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Phytoplankton Communities Exhibit a Stronger Response to Environmental Changes than Bacterioplankton in Three Subtropical Reservoirs.

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

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ABSTRACT

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

Bacterioplankton and phytoplankton are critical components of aquatic microbial food webs, and play essential roles in the structure and function of aquatic ecosystems.\textsuperscript{1, 2} Understanding the processes and mechanisms that underlie the abundance and/or biovolume variations of bacterioplankton and phytoplankton communities, are major goals in both pure and applied microbial community ecology. Many previous studies of bacterioplankton and phytoplankton variation have focused on traditional biogeographical concepts, such as distance decay relationships,\textsuperscript{3, 4} species-area relationships,\textsuperscript{5, 6} and the niche vs. neutral debate.\textsuperscript{7, 9} Data on the temporal variation in aquatic microbial community composition is more mixed and limited. There is a long history of monitoring phytoplankton in lakes in some parts of the world – for example, such studies have a history of over 100 years in the English Lake District, with extensive data sets existing from the mid-twentieth century onwards.\textsuperscript{10} However, bacteria (excluding cyanobacteria) were much more challenging to study until the rise of molecular methods in the late 1980’s, so even in well studied regions such as the English Lake District there is much less known about the patterns in bacterial abundance, compared with the data on phytoplankton.\textsuperscript{11} Macan and Worthington (p83) summarized the mid-twentieth century position in writing that ‘The bacteria… play an important, though as yet little understood role in the economy of all fresh waters.’\textsuperscript{12} Even today the global distribution of such studies is very patchy with the vast majority of molecular studies of freshwater bacterial communities being confined to Europe, and to a lesser extent North America. The rest of the world is covered by a very small number of studies\textsuperscript{13} with the tropics and sub-tropics being particularly poorly sampled - but see Dalu et al. for a rare African example.\textsuperscript{14} Arguably, this lack of understanding of the temporal behavior of both bacterioplankton and phytoplankton communities hinders the development of theories about how the stability of microbial community structure and function is maintained across time.\textsuperscript{14}

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

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

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

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

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

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

The comprehensive trophic state index was calculated according to classical Carlson TSI based on three limnological parameters namely chlorophyll a, Secchi disk transparency, and total phosphorus. Where 0 < TSIc ≤ 30 = oligotrophic, 30 < TSIc ≤ 40 = oligo-mesotrophic, 40 < TSIc ≤ 50 = mesotrophic, 50 < TSIc ≤ 60 = light eutrophic, 60 < TSIc ≤ 70 = middle eutrophic, and 70 < TSIc ≤100 = hypereutrophic.

Phytoplankton analysis. Phytoplankton were identified and counted using an inverted microscope (Motic, Xiamen, China) according to Shen et al., Zhang and Huang, and Hu and Wei. Three subsamples were investigated for each sample and at least 500 individuals were counted for each subsample. To compare the bacterioplankton and phytoplankton communities, we transformed the phytoplankton abundance to biovolume according to Paver et al. Biovolume was estimated from
cell numbers and cell size measurements. In using denaturing gradient gel electrophoresis it is difficult to detect microbes with abundances of < 1% of the total community. Therefore, to improve the comparability between bacterioplankton and phytoplankton, we performed the statistical analyses using only abundant phytoplankton species (> 1% biovolume in a sample).

**DNA extraction and PCR amplification.** Total DNA was extracted directly from the filter using an E.Z.N.A. DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s instructions. The extracted DNA was dissolved in 50 µl TE buffer, quantified by spectrophotometer and stored at −20 °C until further use.

