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Phytoplankton communities exhibit a stronger response to environmental changes than bacterioplankton in three subtropical reservoirs

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2 **environmental changes than bacterioplankton in three**
3 **subtropical reservoirs**

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19 **ABSTRACT**

20 The simultaneous analysis of multiple components of ecosystems is crucial for
21 comprehensive studies of environmental changes in aquatic ecosystems - but such
22 studies are rare. In this study, we analyzed simultaneously the bacterioplankton and
23 phytoplankton communities in three Chinese subtropical reservoirs, and compared the
24 response of these two components to seasonal environmental changes. Time-lag
25 analysis indicated that the temporal community dynamics of both bacterioplankton
26 and phytoplankton showed significant directional changes, and variance partitioning
27 suggested that the major reason was the gradual improvement of reservoir water
28 quality from middle eutrophic to oligo-mesotrophic levels during the course of our
29 study. In addition, we found a higher level of temporal stability or stochasticity in the
30 bacterioplankton community than in the phytoplankton community. Potential
31 explanations are that traits associated with bacteria such as high abundances,
32 widespread dispersal, potential for rapid growth rates and rapid evolutionary
33 adaptation may underlie the different stability or stochasticity of bacterioplankton and
34 phytoplankton communities to the environmental changes. In addition, the indirect
35 response to nitrogen and phosphorus of bacterioplankton may result in the fact that
36 environmental deterministic selection was stronger for the phytoplankton than for the
37 bacterioplankton communities.

38 **INTRODUCTION**

39 Bacterioplankton and phytoplankton are critical components of aquatic microbial food
40 webs, and play essential roles in the structure and function of aquatic ecosystems.^{1,2}
41 Understanding the processes and mechanisms that underlie the abundance and/or
42 biovolume variations of bacterioplankton and phytoplankton communities, are major
43 goals in both pure and applied microbial community ecology. Many previous studies
44 of bacterioplankton and phytoplankton variation have focused on traditional
45 biogeographical concepts, such as distance decay relationships,^{3, 4} species-area
46 relationships,^{5,6} and the niche vs. neutral debate.⁷⁻⁹ Data on the temporal variation in
47 aquatic microbial community composition is more mixed and limited. There is a long
48 history of monitoring phytoplankton in lakes in some parts of the world – for example,
49 such studies have a history of over 100 years in the English Lake District, with
50 extensive data sets existing from the mid-twentieth century onwards.¹⁰ However,
51 bacteria (excluding cyanobacteria) were much more challenging to study until the rise
52 of molecular methods in the late 1980's, so even in well studied regions such as the
53 English Lake District there is much less known about the patterns in bacterial
54 abundance, compared with the data on phytoplankton.¹¹ Macan and Worthington (p83)
55 summarized the mid-twentieth century position in writing that ‘The bacteria... play an
56 important, though as yet little understood role in the economy of all fresh waters.’¹²
57 Even today the global distribution of such studies is very patchy with the vast
58 majority of molecular studies of freshwater bacterial communities being confined to
59 Europe, and to a lesser extent North America. The rest of the world is covered by a
60 very small number of studies¹³ with the tropics and sub-tropics being particularly
61 poorly sampled - but see Dalu *et al.* for a rare African example.¹⁴ Arguably, this lack
62 of understanding of the temporal behavior of both bacterioplankton and
63 phytoplankton communities hinders the development of theories about how the
64 stability of microbial community structure and function is maintained across time.¹⁴

65 In aquatic ecosystems, it has become increasingly clear that the temporal variation
66 in composition of bacterioplankton and phytoplankton communities primarily
67 depends on environmental changes through space and time.¹⁴⁻²⁰ However, the
68 response of these microbial communities to such changes are also mediated by their
69 properties, including their history, metabolic flexibility, physiological tolerance,
70 dispersal capacity, and taxonomic and functional diversity.^{3, 21, 22} Therefore, we
71 hypothesize that bacterioplankton and phytoplankton will have different sensitivities
72 to environmental changes. To date, most aquatic microbial diversity studies have
73 focused on just bacterioplankton or phytoplankton; comprehensive studies
74 simultaneously analyzing bacterioplankton and phytoplankton components of
75 microbial communities across time are much rarer – especially in regions other than
76 Europe.^{13,23} Analyzing simultaneously the dynamics of different microbial groups is
77 likely to be crucial for understanding the dynamics and responses of the ecosystems to
78 environmental changes.

79 Additionally, to date, studies estimating temporal variation of microbial
80 communities have mainly used multivariate statistics to illustrate the lack of variation

81 in rates and patterns of community change.²⁴ Few studies have used metrics to
82 specifically quantify temporal variation of microbial communities.^{25, 26} Time-lag
83 analysis (TLA) has proved a useful diagnostic tool to quantify the temporal variation
84 of ecological communities, and can be considered as an extension of autocorrelation
85 analysis for short time series (fewer than 20 time points) of community data.²⁷ A
86 significant and positive regression slope denotes a community undergoing directional
87 change; while a significant and negative regression slope indicates a community with
88 convergent dynamics (e.g., the species composition is becoming more similar to a
89 community type characteristic of the earlier samples in the series). Moreover, a
90 non-significant slope for the regression implies that there is either stochastic variation
91 or high stability over time. Furthermore, the slope of the regression and the regression
92 R^2 can be used as a measure of rate of change across sampling intervals. TLA has
93 been successfully used for estimating anthropogenically perturbed fish,²⁸ plant, and
94 freshwater zooplankton assemblages over a time scale of one to a few decades.²⁷
95 However studies on freshwater bacterioplankton and/or phytoplankton communities
96 using this approach are rare. Although questions have been raised about the power,
97 and so usefulness of time lag analyses,²⁹ it has been widely applied and has the
98 advantages of ‘computational ease, its easy comprehensibility... and the possibility of
99 characterizing and comparing the temporal dynamics of large numbers of
100 communities with a single measure’.³⁰

