



1 **Diversity and assembly processes of microeukaryotic**
2 **community in Fildes Peninsula Lakes (West Antarctica)**

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15



16 **Abstract**

17 The diversity, co-occurrence patterns and assembly processes of microeukaryotes in
18 Antarctic freshwater lakes are not well understood, despite its wide distribution and
19 ecological importance. This study used Illumina high-throughput sequencing to
20 explore five freshwater lakes' microeukaryotic communities on the Fildes Peninsula
21 during three summer seasons. A total of 33 phyla were detected, with the
22 phytoplankton occupying the highest percentage of sequences (accounting for up to
23 98%). Meanwhile, the main dominant taxa were Chrysophyta, Chlorophyta, and
24 Cryptophyta. Alpha diversity varied among lakes, with Changhu (CH), Kitec (KT)
25 lake having higher values, and Yue Ya (YY) lake having the lowest value. There were
26 significant differences in microeukaryotic communities between lakes, with spatial
27 and temporal variation in the relative abundance of dominant taxa ($P < 0.05$).
28 Environmental variables only explained about 30% of the variation in community
29 structure. In the co-occurrence network, microeukaryotes tended to be more symbiotic
30 than competitive with each other (positive correlation 82% vs. negative correlation
31 18%), with only 8% of OTUs significantly associated with environmental factors. The
32 neutral community model found that neutral processes explained more than 56% of
33 the community variation. The stochastic processes (e.g., homogenizing dispersal and
34 undominated process) predominated in community assembly than the deterministic
35 processes. These findings revealed the diversity of the microeukaryotic community
36 and have important implications for understanding the community assembly in the
37 freshwater lakes of the Fildes Peninsula (Antarctica).

38 **Keywords:** Microeukaryotic community; Diversity; Co-occurrence network;
39 Stochastic processes; Fildes Peninsula lakes.

40



41 **1 Introduction**

42 The Fildes Peninsula locates in the southwestern part of King George Island of
43 the West Antarctic region, which has a high concentration of Antarctic scientific
44 research stations worldwide and is commonly free of ice during summer. It is the
45 largest ice-free area (40 km²) on King George Island, South Shetlands. This area falls
46 within the “maritime Antarctic” with precipitation of 400-600 mm year⁻¹ and an
47 average temperature of -3 °C (Holdgate 1977). Nevertheless, permafrost and
48 periglacial processes occur (Barsch and Caine 1984). The waters are affected by
49 natural factors such as the sea, animals, and anthropogenic pressures in solid, volatile,
50 and fluid wastes (Kawecka et al., 1998). Lakes in Fildes Peninsula, along with those
51 found in other ice-free areas in Antarctica, represent the year-round liquid water
52 reservoirs on the continent (Lyons W B et al., 2007; Priscu 2010). Antarctica lake
53 systems are sentinels for climate change and contain chemical elements and
54 microorganisms of global relevance (Marsh et al., 2020; Wilkins et al., 2013).
55 Because of their physical stability, such lakes have been identified as model systems
56 for inferring biogeochemical processes within water columns (Comeau et al., 2012).
57 Most Antarctic lakes are ultra-oligotrophic to oligotrophic, which only allow a few
58 species to adapt to such extreme environments resulting in truncated simplified food
59 webs (Izaguirre et al., 2020).

60 As an essential component of microorganisms in Antarctic freshwater lake
61 systems, microeukaryotes has shown critical roles in the ecosystem services, acting as
62 the main food source and the primary contributors to material circulation (Mo et al.,
63 2018b; Moreno-Pino et al., 2016; Zeng et al., 2014). The small size, short generation
64 time, rapid growth, sensitivity to environmental conditions, and genetic plasticity
65 render them capable of quick reflection to environmental changes (Karimi et al.,
66 2017). Hence, they are excellent bioindicators of the impact of environmental
67 perturbations and ecosystems quality (Bouchez et al., 2016). The Ciliophora,
68 Cryptomycota, Chlorophyta, and Bacillariophyta have been detected in the
69 Freshwater Glacier Lake, East Antarctica, the biodiversity seem to be affected by the



70 temperature and salinity (Lopez-Garcia et al., 2001). A pretty low number of taxa, the
71 abundance of diatom genera such as *Nitzschia*, *Achnanthes*, and *Navicula*, etc., have
72 been investigated from the periodically brackish water ponds near the Polish Antarctic
73 Station on King George Island (Kawecka et al., 1998). The microeukaryotic
74 communities of shallow lakes from the Antarctic Peninsula are influenced by nutrient
75 and surrounding inputs (Mataloni et al., 2000). However, the spatio-temporal
76 variation, co-occurrence pattern, and community assembly of microeukaryotes in
77 Antarctic (Fildes Peninsula) freshwater lakes have been rarely reported.

78 Deterministic and stochastic processes have been considered the two main
79 ecological processes in community assembly (Ofiteru et al., 2010). Deterministic
80 processes are based on ecological niche theory; some deterministic factors
81 (environmental conditions and species interactions) influence and determine
82 community assembly (Powell et al., 2015). Stochastic processes are based on the
83 neutral theory, which believes that random birth or death, drift, and dispersal events
84 also play an essential role in community composition (Bahram et al., 2016).
85 Deterministic processes (selection) are prevalent in building whole ecosystem
86 communities (Liu et al., 2020a), selection leads to species classification, and applying
87 similar habitats results in similar community assemblages. Although other studies
88 support a role for stochastic processes (drift and dispersal) in community assembly,
89 dispersal is the movement of species in spatial location, and drift is associated with
90 the relative abundance of species (Massana and Logares 2013; Wu et al., 2019).
91 Stochastic processes account for up to 95% of the microeukaryotic community
92 assembly mechanism in a set of lakes in Eastern Antarctica (Logares et al., 2018).
93 Nonetheless, the study of microeukaryotic diversity and its community assembly
94 processes in Antarctica still require further investigations for a more comprehensive
95 view.

