Bacterial Community Composition of Size-Fractioned Aggregates within the Phycosphere of Cyanobacterial Blooms in a Eutrophic Freshwater Lake

Haiyuan Cai¹, Helong Jiang¹*, Lee R. Krumholz², Zhen Yang¹

¹ State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China, ² Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma, United States of America

Abstract

Bacterial community composition of different sized aggregates within the Microcystis cyanobacterial phycosphere were determined during summer and fall in Lake Taihu, a eutrophic lake in eastern China. Bloom samples taken in August and September represent healthy bloom biomass, whereas samples from October represent decomposing bloom biomass. To improve our understanding of the complex interior structure in the phycosphere, bloom samples were separated into large (>100 μm), medium (10–100 μm) and small (0.2–10 μm) size aggregates. Species richness and library coverage indicated that pyrosequencing recovered a large bacterial diversity. The community of each size aggregate was highly organized, indicating highly specific conditions within the Microcystis phycosphere. While the communities of medium and small-size aggregates clustered together in August and September samples, large- and medium-size aggregate communities in the October sample were grouped together and distinct from small-size aggregate community. Pronounced changes in the absolute and relative percentages of the dominant genus from the two most important phyla Proteobacteria and Bacteroidetes were observed among the various size aggregates. Bacterial species on large and small-size aggregates likely have the ability to degrade high and low molecular weight compounds, respectively. Thus, there exists a spatial differentiation of bacterial taxa within the phycosphere, possibly operating in sequence and synergy to catalyze the turnover of complex organic matters.

Introduction

Due to climate change and anthropogenic carbon and nitrogen runoff, cyanobacterial blooms are becoming more common in freshwater lakes and estuaries throughout the world and threaten the sustainability of aquatic ecosystems [1]. The formation of large mucilaginous cyanobacterial blooms in freshwater lakes restricts light penetration, depleting oxygen levels, thereby reducing water quality adversely affecting the ecosystem [1]. These changes can result in reduction in the numbers of submerged plants, killing of aquatic animals, and alteration in food web dynamics [2]. Furthermore, massive cyanobacterial blooms in eutrophic lakes are dominated by Microcystis spp., which produces toxic microcystins that can prevent water consumption [3].

In order to better understand the process and mechanism of cyanobacterial bloom formation, previous studies usually focused on cyanobacterial species composition and chemical and physical factors influencing cyanobacterial growth [4]. However, numerous heterotrophic bacteria were found to be associated with cyanobacteria, and had an important impact on cyanobacterial growth. In fact, cyanobacterial-heterotrophic bacterial associations are commonly observed both inside cyanobacterial colonies/aggregates and within extracellular polymers outside cyanobacterial cell walls. Collectively, these microhabitats constitute the cyanobacterial phycosphere. In the phycosphere, bacteria can live freely, attached to the algal surface, or extracellular products [5].

The phycosphere was a niche that might provide a suitable microenvironment for a diverse subset of bacteria [6]. The cyanobacteria excreted an abundance of extracellular organic matter that likely provides energy for associated bacteria [7]. In turn, bacterial partners may play a role in providing CO₂, nitrogen, phosphorus, sulfur and trace elements to the cyanobacteria [8]. Thus, investigation of microbial communities in the phycosphere of cyanobacterial blooms may help us to understanding why cyanobacteria often dominate phytoplankton communities in eutrophic freshwater ecosystems.

While the microbial community associated with cyanobacterial aggregates/colonies has been widely investigated in recent years [9–13], few studies investigated the complex interior structure in the phycosphere of cyanobacterial blooms. In eutrophic lakes and estuaries, cyanobacterial Microcystis colonies usually aggregate, and then form mucilaginous Microcystis blooms through coagu-
loration of extracellular polymeric substances [14], moreover, cyanobacterial debris and other particle organic matter also was released from the *Microcystis* colonies. As a result, there exist microbial aggregates of various sizes in cyanobacterial blooms due to of nutrient availability [15]. Notably, distinct microcystin production and genotype compositions among size-fractionated *Microcystis* aggregates were observed [15], emphasizing the need to partition aggregates by size for microbial community analysis.

In addition, the non-cyanobacterial community associated with blooms has been intensively studied by the application of various molecular methods, including polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) [13] and terminal restriction fragment length polymorphism (T-RFLP) [16]. Because *Microcystis* spp. dominated bloom samples, the above molecular methods lacked sensitivity and could not accurately reflect the bacterial diversity within cyanobacterial blooms. In comparison, parallel 454 pyrosequencing is a high-throughput analytical method that can generate much more information on community composition [17]. This technology has been used widely to analyze the microbial community in various environmental samples, but has not been previously used to study cyanobacterial bloom communities.

