



Community dynamics of free-living and particle-attached bacteria following a reservoir *Microcystis* bloom

Min Liu^{a,b,c}, Lemian Liu^{a,d}, Huihuang Chen^a, Zheng Yu^{a,e}, Jun R. Yang^{a,f}, Yuanyuan Xue^{a,b,c}, Bangqin Huang^c, Jun Yang^{a,*}

^a Aquatic Ecohealth Group, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c College of the Environment and Ecology, Xiamen University, Xiamen 361102, China

^d Technical Innovation Service Platform for High Value and High Quality Utilization of Marine Organism, Fuzhou University, Fuzhou 350108, China

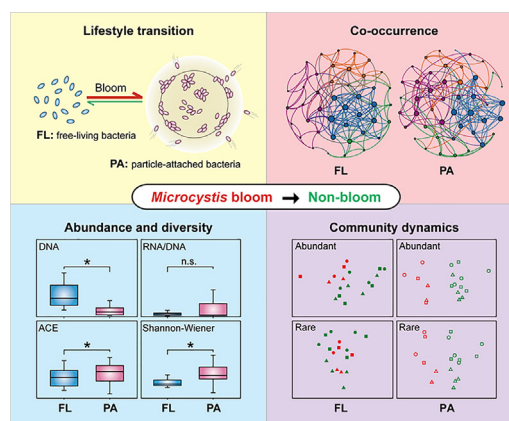
^e Department of Chemical Engineering, University of Washington, Seattle, WA 98105, USA

^f Engineering Research Center of Ecology and Agricultural Use of Wetland, College of Agriculture, Yangtze University, Jingzhou 434025, China

HIGHLIGHTS

- Bacteria showed lifestyle-specific succession following *Microcystis* bloom.
- Bacteria exhibited a preference for a particle-attached lifestyle during the bloom.
- Free-living and particle-attached bacteria shaped by different ecological factors.
- Abundant and rare bacteria presented contrasting variations across depth and time.

GRAPHICAL ABSTRACT



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ABSTRACT

The composition of microbial communities can vary at the microspatial scale between free-living (FL) and particle-attached (PA) niches. However, it remains unclear how FL and PA bacterial communities respond to cyanobacterial blooms across water depths. Here, we examined the community dynamics of the FL (0.2–3 μm) and PA (>3 μm) bacterioplankton based on 16S rRNA gene high-throughput sequencing in a subtropical stratified reservoir under *Microcystis aeruginosa* bloom and non-bloom conditions. Both FL and PA bacterioplankton communities showed different responses in alpha- and beta-diversities to the bloom, suggesting the idea that the responses of bacterial community could depend on lifestyle. Specifically, abundant PA subcommunities showed a greater variation between bloom and non-bloom groups than abundant FL ones. In contrast, rare FL subcommunities exhibited a stronger response to water depth than rare PA ones. Furthermore, the rare taxa exhibited a preference for PA status, shaped and stimulated by the *M. aeruginosa* bloom. Our analyses also showed that PA bacterial communities were generally more diverse and appeared to be more responsive to routinely measured environmental variables than FL bacteria. *Microcystis* blooms had a facilitative influence on specific bacteria by mediating the transitions from free-living to particle-attached lifestyles. Altogether, these findings highlight

* Corresponding author.

E-mail address: jyang@iue.ac.cn (J. Yang).

the importance of bacterial lifestyle and abundance in understanding the dynamics of microbial community in cyanobacterial bloom aquatic ecosystem.

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1. Introduction

Cyanobacterial blooms occur in aquatic ecosystem around the world and have adverse effects on water quality, recreation and food web dynamics (Berry et al., 2017; Huisman et al., 2018). A cascade of changes in planktonic microbial communities have been observed during cyanobacterial blooms (Woodhouse et al., 2016; Xue et al., 2017; Huisman et al., 2018). Monitoring the dynamics of cyanobacterial and bacterial populations, and investigating their interaction and driving factors will help in mitigating and prevention of harmful cyanobacterial blooms, and enhance the ability to predict microbial response to environmental change (Ramanan et al., 2016; Woodhouse et al., 2016; Huisman et al., 2018).

Attempts have been made to study bacterioplankton succession during algal blooms in both experimental mesocosms (Riemann et al., 2000; von Scheibner et al., 2014) and field studies (Teeling et al., 2012; Yang et al., 2015; Woodhouse et al., 2016; Berry et al., 2017). However, these studies were mainly based on either a single biomass size fraction or on bulk water samples. In fact, bacterioplankton can be classified into two types of communities depending on their relationship with the particulate matter in the water (e.g. free-living (FL) and particle-attached (PA) lifestyles) (Stocker, 2012; Satinsky et al., 2014; Simon et al., 2014; Tang et al., 2015). Increasing evidence indicates that mode of bacterial life influences the utilization of dissolved organic matter (Luo and Moran, 2015), the relationship between phytoplankton-bacteria (Ramanan et al., 2016) and environmental sensitivity (Yung et al., 2016). Recently, contrasting dynamics of FL and PA bacteria at chlorophyll-*a* maximum layer were observed during the succession of diatom blooms (Rösel and Grossart, 2012). Synchronized growth of *Microcystis* sp., and attached bacteria as well as *Pediastrum* (chlorophyta) and FL bacteria have been found in microcosm experiments (Cao et al., 2016). So far, we have limited knowledge about the dynamics of bacterioplankton community following cyanobacterial blooms in inland waters at micron-scale distance.

Free-living and particle-attached bacteria have been regarded as interacting assemblages (Riemann and Winding, 2001; Grossart, 2010). The switching of bacterial lifestyles is a key factor shaping particle remineralization rates in the aquatic ecosystem. The exchange or transition of bacterial lifestyles, which was influenced by chemical triggers, substrate availability, turbulence and eutrophication (Grossart, 2010; Tang et al., 2017), has been shown during a marine phytoplankton bloom (Rinta-Kanto et al., 2012; Teeling et al., 2012). The biogeochemical process of microbial food webs depends strongly on the properties of suspended particles (Mestre et al., 2017). Hence, there is a need for understanding of the transition between two types of bacterial lifestyle to help clarify the influence of cyanobacterial bloom on the microbial community dynamics.

In this study, we investigated the community variation of both FL and PA bacteria using quantitative real-time PCR and 16S rRNA gene sequencing following a *Microcystis* bloom in a subtropical stratified reservoir. The main objectives of this study were to: 1) explore whether or not FL and PA bacteria present different variations in abundance, diversity and community composition across water layers following a *Microcystis* bloom; 2) investigate the transition of lifestyles for different bacterial taxa between the bloom and non-bloom stages; 3) investigate the relationship between environment and bacterial community, and species co-occurrence patterns of FL and PA bacteria. We hypothesized that 1) bacterial lifestyle influenced the community response to the *Microcystis* bloom, 2) cyanobacterial bloom would facilitate a lifestyle

switch from FL to PA bacteria, and 3) community assembly of FL to PA bacteria could be shaped by different ecological factors.

2. Materials and methods

2.1. Study site and sampling

Xidong Reservoir is located in the north of Xiamen city, southeast China, with a capacity of 14.0×10^6 m³. The area has a subtropical humid monsoon climate with an annual mean precipitation of 1468 mm and an annual mean temperature of 21 °C (Xue et al., 2017). The sampling station is located in the main lacustrine zone (24°49'N, 118°10'E) near the dam (Fig. 1). Water samples were collected twice a month from October to December 2014 at three layers namely the surface (0.5 m), oxycline (12–20 m) and bottom (25 m) layers; as described in our previous study (Xue et al., 2017). The sampling periods were divided into bloom (days 297, 304) and non-bloom (days 325, 332, 346 and 363) stages. Each water sample (maintained at the temperature of 4 °C) was immediately transported to the laboratory and was subsequently divided into two subsamples: one for water chemistry analyses, the other for bacterioplankton analyses. Although there is no standard definition of pore-size to distinguish free-living bacteria from particle-attached ones, the 3 µm pore-size has been the most widely used in previous studies (e.g., Teeling et al., 2012; Simon et al., 2014; Schmidt et al., 2016). Therefore, to facilitate comparisons with other studies, we used the 3 µm pore-size to separate both types of bacteria. Filtering was carried out by pumping 300–400 mL water serially through 3 µm and 0.2 µm pore-size polycarbonate membranes (Millipore, Bedford, MA, USA) within 60 min. The filters were then stored at –80 °C until DNA and RNA extraction.

