT (Fig. 3f, $P<0.05$). KT had the highest Shannon index, richness, and NTI, and CH also exhibited relatively~~had the~~ higher richness value (Fig. 3b, d, f).

The total number of OTUs shared between~~in~~ 2017-2019 was 239, and 32, 49, and 66 ~~the~~ unique OTUs were identified for 2017, 2018, and 2019, respectively~~32 (2017), 49 (2018), 66 (2019)~~ (Fig. 3g). The ~~Venn diagram showed that the~~ total number of OTUs shared by the five lakes was 103, and ~~the unique OTUs were~~ 59 (CH),43 (KT),9 (XH),14 (YO), and 15 (YY) unique OTUs were distinguished among the lakes (Fig. 3h).

The NMDS results divided the samples into five clusters according to their similarity of microbial eukaryotic community (stress value = 0.12) (Fig. 4a). In addition, the ~~analysis of similarity (~~ANOSIM~~)~~ based on Bray-Curtis distance indicated that the differences between the lakes were significant (Global R = 0.70, $P<0.01$). Meanwhile, no significant differences were detected by ANOSIM among the interannual variation (R=0.013, $P=0.393$).

The 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~~, respectively~~. ~~For other lakes, CH_19, and YY_19 clustered together.~~ YO lake was distant from the other lakes and clustered into a separate ~~one~~group.

3.3 Driving factors and co-occurrence patterns

Canonical correspondence analysis (CCA) demonstrated that the first two sequencing axes explained 21.84% and 17.11% of the community variation, respectively (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 ~~WT~~water temperature and PO_4^{3-} significantly affected the microbial eukaryotic communities~~y~~ ($P<0.05$). The ~~variation partition analysis (~~VPA~~)~~ indicated that the monitored environmental factors~~ monitored~~ explained 9.34% of the microbial eukaryotic community variability among lakes, but there was still~~and still had~~ a large amount of unexplained ~~community ~~variation (90.66%, Fig. 5b).

A total of 220 nodes linked by 1521 edges comprised the~~were made up~~ microbial eukaryotic community network. The majority of nodes in the network had many connections. Notably, the positive associations among species were predominant in the network (Fig. 5c), comprising with 81.82% of the associations, whereas the portion of negative association was only 18.18%. In addition, the positive interactions were mainly found within the same taxonomic affiliations, such as Chrysophyta, or between a few different taxonomic affiliations, such as Chrysophyta and Chlorophyta. ~~While t~~The negative correlations on the other hand mainly were reflected between Chrysophyta and Chlorophyta. ~~We found that o~~Only ~~about~~around 8% of OTUs ~~directly ~~correlated directly with environmental factors ($P<0.05$). Meanwhile, only two of the top 20 OTUs with the highest degree centrality were directly associated with environmental factors (~~WT~~water temperature and PO_4^{3-});~~,~~ ~~and ~~one belonged to Chrysophyta and ~~one ~~the other to Chytridiomycota.

3.4 Community assembly processes

The Sloan neutral community model (NCM) showed the importance of neutral processes for microbial eukaryotic communities (Fig. 6a), with the neutral processes explaining 55.5% of the community variation. In addition, the NCM classified microbial eukaryotic taxa into three groups (above prediction, below prediction, and neutral prediction). In addition, compared to the above and below prediction groups, we observed a higher contribution of neutral prediction group to community abundance (86.4%) and richness (80%), ~~We found that the neutral group (within 95% confidence interval), with richness and abundance ratios of 80.0% and 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 10.58% of the microbial eukaryotic richness but corresponded to only 0.96% of the abundance, and was dominated by Chrysophyta, Chlorophyta, and Cercozoa. Cryptophyta accounted for 16.58% of the abundance in the neutral prediction group but was almost absent in the above prediction group. In contrast, Cercozoa was present with a~~in~~ 9.38% abundance in the above prediction group, but only comprised 0.32% ~~in~~ of the neutral prediction group.

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 analysis of community assembly processes ~~analysis~~ showed that stochastic rather than deterministic processes controlled the community assembly. Among them, homogenizing dispersal dominated, with a proportion of 59.05%, followed by undominated processes and dispersal limitation (Fig. 6d).

