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

Chunmei Zhang[a,b], Huirong Li[a,c,d], Yinxin Zeng[a,c,d], Haitao Ding[a,c,d], Bin Wang[e],
Yangjie Li[e], Zhongqiang Ji[e], Yonghong Bi[b*], Wei Luo[a,c,d*]

[a] Key Laboratory for Polar Science, Polar Research Institute of China, Ministry of Natural Resources, Shanghai 200136, China

[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

[c] Antarctic Great Wall Ecology National Observation and Research Station, Polar Research Institute of China, Ministry of Natural Resources, Shanghai 200136, China

[d] School of Oceanography, Shanghai Jiao Tong University, Shanghai 200030, China

[e] Key Laboratory of Marine Ecosystem Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

*Corresponding Author: biyh@ihb.ac.cn, luowei@pric.org.cn

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 heterogenicity 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.

1 Introduction

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

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

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 µm) 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

conditions and species interactions) influence and determine community assembly

(Powell et al., 2015). Stochastic processes are based on the neutral theory, which

states that random birth or death, drift, and dispersal events also play an essential role

in community assembly (Bahram et al., 2016). Deterministic processes are prevalent

in building whole ecosystem communities (Liu et al., 2020) where selection leads to

species classification, and applying similar habitats thus results in similar community

assemblages. However, other studies have supported an important role of stochastic

processes (e.g., drift and dispersal) in community assembly, where dispersal is the

movement of species over spatial locations and drift is associated with the relative

abundance of the species (Massana and Logares, 2013; Wu et al., 2019). Stochastic

processes have been shown to account for up to 95% of the microbial eukaryotic

community assembly in a set of lakes in Eastern Antarctica (Logares et al., 2018).

Stochastic processes have also been found to prevail in micro-and nanoplankton

eukaryotic communities in the intertidal zones of southeast Fujian, China (Kong et al.,

2019). Nonetheless, the study of microbial eukaryotic community assembly processes

in Antarctica still requires further investigations for a more comprehensive view.

Few studies have been conducted on the microbial eukaryotic diversity and

community assembly processes of the freshwater lakes on the Fildes Peninsula,

Antarctica. Our study attempted to analyze the microbial eukaryotic samples of five

freshwater lakes collected over three summers using high-throughput 18S rRNA

sequencing. We aimed to (I) understand the diversity and co-occurrence of microbial

eukaryotes and (II) explore the influencing factors and their community assembly

processes.

2 Material and Method

2.1 Study area, sample collection and measurement of environmental
variables

Field samples were collected on the 34th (2017/2018), 35th (2018/2019), and

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 μm, Whatman), and Chl *a* was then

146 extracted with 90% acetone over 24 h and measured spectrophotometrically. Nutrients,

147 including ammonia (NH_4^+), nitrite (NO_2^-), silicate (SiO_3^{2-}), and phosphate (PO_4^{3-})

148 were measured spectrophotometrically with a San^{++} 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 μm 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, $1 \times$ 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 ($2\times$

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   Nm 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 β-nearest taxon index (βNTI) 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 βNTI 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   five lakes showed lower values of 0.00-0.15 µM (NO_2^-), 0.05-0.74 µM (NH_4^+) and

263   0.02-2.29 µM (PO_4^{3-}). Relatively higher and lower levels of nutrients were identified

264   in YY and XH, respectively. The lowest value of SiO_3^{2-} was recorded in YY (1.43 µM)

265   and the highest in CH (51.5 µM). The highest and lowest Chl *a* were reached in YY

266   and CH (2.11 and 0.25 µg L^{-1}, respectively). pH showed minimum and maximum

267   values in YY (7.65) and CH (8.27). Sal values ranged from 0.00-0.14 PSU and were

268   significantly lower in YO than in the other lakes ($P<0.05$).

269   3.2 Diversity and composition of microbial eukaryotic communities

270   A total of 9 dominant phyla were identified, accounting for over 98% of the

271   sequences. These dominant phyla mainly consisted of microbial eukaryotic

272   phytoplankton, with various relative abundances between different lakes (Fig. 2a).

273   Chrysophyta (34.91% in CH–78.88% in XH), Chlorophyta (15.17% in KT–35.88% in

274   YY), and Cryptophyta (0.01% in YO–23.71% in CH) were most abundant in the

275   studied lakes. Cryptophyta in KT was significantly more abundant than in XH and YO,

276   and Pyrrophyta in XH was significantly more abundant than in YY ($P<0.05$) (Fig. 2b).

277   Haptophyta represented 0.09% of the sequence in KT and up to 4.93% in YO;

278   Alveolates represented 0.02% of the sequence in XH and up to 1.00% in CH, while

279   unclassified Stramenopiles-Alveolates-Rhizaria (SAR) represented 1.12 % in XH and

280   8.05 % in YO.

281   The relative abundances of the dominant taxa in the same lake exhibited some

282   interannual variations. The relative abundances of Chrysophyta in CH_19, YO_19,

283   and YY_19 samples were lower than in the other samples, while the relative

284   abundances of Cryptophyta in CH_19 and YY_19 were lower than in the other

285   samples of CH and YY, respectively (Fig. 2a).

286   A total of 21 dominant genera were identified (Fig. 2c), accounting for over 87%

287   of the sequences. The dominant genera included *Hydrurus*, *Paraphysomonas*,

288   *Ochromonas*, *Synura,* and *Monochrysis* belonging to Chrysophyta, *Komma* in

289   Cryptophyta, *Monomastix*, *Chlamydomonas*, and *Mantoniella* belonging to

290   Chlorophyta.

291   As shown in Fig. 2c, the abundance of the dominant genera differed
292 interannually among the investigated lakes. The relative abundance of *Komma* varied
293 from 0 to 48.52%, which showed an increasing trend over the years in CH and YY.
294 The ranges of *Paraphysomonas* and *Ochromonas* were 3.06–56.88% and 0.06–
295 18.86%, showing an increase followed by a decrease in XH over the years. The
296 highest relative abundances of *Hydrurus* were in samples XH_18 and YO_18 than in
297 the other samples. *Ochromonas* in CH was significantly lower than in YO and XH
298 (P<0.05, Table S2).

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

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

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

319   The UPGMA clustering analysis (Fig.4b) showed the same lakes in a different

320   year, such as CH_17 and CH_18, YY_17 and YY_18, YO_17 and YO_18 clustered

321   together. YO lake was distant from the other lakes and clustered into a separate group.

322   3.3 Driving factors and co-occurrence patterns

323   Canonical correspondence analysis (CCA) demonstrated that the first two

324   sequencing axes explained 21.84% and 17.11% of the community variation,

325   respectively (Fig. 5a). The samples from the same lake were closer, with a more

326   similar community structure. More importantly, the Monte Carlo analysis confirmed

327   that the water temperature and PO_4^{3-} significantly affected the microbial eukaryotic

328   communities ($P<0.05$). The VPA indicated that the monitored environmental factors

329   explained 9.34% of the microbial eukaryotic community variability among lakes, but

330   there was still a large amount of unexplained variation (90.66%, Fig. 5b).

331   A total of 220 nodes linked by 1521 edges comprised the microbial eukaryotic

332   community network. The majority of nodes in the network had many connections.

333   Notably, the positive associations among species were predominant in the network

334   (Fig. 5c), comprising with 81.82% of the associations, whereas the portion of negative

335   association was only 18.18%. In addition, the positive interactions were mainly found

336   within the same taxonomic affiliations, such as Chrysophyta, or between a few

337   different taxonomic affiliations, such as Chrysophyta and Chlorophyta. The negative

338   correlations on the other hand mainly were reflected between Chrysophyta and

339   Chlorophyta. Only around 8% of OTUs correlated directly with environmental factors

340   ($P<0.05$). Meanwhile, only two of the top 20 OTUs with the highest degree centrality

341   were directly associated with environmental factors (water temperature and PO_4^{3-});

342   one belonged to Chrysophyta and the other to Chytridiomycota.

