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## Microbial community day-to-day dynamics during a spring algal bloom event in a tributary of Three Gorges Reservoir

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# The daily dynamics of algal blooms: A case study in a tributary of Three Gorges Reservoir

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### 30 **Highlight**

- 31 1. Pengxi River, the largest tributary in the northern bank of the Three Gorges Reservoir,  
32 experienced an algal bloom with a peak chlorophyll a concentration as high as 219.5  
33  $\mu\text{g L}^{-1}$  from May 16 to 23, 2018
- 34 2. Main algal species of the bloom belonged to cyanobacteria and green algae; *microcystis*  
35 dominated the prokaryotic group
- 36 3. Along with it diurnal evolving, the algal bloom has brought diurnally considerably  
37 changes on nutrients, biodiversity and microbial loop in the aquatic system
- 38 4. Based on DNA high throughput sequencing, fungi and microzooplankton were strongly  
39 negatively related with each other during the whole algal bloom process

40  
41

42 **Abstract:** Construction of dams increases water residence time of tributaries in the dam  
43 catchments, leading to conditions in favour of algal growth. We hypothesized that the  
44 microbial community in dammed rivers would experience fast successions in biodiversity  
45 triggered by the process of algal blooms. To test this hypothesis, we investigated the impacts  
46 of algal blooms on microbial community during a 7-day algal bloom event in the Three  
47 Gorges Reservoir in May 2018. The chlorophyll a concentration, microscopic identification,  
48 nutrient concentration including total phosphorus and nitrogen, dissolved total phosphorus  
49 and nitrogen, and DNA high throughput sequencing were measured for the daily surficial  
50 water samples. Throughout the bloom, we found significant diurnal changes in nitrogen,  
51 phosphorus, plankton biodiversity, as well as in the composition of the microbial community.  
52 The algal bloom, both algal density and chlorophyll a, peaked on May 20-21, when the  
53 phytoplankton community was dominated by *Chlorella* sp. and *Microcystis* spp., with  
54 chlorophyll a concentration as high as 219  $\mu\text{g L}^{-1}$ . Both the concentrations of dissolved total  
55 nitrogen and phosphorus fluctuated, and at the bloom peak the dissolved total nitrogen  
56 declined to the second lowest, and the dissolved phosphorus the lowest, respectively during  
57 the whole bloom period. Based on DNA high throughput sequencing data, the Shannon and  
58 ACE indexes of both prokaryotic and eukaryotic microbes were significantly different on  
59 daily basis ( $P < 0.01$ ); so were the DNA proportions of eukaryotic and prokaryotic  
60 phytoplankton, microzooplankton (20~200 $\mu\text{m}$ ), mesozooplankton (>200 $\mu\text{m}$ ), and fungal  
61 communities. By capturing the inherent system variability and dynamics, this research  
62 demonstrates that the algal blooms cause major changes in the microbial food loop in  
63 lakes/rivers, which can be used to inform management of HABs.

64       **Keywords:** Algal bloom; Dammed rivers; Three Gorges Reservoir; Aquatic microbial  
65 loop; Nutrients

66

## 67 1. Introduction

68       Inland lakes and coastal areas close to anthropogenic activities are usually the most  
69 common domains for algal blooms (Anderson and Burkholder, 2002; Ho et al., 2019).  
70 However, it has become clear that dammed waterways provide a significant hotspot for  
71 formation of algal blooms (Acharyya et al., 2012; Xin et al., 2020). Although there are  
72 substantive benefits for flood regulation and hydropower, dams modify the natural landscape  
73 of ecological functions across whole river basins (Simon, 2002; Maavara et al., 2020). Altered  
74 tributaries in the catchment virtually change from lotic to lentic ecosystems (Resh et al.,  
75 1988), which with nutrient loading provides favorable conditions for massive harmful algal  
76 blooms (HABs) composed of species such as *Microcystis*, *Dolichospermum*, *Anabaena* and  
77 *Aphanizomenon* (Cirés et al., 2013; Zhang et al., 2018; Xin et al., 2020).

78       The microbial loop is a food chain based on autotrophic microorganisms (Fenchel, 2008).  
79 Heterotrophic microorganisms convert dissolved nutrients released during photosynthesis into  
80 themselves, which are then grazed by microscopic zooplankton (Buchan, 2014). During the  
81 development and collapse of algal blooms, large amounts of dissolved carbon and nutrients  
82 are captured in algal biomass, which are then either released back into water or lost to  
83 sediment. The released nutrients can be biologically available, and thereby fuel a secondary  
84 succession of other microbial and/or planktonic communities (Kim and Kim, 2013; Zhang et  
85 al., 2018). Aquatic heterotrophic bacteria are decomposers, e.g. *Roseobacter* or *Flavobacter*,