2.2. Measurement of environmental parameters

The physical and chemical characteristics of the water were measured as described in Liu et al. (2013) and Xue et al. (2017). Water temperature, electrical conductivity (EC), pH, dissolved oxygen (DO), turbidity, salinity and oxidation reduction potential (ORP) were measured in situ at 1-increments using a multi-parameter water quality analyzer (Hydrolab DS5, Hach Company, Loveland, CO, USA). Total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) were measured by a Shimadzu TOC-VCPH analyzer (Shimadzu, Japan). Ammonium nitrogen (NH₄-N), nitrite and nitrate nitrogen (NO_x-N) and phosphate phosphorus (PO₄-P) were measured with a Lachat QC8500 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA). Total phosphorus (TP) was determined by spectrophotometry after digestion. Transparency was measured by a 30 cm Secchi disk. Chlorophyll *a* (Chl-*a*) was analyzed by a PHYTO-PAM Phytoplankton Analyzer (Heinz Walz GmbH, Eichenring, Germany). The comprehensive trophic state index (TSIc) was calculated based on three limnological parameters namely chlorophyll-*a*, transparency, and total phosphorus (TP) (Carlson, 1977; Yang et al., 2012). The TSIc values correspond with different trophic state conditions: 40 < TSIc ≤ 50 mesotrophic, 50 < TSIc ≤ 60 light eutrophic, 60 < TSIc ≤ 70 middle eutrophic. In this study, a bloom was defined based on the chlorophyll-*a* concentration, cyanobacteria abundance and water status (Eiler and Bertilsson, 2004; Woodhouse et al., 2016). The phytoplankton species were identified and counted using an inverted microscope following Yang et al. (2016).

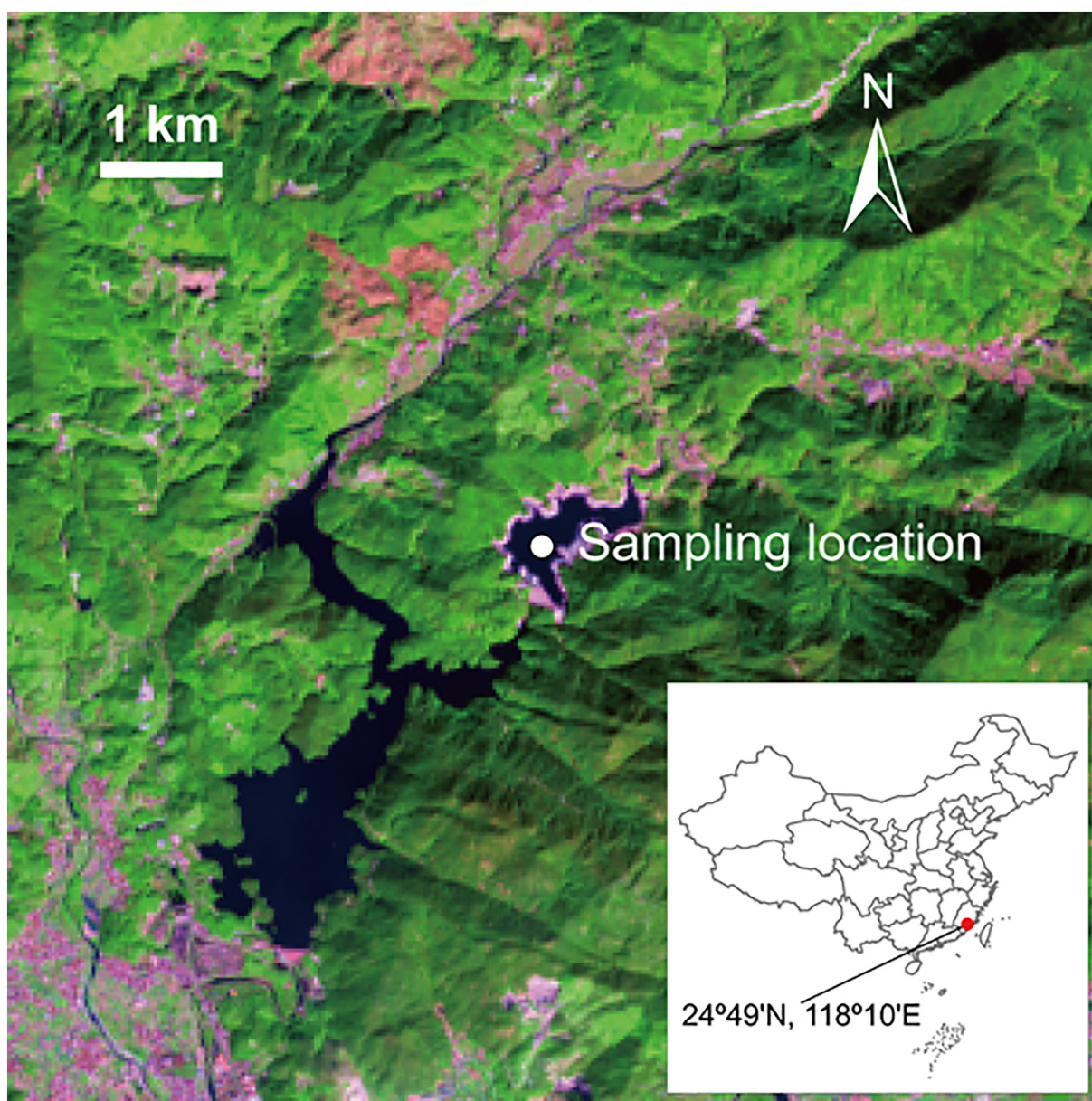


Fig. 1. Map of China showing the sampling site in Xidong Reservoir (Guo et al., 2018).

2.3. DNA and RNA extraction

Total DNA and RNA were extracted using a FastDNA spin kit (MP Biomedicals, Santa Ana, CA, USA) and E.Z.N.A. total RNA kit II (Omega Bio-Tek, Doraville, GA, USA), separately. Then RNA was transcribed into complementary DNA (cDNA) using the Takara OneStep RT-PCR kit Version 2.0. Reverse transcription was performed with a 15 min reaction at 37 °C and terminated by 5 s incubation at 85 °C. The concentration and quality of extracted DNA were determined through spectrophotometric analysis using a NanoDrop ND-1000 device (Thermo Fisher Scientific, Waltham, MA, USA). Then, purified DNA and cDNA were stored at −20 °C until further use.

2.4. Real-time quantitative PCR and RT-PCR

Standard curves were prepared in triplicate from linearized plasmid serial dilutions containing between 10^9 and 10^3 16S rRNA gene copies following Yu et al. (2014). A standard curve was generated by plotting

the threshold cycle values vs $\log_{10}$ of the gene copy numbers. The efficiency of PCR should be between 90% and 110% for further analysis. The quantitative PCR and RT-PCR assays were carried out in a volume of 20 μ L including 10 μ L SYBR Premix ExTaq™ (Takara Bio Inc., Kusatsu, Japan), 0.25 μ M of each primer (341F and 515R) (López-Gutiérrez et al., 2004), RNase-free water and 2 μ L of template DNA or cDNA. Thermal cycling involved incubation at 95 °C for 3 min, followed by 40 cycles of 15 s at 95 °C and 34 s at 56 °C. Negative and positive controls were used throughout the experiment. All the measurements were performed in triplicate. We also calculated the RNA/DNA ratio to roughly estimate the cell activity of the bacterial community (Yu et al., 2014).

2.5. High-throughput sequencing and bioinformatics

Bar-coded fragments of the 16S rRNA gene, spanning the V3 and V4 hypervariable regions, were amplified using primer 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3') and sequenced using Illumina HiSeq platform (Illumina, Inc., San Diego, CA, USA) using a paired-end (2×250 bp) sequencing strategy.