343   3.4 Community assembly processes

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

345   processes for microbial eukaryotic communities (Fig. 6a), with the neutral processes

346 explaining 55.5% of the community variation. In addition, the NCM classified

347 microbial eukaryotic taxa into three groups (above prediction, below prediction, and

348 neutral prediction). In addition, compared to the above and below prediction groups,

349 we observed a higher contribution of neutral prediction group to community

350 abundance (86.4%) and richness (80%), which was dominated by Chrysophyta,

351 Chlorophyta, and Cryptophyta (Fig. 6b, c). The above prediction group accounted for

352 10.58% of the microbial eukaryotic richness but corresponded to only 0.96% of the

353 abundance, and was dominated by Chrysophyta, Chlorophyta, and Cercozoa.

354 Cryptophyta accounted for 16.58% of the abundance in the neutral prediction group

355 but was almost absent in the above prediction group. In contrast, Cercozoa was

356 present with a 9.38% abundance in the above prediction group, but only comprised

357 0.32% of the neutral prediction group.

358 The βNTI was mainly distributed in the region of stochastic processes with a

359 mean value of -0.48 and supported the results of the neutral model. The analysis of

360 community assembly processes showed that stochastic rather than deterministic

361 processes controlled the community assembly. Among them, homogenizing dispersal

362 dominated, with a proportion of 59.05%, followed by undominated processes and

363 dispersal limitation (Fig. 6d).

364 **4 Discussion**

365 4.1 Dominant taxa and diversity

366 The environmental conditions (e.g., low light and low nutrient contents, etc.) in

367 Antarctic freshwater lakes differ from those of temperate lakes. These special features

368 and their relative isolation has resulted in the development of unique communities and

369 survival strategies of the species adapted to such conditions (Pearce and Galand,

370 2008). In our study, the survival of taxa (Chrysophyta, Chlorophyta, and Cryptophyta)

371 might have depended on their survival strategies and adaptation to harsh conditions,

372 which allowed them to become the predominant species.

373 Chrysophyta dominated in the five lakes examined in our study, including

374   *Hydrurus*, *Paraphysomonas*, *Ochromonas*, and *Monochrysis*. First, their dominance

375   might have been due to their adaptation to a low nutrient availability. Chrysophyta

376   have been widely distributed in oligo- and mesotrophic lakes from both the maritime

377   and continental regions of Antarctica (Allende, 2009; Allende and Izaguirre, 2003;

378   Izaguirre et al., 2020; Richards et al., 2005). Secondly, Chrysophyta can still retained

379   a high cell density under low light conditions, since they can adapt to changing light

380   conditions (Yubuki et al., 2008). Furthermore, Chrysophyta is mixotrophic and can

381   even swim, allowing them to obtain available nutrients from other microorganisms,

382   and thus reducing the need for dissolved nutrients in the water (Pick and Lean, 1984;

383   Katechakis and Stibor, 2006). In addition, when environmental conditions change

384   dramatically, such as through freezing and nutrient changes, Chrysophyta can form

385   cysts (Nicholls, 1995), protecting their cells from resisting an unsuitable environment.

386   All these characteristics allow Chrysophyta to be the predominant species in the five

387   Antarctic lakes.

388   Chlorophyta was the second most dominant taxon in our study (15.17%–

389   35.88%), mainly consisting of the *Monomastix*, *Chlamydomonas*, and *Mantoniella*.

390   Chlorophyta was typically represented by flagellated species such as *Chlamydomonas*

391   spp., which dominate phytoplankton communities in different trophic statuses and

392   respond to adverse environmental conditions by forming temporary, non-swimming

393   cell populations encased in a gelatinous mother cell membrane (Izaguirre et al., 2003;

394   Allende and Mataloni, 2013; Toro et al., 2007). Several unicellular algae can undergo

395   mix acid fermentation, and some obligate photoautotrophic species can increase their

396   light capture efficiency through photoadaptation processes involving chlorophyll

397   accumulation (Morgan-Kiss et al., 2016; Atteia et al., 2013). These characteristics

398   might partially explain how Chlorophyta survived and occupied a specific advantage

399   in the lakes studied here.

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

401   dominance has been interpreted as evidence of heterotrophic growth in winter and

402   mixotrophic growth throughout the year (Unrein et al., 2014). Cryptophyta dominated

403   under perennially ice-covered and coastal saline lakes in continental Antarctica

404   (Izaguirre et al., 2020). The ingestion of bacteria by mixotrophic Cryptophyta has

405   previously been observed in two perennially ice-covered lakes (Fryxell and Hoare) in

406   the McMurdo Dry Valleys (Roberts and Laybourn-Parry, 1999).

407   Compared to other aquatic ecosystems (Wang et al., 2021; Wang et al., 2020b;

408   Hernandez-Ruiz et al., 2018), the diversity of microbial eukaryotes in the Antarctic

409   lakes studied here was significantly lower (Shannon index 1.70–3.50, richness 113–

410   268). The diversity of the microorganisms reported here decreases from mid-latitude

411   to the poles (Santos et al., 2020). The isolation and harsh conditions, especially the

412   lower temperatures and nutrients availability, prevailing in Antarctic lakes accounted

413   for a low microbial eukaryotic diversity. In addition, the species-area relationships

414   model (SAR) states that there should be an increased species number as habitat area

415   increases with a specific area (Ma, 2018). An increase in the ice-free area may

416   drastically modify the biodiversity of an area (Pertierra et al., 2017; Duffy et al., 2017;

417   Lee et al., 2017). Our results support the SAR model since a greater alpha diversity

418   was observed in the CH and KT where the habitat areas were much larger than the YY

419   and YO.

420   4.2 Influence of environmental factors on the community

421   Previous studies have demonstrated that abiotic factors affect microbial diversity

422   and community composition (Quiroga et al., 2013; Sun et al., 2017). In this study, we

423   found that water temperature and PO_4^{3-} were significant factors driving community

424   changes among the abiotic factors analysed. Some microorganisms have evolved to

425   grow under defined temperatures, allowing differences in the temperature adaptation

426   of different species (Wilkins et al., 2013). Water temperature has become a major

427   driving factor for microbial eukaryotic community variation since it can regulate

428   cellular activity and metabolic rates (Margesin and Miteva, 2011). The retreat of

429   glaciers due to global warming had the risk of reducing the abundance and diversity

of microorganisms, and more attention should be paid to the impact of temperature changes on community structure (Garcia-Rodriguez et al., 2021). The water temperature in the YO was significantly higher than in the other lakes, and the community of YO clustered into a separate group (Fig. 5b). Microorganisms are highly sensitive to nutrients, and indeed, nutrients have been shown to represent important factors of community differences in microbial eukaryote communities in different areas (Zouari et al., 2018). In addition, PO_4^{3-} has been demonstrated to be one of the environmental variables that "best" explained the picoeukaryotic distributions in the Western Pacific Ocean (Liu et al., 2021).

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

4.3 Co-occurrence patterns

Network analysis can help us understand complex biological interactions and ecological rules for community assembly within a specific ecological niche (Li and

458 Hu, 2021; Lupatini et al., 2014). Microorganisms can form various ecological

459 relationships, ranging from mutualism to competition, ultimately shaping the

460 microbial abundances (Faust and Raes, 2012). Positive associations in a network often

461 indicate common preferred environmental conditions or niche-overlapping, whereas

462 negative associations imply competition or niche division (Faust and Raes, 2012). Our

463 network analysis revealed that the positive correlations were much more common

464 (82%) than negative ones (18%). These results suggested that positive relationships

465 (e.g., due to cross-feeding, niche overlap, mutualism, or commensalism) might play a

466 more important role in the lake ecosystems of Antarctica than negative relationships

467 (e.g., predator-prey, host-parasite, or competition) (Chen and Wen, 2021). Similar

468 results have been obtained through the study of small planktonic eukaryotes (0.2–20

469 µm) inhabiting the surface waters of a coastal upwelling system (Hernandez-Ruiz et

470 al., 2018). Notwithstanding, further studies are needed to corroborate the biological

471 interactions and other nonrandom processes (for example, cross-feeding versus niche

472 overlap) between the species pairs detected by network analyses. In addition, only 8%

473 of the OTUs were significantly correlated with environmental factors, suggesting that

474 microbial eukaryotes had a relatively lower response to environmental factors.

