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 28 phyla were detected, with the phytoplankton occupying the highest percentage of sequences (accounting for up to 98%). The main dominant taxa were Chrysophyta, Chlorophyta, and Cryptophyta. 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. There were significant differences in microbial eukaryotic communities among lakes, with spatial and temporal heterogenicity in the relative abundance of 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 found that neutral processes explained more than 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). 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).

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), as well as contributing to plankton biomass and carbon export (Hernandez-Ruiz et al., 2018; Leblanc et al., 2018). 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 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 and dinoflagellates have been detected in the size fraction 0.2~5 μm of Antarctica plankton eukaryotes, the biodiversity was affected by 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 microphytoplankton communities of shallow lakes from the Antarctic Peninsula, 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; (II) 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 (XH), Yan Ou (YO), and Yue Ya (YY) (Fig. 1). Moreover, the physio-chemical parameters were measured synchronously.

CH is 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, near the airport for access to the Fildes Peninsula in Antarctica. The KT is the largest lake in this investigation area. XH is the drinking water source area for the Great Wall Station scientific expedition station. YO is surrounded by mountains and snow-covered, with moss and lichen growing in the soil. It is the smallest lake in this investigation area and is relatively sensitive to the effects of scientific expeditions. YY, situated on Ardley Island, is far from human activities but 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). 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

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. 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).

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 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 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 (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. To reduce the complexity of the data sets, OTUs 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).

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 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 ($|\beta NTI| < 2$ and $RC_{bray} < -0.95$), dispersal limitation ($|\beta NTI| < 2$ and $RC_{bray} > +0.95$) and undominated processes (i.e. weak selection, weak dispersal, diversification, and drift processes) with $|\beta NTI| < 2$ 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 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 NO_2-N, NH_4^+, and PO_4^{3-} concentrations with 0.00~0.15 μM, 0.05~0.74 μM, and 0.02~2.29 μM, respectively. YY had higher concentrations of PO_4^{3-}, NH_4^+, and NO_2^-, while XH had lower NO_2^- and PO_4^{3-}. SiO_3^{2-} 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

A total of 9 dominant phyla were identified, accounting for over 98% of the sequences. These dominant phyla were mainly 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 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% in KT~4.93% in YO; Alveolate represented 0.02% in XH~1.00% in CH, and unclassified Stramenopiles-Alveolates-Rhizaria (SAR) represented 1.12 % in XH~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 (Fig. 2a).

A total of 21 dominant genera were identified (Fig. 2c), accounting for over 87% of the sequences. The dominant genera were mainly *Hydrurus*, *Paraphysomonas*, *Ochromonas*, *Synura*, and *Monochrysis* belonging to Chrysophyta, *Komma* in Cryptophyta, *Monomastix*, *Chlamydomonas*, and *Mantoniella* 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 48.52%, which showed an increasing trend over the year 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 year. *The Hydrurus* in XH_18 and YO_18 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 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; Richness were highest in 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 value and was significantly lower than KT (Fig. 3b, d, $P<0.05$). The range of NTI was 0.62~1.56, 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, and CH also had the higher richness (Fig. 3b, d, f).

The total number of OTUs shared in 2017-2019 was 239, and the unique OTUs were 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) (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 lakes were significant (Global R = 0.70, $P<0.01$). 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, respectively. For other lakes, CH_19, and YY_19 clustered together. 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 21.84% and 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 WT and PO_4^{3-} significantly affected the microbial eukaryotic community ($P<0.05$). The variation partition analysis (VPA) indicated that environmental factors monitored explained 9.34% of microbial eukaryotic community variability among lakes and still had a large amount of unexplained community variation (90.66%, Fig. 5b).

A total of 220 nodes linked by 1521 edges 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 81.82%, whereas the portion of negative association was only 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 two of the top 20 OTUs with the highest degree centrality were directly associated with environmental factors (WT and PO_4^{3-}), and one belonged to Chrysophyta and one to Chytridiomycota.