96 Few studies have been conducted on microeukaryotic diversity and community
97 assembly processes of the freshwater lakes in the Fildes Peninsula, Antarctica. Our
98 study attempted to analyze the microeukaryotic samples of five freshwater lakes from



99 three summers using high-throughput 18S rRNA sequencing. We aim to (I)
100 understand the diversity and co-occurrence of microeukaryotes; and (II) to explore the
101 influencing factors and their community assembly processes.

102 **2 Material and Method**

103 **2.1 Sampling collecting**

104 Field samples were collected on the 34th (2017/2018), 35th (2018/2019), and
105 36th (2018/2019) Chinese Antarctic Research Expedition (CHINARE) in January
106 2018 (34th), December 2018 (35th), and December 2019 (36th), respectively. The
107 investigations were conducted in the Chinese Great Wall Station area at King George
108 Island, the largest island in southern Shetland Island. Surface water samples were
109 collected from five lakes, Changhu (CH), Kitec (KT), Xihu (XH), Yanou (YO), and
110 YueYa (YY) (Fig. 1). Moreover, the physio-chemical parameters were measured
111 synchronously.

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

123 Water temperature (WT), pH, and salinity (Sal) were measured using a YSI
124 Model 30 (Yellow Springs Instruments, Yellow Springs, USA). Chlorophyll a (Chl a)
125 was extracted with acetone and measured spectrophotometrically. Nutrient, including
126 ammonia (NH_4^+), Nitrite (NO_2^-), silicate (SiO_3^{2-}) and phosphate (PO_4^{3-}) were



127 measured spectrophotometrically with a continuous flow autoanalyzer Scan++ (Skalar,
128 the Netherlands) after filtering water through 0.45 μm cellulose acetate membrane
129 filters (Whatman) as described by (HP Hansen and F Koroleff 1999).

130 2.2 PCR and Illumina MiSeq

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

136 PCR was performed using primers with barcode flanking the hypervariable V4
137 region of the 18S rRNA gene: 3Ndf (Charvet et al., 2012) with the reverse primer
138 V4_euk_R2 (Brate et al., 2010). Polymerase chain reactions (PCRs) were conducted
139 in 20 μL reactions with 0.2 μM each primer, 10 ng of template DNA, 1 \times PCR buffer,
140 and 2.5 U of Pfu DNA Polymerase (Promega, USA). The amplification program
141 consisted of an initial denaturation step at 95°C for 2 mins, followed by 30 cycles of
142 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
143 min. PCR products were pooled and purified using the DNA gel extraction kit
144 (Axygen, Hangzhou, China). The DNA concentration of each PCR product was
145 determined using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen,
146 Germany) and was quality controlled on a TBS-380 Mini-Fluorometer (Turner
147 Biosystems, Sunnyvale, CA, USA). Finally, amplicons of all samples were pooled in
148 equimolar concentrations.

149 We used QIIME default parameters for quality filtering (reads truncated at first
150 low-quality base and excluded if: (1) overlap $\leq 10\text{bp}$ while the coupled reads were
151 assembled into one single sequence, (2) less than 80% of reading length was
152 consecutive high-quality base calls, (3) more than 1 errors were present in the bar
153 code, (4) the length was less than 50 bases (Caporaso et al., 2010). We picked
154 operational taxonomic units (OTUs) with a 97% similarity cut-off using available



155 reference UPARSE version7.1 (<http://drive5.com/uparse/>). Reads that did not match
156 any sequences in the reference database at $\geq 97\%$ identity were clustered de novo.
157 The taxonomic identity of eukaryotic representative sequences was performed using
158 RDP classifier against the SILVA database (version 132 NR) (Quast C et al., 2013) at
159 a bootstrap cutoff of 80%.

160 2.3 Community composition and diversity

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

169 Non-metric multidimensional scaling (NMDS) of microeukaryotic communities
170 was performed with the relative abundance of OTUs (Roberts 2013). Analysis of
171 similarity (ANOSIM) investigated differences in the microeukaryotic communities
172 between groups. The unweighted pair-group method with arithmetic means (UPGMA)
173 was used to determine the similarity between samples by clustering analysis
174 according to community composition similarity. These analyses were performed in the
175 R package “Vegan” and “Phangorn”. All calculations were based on similarity
176 matrices calculated with the Bray-Curtis similarity index.

177 2.4 Influencing factors of the community structure

178 Canoco 4.5 software (Braak and Smilauer 2002) was used to rank species and
179 environmental factor data, and the ranking model was determined by de-trending
180 correspondence analysis (DCA) of OTUs data. All environmental factors, except pH,
181 were $\log(x+1)$ transformed before analysis to improve normality and



182 homoscedasticity. To reduce multicollinearity among environmental factors, all
183 variance inflation factors (VIFs) were kept below 10. The environmental factors
184 influencing the composition of the microeukaryotic community were selected by 999
185 Monte Carlo permutation tests at the significant level ($P < 0.05$). In addition, the
186 relative importance of water temperature, physicochemical factors, and nutrients was
187 assessed using the variation partitioning analysis (VPA).

188 2.5 Co-occurrence Network Analysis

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

196 2.6 Ecological community assembly analysis

197 The Neutral community model (NCM) can measure the potential role of
198 stochastic processes in the assembly of microeukaryotic communities based on the
199 relationship between OTUs frequency and relative abundance (Chen et al., 2019). The
200 model is derived from neutral theory (Zhou et al., 2014). The parameter N_m
201 represents the metacommunity size, and R^2 represents the degree of fit to a neutral
202 model.