The objective of the present study was to describe and compare the phylogenetic diversity of the microbial communities in various size-fractioned aggregates within the phycosphere of cyanobacterial blooms. In this study, *Microcystis* blooms in the eutrophic Lake Taihu, were taken from on three dates (10th August, 9th September and 11th October 2012). The bloom samples were filtered to separate communities into three fractions (>100 μm, 10–100 μm, and 0.2–10 μm). Bacterial community composition in the bloom phycosphere was characterized using high throughput sequencing and a well-established β-diversity analytical tool. This study revealed the complex bacterial communities of the phycosphere within *Microcystis* blooms, and suggests possible ecological roles in catalyzing the turnover of complex organic matter released from the cyanobacterial aggregates. These diversity analyses will facilitate current understanding of the distribution and the ecological roles of bacterial communities associated with *Microcystis* blooms in eutrophic lakes.

**Materials and Methods**

**Ethics statement**

No specific permits were required for the described field studies. The location studied is not privately-owned or protected in any way and our studies did not involve any endangered or protected species.

**Sample collection**

Lake Taihu, located in the Changjiang (Yangtze) River delta in eastern China, is a large shallow, eutrophic and temperate lake. The lake has a surface area of 2338 km², and an average depth of 1.9 m. *Microcystis* spp. blooms have been observed in Lake Taihu since the 1990s, usually between June and October. When cyanobacterial blooms occur in Lake Taihu, large *Microcystis* biomass accumulates on and below the water surface.

*Microcystis* bloom samples were collected from the surface water of Meiliang Bay (site A: 31°30’N, 120°11’E; site B: 31°44’N, 120°18’E) within Lake Taihu on 10th August, 9th September, and 11th October 2012. Equal volume bloom samples (5 L) taken from site A and B were mixed. Physiochemical parameters were determined in situ by Yellow Spring Instruments (YSI, 6600, USA). Total nitrogen (TN) and total phosphorus (TP) were analyzed according to standard methods [18]. Chlorophyll a (Chl a) was determined according to Asai and colleagues [19]. Bloom samples were retrieved by dipping a sterile beaker off the side of a boat from the surface down to a depth of about 5 cm. In August and September, *Microcystis* blooms were driven by the wind to accumulate in Meiliang Bay, forming a dense layer (nearly 20 cm in thickness). However, thin (less than 5 cm in thickness) and brown-yellow bloom layers formed in October, due to lower water temperature. *Microcystis* blooms in August and September were mostly intact and green. However, in October, they were broken and brown-yellow, indicating that cyanobacterial aggregates was decomposing. Therefore, the two blooms samples taken in August and September, represented healthy cyanobacterial bloom biomass and the sample taken in October represented decomposing cyanobacterial bloom biomass.

The phycosphere sample was obtained by taking advantage of the relative buoyancy of the cyanobacterial aggregates. After transferring to the laboratory within a few hours, bloom samples were put into in 50 mL sterile centrifuge tubes, and the tubes were left at room temperature for 2 hours. This process resulted in a layer of the concentrated cyanobacterial aggregates at the top surface of the centrifuge tube. This top layer was regarded as the phycosphere sample of cyanobacterial blooms.

Sample biomass was stored at -20°C prior to DNA extraction. For simplification, the samples (BCA, MCA, and SC) in cyanobacterial blooms taken in August, September and October were named as 08BCA, 08MCA, 08SC, 09BCA, 09MCA, 09SC, 10BCA, 10MCA, and 10SC, respectively.

**Nucleic acid extraction and 454 pyrosequencing**

Bacterial genome DNA was extracted using two methods in parallel: one using an UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s directions, and another one using a phenol-chloroform protocol as previously described [20]. DNA concentration and purity were then determined using a Nanodrop UV-Vis spectrophotometer (Nanodrop, Wilmington, DE, USA). Purified DNA extracts were stored at -20°C until use. In order to minimize putative bias due to the DNA extraction methods, the DNA obtained by both methods were pooled together in equal concentrations. 16S rRNA genes were amplified using 341F [21] and 907R [22] primers. The PCR amplifications, performed in triplicate for each DNA extract, and a smaller number of PCR cycles were employed in this study. The thermo cycling steps were as follows: 95°C for 4 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension step at 72°C for 5 min. The amplicons were quantified by fluorimetry with PicoGreen dsDNA quantitation kit (Invitrogen, Life Technologies, Carlsbad, CA) and pooled at equimolar concentrations. Roche GS-FLX 454 pyrosequencing was conducted by Meiji Biotechology Company (Shanghai, China). Pyrosequencing sequence data from this study were submitted to the NCBI Sequence Read Archive (SRA) under accession number SRP042642.
### Results and Discussion

#### Environmental Conditions

Biologically available nutrients in August were more abundant than in September or October, consistent with the highest primary production, indicated by chlorophyll a levels, occurring in August (Table 1).

#### Diversity of microbial communities

The quality-filtering process removed low quality raw sequence reads, leaving 92,793 high-quality target tags. The average read length was improved to about 494 bp, and the number of reads per sample ranged from 6,951 to 15,653 (Table 2). By performing the alignment at a uniform length of 450 bp, OTUs were clustered at 5% and 5% distances. As species richness increases with the number of sequences in a given sample, all the samples were subsampled using MOTHUR randomly to the same size based on the sample with the smallest sequences number. Coverage analyses showed that the cyanobacterial bloom libraries contained at least 97.6% of the total number OTUs that exist in samples (Table 2). The rarefaction curves appear to almost reach the saturation level (Fig. 1). Hence the 454 pyrosequencing libraries provide us with a nearly complete inventory of the bacterial 16S rRNA sequences present in the samples. On the basis of Richness and Shannon’s index, diversity in BCA samples was higher than that in the MCA samples, indicated that *Microcystis* containing aggregates harbored a more diverse bacterial community than that in other organic particles. This may be due to the fact that freshly formed, labile extracellular organic matter is able to sustain a highly diverse community.

