Spatiotemporal dynamics of bacterial community composition in large shallow eutrophic Lake Taihu: High overlap between free-living and particle-attached assemblages

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Abstract
In eutrophic lakes, heterotrophic bacteria are closely associated with algal detritus and play a crucial role in nutrient cycling. However, the seasonal and spatial dynamics of free-living (FL) and particle-attached (PA) bacteria and the environmental factors shaping this relationship remain poorly understood. To address this issue, we explored the spatiotemporal patterns of bacterial community composition (BCC) in Lake Taihu, China, using terminal restriction fragment length polymorphism (T-RFLP) and 454-tag pyrosequencing of 16S rRNA gene. We generated a total of 218,027 high quality non-cyanobacterial sequence reads that resulted in 4940 OTUs (97% cutoff), with Actinobacteria, β- and α-proteobacteria being the predominant taxa. Although PA communities contained significantly higher alpha-diversity than FL ones, we found that 59% of OTUs, that accounted for 96% of the total reads, were shared by both communities. The high degree of overlap between FL and PA communities indicates a high rate of dispersal potential, highlighting an underestimated connectivity and potentially similar ecological role for these two components. Distinct seasonal trends were recorded in both FL and PA communities, while spatial differences in BCC were small. In addition, both FL and PA bacterial communities exhibited similar patterns and synchrony, correlated to water temperature, nitrate and total suspended solids (TSS). Accordingly, the effects of eutrophication and hydrodynamics on the phylogenetic overlap and diversity between FL and PA communities were discussed.

In aquatic systems, heterotrophic bacteria are the major drivers of organic matter mineralization, nutrient regeneration, and energy flow (Cotner and Biddanda 2002; Azam and Malfatti 2007; Stocker 2012). The diversity of heterotrophic bacteria and resulting community composition remain a fundamental research area in aquatic microbial ecology, as inference on the role of microbial community members are often made from their identity. Due to chemotactic behavior and motility (Stocker and Seymour 2012), bacteria can rapidly exploit organic particles in water, creating “hotspots” of bacterial growth and nutrient cycling (Azam and Malfatti 2007). Thus, compared to free-living (FL) bacteria in the surrounding water, particle-attached (PA) bacteria are often larger, concentrated and have much higher metabolic activity (Simon et al. 2002; Grossart et al. 2007; Kellogg and Deming 2014).

Previous studies of alpha-diversity of FL and PA bacteria in aquatic systems have resulted in conflicting observations. Generally, the diversity of FL bacteria is much higher than that of the PA fraction in oligotrophic marine systems (Aciñas et al. 1999; Ghiglione et al. 2007; Rink et al. 2011; Lecleir et al. 2014; Li et al. 2015a). In some mesotrophic and eutrophic lakes (Parveen et al. 2011; Rösel et al. 2012; Tang et al. 2015), however, higher diversity has been observed in PA bacterial assemblages compared to free-living counterparts. In addition, using pyrosequencing technology, PA bacteria have been found to be more diverse than FL bacteria in lakes, rivers and even in the open seas (Crespo et al. 2013; Ortega-Retuerta et al. 2013; Bizić-Ionescu et al. 2015).

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Bacterial community composition (BCC) of PA communities has been compared to that of FL ones in various aquatic environments. Studies on macroaggregates (> 500 μm, such as marine and lake snow) have shown that they usually harbor specific aggregate-adapted microbial populations because of the nutritious microhabitats suitable for biogeochemical processes (Delong et al. 1993; Rath et al. 1998; Grossart et al. 2003; Grossart et al. 2007). Distinct or significant differences between FL and PA bacterial communities are normally observed in marine systems and oligotrophic to mesotrophic deep lakes (see Supporting Information Table S1 for details). On the contrary, similar bacterial compositions of these two fractions has been found in turbulent estuaries, such as Chesapeake Bay (summer, Noble et al. 1997), San Francisco Bay (Hollibaugh et al. 2000), the Weser Estuary (Selje and Simon 2003), and the Mackenzie River estuary (Ortega-Retuerta et al. 2013), as well as in the eutrophic Melliang Bay, Lake Taihu (Tang et al. 2015).

To date, the debate on which is more diverse and how extend in these fractions is related between FL and PA bacteria in aquatic ecosystems has not been resolved. Contrasting observations concerning diversity could be due to differences in system characteristics (hydrodynamic forcing and trophic levels which affect the size, nature, and concentration of particles), in protocols and standards employed to separate PA bacteria from FL cells, in methods used to measure diversity and BCC (fingerprinting methods, normal cloning and sequencing or high-throughput next-generation sequencing), as well as in spatiotemporal dynamics (Ghiglione et al. 2007; Crespo et al. 2013; Ortega-Retuerta et al. 2013; Bizić-Ionescu et al. 2015), which are summarized in Supporting Information Table S1. Based on previous examinations of bacterial diversity and community composition, we hypothesized that the overlap between PA and FL assemblages increases with hydrodynamic forcing and trophic levels.

Lake Taihu is a large, shallow, eutrophic subtropical lake with an area of 2338 km² and mean depth of 1.9 m (Qin 2008). Wind-induced sediment resuspension and eutrophication induced cyanobacterial blooms represent two major characteristics of this ecosystem (Qin 2008; Qin et al. 2015). Although many studies focus on the diversity and composition of bacterioplankton (Niu et al. 2011; Li et al. 2015b) and sometimes the total bacterial communities (Wu et al. 2007b; Tian et al. 2009; Wilhelm et al. 2011) in Lake Taihu, surprisingly few have examined the FL and PA bacterial communities separately (Tang et al. 2015). Moreover, the seasonal and spatial dynamics of FL and PA bacteria and environmental factors driving this relationship are poorly understood. Due to strong hydrodynamic stress and eutrophication, abundant organic particles/microaggregates (5–500 μm) dominate this shallow and turbid system (Tang et al. 2009, 2010), which likely influence the dynamics of FL and PA bacterial communities.

In this study, we explored the diversity and phylogenetic composition of FL and PA bacterial assemblages from Lake Taihu at different seasons and stations by applying the community fingerprinting method of terminal restriction fragment length polymorphism (T-RFLP) and high-throughput 454-tag pyrosequencing V1–V3 regions of 16S rRNA genes. T-RFLP analyses of 96 samples yield a detailed depth profile and provide insight on within-site variation, whereas the large number of high quality heterotrophic bacterial reads (c. 6800 per sample) allows deep comparison of diversity and community similarities between FL and PA assemblages. The results provide evidence for a strong community similarity between PA and FL assemblages in turbulent eutrophic Lake Taihu, with new insights into bacterial partitioning and the potential role of PA bacteria related to nutrients recycling in the process of cyanobacterial blooms.

