Annals of Microbiology (2018) 68:493–503
https://doi.org/10.1007/s13213-018-1354-y

ORIGINAL ARTICLE

Composition and diversity of cyanobacteria-associated and free-living bacterial communities during cyanobacterial blooms

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Received: 11 December 2017 / Accepted: 28 June 2018 / Published online: 6 July 2018
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Abstract

Lakes undergoing cyanobacterial blooms often exhibit differences between free-living (FL) and cyanobacteria-associated (CA) bacterial assemblages, but previous studies have not compared distinct FL and CA communities across multiple lakes. This project investigated whether FL and CA communities differ from each other in consistent ways across lakes. FL and CA communities were collected from three Ohio (USA) lakes on two sampling dates during cyanobacterial blooms. High-throughput sequencing was used to characterize the communities, and comparisons were made of the composition and diversity of FL and CA communities within and across lakes. Diversity estimates did not vary significantly among lakes nor between CA and FL assemblages. The taxonomic composition of CA communities differed significantly from that of FL communities in Buckeye and Harsha Lakes and in Maumee Bay on one of two sampling dates. CA communities from Buckeye and Harsha Lakes were more similar to each other than to their respective FL communities. Community composition in Maumee Bay on August 18 did not differ between FL and CA habitats. As the bloom progressed, the FL community remained similar in composition to samples collected on August 18, while the CA community became significantly dissimilar. This study is the first cross-lake comparisons of CA and FL communities, uncovering the impacts of habitat type, lake, and sampling date in determining community composition.

Keywords Cyanobacterial bloom · Community composition · Free-living bacteria · Cyanobacteria-associated bacteria

Introduction

Cyanobacterial harmful algal blooms (CyanoHABs) are common in growing numbers of freshwater ecosystems around the world due to climate change and nutrient loading (Paerl 1996; Paerl and Paul 2012). Typically, bloom-forming cyanobacteria occur as colonies embedded in mucilaginous matrices or as filaments within mucilaginous sheaths. The surfaces of cyanobacteria and their surrounding mucilage form microenvironments that make up the phycosphere, a distinctive habitat which supports heterotrophic bacterial communities that generally differ from the surrounding bacterioplankton (Li et al. 2011; Louati et al. 2015; Niu et al. 2011; Parveen et al. 2013a, b; Shi et al. 2012). These microenvironments are protected from physico-chemical fluctuations in the water column (Paerl 1996) and are rich in organic compounds, including polysaccharides (Parikh and Madamwar 2006; Pereira et al. 2009; Plude et al. 1991; Xu et al. 2013) and oligopeptides (such as microcystins and nodularins) that can be used as carbon sources by some bacteria (Imanishi et al. 2005; Jones et al. 1994; Maruyama et al. 2003).

Previous studies of the composition and diversity of heterotrophic bacteria living on mucilaginous cyanobacteria have focused on the differences between cyanobacteria-associated (CA) communities and free-living (FL) bacterioplankton communities within a single lake rather than comparing CA bacterial assemblages from different lakes (Louati et al. 2015; Niu

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-018-1354-y) contains supplementary material, which is available to authorized users.

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et al. 2011; Parveen et al. 2013a; Shi et al. 2012). Comparisons across multiple lakes are necessary to understand whether differences between FL and CA bacterial communities are attributable to innate selective properties imposed by the cyanobacteria-microhabitat. Alternatively, such differences may result from stochastic community assembly in combination with differences among lakes in physicochemical conditions and pools of potential bacterial colonizers. Ultimately, such information will reveal whether or not CA communities share common characteristics that could potentially serve as predictive or management tools.