101 In this study, we used classical denaturing gradient gel electrophoresis (DGGE) to
102 analyze the abundant bacterioplankton community and used microscopy to investigate
103 the abundant phytoplankton community in three subtropical reservoirs, southeast
104 China. Although DGGE is starting to be superseded by sequencing techniques, it has
105 advantages of lower cost and is a reasonable approach for characterizing the more
106 dominant taxa. These three drinking water reservoirs provide interesting systems for
107 investigating the response of microbial communities to environmental changes,
108 because, these reservoirs were exhibiting an early stage of eutrophication - their
109 trophic state was unstable, and it declined gradually from middle eutrophic to
110 oligo-mesotrophic levels during the study period. We further quantified the temporal
111 variation of both bacterioplankton and phytoplankton communities using TLA, and
112 compared the response of both communities to the changed environment.
113 Cyanobacteria have in the past been considered ‘algae’ (blue green algae) and
114 considered alongside the photosynthetic eukaryotic plankton, indeed because they can
115 be identified and counted in water samples using microscopy they are usually
116 considered part of the phytoplankton – while in freshwater ecology ‘bacteria’ often
117 mainly refers to heterotrophic bacteria.³¹ In our analyses we treat cyanobacteria
118 mainly as phytoplankton (a functional/ecological classification that makes sense
119 because of their photosynthetic nature) but also explore the implications of classing
120 them as bacteria (the correct phylogenetic classification). The aims of this study were
121 (1) to quantify and compare the temporal patterns between bacterioplankton and
122 phytoplankton communities in three subtropical reservoirs; (2) to reveal the response
123 mechanisms of bacterioplankton and phytoplankton communities to the reservoir
124 environmental changes.

125 MATERIAL AND METHODS

126 **Sample collection.** This study was carried out in three reservoirs near Xiamen city,
127 southeast China (Shidou Reservoir - SD, 118°00'E, 24°42'N; Bantou Reservoir - BT,
128 118°01'E, 24°40'N, Tingxi Reservoir - TX, 118°08'E, 24°48'N); full details of study
129 reservoirs information were showed in our previous study.³² The main purposes of
130 these reservoirs are flood control, hydroelectric power, irrigation, and water supply
131 for the city of Xiamen. This area has a subtropical humid monsoon climate with an
132 annual mean precipitation of 1350 mm and an annual mean temperature of 20 °C. The
133 rainfall is concentrated in warm months (April to September), while in cold months
134 (October to March) rainfall is much lower.³³

135 Three sampling stations were selected at each reservoir in the riverine zone,
136 transitional zone, and lacustrine zone, respectively. Surface water samples (upper 50
137 cm) were collected bi-monthly in each station from May 2010 to March 2011,
138 therefore 18 samples were collected in total from each reservoir. Water samples were
139 subsequently divided into three subsamples: one for water chemistry analyses, the
140 others for bacterioplankton and phytoplankton analyses, respectively. All water
141 samples were stored in the dark at 4 °C and returned to the laboratory within two
142 hours for further processing. For the phytoplankton analysis, a total of 2.5 L surface
143 water samples were fixed in situ with 1% Lugol's iodine solution and were
144 concentrated to a final volume of 50 mL.¹⁹ For the bacterioplankton analysis, 400 ml
145 water was filtered through a 0.22- μ m pore size polycarbonate filter (47 mm diameter,
146 Millipore, Billerica, MA, USA). The filters were stored at -80 °C until further use.

147 **Environmental analysis.** Water temperature (WT), pH, dissolved oxygen (DO),
148 electrical conductivity (EC) and chlorophyll *a* (Chl *a*) were measured in situ with a
149 Hydrolab DS5 multi-parameter water quality analyzer (Hach, Loveland, CO, USA).
150 Water transparency was determined with a 30 cm Secchi disk. Total nitrogen (TN),
151 ammonium nitrogen (NH₄-N), nitrite and nitrate nitrogen (NO_x-N), total phosphorus
152 (TP), and phosphate phosphorus (PO₄-P) were measured following methods used in
153 our previous study.²³

154 The comprehensive trophic state index was calculated according to classical
155 Carlson TSI based on three limnological parameters namely chlorophyll *a*, Secchi
156 disk transparency, and total phosphorus.^{32, 34} Where $0 < \text{TSIc} \leq 30 =$ oligotrophic, 30
157 $< \text{TSIc} \leq 40 =$ oligo-mesotrophic, $40 < \text{TSIc} \leq 50 =$ mesotrophic, $50 < \text{TSIc} \leq 60 =$
158 light eutrophic, $60 < \text{TSIc} \leq 70 =$ middle eutrophic, and $70 < \text{TSIc} \leq 100 =$
159 hypereutrophic.

160 **Phytoplankton analysis.** Phytoplankton were identified and counted using an
161 inverted microscope (Motic, Xiamen, China) according to Shen *et al.*, Zhang and
162 Huang, and Hu and Wei.³⁵⁻³⁷ Three subsamples were investigated for each sample and
163 at least 500 individuals were counted for each subsample. To compare the
164 bacterioplankton and phytoplankton communities, we transformed the phytoplankton
165 abundance to biovolume according to Paver *et al.*³⁸ Biovolume was estimated from

166 cell numbers and cell size measurements.^{19, 39} In using denaturing gradient gel
167 electrophoresis it is difficult to detect microbes with abundances of < 1% of the total
168 community.^{40, 41} Therefore, to improve the comparability between bacterioplankton
169 and phytoplankton, we performed the statistical analyses using only abundant
170 phytoplankton species (> 1% biovolume in a sample).

171 **DNA extraction and PCR amplification.** Total DNA was extracted directly from
172 the filter using an E.Z.N.A. DNA Kit (Omega Bio-Tek, Norcross, GA, USA)
173 according to the manufacturer's instructions. The extracted DNA was dissolved in 50
174 μ l TE buffer, quantified by spectrophotometer and stored at $-20\text{ }^{\circ}\text{C}$ until further use.