Cyanobacterial reads were abundant in the BCA and MGA samples, and were detected at lower percentages in the smaller aggregates (less than 1.5% in the SC samples) (Table 2). More than 99% of the cyanobacterial reads were classified as *Microcystis* spp., with the others assigned to GpVI and GpIIa. The percentage of cyanobacterial reads in the 08BCA sample was the highest at 68.5%, while the cyanobacterial percentage in 10BCA decreased to 27.3%. The cyanobacterial percentage in 10MCA was the highest among the medium sized aggregates at 12.4% compared to 4.2% in 08MCA and 5.9% in 09MCA samples.

#### Functional organization analysis

Pareto–Lorenz curve distribution patterns were plotted after the cyanobacterial OTUs were excluded for all samples (Fig. 2). When all samples were grouped together, 40.8% of sequences were assigned to the top 10 most abundant OTUs and this number increased to 85.3% for the top 100 most abundant OTUs. For individual samples, 20% of the OTUs contained 85 to 95% (on average 91%) of the cumulative sequence abundance. The latter number is the $F_0$ index, which when higher than 85% represents a specialized community in which a small amount of the species is dominant and all the others are present in low numbers [25]. Thus, all these communities were highly specialized, and as there were major differences among communities, we conclude that they were affected by the phycosphere conditions created by cyanobacteria. Others have also observed the selection of specific bacterial species within the *Microcystis* phycosphere by comparing *Microcystis*-attached bacteria (phycosphere bacteria) with other microbial communities living in the same aquatic ecosystem [11,13].

#### Similarity analysis

The abundance and diversity of OTUs in our samples were compared using multivariate ordinations (Fig. 3). When all
cyanobacterial reads were included, BCA communities at all three sampling times clustered together and separated from other two size fraction communities (Fig. 3a). This may be due to the high relative abundance of *Microcystis* spp. in the large aggregates (Table 2). Thus, *Microcystis* sequences were excluded in order to compare non-cyanobacterial bacterial community composition within the blooms. When all cyanobacterial reads were excluded, BCAs were much less related to each other (Fig. 3b), indicating that large aggregate communities differed over the sampling times. In addition, 08MCA and 08SC clustered together and were separate from 08BCA and 09MCA and 09SC clustered together and were separate from 09BCA. However, 10BCA clustered together with 10MCA and both were separate from 10SC.

Associated bacteria within the cyanobacterial phycosphere are strongly affected by the physiological status of cyanobacteria inside [26]. Thus, the dominance of *Microcystis* cells and microenvironments created within *Microcystis* aggregates were likely the major contributing parameter for the spatial variation observed in the bacterial community structure. *Microcystis* blooms in August and September were green, healthy and intact, whereas in October, they were yellow and broken up, likely as a result of low water temperature and lack of nutrient availability (Table 1). The overall abundance of cyanobacterial cells in BCA in August and

### Table 1. Environmental variables at the three sampling times.

| Time    | Temp (°C) | pH   | TN(mg/L) | TP (mg/L) | Chla(µg/L) |
|---------|-----------|------|----------|-----------|------------|
| August  | 31        | 8.31 | 9.53     | 0.64      | 102.06     |
| September | 21       | 8.22 | 1.68     | 0.14      | 27.91      |
| October | 18        | 8.24 | 1.34     | 0.13      | 16.92      |

doi:10.1371/journal.pone.0102879.t001

Figure 1. Rarefaction analysis of the 16S rRNA gene sequences among phycosphere samples using an evolutionary distance threshold of 3% (i.e., 97% similarity).

doi:10.1371/journal.pone.0102879.g001
September then led to distinct bacterial communities in BCA compared to MCA and SC. In comparison, for cyanobacterial bloom biomass taken in October, the cyanobacterial percentage in BCA was only around two fold of that in MCA. It seems likely that MCA in October were derived from the decomposition of BCA. As a result, in October, there was a similarity between the bacterial community in BCA and MCA.

General bacterial composition

All of our non-cyanobacterial sequences were affiliated with at least 22 bacterial divisions: Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Verrucomicrobia, Acidobacteria, Armatimonadetes, Chloroflexi, Chlorobi, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospirae, Planctomycetes, Spirochaetes, Verrucomicrobia and Candidate divisions OD1, SR1 and TM6. The majority of phycosphere sequences belonged to the five major phyla: Bacteroidetes, Proteobacteria, Actinobacteria, Firmicutes and Verrucomicrobia, and these major groups varied in relative abundance among the samples (Fig. 4).