Composition of bacterial communities associated with mucilaginous cyanobacteria varies but often includes *Bacteroidetes* and *Actinobacteria* (Cai et al. 2014; Li et al. 2011; Louati et al. 2015; Niu et al. 2011; Parveen et al. 2013a; Shen et al. 2011; Shi et al. 2012). *Actinobacteria* tend to be predominantly free-living cells incidentally co-occurring with cyanobacteria (Louati et al. 2015; Parveen et al. 2013b), although some members of this phylum live within the mucilage (Zhang et al. 2016). *Bacteroidetes* are found embedded in the mucilage (Parveen et al. 2013b) or associated with surfaces of non-mucilaginous cells (Velichko et al. 2015). *Gammaproteobacteria*, uncommonly found as free-living organisms in freshwater (Niu et al. 2011; Parveen et al. 2013a; Shi et al. 2012), are often abundant in communities attached to cyanobacteria (Parveen et al. 2013a; Velichko et al. 2015). *Betaproteobacteria* are typically well-represented in free-living bacterial communities in freshwater lakes and have even higher abundances in communities associated with mucilaginous cyanobacteria (Louati et al. 2015; Parveen et al. 2013a). While taxonomically coarse comparisons can be made among CA bacterial communities across studies, whether or not there is a characteristic CA bacterial community is unknown.

In this study, the composition and diversity of CA and FL bacterial communities from three temperate lakes (OH, USA) during toxic cyanobacterial blooms were investigated. We hypothesized that the protected and resource-rich microenvironments associated with mucilaginous cyanobacteria would select for a subset of the bacteria available in the water column. Thus, we anticipated that CA bacterial communities would be compositionally distinct from FL bacterial communities within the same lakes and would have lower alpha diversity than FL communities as suggested by prior studies (Li et al. 2011; Niu et al. 2011; Parveen et al. 2013a, b; Shi et al. 2012). Furthermore, we anticipated that the conditions of CA microhabitats would select for a consistent subset of taxonomic groups from among the pool of potential colonizers. Therefore, we hypothesized that CA communities from different lakes would be more similar in composition to each other than to FL communities from the same lakes and that CA communities would exhibit a greater degree of cross-lake similarity than would FL communities.

Methods

Study sites

In the summer of 2014, three lakes were examined based on the occurrence of cyanoHABs as reported by the Ohio Environmental Protection Agency (2014) and the presence of dense, visible green surface scum. High cyanobacterial cell counts, cell biovolumes, and gene sequence abundances in these lakes were also reported by Francy et al. (2015). In recent years, the selected lakes have all developed annually recurring toxic cyanobacterial blooms which typically persist throughout the summer and into early autumn. The lakes were located in northeast, central, and southern Ohio (Fig. 1). Buckeye Lake (39.93° N, 82.48° W; mean depth 2.5 m, maximum depth 7 m, surface area 11.6 km^2) is a reservoir in central OH (Francy et al. 2016). For most of the year, the reservoir is fed by a small watershed (~ 70 km^2) with 60% agricultural, 14% forest, and 15% urban land use (Francy et al. 2016; Taylor and Governor 2012). At times of high precipitation, it receives overflow from the headwaters of South Fork Licking (Taylor and Governor 2012) and runoff from additional areas, draining a total of 127 km^2 of predominately agricultural land (Francy et al. 2016; Taylor and Governor 2012).

William Harsha Lake (39.02° N, 84.11° W, mean depth 12.9 m, maximum depth 30 m, surface area 8 km^2), formerly known as East Fork Lake, is a monomictic reservoir in southern OH (Beaulieu et al. 2014; Francy et al. 2016). Constructed on the East Fork of the Little Miami River, it drains a watershed of about 886 km^2. Land use is 64% agricultural and 27% forest, with the rest lightly urbanized (Beaulieu et al. 2014; Francy et al. 2016).