475 4.4 Community assembly processes

476 In general, deterministic and stochastic processes exist simultaneously in a

477 community assembly (He et al., 2021; Chase, 2010). Several factors such as habitat

478 connectivity and size (Orrock and Watling, 2010), productivity (Chase, 2010),

479 disturbance (Liang et al., 2020), predation (Chase et al., 2009), and resource

480 availability (Kardol et al., 2013) can influence the relative importance of stochastic

481 and deterministic processes in a community assembly. The importance of stochastic

482 processes has been previously illustrated for other microbial eukaryotic communities

483 in aquatic ecosystems (Chen et al., 2019; Wang et al., 2020a). For example,

484 picoeukaryotic communities in the lower oxygen layer of the Bengal Bay were shown

485 to be dominated by stochastic processes (Chen et al., 2022). Results from our study

486 supported a more prominent role of stochastic processes over deterministic ones in

487 shaping the assembly of microbial eukaryotic communities. Hence, environmental

488 variables explained only a small amount of variation in the microbial eukaryotic

489 community studied here, and a small number of taxa were significantly correlated

490 with environmental factors.

491   In our study, the microbial eukaryotic community showed a good fit with the

492 neutral model (Fig. 6a), suggesting that community variation could be explained by

493 stochastic processes such as birth, death, and migration to a large extent. The NCM

494 can also attribute the patterns observed of community assembly to different

495 population behavior (Zhang et al., 2021). Here, the NCM separated the studied taxa

496 into three groups which had different community structures (Fig. 6), indicating that

497 these taxa might differ in their adaptability to the environment or their dispersal rates

498 (Chen et al., 2019). In addition, the neutral prediction group contributed a high

499 proportion to both the species abundance (86.4%) and richness (80.0%) in our study.

500 Previous studies have shown that the neutral prediction group also dominated the

501 microbial eukaryotic community in a subtropical river in terms of richness and

502 abundance (Chen et al., 2019). Similar results in this study suggest that microbial

503 eukaryotes studied here were more susceptible to stochastic processes.

504   The importance of stochastic processes has been previously illustrated by the

505 microbial eukaryotic communities of lakes in East Antarctica (Logares et al., 2018).

506 Antarctic freshwater lakes can receive external microbial colonies from the input of

507 microorganisms from the surrounding ice melt, atmospheric transport, human

508 activities, or bird migration (Unrein et al., 2005). Zeng et al. (2019) showed that

509 homogenizing dispersal occupied a high proportion of community assembly processes

510 in freshwater lakes, and similar results were obtained in our study. Most

511 microorganisms detected in the sea have also been found in lakes in East Antarctica,

512 suggesting that some marine taxa in the lakes could be the product of homogenizing

513 dispersal from the sea to the lakes (Logares et al., 2018). In addition, the five studied

514 lakes were covered in ice for most of the year and were limited by geographical

515 distance, resulting in the limited dispersal of microorganisms (0.95%). Undominant

516 processes accounted for 40% of the community assembly in our study, including

517 ecological drift and other complex processes that have not been fully quantified, such

518 as weak selection and diffusion (Mo et al., 2018). This suggest that microbial

519 eukaryotic communities might be formed by some highly complex assembly

520 mechanisms in Antarctic freshwater lakes.

5 Conclusion

522 In conclusion, a unique microbial eukaryotic community structure and low alpha

523 diversity (richness and Shannon index) have been demonstrated in five freshwater

524 lakes of the Fildes Peninsula, Antarctica. Water temperature and PO_4^{3-} were identified

525 as important factors driving the variation of community structures ($P<0.05$).

526 Stochastic processes and biotic co-occurrence patterns were shown to be important in

527 shaping microbial eukaryotic communities in the area. Our study provides a better

528 understanding of the dynamic patterns and ecological assembly processes of microbial

529 eukaryotic communities in Antarctic oligotrophic lakes (Fildes Peninsula).

Data Availability Statement

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

532 database with the accession numbers PRJNA805287.

Author Contribution Statement

534 Conceptualization: Chunmei Zhang and Yonghong Bi; methodology: Chunmei

535 Zhang and Wei Luo; molecular technique:Huirong Li; physico-chemical properties:

536 Bin Wang, Yangjie Li, and Zhongqiang Ji; sample collection:Yinxin Zeng and

537 Haitao Ding; funding acquisition: Yonghong Bi and Wei Luo; supervision: Yonghong

538 Bi and Wei Luo; writing - original draft: Chunmei Zhang; writing-review & editing:

539 Yonghong Bi and Wei Luo.

Competing interests

541 The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge to the 34th, 35th, and 36th Chinese Antarctic Research Expedition (CHINARE) and the Antarctic Great Wall Ecology National Observation and Research Station (PRIC) for their strong logistic support of this field survey in the summer season of 2017/2018, 2018/2019, and 2019/2020. The field samples were approved by the Chinese Arctic and Antarctic Administration (CAA). This research was supported by the National Natural Science Foundation of China (No. 91851201; No.31971477) and the Open Fund from Hubei Key Laboratory of Three Gorges Project for Conservation of Fishes (SXSN/4194).

Reference

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

Allende, L. and Izaguirre, I.: The role of physical stability on the establishment of steady states in the phytoplankton community of two Maritime Antarctic lakes, Hydrobiologia, 502, 211-224, https://doi.org/10.1023/B:HYDR.0000004283.11230.4a, 2003.

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

Atteia, A., van Lis, R., Tielens, A. G. M., and Martin, W. F.: Anaerobic energy metabolism in unicellular photosynthetic eukaryotes, BBA-BIOENERGETICS, 1827, 210-223, https://doi.org/10.1016/j.bbabio.2012.08.002, 2013.

Bahram, M., Kohout, P., Anslan, S., Harend, H., Abarenkov, K., and Tedersoo, L.: Stochastic distribution of small soil eukaryotes resulting from high dispersal and

drift in a local environment, ISME J., 10, 885-896, https://doi.org/10.1038/ismej.2015.164, 2016.

Barsch, D. and Caine, N.: The nature of mountain geomorphology, Mt. Res. Dev., 4, 287-298, https://doi.org/10.2307/3673231, 1984.

Bastian, M., Heymann, S., and Jacomy, M.: Gephi: an open source software for exploring and manipulating networks, Int. Conf. Weblogs Soc. media., 361-362,

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

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

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

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

Chase, J. M.: Stochastic community assembly causes higher biodiversity in more productive environments, Science, 328, 1388-1391, https://doi.org/10.1126/science.1187820, 2010.

Chase, J. M., Biro, E. G., Ryberg, W. A., and Smith, K. G.: Predators temper the relative importance of stochastic processes in the assembly of prey

metacommunities, Ecol. Lett., 12, 1210-1218, https://doi.org/10.1111/j.1461-0248.2009.01362.x, 2009.

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

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

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

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

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

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

Edgar, R. C.: UPARSE: highly accurate OTU sequences from microbial amplicon reads, Nat. Methods., 10, 996-998, https://doi.org/10.1038/Nmeth.2604, 2013.

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

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

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

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

Glassman, S. I. and Martiny, J. B. H.: Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units, mSphere., 3, e00148-00118, https://doi.org/10.1128/mSphere, 2018.

Grob, C., Ulloa, O., Li, W. K. W., Alarcon, G., Fukasawa, M., and Watanabe, S.: Picoplankton abundance and biomass across the eastern South Pacific Ocean along latitude 32.5 degrees S, Mar. Ecol. Prog. Ser., 332, 53-62, https://doi.org/10.3354/meps332053, 2007.

Hansen, H. P. and Koroleff, F.: Determination of nutrients, Methods Seawater Anal., 23, 159–228, https://doi.org/10.1002/9783527613984.ch10, 1999.

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

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

654 Holdgate, M. W.: Terrestrial ecosystems in Antarctic, Philos. Trans. R. Soc. Lond., B,

655   Biol. Sci., 279, 5-25, https://doi.org/10.1098/rstb.1977.0068, 1977.

656 Horner-Devine, M. C. and Bohannan, B. J. M.: Phylogenetic clustering and

657   overdispersion in bacterial communities, Ecology, 87, S100-S108,

658   https://doi.org/10.1890/0012-9658(2006)87[100:Pcaoib]2.0.Co;2, 2006.