Each DNA sample was individually PCR-amplified in 30 μ L reactions including an initial denaturation at 98 °C for 1 min, followed by 30 cycles of 10 s at 98 °C, 30 s at 50 °C, and 30 s at 72 °C. At the end of the amplification, the amplicons were subjected to a final 5 min extension at 72 °C. Each reaction contained 15 μ L of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Beverly, MA, USA), 0.2 μ L of forward and reverse primers, and about 10 ng template DNA.

Paired-end reads from the original DNA sequences were merged by using FLASH (Magoč and Salzberg, 2011) and then assigned to each sample according to the unique barcodes. A total of 2,665,882 raw sequences were obtained, ranging from 59,695 to 83,572 with a mean of 74,052 sequences per sample. Sequence data were processed using QIIME 1.2.0 software following standard protocols: maximum number of consecutive low-quality base = 3; minimum of continuous high-quality base = 75% of total read length; maximum number of ambiguous bases = 0 (Caporaso et al., 2010). Chimera sequences were discarded prior to further analysis (Edgar et al., 2011). The UPARSE pipeline was used to pick operational taxonomic units (OTUs) at 97% similarity level (Edgar, 2013). Representative sequences were taxonomically classified by the RDP classifier using an 80% confidence threshold against the Greengenes database (DeSantis et al., 2006). All eukaryota, chloroplasts, archaea, mitochondria and unknown sequences were excluded. To minimize inclusion of sequencing errors, singletons (OTUs with only one sequence) were also eliminated before statistical analysis. From the 36 samples, we obtained 1,854,751 high quality sequences, ranging from 36,152 to 61,477, with a mean of 51,520 sequences per sample. Finally, sequences data were normalized to 36,152 sequences per sample using the 'sub.sample' command in MOTHUR v.1.33.3 (Schloss et al., 2009).

Considering that the OTU definition at 97% similarity with UPARSE approach is not a specific and accurate estimation of the species level diversity, we also used the DADA2 approach based on exact sequence variants to define the OTU. The DADA2 method was run using scripts found in Microbiome Helper (Callahan et al., 2016). All DADA2 wrapper scripts were run with default settings. Only the results of indicator OTUs with both approaches were showed in this study, because more indicator taxa were generated by DADA2 than UPARSE. However, other community statistical results based on UPARSE were very similar to those of DADA2, thus we only showed UPARSE-based results.

2.6. Definition of abundant and rare taxa

Abundant OTUs were defined as those with a representation $\geq 1\%$ within a sample but never rare ($<0.01\%$), and rare OTUs were defined as having an abundance $<0.01\%$ within a sample but never $\geq 1\%$ based on all samples ($n = 36$). Our definition could avoid overlaps between abundant and rare OTUs compared with previous studies (Galand et al., 2009; Liu et al., 2015b).

2.7. Particle-association niche index

We used a 'particle-association niche index' (PAN index) as a measure of the position of an OTU in a continuous niche space ranging from a completely free-living to a completely particle-attached lifestyle. Only the OTUs with >10 sequences were considered in this study. PAN index was computed by an abundance-weighted mean: for a given OTU, we recorded its abundance in every sample and recorded the size fraction to which each sample belonged (Salazar et al., 2015). FL and PA bacteria were given a value of 0 and a value of 1, respectively. Then, abundance-weighted mean of these values were calculated. Thus, values of PAN index ranges from 0 to 1, and each number reflects the size preference of a given OTU as follows: 0, preference for FL lifestyle; 0.5, equally distributed across FL and PA lifestyle; 1, preference for PA lifestyle.

2.8. Statistical analyses

The ACE, Chao 1, Shannon-Wiener, Simpson, and Pielou's evenness indices were calculated in the vegan package (R Development Core Team, 2017). One-way analysis of variance (ANOVA) was used to test the effect of stratification, size fraction and bloom on the microbial abundance, activity and diversity. Spearman's rank correlations were used to determine the relationships between environmental factors and diversity, abundance of bacterioplankton community.

The Bray-Curtis dissimilarity matrix was computed with the bacterial absolute abundance data which was $\log(x + 1)$ transformed. Then, bacterioplankton community composition was visualized using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities (Clarke and Gorley, 2015). Analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) were used to evaluate whether or not community difference is significant between groups. To identify potential temporal variation in environment conditions and community dynamics, we performed linear regressions on Euclidean distance of all environmental variables and Bray-Curtis dissimilarity of community composition versus the square root of the time lags through time-lag analysis (Liu et al., 2015a). Mann-Whitney U tests were used to determine the significant difference between groups using SPSS 22.0 (IBM Corp., Armonk, NY, USA).

Species that characterize a given habitat or period are known as indicator species. Indicator species analysis (ISA) was used to identify phylotypes characteristic of cyanobacterial bloom. Those indicator OTUs with a P -value < 0.05 and both, a fidelity and specificity value ≥ 0.8 , were considered valid (Dufrene and Legendre, 1997; Salazar et al., 2015). In order to assess if our results were biased by the OTU definition approach, both DADA2 and UPARSE-produced OTUs were included for indicator species analysis.

To determine the relative contribution of environmental variables to the distribution of bacterial communities, partial Mantel tests were performed (Legendre and Legendre, 2012). The dissimilarity matrices of bacterial community composition were obtained using the Bray-Curtis similarity, while the environmental matrices were obtained using the Euclidean distance. Network analyses were performed to gain a better understanding of the species interaction. Spearman correlation coefficients were calculated for each abundant OTU and cyanobacterial indicator OTU using the ltm package (Woodhouse et al., 2016). The P -values for each correlation were generated. Any pairwise correlation > 0.6 or < -0.6 at $P < 0.01$ was selected for further analysis (Dini-Andreote et al., 2014). CIRCOS plots (<http://circos.ca/software/>) were used to display taxonomic pattern within the co-occurrence networks.

All the analyses were made using the R (version 3.3.1) (R Development Core Team, 2017), PRIMER 7.0, and SPSS 22.0.

2.9. Accession number

All raw sequence data were deposited in the sequence read archive (SRA) database at public NCBI (<http://www.ncbi.nlm.nih.gov/>) under the BioProject number PRJNA315049 and the accession number SRP071908.

3. Results

3.1. Cyanobacterial bloom and environmental characterization

Xidong Reservoir is a typical subtropical warm-monomictic reservoir. At the beginning of the sampling period the reservoir was experiencing a typical cyanobacterial bloom. The most dominant species was *Microcystis aeruginosa*, which accounted for about 80% of total phytoplankton biomass during the bloom period (Supporting information Fig. S1). The depth-time profiles of temperature and dissolved oxygen (DO) demonstrated that the water column changed from stratified (days 297–346) to well mixed states (day 363) during

the sampling period (Fig. 2). Environmental parameters in the bottom layer were quite different from the surface and middle layers during stratification period (Supporting information Fig. S2). During the stratification period, temperature, DO and oxidation reduction potential (ORP) were significantly higher in the epilimnion and rapidly dropping down in the hypolimnion ($P < 0.05$). In contrast, electrical conductivity (EC), total nitrogen (TN), ammonium nitrogen ($\text{NH}_4\text{-N}$) and total phosphorus (TP) were significantly higher in the hypolimnion compared with epilimnion ($P < 0.05$). The concentration of total carbon (TC), TOC and chlorophyll-*a* throughout the water column decreased gradually with the time (Fig. 2, Supporting information Fig. S2), and all three factors were significantly higher during bloom than non-bloom periods ($P < 0.05$). Chlorophyll-*a* in the surface water ranged from 29.58 to $46.28 \mu\text{g L}^{-1}$ during the bloom period, but dropped to 6.69 – $14.87 \mu\text{g L}^{-1}$ during the non-bloom period. Meanwhile, the comprehensive trophic state index (TSIc) decreased from middle eutrophic (61.3 – 62.2) to mesotrophic and light eutrophic (46.1 – 52.4) levels.