86 and are often associated with the termination of HAB events, converting the collapsed algal  
87 biomass into bioavailable nutrients that may hence promote secondary growths of other algae  
88 (Buchan et al., 2014). The secondary or even tertiary growths create new natural capital and  
89 trophic ecosystem structure, especially at the base of the food web (Engström-Öst et al., 2002;  
90 Engström-Öst et al., 2013; Long et al., 2019). For example, bacteria growing on cell-lysates  
91 can serve as a food source for micro-zooplankton (Work & Haven, 2003; Long et al., 2019).  
92 Therefore, algal blooms are not the end of carbon and nutrient cycling in aquatic habitats, but  
93 rather an intermediate temporal process of growth, biodiversity and ultimately nutrient  
94 recycling (Wilk-Wozniak, 2020). However, studies on these complex spatial and temporal  
95 cycles of microbial succession at the base of food web in response to HABs are still lacking  
96 (Xiao et al., 2016; Wilk-Wozniak, 2020).

97 As the largest hydroelectric project in the world to date, the Three Gorges Dam has been  
98 impounding water since 2003, reducing water flow, i.e. from 0.5–1 m s<sup>-1</sup> to 0.05 m s<sup>-1</sup> in the  
99 tributaries (Zhang et al., 2020), thus increasing residence time in the main channel and  
100 tributaries trapped behind the dam (Sha et al. 2015). Nutrients and organic matter constrained  
101 in these tributaries have led to serious eutrophication in the reservoir catchment (Yang et al.,  
102 2010). As a result, more than 50% of the first-order tributaries, i.e. 20 tributaries, in the Three  
103 Gorges Reservoir (TGR) area, have reported different degrees of HABs recurring each year  
104 (Ji et al., 2017; Zhang et al., 2020). One of those tributaries is the Pengxi River, the largest  
105 tributary on the northern bank of TGR, often suffers from intense cycling algal blooms for  
106 periods of up to 3 months from late spring till early summer (Zhang et al., 2020; Yang et al.,  
107 2020).



108 The objective of this study is to determine the impact of HAB events on nutrient and the  
109 microbial food loop in order to better understand and manage HABs in impounded waterways  
110 of lakes and reservoirs.

## 111 **2. Materials and Methods**

### 112 **2.1 Study area**

113 The TGR is located in the mid-reach of the Yangtze River, China. Constructed in 2003,  
114 the Yangtze River reached the planned impoundment level of 175 m above sea level (ASL) in  
115 2009 with a total reservoir capacity of 40 billion m<sup>3</sup> (Pan et al., 2018). As a result of the  
116 raising water level, a 660-km stretch of the Yangtze was transformed from a river into a  
117 reservoir, with an increase in water surface area from 645 to 1200 km<sup>2</sup>. For most of the year  
118 the water level in TGR is 175 m ASL to facilitate navigation and increased electricity  
119 generation. However, for flood control during the rainy season, the water table is reduced to  
120 145 m ASL for approximately three months (Three Gorges Corporation) (Ji et al., 2017; Pan  
121 et al., 2018).

122 In this paper we sampled daily during one bloom event from May 16 to May 23, 2018  
123 at monitoring station S1 (31°05'27.4" N, 108°40'41.6" E) in Gaoyang Lake (~25km<sup>2</sup>) of  
124 Pengxi River (Fig.1C). At this site, algal blooms have been observed regularly from the  
125 middle of April to the end of June (Zhang et al., 2010; Xiao et al., 2016; Zhang et al., 2020;  
126 Yang et al., 2020). The main stream of Pengxi River is 182.4 km long, with an average  
127 volume of 3.4 billion m<sup>3</sup> and a basin catchment area of 5173 km<sup>2</sup> (Zhang et al., 2020). Due to  
128 the seasonal water flows at the Three Gorges Dam, water levels in Gaoyang Lake fluctuate

129 with a water depth approaching 10 m during summer (i.e. June to August), and >40 m depth  
130 in the rest of the year (Xiao et al., 2016; Zhang et al., 2020). The velocity in May was  
131 averagely  $0.2 \text{ m s}^{-1}$  (Zhang et al., 2020). Therefore, the water retention time in Pengxi River  
132 in spring can be as long as 63 days (Xiao et al., 2016). In May 2018, the water retention time  
133 at the study site of Gaoyang Lake was approximately 33 days (Hu, 2020). The hydrodynamic  
134 conditions in the study area during the event period are considered relatively stable.

135 Weather data during the sampling period was collected from two nearby weather stations  
136 (# 57339 and 57432) in Yunyang County (China Meteorological Data Service Center,  
137 CMDC, <http://data.cma.cn/en/>) and mean daily values were calculated. The duration of  
138 sunlight was 10 hours during May 16-18, 4 hours on May 19-21 and 23, and 0 h on May 22  
139 due to the rain. The average daily temperature increased from  $24.2 \text{ }^{\circ}\text{C}$  to  $28.6 \text{ }^{\circ}\text{C}$  from 16 to  
140 21 May; from 17 to 18 May, there was light precipitation ( $< 9 \text{ mm}$ ). On 21 and 22 May, 2018  
141 a heavy rainstorm hit the area with precipitation of 152 mm within 24 h.