The 16S rRNA gene fragments were amplified with the primers 341F-GC (5′-CGC CCG CGC CCC GCG CCC GTC CCG CCC CCC GCC TAC GGG AGG CAG CAG-3′) and 907R (5′-CGC TCA ATT CMT TTG AG T TTS3′) under the following PCR conditions: 5 min denaturation at 94°C and 10 touchdown cycles at 94°C for 0.5 min, 67°C (with the temperature decreasing 1°C each cycle) for 0.5 min, 72°C for 1 min; followed by 20 cycles at 94°C for 0.5 min, 57°C for 0.5 min, 72°C for 1 min and a final extension at 72°C for 10 min. Each 50 µl PCR reaction contained 0.3 µM each primer, 2.5 U Taq DNA polymerase (TaKaRa, Otsu, Shiga, Japan), 1.5 mM MgCl₂, 200 µM each deoxynucleoside triphosphate, and approximately 40 ng of template DNA in 1× PCR buffer.

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

Dominant DGGE bands were excised from the gels and eluted overnight in autoclaved Milli-Q water at 4 °C. The eluted DNA was reamplified with the original primer set (without GC clamp). PCR products were purified with the TaKaRa Agarose Gel DNA Purification Kit (Takara, Otsu, Shiga, Japan), then cloned into a pMD18-vector (Takara, Otsu, Shiga, Japan) and transformed into Escherichia coli DH5α competent cells (Takara, Otsu, Shiga, Japan). The successfully inserted plasmids were sequenced unidirectionally using an automated sequencer (ABI 3730 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). All bacterial 16S
rRNA sequences were manually checked and modified with BIOEDIT v7.0.9\(^44\) and then compared with the GenBank database using BLASTN\(^45\). The bacterial 16S rRNA sequences were classified by the Ribosomal Database Project Classifier with 80% confidence.\(^46\)

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

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

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

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

We divided the twelve environmental variables into three groups: the first group which is related to eutrophication includes TN, NH\(_4\)-N, NO\(_x\)-N, TP, PO\(_4\)-P, transparency, Chl \(a\) and TSIc; the second group constitutes of three variables (EC, pH, DO) which are related to physico-chemical factors; the third group comprised only water temperature. We then used a forward-selection procedure with Monte Carlo permutation tests to select the environmental variables which explained a significant (\(P < 0.05\)) variation of the bacterial and phytoplankton data in each group.\(^49\) To eliminate collinearity among variables within each group, explanatory variables with the highest variance inflation factor (VIF) were sequentially removed until all VIFs were less than 20.\(^49\) Finally, significant variables in each group were selected to perform variance partitioning using varpart function with adjusted \(R^2\) (vegan package in R software). Before the forward-selection procedure, the microbial data were Hellinger transformed. We used principal component analysis (PCA) to show main gradients in explained variance.

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

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

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

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

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

Temporal variability of community composition. We obtained NMDS ordination plots for bacterioplankton and phytoplankton separately (Supporting Information Figure S4). Both bacterioplankton and phytoplankton communities showed a seasonal succession from May 2010 to March 2011 in all three reservoirs. Further, the variation in phytoplankton community between months was larger than in the bacterioplankton
community. Also, both the coefficient of variation (CV) of abundant phytoplankton taxa and median absolute deviation (MAD) of phytoplankton Bray-Curtis dissimilarity were pronounced higher than those of bacterioplankton community (Figure 2).

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

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

DISCUSSION
In general, the TN, TP, NO$_3$-N, Chl $a$, and TSIc, which are closely related to eutrophication, decreased gradually during the study period. In particular, the TSIc values decreased from middle eutrophic to oligo-mesotrophic levels. In addition, the EC and pH decreased (note that the pH gradually declined to close to 7), while the transparency and DO increased. These results indicated that reservoir water quality gradually improved during the study (Supporting Information Figure S2). There are two potential explanations for this. First, the monsoon climate brought large precipitation in the warmer months, which would have resulted in large nutrient runoff from the reservoir watershed. Following the monsoon, the nutrients concentration decreased due to reduced precipitation. Second, the Cyanophyta dominated the phytoplankton communities in the warm months due to their higher optimum growth temperature. These Cyanophyta blooms resulted in the high phytoplankton biovolume in warm months. However, the phytoplankton biovolume decreased with the decreasing of Cyanophyta in the SD and BT reservoirs associated with the decline of water temperature (Supporting Information Figure S3). These two explanations are not entirely independent as it is well known the high water
temperature and nutrient level can combine to increase the likelihood of cyanobacterial blooms – indeed modifying nutrient levels has been suggested as a potentially tractable approach to reducing the incidence of such blooms in a warming world.\textsuperscript{55}