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 55.5% community variation. In addition, the 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 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, dominated by Chrysophyta, Chlorophyta, and Cercozoa. Cryptophyta accounted for 16.58% of the abundance in the neutral group but was almost absent in the above group. In contrast, Cercozoa was present in 9.38% abundance in the above group, but only 0.32% in the neutral 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 community assembly process 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 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 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). Secondly, Chrysophyta still retained high cell density under low light conditions, as they could adapt to changing light conditions (Yubuki et al., 2008). Furthermore, Chrysophyta was mixotrophic and even could swim, which allowed 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 could form cysts (Nicholls 1995), protecting cells from resisting an unsuitable environment. All these aspects made 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 *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 (Allende and Mataloni 2013; Izaguirre et al., 2003; Toro et al., 2007). Several unicellular algae 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 dominated 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 1.70~3.50, richness 113~268). 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 modified biodiversity (Duffy et al., 2017; Lee et al., 2017; Pertierra et al., 2017). Our results supported the SAR model, observing more alpha diversity 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 WT and PO_4^{3-} were 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). WT has become a major driving factor for microbial eukaryotic community 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 temperature changes on community structure (Garcia-Rodriguez et al., 2021). The WT in the YO 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). 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 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). Thirdly, stochastic processes such as ecological drift (birth, death) might cause unexplained community variation (Zhang et al., 2018).

4.3 Co-occurrence patterns

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 (82% vs. 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. 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 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 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 to the neutral model (Fig. 6a), which suggested community variation could 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 (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 (86.4%) and richness (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 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 by the input of microorganisms from the surrounding ice melt, atmospheric transport, human activities, or bird migration (Unrein et al., 2005). 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 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 (0.95%). Undominant processes accounted for 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 five freshwater lakes on the Fildes Peninsula, Antarctica. 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). Stochastic processes played a very prominent role in microbial eukaryotic community assembly. 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.

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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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: *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 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-}: phosphate. (c) Network 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) 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