## 142 **2.2 Sampling collection and handling**

143 Local residents observed changes of water color in the Gaoyang Lake since May 11,  
144 2018. Our sampling was then initiated from 16 May 2018 onwards until 23 May when the  
145 bloom disappeared following a heavy rainstorm. Daily sampling was performed between 8:30  
146 am and noon, except May 17<sup>th</sup> (instrument malfunction) and May 21 (heavy rainstorm).  
147 Sample collection and analysis were carried out following Ban et al. (2013). In summary,  
148 three locations were sampled on a transect at monitoring station S1, i.e. one sample site on  
149 S1, and the other two sample sites 50 m away on each side of S1. Depth-integrated (top 50

150 cm) water samples were collected using a plexi-glass tube sampler (13 cm in diameter × 30  
151 cm in length) (Xing Changyuan Ltd. Henan Province, China). On each sampling day, samples  
152 from the three locations were pooled and mixed in a larger glass container on board before  
153 samples were collected into new glass bottles. Four 1-liter water samples were collected from  
154 the larger glass container, among which 1L sample used for phytoplankton community  
155 composition analysis was added with Lugol's iodine reagent to a final concentration of 1%  
156 on-site to immobilized the algae; 1L sample for analysis of nutrients; 1L for chlorophyll-a  
157 (Chl a) was transported on ice in dark to laboratory within 3 h of collection and stored at 4 °C  
158 before chemical analysis; 1L for DNA high throughput sequencing was kept in a sterilized  
159 bottle, and filtered through a 0.22-μm membrane filter (Xingya, Shanghai, China) on the boat  
160 immediately. The membranes were then placed on dry ice afterwards. In the lab, each  
161 membrane was then stored at -80 °C until DNA extractions by following Wang et al. (2018).

### 162 ***2.3 Nutrients and Chlorophyll a measurement***

163 Within 24 hours after collection, total nitrogen (TN), total phosphorus (TP), dissolved  
164 total nitrogen (DTN) and dissolved total phosphorus (DTP) were measured  
165 spectrophotometrically using the State Environmental Protection Administration (SEPA  
166 2002) guidelines.

167 Upon return to the lab the Chl a samples were filtered through a 0.45-μm cellulose  
168 membrane filter (Shanghai Xingya Plant) and stored under -80 °C before extraction. The  
169 filtered membranes were then extracted with 90% acetone for 24 h in dark at 0~4 °C. This  
170 extract was then centrifuged at 3500~4000 RPM for 10~15 min before the supernatant was

171 measured spectrophotometrically respectively (Cheng & Xue, 2012).

## 172 ***2.4 Phytoplankton community composition analysis***

173 In laboratory, the 1-L sub-samples were precipitated and concentrated to 0.1 ml before  
174 counting. Phytoplankton species were determined following keys in the "Freshwater algae in  
175 China" (Hu & Wei, 2006) and "Freshwater plankton research methods" (Zhang & Huang,  
176 1995). Algae counts were performed using an Olympus BX53 LED microscope under 14140  
177 or 16140 magnification with a 0.1ml plankton chamber (surface area: 20120 mm) (Hamilton  
178 et al., 2001). Samples were prepared in duplicate and 50-100 microscopic fields were counted  
179 for each. A third sample was considered if the difference of total counts between the two  
180 samples was greater than 15%.

181 The algal density was calculated using the following formula:

$$182 \quad N = \frac{C_s}{F_s \cdot F_n} \times \frac{V}{U} \times P_n$$

183 Where N is the algal concentration (ind L<sup>-1</sup>); C<sub>s</sub> is the count chamber area (mm<sup>2</sup>), 400  
184 mm<sup>2</sup> in this study; F<sub>s</sub> is the area of each field of view (mm<sup>2</sup>); F<sub>n</sub> is the number of fields that  
185 have been counted; V is the volume (ml) of a liter of water sample after precipitation and  
186 concentration; U is the volume (ml) of the chamber, which was 0.1 ml in this study; P<sub>n</sub> is the  
187 number of phytoplankton individuals that were counted.