203 To further evaluate the contributions of deterministic and stochastic processes to
204 community assembly, the Stegen null model was used (Stegen et al., 2012). The β -
205 nearest taxon index (β NTI) was calculated using phylogenetic distance and OTUs
206 abundance (Stegen et al., 2013; Webb et al., 2002). The relative contribution of
207 variable selection and homogeneous selection was estimated from the percentage of
208 pairwise comparisons whose β NTI were > 2 and < -2 , respectively. We further



209 calculated the Bray-Curtis-based Raup-Crick index (RC_{bray}) to investigate pairwise
210 comparisons that deviated from selection (Evans et al., 2017; Stegen et al., 2013).
211 Integrated with the value of $|RC_{\text{bray}}|$, the underlying community assembly processes
212 could be homogenizing dispersal ($|\beta\text{NTI}| < 2$ and $RC_{\text{bray}} < -0.95$), dispersal limitation
213 ($|\beta\text{NTI}| < 2$ and $RC_{\text{bray}} > +0.95$) and undominated processes (i.e. weak selection,
214 weak dispersal, diversification, and drift processes) with $|\beta\text{NTI}| < 2$ and $|RC_{\text{bray}}| <$
215 0.95. The null community of all the samples was randomized 999 times to obtain
216 average null expectations.

217 **3 Result**

218 **3.1 Physico-chemical properties**

219 The water temperatures (WT) of all five lakes had similar values as 0.90°C to
220 7.14°C (Table S1), while the YO lake was significantly higher than other lakes
221 ($P < 0.05$). Nutrient values were low with nitrite ($\text{NO}_2\text{-N}$), ammonium nitrogen (NH_4^+),
222 and phosphate ($\text{PO}_4\text{-P}$) concentrations with 0.00~0.15 $\mu\text{M L}^{-1}$, 0.05~0.74 $\mu\text{M L}^{-1}$, and
223 0.02~2.29 $\mu\text{M L}^{-1}$, respectively. YY lake had higher concentrations of phosphate,
224 ammonium nitrogen, and nitrite, while XH had lower nitrite and phosphate. Silicate
225 (SiO_3^{2-}) varied from 1.43 to 51.5 $\mu\text{M L}^{-1}$, with the highest value in CH and lowest
226 value in YY. The range of Chl a was 0.25~2.11 $\mu\text{g L}^{-1}$, with the YY highest and the
227 CH lowest. pH ranged from 7.65 to 8.27. Salinity was 0.00-0.14, which in YO lake
228 exhibited significantly lower ($P < 0.05$).

229 **3.2 Diversity and composition of microeukaryotic community**

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

235 A total of 10 dominant phyla were identified, accounting for 96.02% sequences



236 in CH, 97.01% in KT, 98.30% in XH, 94.19% YO, and 98.27% YY. These dominant
237 phyla were mainly composed of microeukaryotic phytoplankton, with various relative
238 abundances between different lakes (Fig. 2a). The Chrysophyta (35.04% in
239 CH~76.69 % in XH), Chlorophyta (13.94% in KT~35.37% in YY), and Cryptophyta
240 (0.01% in YO~23.73% in CH) were most abundant in lakes. The Cryptophyta in KT
241 was significantly more abundant than in XH and YO, and Alveolate in KT was
242 significantly more abundant than in YO ($P < 0.05$) (Fig. 2b). Meanwhile, it was noticed
243 that the relative abundances of some phyla varied between lakes but not significantly,
244 with Chytridiomycota, Cercozoa, and Cryptophyta in XH being higher than those in
245 YO. The Arthropoda represented 0.10% in CH~4.11% in YO; Alveolate represented
246 0.03% in XH~1.01% in CH, and unclassified SAR represented 1.07 % in XH~5.27 %
247 in YO.

248 The relative abundance of the dominant taxa in the same lake had some
249 interannual variation. The Chrysophyta in CH₁₉, YO₁₉, and YY₁₉ samples were
250 lower than the other samples, while the Cryptophyta in CH₁₉ and YY₁₉ were
251 lower than the other samples in CH, YY, respectively. The proportion of Arthropoda
252 in YO₁₉ reached 70.09%, which was remarkably higher than the different samples
253 (Fig. 2a).

254 A total of 24 dominant genera were identified (Fig. 2c), accounting for 81.22%
255 sequences in CH, 79.43% in KT, 61.22% in XH, 65.95% in YO, and 59.06% in YY.
256 The dominant genera were mainly *Hydrurus*, *Paraphysomonas*, *Ochromonas*, and
257 *Monochrysis* belonging to Chrysophyta, *Komma* in Cryptophyta, *Monomastix*,
258 *Chlamydomonas*, and *Raphidonema* in Chlorophyta.

259 As shown in Fig. 2c, the abundance of the dominant genera differed among the
260 lakes investigated interannually. The relative abundance of *Komma* varied from 0 to
261 48.49%, which showed an increasing trend over the year in CH and YY. The ranges of
262 *Paraphysomonas* and *Ochromonas* were 0.28~41.98% and 0.22~15.82%, showing an
263 increase followed by a decrease in XH and YO over the year. *The Hydrurus* in XH₁₈
264 and YO₁₈ was higher than the other samples. *Raphidonema* was significantly more



265 abundant in CH than in other lakes. *Chryso-sphaerell* and *Synura* in KT, except for
266 compared with CH, were significantly higher than other lakes ($P < 0.05$, Table S2).

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

275 The total number of OTUs shared in 2017-2019 was 276, and the unique OTUs
276 were 31 (2017), 34 (2018), 70 (2019) (Fig. 3g). The Venn diagram showed that the
277 total number of OTUs shared by the five lakes was 129, and the unique OTUs were
278 62 (CH), 37 (KT), 5 (XH), 14 (YO), and 14 (YY) (Fig. 3h).

279 The NMDS results divided the samples into five clusters according to their
280 similarity of microeukaryotic community (stress value = 0.14) (Fig. 4a). In addition,
281 the analysis of similarity (ANOSIM) based on Bray-Curtis distance indicated that the
282 differences between lakes were significant (Global R = 0.613, $P < 0.01$). Meanwhile,
283 no significant differences were detected by ANOSIM among interannual variations
284 ($R = 0.013$, $P = 0.393$).