Maumee Bay (41.68° N, 83.38° W, mean depth < 3 m, maximum depth ~ 3 m except for a dredged shipping channel of 8.5 m, surface area 70 km^2) is a shallow embayment on the southwestern shore of Lake Erie (mean depth 7.4 m, max depth 19 m, surface area 19,830 km^2). The 2014 HAB in Maumee Bay was part of a larger bloom in which cyanobacterial surface scum, consisting predominately of *Microcystis* spp., covered much of the lake's western basin (3284 km^2). The Maumee River drains a watershed of 16,388 km^2, of which 73.3% is agricultural land and 10.6% is urban, including the city of Toledo, OH (Baker et al. 2014; Moorhead et al. 2008). The river discharges directly into Maumee Bay from the southwest (Francy et al. 2015; Michalak et al. 2013, Moorhead et al. 2008). To the northeast, the bay opens onto the western basin, but water flow patterns permit little mixing within Maumee Bay, leaving the Maumee River as the primary conduit of water, dissolved nutrients, and suspended sediment into the bay.

Fig. 1 Locations of three eutrophic lakes sampled during cyanobacterial blooms in 2014

Sample collection and processing

Three replicate 1-l water samples were collected from the top 5 cm of each lake in 2014. Each lake was sampled once in the period from July to August, when the cyanobacterial bloom season is at its height in temperate North America due to high temperatures and strong thermal stratification of lakes, and again in September, when cyanobacterial blooms in the region are generally on the decline. Buckeye Lake was sampled on July 24 and September 19, William Harsha Lake was sampled on July 17 and September 2, and Maumee Bay was sampled on August 18 and September 28. At the time of sampling, temperature, conductivity, and dissolved oxygen were measured with a HQ40d multiprobe (Hach, Loveland, CO, USA). Samples were transported on ice to the lab, where they were first filtered through 3-μm nitrocellulose membranes (Millipore, Darmstadt, Germany) under vacuum to collect cyanobacteria-associated bacteria (CA) associated with larger sized cyanobacteria cell surfaces or embedded in cyanobacterial mucilage, and then through 0.2-μm polycarbonate membranes (Millipore, Darmstadt, Germany) (modified from Li et al. 2011 by using filters with a smaller pore size) to collect small sized free-living bacterial (FL) fractions. Membranes were stored at − 80 °C until DNA extraction.

Inorganic nitrogen was measured with a Synergy 2 plate reader (BioTek, Winooski, VT, USA) following the indophenol blue method for ammonium and the sulfanilamide method for nitrate/nitrite as adapted for microplates (Ringuet et al. 2011). Soluble reactive phosphorus (SRP) was assayed by the ascorbic acid method (Murphy and Riley 1962), and absorbance measured with a DU 730 UV/visible spectrophotometer (Beckman Coulter, Brea, CA, USA). Nutrient data were tested for normality with the Shapiro-Wilkes test and the Kruskal-Wallis test was used to check for significant differences in the event of non-normal distribution. Statistical analyses were carried out in JMP (SAS, Inc., Cary, NC, USA).

Bacterial community analysis

DNA was extracted from filters using the Power Soil DNA extraction kit (MoBio, Carlsbad, CA, USA) according to manufacturer's protocol. The presence of 16S rRNA genes was confirmed and samples were subsequently submitted for high-

throughput 2 × 300 bp paired-end sequencing of the V4-V5 hypervariable region (Sun et al. 2013) using an Illumina MiSeq Series System (Illumina Inc., San Diego, CA, USA) at the Ohio State University Molecular and Cellular Imaging Center (Wooster, OH, USA).

Following sequencing, paired reads were assembled into iTags (Degnan and Ochman 2012). iTags were sorted by length, filtered for chimeras, and quality filtered in the pick_open_reference workflow with usearch61_ref as the operational taxonomic unit (OTU) picking and classification method in QIIME Version 1.9.1 (Caporaso et al. 2010). OTU clustering was performed at the 97% similarity level and taxonomy assigned based on partial 16S rRNA sequences in the 16S rRNA SSU_Ref_NR_99_128.1 reference database (SILVA_SSU_128.1, Release date, September 29, 2016) (Caporaso et al. 2010). A total of 1,564,141 "iTags" and 44,296 OTUs were obtained. Two samples with low OTU counts, Maumee Bay Aug. 18 FL (33 iTags) and William Harsha Lake Sept. 2 FL (52 iTags), were excluded from subsequent analysis. Singletons, OTUs unassigned at the basal level (D1), and OTUs assigned to Archaea, mitochondria, chloroplast, and cyanobacteria lineages were removed from all samples in the resulting OTU table, yielding 968,520 iTags with 19,306 OTUs. The final filtered OTU table was then summarized to 445 bacterial phylotypes at the family level.