3.2. General patterns of bacterial abundance, alpha diversity and activity

In total, 3014 bacterial OTUs with 1,301,472 sequences were obtained in this study. Rarefaction analysis for free-living (FL) and particle-attached (PA) bacteria suggested that species richness approached an asymptote (Supporting information Fig. S3). In the comparison between size fractions, the richness (mean $\pm$ s.e., ACE (FL, 1597 ± 53 ; PA, 1863 ± 60), Chao 1 (FL, 1461 ± 50 ; PA, 1684 ± 59)), and Shannon-Wiener indices (FL, 4.31 ± 0.04 ; PA, 4.60 ± 0.09) were higher for PA than FL bacteria (Supporting information Table S1). However, the bacterial abundance was higher for FL (3.68×10^{10} copies/L) than PA (1.02×10^{10} copies/L) bacteria (Fig. 3 and Supporting information Table S1). In the comparison between bloom and non-bloom communities, the activity (RNA/DNA) of PA bacteria was significantly higher during bloom (22.9 ± 4.4) than non-bloom (1.5 ± 0.2) periods, whereas the richness (OTU number, ACE, Chao 1) and Shannon-Wiener indices showed no significant difference ($P > 0.05$) (Fig. 3 and Supporting information Table S1). In the comparison between stratification layers, Chao

1 and Shannon-Wiener indices were highest in bottom (Chao 1, 1796 ± 47 ; Shannon-Wiener, 4.55 ± 0.06) compared with surface (Chao 1, 1414 ± 64 ; Shannon-Wiener, 4.35 ± 0.09) and middle layers (Chao 1, 1507 ± 60 ; Shannon-Wiener, 4.45 ± 0.11), regardless of the bacterial size-fraction (Fig. 3 and Supporting information Table S1). Furthermore, richness and diversity indices, abundance and activity for FL and PA bacteria showed different correlations with environmental factors, with FL bacteria being relatively more strongly correlated with environmental variables than PA bacteria (30 factors for FL, 10 factors for PA; Supporting information Table S2), indicating that FL bacteria richness was more sensitive to the environmental variations.

Totally, 46 abundant and 2872 rare OTUs were identified in this study. The always abundant taxa (AAT) and the conditionally abundant taxa (CAT) contributed 29.86% and 37.42% of the total relative abundance, respectively, while the always rare taxa (ART) and conditionally rare taxa (CRT) accounted for 52.75% and 42.57% richness, respectively (Supporting information Fig. S4).

3.3. Effects of size-fraction, stratification and bloom on bacterial community

We found that bacterial communities clustered according to bacterial lifestyles, water stratification and bloom status in Xidong Reservoir (Fig. 4). Overall, both ANOSIM and PERMANOVA showed that the most significant differences in communities were between depths (Global $R = 0.371$ for ANOSIM, $R^2 = 0.166$ for PERMANOVA, $P < 0.01$), then size-fraction (Global $R = 0.281$, $R^2 = 0.088$, $P < 0.01$) and bloom status (Global $R = 0.198$, $R^2 = 0.082$, $P < 0.01$) for the whole bacterial community (Table 1). We also found that water stratification had the higher influence on FL bacterial communities, while bloom status exhibited the stronger impact on PA bacteria (Fig. 4, Table 1). When we consider the relative abundance of taxa, we found that the abundant PA subcommunity was largely influenced by bloom status (Global $R = 0.831$, $R^2 = 0.360$, $P < 0.01$). In contrast, the rare FL bacterial subcommunity was highly variable at spatial scale (Global $R = 0.765$, $R^2 = 0.242$, $P < 0.01$). Furthermore, the time-lag analysis of abundant taxa for both size-fractions had significant positive slopes, indicating that these

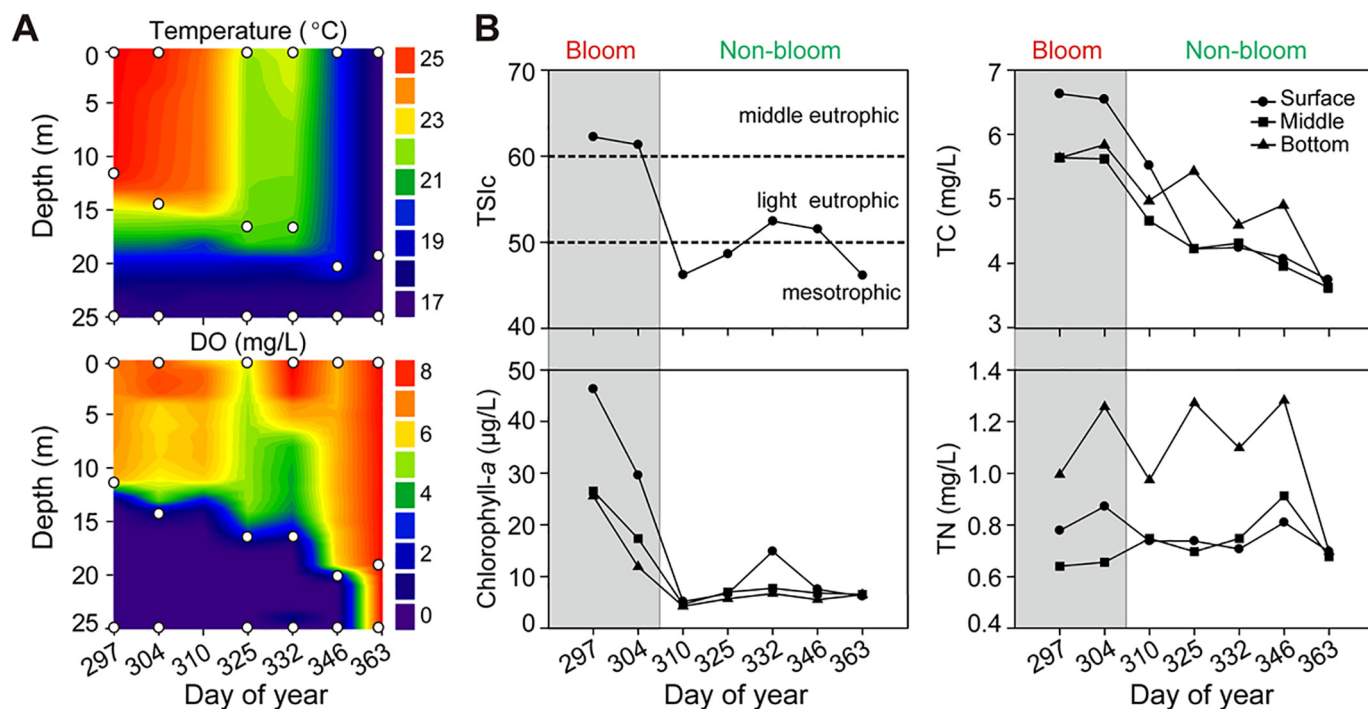


Fig. 2. Environmental parameters measured in Xidong Reservoir from 24 October 2014 (day 297) to 29 December 2014 (day 363). (A) Depth-time profiles of water temperature and dissolved oxygen (DO). White dots indicate time and depths of sampling for molecular work. (B) Comprehensive trophic state index (TSIc), chlorophyll-*a*, total carbon (TC), and total nitrogen (TN) in different depth layers during cyanobacteria bloom (day 297 and day 304) and non-bloom periods (day 310–day 363).