175 The 16S rRNA gene fragments were amplified with the primers 341F-GC (5'-CGC
176 CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG
177 AGG CAG CAG-3') and 907R (5'-CCG TCA ATT CMT TTG AGT TT-3')⁴² under
178 the following PCR conditions: 5 min denaturation at $94\text{ }^{\circ}\text{C}$ and 10 touchdown cycles
179 at $94\text{ }^{\circ}\text{C}$ for 0.5 min, $67\text{ }^{\circ}\text{C}$ (with the temperature decreasing $1\text{ }^{\circ}\text{C}$ each cycle) for 0.5
180 min, $72\text{ }^{\circ}\text{C}$ for 1 min; followed by 20 cycles at $94\text{ }^{\circ}\text{C}$ for 0.5 min, $57\text{ }^{\circ}\text{C}$ for 0.5 min,
181 $72\text{ }^{\circ}\text{C}$ for 1 min and a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. Each 50 μ l PCR reaction
182 contained 0.3 μ M each primer, 2.5 U *Taq* DNA polymerase (TaKaRa, Otsu, Shiga,
183 Japan), 1.5 mM MgCl_2 , 200 μ M each deoxynucleoside triphosphate, and
184 approximately 40 ng of template DNA in $1\times$ PCR buffer.

185 **Denaturing gradient gel electrophoresis and sequencing.** Denaturing gradient
186 gel electrophoresis (DGGE) was performed using a DCode mutation detection system
187 (Bio-Rad, Hercules, CA, USA). Samples containing equal amounts of PCR amplicons
188 were loaded onto 6% (w/v) polyacrylamide gels (37.5 : 1 acrylamide : bisacrylamide)
189 in $1\times$ Tris-acetate-EDTA (TAE) buffer. The denaturing gradient of 30%–60% was
190 applied for separation of the 16S rRNA genes and 100% denaturant is defined as 7 M
191 urea and 40% (v/v) deionized formamide, respectively. Electrophoresis was
192 performed at $60\text{ }^{\circ}\text{C}$ with a constant voltage of 100 V for 16 h. The DGGE gels were
193 stained with SYBR Green I nucleic acid stain for 30 min in $1\times$ TAE buffer, rinsed in
194 distilled water, and then visualized with UV radiation by using Gel Doc EQ imager
195 (Bio-Rad, Hercules, CA, USA). DGGE patterns were analyzed using the Quantity
196 One software (Bio-Rad, Hercules, CA, USA)⁴³, and were carefully checked and
197 corrected manually. The bands occupying the same position in the different lanes of
198 the gel were identified. The relative abundance matrix was constructed for all lanes,
199 taking into account the relative intensity of individual bands in each lane.

200 Dominant DGGE bands were excised from the gels and eluted overnight in
201 autoclaved Milli-Q water at $4\text{ }^{\circ}\text{C}$. The eluted DNA was reamplified with the original
202 primer set (without GC clamp). PCR products were purified with the TaKaRa Agarose
203 Gel DNA Purification Kit (Takara, Otsu, Shiga, Japan), then cloned into a
204 pMD18-vector (Takara, Otsu, Shiga, Japan) and transformed into *Escherichia coli*
205 DH5 α competent cells (Takara, Otsu, Shiga, Japan). The successfully inserted
206 plasmids were sequenced unidirectionally using an automated sequencer (ABI 3730
207 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). All bacterial 16S

208 rRNA sequences were manually checked and modified with BIOEDIT v7.0.9⁴⁴ and
209 then compared with the GenBank database using BLASTN⁴⁵. The bacterial 16S rRNA
210 sequences were classified by the Ribosomal Database Project Classifier with 80%
211 confidence.⁴⁶

212 **Data analysis.** We used principal component analysis (PCA) to display the overall
213 trends in environmental variables.

214 Two Bray-Curtis dissimilarity matrices were constructed using the bacterioplankton
215 relative abundance data and phytoplankton relative biovolume data generated from
216 each sample, respectively. The non-metric multidimensional scaling (NMDS)
217 ordination was used to investigate differences in microbial communities among
218 samples based on Bray-Curtis dissimilarity.⁴⁷

219 The coefficient of variation (CV) was calculated to compare the temporal
220 variability between the relative abundance of bacterioplankton OTUs and relative
221 biovolume of phytoplankton taxa in the reservoirs. Median absolute deviation (MAD)
222 was used to compare the temporal variability in Bray-Curtis dissimilarity between
223 bacterioplankton and phytoplankton communities.⁴⁸

224 To explore the temporal patterns of environment and community dynamics, we
225 performed linear regressions on Bray-Curtis dissimilarity of community composition
226 (dependent variables) versus the square root of the time lags (independent variables)
227 and Euclidean distance of all environmental variables (dependent variables) versus the
228 square root of the time lags (independent variables) through TLA.²⁷

229 We divided the twelve environmental variables into three groups: the first group
230 which is related to eutrophication includes TN, NH₄-N, NO_x-N, TP, PO₄-P,
231 transparency, Chl *a* and TSIC; the second group constitutes of three variables (EC, pH,
232 DO) which are related to physico-chemical factors; the third group comprised only
233 water temperature. We then used a forward-selection procedure with Monte Carlo
234 permutation tests to select the environmental variables which explained a significant
235 ($P < 0.05$) variation of the bacterial and phytoplankton data in each group.⁴⁹ To
236 eliminate collinearity among variables within each group, explanatory variables with
237 the highest variance inflation factor (VIF) were sequentially removed until all VIFs
238 were less than 20.⁴⁹ Finally, significant variables in each group were selected to
239 perform variance partitioning using varpart function with adjusted R² (vegan package
240 in R software). Before the forward-selection procedure, the microbial data were
241 Hellinger transformed. We used principal component analysis (PCA) to show main
242 gradients in explained variance.