659 Izaguirre, I., Allende, L., and Marinone, M. C.: Comparative study of the planktonic

660   communities from lakes of contrasting trophic status at Hope Bay (Antarctic

661   Peninsula), J. Plankton Res., 25, 1079–1097,

662   https://doi.org/10.1093/plankt/25.9.1079, 2003.

663 Izaguirre, I., Allende, L., and Romina Schiaffino, M.: Phytoplankton in Antarctic

664   lakes: biodiversity and main ecological features, Hydrobiologia, 848, 177–207,

665   https://doi.org/10.1007/s10750-020-04306-x, 2020.

666 Kardol, P., Souza, L., and Classen, A. T.: Resource availability mediates the

667   importance of priority effects in plant community assembly and ecosystem

668   function, Oikos., 122, 84-94, https://doi.org/10.1111/j.1600-0706.2012.20546.x,

669   2013.

670 Karimi, B., Maron, P. A., Chemidlin-Prevost Boure, N., Bernard, N., Gilbert, D., and

671   Ranjard, L.: Microbial diversity and ecological networks as indicators of

672   environmental quality, Environ. Chem. Lett., 15, 265-281,

673   https://doi.org/10.1007/s10311-017-0614-6, 2017.

674 Katechakis, A. and Stibor, H.: The mixotroph Ochromonas tuberculata may invade

675   and suppress specialist phago- and phototroph plankton communities depending

676   on nutrient conditions, Oecologia., 148, 692-701,

677   https://doi.org/10.1007/s00442-006-0413-4, 2006.

678 Kawecka, B., Olech, M., Nowogrodzka-Zagorska, M., and Wojtun, B.: Diatom

679   communities in small water bodies at H. Arctowski Polish Antarctic Station

680   (King George Island, South Shetland Islands, Antarctica), Polar Biol., 19, 183-

681   192, https://doi.org/10.1007/s003000050233, 1998.

682 Kong, J., Wang, Y., Warren, A., Huang, B., and Sun, P.: Diversity distribution and
683   assembly mechanisms of planktonic and benthic microeukaryote communities in
684   intertidal zones of southeast Fujian, China, Front. Microbiol., 10, 2640,
685   https://doi.org/10.3389/fmicb.2019.02640, 2019.

686 Leblanc, K., Queguiner, B., Diaz, F., Cornet, V., Michel-Rodriguez, M., Durrieu de
687   Madron, X., Bowler, C., Malviya, S., Thyssen, M., Gregori, G., Rembauville, M.,
688   Grosso, O., Poulain, J., de Vargas, C., Pujo-Pay, M., and Conan, P.:
689   Nanoplanktonic diatoms are globally overlooked but play a role in spring blooms
690   and carbon export, Nat. Commun., 9, 953, https://doi.org/10.1038/s41467-018-
691   03376-9, 2018.

692 Lee, J. R., Raymond, B., Bracegirdle, T. J., Chades, I., Fuller, R. A., Shaw, J. D., and
693   Terauds, A.: Climate change drives expansion of Antarctic ice-free habitat,
694   Nature, 547, 49-54, https://doi.org/10.1038/nature22996, 2017.

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

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

702 Liang, Y., Ning, D., Lu, Z., Zhang, N., Hale, L., Wu, L., Clark, I. M., McGrath, S. P.,
703   Storkey, J., Hirsch, P. R., Sun, B., and Zhou, J.: Century long fertilization
704   reduces stochasticity controlling grassland microbial community succession, Soil
705   Biol. Biochem., 151, 108023, https://doi.org/10.1016/j.soilbio.2020.108023,
706   2020.

707 Liu, K., Liu, Y., Hu, A., Wang, F., Chen, Y., Gu, Z., Anslan, S., and Hou, J.: Different
708   community assembly mechanisms underlie similar biogeography of bacteria and
709   microeukaryotes in Tibetan lakes, FEMS Microbiol. Ecol., 96, fiaa071,

https://doi.org/10.1093/femsec/fiaa071, 2020.

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

Lizotte, M. P.: Phytoplankton and primary production, in: Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems, edited by: Warwick F. Vincent, and Johanna Laybourn-Parry, Oxford, 9, 157-178, https://doi.org/10.1093/acprof:oso/9780199213887.003.0009, 2008.

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

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

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

Lupatini, M., Suleiman, A. K. A., Jacques, R. J. S., Antoniolli, Z. I., de Siqueira Ferreira, A. o., Kuramae, E. E., and Roesch, L. F. W.: Network topology reveals high connectance levels and few key microbial genera within soils, 2, 10, Front. Environ. Sci., https://doi.org/10.3389/fenvs.2014.00010, 2014.

Lyons, W. B., Laybourn-Parry J., Welch K A., and Priscu, J. C.: Antarctic Lake Systems and Climate Change, in: Trends in Antarctic Terrestrial and Limnetic Ecosystems, edited by: Bergstrom, D.M., Convey, P., Huiskes, A.H.L., Springer,

738   Dordrecht, 273-295, https://doi.org/10.1007/1-4020-5277-4_13, 2007.

739   Ma, Z. S.: DAR (diversity-area relationship): Extending classic SAR (species-area

740   relationship) for biodiversity and biogeography analyses, Ecol. Evol., 8, 10023-

741   10038, https://doi.org/10.1002/ece3.4425, 2018.

742   Magoc, T. and Salzberg, S. L.: FLASH: fast length adjustment of short reads to

743   improve   genome   assemblies,   Bioinformatics,   27,   2957-2963,

744   https://doi.org/10.1093/bioinformatics/btr507, 2011.

745   Margesin, R. and Miteva, V.: Diversity and ecology of psychrophilic microorganisms,

746   Res. Microbiol., 162, 346-361, https://doi.org/10.1016/j.resmic.2010.12.004,

747   2011.

748   Marquardt, M., Vader, A., Stubner, E. I., Reigstad, M., and Gabrielsen, T. M.: Strong

749   Seasonality of Marine Microbial Eukaryotes in a High-Arctic Fjord (Isfjorden, in

750   West Spitsbergen, Norway), Appl. Environ. Microbiol., 82, 1868-1880,

751   https://doi.org/10.1128/AEM.03208-15, 2016.

752   Marsh, N. B., Lacelle, D., Faucher, B., Cotroneo, S., Jasperse, L., Clark, I. D., and

753   Andersen, D. T.: Sources of solutes and carbon cycling in perennially ice-

754   covered   Lake   Untersee,   Antarctica,   Sci.   Rep.,   10,   12290,

755   https://doi.org/10.1038/s41598-020-69116-6, 2020.

756   Massana, R. and Logares, R.: Eukaryotic versus prokaryotic marine picoplankton

757   ecology, Environ. Microbiol., 15, 1254-1261, https://doi.org/10.1111/1462-

758   2920.12043, 2013.

759   Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., Chambouvet, A.,

760   Christen, R., Claverie, J. M., Decelle, J., Dolan, J. R., Dunthorn, M., Edvardsen,

761   B., Forn, I., Forster, D., Guillou, L., Jaillon, O., Kooistra, W. H. C. F., Logares,

762   R., Mahe, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M. C., Probert, I.,

763   Romac, S., Richards, T., Santini, S., Shalchian-Tabrizi, K., Siano, R., Simon, N.,

764   Stoeck, T., Vaulot, D., Zingone, A., and de Vargas, C.: Marine protist diversity in

765   European   coastal   waters   and   sediments   as   revealed   by   high-throughput

sequencing, Environ. Microbiol., 17, 4035-4049, https://doi.org/10.1111/1462-2920.12955, 2015.

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

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

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

Nicholls, K. H.: Chrysophyte Blooms in the Plankton and Neuston of Marine and Freshwater Systems, in: Chrysophyte Algae: Ecology, Phylogeny and Development, edited by: C. Sandgren, J. Smol, & J. Kristiansen, Cambridge: Cambridge University Press, 181-213, https://doi.org/10.1017/CBO9780511752292.010, 1995.

Ofiteru, I. D., Lunn, M., Curtis, T. P., Wells, G. F., Criddle, C. S., Francis, C. A., and Sloan, W. T.: Combined niche and neutral effects in a microbial wastewater treatment community, Proc. Natl. Acad. Sci. U. S. A., 107, 15345-15350, https://doi.org/10.1073/pnas.1000604107, 2010.