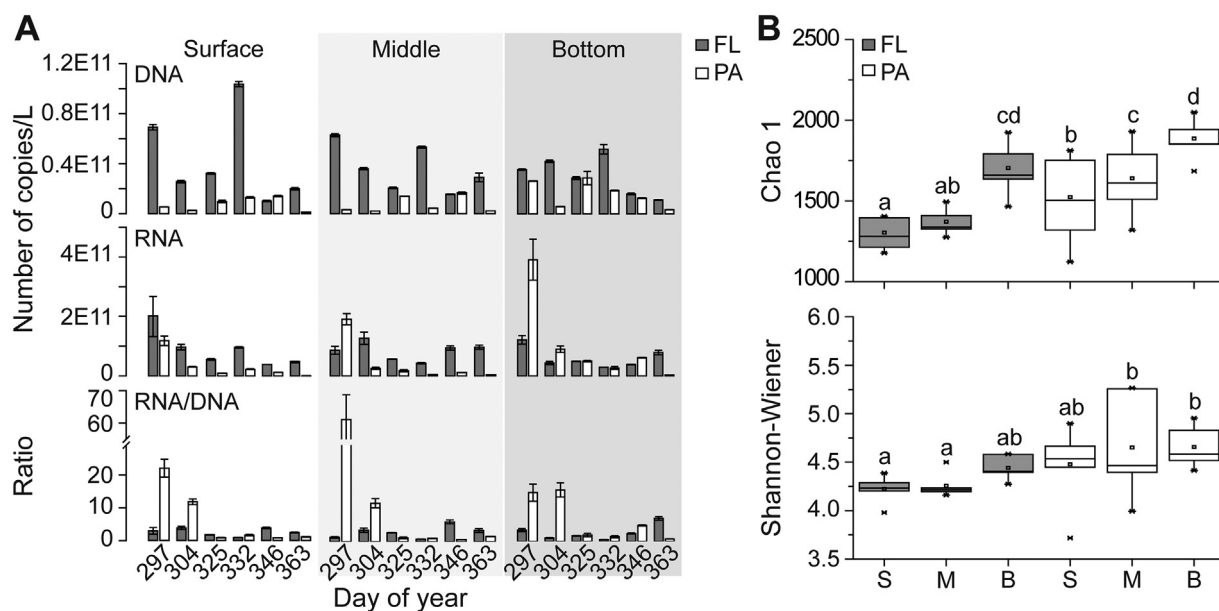


Fig. 3. Abundance, activity and diversity of bacterioplankton communities in Xidong Reservoir based on 16S rRNA gene across space and time. (A) Number of 16S rDNA and 16S rRNA copies per liter and RNA/DNA ratio for free-living (FL, 0.2–3 μ m) and particle-attached (PA, >3 μ m) bacterioplankton from different depth layers. Error bars indicate standard errors of the three replicates. (B) Chao 1 and Shannon-Wiener indices across different depth layers. S, surface; M, middle; B, bottom. Analysis of variance (ANOVA) was used in combination with Scheffe's F multiple-comparison test to examine differences among the sampling depths. Different lower case letters indicate significant differences among the depths. The ends of the box represent the 25th and 75th percentiles, the whiskers represent minimum and maximum range, black dots represent outliers and the centers represent the median.

abundant microbial subcommunities were undergoing a directional change (Supporting information Table S3).

At the phylum level, Actinobacteria was generally the most dominant in all samples with the relative abundance ranging from 25% to 67% (Supporting information Fig. S5). Distribution patterns of many bacterial taxonomic groups varied substantially among size-fractions, water depths and bloom status, although we did not find any significant difference for rare FL bacteria between *Microcystis* bloom and non-bloom periods (Supporting information Figs. S5–S7). Except for cyanobacteria, no significant difference for abundant FL and PA bacterial groups was found between epilimnion and hypolimnion layers (Supporting information Fig. S7). Further investigations at the OTU level found a higher number of indicator OTUs identified based on the output OTUs generated by DADA 2 (60) than UPARSE (32) approaches (Supporting information Tables S4–S5). However, the relative ratio of

each taxon in both methods appeared identical at high taxonomic level (e.g. Bacteroidetes (25.0% for DADA2, 25.0% for UPARSE); Proteobacteria (25.0%, 25.0%); Cyanobacteria (10.0%, 12.5%)) (Supporting information Fig. S8). Indicator OTUs showed high abundance following the bloom and declined quickly during post-bloom stage (Supporting information Fig. S9).

3.4. Lifestyle transition of bacterioplankton

Bacterial taxa showed a transition or switching phenomenon between the two different lifestyles at the OTU and broad taxonomic levels (Fig. 5). Overall, the majority of phyla/classes in the rare biosphere had mean PAN index > 0.5 supporting a preference for the particle-associated lifestyle during the sampling period. Further, Actinobacteria, Bacteroidetes, and Firmicutes showed a strong transition from free-

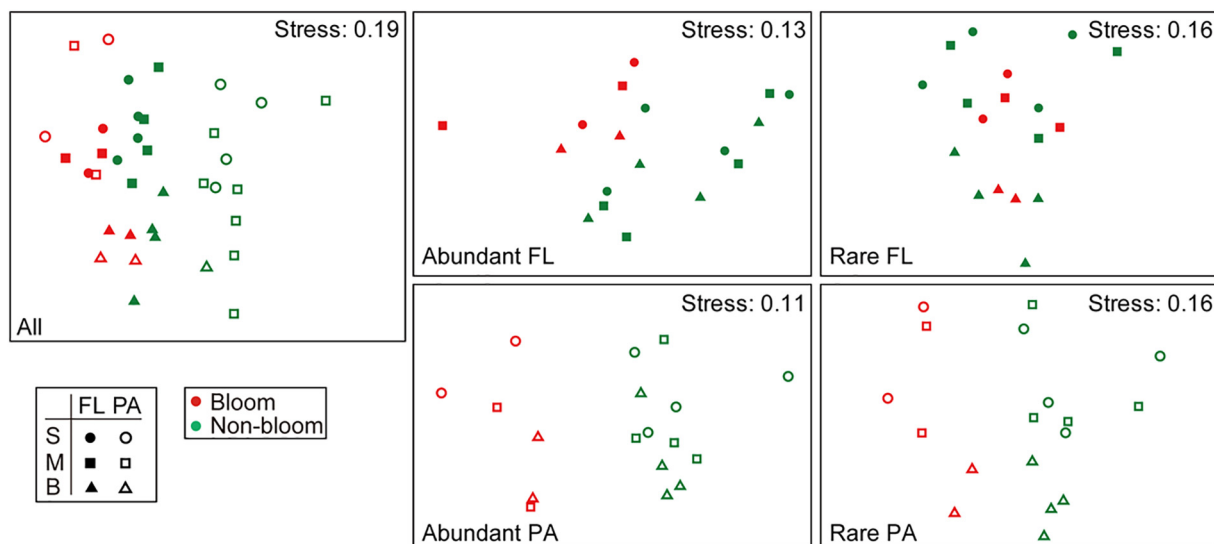


Fig. 4. Non-metric multidimensional scaling (NMDS) ordination of bacterial communities based on Bray-Curtis dissimilarity. All represents the whole bacterioplankton community including both free-living (FL) and particle-attached (PA) bacteria. S, surface; M, Middle; B, bottom.

Table 1

Pairwise comparisons of bacterioplankton communities based on two different statistical approaches.

Factors	ANOSIM					PERMANOVA				
	All	Abundant		Rare		All	Abundant		Rare	
		FL	PA	FL	PA		FL	PA	FL	PA
Size-fraction	0.281**	–	–	–	–	0.088**	–	–	–	–
Bloom/non-bloom	0.198**	0.290*	0.831**	–0.027	0.562**	0.082**	0.192**	0.360**	0.071	0.171**
Depth	0.371**	–0.052	–0.031	0.455**	0.290**	0.166**	0.090	0.105	0.223**	0.201**
Surface vs middle	–0.024	–0.139	–0.115	–0.107	–0.102	0.039	0.031	0.054	0.073	0.067
Surface vs bottom	0.639**	0.05	0.117	0.765**	0.550**	0.190**	0.124	0.137	0.242*	0.224*
Middle vs bottom	0.496**	–0.054	–0.098	0.639**	0.413**	0.156**	0.063	0.044	0.211**	0.185**

ANOSIM, analysis of similarity; PERMANOVA, permutational multivariate analysis of variance; All, both free-living and particle-associated bacteria; FL, free-living bacteria; PA, particle-associated bacteria.

Both tests are non-parametric multivariate analyses based on Bray-Curtis dissimilarities between samples.

Values show the R and R² values for ANOSIM and PERMANOVA, respectively. Boldface indicates significant difference (**P* < 0.05, ***P* < 0.01).