## 188 ***2.5 DNA high throughput sequencing***

189 DNA high throughput sequencing (HTS) was performed by Majorbio Bio-pharm  
190 Technology Co., Ltd., Shanghai, China (<http://www.majorbio.com>), where the Illumina

191 MiniSeq platform was implemented by using primer sets 338F\_806R and SSU0817F\_1196R  
192 for 16S and 18S rDNA (Kumari et al., 2021), respectively to amplify relative genes in  
193 prokaryotic and eukaryotic microbes. The databases used for analyzing the rDNA data were  
194 silva 128/16S\_bacteria and silva 128/18S\_eukaryota respectively (<http://www.arb-silva.de>).  
195 Prokaryotes identified by 16S rDNA were divided into prokaryotic phytoplankton and  
196 heterotrophic bacteria at the phylum level (Garrity & Holt, 2001). Based on trophic  
197 classification and the regular size of species ((Hu & Wei, 2006), eukaryotes in the 18S rDNA  
198 profile were classified into four phyla: eukaryotic phytoplankton, micro-zooplankton, meso-  
199 zooplankton, and fungi.

## 200 ***2.7 Statistical analysis***

201 The DNA sequencing data were analyzed via the online Cloud Platform provided by  
202 Majorbio Ltd. Then the raw 16S and 18S sequences were sub-sampled to 1084289 and  
203 1018169 reads, respectively, in order to keep the same number of reads among the samples  
204 for further comparison. There are thousands of sequences obtained in high-throughput  
205 sequencing, which complicates data analysis. The operational taxonomic units (OTUs) were  
206 introduced in the analysis to cluster similar sequences into a small number of taxa and  
207 annotate species based on taxa (Edgar, 2013). In this way, not only the work can be  
208 simplified, but also some wrong sequences can be removed in the clustering process and  
209 improve the accuracy.

210 Alpha diversity analyses, i.e. Shannon diversity index and abundance-based coverage  
211 estimator (ACE) indexes were used to reflect the diversity and abundance of microbial

212 communities (Baczkowski et al., 1998). The Shannon index is used to express community  
 213 diversity, and in contrast the ACE index is positively correlated with taxa richness (Hunter,  
 214 1988). The ACE and Shannon index were calculated using the following formula:

215 (1) ACE index

$$216 \quad S_{ACE} = \begin{cases} S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{n1}{C_{ACE}} \hat{\gamma}_{ACE}^2, \text{ for } \hat{\gamma}_{ACE} < 0.80 \\ S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{n1}{C_{ACE}} \tilde{\gamma}_{ACE}^2, \text{ for } \tilde{\gamma}_{ACE} \geq 0.80 \end{cases}$$

$$217 \quad N_{rare} = \sum_{i=1}^{abund} i n_i, \quad C_{ACE} = 1 - \frac{n1}{N_{rare}}$$

$$218 \quad \hat{\gamma}_{ACE}^2 = \max \left[ \frac{N_{rare}}{C_{ACE}} \frac{\sum_{i=1}^{abund} i(i-1)n_i}{N_{rare}(N_{rare}-1)} - 1, 0 \right]$$

$$219 \quad \tilde{\gamma}_{ACE}^2 = \max \left[ \hat{\gamma}_{ACE}^2 \left( 1 + \frac{N_{rare}(1-C_{ACE}) \sum_{i=1}^{abund} i(i-1)n_i}{N_{rare}(N_{rare}-C_{ACE})} \right), 0 \right]$$

220 Where  $n_i$  represents the number of the OTUs containing 1 sequence;  $S_{rare}$  indicates the  
 221 number of OTUs containing “abund” or less than “abund”;  $S_{abund}$  refers to the number of  
 222 OTUs containing more than "abund" number of sequences. “Abund” is the threshold of a  
 223 "dominant" OTU and the default value is 10.

224 (2) Shannon index

$$225 \quad H_{shannon} = - \sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

226  $S_{obs}$  is the number of OTU actually observed;  $n_i$  is the sequence number contained in the  
 227 concerned OTU;  $N$  is the number of all sequences.

228 One-way ANOVA was used to analyze the differences within the Alpha diversity indexes

229 among prokaryotic and eukaryotic microbial communities for each sampling event. The  
230 relationships between bacteria and fungi were shown with one standard deviation ( $\sigma$ ) based  
231 on the bacterial and fungal DNA proportions during the bloom's naturally developing period  
232 (from May 16 to 21, 2018). All analysis was performed using IBM SPSS 23.0.

### 233 **3. Results**

#### 234 **3.1 Weather and algal bloom**

235 During the sampling period, an algal bloom developed along with increasing water  
236 temperature leading to high pH and water turbidity (Table 1; Fig. 2). Subsequently, on the  
237 evening of May 21 a heavy rainstorm (152 mm) over 24 hours resulted in high sediment  
238 loadings which terminated the bloom.