Of these major phyla, Bacteroidetes was the most abundant phylum in all samples, accounting for 2.4–74% of total non-cyanobacterial sequences (Fig. 4). This is in line with other studies [27–28] and thus indicates that members of the clade appear to be particularly adapted to bloom conditions, most likely as a result of their ability to degrade complex bio-macromolecules [29] and alga-derived metabolites [27]. Proteobacteria was also abundant and dominated 08MCA with a relative abundance of 77%. Other studies have shown Proteobacteria as a significant fraction of bloom associated bacteria [9,12,13]. Alpha-, Beta-, Gamma- and Deltaproteobacteria were all detected in all nine samples, but relative abundances varied. Alphaproteobacteria was the dominant class in the 08SC (32% of the total). Actinobacteria was the third most abundant phylum and dominated in the SC libraries, but was rarely detected in the BCA samples. Actinobacteria have been previously shown to be the most abundant phylum in 16S rRNA clone libraries prepared from bacterioplankton communities during the period of cyanobacterial bloom in Lake Taihu [30]. In contrast, the phylum Firmicutes was most abundant in the BCA and MCA samples rather than in the SC samples. The phylum Verrucomicrobia was abundant in September and its relative abundance was as high as 14% in the 09SC samples. However, sequences belonging to Verrucomicrobia were rare in August and October.

Comparative bacterial composition of different sized aggregates at genus level

To further compare the bacterial composition within the Microcystis phycosphere, bacterial composition determined at genus level and those with a relative percent greater than 1% of the sample reads are shown (Table 3). With the exception of 08MCA, 08SC and 10SC, Flavobacterium sp. was most abundant in all phycosphere samples comprising 20% of all non-cyanobacterial sequences and it was also very unevenly distributed. This genus was most abundant in September with 66% of all sequences in the 09MCA. Previously, Flavobacteria were found to be abundant during phytoplankton blooms, particularly during cyanobacterial blooms in freshwater environment [10,28,31]. Flavobacterium sp. are likely well adapted to the cyanobacterial phycosphere, especially that of Microcystis, and may play an important role in degrading the chemically stable cyanobacterial hepatotoxins [32] and enhancing aggregation of M. aeruginosa [26].

We observed obligate gram-negative predatory bacteria belonging to the Bdellovibrio-and-like organisms (BALOs), which
include Bdellovibrio and Peredibacter (Table 3). These organisms may lyse Microcystis releasing the cell contents to the phycosphere. The representative 454 sequences of Bdellovibrio are closely related to B. bacteriovorus (97% similarity), which is a gram-negative, vibrio-shaped bacterium that preys on other gram-negative bacteria. It was previously reported that Microcystis cells were lysed by Bdellovibrio-like bacteria through breakdown of cell structures [33]. Peredibacter sp. consists of predatory, gram-negative, bacteriovorous organisms that require a gram-negative host as prey to complete a biphasic life cycle. Members of the genus Peredibacter are generally regarded as soil-dwellers [34], and were not previously shown to exist with high abundance in cyanobacterial phycosphere.

All phycosphere samples included some obligate or facultative anaerobic microbes (Table 3). For example, September samples were dominated by Opitutus, which is an obligatory anaerobic member of the phylum Verrucomicrobia, usually inhabiting anoxic environments. Microcystis bloom forms a dense scum that can be 10–30 cm in thickness in September, when local temperatures are higher than 30°C. At this time, dissolved oxygen is rapidly exhausted below the surface of the bloom and the microenvironment quickly becomes anoxic [35]. The metabolism of Opitutus sp. is suited for growth on plant-derived (poly)saccharides [36]. Thus, Opitutus may decompose organic matter released by Microcystis, allowing it to be abundant in anaerobic environments created by the cyanobacterial bloom. In August and October samples, Clostridium and Dechloromonas were abundant. The representative 454 sequences of Dechloromonas are closely related to D. agitata (96% similarity), which has the ability to reduce (per)chlorate [37]. The relatively high abundance of Dechloromonas indicated that there exist anaerobic niches in August phycosphere. Clostridium sp. was also observed to be highly abundant, coexisting with Microcystis and is likely involved in the hydrolysis of Microcystis biomass [38].

Fig. 3B shows that 08MCB and 08SC cluster together and 09MCB and 09SC clustered together. Azospirillum, Pelagibacter, Pseudomonas and ACK-M1 were abundant in the 08MCB and 08SC samples and Aeromonas, Legionella, Methylophilus and Methylovorus were abundant in the 09MCB and 09SC (Table 3). These genera seem to have ability to degrade a range of high to low molecular weight (MW) compounds coming from cyanobacterial debris and extracellular complexes. Pseudomonas is a
widespread genus, made up of versatile aerobic bacteria [39] can utilize various substrates, including a variety of macromolecules such as hydrocarbons and aromatic compounds [40]. *Legionella* strains have been previously observed in cyanobacterial biofilms [41] and are thought to grow on organic compounds produced by the cyanobacteria [42]. *Pelagibacter* had a pronounced preference for glutamine and glutamate over 7 other amino acids in situ [43]. In addition, *Pelagibacter* was also able to exploit other mononermic sources of organic carbon including glucose, fructose or acetate [43]. *Methylphilus* is a restricted facultative methanol-utilizing bacterium and has been observed in high abundance in *Microcystis* blooms [9]. *Methylovorus* is facultatively methylotrophic bacterium [44], but has been rarely reported to be abundant in cyanobacterial bloom samples.

