

1 **Diversity and assembly processes of microbial eukaryotic**  
2 **communities in Fildes Peninsula Lakes (West Antarctica)**

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15

16 **Abstract**

17 The diversity, co-occurrence patterns, and assembly processes of microbial eukaryotes  
18 (0.2–20 µm) in Antarctic freshwater lakes are not well understood, despite their wide  
19 distribution and ecological importance. This study used Illumina high-throughput  
20 sequencing to investigate the microbial eukaryotic communities of five freshwater  
21 lakes on the Fildes Peninsula over three summer seasons. A total of 28 phyla were  
22 detected, with phytoplankton occupying the highest percentage of sequences  
23 (accounting for up to 98%). The dominant taxa consisted of Chrysophyta,  
24 Chlorophyta, and Cryptophyta. The species richness (113–268) and Shannon index  
25 (1.70–3.50) varied among the lakes, with higher values recorded in Lake Chang Hu  
26 and Lake Kitec and the lowest value obtained for Lake Yue Ya. There were significant  
27 differences between the microbial eukaryotic communities of the lakes, with spatial  
28 and temporal heterogeneity in the relative abundance of the dominant taxa ( $P < 0.05$ ).  
29 Environmental variables explained about 39% of the variation in community  
30 structures, with water temperature and phosphate identified as the driving factors  
31 ( $P < 0.05$ ). Network analysis revealed comprehensive co-occurrence relationships  
32 (positive correlation 82% vs. negative correlation 18%). The neutral community  
33 model revealed that neutral processes explained more than 55% of the community  
34 variation. Stochastic processes (e.g., homogenising dispersal and undominated  
35 processes) predominated in community assembly over the deterministic processes.  
36 These findings demonstrate the diversity of the microbial eukaryotic communities in  
37 the freshwater lakes of the Fildes Peninsula and have important implications for  
38 understanding the community assembly in these ecosystems.

39 **Keywords:** Microbial eukaryotic communities; Diversity; Co-occurrence network;  
40 Stochastic processes; Fildes Peninsula lakes.

## 41 **1 Introduction**

42       The Fildes Peninsula—which makes up the southwestern end of King George  
43 Island, South Shetland Islands, Antarctica—is home to a relatively high density of  
44 scientific research stations. The peninsula is commonly ice-free throughout the austral  
45 summer, making it the largest ice-free area (40 km<sup>2</sup>) on King George Island. Located  
46 within the maritime Antarctic, the peninsula experiences 400-600 mm of precipitation  
47 each year and has an average annual temperature of -3°C (Holdgate, 1977).  
48 Nevertheless, permafrost and periglacial processes also occur in the region (Barsch  
49 and Caine, 1984). Lakes on the Fildes Peninsula, along with those found in other ice-  
50 free areas of Antarctica, represent the year-round liquid water reservoirs on the  
51 continent (Priscu, 2010; Lyons et al., 2007). The availability and quality of water are  
52 impacted by sea conditions, macro-fauna usage, and anthropogenic influences, such  
53 as solid, volatile, and fluid waste production and disposal (Kawecka et al., 1998).  
54 Antarctic lake systems are sentinels for climate change and host globally-relevant  
55 microbes and biogeochemical cycles (Marsh et al., 2020; Wilkins et al., 2013), thus  
56 making a more complete understanding of the processes shaping microbial  
57 communities there a priority. Moreover, the physical stability observed in these lakes  
58 makes them a good model system for interrogating biogeochemical processes within  
59 water columns (Comeau et al., 2012).

60       Microbial eukaryotes (0.2–20 μm, pico-/nanoeukaryotes) are important  
61 components in microbial food webs and play a significant role in the biogeochemical  
62 cycles (Massana et al., 2015; Unrein et al., 2014; Grob et al., 2007), as well as  
63 contributing to plankton biomass and carbon export (Hernandez-Ruiz et al., 2018;  
64 Leblanc et al., 2018). However, microbial eukaryotes are generally difficult to study  
65 due to their small size and common lack of distinguishing morphological features,  
66 especially among pico- and nanoeukaryotes. A molecular approach can be used to  
67 compensate for the lack of traditional microscopic methods, providing us with a  
68 convenient way to study these small-sized eukaryotes. The application of 18S rRNA

69 gene-based molecular tools has revealed a high taxonomic diversity of microbial  
70 eukaryotes in some oligotrophic and extreme regions (Richards et al., 2005; Zhao et  
71 al., 2011; Marquardt et al., 2016). Nevertheless, previous research exploring the  
72 molecular diversity and the population fluctuations in these far cold and oligotrophic  
73 lakes of Antarctica is limited.

74 The small size, short generation time, quick reflection of environmental  
75 conditions and genetic plasticity of microbial eukaryotes make them excellent  
76 bioindicators reflecting environmental disturbances and the quality of ecosystems  
77 (Karimi et al., 2017; Bouchez et al., 2016). A previous study revealed the presence of  
78 Alveolates and Dinoflagellates among small deep-sea Antarctic plankton eukaryotes  
79 (0.2–5  $\mu\text{m}$ ) and demonstrated that their diversity was affected by temperature and  
80 salinity (Lopez-Garcia et al., 2001). Microscopic observations have also been used to  
81 show that diatoms in the lakes of Fildes Peninsula region accounted for 59.8% of the  
82 total number of phytoplankton species, and that water temperature and nutrients  
83 resulted in the variations in nano-and microphytoplankton abundance and  
84 composition (Zhu et al., 2010). A relatively low number of taxa, such as *Nitzschia*,  
85 *Achnanthes*, and *Navicula*, etc., have also been investigated from periodically  
86 brackish water ponds near the Polish Antarctic Station on King George Island, whose  
87 abundance were influenced by the nutrients in the water (Kawecka et al., 1998).  
88 Indeed, the microphytoplankton communities of the shallow lakes of the Antarctic  
89 Peninsula, are influenced by nutrients and surrounding inputs (Mataloni et al., 2000).  
90 Although several studies on other regions have explored the fluctuations of microbial  
91 eukaryotes and their relationship with environmental factors, the spatio-temporal  
92 variation, co-occurrence pattern, and community assembly of microbial eukaryotes in  
93 Antarctic (Fildes Peninsula) freshwater lakes have been rarely investigated.

94 Deterministic and stochastic processes are considered the two main ecological  
95 processes in community assembly (Ofiteru et al., 2010). Deterministic processes are  
96 based on the ecological niche theory; some deterministic factors (environmental

97 conditions and species interactions) influence and determine community assembly  
98 (Powell et al., 2015). Stochastic processes are based on the neutral theory, which  
99 states that random birth or death, drift, and dispersal events also play an essential role  
100 in community assembly (Bahram et al., 2016). Deterministic processes are prevalent  
101 in building whole ecosystem communities (Liu et al., 2020) where selection leads to  
102 species classification, and applying similar habitats thus results in similar community  
103 assemblages. However, other studies have supported an important role of stochastic  
104 processes (e.g., drift and dispersal) in community assembly, where dispersal is the  
105 movement of species over spatial locations and drift is associated with the relative  
106 abundance of the species (Massana and Logares, 2013; Wu et al., 2019). Stochastic  
107 processes have been shown to account for up to 95% of the microbial eukaryotic  
108 community assembly in a set of lakes in Eastern Antarctica (Logares et al., 2018).  
109 Stochastic processes have also been found to prevail in micro-and nanoplankton  
110 eukaryotic communities in the intertidal zones of southeast Fujian, China (Kong et al.,  
111 2019). Nonetheless, the study of microbial eukaryotic community assembly processes  
112 in Antarctica still requires further investigations for a more comprehensive view.