Species richness (alpha diversity) across samples was assessed using Shannon diversity, Simpson's index, and the unique OTU count (observed_species metric in QIIME), following rarefaction of the family-level OTU table to 6940 iTags per sample. Samples were sorted into 12 a priori FL and CA groups representing bacterial communities collected from the three lakes on two sampling dates per lake. Differences in Shannon diversity, Simpson's index, and OTU richness were evaluated using the Wilcoxon non-parametric test. To estimate beta diversity, a Bray-Curtis distance matrix was generated using the rarefied family-level table (Bray and Curtis 1957; Anderson et al. 2006). A non-metric multidimensional scaling (NMDS) plot was generated to visualize dissimilarity in community composition among samples following NMDS analysis on the distance matrix (Kruskal 1964). Differences in community composition among samples were evaluated using the Multivariate Response Permutation Procedure (MRPP) (Mielke 1984) on the distance matrix via the "compare_categories.py" command in QIIME with 1000 permutations.

An additional test of dissimilarity in microbial composition among samples (permutational multivariate analysis of variance, PERMANOVA) was performed (Anderson et al. 2006). An underlying assumption of PERMANOVA is that all groups are made up of replicates which exhibit the same level of dispersion around their group centroids (Anderson and Walsh 2013). Homogeneity of within-group dispersions was assessed using PERMDISP (Anderson and Walsh 2013), and a PERMANOVA-based F value calculated from average distances among groups relative to average distances within groups for actual and permutated data. A pseudo-F statistic then tested the likelihood that permutated F values were larger than the observed F value. Finally, differences in abundance values for each OTU among sampling groups and among *a posterior* clusters were examined using the group_significance.py command followed by the default non-parametric Kruskal-Wallis test of significance in QIIME (Caporaso et al. 2010). The P values were adjusted using the False Discovery Rate (FDR) approach.

Results

Sequences of cyanobacterial taxa dominated the 3-μm pore size fraction of each sample. The bloom in Buckeye Lake consisted almost entirely of *Planktothrix* on both sampling dates. Maumee Bay was strongly *Microcystis*-dominated on August 18, but bloom composition shifted to a mix of *Microcystis* and *Dolichospermum* by September 24. William Harsha Lake had the most diverse cyanobacterial assemblage, including *Cylindrospermopsis*, *Dolichospermum*, *Synechococcus*, and *Microcystis*, although *Microcystis* became much less abundant in September than in July.

In contrast to our prediction, there were no significant differences in bacterial diversity (cyanobacteria excluded) between CA and FL communities across the three lakes (OTU richness, Wilcoxon chi-square = 11.6, df = 11, $P = 0.40$; Shannon's index, Wilcoxon chi-square = 9.2, df = 11, $P = 0.60$; Simpson's index, Wilcoxon chi-square = 10.2, df = 11, $P = 0.52$), although FL community diversity indices were generally larger than CA indices (Fig. 2). The CA community of Maumee Bay became slightly less diverse over time, with fewer unique OTUs detected in September than in August (Fig. 2a), but the change was statistically non-significant.