In comparison to MCA and SC samples in August and September, BCA samples from August and September had high levels of high MW organic matter degrading genera including *Alkaliflexus*, *Dechloromonas*, *Clostridium* and *Gemmatimonas*. *Gemmatimonas* belongs to the phylum *Gemmatimonadetes* and is frequently associated with cyanobacterial mats [45].

October samples were also dominated by various organic matter degraders. 10BCA and 10MCA bacteria that have ability to degrade high MW and/or compounds with a complex structure were abundant during October and include *Alkaliflexus*, *Propionivibrio*, *Pseudomonas* and *Clostridium*. The representative 16S sequences of *Alkaliflexus* are closely related to *A. shenetskii*, which is capable of decomposing plant polymers (xylan and starch), as well as mono- and disaccharides [46]. The representative 16S sequences of *Propionivibrio* were closely related to *P. limicola*, which degrades hydroyaromatic compounds [47].

It is important to point out that *Aeromonas* sp. dominated both the 10BCA and 10MCA. A recent study suggested that the *Aeromonas* strains could be responsible for gastrointestinal symptoms declared following recreational exposure to cyanobacterial bloom [48]. The water from Lake Taihu is used as a source of drinking water, and therefore could transfer pathogens like *Aeromonas* during cyanobacterial blooms.

The most abundant genera in 10SC were in the *Pelagibacter*, *Methyllobacterium* and *Methylphilus*, which all have a small cell size and the ability to degrade low MW carbon organic compounds. *Methylobacterium* can use methanol and methylamine as well as C2, C3 and C4 compounds to grow [49]. Nearly 10% of non-cyanobacterial reads in 10SC were classified as *Methyllobacterium*. Few studies have detected this genus at high abundance in the cyanobacterial phycosphere. The closest relative of the 16S sequence of *Methyllobacterium* was *M. hispanicum* (99% similarity). Also, the genus *Methylphilus* that only degrades acetate and single-carbon compounds [50] was detected in 10SC with 8.0% of the reads.

Spatial differentiation of bacterial taxa within the phycosphere was observed in three months bloom samples in Lake Taihu. This variation could be a result of a specific metabolic sequence includes sequential processing and degradation of specific components within extracellular organic matter (EOM) released by cyanobacteria. In previous chemical analyses of bloom samples, high MW and hydrophilic organic compounds accounted for the majority of *Microcystis aeruginosa* EOM which was comprised of protein-like, polysaccharide-like and humic-like substances [51]. Our present findings suggest that BALOs may lyse *Microcystis* cells and then *Flavobacterium*, *Gemmatimonas*, *Aeromonas*, *Pseudomonas* and others, perhaps including anaerobic microbes might metabolize high MW EOM and/or *Microcystis* cell contents to single- or low-carbon organic compounds, and carbon dioxide. The single- or low MW carbon compounds could be further utilized by *Pelagibacter* sp., *Methyllobacterium* sp., *Methylphilus* sp. and *Methyllobacterium* sp. in the terminal portion of this aerobic food chain. Although confirmation awaits further experimentation and chemical analyses, if correct, this metabolic pathway may provide a partial explanation for the ubiquitous presence of methlytrophs in association with cyanobacterial blooms [9,32].

**Possible ecological role of bacterial communities in different-size aggregates within the phycosphere of cyanobacterial blooms**

In this study, it was observed that phylotypes capable of utilizing high molecular weight (HMW) compounds, were mainly present in BCA within the phycosphere of cyanobacterial blooms, while phylotypes capable of utilizing single-carbon or low MW organic compounds, were only present in SC. As one of the most common...
bloom-forming cyanobacteria, *Microcystis aeruginosa* produces numerous secondary metabolites and EOM during normal growth [14,51]. Compared to exudates from cyanobacteria, lysates from cyanobacteria consist of more complex organic compounds [52]. These high MW compounds are degraded and recycled by cooperation from a variety of bacterial species [53]. As cyanobacteria are at highest abundance in the BCA phycosphere, attached bacteria in BCA are likely responsible for initial degradation of HMW to lower MW compounds, utilized by bacterial communities in MCA and SC. The varied organic carbon compounds within different-size particles in the cyanobacteria blooms resulted in differentiation of bacterial communities among BCA, MCA and SC.

Based on above analyses, we have proposed a conceptual model regarding roles for bacteria in using organic compounds in different-size aggregates within the phycosphere of cyanobacterial blooms. As illustrated (Fig. 5), HMW organic compounds in the phycosphere are degraded by bacterial communities from large-size aggregates resulting in small-size aggregate. Bacterial phyotypes for utilization single-carbon compounds, including *Pelagibacter* sp., *Methyllobacterium* sp., *Methylophilus* sp. and *Methylovorus* sp., are present in SC at higher than 2% concentration, indicated that at least a fraction of organic matter within the phycosphere is well utilized. Thus, bacteria within the phycosphere could efficiently provide nutrients and trace elements to the cyanobacteria through recycling the organic matter, which allow persistence of cyanobacterial blooms in freshwater lakes.