243 For all the statistical analyses, the cyanobacteria (bands 7 and 28) and Chloroplast
244 bands (bands 32-34) from the DGGE profile were removed from the bacterioplankton
245 data sets (Supporting Information Figure S1). Before the PCA, forward-selection, and
246 TLA, the environmental variables were log($x+1$) transformed, with the exception of
247 pH, to improve normality and homoscedasticity. All the statistical analyses were
248 performed in CANOCO 4.5, PRIMER 5.0 and R language environment.⁵⁰⁻⁵²

249 **Accession number.** The 16S rRNA gene sequences from this study were deposited
250 in the GenBank under the accession numbers KP721939 to KP721985.

251 **RESULTS**

252 **Variations in environmental variables.** All of the 12 physico-chemical and
253 biological parameters generally showed clear temporal variations and represented a
254 wide range of environmental conditions (Figure 1 and Supporting Information Figure
255 S2). $\text{NH}_4\text{-N}$ showed seasonal cycle patterns. However, water temperature, EC, pH,
256 TN, TP, and Chl *a* decreased, while DO, transparency, and $\text{NO}_x\text{-N}$ increased gradually
257 during the study period. The trend of $\text{PO}_4\text{-P}$ was irregular in three reservoirs. In
258 addition, the comprehensive trophic state index (TSIc) decreased from middle
259 eutrophic to oligo-mesotrophic levels in the three reservoirs. The highest TSIc (62.3)
260 appeared in the BT riverine zone in May 2010, and the lowest TSIc (34.3) appeared in
261 the SD transitional zone in March 2011 (Supporting Information Figure S2).

262 **Microbial diversity and taxonomic composition.** There were 49, 36, and 48
263 distinct bacterial DGGE bands in the Shidou Reservoir (SD), the Bantou Reservoir
264 (BT), and the Tingxi Reservoir (TX), respectively. The average band number per
265 month was 26 in all three reservoirs (the mean band numbers of SD, BT, and TX were
266 28, 21, and 30, respectively) (Supporting Information Figure S1). For the
267 phytoplankton, 221 taxa were detected in all three reservoirs, and 50 of them were
268 abundant ($> 1\%$ biovolume in a sample). Further, 35, 28, and 33 abundant
269 phytoplankton taxa were found in the SD, BT, and TX, respectively. The mean
270 richness for abundant taxa was 19 in all three reservoirs. The mean abundant taxa
271 richness of BT (21) was the highest, followed by TX (20) and SD (16).

272 There were 18, 13, and 16 prominent DGGE bands that were successfully
273 sequenced in SD, BT, and TX, respectively. They were affiliated with the divisions
274 Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Chloroplast, Cyanobacteria,
275 Proteobacteria, and Verrucomicrobia. In general, Actinobacteria and Proteobacteria
276 were the dominant groups, but they showed no pronounced seasonal variation. In
277 contrast, the phytoplankton community showed a distinct seasonal shift at phylum
278 level (Supporting Information Figure S3). Cyanophyta dominated the phytoplankton
279 communities in the warmest months, but were subsequently replaced by
280 Bacillariophyta and Chlorophyta. In March, Euglenophyta and Cryptophyta were
281 dominant taxa in the SD and TX reservoirs, respectively. The most dominant
282 Cyanophyta species in the SD and BT reservoirs was *Cylindrospermopsis raciborskii*.
283 However, in the TX reservoir, *Raphidiopsis* sp. and *Microcystis flosaquae* were the
284 dominant Cyanophyta species. Interestingly, one sequenced band was affiliated with
285 *Cylindrospermopsis raciborskii* in the SD reservoir (band 7, Supporting Information
286 Figure S1).

287 **Temporal variability of community composition.** We obtained NMDS ordination
288 plots for bacterioplankton and phytoplankton separately (Supporting Information
289 Figure S4). Both bacterioplankton and phytoplankton communities showed a seasonal
290 succession from May 2010 to March 2011 in all three reservoirs. Further, the variation
291 in phytoplankton community between months was larger than in the bacterioplankton

292 community. Also, both the coefficient of variation (CV) of abundant phytoplankton
293 taxa and median absolute deviation (MAD) of phytoplankton Bray-Cutis dissimilarity
294 were pronounced higher than those of bacterioplankton community (Figure 2).

295 **Quantified temporal change in microbial communities and environmental**
296 **factors.** In general, the TLA regressions had significant positive slopes, indicating the
297 microbial communities and environmental conditions were undergoing a directional
298 change (Figure 3). In addition, a steeper regression slope (faster rate of change) and
299 higher R^2 (lower stochasticity or stability) for the phytoplankton communities were
300 detected compared to the bacterioplankton communities (Figure 3). Interestingly, the
301 regression R^2 were slightly lower (All bacterioplankton: 0.111, SD: 0.076, BT: 0.112,
302 TX: 0.157) if the Cyanobacteria and Chloroplast bands were included in the
303 bacterioplankton data sets.

304 **Relationships between microbial communities and environmental factors.** The
305 variables which were significantly related to bacterioplankton or phytoplankton
306 communities in each group are shown in Supporting Information Table S1. Results of
307 the variance partitioning showed that the environmental variables explained 72-90%
308 variation of phytoplankton community, but only explained 36-67% variation of the
309 bacterioplankton community. Moreover, the phytoplankton community had a greater
310 explained variance than the bacterioplankton community for eutrophic related factors
311 (pure eutrophic factors + eutro-physico covariation + eutro-temp covariation +
312 covariation of all variables) (Figure 4 and Supporting Information Figure S5). The
313 first two axes of PCA explained 94.8 % of the total variability and effectively
314 captured the main patterns of variation in the original variables.