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

## 120 **2 Material and Method**

### 121 **2.1 Study area, sample collection and measurement of environmental** 122 **variables**

123 Field samples were collected on the 34th (2017/2018), 35th (2018/2019), and  
124 36th (2018/2019) Chinese Antarctic Research Expedition (CHINARE) in January

125 2018 (34th), December 2018 (35th), and December 2019 (36th). The investigations  
126 were conducted in the Chinese Great Wall Station area on King George Island, the  
127 largest of the South Shetland Islands. Surface water samples were collected from five  
128 lakes, namely Chang Hu (CH), Kitec (KT), Xi Hu (XH), Yan Ou (YO), and Yue Ya  
129 (YY) (Fig. 1). Moreover, the physio-chemical parameters of water samples were  
130 measured synchronously.

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

141 Water temperature (WT), pH, and salinity (Sal) were measured using an  
142 RBRconcerto C.T.D (Canada). In addition to the in-situ measurements, a water  
143 sample obtained 0.5 m below the surface was collected at each monitoring site using a  
144 5 L plexiglass sampler. For measurements of chlorophyll *a* (Chl *a*) contents, 1 L water  
145 samples were filtered using GF/F filters (0.70  $\mu\text{m}$ , Whatman), and Chl *a* was then  
146 extracted with 90% acetone over 24 h and measured spectrophotometrically. Nutrients,  
147 including ammonia ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), silicate ( $\text{SiO}_3^{2-}$ ), and phosphate ( $\text{PO}_4^{3-}$ )  
148 were measured spectrophotometrically with a San<sup>++</sup> continuous flow auto-analyser  
149 (Skalar, the Netherlands) after filtering following the procedure described by Hansen  
150 and Koroleff (1999).

## 151 2.2 Polymerase chain reaction (PCR) and Illumina MiSeq

152 1 L surface water was collected and prefiltered through a 20  $\mu\text{m}$  mesh sieve to

153 remove most of the mesozooplankton and large particles, then directly filtered  
154 through a 0.2 µm pore size nucleopore membrane filter (Whatman). The filters were  
155 frozen at -80°C in CTAB buffer prior to the laboratory experiments. The DNA  
156 extraction was performed as described by Luo et al. (2015).

157 PCR was performed using primers by barcode flanking the hypervariable V4  
158 region of the 18S rRNA gene: 3NDf (Charvet et al., 2012) with the reverse primer  
159 V4\_euk\_R2 (Brate et al., 2010). The PCRs were conducted in 20 µL reactions with  
160 0.2 µM of each primer, 10 ng of template DNA, 1 × PCR buffer, and 2.5 U of Pfu  
161 DNA Polymerase (Promega, USA). The amplification program consisted of an initial  
162 denaturation step at 95 °C for 2 mins, followed by 30 cycles of 95 °C for 30 s, 55°C  
163 for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min. The PCR  
164 product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel  
165 Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to  
166 manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega,  
167 USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2×  
168 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the  
169 standard protocols described by Wefind Biotechnology Co., Ltd. (Wuhan, China).

170 The raw 18S rRNA gene sequencing reads were demultiplexed, quality-filtered  
171 by fastp version 0.20.0 (Chen et al., 2018) and merged by FLASH version 1.2.7  
172 (Magoc and Salzberg, 2011) with the following criteria: (i) the 300 bp reads were  
173 truncated at any site receiving an average quality score of <20 over a 50 bp sliding  
174 window, and the truncated reads shorter than 50 bp were discarded, reads containing  
175 ambiguous characters were also discarded; (ii) only overlapping sequences longer  
176 than 10 bp were assembled according to their overlapped sequence. The maximum  
177 mismatch ratio of overlap region is 0.2. Reads that could not be assembled were  
178 discarded; (iii) Samples were distinguished according to the barcode and primers, and  
179 the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch  
180 in primer matching.

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

186 The analysis of the OTUs obtained using the UPARSE clustering method have  
187 been widely used for high-throughput sequencing analysis (Gad et al., 2022; Xu et al.,  
188 2022; Zhang et al., 2022; Reboul et al., 2021; Sun et al., 2022). Some of the diversity  
189 indices in our study were more comparable to previous research using 97% sequence  
190 similarity OTUs (Chen et al., 2022; Wang et al., 2021; Wang et al., 2020b), as  
191 comparisons of these statistics using the same bioinformatics tool seem to remain  
192 persuasive (Li et al., 2019; Glassman and Martiny, 2018).

193 A total of 726,700 valid sequences of the 18S rRNA gene in all samples were  
194 obtained, and the average length of the acquired reads was 443 base pairs. The OTUs,  
195 classified as metazoa, unassigned and unclassified sequences, were filtered based on  
196 taxonomic metadata. Finally, the sequences were normalised at the lowest sequences  
197 depth and rarefied at 16,717 reads, yielding a total of 520 OTUs, distributed among  
198 28 phyla. The Good's coverage values were above 99.9%, confirming that the  
199 libraries could represent most species in these lakes, with rarefaction curves reaching  
200 saturation (Fig. S1).

### 201 2.3 Community composition and diversity

202 The richness and Shannon index (H) were measured using the “vegan” R  
203 package based on the OTUs table, respectively. The nearest-taxon index (NTI) was  
204 used to measure the degree of phylogenetic clustering of the taxa on a within-  
205 community scale for communities. High or positive values indicated clustering taxa  
206 across the overall phylogeny, while lower negative values indicated an overdispersion  
207 of taxa across the phylogeny (Horner-Devine and Bohannan, 2006). The NTI  
208 quantifies the number of standard deviations that the observed mean nearest taxon



209 distance (MNTD) is from the mean of the null distribution with 999 randomisations  
210 determined in the “Picante” R package.

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

## 220 2.4 Influencing factors of the community structure

221 The Canoco 4.5 software (Ter Braak and Smilauer, 2002) was used to rank  
222 species and environmental factor data, and the ranking model was determined by the  
223 de-trending correspondence analysis (DCA) of OTUs data. All environmental factors,  
224 except pH, were  $\log(x+1)$  transformed prior to analysis to improve normality and  
225 homoscedasticity. To reduce multicollinearity among the environmental factors, all  
226 variance inflation factors (VIFs) were kept below 10. The environmental factors  
227 influencing the composition of the microbial eukaryotic community were selected by  
228 999 Monte Carlo permutation tests at the significant level ( $P < 0.05$ ). In addition, the  
229 relative importance of water temperature, physicochemical factors, and nutrients was  
230 assessed using the variation partitioning analysis (VPA).