285 UPGMA clustering analysis (Fig. 4b) showed the same lakes in a different year,
286 such as CH₁₇ and CH₁₈, YY₁₇ and YY₁₈, YO₁₇ and YO₁₈ clustered into
287 one clade, respectively. For other lakes, KT₁₈ and XH₁₈ clustered as one clade,
288 CH₁₉, and YY₁₉ clustered as one clade, CH₁₇, and XH₁₉ clustered as one clade.
289 YO lake was distant from other lakes and clustered into a separate one.

290 3.3 Driving factors and co-occurrence patterns

291 Canonical correspondence analysis (CCA) demonstrated that the first two
292 sequencing axes explained 16.7% and 15.5% of community variation (Fig. 5a). The



293 samples from the same lake were closer, with a more similar community structure.
294 More importantly, the Monte Carlo analysis confirmed that only the water
295 temperature significantly affected the microeukaryotic community ($P < 0.01$). The
296 variation partition analysis (VPA) indicated that environmental factors monitored
297 explained 14.19% of microeukaryotic community variability among lakes and still
298 had a large amount of unexplained community variation (85.8%, Fig. 5b).

299 A total of 223 nodes linked by 1941 edges was made up microeukaryotic network.
300 The majority of nodes in the network had many connections. Notably, the positive
301 associations among species were predominant in the network (Fig. 5c), with 82.25%,
302 whereas the portion of negative association was only 17.75%. In addition, the positive
303 interactions were mainly within the same taxonomic affiliations, such as Chrysophyta,
304 or between a few different taxonomic affiliations, such as Chrysophyta and
305 Chlorophyta. While the negative correlations mainly were reflected between
306 Chrysophyta and Chlorophyta. We found that only about 8% of OTUs directly
307 correlated with environmental factors. Meanwhile, only four of the top 20 OTUs with
308 the highest degree centrality were directly associated with environmental factors (WT,
309 PO_4 -P), and three belonged to Chrysophyta and one to Cercozoa.

310 24 nodes were identified as potential keystone species (Table S3), which
311 contained *Heteromita* belonging to Cercozoa, seven genera belonging to Chrysophyta,
312 such as *Spumella*, *Ochromonas*, and *Chromulina*. The Chlorophyta keystone genera
313 included *Chloromonas* and *Chlamydomonas*, and other genera were from
314 Bacillariophyta and Alveolata.

315 3.4 Community assembly processes

316 The Sloan neutral community model (NCM) showed the importance of
317 stochastic processes for microeukaryotic communities (Fig. 6a), with the neutral
318 processes explaining 56.8% community variation. In addition, the Sloan neutral
319 model classified microeukaryotic taxa into three groups (above prediction, below
320 prediction, and neutral prediction). We found that the neutral group (within 95%



321 confidence interval), with richness and abundance ratios of 79.7% and 90.4%,
322 respectively, were both much higher than the above and below prediction groups,
323 which was dominated by Chrysophyta, Chlorophyta, and Cryptophyta (Fig. 6b, c).
324 The above prediction group accounted for 8.8% of the microeukaryotic richness but
325 corresponded to only 0.75% of the abundance, dominated by Chrysophyta,
326 Chlorophyta, and Chytridiomycota. Cryptophyta accounted for 13.3% of the
327 abundance in the neutral group but was almost absent in the other two groups. In
328 contrast, Chytridiomycota was present in 10.4% abundance in the two groups
329 mentioned above, but only 0.1% in the neutral group.

330 The variation of β NTI ranged from -1.65~1.31 with a mean value of -0.48 (Fig.
331 6d), which was mainly distributed in the region of stochastic processes and supported
332 the results of the neutral model. The community assembly process analysis showed
333 that stochastic rather than deterministic processes controlled the community assembly.
334 Among them, homogenizing dispersal dominated, with a proportion of 64.76%,
335 followed by undominated process and dispersal limitation, with 32.38% and 2.86%,
336 respectively (Fig. 6e).

337 **4 Discussion**

338 **4.1 Diversity and dominant taxa**

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

345 Chrysophyta dominated in five lakes examined in our study, including *Hydrurus*,
346 *Paraphysomonas*, *Ochromonas*, and *Monochrysis*. Firstly, the dominance may be due
347 to the adaptation to low nutrient availability; the relatively high surface to volume
348 ratio contributes to the uptake of nutrients at low concentrations, Which have been



349 reported in high latitude polar lakes (Charvet et al., 2012) . Secondly, Chrysophyta
350 still keeps a high proportion under low light conditions, as they can adapt to changing
351 light conditions (Yubuki et al., 2008). Furthermore, Chrysophyta is mixotrophic and
352 even can swim, which allows them to get available nutrients from other
353 microorganisms, reducing the need for dissolved nutrients in the water (F R Pick and
354 Lean 1984; Katechakis and Stibor 2006). In addition, when the environmental
355 conditions change dramatically, such as freezing and nutrient changes, Chrysophyta
356 can form cysts (Nicholls 1995), protecting cells from resisting an unsuitable
357 environment. All these aspects make Chrysophyta has the advantage to be the
358 predominant species in the five Antarctic lakes.

359 Chlorophyta was the second most dominant taxon in our study (13.94%~
360 35.37%), containing mainly *Monomastix*, *Chlamydomonas*, and *Raphidonema*.
361 Chlorophyta is typically represented by flagellated species such as *Chlamydomonas*
362 spp., which dominate the phytoplankton in different trophic statuses and respond to
363 adverse environmental conditions by forming temporary groups (Allende and
364 Mataloni 2013; Izaguirre et al., 2003; Toro et al., 2007). Several unicellular algae can
365 mix acid fermentation, and some obligate photoautotrophic species respond by photo-
366 acclimation processes involving the accumulation of chlorophyll to increase the light
367 capture efficiency (Atteia et al., 2013; Morgan-Kiss et al., 2016). These characteristics
368 might partially be explained how Chlorophyta survived and occupied a specific
369 advantage of the important reason in lakes we studied.