Community composition differed significantly among the 12 sampling groups representing three lakes, two sampling dates, and two community types ([FL and CA]; MRPP, within-group agreement, effect size, $A = 0.67$, observed delta = 0.18, and expected delta = 0.54, $P = 0.001$, 1000 permutations; PERMANOVA, Pseudo-F = 21.5, $P = 0.001$, 1000 permutations). The sampling groups fell into four clusters, with cluster 1 made up of CA communities from Buckeye and Harsha Lakes and cluster 2 composed of FL communities from the same two lakes (Fig. 3). Both CA and FL communities sampled from Maumee Bay in August were represented by cluster 3, along with the FL community from Maumee Bay sampled in September. Cluster 4 consisted of only the CA community from the September sampling date in Maumee Bay. The four clusters differed significantly in community composition (MRPP, within-group agreement, effect size, $A = 0.35$, observed delta = 0.35, and expected delta = 0.54,

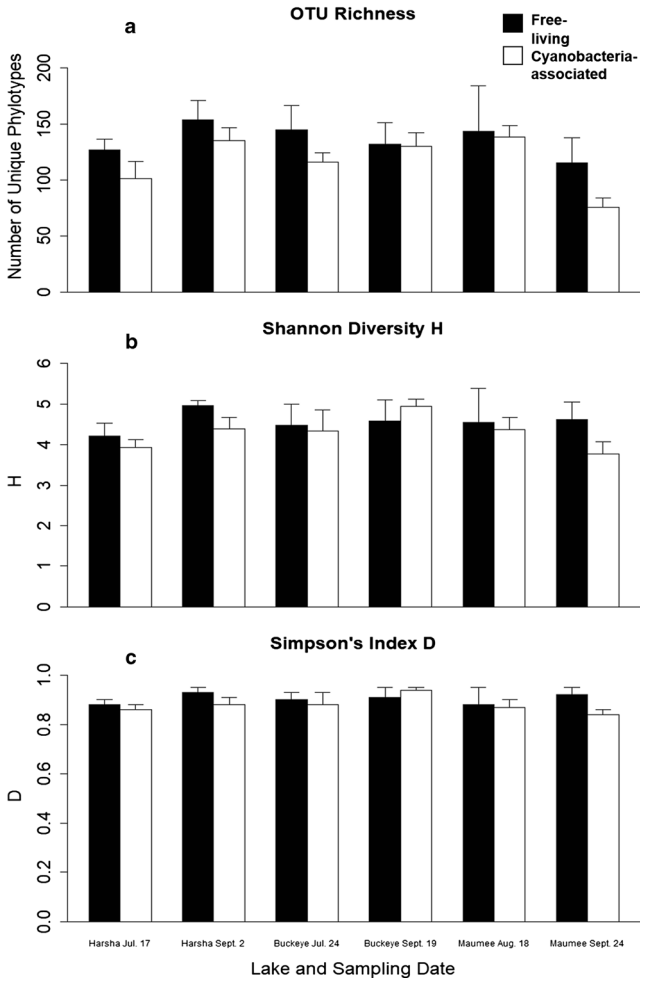

Fig. 2 Bar plots of average values, with standard error, of **a** OTU richness, **b** Shannon diversity, and **c** Simpson's Index for free-living (black bars) and cyanobacteria-associated (white bars) bacterial communities from William Harsha Lake, Buckeye Lake, and Maumee Bay

$P = 0.001$, 1000 permutations), confirming differences between FL and CA habitat types and similarities among samples within habitat types for two of three lakes.

Out of 445 family-level bacterial taxa, 178 differed significantly in abundance among sampling groups (Table S1), including 80 Proteobacteria taxa (Fig. 4) and 98 other taxa (Fig. 5), and 128 family-level groups differed among the four clusters in Fig. 3 at $\alpha < 0.05$ (Table S2). With the exception of the CA community collected from Maumee Bay in August, CA communities were separated from FL communities along the first axis of the NMDS plot (Fig. 3). Multiple taxa were abundant in clusters 2 and 3, which consisted entirely or mostly of FL samples, that were relatively depleted in clusters 1 and 4, made up exclusively of CA communities. The greatest difference was in the abundance of *Sporichthyaceae* (*Actinobacteria*), a family more than ten times as abundant in the FL-dominated clusters than in the CA-only clusters (Table S2). Most other taxa that were more abundant in the FL clusters were *Alphaproteobacteria* or *Betaproteobacteria* (Table S2). However, unassigned *Acidimicrobiales* (*Actinobacteria*), *Sphingobacteriaceae* (*Bacteroidetes*), and *Leptospiraceae* (*Spirochaetes*) were also found in the FL-dominated clusters at abundances that were, while low, still significantly greater than their abundances in clusters 1 and 4 (Table S2). No taxa that were found to be abundant in clusters 1 and 4 were significantly less abundant in clusters 2 and 3.