4 Discussion

4.1 ~~Diversity and d~~Dominant taxa and diversity

The environmental conditions (e.g., low light and low nutrient contents, etc.) in Antarctic freshwater lakes ~~differed~~ differ from those of temperate lakes. These special features and their relative isolation has resulted in the development of unique communities and ~~the~~ survival strategies of the species adapted to such conditions (Pearce and Galand, 2008). In our study, the survival of taxa (Chrysophyta, Chlorophyta, and Cryptophyta) might have depended on their survival strategies and adaptation to harsh conditions, which allowed them to become~~made them as~~ the predominant species.

Chrysophyta dominated in the five lakes examined in our study, including *Hydrurus*, *Paraphysomonas*, *Ochromonas*, and *Monochrysis*. First~~ly~~, ~~the~~ their dominance might have been~~be~~ due to their adaptation to a low nutrient availability. Chrysophyta have been widely distributed~~well represented mostly~~ in oligo- and mesotrophic lakes from both the maritime and continental regions of Antarctica (Allende, 2009; Allende and Izaguirre, 2003; Izaguirre et al., 2020; Richards et al., 2005). Secondly, Chrysophyta can still retain~~ed~~ a high cell density under low light conditions, since they can~~as~~ ~~they could~~ adapt to changing light conditions (Yubuki et al., 2008). Furthermore, Chrysophyta ~~was~~ is mixotrophic and can even ~~could~~ swim, allowing~~which allowed~~ them to ~~get~~ obtain available nutrients from other microorganisms, and thus reducing the need for dissolved nutrients in the water (Pick and Lean, 1984; Katechakis and Stibor, 2006). In addition, when ~~the~~ environmental conditions change~~d~~ dramatically, such as through freezing and nutrient changes, Chrysophyta ~~could~~ can form cysts (Nicholls, 1995), protecting their cells from resisting an unsuitable environment. All these characteristics~~aspects~~ ~~made~~ allow Chrysophyta ~~have the advantage~~ to be the predominant species in the five Antarctic lakes.

Chlorophyta was the second most dominant taxon in our study (15.17%~~~~~–35.88%), ~~containing~~ mainly consisting of the *Monomastix*, *Chlamydomonas*, and *Mantoniella*. Chlorophyta was typically represented by flagellated species such as *Chlamydomonas* spp., which dominate 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 (Izaguirre et al., 2003; Allende and Mataloni, 2013; Toro et al., 2007). Several unicellular algae ~~could~~ can undergo mix acid fermentation, and some obligate photoautotrophic species can increase their light capture efficiency through photoadaptation processes involving chlorophyll accumulation~~responded by photo-acclimation processes involving the accumulation of chlorophyll to increase the light capture efficiency~~ (Morgan-Kiss et al., 2016; Atteia et al., 2013). These characteristics might partially ~~be~~ explain~~ed~~ how Chlorophyta survived and occupied a specific advantage ~~of the important reason~~ in the lakes ~~we~~ studied here.

Cryptophyta was the third most abundant~~dominant~~ taxon observed. Indeed, their dominance has been interpreted as evidence of heterotrophic growth in winter and mixotrophic growth throughout the year (Unrein et al., 2014). Cryptophyta dominated under perennially ice-covered and coastal saline lakes in continental Antarctica

(Izaguirre et al., 2020). The ingestion of bacteria by mixotrophic Cryptophyta has previously been observed in two perennially ice-covered lakes (Fryxell and Hoare) in the McMurdo Dry Valleys (Roberts and Laybourn-Parry, 1999).

Compared ~~with~~ to other aquatic ecosystems (Wang et al., 2021; Wang et al., 2020b; Hernandez-Ruiz et al., 2018), the diversity of microbial eukaryotes in the Antarctic lakes studied here was significantly lower (Shannon index 1.70~~∼~~–3.50, richness 113~~∼~~–268). The diversity of the microorganisms reported here decreases from mid-latitude to the poles (Santos et al., 2020). The isolation and harsh conditions, especially the lower temperatures and nutrients availability, prevailing in Antarctic lakes accounted for a low microbial eukaryotic diversity. In addition, the species-area relationships model (SAR) states that there should be an increased species number as habitat area increases with~~increased species number with increasing habitat area within~~ a specific area (Ma, 2018). An increase in the ice-free area may drastically modify the ~~modified~~ biodiversity of an area (Pertierra et al., 2017; Duffy et al., 2017; Lee et al., 2017). Our results support~~ed~~ the SAR model~~, observing more~~ since a greater alpha diversity was observed in the CH and KT ~~,~~where the habitat areas were much larger than the YY and YO.