315 DISCUSSION

316 In general, the TN, TP, $\text{NO}_x\text{-N}$, Chl *a*, and TSIC, which are closely related to
317 eutrophication,⁵³ decreased gradually during the study period. In particular, the TSIC
318 values decreased from middle eutrophic to oligo-mesotrophic levels. In addition, the
319 EC and pH decreased (note that the pH gradually declined to close to 7), while the
320 transparency and DO increased. These results indicated that reservoir water quality
321 gradually improved during the study (Supporting Information Figure S2). There are
322 two potential explanations for this. First, the monsoon climate brought large
323 precipitation in the warmer months, which would have resulted in large nutrient
324 runoff from the reservoir watershed. Following the monsoon, the nutrients
325 concentration decreased due to reduced precipitation. Second, the Cyanophyta
326 dominated the phytoplankton communities in the warm months due to their higher
327 optimum growth temperature.⁵⁴ These Cyanophyta blooms resulted in the high
328 phytoplankton biovolume in warm months. However, the phytoplankton biovolume
329 decreased with the decreasing of Cyanophyta in the SD and BT reservoirs associated
330 with the decline of water temperature (Supporting Information Figure S3). These two
331 explanations are not entirely independent as it is well known the high water

332 temperature and nutrient level can combine to increase the likelihood of
333 cyanobacterial blooms – indeed modifying nutrient levels has been suggested as a
334 potentially tractable approach to reducing the incidence of such blooms in a warming
335 world.⁵⁵

336 Microbial communities are considered one of the most promising indicators of
337 environmental changes and aquatic ecosystem states due to their rapid response to
338 environmental changes compared with larger animals and plants.^{56, 57} However,
339 microbial communities with different properties may have different and diverse
340 responses to the environmental changes. Therefore, quantifying the response patterns
341 of bacterioplankton and phytoplankton communities to environmental changes is
342 essential for quantifying and understanding the ecosystem recovery process from
343 water pollution (e.g., eutrophication). In this study, we demonstrated that TLA is a
344 useful diagnostic tool to evaluate the direction, rates, and patterns of community
345 change that were not obvious from our more conventional multivariate methods.

346 We found that environmental conditions changed gradually over time - however, it
347 is interesting to note that the direction and variation of community dynamics were
348 stronger in the phytoplankton than in the bacterioplankton (Figure 3). Clearly, the
349 environmental effects measured in this study were stronger for the phytoplankton than
350 for the bacterioplankton (Figure 4). In other words, the temporal stability (or
351 stochasticity) of the community was stronger for the bacterioplankton than for the
352 phytoplankton in these reservoirs. A possible explanation is that the dispersal
353 probability of bacteria is greater than that of phytoplankton. Jones *et al.* investigated
354 the spatial and temporal dynamics of bacterioplankton beta diversity based on the
355 decay of similarity across time and space, and identified equivalent temporal (1 day)
356 and spatial (10 m) scales of variation in bacterial community composition.⁵⁸ The
357 equivalence of a day and a few meters in their impact on bacterial community
358 similarity suggests similar ecological processes driving community assembly occur
359 over both space and time.^{58, 59} Sojininen highlighted factors, such as dispersal rate, as
360 likely drivers of bacterial community turnover in both space and time.⁵⁹ Over time,
361 dispersal may have important effects on the temporal dynamics of microorganisms.^{21,}
362 ²⁵ High dispersal ability allows the microorganisms to have a higher probability of
363 colonizing suitable habitats from regional pools, therefore potentially reducing the
364 variation of community composition through time.^{60, 61} Therefore, it is possible that
365 some, or many, of these microbial populations in reservoirs should be thought of as
366 metapopulations – an idea that is now widely applied to the populations of many
367 macroscopic organisms.^{21, 62} In most cases, the cell size of bacteria is smaller than the
368 size of phytoplankton. Due to their small bodies, free-living bacteria are often
369 assumed to be ubiquitous dispersers and they are presumably more likely to become
370 widely dispersal – possibly by mechanisms such as becoming airborne as waves
371 break.⁶³ It has often been suggested that smaller cell size should lead to the wider
372 dispersal probability in microbes.^{60, 64, 65} It follows that one reason the bacterial
373 community may be more stable in the face of environmental change than the
374 phytoplankton community is because of recolonization after extinction and/or the
375 supplementation of populations by individuals dispersing from elsewhere.

376 In addition to dispersal, other factors such as high abundance, the potential for
377 rapid growth rates, and rapid evolutionary adaptation through mutations and/or
378 horizontal gene transfer could also allow bacteria to quickly adapt to new
379 environmental conditions and maintain the stability of community composition.³
380 Indeed, the potential for widespread horizontal gene transfer potentially blurs the
381 distinction between individual and community in prokaryotic ecology.^{66, 67} Therefore,
382 the response of bacterial communities to environmental change may be less sensitive
383 than that of the eukaryotic microbial community. Recently, Jones and colleagues
384 compared the seasonality of bacterioplankton and micro-eukaryotic planktonic
385 communities in a freshwater lake, and found that the eukaryotic species richness at
386 both sampling locations exhibited strong fluctuations with algal blooms and other
387 environmental changes, whereas the annual fluctuations in the numbers of bacterial
388 OTUs were relative stable.⁶⁸ Furthermore, these authors suggested that the bacterial
389 taxonomic richness was less sensitive to seasonal forcing factors (i.e. temperature,
390 salinity, *Prymnesium parvum* cell abundances, and large spring rain event) than the
391 micro-eukaryotic diversity.⁶⁸ In another similar study, Lear and colleagues compared
392 the epilithic bacterial and benthic macroinvertebrate communities as indicator of
393 ecological health in New Zealand streams.⁶⁹ Although these authors considered that
394 the relationship between localized influences and sessile bacteria may be closer than
395 that between localized influences and bacterioplankton, they found that
396 macroinvertebrate community composition showed a clear gradient with the
397 increasing localized human impact, while epilithic bacterial communities were only
398 different at the most impacted sites.⁶⁹ It appears that bacterial communities provided a
399 less sensitive indicator of the prevailing environmental conditions, than did
400 macroinvertebrates at community level.⁶⁹