The ANOSIM statistic compares the mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups. An R value close to “1” suggests dissimilarity between groups while an R value near “0” suggests an even distribution of high and low ranks within and between groups. Negative R values indicate that dissimilarities are greater within groups than between groups.

The PERMANOVA was used to test if size-fraction, bloom/non-bloom and depth could significantly explain variation in the bacterioplankton communities.

The operational taxonomic units (OTUs) were defined at 97% sequence similarity threshold.

Abundant indicates always abundant taxa and conditionally abundant taxa, while rare includes always rare taxa and conditionally rare taxa.

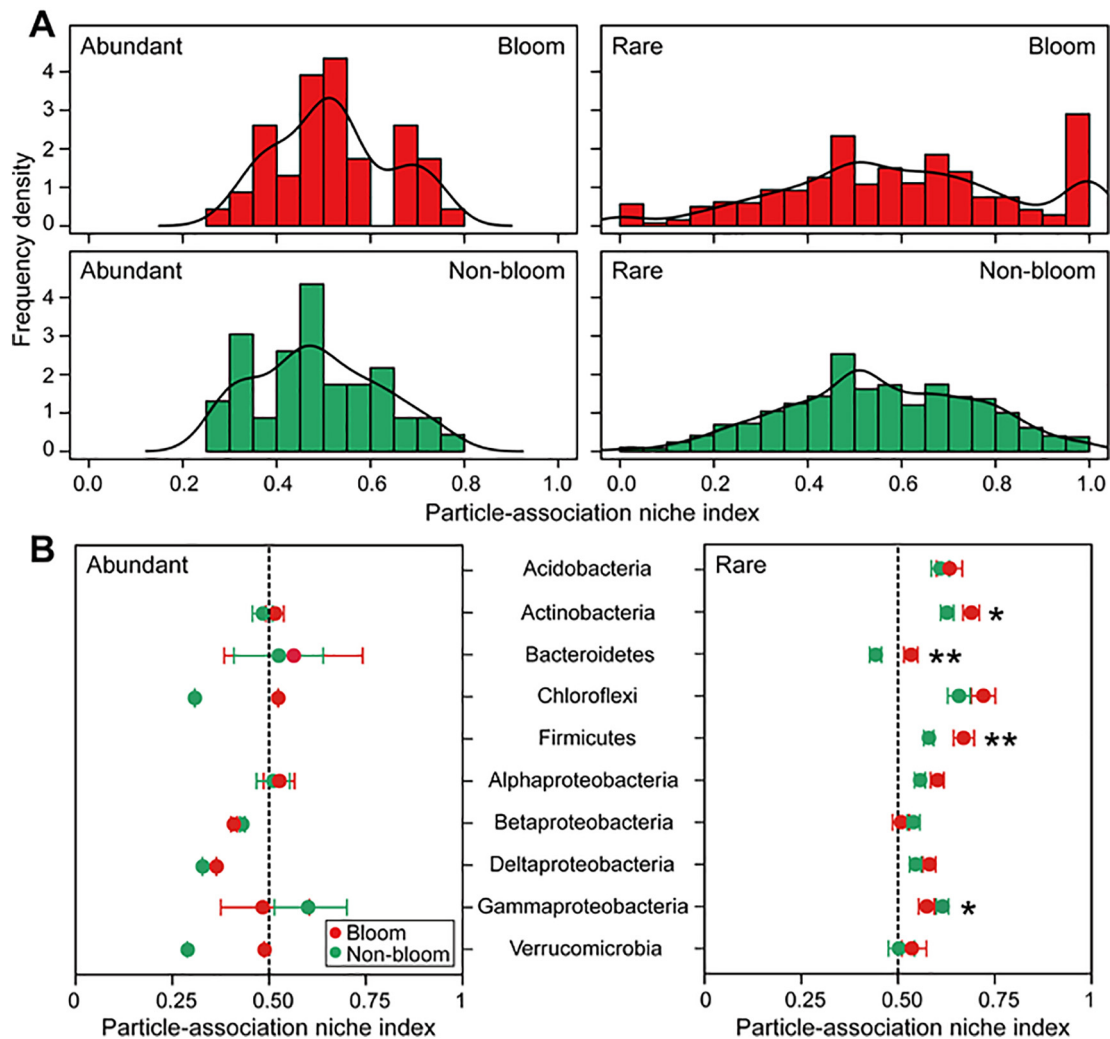


Fig. 5. Comparison of abundant and rare bacterioplankton frequency between the bloom and non-bloom samples. (A) Histogram presenting the distribution of particle-associated niche (PAN) index for abundant and rare bacterial taxa during bloom and non-bloom stages. The lines correspond to frequency estimates. (B) PAN index of the abundant and rare bacterial taxa at phylum/class level during bloom and non-bloom stages. Only phyla/classes containing >40 OTUs in abundant or rare taxa are presented. The vertical dashed line corresponds to a 0.5 PAN index, which indicates that the relative abundance of free-living bacteria is equal to that of particle-associated bacteria. Error bars indicate standard errors of the PAN index of OTUs belong to each phylum/class. Significant difference is calculated by nonparametric Mann-Whitney U test. **P* < 0.05, ***P* < 0.01. Data are expressed as means ± standard error (error bars).

living to particle-attached lifestyle during the *Microcystis* bloom period (Fig. 5B). In contrast, Gammaproteobacteria had significant lifestyle transition from particle-attached to free-living lifestyle following the *Microcystis* bloom. Further, the lifestyle of some indicator taxa (e.g. Bacteroidetes (12 OTUs for DADA2, 5 OTUs for UPARSE), Proteobacteria (10, 5), Gemmatimonadetes (4, 2)) clearly showed a significant transition from FL to PA states stimulated by *Microcystis* bloom (Supporting information Tables S4–S5).

3.5. Factors related to variation of free-living and particle-attached bacterial communities

Our partial Mantel tests illustrated that the dynamics of all, FL and PA bacterial communities were significantly related to different environmental variables (Table 2). The whole community was primarily influenced by temperature ($r = 0.20$, $P < 0.01$), pH ($r = 0.36$, $P < 0.01$), total carbon ($r = 0.24$, $P < 0.01$), total organic carbon ($r = 0.33$, $P < 0.01$) and chlorophyll-*a* ($r = 0.39$, $P < 0.01$) (Table 2). For abundant taxa, no detected environmental factor had significant effect on the abundant FL bacterial communities ($P > 0.05$), whereas five environmental variables (including temperature, pH, total carbon, total organic carbon and chlorophyll-*a*) significantly correlated with the abundant PA bacterial communities ($P < 0.01$). For rare taxa, we found that only nitrate nitrogen ($r = 0.31$, $P < 0.05$) exhibited significant and positive effect on the FL bacterial communities, whereas six environmental variables, including temperature ($r = 0.36$, $P < 0.01$), turbidity ($r = 0.32$, $P < 0.01$), pH ($r = 0.50$, $P < 0.01$), total carbon ($r = 0.38$, $P < 0.01$), total organic carbon ($r = 0.51$, $P < 0.01$) and chlorophyll-*a* ($r = 0.58$, $P < 0.01$) significantly correlated with the PA bacterial communities.

Three (all, FL and PA bacteria) taxon-taxon co-occurrence networks were constructed, representing the combinations of three depths (Fig. 6, Supporting information Fig. S10). Co-occurrence patterns demonstrated that association patterns changed across the bacterial size fractions. The interactions between bacteria were most complex for the whole bacterial network (195 edges, average degree of 8.57) compared with FL (168, 7.81) and PA (149, 6.48) bacterial networks, with a much higher ratio of strong positive correlations (61.5%) observed than negative ones (38.5%). Interestingly, more correlations among cyanobacterial indicator OTUs and cyanobacteria-associated bacteria were found in PA (10.7%) than FL (2.2%) bacterial networks. Further, cyanobacteria showed more positive relationships with Alphaproteobacteria (5.0%),

and more negative correlations with Actinobacteria (6.4%) for PA bacterial communities. A total of 5, 4 and 4 modules were generated for the all, FL and PA bacterial networks, respectively (Supporting information Fig. S10A, B). Unique node-level topological features of three communities were also compared (Supporting information Fig. S10C), and both the degree and closeness centrality values showed a significant difference. However, no significant difference was found for betweenness centrality and eigenvector centrality.