4.2 Influence of environmental factors on the community

Previous ~~great efforts~~studies have demonstrated that abiotic factors affect microbial diversity and community composition (Quiroga et al., 2013; Sun et al., 2017). ~~Our study found~~In this study, we found that ~~WT~~water temperature and PO_4^{3-} were significant ~~driving~~ factors driving~~for~~ community changes among the abiotic factors analy~~z~~sed. Some microorganisms have evolved to grow under ~~a~~ defined temperatures, allowing differences in the temperature adaptation of different species (Wilkins et al., 2013). Water temperature~~WT~~ has become a major driving factor for microbial eukaryotic community variation since it can~~by~~ regulate~~ing~~ 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 temperature changes on community structure (Garcia-Rodriguez et al., 2021). The ~~WT~~ water temperature in the YO was significantly higher than in the other lakes, and the community of YO clustered into a separate ~~clade~~ group (Fig. 5b). Microorganisms are~~have been~~ highly sensitive to nutrients, and indeed, nutrients have been shown to represent~~were~~ important factors of community differences in microbial eukaryote~~s~~ communities in different areas (Zouari et al., 2018). In addition, 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 the ~~measured~~ environmental variables measured in our analysis. Our results~~This~~ indicate~~d~~ that these environmental factors played a minor role in shaping the microbial eukaryotic community structures. A substantial amount of variation remained unaccounted for~~There were many unexplained variations~~ (Fig. 5), which could be due to several reasons.~~and some possible causes have been indicated.~~ First~~ly~~, it is impossible to measure all the environmental factors that could influence microbial communities and, thus, some significant driving factors may not have been included in our study~~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). Potentially vital abiotic factors in Antarctic lakes may include the solar cycle, light availability, ice cover (thickness and duration), physical mixing of snow melt, and other hydrological processes~~There 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 relationships between the microorganisms considered here could not be quantified, and these relationships could represent potentially essential factors shaping the community structures in the studied lakes. For example, predation pressure can manifest as a top-down control of microbial eukaryotes~~Secondly, the relationship between microorganisms couldn't be quantified, which was an essential factor influencing community structure. Predation pressure manifested itself in lakes as a top-down control of microbial eukaryotes~~ (Blomqvist, 1997). Finally~~Thirdly~~, stochastic processes such as ecological drift (birth, death) might also cause unexplained community variations (Zhang et al., 2018).

4.3 Co-occurrence patterns

Network analysis ~~could~~ can 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 can form various ecological relationships, ranging from mutualism to competition, ultimately shaping the microbial abundances (Faust and Raes, 2012). Positive associations in a network often indicate common preferred environmental conditions or niche-overlapping, whereas negative associations imply~~mean~~ competition or niche division (Faust and Raes, 2012). Our network analysis revealed~~By analyzing the network, we found~~ that the positive correlations were much more common (82%) than ~~the~~ negative ones ~~correlations~~(18%). ~~in the co-occurrence network (82% vs. 18%),~~ These results suggested that positive relationships (e.g., due to cross-feeding, niche overlap, mutualism, or commensalism) might play a more important role in the lake ecosystems of Antarctica than negative relationships (e.g., predator-prey, host-parasite, or competition) ~~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 results ~~has~~ have been obtained through the study of~~found~~ ~~in~~ small planktonic eukaryotes (0.2~~∼~~–20 μm) inhabiting the surface waters of a coastal upwelling system (Hernandez-Ruiz et al., 2018). Notwithstanding, further studies are needed~~necessary~~ to corroborate the biological interactions and other nonrandom processes (for example, cross-feeding versus niche overlap) between the species pairs detected by network analyses. In addition, only 8% of the OTUs were significantly correlated with environmental factors, suggesting that microbial eukaryotes had a relatively lower response to environmental factors.~~and these 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).~~