**Materials and methods**

**Study site and sampling**

Lake Taihu (30°55′40″–31°32′58″ N, 119°52′32″–120°36′10″ E) is the third largest freshwater lake in China, located on the south side of the Yangtze delta. Due to massive external nutrient loads from northern and western watersheds in the past 30 yr, Lake Taihu has become eutrophic. The high concentrations of nitrogen and phosphorus entering the system have resulted in massive algal blooms consisting mainly of *Microcystis* spp. (Chen et al. 2003; Qin 2008; Niu et al. 2011; Xu et al. 2013). These nutrient inputs combined with lake hydrology result in a decreasing trophic gradient from northwestern to southeastern in Lake Taihu.

Samples were collected at four stations (Fig. 1) that could be distinctly characterized by different trophic states (Qin 2008; Tang et al. 2009). Station A is located in Melliang Bay, a region where thick *Microcystis* blooms commonly occur. Station B is situated on the nearshore of the mouth of Dapu River, one of the main river inputs: it is dominated by wastewater in the upstream region of the northwestern plain. Station C is located in the open lake, where the water is less enriched with nutrients but exposed to more frequent wind-induced sediment resuspension. Station D is located in Eastern Taihu Bay, a typical macrophyte zone with relatively low phytoplankton concentrations.

At each station, three independent samples were collected (about 1 km apart, Fig. 1) on February 27 (winter), May 18 (spring), August 06 (summer) and November 08 (fall) of 2010, respectively. Surface water (top 50 cm) was collected with a 5 L Schindler sampler and then transported to laboratory in dark cooling boxes and processed 3–5 h after sampling. A 50 mL subsample to determine bacterial abundance was fixed in situ with a final concentration of 2.0% formaldehyde (pre-filtered through 0.2 μm polycarbonate membrane).
Measurement of environmental parameters and bacterial enumeration

Water column depth and Secchi depth (SD) were measured using a water depth gauge (Uwitec, Austria) and Secchi disk, respectively. Water temperature (Temp), dissolved oxygen (DO), electrical conductivity (EC), and pH were measured in situ using a multiparameter water quality sonde (YSI 6600 V2, Yellow Springs Instruments, U.S.A.). Total nitrogen (TN), dissolved total nitrogen (DTN), ammonium (NH\textsubscript{4}+), nitrate (NO\textsubscript{3}-), total phosphorus (TP), dissolved total phosphorus (DTP), orthophosphate (PO\textsubscript{4}\textsuperscript{3-}), total suspended solids (TSS), and chlorophyll a (Chl a) were determined using standard methods (Jin and Tu 1990).

A volume of water, ranging from 100 mL to 150 mL for each sample, was used to filter size-fractionate bacterial communities. Water samples were sequentially filtered through 5.0-µm, 2-µm, and 0.2-µm polycarbonate membranes (47-mm diameter, Millipore), respectively, under the vacuum pressure of < 20 mm Hg (Allgaier and Grossart 2006; Parveen et al. 2011) to separate particle-attached (PA, 0.2–2 µm) and free-living bacteria (FL, 0.2–2 µm). Unicellular Microcystis spp., retained on 2-µm filters, were discarded because this study focused on heterotrophic bacteria.

Total bacterial abundance (TBA) and free-living bacterial abundance (FBA) were determined by epifluorescence microscopy. For enumeration of TBA, formaldehyde preserved subsamples were sonicated (using a BioSonik II sonicator with a 4 mm probe) at a power level of 100 W for 30–60 s before staining (Velji and Albright 1993). The prefiltered (< 2.0 µm) water samples were used for enumeration of FBA. Samples were stained using 4′, 6′-diamidino-2-phenylindole (DAPI, final concentration 2 µg mL\textsuperscript{-1}) for 10 min in the dark and filtered onto black polycarbonate filters (0.2 µm pore size; Poretics\textsuperscript{TM}) with a vacuum pressure of < 10 mm Hg (Porter and Feig 1980). Bacterial cells were enumerated using a Zeiss Axiovert 135 M epifluorescence microscope as described by Tang et al. (2015). The particle-attached bacterial abundance (PBA) was calculated as the value of TBA minus FBA. Since not all cells have been detached by sonication, the PBA may be underestimated in the present study.

DNA extraction, PCR amplification, and T-RFLP analysis

Total nucleic acids were extracted for 96 samples (48 FL samples and 48 PA samples) using the FastDNA\textsuperscript{®} Spin Kit for Soil (MP Biomedicals) according to the manufacturer’s instruction. Polymerase chain reaction (PCR) was performed with a Cy5-labeled forward bacterial primer 8F (5′-AGAGTTTGTGCTCAG-3′) and 1492R (5′-GGTACCTTGTACGACT-3′) (Newton et al. 2006). Amplification was carried out using a touchdown program (Tang et al. 2010). The quality and quantity of the amplified DNA fragment was checked by agarose gel electrophoresis and by by using a NanoDrop ND-1000 UV/Vis spectral photometer. Triplicate PCR products were then combined and purified immediately with the E.Z.N.A.\textsuperscript{®} Cycle-Pure Kit (Omega).

Enzymatic digestions were performed simultaneously with the two restriction enzymes HaeIII and MvnI (Fermentas) and the terminal restriction fragments (T-RFs) were separated on a Beckman Coulter CEQ\textsuperscript{TM} 8000 capillary electrophoretic sequencer, as described previously (Tang et al. 2010). T-RF sizes between 60 bp and 640 bp, with peak area of > 100 fluorescence units, were binned and standardized for subsequent analysis (Boucher et al. 2006).