**Conclusion**

This study separated the phycosphere communities into three fractions and illustrated the complex and highly organized bacterial communities of the phycosphere within *Microcystis* blooms. Functional organization analysis suggested that bacterial composition was highly influenced by phycosphere conditions.

**Figure 4.** Relative abundance and bacterial composition obtained by pyrosequencing from phycosphere samples in September and October, by phylum. Phylogenetic classification for the pyrosequencing analysis obtained from Ribosomal Database Project Classifier analyses. doi:10.1371/journal.pone.0102879.g004
Table 3. The relative abundance of the predominant phylogenetic groups at the genus level.

| Taxa            | Genus       | 08BCA | 08MCA | 08SC | 09BCA | 09MCA | 09SC | 10BCA | 10MCA | 10SC |
|-----------------|-------------|-------|-------|------|-------|-------|------|-------|-------|------|
| Bacteroidetes   | Alkaliflexus| 13.16 | L     | L    | L     | L     | L    | 3.52  | L     | L    |
|                 | Chryseobacterium | 0.62  | L     | L    | L     | L     | L    | 2.26  | L     | L    |
|                 | Flavobacterium | 3.87  | 0.66  | 0.15 | 44.29 | 66.82 | 25.48| 33.06 | 21.08 | L    |
|                 | Sediminibacterium | L    | 0.34  | 0.43 | L     | L     | L    | 25.62 | L     | L    |
|                 | Solitalea    | 0.65  | 0.23  | L    | L     | 4.92  | 5.54 | L     | L     | L    |
| Alpha           | Azospirillum | L     | 15.85 | 1.80 | L     | 0.31  | L    | 5.50  | L     | L    |
|                 | Methylobacterium | L    | L     | L    | L     | L     | L    | 8.38  | L     | L    |
|                 | Pelagibacter | L     | 3.58  | 16.47| L     | L     | 0.65 | L     | 4.40  | L    |
|                 | Rhodocista   | L     | 0.19  | L    | 1.23  | L     | 0.33 | L     | 3.13  | L    |
|                 | Ralsononas   | 1.97  | 0.51  | 0.63 | 1.54  | 1.17  | 0.73 | 0.70  | 0.31  | L    |
| Beta            | Dechloromonas| 5.45  | L     | L    | L     | L     | L    | 0.73  | L     | L    |
|                 | Limnobacter  | L     | 0.50  | L    | 0.74  | 1.70  | L    | 1.12  | 3.37  | 0.64 |
|                 | Methylophilus| L     | 0.73  | 0.49 | L     | 0.51  | 2.13 | L     | 0.13  | 4.84 |
|                 | Methylovarus | L     | L     | L    | L     | 0.31  | 2.07 | L     | L     | L    |
|                 | Propionivibrio| 0.22 | L     | L    | L     | L     | L    | 5.05  | L     | L    |
|                 | Vorgesella   | L     | L     | L    | L     | 0.78  | 0.29 | L     | 0.97  | 6.92 |
| Gamma           | Aeromonas    | 0.54  | 2.67  | 1.01 | 0.56  | 1.26  | 1.92 | 4.98  | 12.53 | L    |
|                 | Legionella   | L     | L     | L    | L     | 0.52  | 1.87 | L     | 0.79  | L    |
|                 | Pseudomonas  | 0.17  | 30.52 | 15.42| 0.94  | 1.75  | 0.35 | 5.28  | 5.76  | 0.48 |
|                 | Rheinheimera| 0.18  | 0.34  | 0.37 | 0.18  | L     | L    | L     | 0.79  | 1.75 |
| Delta           | Bidellivibrio| L     | L     | L    | L     | 0.42  | L    | 0.51  | L     | L    |
|                 | Peledibacter | L     | L     | L    | L     | 0.21  | 0.47 | L     | L     | L    |
|                 | Gemmatimonadetes | 0.84 | 0.34  | L    | 4.06  | L     | L    | 0.97  | L     | L    |
|                 | Fiscutes     | 0.48  | 3.21  | L    | L     | 0.33  | L    | 2.12  | 3.56  | L    |
|                 | Verrucomicrobia | 0.65 | 0.23  | L    | L     | 4.92  | 5.54 | L     | L     | L    |

Relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%). Taxa represented occurred at >1% abundance in at least one sample. “L” represented low abundance (<0.1%).

doi:10.1371/journal.pone.0102879.t003
created during bloom formation, persistence and subsequent decomposition. The bacterial communities on large-to-small size aggregates were able to degrade high-to-low MW compounds, respectively. HMW organic compounds in the phycosphere were degraded in a stepwise manner by bacterial communities from large-size aggregates to small-size aggregates. With the coordinated utilization of complex organic matter, nutrients and trace elements are efficiently recycled within the phycosphere to facilitate the maintenance of cyanobacterial blooms in aquatic environments.