#### 239 **3.2 Chl a and phytoplankton communities**

240 Chl a concentration among the sampling period was in the range of 10-219  $\mu\text{g L}^{-1}$ , with  
241 the lowest on May 24, after the heavy rain while the highest on May 20. From May 16 to 20,  
242 Chl a concentration gradually increased with the pace of 30-40% addition from the previous  
243 day (Fig. 3A). Chl a concentrations were consistent with cell densities ( $r=0.94^{**}$ ,  $P=0.004$ )  
244 during the bloom. These figures illustrate that Gaoyang Lake experienced an intense algal  
245 bloom which peaked on May 20 at 219  $\mu\text{g L}^{-1}$  (Fig. 3A).

246 Algal density fluctuated during the bloom (Fig. 3B). The maximum density ( $1.228 \times 10^9$   
247 ind  $\text{L}^{-1}$ ) was observed on May 20, three times higher than that of May 19. A total of 71  
248 species belonging to 6 phyla, 14 orders, 24 families, and 31 genera (Table S1, including

249 varieties and variants) of phytoplankton were identified during the bloom event. The  
250 dominant phylum was Chlorophyta (28%~97%) composed mainly of *Chlorella* sp. The  
251 second dominant phylum was Cyanobacteria (1% ~26%) dominated by *Anabaena* spp.,  
252 *Doliospermum* spp. and *Microcystis* spp.. Bacillariophyta were first detected on May 18, with  
253 4 orders, 4 families, 5 genera and 5 species mainly composed of *Aulacoseira* sp. and  
254 *Fragilaria* sp.. Cryptophyta density reached their highest value on May 20, mainly composed  
255 of *Chroomonas* spp and *Cryptomonas* spp. Pyrrophyta were abundant on May 18, represented  
256 by *Ceratium hirundinella*. Euglenophyta numbers were low throughout the study, mainly  
257 composed of *Trachelomonas* and *Euglena*.

### 258 3.3 Nutrient Dynamics

259 Nutrient loading in the Pengxi River were high throughout the study period with the  
260 highest concentrations observed after the rain event at the end of the sampling period (Fig. 4).  
261 TN increased from 3 mg L<sup>-1</sup> (May 18) to 5 mg L<sup>-1</sup> (May 23), in line with the process of the  
262 bloom (Fig. 4 A). TP demonstrated a declining trend from 0.3 to 0.2 mg L<sup>-1</sup> until a sharp  
263 increase up to 0.42 mg L<sup>-1</sup> following the rainstorm (Fig. 4B). DTN and DTP increased from 0  
264 and 0.05 mg L<sup>-1</sup> to 2.5 and 0.18 mg L<sup>-1</sup>, respectively, from May 16 to May 18 and then  
265 declined to close their level of May 16 prior to the rain event. The multiple regression  
266 analysis of TP, TN, DTN, DTP and Chl a showed that TP had a greater impact on Chl a  
267 (Beta=-0.97\*\*), while the others did not. TN/TP molar ratio during the study varied from 20-  
268 36. The value of DTN/DTP molar ratio was varied greatly, reaching 106 at the peak of bloom  
269 on 21<sup>st</sup>. Chl a sharply declined after the rain.



### 270 3.4 HTS-based aquatic microbial community

271 A total of 32 phyla, 65 classes, 126 orders, 242 families, 472 genera, 754 species and  
272 1211 OTUs of prokaryotes were present based on 16S rDNA. Also 19 phyla, 84 classes, 125  
273 orders, 242 families, 152 genera, 197 species and 593 OTUs of eukaryotes were present based  
274 on 18S rDNA, over the 6-day sampling period. The microbial Chlorophyte community was  
275 not monitored due to the limitation of the selected primer sets for the 18S DNA high  
276 throughput sequencing.

#### 277 3.4.1 Microbial community diversity

278 On May 20, ACE reached relatively high values, especially for prokaryotes (556, 16S in  
279 Fig. 5), consistent with the microscopic counts (Fig. 3 A&B). Furthermore, ACE of  
280 prokaryotes was 2-3 times of those of eukaryotes. Diversity, represented by the Shannon  
281 index, was the lowest, 3.23, on May 20 for prokaryotes, and 2.1 on May 19 for eukaryotes,  
282 fitting the contrary relationship between richness and diversity. The Shannon and ACE  
283 indexes for both prokaryotic and eukaryotic microbes were significantly different on a daily  
284 basis ( $P < 0.01$ ). The heavy rain on May 23 led to the highest values in both the Shannon (5.35  
285 for prokaryotes, 3.21 for eukaryotes) and ACE indexes (981.04 for prokaryotes, 367.35 for  
286 eukaryote). The increased richness and diversity by the rain (Fig. 2F) was not for  
287 phytoplankton, compared to previous days (Fig. 3 A&B). Both algal densities ( $6.29 \times 10^6$  ind  
288  $L^{-1}$ ) and Chl a ( $13.71 \text{ mg } L^{-1}$ ) were the lowest on May 23.