Orrock, J. L. and Watling, J. I.: Local community size mediates ecological drift and competition in metacommunities, P. ROY. SOC. B-BIOL. SCI., 277, 2185-2191, https://doi.org/10.1098/rspb.2009.2344, 2010.

Pearce, D. A. and Galand, P. E.: Microbial biodiversity and biogeography, in: Polar lakes and rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems, edited by: Vincent, W. F. & J. Laybourn-Parry, Oxford University Press, Oxford, 213–230, https://doi.org/10.1093/acprof:oso/9780199213887.003.0012, 2008.

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

798 Pick, F. R. and Lean, D.: Diurnal movements of metalimnetic phytoplankton, J.
799   Phycol., 20, 430–436, https://doi.org/10.1111/j.0022-3646.1984.00430.x, 1984.

800 Powell, J. R., Karunaratne, S., Campbell, C. D., Yao, H., Robinson, L., and Singh, B.
801   K.: Deterministic processes vary during community assembly for ecologically
802   dissimilar taxa, Nat. Commun., 6, 8444, https://doi.org/10.1038/ncomms9444,
803   2015.

804 Priscu, J. C.: The biogeochemistry of nitrous oxide in permanently ice‐covered lakes
805   of the McMurdo Dry Valleys, Antarctica., Glob. Chang. Biol., 3, 301-315,
806   https://doi.org/10.1046/j.1365-2486.1997.00147.x, 2010.

807 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and
808   Glöckner, F.: The SILVA ribosomal RNA gene database project: improved data
809   processing and web-based tools, Nucleic Acids Res., 41, 590-596,
810   https://doi.org/10.1093/nar/gks1219, 2013.

811 Quiroga, M. V., Unrein, F., González Garraza, G., Küppers, G., Lombardo, R.,
812   Marinone, M. C., Menu Marque, S., Vinocur, A., and Mataloni, G.: The plankton
813   communities from peat bog pools: structure, temporal variation and
814   environmental factors, J. Plankton Res., 35, 1234-1253,
815   https://doi.org/10.1093/plankt/fbt082, 2013.

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

820 Richards, T. A., Vepritskiy, A. A., Gouliamova, D. E., and Nierzwicki-Bauer, S. A.:
821   The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake

822   reveals diverse, distinctive and globally dispersed lineages, Environ. Microbiol.,

823   7, 1413-1425, https://doi.org/10.1111/j.1462-2920.2005.00828.x, 2005.

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

825 Roberts, E. C. and Laybourn-Parry, J.: Mixotrophic cryptophytes and their predators

826   in the Dry Valley lakes of Antarctica, Freshw. Biol., 41, 737–746,

827   https://doi.org/10.1046/j.1365-2427.1999.00401.x, 1999.

828 Santos, J. A. D., Meyer, E., and Sette, L. D.: Fungal Community in Antarctic Soil

829   Along the Retreating Collins Glacier (Fildes Peninsula, King George Island),

830   Microorganisms., 8, 1145, https://doi.org/10.3390/microorganisms8081145, 2020.

831 Stegen, J. C., Lin, X., Konopka, A. E., and Fredrickson, J. K.: Stochastic and

832   deterministic assembly processes in subsurface microbial communities, ISME J.,

833   6, 1653-1664, https://doi.org/10.1038/ismej.2012.22, 2012.

834 Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J.,

835   Rockhold, M. L., and Konopka, A.: Quantifying community assembly processes

836   and identifying features that impose them, ISME J., 7, 2069-2079,

837   https://doi.org/10.1038/ismej.2013.93, 2013.

838 Sun, D., Bi, Q., Li, K., Dai, P., Yu, Y., Zhou, W., Lv, T., Liu, X., Zhu, J., Zhang, Q.,

839   Jin, C., Lu, L., and Lin, X.: Significance of temperature and water availability for

840   soil phosphorus transformation and microbial community composition as

841   affected by fertilizer sources, Biol. Fertil. Soils, 54, 229-241,

842   https://doi.org/10.1007/s00374-017-1252-7, 2017.

843 Sun, P., Wang, Y., Huang, X., Huang, B., and Wang, L.: Water masses and their

844   associated temperature and cross-domain biotic factors co-shape upwelling

845   microbial communities, Water Res., 215, 118274,

846   https://doi.org/10.1016/j.watres.2022.118274, 2022.

847 ter Braak, C. and Smilauer, P.: CANOCO Reference Manual and CanoDraw for

848   Windows User's Guide: Software for Canonical Community Ordination (version

849   4.5), https://doi.org/edepot.wur.nl/405659, 2002.

850   Toro, M., Camacho, A., Rochera, C., Rico, E., Banon, M., Fernandez-Valiente, E.,

851   Marco, E., Justel, A., Avendano, M. C., Ariosa, Y., Vincent, W. F., and Quesada,

852   A.: Limnological characteristics of the freshwater ecosystems of Byers Peninsula,

853   Livingston Island, in maritime Antarctica, Polar Biol., 30, 635-649,

854   https://doi.org/10.1007/s00300-006-0223-5, 2007.

855   Unrein, F., Gasol, J. M., Not, F., Forn, I., and Massana, R.: Mixotrophic haptophytes

856   are key bacterial grazers in oligotrophic coastal waters, ISME J., 8, 164-176,

857   https://doi.org/10.1038/ismej.2013.132, 2014.

858   Unrein, F., Izaguirre, I., Massana, R., Balague, V., and Gasol, J. M.: Nanoplankton

859   assemblages in maritime Antarctic lakes: characterisation and molecular

860   fingerprinting comparison, Aquat. Microb. Ecol., 40, 269-282,

861   https://doi.org/10.3354/ame040269, 2005.

862   Wang, F., Huang, B., Xie, Y., Cai, S., Wang, X., and Mu, J.: Diversity, Composition,

863   and Activities of Nano- and Pico-Eukaryotes in the Northern South China Sea

864   With Influences of Kuroshio Intrusion, Front. Mar. Sci., 8, 658233,

865   https://doi.org/10.3389/fmars.2021.658233, 2021.

866   Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R.: Naive Bayesian classifier for

867   rapid assignment of rRNA sequences into the new bacterial taxonomy, Appl.

868   Environ. Microbiol., 73, 5261-5267, https://doi.org/10.1128/Aem.00062-07,

869   2007.

870   Wang, W., Ren, K., Chen, H., Gao, X., Ronn, R., and Yang, J.: Seven-year dynamics

871   of testate amoeba communities driven more by stochastic than deterministic

872   processes in two subtropical reservoirs, Water Res., 185, 116232,

873   https://doi.org/10.1016/j.watres.2020.116232, 2020a.

874   Wang, Y., Li, G., Shi, F., Dong, J., Gentekaki, E., Zou, S., Zhu, P., Zhang, X., and

875   Gong, J.: Taxonomic diversity of pico-/nanoeukaryotes is related to dissolved

876   oxygen and productivity, but functional composition is shaped by limiting

877   nutrients in eutrophic coastal oceans, Front Microbiol, 11, 601037,

878   https://doi.org/10.3389/fmicb.2020.601037, 2020b.

879   Webb, C. O., Ackerly, D. D., McPeek, M. A., and Donoghue, M. J.: Phylogenies and

880   Community Ecology, Annu. Rev. Ecol. Syst., 33, 475-505,

881   https://doi.org/10.1146/annurev.ecolsys.33.010802.150448, 2002.

882   Wilkins, D., Yau, S., Williams, T. J., Allen, M. A., Brown, M. V., DeMaere, M. Z.,

883   Lauro, F. M., and Cavicchioli, R.: Key microbial drivers in Antarctic aquatic

884   environments, FEMS Microbiol. Rev., 37, 303-335, https://doi.org/10.1111/1574-

885   6976.12007, 2013.