## 231 2.5 Co-occurrence network analysis

232 We constructed one co-occurrence network based on the samples acquired over  
233 the whole study period. To reduce the complexity of the data sets, OTUs occurring in  
234 at least 5 samples were retained to construct the network. Only robust ( $|r| > 0.6$ ) and  
235 statistically significant ( $P < 0.05$ ) correlations were incorporated into the network

236 analyses. Finally, the network was visualised by Gephi software (Bastian et al., 2009).

## 237 2.6 Ecological community assembly analysis

238 The neutral community model (NCM) was used to measure the potential role of  
239 stochastic processes in the assembly of microbial eukaryotic communities based on  
240 the relationship between the OTUs frequency and their relative abundance (Chen et  
241 al., 2019), which was derived from neutral theory (Zhou et al., 2014). The parameter  
242  $N_m$  represents the metacommunity size, and  $R^2$  represents the degree of fit to a  
243 neutral community model.

244 The Stegen null model was used to further evaluate the contributions of  
245 deterministic and stochastic processes to community assembly (Stegen et al., 2012).  
246 The  $\beta$ -nearest taxon index ( $\beta$ NTI) was calculated using phylogenetic distance and  
247 OTU abundance (Stegen et al., 2013; Webb et al., 2002). The relative contributions of  
248 variable selection and homogeneous selection were estimated from the percentage of  
249 pairwise comparisons whose  $\beta$ NTI were  $> 2$  and  $< -2$ , respectively. We further  
250 calculated the Bray-Curtis-based Raup-Crick index ( $RC_{bray}$ ) to investigate the  
251 pairwise comparisons that deviated from the selection (Evans et al., 2017; Stegen et  
252 al., 2013). When integrated with the value of  $|RC_{bray}|$ , the underlying community  
253 assembly processes could represent a homogenizing dispersal ( $|\beta$ NTI|  $< 2$  and  $RC_{bray}$   
254  $< -0.95$ ), dispersal limitation ( $|\beta$ NTI|  $< 2$  and  $RC_{bray}$   $> +0.95$ ) and undominated  
255 processes (i.e., weak selection, weak dispersal, diversification, and drift processes)  
256 with  $|\beta$ NTI|  $< 2$  and  $|RC_{bray}| < 0.95$ . The null community of all the samples was  
257 randomised 999 times to obtain the average null expectations.

## 258 **3 Results**

### 259 3.1 Physicochemical properties

260 Water temperature ranged from 0.90°C to 7.14°C, and the water temperature in  
261 YO was significantly higher than in the other lakes (Table S1,  $P < 0.05$ ). Nutrients in  
262 the sampled lakes were in general quite low in concentration with values of 0.00-0.15

263  $\mu\text{M}$  ( $\text{NO}_2^-$ ), 0.05-0.74  $\mu\text{M}$  ( $\text{NH}_4^+$ ) and 0.02-2.29  $\mu\text{M}$  ( $\text{PO}_4^{3-}$ ). Relatively higher and  
264 lower levels of nutrients were identified in YY and XH, respectively. The lowest value  
265 of  $\text{SiO}_3^{2-}$  was recorded in YY (1.43  $\mu\text{M}$ ) and the highest in CH (51.5  $\mu\text{M}$ ). The  
266 highest and lowest Chl *a* were reached in YY and CH (2.11 and 0.25  $\mu\text{g L}^{-1}$ ,  
267 respectively). pH showed minimum and maximum values in YY (7.65) and CH (8.27).  
268 Salinity values ranged from 0.00-0.14 PSU and were significantly lower in YO than in  
269 the other lakes ( $P < 0.05$ ).

### 270 3.2 Diversity and composition of microbial eukaryotic communities

271 A total of 9 dominant phyla were identified, accounting for over 98% of the  
272 sequences. These dominant phyla mainly consisted of microbial eukaryotic  
273 phytoplankton, with various relative abundances between different lakes (Fig. 2a).  
274 Chrysophyta (34.91% in CH–78.88% in XH), Chlorophyta (15.17% in KT–35.88% in  
275 YY), and Cryptophyta (0.01% in YO–23.71% in CH) were most abundant in the  
276 studied lakes. Cryptophyta in KT was significantly more abundant than in XH and YO,  
277 and Pyrrophyta in XH was significantly more abundant than in YY ( $P < 0.05$ ) (Fig. 2b).  
278 Haptophyta represented 0.09% of the sequence in KT and up to 4.93% in YO;  
279 Alveolates represented 0.02% of the sequence in XH and up to 1.00% in CH, while  
280 unclassified Stramenopiles-Alveolates-Rhizaria (SAR) represented 1.12 % in XH and  
281 8.05 % in YO.

282 The relative abundances of the dominant taxa in the same lake exhibited some  
283 interannual variations. The relative abundances of Chrysophyta in CH<sub>19</sub>, YO<sub>19</sub>,  
284 and YY<sub>19</sub> samples were lower than in the other samples, while the relative  
285 abundances of Cryptophyta in CH<sub>19</sub> and YY<sub>19</sub> were lower than in the other  
286 samples of CH and YY, respectively (Fig. 2a).

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

291 Chlorophyta.

292 As shown in Fig. 2c, the abundance of the dominant genera differed  
293 interannually among the investigated lakes. The relative abundance of *Komma* varied  
294 from 0 to 48.52%, which showed an increasing trend over the years in CH and YY.  
295 The ranges of *Paraphysomonas* and *Ochromonas* were 3.06–56.88% and 0.06–  
296 18.86%, showing an increase followed by a decrease in XH over the years. The  
297 highest relative abundances of *Hydrurus* were in samples XH\_18 and YO\_18  
298 compared to other samples. *Ochromonas* in CH was significantly lower relative  
299 abundance than in YO and XH ( $P < 0.05$ , Table S2).

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

309 The total number of OTUs shared between 2017-2019 was 239, and 32, 49, and  
310 66 unique OTUs were identified for 2017, 2018, and 2019, respectively (Fig. 3g). The  
311 total number of OTUs shared by the five lakes was 103, and 59 (CH), 43 (KT), 9  
312 (XH), 14 (YO), and 15 (YY) unique OTUs were distinguished among the lakes (Fig.  
313 3h).

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

319 (R=0.013,  $P=0.393$ ).

320 The UPGMA clustering analysis (Fig.4b) showed the same lakes in a different  
321 year, such as CH\_17 and CH\_18, YY\_17 and YY\_18, YO\_17 and YO\_18 clustered  
322 together. YO lake was distant from the other lakes and clustered into a separate group.

### 323 3.3 Driving factors and co-occurrence patterns

324 Canonical correspondence analysis (CCA) demonstrated that the first two  
325 sequencing axes explained 21.84% and 17.11% of the community variation,  
326 respectively (Fig. 5a). The samples from the same lake were closer, with a more  
327 similar community structure. More importantly, the Monte Carlo analysis confirmed  
328 that the water temperature and  $\text{PO}_4^{3-}$  significantly affected the microbial eukaryotic  
329 communities ( $P<0.05$ ). The VPA indicated that the monitored environmental factors  
330 explained 9.34% of the microbial eukaryotic community variability among lakes, but  
331 there was still a large amount of unexplained variation (90.66%, Fig. 5b).