401 Last but not least, the phytoplankton communities had larger variance explained by
402 eutrophic related factors (pure eutrophic factors + eutro-physico covariation +
403 eutro-temp covariation + covariation of all variables) than the bacterioplankton
404 communities in our study (Figure 4 and Supporting Information Figure S5). The
405 eutrophication factors such as nitrogen, phosphorus, and transparency can be directly
406 related to the phytoplankton.^{70, 71} On the other hand, the interaction between the
407 temperature and eutrophication factors or between the physico-chemical and
408 eutrophication factors was also highly and directly related to the phytoplankton. As
409 described above, water temperature has a positive effect increasing eutrophication.
410 For example, Cyanophyta may benefit from high temperature, since they have high
411 optimum growth temperatures.⁵⁴ Additionally, algal growth and bloom have dramatic
412 effects on or closely relationship with EC, DO, and pH.⁷²⁻⁷⁴ In contrast, the
413 eutrophication factors have both direct effects on the bacterial community and also
414 indirect effects through changes in phytoplankton community.^{38, 75} In addition, the
415 bacterioplankton community had larger unexplained variance than the phytoplankton.
416 It is possible that unmeasured carbon flux is an important factor that influences the
417 succession of bacterial community.⁷⁶ However, we can safely conclude that the
418 bacterioplankton were less sensitive to the environmental changes of eutrophication
419 variables comparing to phytoplankton in the subtropical reservoir ecosystems.

420 There are, however, potential limitations in our approach that merit further
421 discussion. We should note the different detectability between DGGE and microscopy.
422 The DGGE is a well-established method and most easily detects microorganisms with
423 abundances in the ecosystem of 1% of the total community. Interestingly, the different
424 in detectability seems to have an obvious influence on bacterial α diversity but not on
425 β diversity, thus DGGE has been most useful to compare community structural
426 changes across time and space.⁴⁰ Galand *et al.* defined the abundant bacterioplankton
427 phylotypes as the phylotypes with a relative abundance >1% within a sample. These
428 authors indicated that the composition of abundant bacterial communities was similar
429 to that of the entire bacterial communities.⁷⁷ Similarly, we previously investigated 42
430 Chinese lakes and reservoirs using high-throughput sequencing, and we also found
431 very similar spatial patterns in both abundant (relative abundance >1% in a sample,
432 and mean relative abundance of > 0.1% in all samples) and entire bacterioplankton
433 communities (RELATE $p_m = 0.934$, $P < 0.01$).⁷⁸ Another limitation of DGGE is that
434 with bacterial 16S rRNA genes it can detect some phytoplankton including
435 prokaryotic Cyanophyta but also a few Chloroplasts (from eukaryotes). However, the
436 relative abundance of such phytoplankton is low in our bacterial data (Supporting
437 Information Figure S3). Further, Niu *et al.* explored the relationship between
438 phytoplankton blooms and temporal variation of bacterioplankton community
439 composition.⁷⁹ These authors found a serious *Microcystis* bloom and high biomass of
440 Bacillariophyta and Cryptophyta using microscopy, whereas only three of seventy
441 eight DGGE bands were found to affiliate with Cyanobacteria using universal
442 bacterial 16S rRNA gene primer.⁷⁹

443 In conclusion, our results demonstrated that the temporal community dynamics of
444 both abundant bacterioplankton and phytoplankton showed a significant directional
445 change, corresponding to the environmental changes in the reservoirs. Due to high
446 levels of dispersal, growth rates, evolutionary adaptation, and indirect response to the
447 nitrogen and phosphorus, the temporal stability or stochasticity of abundant
448 bacterioplankton community was greater than that of phytoplankton community.
449 These indicated that the phytoplankton community was more sensitive to
450 environmental changes (i.e. improvements in water quality from a human perspective)
451 than the bacterioplankton community in these reservoir ecosystems. This is important,
452 because analyzing multi-components of ecosystem (e.g. primary producers and
453 decomposers) simultaneously can provide a more comprehensive picture when
454 identifying an aquatic ecosystem experiencing or recovering from an environmental
455 change or stress. Additional investigations of more microbial components at different
456 time scales and environmental gradients are needed in order to better understand the
457 relative roles of eutrophication and global warming in affecting aquatic ecosystems.

458 ASSOCIATED CONTENT

459 Supporting Information

460 Supplementary Figures S1–S5 and Supplementary Table S1 showing additional study
461 details. The Supporting Information is available free of charge on the ACS

462 Publications website.

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466 **Notes**

467 The authors declare no competing financial interest.

468

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475 **AUTHOR CONTRIBUTIONS**

476 J.Y.* and L.L. designed research; L.L., J.Y., H.L., and X.Y. performed the
477 experiments; L.L., D.M.W., and J.Y.* analyzed data and wrote the paper.

478

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- 666

667 Figure Legends

668 **Figure 1.** PCA plots showing the overall seasonal trends of environmental variables in Shidou
669 (SD), Bantou (BT) and Tingxi (TX) reservoirs. Temp – water temperature, Trans - transparency,
670 EC – electrical conductivity, TSIC – comprehensive trophic state index. The heavy monsoon rains
671 begin from April to September in the study area (see Material and Methods).