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

We found that environmental conditions changed gradually over time - however, it is interesting to note that the direction and variation of community dynamics were stronger in the phytoplankton than in the bacterioplankton (Figure 3). Clearly, the environmental effects measured in this study were stronger for the phytoplankton than for the bacterioplankton (Figure 4). In other words, the temporal stability (or stochasticity) of the community was stronger for the bacterioplankton than for the phytoplankton in these reservoirs. A possible explanation is that the dispersal probability of bacteria is greater than that of phytoplankton. Jones \textit{et al.} investigated the spatial and temporal dynamics of bacterioplankton beta diversity based on the decay of similarity across time and space, and identified equivalent temporal (1 day) and spatial (10 m) scales of variation in bacterial community composition.\textsuperscript{58} The equivalence of a day and a few meters in their impact on bacterial community similarity suggests similar ecological processes driving community assembly occur over both space and time.\textsuperscript{58, 59} Soininen highlighted factors, such as dispersal rate, as likely drivers of bacterial community turnover in both space and time.\textsuperscript{59} Over time, dispersal may have important effects on the temporal dynamics of microorganisms.\textsuperscript{21, 25} High dispersal ability allows the microorganisms to have a higher probability of colonizing suitable habitats from regional pools, therefore potentially reducing the variation of community composition through time.\textsuperscript{60, 61} Therefore, it is possible that some, or many, of these microbial populations in reservoirs should be thought of as metapopulations – an idea that is now widely applied to the populations of many macroscopic organisms.\textsuperscript{21, 62} In most cases, the cell size of bacteria is smaller than the size of phytoplankton. Due to their small bodies, free-living bacteria are often assumed to be ubiquitous dispersers and they are presumably more likely to become widely dispersal – possibly by mechanisms such as becoming airborne as waves break.\textsuperscript{63} It has often been suggested that smaller cell size should lead to the wider dispersal probability in microbes.\textsuperscript{60, 64, 65} It follows that one reason the bacterial community may be more stable in the face of environmental change than the phytoplankton community is because of recolonization after extinction and/or the supplementation of populations by individuals dispersing from elsewhere.
In addition to dispersal, other factors such as high abundance, the potential for rapid growth rates, and rapid evolutionary adaptation through mutations and/or horizontal gene transfer could also allow bacteria to quickly adapt to new environmental conditions and maintain the stability of community composition.\(^3\) Indeed, the potential for widespread horizontal gene transfer potentially blurs the distinction between individual and community in prokaryotic ecology.\(^66,67\) Therefore, the response of bacterial communities to environmental change may be less sensitive than that of the eukaryotic microbial community. Recently, Jones and colleagues compared the seasonality of bacterioplankton and micro-eukaryotic planktonic communities in a freshwater lake, and found that the eukaryotic species richness at both sampling locations exhibited strong fluctuations with algal blooms and other environmental changes, whereas the annual fluctuations in the numbers of bacterial OTUs were relatively stable.\(^68\) Furthermore, these authors suggested that the bacterial taxonomic richness was less sensitive to seasonal forcing factors (i.e., temperature, salinity, *Prymnesium parvum* cell abundances, and large spring rain event) than the micro-eukaryotic diversity.\(^68\) In another similar study, Lear and colleagues compared the epilithic bacterial and benthic macroinvertebrate communities as indicators of ecological health in New Zealand streams.\(^69\) Although these authors considered that the relationship between localized influences and sessile bacteria may be closer than that between localized influences and bacterioplankton, they found that macroinvertebrate community composition showed a clear gradient with the increasing localized human impact, while epilithic bacterial communities were only different at the most impacted sites.\(^69\) It appears that bacterial communities provided a less sensitive indicator of the prevailing environmental conditions, than did macroinvertebrates at community level.\(^69\)