4. Discussion

Our results found that the patterns of community succession following a reservoir *Microcystis* bloom depend on the lifestyle of bacteria. The dynamics of PA bacteria were more closely related to the development of the *Microcystis* bloom compared to FL bacteria (Figs. 3, 4 and Table 1). Lifestyle-specific patterns have been found during a diatom spring bloom based on denaturing gradient gel electrophoresis analysis of 16S rRNA gene (Rösel and Grossart, 2012). Indeed, distinct temporal variations have been recorded for FL and PA communities in other time series studies (Yung et al., 2016; Tang et al., 2017), suggesting that the results of this study can be applied to other similar systems. One explanation for this lifestyle-specific response involves the properties of PA bacteria. The PA bacteria have chemotaxis and motility which allow for the coupling between PA bacteria and phytoplankton, with bacteria gathering within the DOC-rich “phycosphere” surrounding individual algal cells (Stocker and Seymour, 2012). Furthermore, many of the PA bacteria can be part of biofilms or zoogloae (Dang and Lovell, 2016; Berne et al., 2018). This particle-attached lifestyle exhibits plenty of advantages contrasting with that of free-living planktonic bacteria, including protection from predators and many other environmental changes (Berne et al., 2018). The other explanation for lifestyle-specific response involves the genetic properties. PA bacteria generally have large and variable genomes that contain genes enabling a variety of metabolic capabilities which are thought to equip cells to take advantage of patches of organic matter and grow rapidly during phytoplankton blooms (Luo and Moran, 2015). Additionally, lifestyle-specific vertical variation, being greater for FL bacteria (Fig. 4 and Table 1) was found here, although the vertical differentiation of communities is well documented (Yu et al., 2014; Schmidt et al., 2016). Our results concur with a previous study which proved the lowest vertical isolation of large size fractions communities (Mestre et al., 2018). Most of the bacterial groups from the bottom layer can also be detected in surface waters, and this vertical connectivity is higher in PA communities (Supporting information Fig. S7), likely due to their higher sinking rates (Mestre et al., 2018). The results shown here provide evidence that particle sinking forms a dispersal vector of viable bacterial diversity from surface to the bottom waters.

Despite difference between free-living and particle-attached bacteria, we found strong evidence of lifestyles transition between FL and PA bacteria. Bacterial shifts from free-living to the particle-attached states were stimulated and shaped by the *Microcystis* bloom, especially for the rare bacteria (Fig. 5). This possible lifestyles transition has been detected through community composition, production of exoenzymes or community transcription patterns in the marine environments (Rinta-Kanto et al., 2012; Teeling et al., 2012) or in experimental mesocosms (Riemann et al., 2000; von Scheibner et al., 2014). For example, microorganisms affiliated with *Polaribacter* sp. strain Hel1_85 (Bacteroidetes) have been found to lie in-between those of planktonic and algae-associated species (Xing et al., 2015). Xing et al. (2011) had earlier revealed the *Clostridium* (Firmicutes) clusters and their diverse consortiums' function during anaerobic degradation of *Microcystis* by the incubation of *Microcystis* scum. We first used particle-association niche (PAN) index to quantitatively estimate the lifestyle alternation between FL and PA bacteria at phylum or class level during a cyanobacterial bloom. Particularly, Actinobacteria, Bacteroidetes, Firmicutes, and Gammaproteobacteria had significant lifestyle

Table 2
Partial Mantel tests for the correlation between community similarity and environmental factors using Pearson's coefficient. The controlling factor was water depth.

Factors	All	Abundant		Rare	
		FL	PA	FL	PA
Temperature	0.20**	0.02	0.26**	0.09	0.36**
Electrical conductivity	−0.00	−0.00	−0.05	0.14	−0.07
Turbidity	0.14	−0.09	0.22	−0.04	0.32**
pH	0.36**	0.11	0.52**	0.01	0.50**
Oxidation reduction potential	0.04	0.13	0.01	0.10	−0.02
Dissolved oxygen	−0.01	0.05	0.04	0.09	−0.05
Total carbon	0.24**	0.13	0.31**	−0.01	0.38**
Total organic carbon	0.33**	0.08	0.44**	0.06	0.51**
Total nitrogen	−0.05	−0.08	−0.09	0.04	−0.10
Ammonium nitrogen	0.01	−0.05	0.02	0.13	0.04
Nitrate nitrogen	−0.04	−0.03	−0.08	0.31*	0.01
Nitrite nitrogen	−0.01	−0.06	0.03	−0.13	0.04
Total phosphorus	−0.07	−0.04	−0.10	0.02	−0.13
Phosphate phosphorus	−0.05	−0.06	−0.04	0.12	−0.05
Chlorophyll- <i>a</i>	0.39**	0.13	0.51**	0.09	0.58**

FL, free-living bacteria; PA, particle-attached bacteria.

Values show the *r* value, and data in bold indicates significant correlation (* $P < 0.05$, ** $P < 0.01$).

Abundant indicates always abundant taxa and conditionally abundant taxa, while rare includes always rare taxa and conditionally rare taxa.

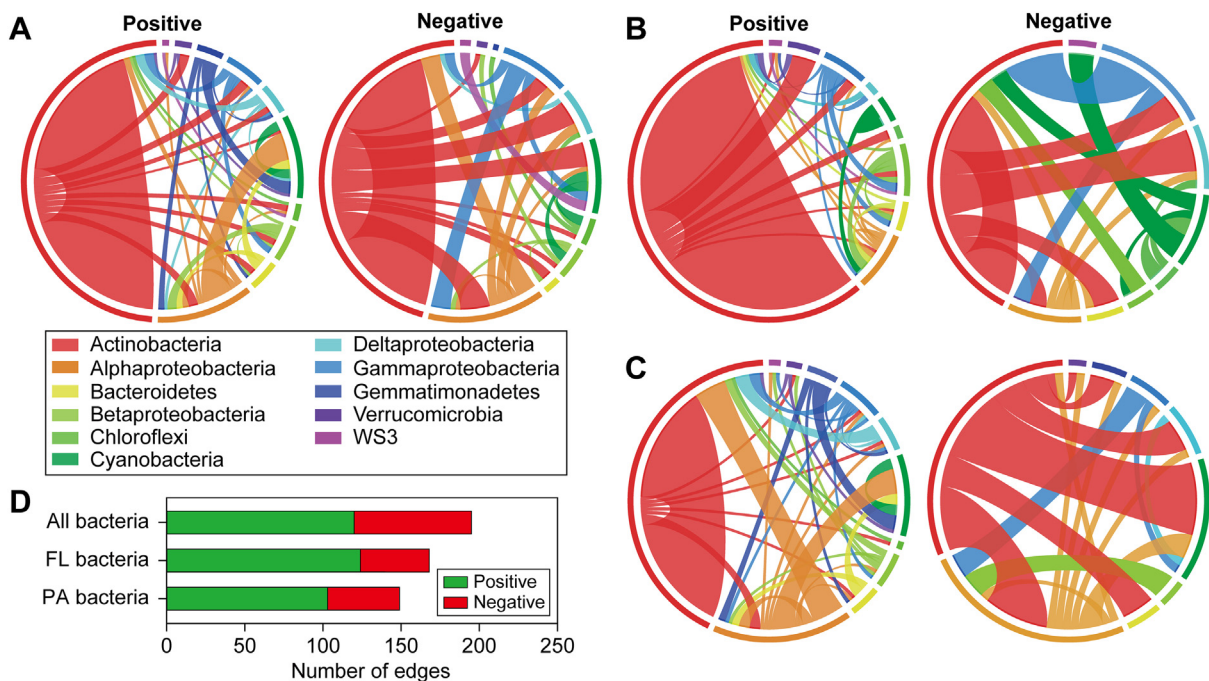


Fig. 6. Taxonomic pattern within the co-occurrence network. Taxonomic patterns of co-occurrences across the all community (A), free-living (FL) bacteria (B), and particle-attached (PA) bacteria (C). Abundant OTUs and Cyanobacteria indicator OTUs (OTUs12, 21, 60 and 197) depicted as colored segments in a CIRCOS plot, in which ribbons connecting two segments indicate co-presence and exclusion links, on the left and right, respectively. (D) Frequency of co-occurrence patterns for all, free-living and particle-attached bacteria. Any pairwise correlation > 0.6 or < -0.6 and $P < 0.01$ was selected for network analysis.