The standardized T-RFLP data was used to compare the differences of bacterial communities across seasons, stations and between bacterial fractions (FL vs. PA). Non-metric multidimensional scaling (NMDS) analysis was performed using standardized and log(x + 1) transformed T-RF data for all samples (n = 96) with the software Plymouth Routines In Multivariate Ecological Research (PRIME, version 6.1.11) (Clarke 1993; Clarke and Gorley 2006). Distances were calculated using the Bray–Curtis algorithm. Then, the comparisons were tested by two-way crossed analysis of similarity (ANOSIM) using the same algorithm. ANOSIM was also used to test the significance between FL and PA bacterial communities. The test statistic, R (ranges between 0 and 1), generated by ANOSIM is indicative of the degree of separation between groups, with a score of 1 indicating complete separation, and 0 indicating no separation (Clarke 1993). Quantitative effects of sampling season, station and their interaction on the bacterial community variance were calculated by permutational multivariate analysis of variance (with ADONIS function).
Bacterial 16S rRNA gene sequencing

The DNA of the three replicate-sites sampled at each station for each season was pooled for subsequent 454-tag pyrosequencing. The V1-V3 regions of bacterial 16S rRNA genes were amplified using the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) primer with the Roche 454 “A” sequencing adapter, and the 533R (5′-TTACCAGGCTGCTGCGAC-3′) primer with a 10 bp barcode sequence and the Roche 454 “B” sequencing adapter. PCR amplifications were performed in triplicate for each sample using 20-μL reaction mixtures containing 1 × PCR buffer, 1.5 mM MgCl₂, 2.5 mM dNTPs, 4 μM of each primer, 1.0 U of TransStart FastPfu DNA Polymerase (TransGen Biotech, China), and 10 ng diluted DNA extract.

Amplification was carried out in GeneAmp® PCR System 9700 (Applied Biosystems, U.S.A.) under the following conditions: denaturation at 95°C for 2 min, 25 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR products were pooled, run on a 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, U.S.A.), and then quantified with QuantiFluor® ONE dsDNA System (Promega, U.S.A.). An equimolar amount of barcoded amplicons for each sample was used for subsequent emulsion-based clonal amplification and sequencing on a Roche 454 FLX pyrosequencer. Reads were produced from the backward direction (533R to 27F) at Majorbio Bio-pharm Technology (Shanghai, China).

Data preprocessing, OTU clustering, and taxonomy assignment

Pyrosequencing reads (further referred to as “reads”) were processed using the Quantitative Insights Into Microbial Ecology (QIIME) v. 1.8.0 pipeline (Caporaso et al. 2010). After demultiplexing, quality filtering, denoising and chimeric removal, bacterial phylotypes were identified and assigned to operational taxonomic units (OTUs, 97% cutoff) using Uclust algorithm (Edgar 2010) to generate stable OTUs (He et al. 2015). The representative read of each phylotype was selected and aligned in the Greengenes database (version 13.5) using PyNAST to identify the taxonomy of each phylotype (Desantis et al. 2006). Reads reassembled with chloroplasts, mitochondria, and Cyanobacteria were excluded from the subsequent analysis because this study focused on the composition of heterotrophic bacterial communities. In addition, low confidence singletons (OTUs with a read count of smaller than 2) were discarded from downstream analysis.

Bacterial diversity and statistical analysis

To normalize the sequencing depth of different samples, we randomly selected a subset of 1069 reads per sample based on the sample with the smallest sequencing effort using QIIME’s alpha_rarefaction.py workflow and beta_diversity_through_plots.py workflow according to QIIME tutorials (http://qiime.org/tutorials/tutorial.html). Then, α- and β-

diversity of the bacterial communities were estimated. Four indices, i.e., observed species, Chao1 (a nonparametric species richness estimator), Shannon index (a combination of richness and evenness) and phylogenetic diversity (PD whole tree) were calculated respectively to measure the α-diversity (Chao 1984; Faith 2006; Gotelli and Colwell 2011). Non-parametric Kruskal-Wallis rank tests were performed to check statistical differences of α-diversity indices among seasons and stations, while paired t-test was used to check the statistical differences between bacterial fractions.

Principal coordinate analysis (PCoA) was applied to visualize the β-diversity of bacterial communities (454 pyrosequencing data; n = 32), using Bray-Curtis distance (a nonphylogenetic metric), weighted UniFrac (a quantitative phylogenetic metric) and unweighted UniFrac (a qualitative phylogenetic metric) (Lozupone and Knight 2005). Then, ADONIS was used to test whether the samples from different seasons, stations and fractions clustered independently of each other using QIIME (He et al. 2015). In addition, Procrustes tests (PROTEST) with Bray-Curtis dissimilarity matrix, weighted UniFrac distance and unweighted UniFrac distance were performed to test if there was synchrony in the variation of PA and FL bacterial communities.

Statistical tests were carried out in R (version 3.2.2, http://www.r-project.org) using the community analysis package Vegan version 2.3-1 (Oksanen et al. 2015). Most data were visualized with the packages “ggplot2” (Wickham 2009) or base graphics. Analysis of variance (ANOVA) was performed to test whether the environment parameters varied significantly among different seasons and stations. Kruskal-Wallis rank tests were used with Bonferroni correction to compare the distribution of phylum and genus among seasons, sampling stations and between bacterial fractions. The Venn diagram was constructed using the “VennDiagram” package (Chen and Boutros 2011) to compare the community between fractions at OTUs level. Heat map of the most abundant bacterial genera (average relative abundance > 1%) were made using “pheatmap” package in the R environment.

Relationships between bacterial communities and environment

Correlations between bacterial communities and environmental factors were analyzed with ordination methods in the R environment using the Vegan package. Redundancy analyses (RDA) was computed using the unimodal species-environment relationship method because detrended correspondence analysis run on the pyrosequencing bacterial OTU (97% similarity) profile matrix indicated that the length of the first axis was < 3 (Lepš and Šmilauer 2003). The average value of each three samples in each station was used as the environmental input, since the OTU data were generated by pyrosequencing the pooled DNA of three samples in each station of each season. For RDA analysis, OTU data were transformed using Hellinger’s transformation (Legendre
and Gallagher 2001) as independent variable and environmental variables were log-transformed and standardized as explanatory variables. Environmental variables that significantly explained parts of the variation in BCC were identified by forward selection. Based on a Monte Carlo test with 999 permutations, only variables that explained a significant \( p < 0.05 \) additional proportion of total variance were included in the subset of forward selected variables (Leps and Smilauer 2003).

### Nucleotide sequence accession number

The raw pyrosequencing data generated in the present study were submitted to the National Center for Biotechnology Information (NCBI) Sequence/Short Read Archive (SRA) under accession number SRP070550.