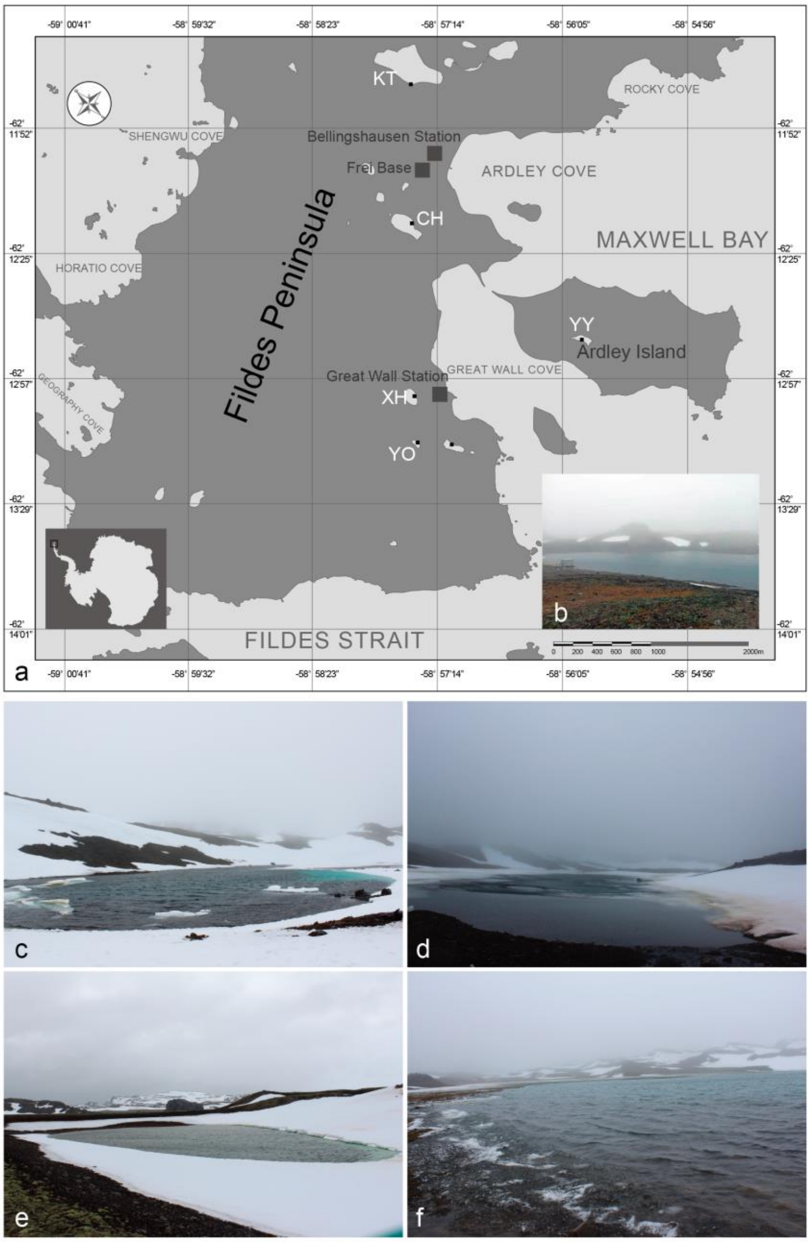

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Fig. 2

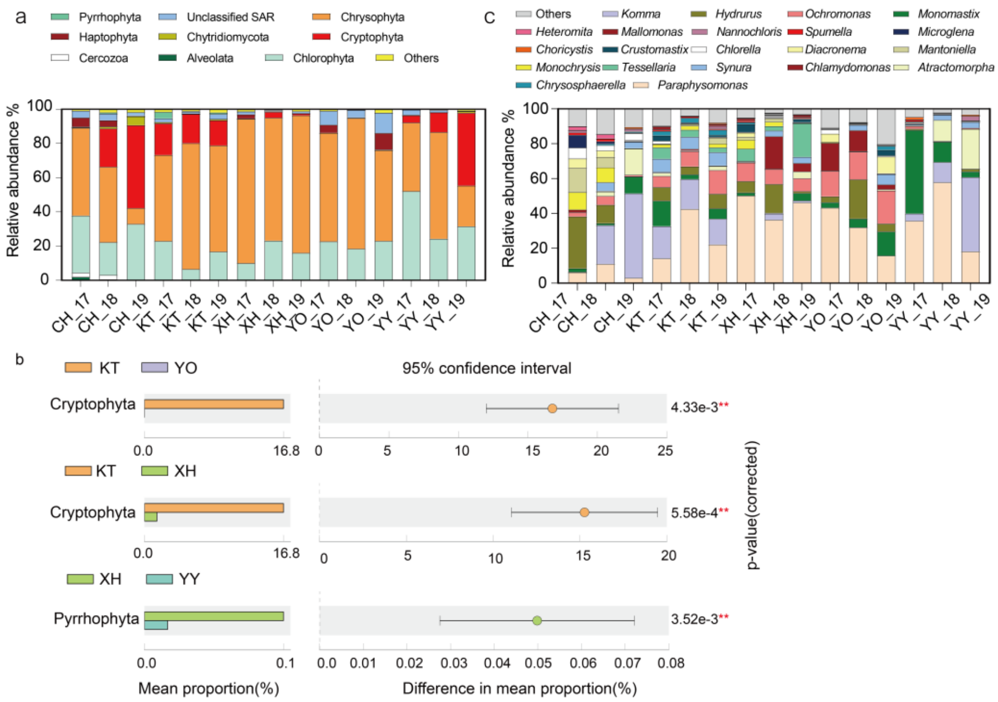

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Fig. 3