4.4 Community assembly processes

In general, deterministic and stochastic processes exist~~ed~~ simultaneously in ~~the~~ a community assembly (He et al., 2021; Chase, 2010). 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) can influence~~d~~ the relative importance of stochastic and deterministic processes in ~~the~~ a community assembly. The importance of stochastic processes has been previously illustrated for other microbial eukaryotic communities ~~from~~ in ~~the~~ aquatic ecosystems (Chen et al., 2019; Wang et al., 2020a). For example,~~The~~ picoeukaryotic communities in the lower oxygen layer of the Bengal Bay were shown to be dominated by stochastic processes (Chen et al., 2022). Results from our study supported a more prominent role of stochastic processes over deterministic ones in shaping the assembly of microbial eukaryotic communities. ~~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 amount ~~number~~ of variation~~s~~ in the~~our study's~~ microbial eukaryotic community studied here, and a small number of taxa were significantly correlated with environmental factors.

In our study, the microbial eukaryotic community showed a good fit with~~to~~ the neutral model (Fig. 6a), ~~which suggested~~ suggesting that community variations could be explained by stochastic processes such as birth, death, and migration to a large extent. The NCM can also attribute~~ed~~ the ~~observed~~ patterns observed of community assembly to different population behavior (Zhang et al., 2021). Here, ~~The~~ the NCM separated the studied taxa into three groups~~,~~ which ~~and these groups were~~had different ~~in~~ community structures (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 (Zhang et al., 2021),~~ indicating that these taxa might differ in their adaptability to the environment or their dispersal rates (Chen et al., 2019). In addition, the neutral prediction group contributed a high proportion to both the species abundance (86.4%) and richness (80.0%) in our study. Previous studies have shown that~~In a subtropical river,~~ the neutral prediction group also dominated the microbial eukaryotic community in a subtropical river in terms of richness and abundance (Chen et al., 2019). Similar results in this study suggest~~ed~~ that microbial eukaryotes studied here were more susceptible to stochastic processes.

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 from~~by~~ the input of microorganisms from the surrounding ice melt, atmospheric transport, human activities, or bird migration (Unrein et al., 2005). Zeng et al. (2019) showed that ~~Homogenizing~~ homogenizing dispersal occupied a high proportion of community assembly processes in freshwater lakes~~water bodies~~ (Zeng et al., 2019), and ~~the~~ similar results ~~was~~ were obtained~~found~~ in our study. Most microorganisms detected in the sea ~~also~~ have also been found ~~present~~ in lakes in East Antarctica, suggesting~~pointing to~~ that some marine taxa in the lakes could be the product of homogenizing dispersal from the sea to the lakes (Logares et al., 2018). In addition, the five studied lakes were covered in ice for most of the year and were limited by geographical distance, resulting in the limited dispersal ~~limitation~~ of microorganisms (0.95%). Undominant processes accounted for 40~~.00~~% of the 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., 2018). This suggest that microbial eukaryotic communities might be formed by some highly complex assembly mechanisms in Antarctic freshwater lakes.

5 Conclusion

In conclusion, ~~the~~ a unique microbial eukaryotic community structure and low alpha diversity (richness and Shannon index) have been~~were~~ demonstrated in five freshwater lakes ~~on~~ of the Fildes Peninsula, Antarctica. ~~Importance of stochastic processes and co-occurrence patterns in shaping the microbial eukaryotic community of this area were proved.~~ ~~WT~~Water temperature and PO_4^{3-} were identified as important ~~driving~~ factors driving the~~for~~ variation of community structures (P<0.05). Stochastic processes and biotic co-occurrence patterns were shown to be important in shaping microbial eukaryotic communities in the area. ~~Stochastic processes played a very prominent role in microbial eukaryotic community assembly.~~ Our study provides a better understanding of the dynamic patterns and ecological assembly processes of microbial eukaryotic ~~community~~ communities ~~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.

Author Contribution Statement

Conceptualization: Chunmei Zhang and Yonghong Bi~~.~~; m~~M~~ethodology: Chunmei Zhang and Wei Luo~~.~~; ~~M~~molecular technique：Huirong Li~~.~~; ~~P~~physico-chemical properties: Bin Wang, Yangjie Li, and Zhongqiang Ji~~.~~; ~~S~~sample collection：Yinxin Zeng and Haitao Ding~~.~~; ~~F~~funding acquisition: Yonghong Bi and Wei Luo~~.~~; ~~S~~supervision: Yonghong Bi and Wei Luo~~.~~; ~~W~~writing - original draft: Chunmei Zhang~~.~~; ~~W~~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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Figure Captions