### Results

#### Dynamics of environmental parameters and bacterial abundance

Most environmental parameters in Lake Taihu varied among stations and seasons (Supporting Information Fig. S1). Seasonal variations in TN, DTN, NO\(_3^-\), Temp, pH, DO, TSS, and SD were significant (ANOVA, \( p < 0.05 \)). Water temperature increased from mean value of 9.6°C in winter to a maximum of 31.4°C in summer. The concentrations of TN and NO\(_3^-\) were much lower in summer and fall than in winter and spring. Stations A to D have depths of approximately 2.5 m, 2.0 m, 2.8 m, and 1.9 m, respectively. Station B (near the mouth of Dapu River) had the highest concentrations of nitrogen (mean TN = 5.24 mg L\(^{-1}\)) and phosphorous (mean TP = 0.22 mg L\(^{-1}\)) compared to other stations with mean concentrations of TN and TP 2.91 and 0.37 mg L\(^{-1}\), respectively. The concentration of Chl \( a \) was much higher in the mouth of Dapu River (Station B, mean = 15.85 g L\(^{-1}\)) and in Meiliang Bay (Station A, mean = 13.45 g L\(^{-1}\)) than in Eastern Taihu Bay (Station D, mean = 4.14 g L\(^{-1}\)) and in the open lake (Station C, mean = 3.48 g L\(^{-1}\)). PBA and FBA varied significantly across seasons and both peaked in spring (Supporting Information Fig. S1) with the mean abundance of 5.71 \( \times 10^6 \) and 3.04 \( \times 10^6 \) cells mL\(^{-1}\), respectively. On average, the abundance of PA bacteria was 1.87 times higher than those of FL bacteria in Lake Taihu. Spearman rank correlation analysis (Supporting Information Table. S2) showed that nitrogen, phosphorus, and TSS were significantly related. Chl \( a \) was significantly related to phosphorus and NH\(_4^+\), while PBA and FBA were positively related to water temperature.

#### Bacterial community structure measured by T-RFLP

T-RFLP data were used to generate the overall pattern of bacterial community structure. In total, 180 T-RFs were detected from the 96 samples with an average number of 22 for each sample. Based on the T-RFs data, an NMDS ordination analysis revealed distinct seasonal succession of bacterial communities with the largest heterogeneity recorded between spring and summer (Fig. 2).

Two-way crossed ANOSIM showed that season and station were both significant factors in shaping the BCC (Table 1), and season (global \( R > 0.61, p = 0.001 \)) had much greater influence on community variation than station (global \( R \approx 0.24, p = 0.001 \)) did. Pairwise ANOSIM comparisons also showed the most significant differences in communities between summer and spring (Table 1). However, the bacterial community structure was more similar within sampling dates in summer and spring than that in fall or winter (Fig. 2). Furthermore, the bigger ANOSIM \( R \) values appeared when Station B was in comparison (Table 1), indicating greater dissimilarity of Station B in BCC compared with other stations.

The separation of PA and FL bacterial assemblages was not robust (global \( R = 0.158, p = 0.001 \) for T-RF abundance data; \( R = 0.113, p = 0.002 \) for T-RF presence-absence data), especially in summer and spring (Fig. 2). Furthermore, ADONIS indicated that sampling season and station were both significant factors in shaping the PA and FL bacterial communities, respectively (Table 2). Sampling season constrained 41.9% and 36.8% of PA and FL community variances, respectively; while sampling station accounted for only 10.0% and 11.6% variations of PA and FL communities, respectively (Table 2). The interaction of season and station constrained an additional about 31% of both PA and FL community variances (Table 2).

#### Taxonomic identification and variation measured by pyrosequencing

We generated 423,833 reads with average length of 474 bp were obtained from the 32 PA and FL bacterial samples. After demultiplexing, quality filtering, denoising, and removing of chimera, cyanobacterial reads (which accounted for 22.3% and 4.2% of total PA and FL bacterial reads, respectively; more than 90% of them belong to Microcystis sp. and Synechococcus spp.) and singletons OTUs, a total of 218,027 reads (94,914 from PA samples and 122,113 from FL samples) and 4940 unique OTUs (97% cutoff) were obtained. Overall, the reads were classified and grouped under 43 phylum-level taxonomic groups. Across all seasons and all sampling stations, the most seven abundant phyla of bacteria were Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Verrucomicrobia, and Chlorobi (Fig. 3). Within the Proteobacteria phylum, \( \beta - \) and \( \varepsilon - \)proteobacteria were the two most abundant groups, accounting for 21.5% and 11.8% of all clean reads. In winter and spring, a decreasing proportion of reads from Station A to Station D was assigned to the phylum Actinobacteria in the FL size fraction. In contrast, reads assigned to Proteobacteria increased distinctly from Station A to Station D in the FL fraction. In the PA fraction, there was an increasing proportion of Actinobacteria from Station A to Station D in both spring and fall, while a decreasing reads assigned to Proteobacteria from
Station A to Station D was documented in fall (Fig. 3a). Using Kruskal–Wallis rank tests, we found that there were no significant variations of the main seven bacterial phyla among sampling stations, while only the relative abundance of Chlorobi varied significantly (Bonferroni corrected \( p < 0.05 \) among seasons with the highest abundance recorded in summer.

At the phylum and class levels, both FL and PA bacterial communities displayed a similar composition (Fig. 3b). The FL sample was dominated by Actinobacteria (43.6%), \( \beta \)-proteobacteria (23.4%), \( \alpha \)-proteobacteria (11.5%), and Bacteroidetes (7.8%). The PA fraction was also dominated by Actinobacteria (37.1%) and \( \beta \)-proteobacteria (20.6%), followed by \( \alpha \)-proteobacteria (13.5%) and Bacteroidetes (10.6%). The only differences observed between the two fractions were shown for rare phyla. For example, the relative abundance of Planctomycetes and Chlorobi was significantly higher in PA than in FL fraction (Kruskal–Wallis rank test: \( p < 0.001 \) for Planctomycetes; \( p < 0.05 \) for Chlorobi).

At the genus level, the 15 most abundant genera (Fig. 4) comprised 65% of the total reads. They were represented by lineages or clades of known typical freshwater bacteria (Newton et al. 2011), including the Actinobacteria ACK-M1 (acI-A) and C111 (acIV) represented 37.5% of the total reads. This fits the observation that members of ACK-M1 and C111 were the dominants of planktonic bacterial community (\(< 15 \mu m\)) in Lake Taihu (Li et al. 2015b). Abundant Bacteroidetes genera comprised Fluvicola (bacV) and Flavobacterium (bacII-A). The most abundant Proteobacteria were Limnohabitans (betI-A), Polynucleobacter (Pnec), Hydrogenophaga and an unclassified
genus that belongs to the family *Pelagibacteraceae* (LD12). All genera/lineages were presented in all samples (n = 32) except for *Hydrogenophaga* (31/32). Kruskal–Wallis rank tests showed that 12 of the 15 most abundant genera were significantly different (Bonferroni corrected *p* < 0.05) among seasons (Fig. 4), but none of them varied significantly among sampling stations. These 15 genera comprised on average of 68% and 62% the reads in FL and PA communities, respectively. Among them, only two genera were identified to be significantly different between FL and PA fractions. The proportion of ACK-M1 was notably higher (Kruskal–Wallis rank test, *p* < 0.05) in the FL than in the PA fraction, while an unclassified genus belong to the Family *Pirellulaceae* of *Planctomycetes* (Fig. 4) was more abundant (Kruskal–Wallis rank test, *p* < 0.01) in PA compared to FL fraction.