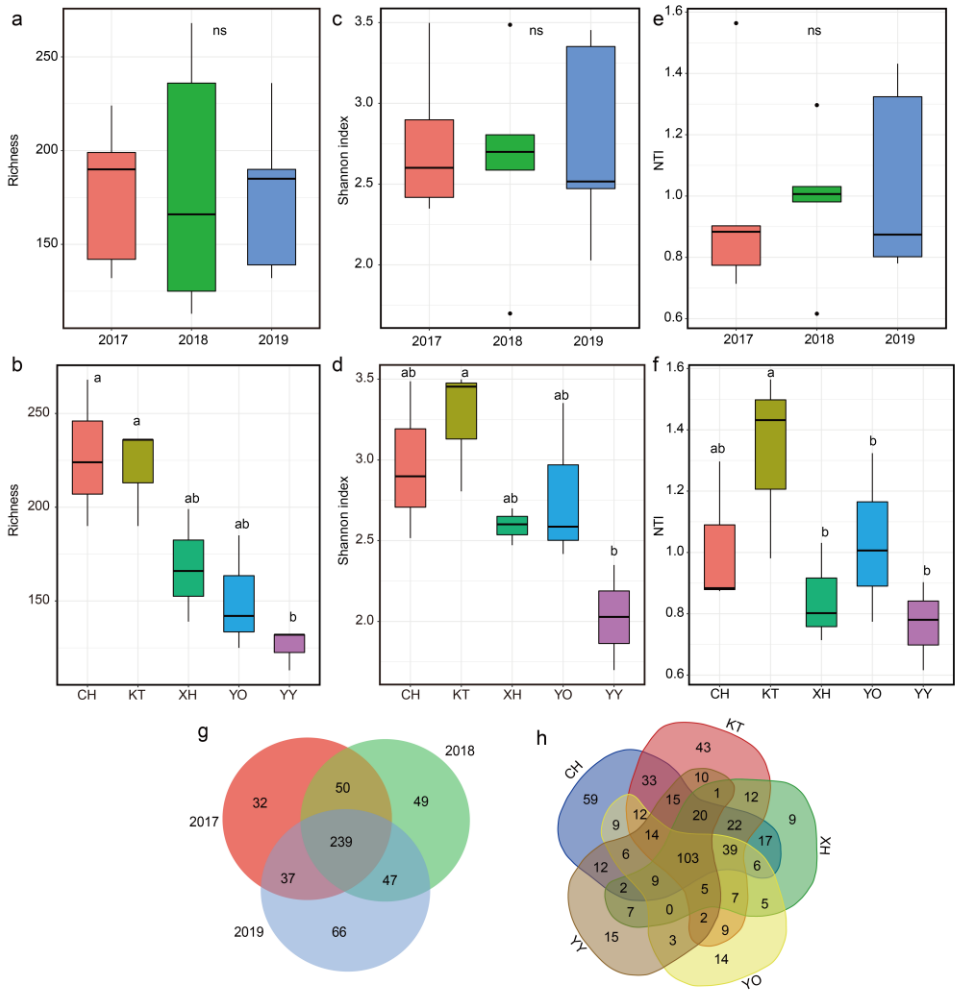

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Fig. 4

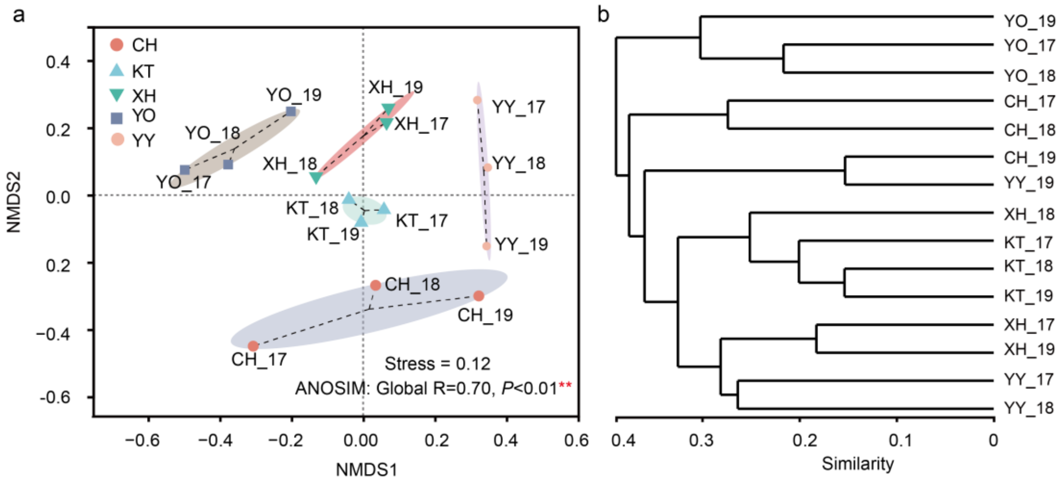

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Fig. 5