Fig. 1 (a) Location of the five studied lakes ~~in~~ on the 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), and (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. The 17, 18, and 19 represent the expedition season ~~of~~ 2017/2018, 2018/2019, and 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different lakes. The Lakes that showed no significant differences ~~were~~ are not shown ($P>0.05$). (c)Temporal and spatial dynamics of relative abundance on ~~Genus~~ the genus level in the studied five lakes. (~~Note~~note: **$P < 0.01$, *$P < 0.05$). (Chlorophyta: *Atractomorpha*, *Chlamydomonas*, *Mantoniella*, *Chlorella*, *Choricystis*, *Crustomastix*, *Microglena*, *Monomastix*, *Nannochloris*; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*, *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*; Cryptophyta: *Komma*; Haptophyta: *Diacronema*; Cercozoa: *Heteromita*. The relative abundance ~~at~~ in any lake was less than 1% ~~was~~ is defined as others).

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

Fig. 4 Temporal variability analysis of ~~Non~~non-metric multidimensional scaling (NMDS) ordination of the 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~~. –~~: (a) Canonical correlation analysis plots~~(a)~~ and (b) variance partitioning analysis~~ (b), respectively~~. Sal: salinity; WT: water temperature; NO_2^-: nitrite nitrogen; PO_4^{3-}: phosphate. (c) Network analysis revealing the interspecies associations between microbial eukaryotic operational taxonomic units (OTUs), and the correlation between environmental factors and OTUs in the 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 NCM~~neutral model~~. Neutral prediction ~~is~~ was within a 95% confidence interval (black), while non-neutral processes included the above and below prediction (dark green and red). (b) Proportions ~~in~~ of the richness and abundance of the three groups (above prediction, below prediction, and neutral prediction) based on the NCM. (c) Abundance ~~Composition~~ composition of the three groups ~~in abundance~~ in the~~for~~ microbial eukaryotic community. (d) Null model analysis revealing the fraction of ecological processes. The percent of community assembly is governed primarily by various deterministic processes, such as~~including~~ homogenous and heterogeneous selections, and stochastic processes, including dispersal limitations, ~~and~~ homogenizing dispersal, and undominated processes (i.e., weak selection, weak dispersal, diversification, and drift processes~~mainly ecological drift~~).