332 A total of 220 nodes linked by 1521 edges comprised the microbial eukaryotic  
333 community network. The majority of nodes in the network had many connections.  
334 Notably, the positive associations among species were predominant in the network  
335 (Fig. 5c), comprising 81.82% of the associations, whereas the portion of negative  
336 association was only 18.18%. In addition, the positive interactions were mainly found  
337 within the same taxonomic affiliations, such as Chrysophyta, or between a few  
338 different taxonomic affiliations, such as Chrysophyta and Chlorophyta. The negative  
339 correlations on the other hand mainly were reflected between Chrysophyta and  
340 Chlorophyta. Only around 8% of OTUs correlated directly with environmental factors  
341 ( $P<0.05$ ). Meanwhile, only two of the top 20 OTUs with the highest degree centrality  
342 were directly associated with environmental factors (water temperature and  $\text{PO}_4^{3-}$ );  
343 one belonged to Chrysophyta and the other to Chytridiomycota.

### 344 3.4 Community assembly processes

345 The Sloan neutral community model (NCM) showed the importance of neutral

346 processes for microbial eukaryotic communities (Fig. 6a), with the neutral processes  
347 explaining 55.5% of the community variation. In addition, the NCM classified  
348 microbial eukaryotic taxa into three groups (above prediction, below prediction, and  
349 neutral prediction). In addition, compared to the above and below prediction groups,  
350 we observed a higher contribution of neutral prediction group to community  
351 abundance (86.4%) and richness (80%), which was dominated by Chrysophyta,  
352 Chlorophyta, and Cryptophyta (Fig. 6b, c). The above prediction group accounted for  
353 10.58% of the microbial eukaryotic richness but corresponded to only 0.96% of the  
354 abundance, and was dominated by Chrysophyta, Chlorophyta, and Cercozoa.  
355 Cryptophyta accounted for 16.58% of the abundance in the neutral prediction group  
356 but was almost absent in the above prediction group. In contrast, Cercozoa was  
357 present with a 9.38% abundance in the above prediction group, but only comprised  
358 0.32% of the neutral prediction group.

359 The  $\beta$ NTI was mainly distributed in the region of stochastic processes with a  
360 mean value of -0.48 and supported the results of the neutral model. The analysis of  
361 community assembly processes showed that stochastic rather than deterministic  
362 processes controlled the community assembly. Among them, homogenizing dispersal  
363 dominated, with a proportion of 59.05%, followed by undominated processes and  
364 dispersal limitation (Fig. 6d).

## 365 **4 Discussion**

### 366 **4.1 Dominant taxa and diversity**

367 The environmental conditions (e.g., low light and low nutrient contents, etc.) in  
368 Antarctic freshwater lakes differ from those of temperate lakes. These special features  
369 and their relative isolation has resulted in the development of unique communities and  
370 survival strategies of the species adapted to such conditions (Pearce and Galand,  
371 2008). In our study, the survival of taxa (Chrysophyta, Chlorophyta, and Cryptophyta)  
372 might have depended on their survival strategies and adaptation to harsh conditions,  
373 which allowed them to become the predominant species.

374 Chrysophyta dominated in the five lakes examined in our study, including  
375 *Hydrurus*, *Paraphysomonas*, *Ochromonas*, and *Monochrysis*. First, their dominance  
376 might have been due to their adaptation to a low nutrient availability. Chrysophyta  
377 have been widely distributed in oligo- and mesotrophic lakes from both the maritime  
378 and continental regions of Antarctica (Allende, 2009; Allende and Izaguirre, 2003;  
379 Izaguirre et al., 2020; Richards et al., 2005). Secondly, Chrysophyta can still retained  
380 a high cell density under low light conditions, since they can adapt to changing light  
381 conditions (Yubuki et al., 2008). Furthermore, Chrysophyta is mixotrophic and can  
382 even swim, allowing them to obtain available nutrients from other microorganisms,  
383 and thus reducing the need for dissolved nutrients in the water (Pick and Lean, 1984;  
384 Katechakis and Stibor, 2006). In addition, when environmental conditions change  
385 dramatically, such as through freezing and nutrient changes, Chrysophyta can form  
386 cysts (Nicholls, 1995), protecting their cells from resisting an unsuitable environment.  
387 All these characteristics allow Chrysophyta to be the predominant species in the five  
388 Antarctic lakes.

389 Chlorophyta was the second most dominant taxon in our study (15.17%–  
390 35.88%), mainly consisting of the *Monomastix*, *Chlamydomonas*, and *Mantoniella*.  
391 Chlorophyta was typically represented by flagellated species such as *Chlamydomonas*  
392 spp., which dominate phytoplankton communities in different trophic statuses and  
393 respond to adverse environmental conditions by forming temporary, non-swimming  
394 cell populations encased in a gelatinous mother cell membrane (Izaguirre et al., 2003;  
395 Allende and Mataloni, 2013; Toro et al., 2007). Several unicellular algae can undergo  
396 mix acid fermentation, and some obligate photoautotrophic species can increase their  
397 light capture efficiency through photoadaptation processes involving chlorophyll  
398 accumulation (Morgan-Kiss et al., 2016; Atteia et al., 2013). These characteristics  
399 might partially explain how Chlorophyta survived and occupied a specific advantage  
400 in the lakes studied here.

401 Cryptophyta was the third most abundant taxon observed. Indeed, their

402 dominance has been interpreted as evidence of heterotrophic growth in winter and  
403 mixotrophic growth throughout the year (Unrein et al., 2014). Cryptophyta dominated  
404 under perennially ice-covered and coastal saline lakes in continental Antarctica  
405 (Izaguirre et al., 2020). The ingestion of bacteria by mixotrophic Cryptophyta has  
406 previously been observed in two perennially ice-covered lakes (Fryxell and Hoare) in  
407 the McMurdo Dry Valleys (Roberts and Laybourn-Parry, 1999).

408 Compared to other aquatic ecosystems (Wang et al., 2021; Wang et al., 2020b;  
409 Hernandez-Ruiz et al., 2018), the diversity of microbial eukaryotes in the Antarctic  
410 lakes studied here was significantly lower (Shannon index 1.70–3.50, richness 113–  
411 268). The isolation and harsh conditions, especially the lower temperatures and  
412 nutrients availability, prevailing in Antarctic lakes accounted for a low microbial  
413 eukaryotic diversity. In addition, the species-area relationships model (SAR) states  
414 that there should be an increased species number as habitat area increases within a  
415 specific area (Ma, 2018). An increase in the ice-free area may drastically modify the  
416 biodiversity of an area (Pertierra et al., 2017; Duffy et al., 2017; Lee et al., 2017). Our  
417 results support the SAR model since a greater alpha diversity was observed in the CH  
418 and KT where the habitat areas were much larger than the YY and YO.