Communities in Maumee Bay separated from communities in the two other lakes along the second NMDS axis (Fig. 3). Clusters 1 and 2 were enriched in *Chthoniobacterales* (*Verrucomicrobia*), unclassified *Verrucomicrobia*, *Planctomycetes* families *Phycisphaerae* and *Planctomycetaceae*, *Rickettsiaceae* (*Alphaproteobacteria*), *Oceanospirillaceae* (*Gammaproteobacteria*), and several families belonging to the *Deltaproteobacteria* orders *Bdellovibrionales* and *Oligoflexales*, whereas these taxa were much less common in clusters 3 and 4 (Table S2). Other *Gammaproteobacteria* families were rare overall but significantly more abundant in the Buckeye-Harsha clusters than in the Maumee Bay clusters (Table S2). In contrast, Maumee Bay clusters were relatively enriched in *Caulobacteraceae* and *Hyphomonadaceae* (*Alphaproteobacteria*), *Nitrosomonadaceae* (*Betaproteobacteria*), and *Chromatiaceae* (*Gammaproteobacteria*), while unassigned *Rickettsiales* (*Alphaproteobacteria*) sequences were rare but most abundant in Maumee Bay (Table S2).

The abundance of unclassified *Verrucomicrobia* was much greater in cluster 1 than in cluster 2. Cluster 1 was further separated from all other clusters by high abundances of *Blastocatellaceae* (*Acidobacteria*), group OPB35 (*Verrucomicrobia*), *Flavobacteriales* NS9 marine group (*Bacteroidetes*), and several members of the order *Sphingobacterales* (*Bacteroidetes*), especially *Saprospiraceae* and group env. OPS 17 (Table S2). Cluster 1 also had significantly more *Chloroflexi* than other clusters, especially *Caldilinaceae* and unassigned *Chloroflexi*, but also the relatively rare *Anaerolineaceae* (Table S2). Two families within the *Bacillales* (*Firmicutes*), *Bacillaceae* and *Paenibacillaceae*, also contributed to the separation of cluster 1, as did an unassigned *Planctomycetes* group OM190. This cluster was further distinguished by high abundances of a wide variety of rare taxa, including *Fusobacteriaceae* (*Fusobacteria*), *Ignavibacteriaceae* (*Ignavibacteriae*), unassigned *Hydrogenedentes*, *Rhizobiales* group A0839 (*Alphaproteobacteria*), unclassified *Proteobacteria*, and many *Deltaproteobacteria* such as unassigned *Bradymonadales*, various members of the order *Myxococcales*, and clade Sva0485 (Table S2).

Phycisphaerae and *Planctomycetaceae* were highly enriched in cluster 2 but less enriched in cluster 1 and relatively depleted in clusters 3 and 4 (Table S2). Cluster 2 communities were also rich in unassigned *Acidobacteria* and