Fig. 1

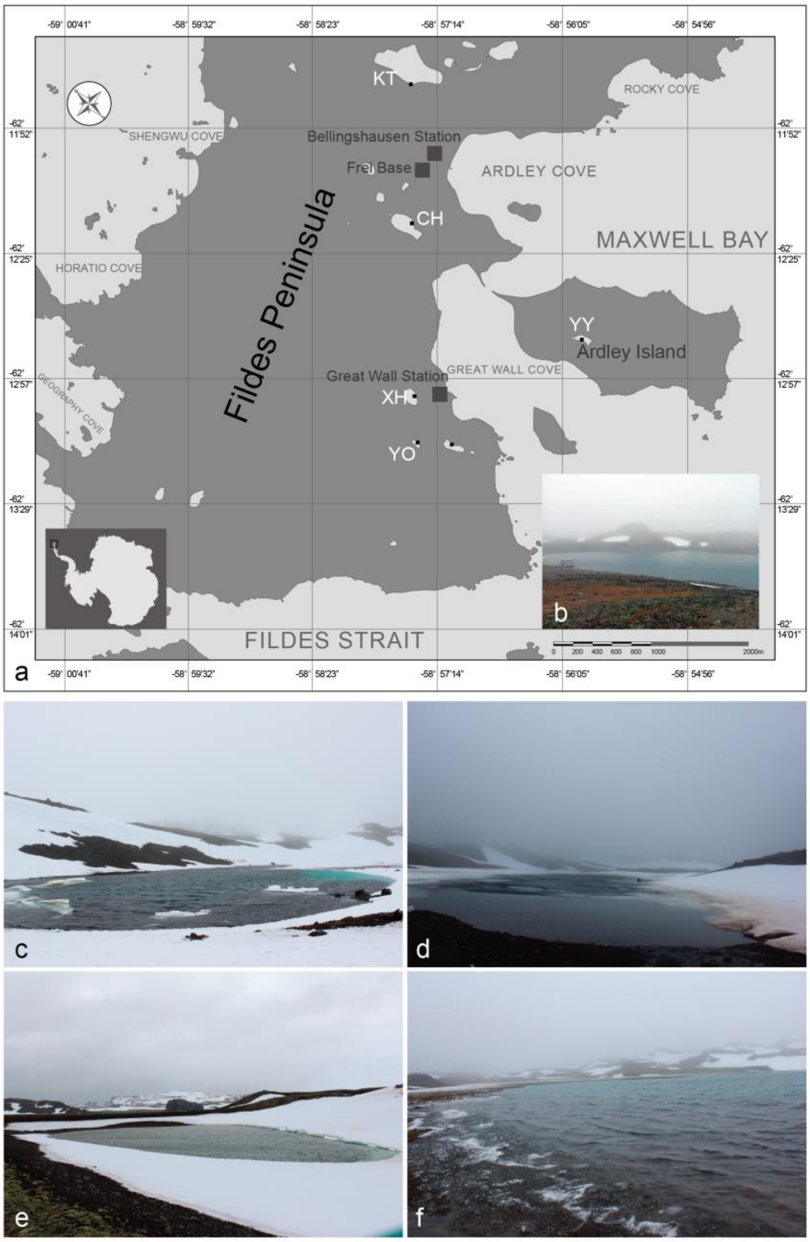

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Fig. 2

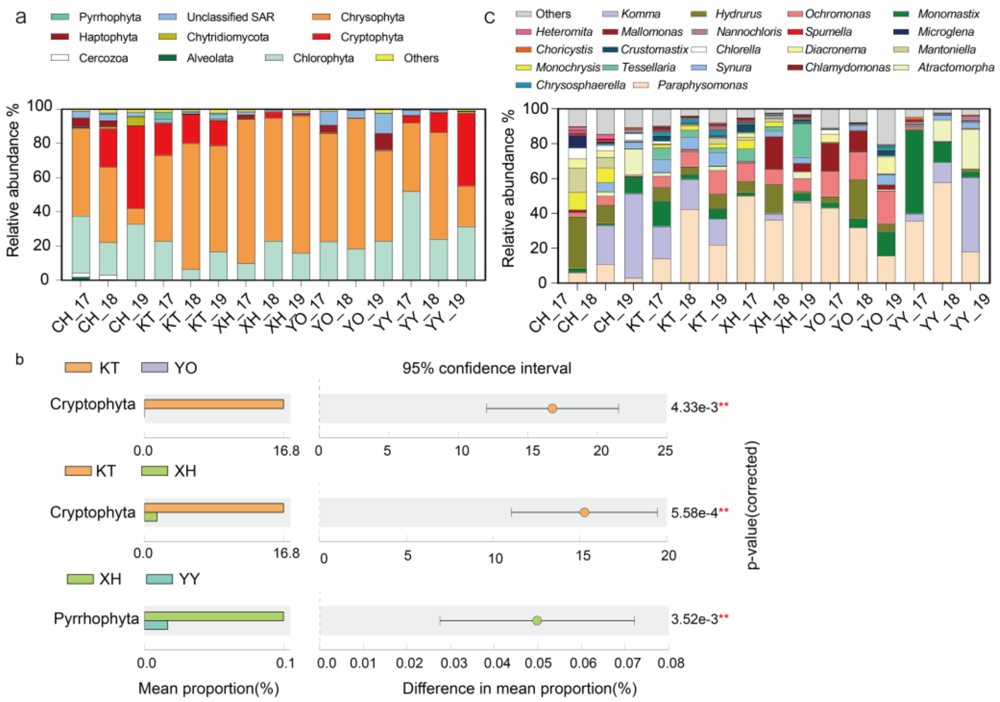