#### 419 4.2 Influence of environmental factors on the community

420 Previous studies have demonstrated that abiotic factors affect microbial diversity  
421 and community composition (Quiroga et al., 2013; Sun et al., 2017). In this study, we  
422 found that water temperature and  $\text{PO}_4^{3-}$  were significant factors driving community  
423 changes among the abiotic factors analysed. Some microorganisms have evolved to  
424 grow under defined temperatures, allowing differences in the temperature adaptation  
425 of different species (Wilkins et al., 2013). Water temperature has become a major  
426 driving factor for microbial eukaryotic community variation since it can regulate  
427 cellular activity and metabolic rates (Margesin and Miteva, 2011). The retreat of  
428 glaciers due to global warming has the risk of reducing the abundance and diversity of  
429 microorganisms, and more attention should be paid to the impact of temperature



430 changes on community structure (Garcia-Rodriguez et al., 2021). The water  
431 temperature in the YO was significantly higher than in the other lakes, and the  
432 community of YO clustered into a separate group (Fig. 5b). Microorganisms are  
433 highly sensitive to nutrients, and indeed, nutrients have been shown to represent  
434 important factors of community differences in microbial eukaryote communities in  
435 different areas (Zouari et al., 2018). In addition,  $\text{PO}_4^{3-}$  has been demonstrated to be  
436 one of the environmental variables that “best” explained the picoeukaryotic  
437 distributions in the Western Pacific Ocean (Liu et al., 2021).

438 Nevertheless, a small amount of community variation could be explained by the  
439 environmental variables measured in our analysis. Our results indicate that these  
440 environmental factors played a minor role in shaping the microbial eukaryotic  
441 community structures. A substantial amount of variation remained unaccounted for  
442 (Fig. 5), which could be due to several reasons. First, it is impossible to measure all  
443 the environmental factors that could influence microbial communities and, thus, some  
444 significant driving factors may not have been included in our study (Wang et al.,  
445 2020a). Potentially vital abiotic factors in Antarctic lakes may include the solar cycle,  
446 light availability, ice cover (thickness and duration), physical mixing of snow melt,  
447 and other hydrological processes (Allende and Izaguirre, 2003; Lizotte, 2008).  
448 Secondly, the relationships between the microorganisms considered here could not be  
449 quantified, and these relationships could represent potentially essential factors shaping  
450 the community structures in the studied lakes. For example, predation pressure can  
451 manifest as a top-down control of microbial eukaryotes (Blomqvist, 1997). Finally,  
452 stochastic processes such as ecological drift (birth, death) might also cause  
453 unexplained community variation (Zhang et al., 2018).

#### 454 4.3 Co-occurrence patterns

455 Network analysis can help us understand complex biological interactions and  
456 ecological rules for community assembly within a specific ecological niche (Li and  
457 Hu, 2021; Lupatini et al., 2014). Microorganisms can form various ecological

458 relationships, ranging from mutualism to competition, ultimately shaping the  
459 microbial abundances (Faust and Raes, 2012). Positive associations in a network often  
460 indicate common preferred environmental conditions or niche-overlapping, whereas  
461 negative associations imply competition or niche division (Faust and Raes, 2012). Our  
462 network analysis revealed that the positive correlations were much more common  
463 (82%) than negative ones (18%). These results suggested that positive relationships  
464 (e.g., due to cross-feeding, niche overlap, mutualism, or commensalism) might play a  
465 more important role in the lake ecosystems of Antarctica than negative relationships  
466 (e.g., predator-prey, host-parasite, or competition) (Chen and Wen, 2021). Similar  
467 results have been obtained through the study of small planktonic eukaryotes (0.2–20  
468  $\mu\text{m}$ ) inhabiting the surface waters of a coastal upwelling system (Hernandez-Ruiz et  
469 al., 2018). Notwithstanding, further studies are needed to corroborate the biological  
470 interactions and other nonrandom processes (for example, cross-feeding versus niche  
471 overlap) between the species pairs detected by network analyses.

#### 472 4.4 Community assembly processes

473 In general, deterministic and stochastic processes exist simultaneously in a  
474 community assembly (He et al., 2021; Chase, 2010). Several factors such as habitat  
475 connectivity and size (Orrock and Watling, 2010), productivity (Chase, 2010),  
476 disturbance (Liang et al., 2020), predation (Chase et al., 2009), and resource  
477 availability (Kardol et al., 2013) can influence the relative importance of stochastic  
478 and deterministic processes in a community assembly. The importance of stochastic  
479 processes has been previously illustrated for other microbial eukaryotic communities  
480 in aquatic ecosystems (Chen et al., 2019; Wang et al., 2020a). For example,  
481 picoeukaryotic communities in the lower oxygen layer of the Bengal Bay were shown  
482 to be dominated by stochastic processes (Chen et al., 2022). Results from our study  
483 supported a more prominent role of stochastic processes over deterministic ones in  
484 shaping the assembly of microbial eukaryotic communities. Hence, environmental  
485 variables explained only a small amount of variation in the microbial eukaryotic

486 community studied here, and a small number of taxa were significantly correlated  
487 with environmental factors.

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

501 The importance of stochastic processes has been previously illustrated by the  
502 microbial eukaryotic communities of lakes in East Antarctica (Logares et al., 2018).  
503 Antarctic freshwater lakes can receive external microbial colonies from the input of  
504 microorganisms from the surrounding ice melt, atmospheric transport, human  
505 activities, or bird migration (Unrein et al., 2005). Zeng et al. (2019) showed that  
506 homogenizing dispersal occupied a high proportion of community assembly processes  
507 in freshwater lakes, and similar results were obtained in our study. Most  
508 microorganisms detected in the sea have also been found in lakes in East Antarctica,  
509 suggesting that some marine taxa in the lakes could be the product of homogenizing  
510 dispersal from the sea to the lakes (Logares et al., 2018). In addition, the five studied  
511 lakes were covered in ice for most of the year and there were long geographical  
512 distances between the lakes, resulting in the limited dispersal of microorganisms  
513 (0.95%). Undominant processes accounted for 40% of the community assembly in our

514 study, including ecological drift and other complex processes that have not been fully  
515 quantified, such as weak selection and diffusion (Mo et al., 2018). This suggest that  
516 microbial eukaryotic communities might be formed by some highly complex  
517 assembly mechanisms in Antarctic freshwater lakes.

## 518 **5 Conclusion**

519 In conclusion, a unique microbial eukaryotic community structure and low alpha  
520 diversity (richness and Shannon index) have been demonstrated in five freshwater  
521 lakes of the Fildes Peninsula, Antarctica. Water temperature and  $\text{PO}_4^{3-}$  were identified  
522 as important factors driving the variation of community structures ( $P < 0.05$ ).  
523 Stochastic processes and biotic co-occurrence patterns were shown to be important in  
524 shaping microbial eukaryotic communities in the area. Our study provides a better  
525 understanding of the dynamic patterns and ecological assembly processes of microbial  
526 eukaryotic communities in Antarctic oligotrophic lakes (Fildes Peninsula).