**Table 1.** Two-way crossed analysis of similarity (ANOSIM) of bacterial community structure in Lake Taihu among different seasons and different sampling stations based on T-RFLP data (n = 96).

| Comparison                  | Sample statistic *R*   |
|-----------------------------|------------------------|
|                             | Peak area data | Presence-absence data |
| **Season**                  |             |                      |
| Global ANOSIM               | 0.619***     | 0.613***             |
| Winter vs. spring           | 0.473***     | 0.449***             |
| Winter vs. summer           | 0.632***     | 0.629***             |
| Winter vs. fall             | 0.448***     | 0.478***             |
| Spring vs. summer           | 0.856***     | 0.821***             |
| Spring vs. fall             | 0.778***     | 0.769***             |
| Summer vs. fall             | 0.574***     | 0.605***             |
| **Station**                 |             |                      |
| Global ANOSIM               | 0.236***     | 0.244***             |
| A vs. C                     | 0.190*       | 0.191**              |
| A vs. B                     | 0.280***     | 0.292***             |
| A vs. D                     | 0.178*       | 0.218**              |
| C vs. B                     | 0.361***     | 0.319**              |
| C vs. D                     | 0.159*       | 0.201**              |
| B vs. D                     | 0.292**      | 0.266***             |

* *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001.

**Bacterial community diversity**

Because 454 pyrosequencing can generate a higher resolution profile of bacterial diversity compared to T-RFLP, pyrosequencing data was used to calculate the diversities and to profile the taxonomic compositions. After normalizing the sequencing depth to the smallest sample (n = 1069 reads), *α*- and *β*-diversities were compared among seasons, sampling stations and between bacterial fractions (FL vs. PA).

For the comparison of *α*-diversity among seasons, observed species (i.e., OTUs), Chao1 and Shannon diversity were slightly lower in summer both for FL and PA bacterial communities, while the lowest phylogenetic diversities were recorded in spring and winter for FL and PA bacterial communities, respectively (Supporting Information Fig. S2). Using Kruskal–Wallis rank tests, however, we found that none of the differences among seasons were significant (*p* > 0.05). For the comparison of diversity among stations, our results showed that Station C (in the open lake) had the lowest of observed species, Chao1 and phylogenetic diversity for FL bacteria, but also had the highest diversity for PA fraction (Supporting Information Fig. S2). However, the differences among stations were insignificant (*p* > 0.05), either. Using paired *t*-test, we found that observed species, Chao1, Shannon index, and phylogenetic diversity (PD whole tree) were all significantly (*p* < 0.05) higher in the PA fraction than in the FL fraction (Fig. 5).

PCoA was performed to evaluate *β*-diversity among seasons and stations and between bacterial fractions using non-phylogenetic (Bray–Curtis distances) and phylogenetic (UniFrac) based ordination (Fig. 6). Our results demonstrated that the major separation in PCoA was caused by season rather than sampling station or bacterial fraction. Season had significant effect on the separation of bacterial communities (ADONIS, *p* = 0.001) regardless of whether phylogenetic or non-phylogenetic ordination was used. Furthermore, the quantitative (weighted UniFrac) *R*² values were much higher than the qualitative (unweighted UniFrac) one with the highest *R*² value reached to 0.396 using Bray–Curtis distances based ordination (Fig. 6).

The effect of sampling station on bacterial communities was not significant (*p* > 0.05) regardless of whether phylogenetic or non-phylogenetic ordination was used (Fig. 6).

**Table 2.** Quantitative effects of sampling season, station and their interaction on the bacterial community variance by permutational multivariate analysis of variance (with ADONIS function) using standardized T-RFLP data.

| Season bacterial communities | FL bacterial communities |
|------------------------------|--------------------------|
| **R²** | *p* | **R²** | *p* | **R²** | *p* | **R²** | *p* |
| Season | 0.419 | <0.001 | 0.368 | <0.001 | 0.313 | <0.001 | 0.316 | <0.001 |
| Station | 0.100 | <0.001 | 0.116 | <0.001 | 0.313 | <0.001 | 0.316 | <0.001 |
| **Season × Station** |                    | **Season × Station** |                    |

* *R*² values represent the proportion of variance constrained by factors.
Fig. 3. Percentage of the dominant bacterial taxa (average relative abundance > 1%) in Lake Taihu separated according to seasons, sampling stations (Station A to Station D, see Fig. 1) and fractions (a) and average percentage of the dominant taxa of free-living (FL) and particle-attached (PA) bacterial communities (b).

Fig. 4. A heat map revealing the dynamics of the 15 most abundant bacterial genera (average relative abundance > 1%) from different seasons, sampling stations (Station A to Station D) and fractions (FL, free-living vs. PA, particle-attached) in Lake Taihu. Typical freshwater bacterial lineages or clades named by Newton et al. (2011) were present in parentheses. At the end of the genera names, asterisk indicates the significances of the variations in their relative abundance among seasons by Kruskal–Wallis rank sum test: * p < 0.05, ** p < 0.01, *** p < 0.001.
addition, bacterial fraction only had significant effect (p = 0.005) on the qualitative evolutionary divergence (unweighted UniFrac) of PA vs. FL bacterial communities with small R² value (0.063). In contrast, the differences between PA and FL assemblages were insignificant when a quantitative factor was used (weighted UniFrac, Fig. 6). Accordingly, among the 4940 OTUs found in this study, 59% were shared by both fractions, 25% and 16% appeared only in PA and FL samples, respectively (Fig. 7a). When the abundance of each OTU was accounted for, 96.4% of the reads occurred in both fractions, 2.0% were exclusive to the PA fraction and 1.6% to the FL fraction (Fig. 7b). This suggests that the fraction exclusive OTUs are rare taxa.