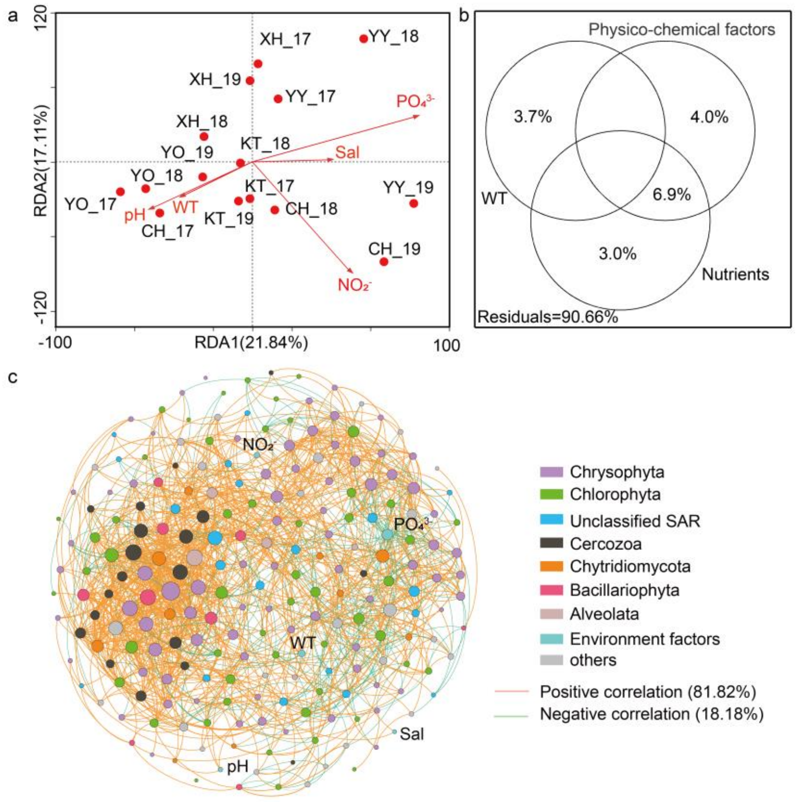

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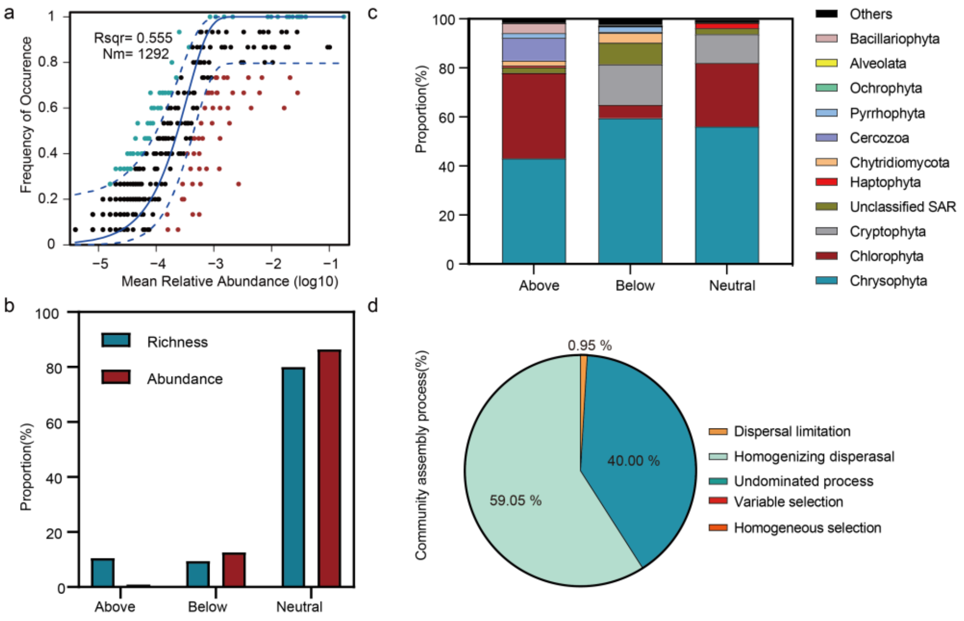
Fig. 6



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