## 527 **Data Availability Statement**

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

## 530 **Author Contribution Statement**

531 Conceptualization: Chunmei Zhang and Yonghong Bi; methodology: Chunmei  
532 Zhang and Wei Luo; molecular technique: Huirong Li; physico-chemical properties:  
533 Bin Wang, Yangjie Li, and Zhongqiang Ji; sample collection: Yinxin Zeng and  
534 Haitao Ding; funding acquisition: Yonghong Bi and Wei Luo; supervision: Yonghong  
535 Bi and Wei Luo; writing - original draft: Chunmei Zhang; writing-review & editing:  
536 Yonghong Bi and Wei Luo.

## 537 **Competing interests**

538 The authors declare that they have no known competing financial interests or  
539 personal relationships that could have appeared to influence the work reported in this  
540 paper.

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## 927 **Figure Captions**

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

931 **Fig.2** Comparison of microbial eukaryotic community composition. (a)Temporal and

932 spatial dynamics of relative abundance on Phylum level in five lakes from 2017 to  
933 2019. The 17, 18, and 19 represent the expedition season 2017/2018, 2018/2019, and  
934 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different  
935 lakes. The Lakes that showed no significant differences are not shown ( $P>0.05$ ).  
936 (c) Temporal and spatial dynamics of relative abundance on the genus level in the  
937 studied five lakes. (note:  $**P < 0.01$ ,  $*P < 0.05$ ). (Chlorophyta: *Atractomorpha*,  
938 *Chlamydomonas*, *Mantoniella*, *Chlorella*, *Choricystis*, *Crustomastix*, *Microglena*,  
939 *Monomastix*, *Nannochloris*; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*,  
940 *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*;  
941 Cryptophyta: *Komma*; Haptophyta: *Diacronema*; Cercozoa: *Heteromita*. The relative  
942 abundance in any lake was less than 1% is defined as others).

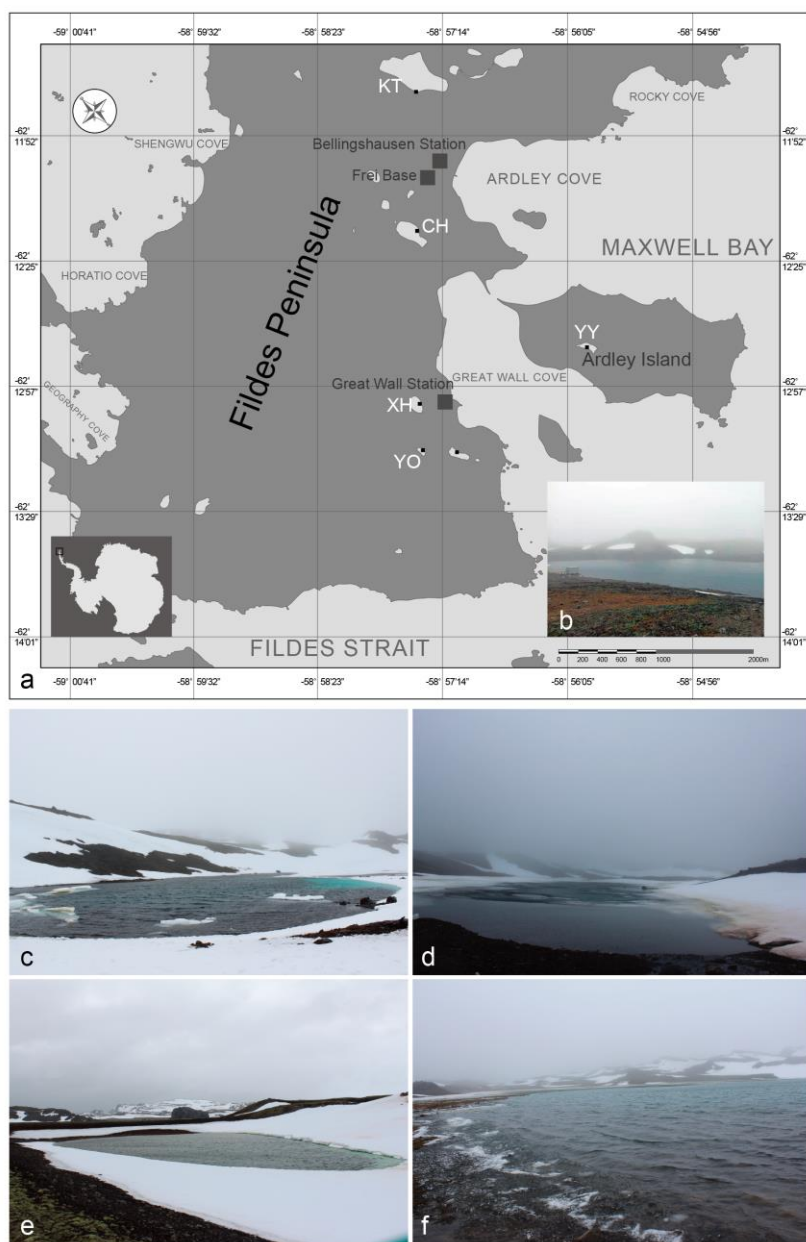
943 **Fig.3** Microbial diversity and Venn diagram for different years and lakes. (a, b)  
944 Variations in the number of microbial operational taxonomic units (OTUs); (c, d)  
945 variations in the microbial Shannon index; (e, f) variations in the within-community  
946 nearest-taxon index (NTI); (g,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

951 **Fig. 4** Temporal variability analysis of non-metric multidimensional scaling (NMDS)  
952 ordination of the microbial eukaryotic communities (a) and clustering of five lakes  
953 based on similarity (b).

954 **Fig.5** The effect of environmental variables on microbial eukaryotic communities, and  
955 co-occurrence pattern: (a) Canonical correlation analysis plots and (b) variance  
956 partitioning analysis. Sal: salinity; WT: water temperature;  $\text{NO}_2^-$ : nitrite nitrogen;  
957  $\text{PO}_4^{3-}$ : phosphate. (c) Network analysis revealing the interspecies associations

958 between microbial eukaryotic operational taxonomic units (OTUs), and the  
959 correlation between environmental factors and OTUs in the lakes' integrated networks.  
960 The size of each OTUs or environmental factor (node) is proportional to the degree  
961 centrality. Others: other phyla and unclassified taxa.

962 **Fig. 6** Relative influences of deterministic and stochastic processes on microbial  
963 eukaryotic community assembly based on the neutral community model (NCM) and  
964 the null model. (a) Fit of the NCM of community assembly. Nm indicates the  
965 metacommunity size and  $R^2$  indicates the fit to the NCM. Neutral prediction was  
966 within a 95% confidence interval (black), while non-neutral processes included the  
967 above and below prediction (dark green and red). (b) Proportions of the richness and  
968 abundance of the three groups (above prediction, below prediction, and neutral  
969 prediction) based on the NCM. (c) Abundance composition of the three groups in the  
970 microbial eukaryotic community. (d) Null model analysis revealing the fraction of  
971 ecological processes. The percent of community assembly is governed primarily by  
972 various deterministic processes, such as homogenous and heterogeneous selections,  
973 and stochastic processes, including dispersal limitations, homogenizing dispersal, and  
974 undominated processes (i.e., weak selection, weak dispersal, diversification, and drift  
975 processes).