886   Wu, L. W., Ning, D. L., Zhang, B., Li, Y., Zhang, P., Shan, X. Y., Zhang, Q. T., Brown,

887   M. R., Li, Z. X., Van Nostrand, J. D., Ling, F. Q., Xiao, N. J., Zhang, Y.,

888   Vierheilig, J., Wells, G. F., Yang, Y. F., Deng, Y., Tu, Q. C., Wang, A. J., Zhang,

889   T., He, Z. L., Keller, J., Nielsen, P. H., Alvarez, P. J. J., Criddle, C. S., Wagner,

890   M., Tiedje, J. M., He, Q., Curtis, T. P., Stahl, D. A., Alvarez-Cohen, L., Rittmann,

891   B. E., Wen, X. H., and Zhou, J. Z.: Global diversity and biogeography of

892   bacterial communities in wastewater treatment plants Nat. Microbiol., 4, 2579-

893   2579, https://doi.org/10.1038/s41564-019-0617-0, 2019.

894   Xu, D., Kong, H., Yang, E. J., Wang, Y., Li, X., Sun, P., Jiao, N., Lee, Y., Jung, J., and

895   Cho, K. H.: Spatial dynamics of active microeukaryotes along a latitudinal

896   gradient: Diversity, assembly process, and co-occurrence relationships, Environ.

897   Res., 212, 113234, https://doi.org/10.1016/j.envres.2022.113234, 2022.

898   Yubuki, N., Nakayama, T., and Inouye, I.: A unique life cycle and perennation in a

899   colorless chrysophyte Spumella sp., J. Phycol., 44, 164-172,

900   https://doi.org/10.1111/j.1529-8817.2007.00441.x, 2008.

901   Zeng, J., Jiao, C., Zhao, D., Xu, H., Huang, R., Cao, X., Yu, Z., and Wu, Q. L.:

902   Patterns and assembly processes of planktonic and sedimentary bacterial

903   community differ along a trophic gradient in freshwater lakes, Ecol. Indic., 106,

904   105491, https://doi.org/10.1016/j.ecolind.2019.105491, 2019.

905   Zhang, L., Yin, W., Wang, C., Zhang, A., Zhang, H., Zhang, T., and Ju, F.: Untangling

microbiota diversity and assembly patterns in the world's largest water diversion canal, Water Res., 204, 117617, https://doi.org/10.1016/j.watres.2021.117617, 2021.

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

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

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

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

Zhu, G. H., Shuji, O., Hu, C. Y., He, J. F., Jin, M., Yu, P. S., and Pan, J. M.: Impact of global climate change on antarctic freshwater algae, China Environ. Sci., 30, 400-404, 2010.

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

Figure Captions

933 **Figure Captions**

934 **Fig. 1** (a) Location of the five studied lakes on the Fildes Peninsula, King George

935 Island, Antarctica, (b) Lake Xi Hu (XH), (c) Lake Yan Ou (YO), (d) Lake Chang Hu

936 (CH), (e) Lake Yue Ya (YY), and (f) Lake Kitec (KT).

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

938 spatial dynamics of relative abundance on Phylum level in five lakes from 2017 to

939 2019. The 17, 18, and 19 represent the expedition season 2017/2018, 2018/2019, and

940 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different

941 lakes. The Lakes that showed no significant differences are not shown ($P>0.05$).

942 (c)Temporal and spatial dynamics of relative abundance on the genus level in the

943 studied five lakes. (note: $**P < 0.01$, $*P < 0.05$). (Chlorophyta: *Atractomorpha*,

944 *Chlamydomonas*, *Mantoniella*, *Chlorella*, *Choricystis*, *Crustomastix*, *Microglena*,

945 *Monomastix*, *Nannochloris*; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*,

946 *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*;

947 Cryptophyta: *Komma*; Haptophyta: *Diacronema*; Cercozoa: *Heteromita*. The relative

948 abundance in any lake was less than 1% is defined as others).

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

950 Variations in the number of microbial operational taxonomic units (OTUs); (c, d)

951 variations in the microbial Shannon index; (e, f) variations in the within-community

952 nearest-taxon index (NTI); (g, h) Venn diagram showing the unique and shared OTUs.

953 Homogeneity and one-way ANOVA analyses of variance were used to test the

954 significance of the indices. "ns" represents no significant differences ($P>0.05$),

955 significant differences ($P<0.05$) are indicated by different letters between lakes, and

956 lakes containing the same letters showed no significant difference ($P>0.05$)

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

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

959   based on similarity (b).

960   **Fig.5** The effect of environmental variables on microbial eukaryotic communities, and

961   co-occurrence pattern: (a) Canonical correlation analysis plots and (b) variance

962   partitioning analysis. Sal: salinity; WT: water temperature; NO_2^-: nitrite nitrogen;

963   PO_4^{3-}: phosphate. (c) Network analysis revealing the interspecies associations

964   between microbial eukaryotic operational taxonomic units (OTUs), and the

965   correlation between environmental factors and OTUs in the lakes' integrated networks.

966   The size of each OTUs or environmental factor (node) is proportional to the degree

967   centrality. Others: other phyla and unclassified taxa.

968   **Fig. 6** Relative influences of deterministic and stochastic processes on microbial

969   eukaryotic community assembly based on the neutral community model (NCM) and

970   the null model. (a) Fit of the NCM of community assembly. Nm indicates the

971   metacommunity size and R^2 indicates the fit to the NCM. Neutral prediction was

972   within a 95% confidence interval (black), while non-neutral processes included the

973   above and below prediction (dark green and red). (b) Proportions of the richness and

974   abundance of the three groups (above prediction, below prediction, and neutral

975   prediction) based on the NCM. (c) Abundance composition of the three groups in the

976   microbial eukaryotic community. (d) Null model analysis revealing the fraction of

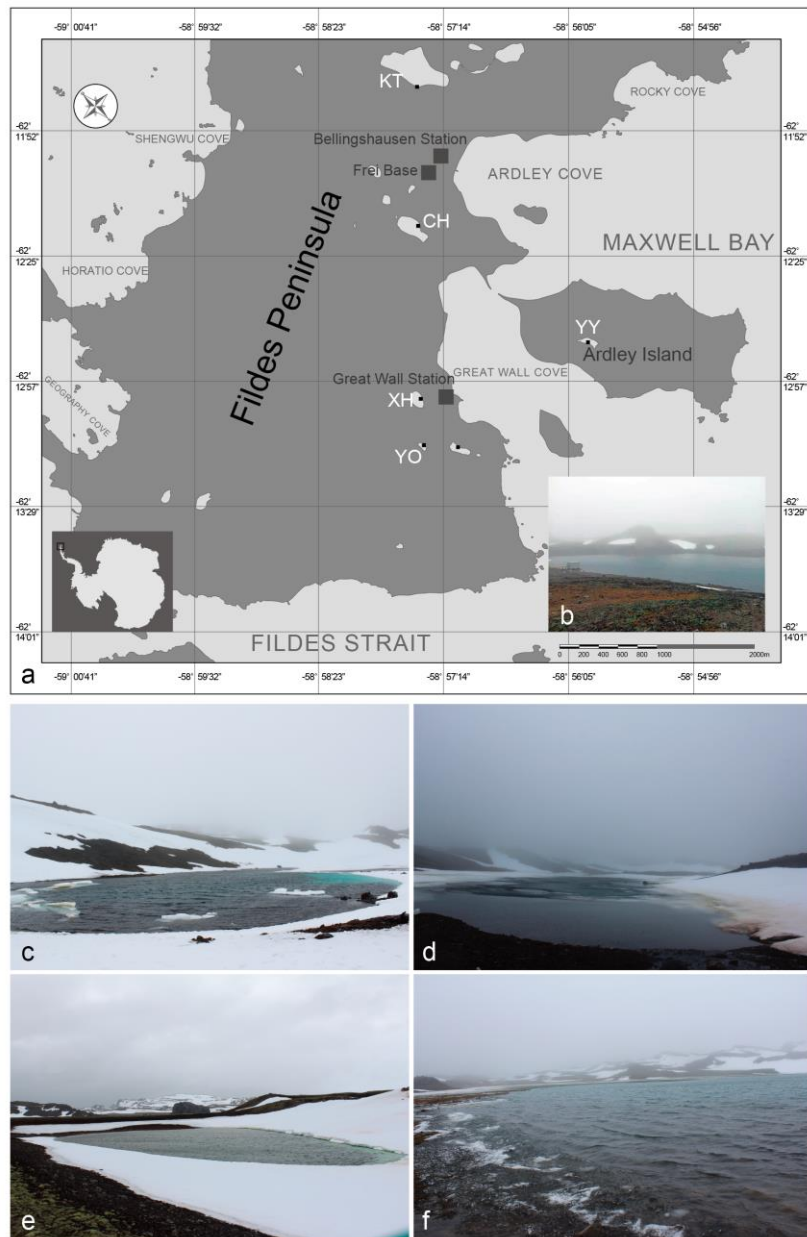
977   ecological processes. The percent of community assembly is governed primarily by

978   various deterministic processes, such as homogenous and heterogeneous selections,

979   and stochastic processes, including dispersal limitations, homogenizing dispersal, and

980   undominated processes (i.e., weak selection, weak dispersal, diversification, and drift

981   processes).