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Fig. 3

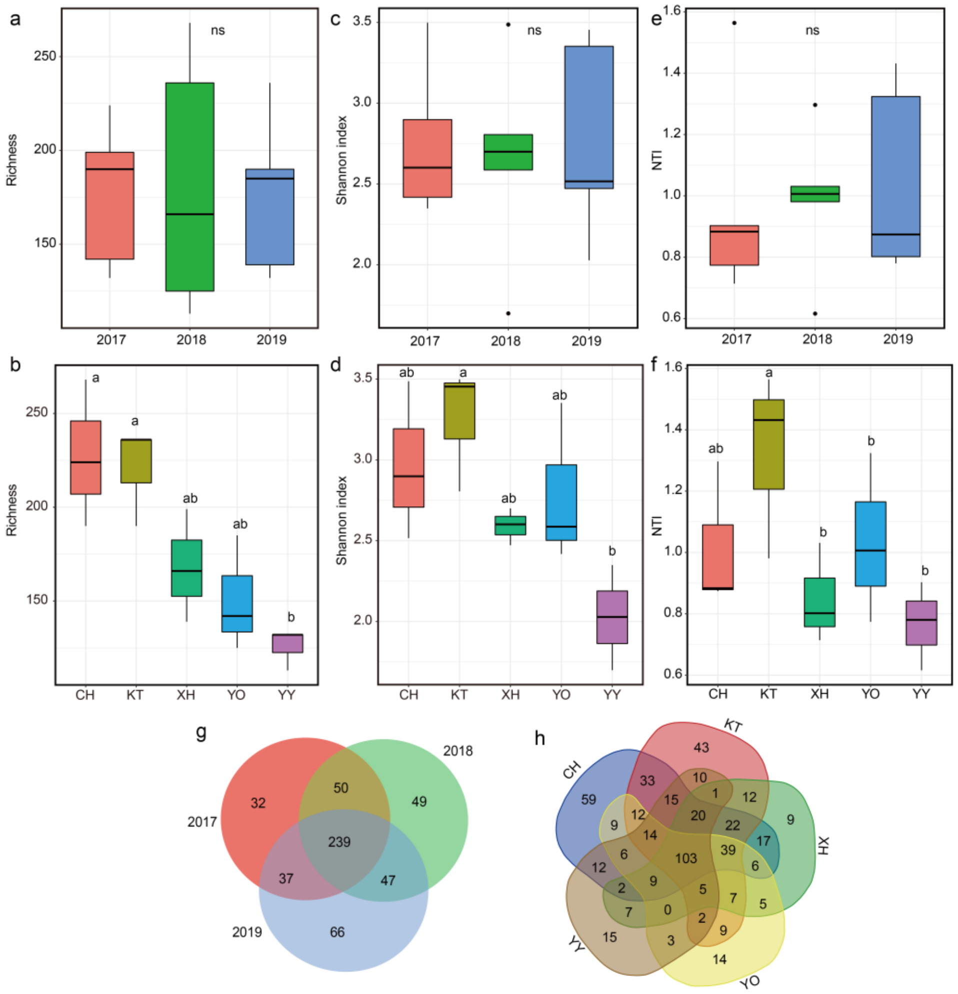

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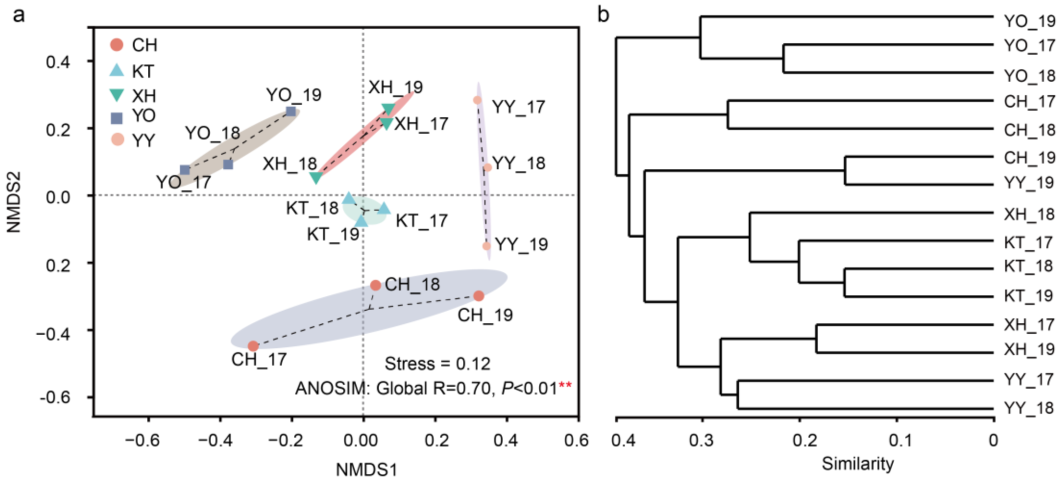
Fig. 4