Furthermore, there appeared to be synchrony in the bacterial community shifts of the two size fractions, which we statistically verified using Procrustes tests with Bray–Curtis dissimilarity matrix (r = 0.85, p = 0.001), weighted UniFrac distance (r = 0.62, p = 0.004) and unweighted UniFrac distance (r = 0.92, p = 0.001) of BCC (Supporting Information Fig. S3).

Environmental drivers of FL and PA bacterial community structure

Results of RDA illustrated that the dynamics of PA and FL bacterial communities are related to similar environmental variables (Fig. 8). Water temperature and NO₃⁻ were the most significant variables (Monte Carlo test, p < 0.01), explaining 28.3% of the changes in FL communities. Water temperature, NO₃⁻ and TSS were the most significant variables (Monte Carlo test, p < 0.01), explaining 27.9% and 36.2% of the changes of the two RDA axes and the total variation, respectively, in PA communities.

Discussion

Overlap between FL and PA bacterial communities

Generally, aquatic bacteria can be classified three categories according to their different lifestyles (Grossart 2010; Rösel and Grossart 2012): (1) truly free-living; (2) truly particle/aggregate-associated (including symbionts) and (3) bacteria possessing a lifestyle alternating between a FL and a PA stage. Among the 4940 OTUs in this study, we found 1249 and 787 OTUs were only exclusive to PA and FL assemblages, respectively (Fig. 7), which indicated that some rare aquatic bacterial species are truly PA or truly FL. In addition, the presence of more unique OTUs in the PA assemblages relative to the FL counterpart suggests that particles may provide more ecological niche space for the colonization of bacterial specialists. For instance, about 47% of the OTUs in the phylum of Planctomycetes were exclusively found in our PA samples, while only 10% OTUs were exclusively found in FL samples, suggesting adaption to PA lifestyle. This result is consistent with those found in recent studies (Shi et al. 2012; Parveen et al. 2013b; Louati et al. 2015), which showed that the phycosphere of cyanobacterial blooms harbors distinct bacterial communities including members of Planctomycetes.

An interesting finding in this study, however, was that we found 59% of the OTUs occupied 96.4% of the shared reads between PA and FL fractions (Fig. 7). In addition, pyrosequencing data showed that there were no significant differences between PA and FL bacterial communities in Lake Taihu using both quantitative phylogenetic (weighted UniFrac) and non-phylogenetic (Bray–Curtis distances) based ordination (Fig. 6). Although ANOSIM comparison between PA and FL fraction using T-RFLP data showed the differences between them were significant, the low statistic R-value (< 0.16) as well as the low resolution of fingerprint method suggested that the differences were not robust. Overall, high degree of overlap between PA and FL bacterial assemblages was found in Lake Taihu, indicating substantial exchanges between the two fractions. This result is similar to previous
observations in San Francisco Bay (Hollibaugh et al. 2000), Chesapeake Bay (summer, Noble et al. 1997), Weser Estuary (Selje and Simon 2003), the Mackenzie River estuary (Ortega-Retuerta et al. 2013), and Meiliang Bay, Lake Taihu (Tang et al. 2015). In addition, highly overlapped OTUs in both FL and PA fractions were also found in Lake Esrum (Riemann and Winding 2001), the euphotic layer of NW Mediterranean Sea (summer, Ghiglione et al. 2009), the coast of Beaufort Sea (Ortega-Retuerta et al. 2013), Canadian Arctic Ocean (Kellogg and Deming 2014) and the mesotrophic coastal of North Sea which have strong riverine influences (Bizić-Ionescu et al. 2015). In contrast to our findings, distinct or significant differences between FL and PA fractions were recorded in marine system and deep lakes (see Supporting Information Table S1 for details).

Based on the fact that most similar BCC in PA and FL fractions were observed in turbulent estuarine and coastal environments and in eutrophic shallow lakes (Supporting Information Table S1), we suggest that eutrophication and hydrodynamic forcing would play vital roles in the high FL/PA bacterial community composition overlap.

With eutrophication, pollution provides the necessary nitrogen (N) and phosphorus (P) for the aquatic bacterial community while carbon appears to be the limiting source. Particulate organic matter (POM) is a readily available carbon source, which can be the limiting factor for bacterial activity and community composition (Bizić-Ionescu et al. 2014). In an oligotrophic system, organic aggregates are hotspots for bacteria activity due to nutrient provision. Hence, it can be assumed that in a eutrophic system like Lake Taihu all bacteria are adapted to the high N and P load, hopping on and off from particles based on carbon needs. The concentration of POM in aquatic system goes up with the increasing nutrient level. Since attached bacteria degrade POM, they will

**Fig. 6.** PCoA based on Bray–Curtis distances (left column), the weighted UniFrac (middle column), and unweighted UniFrac (right column), comparing β-diversity of bacterial communities in Lake Taihu among seasons (upper panels), sampling stations (middle panels) and between bacterial fractions, i.e., particle-attached (PA) vs. free-living (FL) (under panels). ADONIS was used to test whether the partitioning of bacterial communities were affected significantly by season, station, and bacterial fraction. Significant values (p < 0.05) are marked with bold.
release progeny into the surrounding water to explore dissolved organic matter (DOM) as the algal materials are degraded (Noble et al. 1997). Previous studies (Azam and Malfatti 2007; Stocker and Seymour 2012; Taylor and Stocker 2012) have shown that a broad range (5–70%) of aquatic bacteria have evolved the ability of chemotactic behavior and motility to explore and colonize high nutrient habitats, frequently commuting between POM and DOM. In the present study, several freshwater bacterial OTUs (e.g., acIV, bacI-II-A, betl-A, Pnec, and LD12) were found in both PA and FL assemblages (Fig. 4), suggesting that colonization of particles may be mediated by ubiquitous bacteria (generalists). It may be that some typical freshwater lineages are true euryoecious organisms with complex lifestyles, resulting in broadly abundant distributions (Newton and Mclellan 2015). Grossart (2010) highlighted that many bacteria, even the true FL bacteria, may possess switchable lifestyles and spend most of their time dwelling on nutrient particles, depending on substrate availability and the surrounding environmental factors. In support of this assumption, Polynucleobacter (Pnec), well known as single, freely suspended cells, also found to be abundant in PA fraction in the present study (Fig. 4) and in a previously study on organic-aggregates (Tang et al. 2010). Hence, FL and PA bacteria, as suggested by Riemann and Winding (2001), might be considered interacting entities that may move from a FL lifestyle to a PA lifestyle and vice versa. This assumption is consistent with the result of Procrustes tests (Supporting Information Fig. S3), which showed significant synchrony in PA and FL communities. Based on high concentration of POM and the characteristics of bacteria (i.e., motile ability and exchangeable lifestyles), there should be little difference between FL and PA bacterial communities in eutrophic water body.