Author Contributions
Conceived and designed the experiments: HC HJ. Performed the experiments: HC. Analyzed the data: HC HJ LRK. Contributed reagents/materials/analysis tools: HC ZY LRK. Contributed to the writing of the manuscript: HC HJ.

References
1. Carey CC, Belingo BW, Hoffmann EP, Hamilton DP, Brooks J (2012) Ecophysiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Res 46: 1394–1407.
2. Turner AM, Chislock MF (2010) Blinded by the stink: nutrient enrichment impairs the perception of predation risk by freshwater snails. Ecol Appl 20: 2089–2095.
3. Wiegand C, Pfugmacher S (2005) Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicol Appl Pharm 203: 201–218.
4. Oliver RL, Gaido GG (2000) Freshwater blooms. In Whitton BA, Potts M, eds. The ecology of Cyanobacteria 149–194.
5. Jasti S, Sieracki ME, Poulton NJ, Giewat MW, Rooney-Varga JN (2005) Phylogenetic Diversity and Specificity of Bacteria Closely Associated with Alexandrium spp. and Other Phytoplankton. Appl Environ Microbiol 71:3483–3494.
6. Sapp M, Schwaderer AS, Wilshire KH, Hoppe HG, Gerds G, et al. (2007) Species-specific bacterial communities in the phycosphere of microalgae? Microb. Ecol 53: 683–699.
7. Worms J, Sondergaard M (1998) Dynamics of heterotrophic bacteria attached to Microcystis spp. (Cyanobacteria). Aquat Microb Ecol 14:19–28.
8. Havens KE. (2007) Cyanobacteria blooms: effects on aquatic ecosystems, vol. 619. In: Hudnell KH (ed) Cyanobacterial harmful algal blooms: state of the science and research. Springer, New York, pp 675–732.
9. Cai HY, Yan ZY, Wang AJ, Kraumbholz LR, Jiang HL. (2013) Analysis of the attached microbial community on mucilaginous cyanobacterial aggregates in the eutrophic Lake Taihu reveals the importance of Planctomycetes. Microb Ecol 66: 73–83.
10. Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6: 1228–1243.
11. Parveen B, Ravet V, Djediat C, Mary I, Quiblier C, et al. (2013) Bacterial communities associated with Microcystis colonies differ from free-living communities living in the same ecosystem. Environ Microbiol Rep 5: 716–724.
12. Pope PB, Patel BK (2008) Metagenomic analysis of a freshwater toxic cyanobacteria bloom. FEBS Microbiol Ecol 64: 9–27.
13. Shi L, Cai Y, Wang X, Kong FX, Yu Y (2012) Specific association between bacteria and buoyant Microcystis colonies compared with other bulk bacterial communities in the eutrophic Lake Taihu, China. Environ Microbiol Rep 4: 669–678.
14. Xu H, Cai H, Yu G, Jiang H (2013) Insights into extracellular polymeric substances of cyanobacterium Microcystis aeruginosa using fractionation procedure and parallel factor analysis. Water Res 47: 2005–2014.
15. Wang X, Sun M, Xie M, Liu M, Luo L, et al. (2013) Differences in microcystin production and genotype composition among Microcystis colonies of different sizes in Lake Taihu. Water Res 47: 5659–5669.
16. Li H, Xing P, Chen M, Bian Y, Wu QL. (2011) Short-term bacterial community composition dynamics in response to accumulation and breakdown of Microcystis blooms. Water Res 45: 1702–1710.
17. Margulies M, Egholm M, Altman W, Attiya S, Bader J, et al. (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437: 376–380.
18. Jin XC, Tu QY (1990) The standard methods for observation and analysis of lake eutrophication, 2nd edn. Beijing: China Environmental Science Press (in Chinese).
19. Asai R, Horiguchi Y, Yoshida A, McNiven S, Tahira P, et al. (2001) Detection of phycobilin pigments and their seasonal change in Lake Kasumigaura using a sensitive in situ fluorometric sensor. Anal Lett 34: 2521–2533.
20. Cai HY, Wang K, Huang SJ, Jiao NZ, Chen F (2010) Distinct Patterns of Picocyanobacterial Communities in Winter and Summer in the Chesapeake Bay. Appl Environ Microbiol 76: 2953–2960.
21. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase...
chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59: 695–700.

22. Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, et al. (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci USA 82: 4444–4448.

23. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335–336.

24. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537–7541.

25. Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10: 1571–1581.

26. Shen H, Niu Y, Xie P, Tao M, Yang X (2011) Morphological and physiological changes in Microcystis aeruginosa as a result of interactions with heterotrophic bacteria. Fresh Biol 56: 1065–1080.

27. Grossart HP, Levold F, Allgayer M, Simon M, Brinkhoff T (2005) Marine diatom species harbour distinct bacterial communities. Environ Microbiol 7: 860–873.

28. Riemann L, Winding A (2001) Community dynamics of free-living and particle-associated bacterial assemblages during freshwater phytoplankton bloom. Microb Ecol 42: 274–285.