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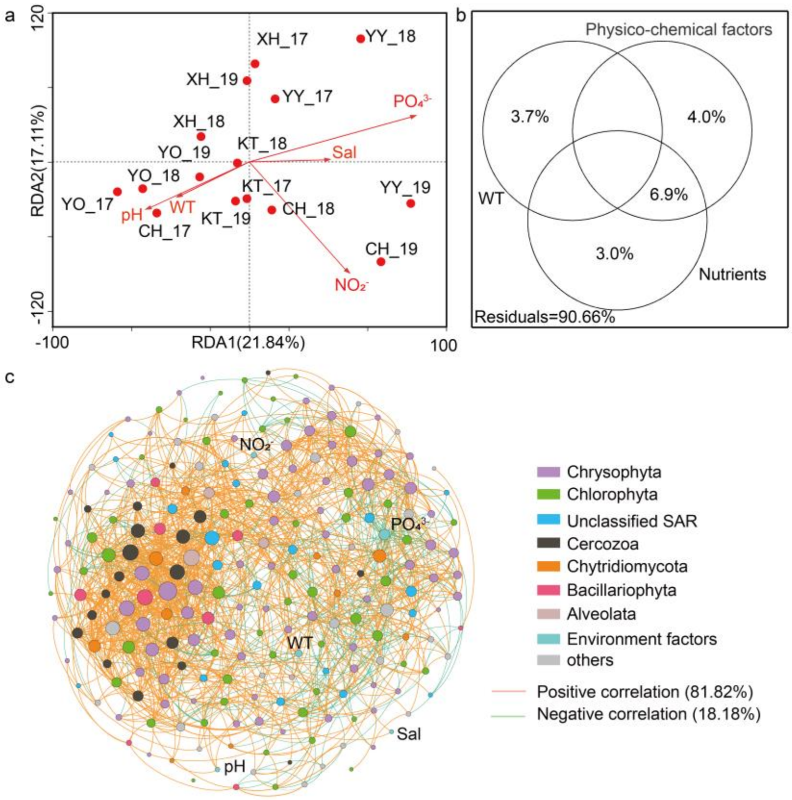

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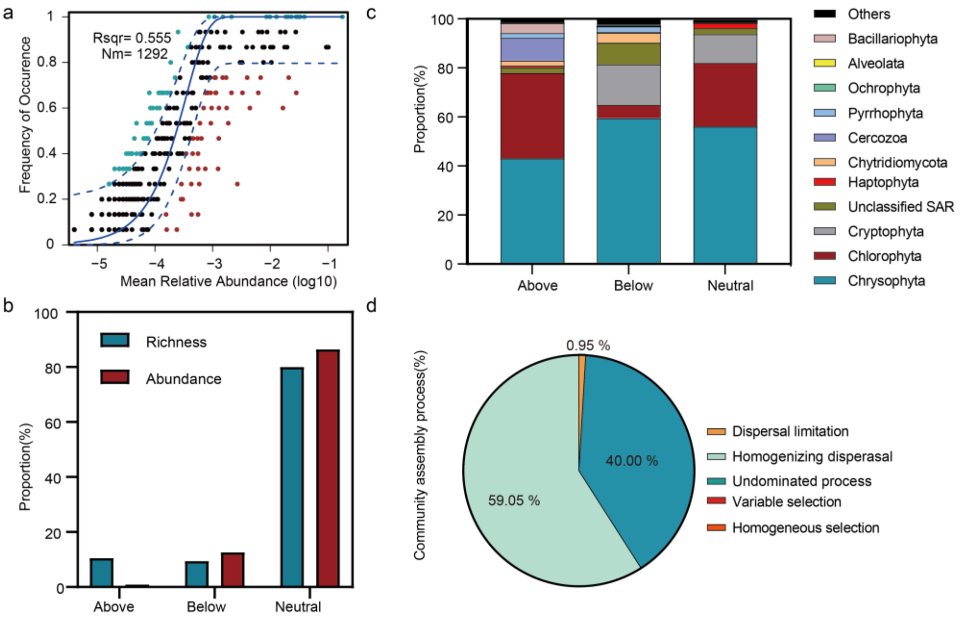

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