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

377 Compared with other aquatic ecosystems (Sun et al., 2021), the diversity of



378 microeukaryotes in Antarctic lakes was significantly lower (Shannon 2.06~3.26,
379 OTUs 151~244). The diversity of microorganisms reported decreases from mid-
380 latitude to the poles (Santos et al., 2020). The isolation and harsh conditions,
381 especially the lower temperatures and nutrients, prevailing in Antarctic lakes account
382 for a low microeukaryotic diversity. In addition, the species-area relationships model
383 (SAR) states that increased species number with increasing habitat area within a
384 specific area (Ma 2018). An increase in the ice-free area drastically modifies
385 biodiversity (Duffy et al., 2017; Lee et al., 2017; Pertierra et al., 2017). Our results
386 supported the SAR model, observing more diversity and richness in CH and KT,
387 where habitat areas were much larger than the YY and YO.

388 4.2 Influence of environmental factors on the community

389 Previous great efforts have demonstrated that abiotic factors affect microbial
390 diversity and community composition (Quiroga et al., 2013; Sun et al., 2017). Our
391 study found that only water temperature was a significant driving factor for
392 community change among the abiotic factors analyzed. Some microorganisms have
393 evolved to grow under a defined temperature, allowing differences in temperature
394 adaptation of different species (Wilkins et al., 2013). Water temperature has become a
395 major driving factor for changes in microeukaryotic communities by regulating
396 cellular activity and metabolic rates (Margesin and Miteva 2011). The retreat of
397 glaciers due to global warming had the risk of reducing the abundance and diversity
398 of microorganisms, and more attention should be paid to the impact of water
399 temperature changes on community structure (Garcia-Rodriguez et al., 2021). The
400 water temperature in the YO lakes was significantly higher than in the other lakes, and
401 YO clustered into a separate clade (Fig. 5b).

402 Nevertheless, a small amount of community variation could be explained by
403 measured environmental variables in our analysis. This indicated that these
404 environmental factors played a minor role in shaping microeukaryotic community
405 structure. There were many unexplained variations (Fig. 5), and some possible causes



406 have been indicated. Firstly, the nonconsecutive of environmental factors among
407 different expedition seasons was deficient in our study. There are also many vital
408 abiotic factors in Antarctic freshwater lakes, including solar cycle, light availability,
409 ice cover (thickness and duration), physical changes as snow melts and mixes, and
410 hydrological changes (Allende and Izaguirre 2003; Lizotte 2008). Secondly, the
411 relationship between microorganisms (symbiotic or competitive) cannot be quantified,
412 which is an essential factor influencing community structure. Predation pressure
413 manifests itself in lakes as a top-down control of microeukaryotes (Blomqvist 1997).
414 Thirdly, stochastic processes such as ecological drift (birth, death) may cause
415 unexplained community variation (Zhang et al., 2018).

416 4.3 Co-occurrence patterns and keystone taxa

417 Network analysis can help us understand complex biological interactions and
418 ecological rules for community assembly within a specific ecological niche (Li and
419 Hu 2021; Lupatini et al., 2014). Microorganisms form various ecological relationships,
420 ranging from mutualism to competition, ultimately shaping microbial abundances
421 (Faust and Raes 2012). Positive associations in a network often indicate common
422 preferred environmental conditions or niche-overlapping, whereas negative
423 associations mean competition or niche division (Faust and Raes 2012). By analyzing
424 the network, we found that the positive correlations were much more than the
425 negative correlations in the co-occurrence network (87% vs. 13%), indicating that
426 species coexistence was achieved mainly by symbiotic relationships between species.
427 In addition, only 8% OTUs were significantly correlated with environmental factors,
428 suggesting that microeukaryotes have a relatively lower response to environmental
429 factors and these could weaken the role of environment selection in community
430 assembly. Previous studies have shown the high response of microeukaryotic
431 communities to mid-and late-stage diatom blooms promotes deterministic processes
432 (Hou et al., 2020).

433 In co-occurrence networks, keystone species play a critical role in maintaining



434 the structure and function of the microbial community, and the loss of essential
435 species may lead to the fracturing of networks (Zhang et al., 2022). The keystone
436 species in this study belonged mainly to Chlorophyta, Chrysophyta, Bacillariophyta,
437 and Cercozoa. *Heteromita* has significant genetic variation and promotes bacterial
438 degradation of alkylbenzenes through predation (Ekelund et al., 2004). *Spumella* is a
439 heterotrophic microorganism commonly found in freshwater and soil (Boenigk et al.,
440 2005). As mixed trophic organisms, *Ochromonas* prey on bacteria and are, therefore, a
441 critical link between bacteria and higher trophic levels (Andersson et al., 1989).
442 *Chloromonas* has motile trophic cells that can grow in the snow to give it a green
443 color and, together with *Chlamydomonas*, are thought to have a strong carbon
444 concentration mechanism (Hu 1998).

445 4.4 Community assembly processes

446 In general, deterministic and stochastic processes exist simultaneously in the
447 community assembly (Chase 2010; He et al., 2021). Several factors such as habitat
448 connectivity and size (Orrock and Watling 2010), productivity (Chase 2010),
449 disturbance (Liang et al., 2020), predation (Chase et al., 2009), and resource
450 availability (Kardol et al., 2013) influence the relative importance of stochastic and
451 deterministic processes in the community assembly. The importance of stochastic
452 processes has been previously illustrated for other microeukaryotic communities from
453 the aquatic ecosystem (Chen et al., 2019; Wang et al., 2020). In our study, the results
454 supported the prominent role of stochastic processes in shaping the microeukaryotic
455 community assembly than deterministic processes. Hence, environmental variables
456 explained only a small number of variations in our study's microeukaryotic
457 community, and a small number of taxa were significantly correlated with
458 environmental factors.