Last but not least, the phytoplankton communities had larger variance explained by eutrophic related factors (pure eutrophic factors + eutro-physico covariation + eutro-temp covariation + covariation of all variables) than the bacterioplankton communities in our study (Figure 4 and Supporting Information Figure S5). The eutrophication factors such as nitrogen, phosphorus, and transparency can be directly related to the phytoplankton.\(^70,71\) On the other hand, the interaction between the temperature and eutrophication factors or between the physico-chemical and eutrophication factors was also highly and directly related to the phytoplankton. As described above, water temperature has a positive effect increasing eutrophication. For example, Cyanophyta may benefit from high temperature, since they have high optimum growth temperatures.\(^54\) Additionally, algal growth and bloom have dramatic effects on or closely relationship with EC, DO, and pH.\(^72-74\) In contrast, the eutrophication factors have both direct effects on the bacterial community and also indirect effects through changes in phytoplankton community.\(^38,75\) In addition, the bacterioplankton community had larger unexplained variance than the phytoplankton. It is possible that unmeasured carbon flux is an important factor that influences the succession of bacterial community.\(^76\) However, we can safely conclude that the bacterioplankton were less sensitive to the environmental changes of eutrophication variables comparing to phytoplankton in the subtropical reservoir ecosystems.
There are, however, potential limitations in our approach that merit further discussion. We should note the different detectability between DGGE and microscopy. The DGGE is a well-established method and most easily detects microorganisms with abundances in the ecosystem of 1% of the total community. Interestingly, the different in detectability seems to have an obvious influence on bacterial $\alpha$ diversity but not on $\beta$ diversity, thus DGGE has been most useful to compare community structural changes across time and space.\(^{40}\) Galand et al. defined the abundant bacterioplankton phylotypes as the phylotypes with a relative abundance $> 1\%$ within a sample. These authors indicated that the composition of abundant bacterial communities was similar to that of the entire bacterial communities.\(^{77}\) Similarly, we previously investigated Chinese lakes and reservoirs using high-throughput sequencing, and we also found very similar spatial patterns in both abundant (relative abundance $> 1\%$ in a sample, and mean relative abundance of $> 0.1\%$ in all samples) and entire bacterioplankton communities (RELATE $\rho_m = 0.934$, $P < 0.01$).\(^{78}\) Another limitation of DGGE is that with bacterial 16S rRNA genes it can detect some phytoplankton including prokaryotic Cyanophyta but also a few Chloroplasts (from eukaryotes). However, the relative abundance of such phytoplankton is low in our bacterial data (Supporting Information Figure S3). Further, Niu et al. explored the relationship between phytoplankton blooms and temporal variation of bacterioplankton community composition.\(^{79}\) These authors found a serious Microcystis bloom and high biomass of Bacillariophyta and Cryptophyta using microscopy, whereas only three of seventy eight DGGE bands were found to affiliate with Cyanobacteria using universal bacterial 16S rRNA gene primer.\(^{79}\)

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

ASSOCIATED CONTENT

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

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**Notes**

The authors declare no competing financial interest.

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**AUTHOR CONTRIBUTIONS**

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

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 ± standard error (error bars); N =18. Statistical analysis is t test (**P < 0.01; *P < 0.05).

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

Figure 4. Results of abundant bacterioplankton and phytoplankton variance partitioning for each reservoir. Eutro (eutrophic factors), Physico (physico-chemical factors), Temp (water temperature).
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180x183mm (300 x 300 DPI)
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58x39mm (300 x 300 DPI)