Hydrodynamics induced exchange between PA and FL bacterial communities may be one of the important mechanisms affecting the similarity between the two types of bacterial habitat, since high overlaps between PA and FL fractions were observed mostly in turbid estuarine and coastal environments with strong hydrodynamic forcing and high density of particles (Selje and Simon 2003; Ortega-Retuerta et al. 2013; Bïzï-Ionescu et al. 2015). Strong hydrodynamic stress can cause frequent collisions between particles, resulting in rapid (yet passive) bacterial attachment and/or detachment from particles (Simon et al. 2002). Due to shallowness (mean water depths of 1.9 m) and the long fetch length, wind has a particularly strong influence on Lake Taihu (Wu et al. 2015). Moreover, Lake Taihu also experiences frequent storms in summer because of the East Asian Monsoon (Zhu et al. 2014). In the present study, we found TSS (an indicator of wind-induced sediment resuspension and phytodetritus) was one of the main factors that structured PA communities (Fig. 8). Strong linkage between bacterial community composition and turbidity or TSS was also found in several turbulent systems (Selje and Simon 2003; Rink et al. 2011). In turbulent aquatic system, the short residence time in PA or FL bacterial community prevent substantial phylogenetic divergence of the bacterial community composition on particles and in water columns.

Overlap between FL and PA assemblages are dependent not only on hydrographic conditions but also on the size, density and quality of particles (Noble et al. 1997; Teeling et al. 2012; Ortega-Retuerta et al. 2013). Smaller particles (mostly < 200 µm) in Lake Taihu (Tang et al. 2009) offer less

**Fig. 7.** Comparison of bacterial community structure between PA and FL. (a) Venn diagram showing operational taxonomic unit (OTU) distribution between fractions. The size of circle is proportional to the number of OTUs. (b) Pie plot showing the percentage of reads in each fraction exclusively and in both fractions.
niches for truly PA bacterial colonization compared to macroaggregates (> 500 μm) in pelagic systems (Simon et al. 2002). This is consistent with a previous study (Kellogg and Deming 2009) which showed greater similarity between FL (0.2–1 μm) and smaller suspended particles (1–60 μm) associated bacterial communities than between FL and large sinking aggregates (> 60 μm) associated communities. In addition, high density of particles in Lake Taihu reduced the distances between particles, leading to higher incidence of particle collisions and the exchanges of bacteria. Ortega-Retuerta et al. (2013) suggested that the quality of particles may provide a major role structuring the exchange of PA and FL bacterial communities. Particle quality were closely related to seasonal succession of phytoplankton and the effect of particle quality on bacterial composition was discussed below.

Seasonal succession of FL and PA bacterial communities

In the present study, we observed clear seasonal patterns in the bacterial composition of both FL and PA communities by using T-RFLP (Fig. 2) and pyrosequencing (Fig. 6). Seasonal succession in BCC have been demonstrated by previous studies in lakes with different trophic levels (Van Der Gucht et al. 2001; Zwisler et al. 2003; Pérez and Sommaruga 2011; Eller et al. 2012). In Lake Taihu, seasonal variation in planktonic bacterial communities (Li et al. 2015b) and in organic aggregates-attached bacterial communities (Tang et al. 2010) were reported recently. In addition, phytoplankton community (Niu et al. 2011), cyanobacterial bloom, and nutrients (Tian et al. 2009) were found to be the main factors that shaped plankton and total bacterial community composition, respectively. Surprisingly, most of the studies focused only on planktonic or total bacterial communities and did not account for different bacterial fractions. Since different bacterial fraction may have different lifestyles and may occupy different ecological niches in aquatic systems, it is important to explore both bacterial compositions of FL and PA separately and to discover the relationship between them in lakes with different trophic levels. So far, a few studies revealed significant seasonal shifts of bacterial communities which treated FL and PA fractions simultaneously. For example, Rösel et al. (2012) revealed contrasting seasonal dynamics of FL and PA bacterial communities in mesotrophic Lake Tiefwaren (NE Germany) using DGGE. Dynamics of FL and PA β-proteobacteria, Actinobacteria, and Verrucomicrobia were found to be related to phytoplankton and zooplankton communities in the mesotrophic deep (maximum depth: 145 m) Lake Bourget located at the edge of the Alps (France) by sequencing of the 16S rRNA gene (Parveen et al. 2013a). However, the study on the dynamics of FL and PA bacterial communities in shallow eutrophic lakes is relatively seldom, except the study in one of the bays (Meiliang Bay) in Lake Taihu which also revealed seasonal pattern of both FL and PA communities (Tang et al. 2015).

Seasonal variations in both FL and PA bacterial communities in this study were probably due to differences in the

**Fig. 8.** Redundancy analyses ordination plot showing the relationship between free-living (FL, a) and particle-attached (PA, b) bacterial community structure and the significant (p < 0.01) environmental variables. NO$_3^-$, nitrate; Temp, water temperature; TSS, total suspended solids. Symbols with adjacent names represent different samples. The first letter of the sample names denote sampling stations and the numbers after the first letters denote sampling months.
nutrient dynamics among seasons and to the quantity and quality of particles. In aquatic ecosystems, seasonal variations of environmental parameters (e.g., water temperature, nutrient loading, and phytoplankton composition) have been shown to have a strong impact on bacterial community composition (Allgaier and Grossart 2006; Kent et al. 2007). RDA analysis demonstrated that water temperature and $\text{NO}_3^-$ were the most significant factors that shaped the dynamics of both FL and PA communities (Fig. 8), indicating that environmental factors in Lake Taihu affect bacterial communities in much the same way regardless of bacterial fractions. A strong Spearman’s rank correlation was found between $\text{NO}_3^-$, TN, and DTN (Supporting Information Table S2). Therefore, $\text{NO}_3^-$ could represent a proxy of nitrogen. The influence of water temperature and nitrogen on BCC was also shown in previous study in mesotrophic Lake Tiefwaren (Rösel et al. 2012).