459 In our study, the microeukaryotic community showed a good fit (57%) to the
460 neutral model (Fig. 6a), which suggested community variation can be explained by
461 stochastic processes such as birth, death, and migration to a large extent. The NCM



462 can also attribute the observed patterns of community assembly to different
463 population behavior (Zhang et al., 2021b). The NCM separated taxa into three groups,
464 and these groups were different in community structure (Fig. 6), similar to the result
465 of microeukaryotic community in the channel (Zhang et al., 2021b), indicating these
466 taxa might differ in their adaptability to the environment or dispersal rate (Chen et al.,
467 2019). In addition, the neutral group contributed a high proportion to both abundance
468 (90.4%) and richness (79.7%) in our study. In a subtropical river, the neutral group
469 also dominates the microeukaryotic community in terms of richness and abundance
470 (Chen et al., 2019). Similar results in this study suggested that microeukaryotes in this
471 study were more susceptible to stochastic processes.

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

489 Our study's microeukaryotic community tended to homogenize during dispersal,
490 and the community compositions were relatively stable. Antarctic freshwater lakes



491 can receive external microbial colonies by the input of microorganisms from the
492 surrounding ice melt, atmospheric transport, human activities, or bird migration
493 (Unrein et al., 2005). Water bodies have been reported occupied with a high
494 proportion of homogenizing dispersal (Zeng et al., 2019). Most microorganisms
495 detected in the sea also have been found present in lakes in East Antarctica, pointing
496 to that some marine taxa in the lake may be the product of homogenizing dispersal
497 from the ocean to the lake (Logares et al., 2018). In addition, the lakes were covered
498 in ice for most of the year and were limited by geographical distance, resulting in the
499 dispersal limitation of microorganisms (2.86%). Undominant processes accounted for
500 32.38% of community assembly in our study, including ecological drift and other
501 complex processes that have not been fully quantified, such as weak selection and
502 diffusion (Mo et al., 2018a), suggesting that microeukaryotic communities might be
503 formed by some highly complex assembly mechanisms in Antarctic freshwater lakes.

504 **5 Conclusion**

505 In conclusion, the microeukaryotic community was dominated by phytoplankton,
506 mainly Chrysophyta, Chlorophyta, and Cryptophyta, with spatial and temporal
507 variation in the relative abundance of dominant taxa from five freshwater lakes on the
508 Fildes Peninsula, Antarctic. This study highlighted the first time the importance of
509 stochastic processes and co-occurrence patterns in shaping the microeukaryotic
510 community of this area. The environmental variables explained only about 30% of the
511 community variation. Microbial interactions were predominantly symbiotic,
512 indicating common preferred environmental conditions or niche-overlapping.
513 Stochastic processes played a very prominent role in microeukaryotic community
514 assembly, and the low response to environmental factors might enhance the
515 proportion of stochastic processes. Our study provides a better understanding of the
516 dynamic patterns and ecological processes of microeukaryotic community structure in
517 Antarctic oligotrophic lakes (Fildes Peninsula).

518 **Data Availability Statement**

519 The raw 18S reads have been deposited into the NCBI Sequence Read Archive



520 database with the accession numbers of SRP359325.

521 **Author Contribution Statement**

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

528 **Competing interests**

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

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884



885 **Figure Captions**

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

889 **Fig.2** Comparison of microeukaryotic community composition. (a) Temporal and
890 spatial dynamics of relative abundance on Phylum level in five lakes from 2017 to
891 2019. 17, 18, and 19 expedition season of 2017/2018, 2018/2019, and 2019/2020,
892 respectively. (b) Differential analysis of microeukaryotes in different lakes. Lakes that
893 showed no significant differences were not shown ($P > 0.05$). (c) Temporal and spatial
894 dynamics of relative abundance on Genus level in five lakes. (Note: $**P < 0.01$, $*P <$
895 0.05). (Chlorophyta: *Aphelida*, *Atractomorpha*, *Chlamydomonas*, *Chloromonas*,
896 *Chlorothrix*, *Choricystis*, *Crustomastix*, *Microglen*, *Monomastix*, *Nannochloris*,
897 *Raphidonema*; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*, *Monochrysis*,
898 *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*; Cryptophyta: *Komma*;
899 Haptophyta: *Diacronema*; Pyrrophyta: *Heterocapsa*; Glissomonadida: *Heteromita*.
900 The relative abundance at any lake was less than 1% was defined as others).

901 **Fig.3** Microbial diversity and Venn diagram in different years and lakes. (a, b)
902 variations in microbial OTUs; (c, d) variations in microbial Shannon index; (e, f)
903 variations in within-community nearest-taxon index (NTI); (g, h) Venn diagram
904 showing the unique and shared operational taxonomic units (OTUs). Homogeneity
905 and one-way ANOVA analysis of variance was used to test the indices' significance.
906 "ns" represents no significant differences ($P > 0.05$). Significant differences ($P < 0.05$)
907 are indicated by different alphabet letters.

908 **Fig. 4** Temporal variability analysis of Non-metric multidimensional scaling (NMDS)
909 ordination of microeukaryotic communities (a) and clustering of five lakes based on
910 similarity (b).