29. Caiola MG, Pellegrini S (1984) Lysis of Microcystis aeruginosa (Kütz) by

30. Wu QL, Zwart G, Wu JF, Kamst-van Agterveld MP, Liu SJ, et al. (2007) Diversity of cultivable heterotrophic bacteria in association with cyanobacterial phycospheres (Gloeotrichia echinulata) and the identity of attached bacteria. Freshwater Biol 51:298–311.

31. Eiler A, Olsson JA, Bertilsson S (2006) Diurnal variation in the auto- and heterotrophic activity of cyanobacterial phycospheres (Gloeotrichia echinulata) and the identity of attached bacteria. Freshwater Biol 51:298–311.

32. Berg KA, Lyra C, Nienmi RM, Heins B, Hoppus K, et al. (2011) Virulence genes of Alcaligenes isolated from drinking water. Int J Syst Evol Microbiol 61: 2199–2206.

33. Achenbach LA, Michaelidou U, Bruce RA, Fryman J, Coates JD (2001) Dechlororomomas agitata gen. nov., sp. nov. and Dechlorosoma saiium gen. nov., sp. nov., two novel environmentally dominant (peri)chlorate-reducing bacteria and their phylogenetic position. Int J Syst Evol Microbiol 51: 527–533.

34. Qu FS, Liang H, Wang ZZ, Wang H, Yu HR, et al. (2012) Ultrafiltration membrane fouling by extracellular organic matters (EOM) of Microcystis aeruginosa in stationary phase: influences of interfacial characteristics of foulants and fouling mechanisms. Water Res 46:1490–1500.

35. Gallego V, Garcia MT, Ventosa A (2005) Methylobacterium sp. nov. and Methylobacterium aquaticum sp. nov., isolated from drinking water. Int J Syst Evol Microbiol 55: 281–287.

36. Jenkins O, Byrom D, Jones D (1987) Methylphilus: a new genus of methanol-utilizing bacteria. Int J Syst Bacteriol 37:335–336.

37. McCartney J, Becker JW, Reppa DJ, Shi YM, Young CR, et al. (2010) Microbial community transcription profiles reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci USA 107: 16420–16427.

38. Berendt RF (1981) Influence of blue-green algae (cyanobacteria) on survival of Legionella pneumophila in aerosols. Infect Immun 32:690–692.

39. Salcher MM, Prenzhaler J, Posch T (2011) Seasonal bloom dynamics and ecophysiology of the freshwater super clade of SAR11 bacteria ‘that rule the waves’ (LD12). ISME J 5: 1242–1252.

40. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335–336.

41. Madigan MT, Martinko JM, Parker J (2003) Brock Biology of Microorganisms. Prentice Hall, Pearson Education Inc., New York.

42. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey’s Manual of Determinative Bacteriology. Williams & Wilkins, Baltimore, MD.

43. Salcher MM, Prenzhaler J, Posch T (2011) Seasonal bloom dynamics and ecophysiology of the freshwater super clade of SAR11 bacteria ‘that rule the waves’ (LD12). ISME J 5: 1242–1252.

44. Gallego V, Garcia MT, Ventosa A (2005) Methylobacterium sp. nov. and Methylobacterium aquaticum sp. nov., isolated from drinking water. Int J Syst Evol Microbiol 55: 281–287.

45. Coman C, Bica A, Deica B, Barbulescu D, Drago N (2011) Methodological constraints in the molecular biodiversity study of a thermomineral spring cyanobacterial mat: a case study. Antonie van Leeuwenhoek 99: 271–281.

46. Detkovski EN, Zachelka MM, Kostov VN (2009) Physiology and biochemistry of alkaliophilic anaerobic hydrolytic bacterium Alkaliflexus uncinatus. Microbiology 78: 310–316.

47. Brune A, Ludwig W, Schink B (2002) Propionivibrio limicola sp. nov., a fermentative bacterium specialized in the degradation of hydroaromatic compounds, reclassification of Propionibacter pelophilus as Propionivibrio pelophilus comb. nov. and amended description of the genus Propionivibrio. Int J Syst Evol Microbiol 52: 441–444.

48. Kwon YS, Kim JS, Kim SU, Kang HJ, Park JH, et al. (2011) Virulence genes of Aeromonas isolates, bacterial endotoxins and cyanobacterial toxins from recreational water samples associated with human health symptoms. J Water Health 9: 670–679.

49. Jenkins O, Byrom D, Jones D (1987) Methylphilus: a new genus of methanol-utilizing bacteria. Int J Syst Bacteriol 37:436–440.

50. Qiu FS, Liang H, Wang ZZ, Wang H, Yu HR, et al. (2012) Ultrafiltration membrane fouling by extracellular organic matters (EOM) of Microcystis aeruginosa in stationary phase: influences of interfacial characteristics of foulants and fouling mechanisms. Water Res 46:1490–1500.

51. Nelsen CE, Carlson CA (2012) Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. Environ Microbiol 14: 1500–1516.

52. McCarren J, Becker JW, Reppa DJ, Shi YM, Young CR, et al. (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci USA 107: 16420–16427.