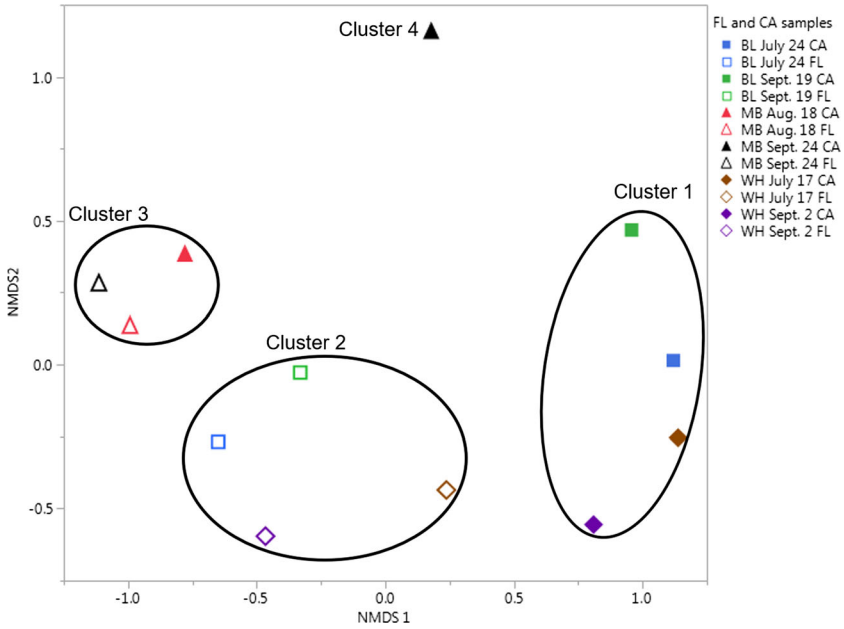

Fig. 3 NMDS plot showing compositional similarity of bacterial communities in free-living (FL) and cyanobacteria-associated (CA) samples collected from Buckeye Lake (BL), William Harsha Lake (WH), and Maumee Bay (MB) based on mean NMDS scores (NMDS stress = 0.07) (MRPP, within-group agreement, effect size, $A = 0.67$, observed delta = 0.18 and expected delta = 0.54, $P = 0.001$, 1000 permutations). Samples close to each other are more similar in composition than those farther apart. Four a posteriori clusters were established based on proximity of samples to each other in the NMDS plot. Cluster 1 is composed of the CA communities from William Harsha Lake (brown and purple filled diamonds for July and September samples, respectively) and Buckeye Lake (blue and green filled squares for July and September samples, respectively). Cluster 2 is made up of the July and September FL communities from William Harsha Lake (brown and purple open diamonds, respectively) and Buckeye Lake (blue and green open squares, respectively). Cluster 3 is composed of August and September FL communities from Maumee Bay (red and black open triangles) and the August CA community (red filled triangle) from Maumee Bay. Cluster 4 represents the September CA community (black filled triangle) from Maumee Bay

several members of the *Actinobacteria*, including the highly abundant *Acidimicrobiaceae* (*Acidimicrobiales*), other *Acidimicrobiales*, *Mycobacteriaceae* (*Corynebacteriales*), and multiple family-level groups within clade PeM15 and class *Thermoleophilia* (Table S2). Two *Gammaproteobacteria* taxa, the *Legionellaceae* family and an unassigned *Xanthomonadales* group, were significantly more abundant in this cluster than elsewhere, as were the *Delatproteobacteria* groups *Bacteriovoraceae* and *Oligoflexales* 0319-6G20, plus a group of bacterial sequences that could not be assigned to any known phylum (Table S2). Rare phylotypes found at their highest abundance in this cluster fell into unassigned groups within *Acidobacteria*, *Actinobacteria*, *Omnitrophica*, *Alphaproteobacteria*, and *Gammaproteobacteria*.

Microbacteriaceae (*Actinobacteria*), *Cyclobacteriaceae* (*Bacteroidetes*), *Chitinophagaceae* (*Bacteroidetes*), *Rhodobacteraceae* (*Alphaproteobacteria*), *Xanthomonadaceae* (*Gammaproteobacteria*), and unassigned *Opitutae* (*Verrucomicrobia*) were all abundant in cluster 3 and significantly less abundant in other clusters (Table S2). The SL56 marine group (*Chloroflexi*) and two family-level groups of *Rhizobiales* (*Alphaproteobacteria*) were moderately abundant in cluster 3 but rare elsewhere (Table S2). *Demequinaceae* and unassigned *Micrococcales*, both belonging to the same order as *Microbacteriaceae*, were rare in all clusters but significantly less rare in cluster 3 (Table S2). Another rare taxon, *Candidatus Azambacteria* (*Parcubacteria*), was also found mostly in this cluster (Table S2).