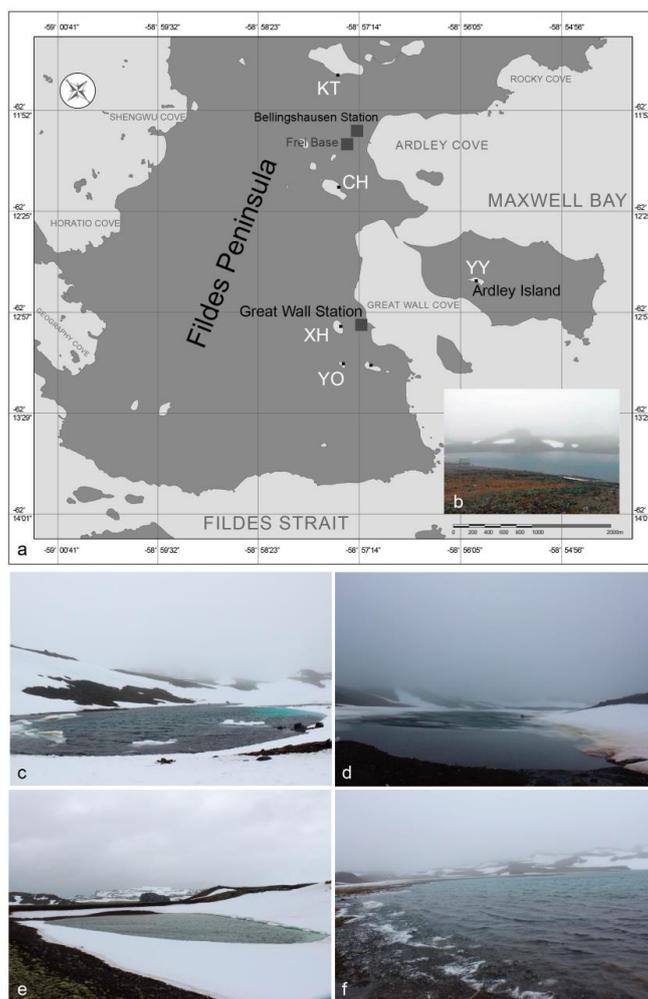
911 **Fig.5** The effect of environmental variables on microeukaryotic communities, and co-
912 occurrence pattern. Canonical correlation analysis plots(a) and variance partitioning
913 analysis (b), respectively. Sal: salinity; WT: water temperature; NO₂ -N: nitrite
914 nitrogen; PO₄ -P: phosphate phosphorus. ** $P < 0.01$. (c) Networks analysis revealing
915 the interspecies associations between microeukaryotic OTUs, and the correlation
916 between environmental factors and OTUs in lakes integrated networks. The size of
917 each OTUs or environmental factor (node) is proportional to the degree centrality.
918 Others: other phyla and unclassified taxa.

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

933



934 **Fig. 1**



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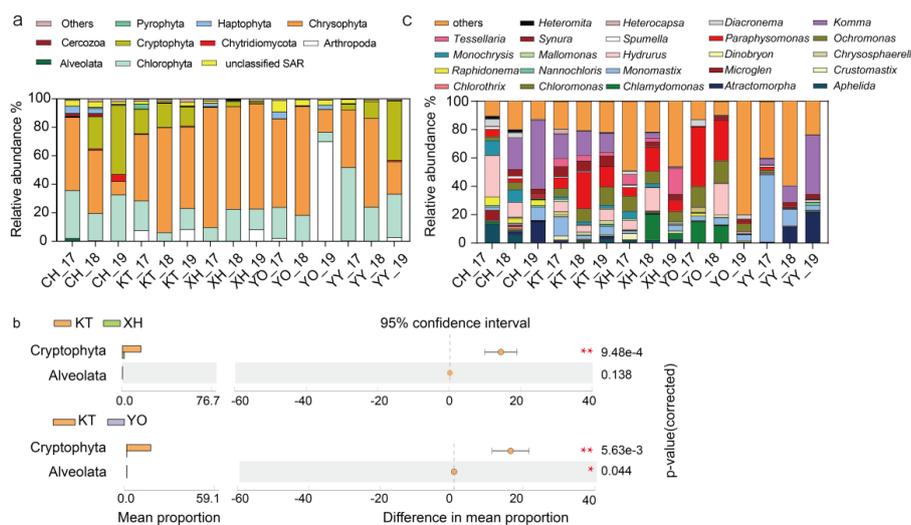
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940



941 **Fig. 2**



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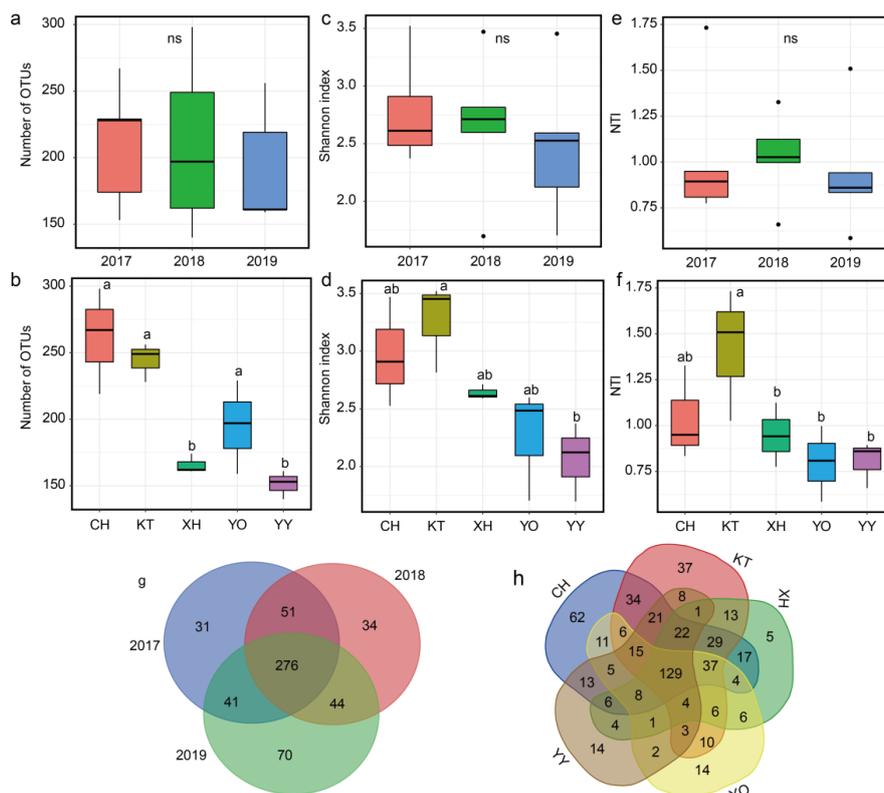
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956