982 **Fig. 1**

983

984

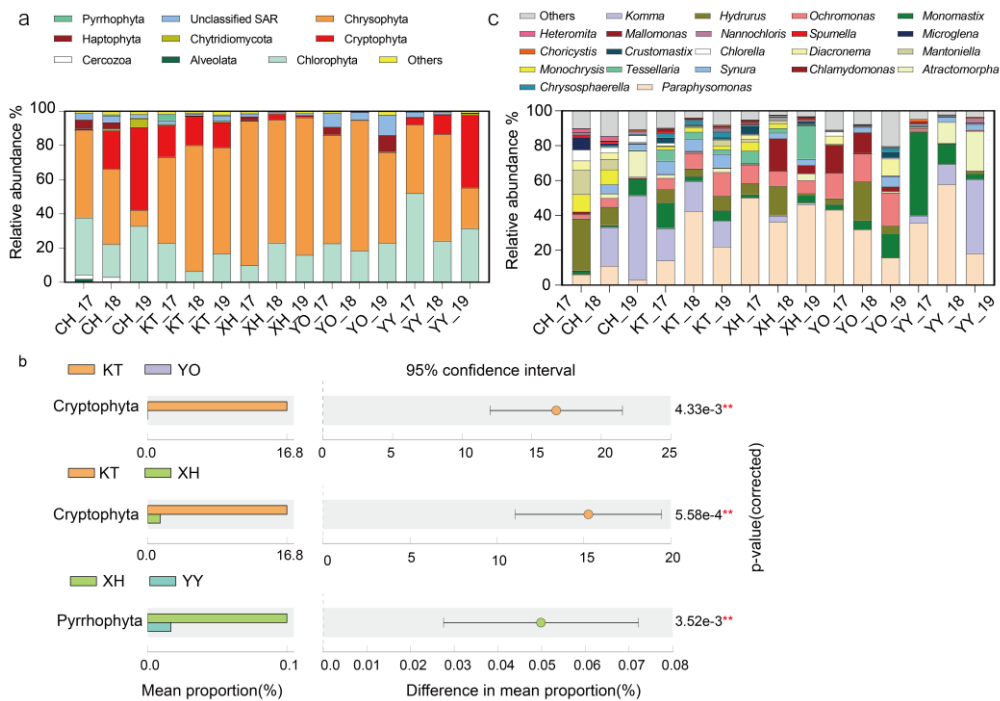
985 **Fig. 1** (a) Location of the five studied lakes on the Fildes Peninsula, King George

986 Island, Antarctica, (b) Lake Xi Hu (XH), (c) Lake Yan Ou (YO), (d) Lake Chang Hu

987 (CH), (e) Lake Yue Ya (YY), and (f) Lake Kitec (KT).

988

989 **Fig. 2**

990

991

992

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

994 spatial dynamics of relative abundance on Phylum level in five lakes from 2017 to

995 2019. The 17, 18, and 19 represent the expedition season 2017/2018, 2018/2019, and

996 2019/2020, respectively. (b) Differential analysis of microbial eukaryotes in different

997 lakes. The Lakes that showed no significant differences are not shown ($P>0.05$).

998 (c)Temporal and spatial dynamics of relative abundance on the genus level in the

999 studied five lakes. (note: **$P < 0.01$, *$P < 0.05$). (Chlorophyta: *Atractomorpha*,

1000 *Chlamydomonas*, *Mantoniella*, *Chlorella*, *Choricystis*, *Crustomastix*, *Microglena*,

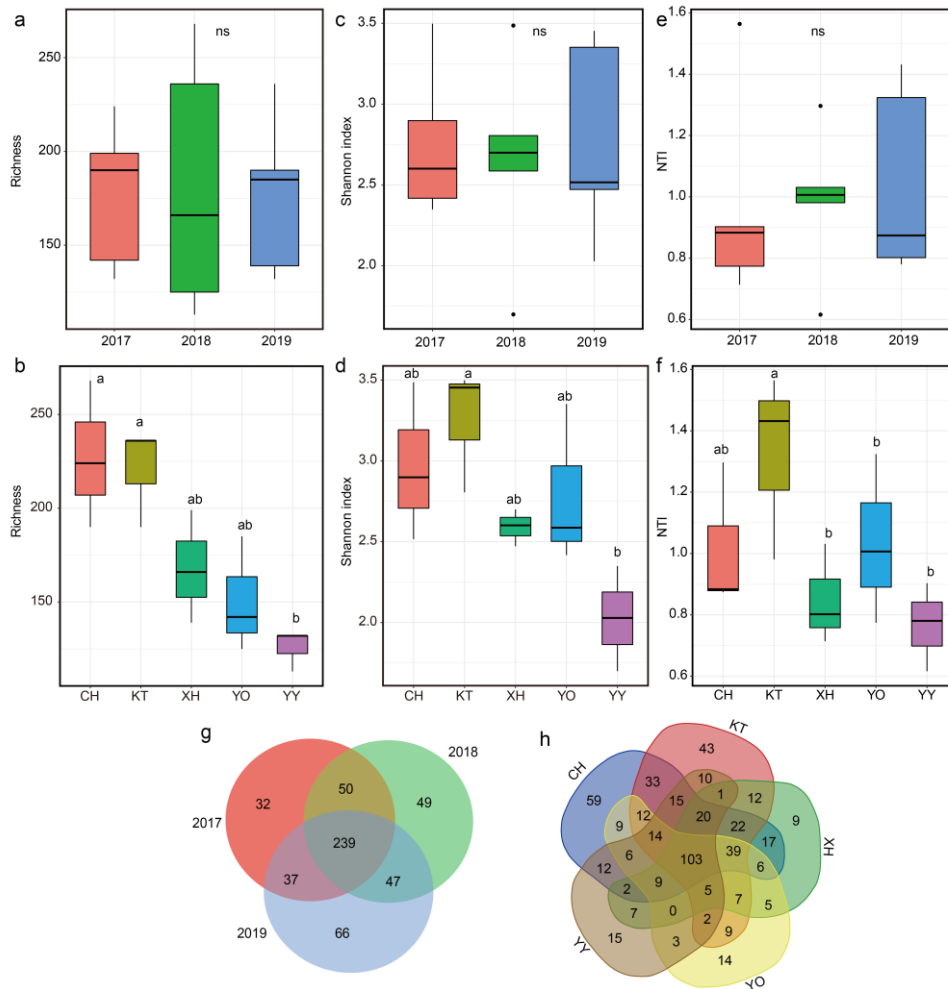
1001 *Monomastix*, *Nannochloris*; Chrysophyta: *Chrysosphaerell*, *Hydrurus*, *Mallomonas*,

1002 *Monochrysis*, *Ochromonas*, *Paraphysomonas*, *Spumella*, *Synura*, *Tessellaria*;

1003 Cryptophyta: *Komma*; Haptophyta: *Diacronema*; Cercozoa: *Heteromita*. The relative

1004 abundance in any lake was less than 1% is defined as others).

1005

1006 **Fig. 3**

1007

1008

1009 **Fig.3** Microbial diversity and Venn diagram for different years and lakes. (a, b)

1010 Variations in the number of microbial operational taxonomic units (OTUs); (c, d)

1011 variations in the microbial Shannon index; (e, f) variations in the within-community

1012 nearest-taxon index (NTI); (g, h) Venn diagram showing the unique and shared OTUs .