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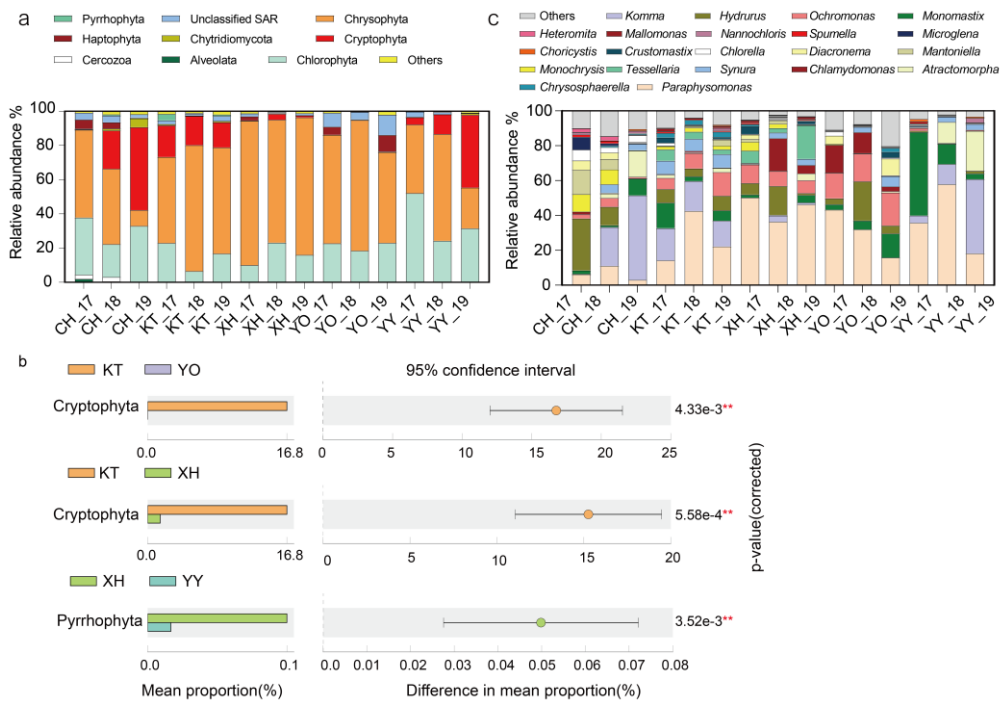
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982

983 **Fig. 2**

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986

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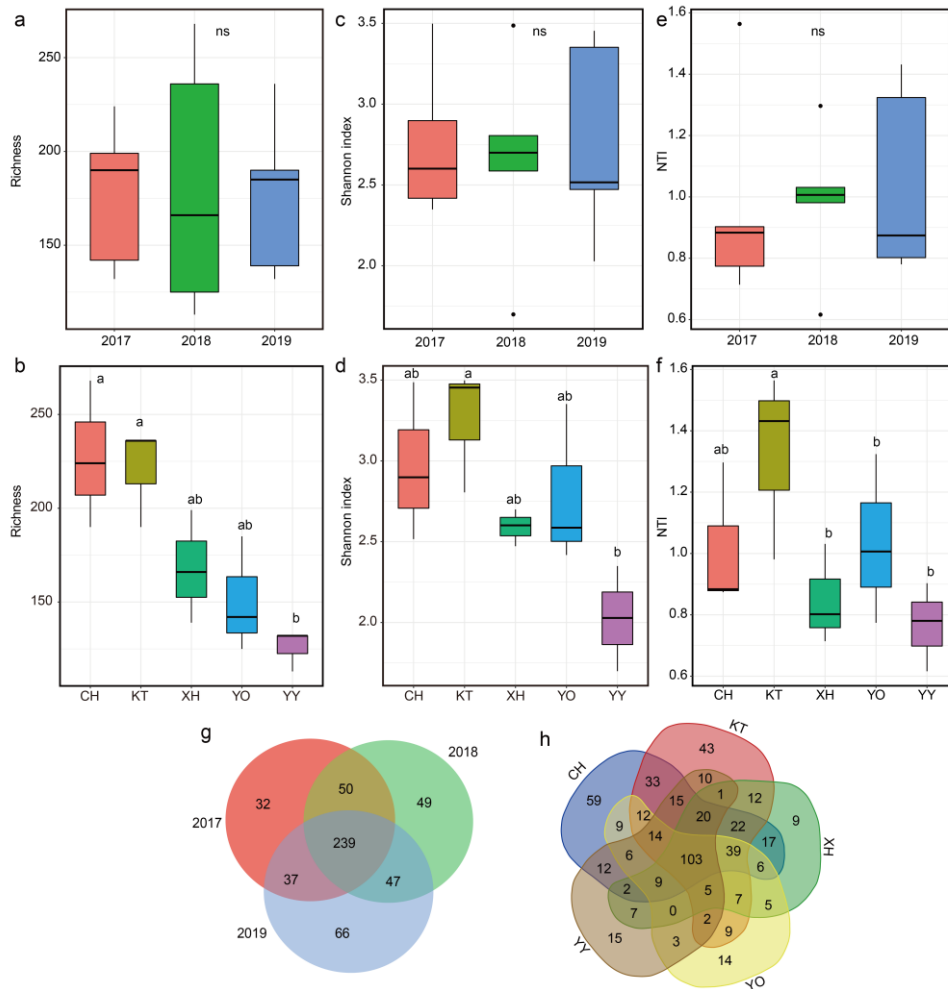
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999

1000 **Fig. 3**



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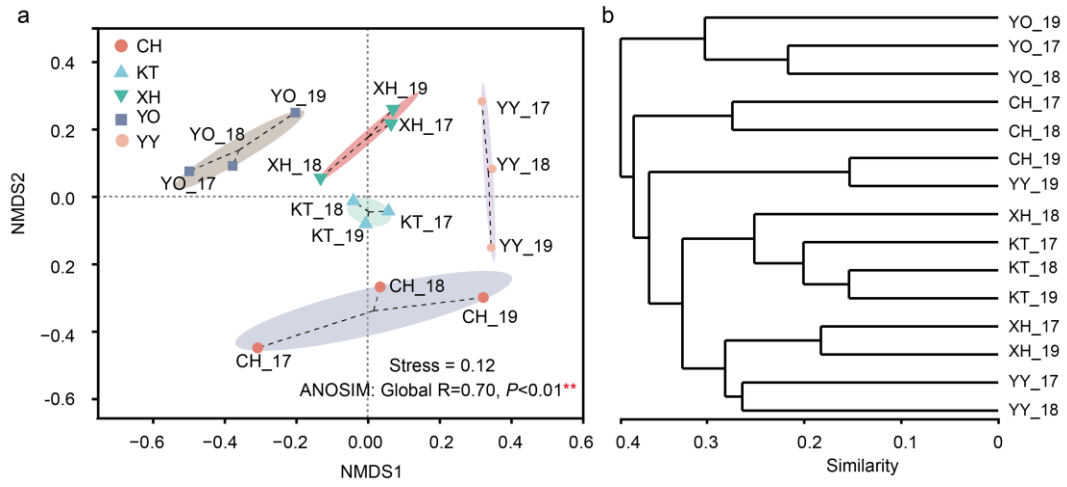
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1003 **Fig.3** Microbial diversity and Venn diagram for different years and lakes. (a, b)  
 1004 Variations in the number of microbial operational taxonomic units (OTUs); (c, d)  
 1005 variations in the microbial Shannon index; (e, f) variations in the within-community  
 1006 nearest-taxon index (NTI); (g, h) Venn diagram showing the unique and shared OTUs .  
 1007 Homogeneity and one-way ANOVA analyses of variance were used to test the  
 1008 significance of the indices. “ns” represents no significant differences ( $P>0.05$ ),  
 1009 significant differences ( $P<0.05$ ) are indicated by different letters between lakes, and  
 1010 lakes containing the same letters showed no significant difference ( $P>0.05$ )