variations during the *Microcystis* bloom compared to the non-bloom period (Fig. 5B). Lifestyle transition can be clarified in several ways. First, bacteria may shift the suite of bioactive compounds assimilated from the DOM pool, decrease energy conservation strategies, and alter cell surfaces in a manner that promotes adhesion and particle formation during bloom succession (Rinta-Kanto et al., 2012). Second, relatively complex metabolic machineries for bacteria in a particle-attached lifestyle can help bacteria exploit microscale hot spots of particulate organic carbon (POC) associated with suspended and sinking particles, indicating their generalist behavior and important role during bloom periods (Salcher, 2014; Salazar et al., 2015). However, there are some potential limitations associated with the selection of filter pore size that merit further discussion. Though the 3 μm size-fractionated method provides new insights into selective factors shaping bacterial communities in aquatic ecosystems and has been widely used, cross-contamination, such as filter clogging, and PA bacterial departure does occur with this differential filtration method (Lapoussière et al., 2011; Simon et al., 2014; Mestre et al., 2017). Therefore, the effect of different pore-size cut-offs on the bacterial community composition deserves further study.

Indeed, PA bacteria exhibited a closer coupling with cyanobacteria development compared with FL bacteria (Table 1), and more numerous positive correlations with high correlation coefficients between bacterial taxa and cyanobacterial OTUs were found (Fig. 6). Other studies showed the complex linkages between bacterioplankton and bloom-forming cyanobacteria in various aquatic systems (lake, reservoir, and sea water), as many isolated bacterial strains are capable of either enhancing, or occasionally inhibiting, the growth of bloom-forming cyanobacteria (Liu et al., 2014; Woodhouse et al., 2016; Callieri et al., 2017; Yang et al., 2017). Few studies have considered the size-fractionated bacteria and found the synchronized growth of *Microcystis* and attached bacteria from both field investigation and microcosm experiment (Cao et al., 2016). In another study, Yang et al. (2017) found that *Microcystis aeruginosa* had high connectivity in the core region of the PA bacterial network, and in both FL and PA bacterial interaction networks, a co-occurrence pattern with positive connection was

predominant. Hence, species interactions between phytoplankton and bacteria are often governed by microscale interactions played out within the algal phycosphere. Further, the crossed relationships between cyanobacteria and bacteria suggest that facilitative effects of cyanobacteria on taxon-specific lineages (e.g. Alphaproteobacteria) are more important for PA than FL bacteria in contributing to microbial community succession patterns during the *Microcystis* bloom.

In addition, contrasting succession patterns of abundant and rare bacteria were found depending on bacterial lifestyle, *Microcystis* bloom status and water depth layer (Fig. 4 and Table 1). However, most previous studies have focused only on the whole community succession during bloom (Eiler and Bertilsson, 2004; Yang et al., 2015; Woodhouse et al., 2016; Yang et al., 2017), as yet no work has distinguished the abundant and rare bacterioplankton following a *Microcystis* bloom. Evidence so far indicates that the rare biosphere is not a random assembly, and that at least in some cases, the observed patterns are comparable to those displayed by abundant taxa (Logares et al., 2015). We found that abundant taxa showed larger variation among bloom/non-bloom groups, while rare taxa were more variable among water depths. The distinct succession patterns may be largely due to the properties of the taxa. On one hand, abundant taxa have larger probability of dispersal (Liu et al., 2015b). In contrast, rare bacterial subcommunities were mainly structured by local environmental variables (Liu et al., 2015b), so leading to the greater variations among stratification layers due to the environmental differences and gradients. On the other hand, abundant taxa have broader niche widths and higher ability to rapidly decompose and utilize organic carbon produced by phytoplankton (Rieck et al., 2015). This species abundance-dependent succession should be tested in future research in more stratified inland waters.

Bloom indicator OTUs (e.g. Bacteroidetes, Proteobacteria, and Cyanobacteria) which have a high abundance closely followed the bloom and decline quickly during post-bloom stage, were detected (Supporting information Fig. S9 and Tables S4–S5). Recent studies addressing the bloom-associated bacterial populations showed taxa being able to 'boom and bust' (i.e. indicator OTUs or opportunistic bacteria), were important contributors for transforming phytoplankton-

derived organic matter (Buchan et al., 2014; Salcher, 2014). One explanation for this rapid change involves the properties of these bacteria. Indicator taxa can be seen as fast-growing r-strategist which specialize on the initial attack of highly complex organic matter during the bloom (Teeling et al., 2012). The other explanation is their fast generation times and short-lived population maxima (Salcher, 2014; Salazar et al., 2015). Furthermore, according to previous research, taxa declining quickly during post-bloom may be due to the loss of particle habitat and being more easily grazed by protists (Salcher, 2014).

Different degrees of response by FL and PA bacterial communities to the environmental changes were found during the *Microcystis* bloom, as we expected. Generally, nutrient concentrations harbored in particle hotspots were up to three orders of magnitude higher than for bulk water (Luo and Moran, 2015). In addition, the properties of particle or ecosystem also can influence the response results (Dang and Lovell, 2016). In this study, the FL bacterial community composition was quite conserved across the environmental changes in surrounding water, while the PA bacterial community was significantly related to the measured environmental variables (Table 2). There is controversy about the correlation between size-fractionated bacterial community and the surrounding environment. Some studies have found that free-living bacteria appear to be more sensitive to environmental variables than bacteria attached with particles (Yung et al., 2016; Yang et al., 2017); while other studies suggested that PA bacterial communities may be more sensitive to global change stressors including land use change, species invasions, changes in geochemical cycles (Schmidt et al., 2016). Recently, Tang et al. (2017) found that environmental factors affect bacterial communities in much the same way regardless of bacterial size-fractions in Lake Taihu China. Here our results clearly showed that the community compositions of both abundant and rare PA bacteria were more sensitive to environmental variation as shown by the more significant correlations between community similarity and environmental factors (Table 2). However, the species richness or diversity of FL bacteria was more correlated with environmental variables than was the case for PA bacteria (30 for FL bacteria, 10 for PA bacteria; Table S2), indicating that FL bacteria richness was more impacted by the environmental variations. These results mean that the multiple and complex responses of bacterioplankton communities to a *Microcystis* bloom are affected by bacterial lifestyles, and particle effects may contribute to bacteria environmental sensitivity in various aspects in complex ways.

5. Conclusion

In conclusion, these results expand our knowledge of the general processes and mechanisms of bacterial community dynamics following a *Microcystis* bloom, and demonstrate that a majority of bacterial groups exhibited a lifestyle transition shaped by the *Microcystis* bloom especially in the rare microbial biosphere. Although this study was based on just one reservoir it is typical of many subtropical reservoirs, suggesting the results of this study may not be site specific. Bacterial communities vary depending on *Microcystis* bloom, lifestyle and taxa abundance in a subtropical stratification reservoir. The *Microcystis* bloom can stimulate bacteria shifts from free-living to particle-attached states for rare members of Actinobacteria, Bacteroidetes, and Firmicutes. The free-living and particle-attached bacterial communities were structured by different ecological drivers and processes. Our results highlight the importance of bacterial lifestyle and taxa abundance, when designing studies to characterize bacterial plankton communities in changing environments.

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Conflict of interests

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary Figs. S1–S10 and Supplementary Tables S1–S5 showing additional study details. The supporting information to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.12.414>.

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