1013 Homogeneity and one-way ANOVA analyses of variance were used to test the

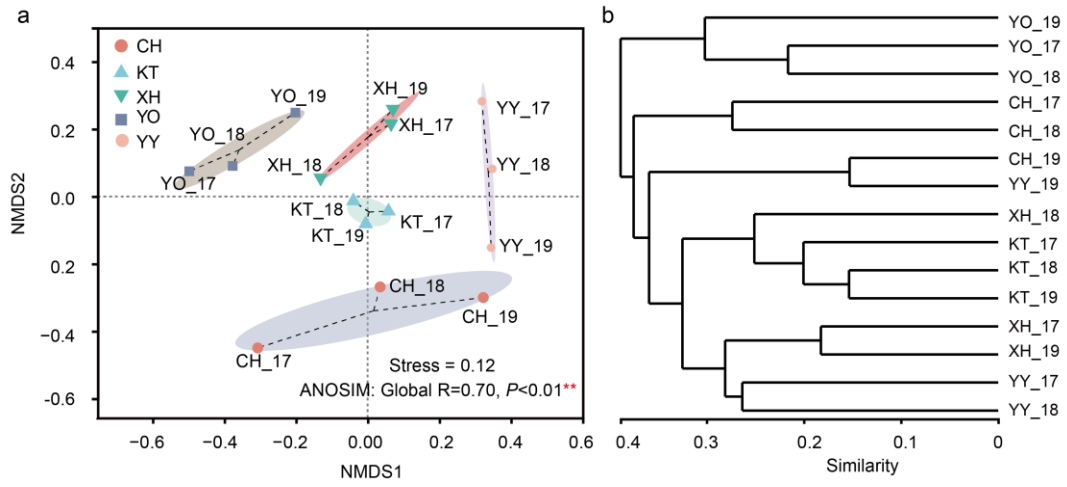
1014 significance of the indices. "ns" represents no significant differences ($P>0.05$),

1015 significant differences ($P<0.05$) are indicated by different letters between lakes, and

1016 lakes containing the same letters showed no significant difference ($P>0.05$)

1017

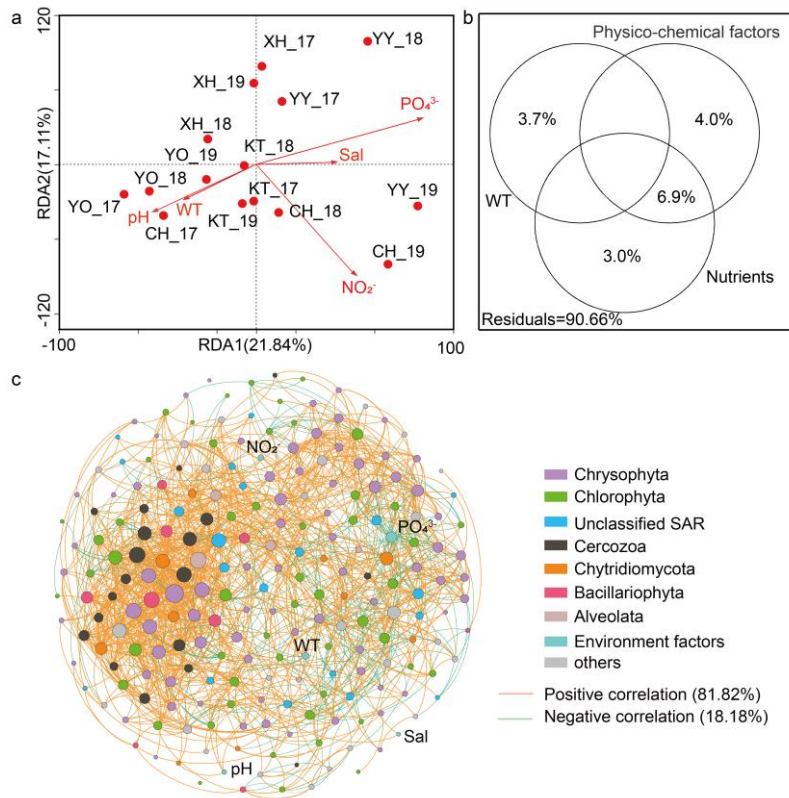
1018

1019 **Fig. 4**

1020

1021 **Fig. 4** Temporal variability analysis of non-metric multidimensional scaling (NMDS)

1022 ordination of the microbial eukaryotic communities (a) and clustering of five lakes

1023 based on similarity (b).

1024

 Fig. 5

1026

1027 **Fig.5** The effect of environmental variables on microbial eukaryotic communities, and

1028 co-occurrence pattern: (a) Canonical correlation analysis plots and (b) variance

1029 partitioning analysis. Sal: salinity; WT: water temperature; NO_2^-: nitrite nitrogen;

1030 PO_4^{3-}: phosphate. (c) Network analysis revealing the interspecies associations

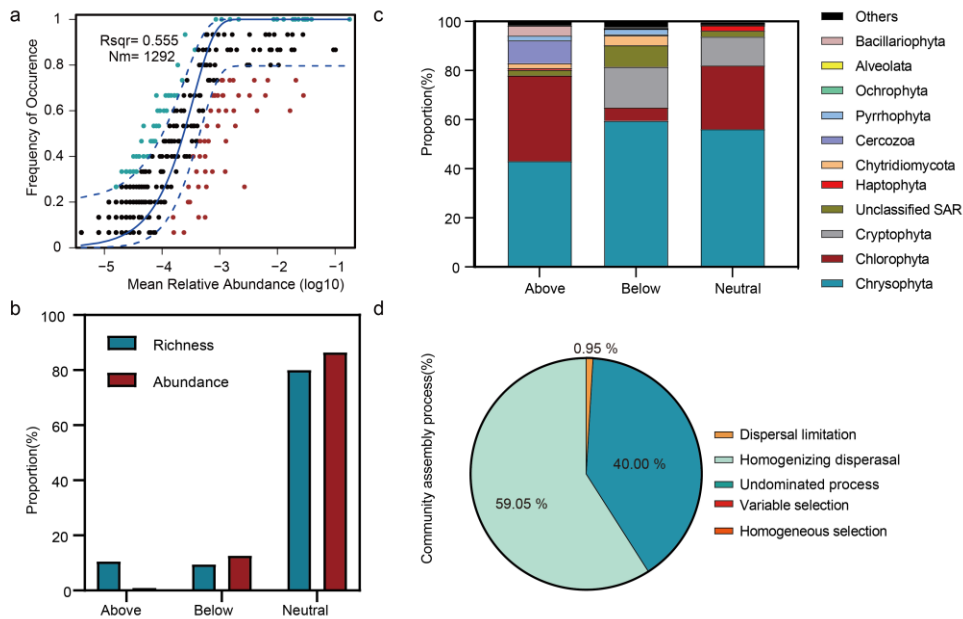
1031 between microbial eukaryotic operational taxonomic units (OTUs), and the

1032 correlation between environmental factors and OTUs in the lakes' integrated networks.

1033 The size of each OTUs or environmental factor (node) is proportional to the degree

1034 centrality. Others: other phyla and unclassified taxa.

1035

1036   **Fig. 6**

1037

1038   **Fig. 6** Relative influences of deterministic and stochastic processes on microbial

1039   eukaryotic community assembly based on the neutral community model (NCM) and

1040   the null model. (a) Fit of the NCM of community assembly. Nm indicates the

1041   metacommunity size and R^2 indicates the fit to the NCM. Neutral prediction was

1042   within a 95% confidence interval (black), while non-neutral processes included the

1043   above and below prediction (dark green and red). (b) Proportions of the richness and

1044   abundance of the three groups (above prediction, below prediction, and neutral

1045   prediction) based on the NCM. (c) Abundance composition of the three groups in the

1046   microbial eukaryotic community. (d) Null model analysis revealing the fraction of

1047   ecological processes. The percent of community assembly is governed primarily by

1048   various deterministic processes, such as homogenous and heterogeneous selections,

1049   and stochastic processes, including dispersal limitations, homogenizing dispersal, and

1050   undominated processes (i.e., weak selection, weak dispersal, diversification, and drift

1051   processes).