#### 289 3.4.2 Microbial community composition based on DNA HTS

290 From May 16 to 19, both prokaryotic and eukaryotic phytoplankton (in green shades,

291 excluding Chlorophyte) gradually increased relative to other groups, reaching their maximum  
292 values on May 20 then decreasing (Fig. 6). This was consistent with the observed algal  
293 densities and Chl a concentrations (Fig 3).

294 In the prokaryotic group (Fig. 6A), cyanobacteria reached a peak level on May 20,  
295 accounting for 97.8% of the prokaryotes (sic passim). In contrast, Chloroflexi and Chlorobi  
296 comprised the remaining proportion within the photosynthetic bacteria (Table S1). From May  
297 16 to 21, the average DNA proportions of Proteobacteria and Bacteroidetes, as major free-  
298 living bacterial groups, were 40.7% and 34.6%, respectively among heterotrophic bacteria  
299 (Table S2). Firmicutes reached a maximum on May 18. The DNA of Actinomycetes  
300 proportionally increased gradually from 1.6% on May 16 to 16.0% on May 21 (Fig. 6A).  
301 After the rainfall on May 22, Chlorobi and Chloroflexi first appeared with OUT number  
302 proportion of 0.01, the two main species of prokaryotic algae second only to cyanobacteria  
303 (Table S2).

304 Compared with prokaryotes, the eukaryotic microbial community (Fig 6B, excluding  
305 Chlorophytes) was temporally more variable. DNA proportion of Ascomycota, Ciliophora,  
306 Katablepharid and p\_P1-31 at the phylum level fluctuated in ranges of 2.5-49.1%, 5.2-47.3%,  
307 0.8-31.8% and 0.1-25.6%, respectively. The dominant eukaryotic phytoplankton phylum was  
308 Cryptophyta (excluding chlorophytes) with a maximum abundance 98.6% (Table S2). Among  
309 the micro-zooplankton (0~200µm), p\_p1-31 and Kathableharidae were dominant on May 16,  
310 accounting for 32% and 24%, respectively; followed by Ciliophora in dominance on May 18  
311 and on May 20, respectively. Meso-zooplankton (>200 µm) in this study mainly included  
312 Arthropoda and Porifera (Table S3). The OTU abundance of Arthropoda increased

313 gradually from 0 on May 16, to 0.03% on May 20 on daily basis, except May 17<sup>th</sup> when data  
314 was not available, and then dropped to 0 on May 23 (Fig. 6B & Table S3). Meanwhile the  
315 proportion of Porifera had the opposite daily dynamics compared to the Arthropoda (Table  
316 S3). Ascomycota was the dominant group among the fungi on most of the sampling days,  
317 except May 17<sup>th</sup> when data was not available and May 21 when its proportion was slightly  
318 surpassed by Cryptomycota (Table S3). Through the mid-part of the bloom, Ascomycota  
319 OTU number proportion accounted for 10-49%, with a distinct decline to 5% on May 20 (Fig.  
320 6B).

### 321 **3.5 Plankton community successions during the bloom**

322 Both prokaryotic and eukaryotic phytoplankton's OTU number relative abundances (Fig.  
323 7 A&B) remained low and stable from May 16 to 19, which then increased several folds from  
324 May 19 to 20 (prokaryotic phytoplankton increased from 5% to 16%, and eukaryotic  
325 phytoplankton from 3.4% to 28%). One day later, on May 21, the percentage of prokaryotic  
326 and eukaryotic phytoplankton decreased by 75% and 42%, respectively (Fig. 7 A&B).

327 The proportion of micro-zooplankton OTU number was still relatively high on May 16  
328 and 18, accounting for approximately 50% OTUs of the whole system (sic passim) (Fig 7 B,  
329 Table S3); from May 18 to 19, it declined from 44.5% to 24.9%. On May 20, OTU number  
330 proportion of micro-zooplankton increased to 51.0% of the total number of OTUs of  
331 eukaryotes, and then dropped by 37.3% from May 20 to 21, keeping a similar trend with  
332 eukaryotic phytoplankton (Fig 7B, Table S2). Within the micro-zooplankton group, the  
333 proportion of Ciliophora OTUs number increased to 35% on May 20, becoming the most

334 dominant group (Table S2).

335 The OTU number proportion of meso-zooplankton increased slightly from 2% to 5%  
336 from May 16 to May 21, while eukaryotic phytoplankton and micro-zooplankton fluctuated  
337 (Fig. 7B).

338 The total OTU number proportion of heterotrophic bacteria was stable overall ( $\sigma=0.059$ ),  
339 while the proportion of fungal number of OTUs fluctuated greatly ( $\sigma=0.22$ ), ranging from  
340 13% to 56% during bloom period (Fig. 7B). The relative proportion of fungi was negatively  
341 correlated with that of micro-zooplankton ( $r=-0.9^*$ ,  $P=0.014$ ).