672

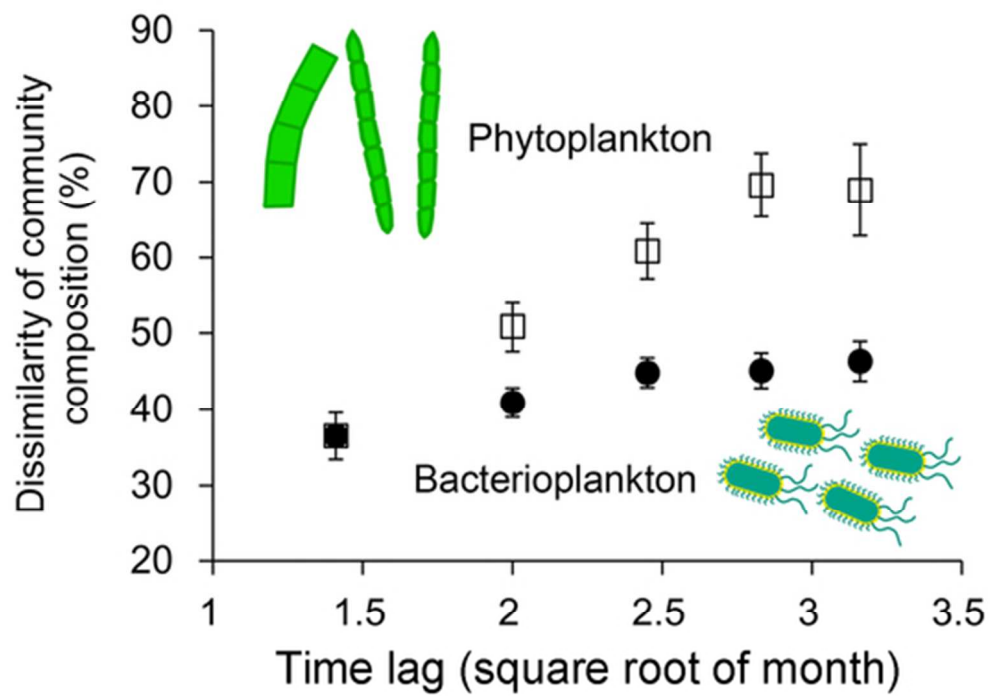
673 **Figure 2.** Temporal variability in abundant bacterial OTU abundance and abundant phytoplankton
674 biovolume, and median absolute deviation (MAD) in Bray-Curtis dissimilarity between samples.
675 Data are means \pm standard error (error bars); $N=18$. Statistical analysis is *t* test (** $P < 0.01$; * $P <$
676 0.05).

677

678 **Figure 3.** Time-lag regression analysis of changes in abundant bacterioplankton, abundant
679 phytoplankton communities, and environmental variables.

680

681 **Figure 4.** Results of abundant bacterioplankton and phytoplankton variance partitioning for each
682 reservoir. Eutro (eutrophic factors), Physico (physico-chemical factors), Temp (water
683 temperature).



Abstract Graphic
45x32mm (300 x 300 DPI)

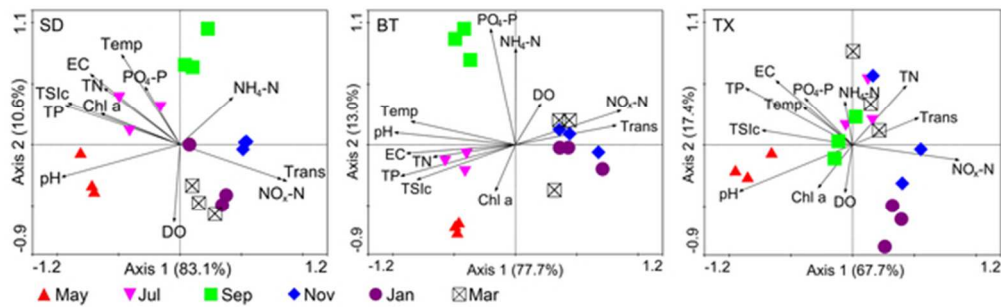


Figure 1. PCA plots showing the overall seasonal trends of environmental variables in Shidou (SD), Bantou (BT) and Tingxi (TX) reservoirs. Temp – water temperature, Trans - transparency, EC – electrical conductivity, TSic – comprehensive trophic state index. The heavy monsoon rains begin from April to September in the study area (see Material and Methods).
 54x16mm (300 x 300 DPI)

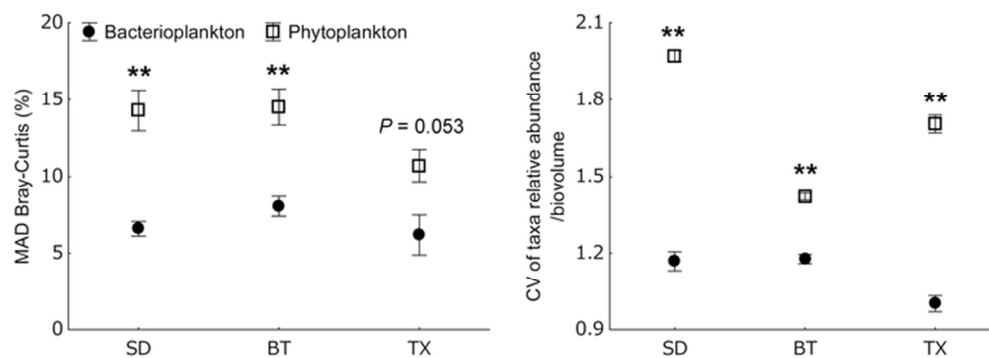


Figure 2. Temporal variability in abundant bacterial OTU abundance and abundant phytoplankton biovolume, and median absolute deviation (MAD) in Bray-Curtis dissimilarity between samples. Data are means \pm standard error (error bars); N = 18. Statistical analysis is t test (**P < 0.01; *P < 0.05). 65x24mm (300 x 300 DPI)