However, water temperature and $\text{NO}_3^-$ contributed only about 30% to the variations of FL and PA communities, which indicates that biotic factors (such as phytoplankton) may also have important role in this process. Since algal exudates can supply important energy and organic matter source, a close coupling between heterotrophic bacteria and autotrophic phytoplankton has been found (Cole 1982; Covey and Wetzel 1995; Grossart et al. 2005; Rösel and Grossart 2012; Teeling et al. 2012). In support of this assumption, previous studies (Ghiglione et al. 2007, 2009) have showed that the contribution of attached bacteria to total bacterial abundance and to total bacterial activity was maximal at the deep chlorophyll maximum layer while, more importantly, the BCC of PA and FL showed the most level of similarity. Although we could not account for dynamics of FL and PA bacterial communities by the succession of phytoplankton, which was known to key role in shaping bacterioplankton community composition in Lake Taihu (Niu et al. 2011; Li et al. 2015b), our data showed higher PA/FL similarity in summer than in other seasons (Figs. 2, 6). This could be related to the quality of particles. In high bloom season, the lability of the particles and the available excreted organic matter due to the massive blooms provide similar environment on and off particles. Since TSS, one of the most significant factors shaped PA communities (Fig. 8), is mainly composed of *Microcystis*-originated particles in summer (Chen et al. 2003; Niu et al. 2011; Xu et al. 2013), the homogeneity of algal particles (i.e., similar chemical components) may inevitably play a vital role in shaping the similarity of PA and FL communities. In other seasons, termination of blooms and subsequent less labile organic matter may recruit more diverse bacteria to particles (Supporting Information Fig. S2), resulting in greater differentiation between PA and FL communities.

**Alpha-diversity of FL and PA bacterial communities**

Our results demonstrated that PA communities in Lake Taihu have significant higher $\alpha$-diversity (observed species, Chao1, Shannon and PD whole tree) compared to their surrounding counterparts (Fig. 5). This fits the observation in mesotrophic and eutrophic lakes (Parveen et al. 2011; Rösel et al. 2012; Tang et al. 2015) and matches recent findings in different aquatic systems performed with comparable pyrosequencing technology (Crespo et al. 2013; Ortega-Retuerta et al. 2013;типичнoе-Іонеску et al. 2015). One explanation for this would be the much higher resolution of the high-throughput sequencing compared to fingerprint methods and common cloning and sequencing. Previous studies used conventional molecular techniques retrieved less than 100 clones and OTUs. In contrast, studies used pyrosequencing (Crespo et al. 2013; Ortega-Retuerta et al. 2013; типичнoе-Іонеску et al. 2015) including the present study, have yielded as much as two orders of magnitude higher clones and OTUs than in those previous studies. The pyrosequencing method has revealed an enormous hidden diversity in PA bacterial assemblages by the detection of many rare taxa (Crespo et al. 2013) as shown in the present study (Fig. 7).

Another explanation for higher bacterial diversity in PA would be related to the enhanced POM concentration and the single particle heterogeneity in Lake Taihu. It has been proposed that POM are nutrient-enriched microhabitat which can provide more favorable nutrients and increased habitat heterogeneity for bacterial colonization (Simon et al. 2002). This idea was supported by Lyons et al. (2010) who demonstrated that organic aggregates were favorable habitats for aquatic pathogens compared to the surrounding water. The traditional simplified view is that particles are colonized by specialists which are able to degrade the particle to certain extent based on their enzymatic arsenal and the current carbon lability of the particle (Kellogg and Deming 2014; типичнoе-Іонеску et al. 2015). Once the carbon is no further available to the current colonizers these will detach and the particle will be colonized by a new set of organisms with a different set of enzymes. This is expected to lead to a reduced diversity on individual particles as compared to the FL fraction. However, since environmental particles are not of the same source and not of the same age, with enhanced POM concentration in Lake Taihu, pooling an entire water sample will eventually lead to an overall higher diversity on particles than in the FL fraction.

Previous researches have shown that PA bacteria are often bigger and more active than their FL counterparts due to concentrated substrates in particle microhabitats (Grossart et al. 2003, 2007; Stocker 2012; Kellogg and Deming 2014). In contrast to oligotrophic marine and deep lake systems (Simon et al. 2002), the overall abundance of PA bacteria distinctly outnumbered those of FL fraction in eutrophic Lake Taihu (Tang et al. 2010, 2015). Consequently, PA bacteria—due to higher $\alpha$-diversity and elevated level of bacterial abundance—may have more important role in biogeochemical element cycling and productivity in large shallow eutrophic lakes like Lake Taihu compared to their FL ones, yet they are
often omitted from the beginning of sampling (Wu et al. 2007a; Niu et al. 2011).

**Conclusions**

In the present study, a major objective was to determine the spatiotemporal dynamics of bacterial diversity and community composition in both FL and PA assemblages in Lake Taihu. Despite the distinct nutrient gradients and spatial heterogeneity within this lake, our examination of the PA and FL bacterial communities across this environmental gradient revealed similar diversity and BCC at different sampling stations in a time scale of 1 yr. We found particles harbor significantly higher bacterial diversity than the surrounding water. However, the difference on bacterial diversity was caused mainly by rare taxa (specialists). The most abundant taxa (generalists) in PA also the most abundant taxa in the FL fraction (Fig. 4). In addition, Procrustes tests confirmed the co-occurrence of BCC between the two components (Supporting Information Fig. S3). These data support the view that free-living and particle-attached bacterial communities are not separate entities but interacting assemblages. Based on our literature survey (Supporting Information Table S1) and the present results, it seems that the differences between FL and PA bacterial communities are less pronounced in turbulent and more productive aquatic systems. In these systems (including Lake Taihu), eutrophication-induced enhanced POM availability, as well as strong hydrodynamics appears to increase the active and passive dispersal potential between the two bacterial fractions. Besides water temperature and nutrients, our study also highlights the potential effect of phytoplankton in the seasonal shifts of both FL and PA bacterial communities. Under the scenario of climate change-induced warmer springs (Deng et al. 2014) and extension of surface cyanobacterial blooms in area (Wu et al. 2015) and duration time (Ma et al. 2016) in Lake Taihu, the interaction between cyanobacterial blooms and heterotrophic bacteria seems to be the key to shed light on the functional connection between them and the theoretical framework for the predictability of cyanobacterial blooms. In addition, higher abundance and diversity made PA bacteria to be an important functional component of this turbid and eutrophic ecosystem. Under a warmer climate in the future, they are likely to play an increasing role in nutrient cycling and cyanobacterial bloom maintenance, which should be considered in the process of sampling strategies and further functional studies.

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Conflict of Interest
None declared.

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