957 **Fig. 3**



958

959

960 **Fig.3** Microbial diversity and Venn diagram in different years and lakes. (a, b)

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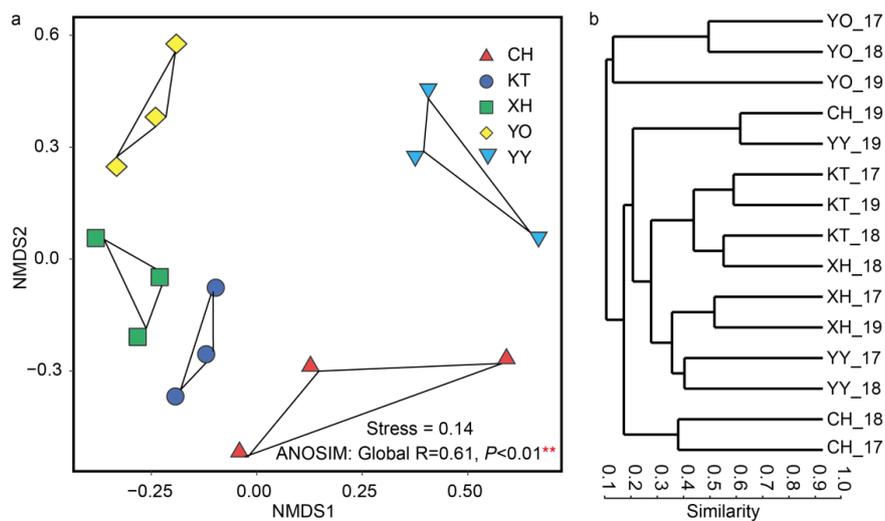
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967



968 **Fig. 4**



969

970 **Fig. 4** Temporal variability analysis of Non-metric multidimensional scaling (NMDS)

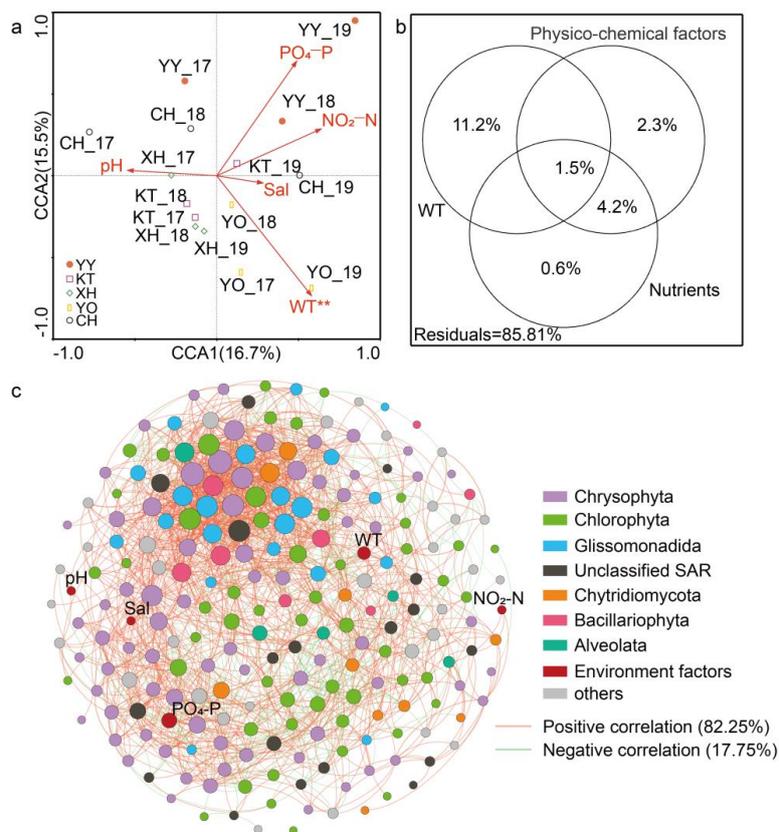
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973



974 **Fig. 5**



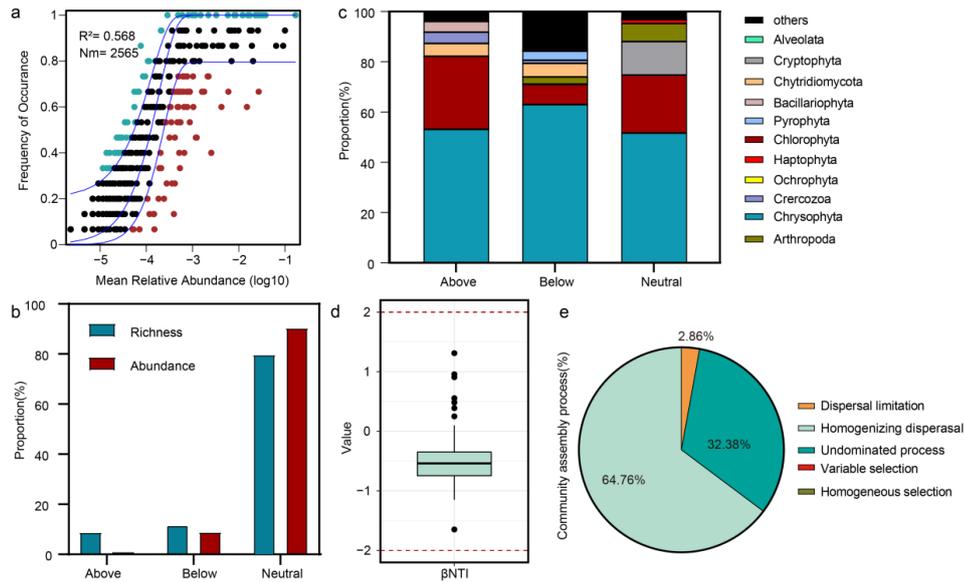
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983



984 **Fig. 6**



985

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