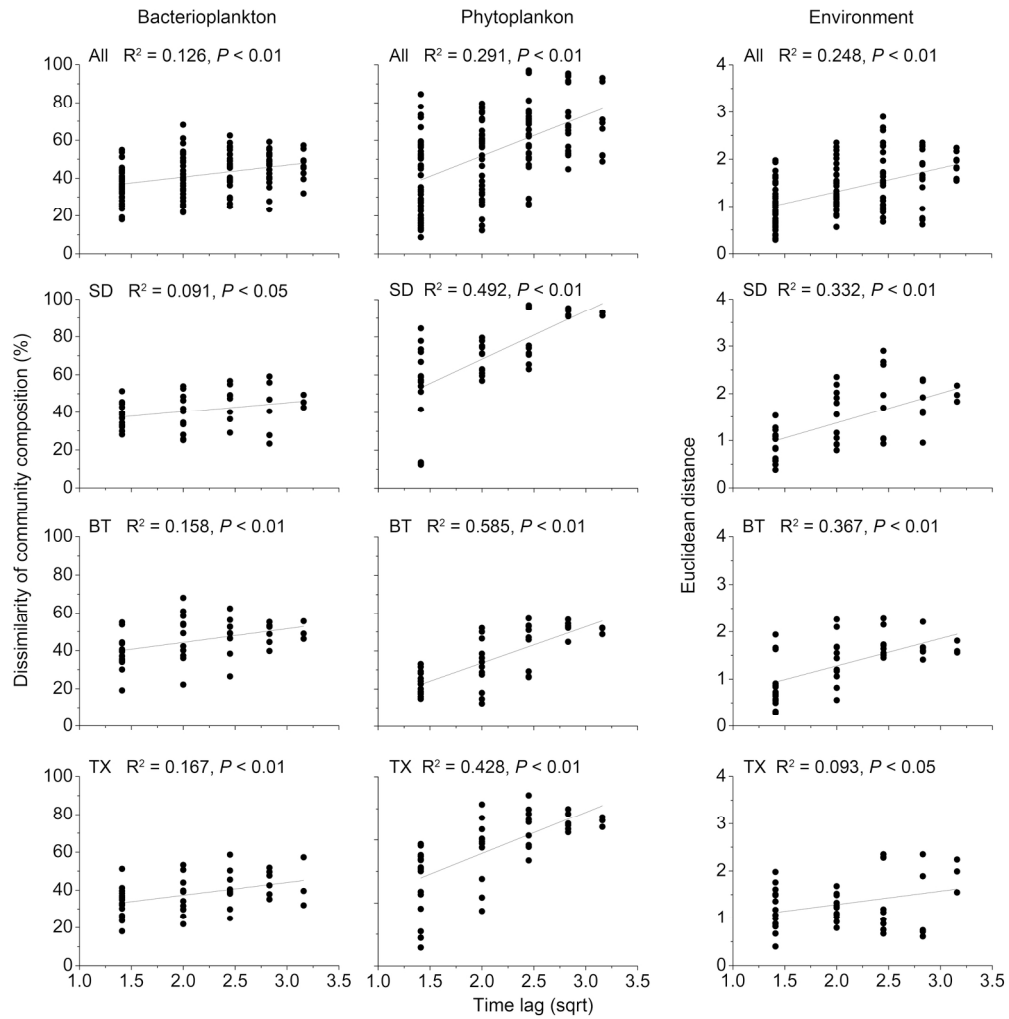


Figure 3. Time-lag regression analysis of changes in abundant bacterioplankton, abundant phytoplankton communities, and environmental variables.
180x183mm (300 x 300 DPI)

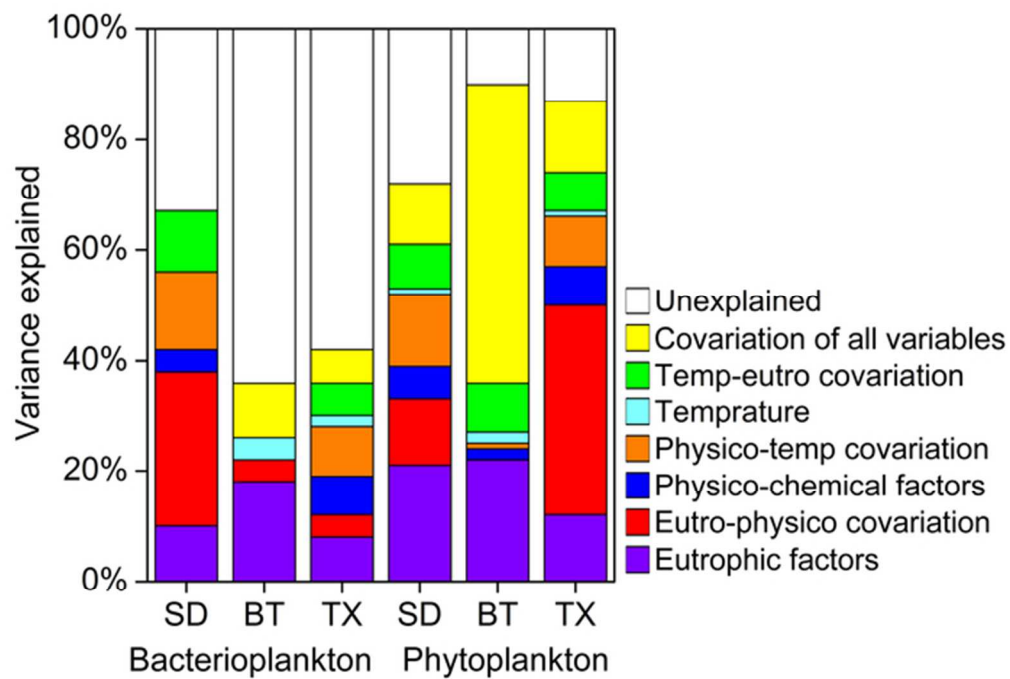


Figure 4. Results of abundant bacterioplankton and phytoplankton variance partitioning for each reservoir. Eutro (eutrophic factors), Physico (physico-chemical factors), Temp (water temperature).
58x39mm (300 x 300 DPI)