342 The heavy rain on May 22 led to a 3.3 times increase of OTU number proportion of the  
343 micro-zooplankton, while those of fungi and meso-zooplankton decreased by 92% and 76%,  
344 respectively and eukaryotic phytoplankton remained stable (Fig. 7B).

#### 345 **4. Discussion**

346 The phenomenon of tributary bloom caused by dam construction is very common all  
347 over the world (Maavara et al., 2020). Currently there are empirical models trying to describe  
348 HABs between nutrients and algal production ignoring the rest of the lake/river ecosystem  
349 (Monchamp et al., 2014; Wang et al., 2020). Our data suggest that this might be incomplete,  
350 since major changes in the microbial loop are occurring during the blooms, and this is  
351 probably true in all lakes/ivers (Buchan, 2014; Wilk-Wozniak, 2020).

##### 352 **4.1 Short-term changes in water chemistry impacted by algal bloom**

353 In this study, based on algal density, Chl a concentration and DNA high throughput  
354 sequencing analysis, from May 16 to May 23, 2018 Gaoyang Lake in the TGR experienced a

355 rapid bloom cycle along with significant changes in the microbial loop. Further, the diversity  
356 of the microbial community changed significantly ( $P<0.01$ ), indicating a possible mechanistic  
357 response to the algal bloom.

358 In aquatic systems, TN and TP loads are often locked in organisms, and it is DTN and  
359 DTP concentrations which wax and wane with HABs (Anderson and Burkholder, 2002). Our  
360 study supports this observation: The DTN and DTP concentrations reduced to only 49% and  
361 85%, respectively from May 18 to 20 (bloom peak), indicating rapid nutrient consumption,  
362 especially the phosphorus during algal bloom formation (Fig.4B). This demonstrated that a  
363 short temporal pattern of dissolved nutrients could trigger an algal bloom which was then  
364 inversely impacted by the bloom over large spatial areas (Giannuzzi et al., 2016). This study  
365 has shown that dissolved nutrients, i.e. DIP and DTN, are better indicators marking fine scale  
366 trends in algal bloom cycles.

## 367 **4.2 Impacts of the algal bloom on the microbial loop**

368 1). Succession in zooplankton.

369 The algal bloom created a short interval modification of the microbial loop in Gaoyang  
370 Lake. Katablepharidea and P1-31 from the class Leucocrypta, were dominant prior to the  
371 bloom, representing a mixotrophic assemblage supported by organic food particulates, algae  
372 and bacteria (Boenigk & Hartmut, 2002; Barlow & Kugrens, 2002; Tsai et al., 2016; Kwon et  
373 al., 2017; Ok et al., 2018). The larger Ciliophora in the micro-zooplankton, were dominant  
374 during the initiation of the bloom (May 18- 20), acting as predators of planktonic algae and  
375 bacteria (Sherr, E. and Sherr, B. 1988; Tan and Su, 1997). Although the DNA signature of the  
376 meso-zooplankton was present, this trophic group was less impactful than the micro-

377 zooplankton, likely because of longer response times associated with their growth cycles. For  
378 example, Diplostraca, an arthropod group with 15 to 20 days life span (Spitze, 1993). In this  
379 study, Diplostraca appeared with the largest DNA signature among meso-zooplankton after  
380 the bloom peak (May 21). Therefore, besides nutrients, grazing pressure was another force in  
381 the diurnal dynamics of the he microbial loop during the bloom (Rose et al., 2017; Ger et al.,  
382 2019).

## 383 2) Micro-zooplankton and fungi

384 In this study, the correlation analysis revealed that the relative DNA signatures of fungi  
385 and micro-zooplankton were negatively significantly correlated ( $r=-0.9^*$ ,  $P=0.014$ , Fig.7 B),  
386 indicating possible food resource partitioning and specialization within the microbial loop  
387 (Grami et al., 2011; Teng et al., 2011; Jia et al., 2013; Frenken et al., 2017; Senga et al.,  
388 2018). We observed that increases in Chytridiomycota and Cryptomycota fungi concurred  
389 with the bloom of the algae, although Cryptomycota continued to increase after the bloom  
390 (Fig. 6B). As common parasites (Kagami et al., 2007; Johnson et al., 2009; Grami et al.,  
391 2011), they may be one of the factors contributing to the death of algae. Ascomycota,  
392 dominated by *Fusarium*, accounted for more than half of the aquatic fungi during bloom  
393 development (May 18-19) (Fig. 6B), supporting the observation that fungi were tracking the  
394 development of the algal bloom (Zhang et al., 2018; Perini et al., 2019). The signatures of the  
395 zooplankton illustrate the importance of food sources in the microbial food webs and how this  
396 translates the bottom-up mechanistic processes into higher level trophic structure (Long et al.  
397 2019). Zooplankton is common host and occasionally preys for a variety of fungal parasites

398 (Grami et al., 2011). Owing to the concurrent blooms of different primary food sources, it is  
399 implied that the relationship between fungi and zooplankton in this study is complex  
400 and competitive.