1011

1012

1013 **Fig. 4**



1014

1015 **Fig. 4** Temporal variability analysis of non-metric multidimensional scaling (NMDS)

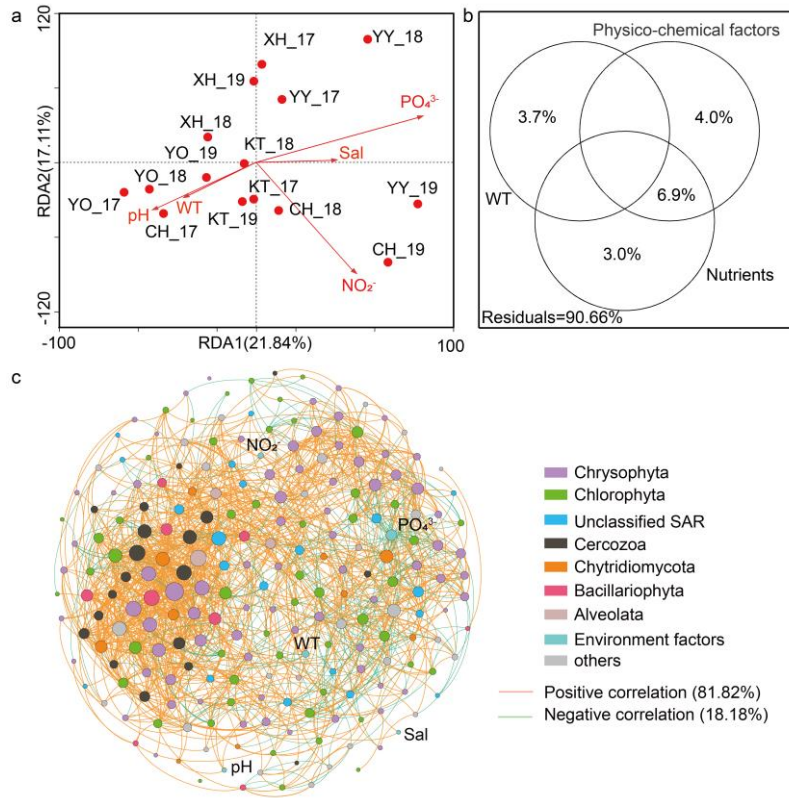
1016 ordination of the microbial eukaryotic communities (a) and clustering of five lakes

1017 based on similarity (b).

1018



1019 **Fig. 5**

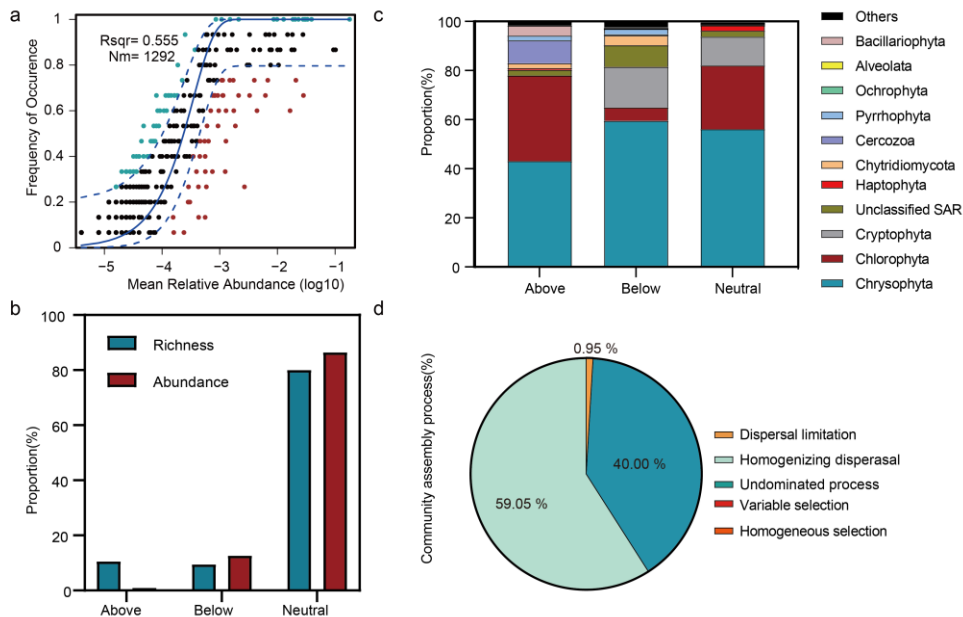


1020

1021 **Fig.5** The effect of environmental variables on microbial eukaryotic communities, and  
1022 co-occurrence pattern: (a) Canonical correlation analysis plots and (b) variance  
1023 partitioning analysis. Sal: salinity; WT: water temperature;  $\text{NO}_2^-$ : nitrite nitrogen;  
1024  $\text{PO}_4^{3-}$ : phosphate. (c) Network analysis revealing the interspecies associations  
1025 between microbial eukaryotic operational taxonomic units (OTUs), and the  
1026 correlation between environmental factors and OTUs in the lakes' integrated networks.  
1027 The size of each OTUs or environmental factor (node) is proportional to the degree  
1028 centrality. Others: other phyla and unclassified taxa.

1029

1030 **Fig. 6**



1031

1032 **Fig. 6** Relative influences of deterministic and stochastic processes on microbial  
1033 eukaryotic community assembly based on the neutral community model (NCM) and  
1034 the null model. (a) Fit of the NCM of community assembly. Nm indicates the  
1035 metacommunity size and  $R^2$  indicates the fit to the NCM. Neutral prediction was  
1036 within a 95% confidence interval (black), while non-neutral processes included the  
1037 above and below prediction (dark green and red). (b) Proportions of the richness and  
1038 abundance of the three groups (above prediction, below prediction, and neutral  
1039 prediction) based on the NCM. (c) Abundance composition of the three groups in the  
1040 microbial eukaryotic community. (d) Null model analysis revealing the fraction of  
1041 ecological processes. The percent of community assembly is governed primarily by  
1042 various deterministic processes, such as homogenous and heterogeneous selections,  
1043 and stochastic processes, including dispersal limitations, homogenizing dispersal, and  
1044 undominated processes (i.e., weak selection, weak dispersal, diversification, and drift  
1045 processes).