### 401 **4.3 Temporal scales of algal blooms**

402 The heavy rain of May 21-22, 2018 terminated the bloom, and introduced additional  
403 allochthonous and bioavailable nutrients into the Pengxi River (Figs. 2&4). This random  
404 event destroyed the developing relationships among the microbial community (Fig. 6 & 7)  
405 that had the potential to recycle nutrient and carbon back into the biogeochemical nutrient  
406 cycle (i.e. a double nutrient injection, Buchan et al., 2014). After the rain fall event and water  
407 transparency would increase as a result of particulate sedimentation and advection, the left  
408 over seed algae have the potential to initiate another bloom (e.g. Michalak et al., 2017;  
409 Ouyang et al., 2021). Thus, both intrinsic dynamics within the primary trophic level as well as  
410 extrinsic events, such as the rainfall, have the potential to regulate the timing and duration of  
411 HAB events.

412 The possibility to use extrinsic events to regulate algal blooms in reservoirs has been  
413 previously studied (Yang et al., 2010; Ji et al., 2017). For example, the TGR dam discharge  
414 has the potential to create a free flowing river preventing HAB events (Ji et al., 2017) as  
415 predicted by Jones & Elliott (2007) and Lee & Lee (2018). The problem with this strategy is  
416 that it affects the entire reservoir and does not specifically address HABs that develop in only  
417 certain locations of the reservoir such as the Pengxi River. There have been rare studies to  
418 determine if it is possible to modify intrinsic dynamics that would allow remedial measures to  
419 be applied to specific blooms without modifying the dynamics of the overall reservoir. The

420 DTN and DTP associations with Chl a and rainfall in this study show that severity of algal  
421 blooms could be mitigated by reducing the diffuse pollution from the land.

#### 422 **4.4 The complexity of trophic structures**

423 Based on 18S rDNA sequencing, non-identifiable fungal sequences accounted for 22.2%  
424 of the total eukaryotes and 41.2% species were detected as no-rank at the Class level. Our  
425 limited ability to classify the aquatic fungal community has been encountered in other studies  
426 (Staay, 2001; Ouyang et al., 2021). We did not capture the Chlorophyte community due to the  
427 limitations of HTS in which one marker gene (primer) is amplified and sequenced for all  
428 organisms, but in contrast, allows only small fragments to be sequenced. The primer biases  
429 also led to chlorophytes underrepresentation in other studies (Tragin et al., 2018; Brown et al.,  
430 2021) . However, the missing data did not dramatically impact this study with respect to the  
431 cross-validation by manual counts, which showed that the Chlorophyte bloom was limited to  
432 a few species within the *Chlorella* species complex. The diversity and complexity of  
433 taxonomic groups identified using high throughput DNA provides the opportunity to observe  
434 and quantify the complexities of the lower trophic food web dynamics with respect to how  
435 fast it can change during bloom events. In the studied bloom event, the primary producers at  
436 the beginning of the study were predominantly mixotrophic, then changed to autotrophic taxa  
437 tracking the developing HABs community. At the end of the bloom, the mixotrophic  
438 community persisted, but the appearance of Chlorobi and Chloroflexi illustrates that the  
439 community did not recover to pre-bloom conditions.

440 Fungi maintained DNA quantity trends through most of the bloom, and became a



441 dominant component of the primary trophic level. Bacteria, however, showed a reverse trend  
442 relative to the algae and fungi, highlighting the importance of rapid community changes in the  
443 microbial food loop over short periods of time. Similar observations have been made for  
444 micro- and meso-zooplankton communities within the food web (Long et al. 2019).

## 445 **Conclusion**

446 Based on algal densities and Chl a concentrations, from May 16 to May 23, 2018,  
447 Gaoyang Lake experienced a short but intense bloom. This 7-day algal bloom event triggered  
448 chemical cycling (plus recycling from algal decomposition), which impacted the structure of  
449 the primary producers. The community composition was analyzed by DNA high-throughput  
450 sequencing data and significant results of this study related to the identification of rapid (<1  
451 day) changes in community and trophic structure through a bloom event. The genetic  
452 observations of the bloom showed high temporal variability within the microbial loop which  
453 was surprising, given the short duration of the bloom. The composition of the microbial  
454 community indicates there was a switch from predominately mixotrophic to autotrophic  
455 species, and then back to mixotrophy during the bloom. It is interesting that the heterotrophic  
456 algae such as cryptophytes and dinophytes were as common as autotrophic primary producers  
457 such as chlorophytes and cyanobacteria (with HABs) during the development of the bloom.  
458 This indicates that mechanistic as well as stochastic processes such as the rain event have the  
459 potential to determine the timing and duration of algal blooms.

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