Few family-level groups were significantly more abundant in cluster 4 than any other cluster. They included the moderately abundant *Cytophagaceae* (*Bacteroidetes*), *Parachlamydiaceae* (*Chlamydiae*), *Nitrosomonadaceae* (*Betaproteobacteria*), and *Chromatiaceae* (*Gammaproteobacteria*) (Table S2). Among the rare taxa, an unassigned *Bacteroidetes* group, *Rhizobiales* group DUNssu044, and *Desulfomonadales* C8S-102 (*Deltaproteobacteria*) were detected mainly in cluster 4 (Table S2).

The lakes exhibited variation in temperature, DO, conductivity, and Secchi depth (Table 1). Inorganic P concentrations were similar across all lakes, below 5 μg/L except for a single sample collected from Buckeye Lake. Ammonium and nitrate/nitrite were below detection limits in all samples.

Discussion

In William Harsha Lake and Buckeye Lake, cyanobacteria-associated communities were compositionally distinct from free-living communities but not significantly different in diversity. The CA communities from these two different lakes

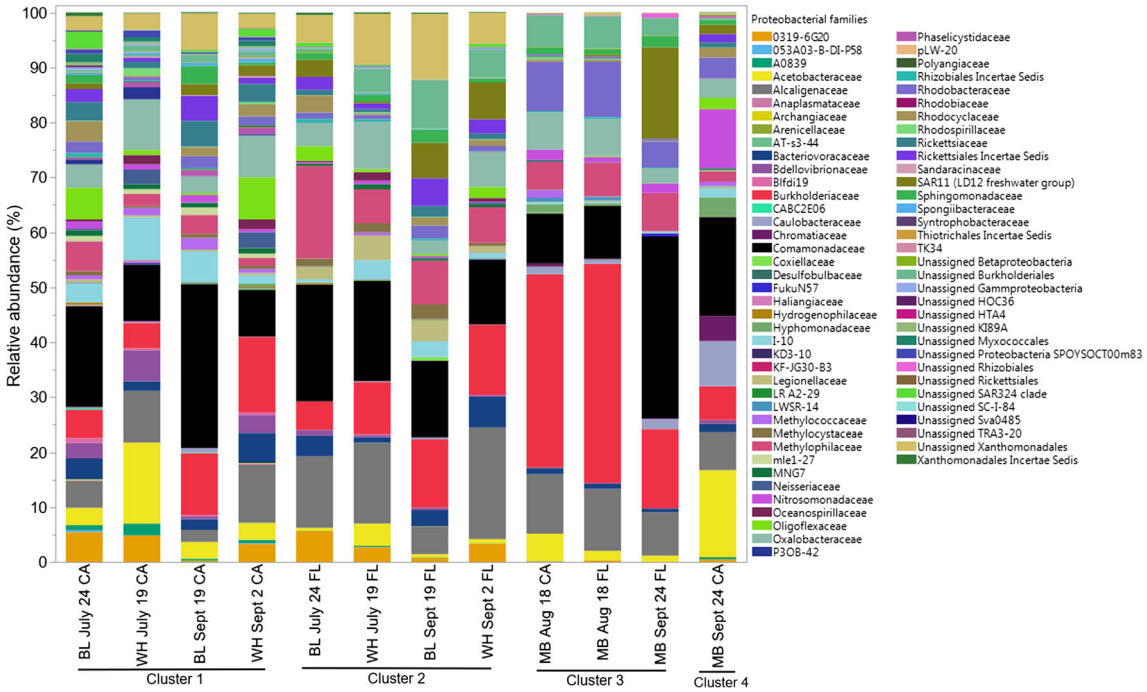

Fig. 4 Relative abundances (%) of the 80 *Proteobacteria* families and family-level groups that differed significantly ($\alpha < 0.05$) among the 12 sampling groups of free-living (FL) or cyanobacteria-associated (CA) communities collected from Buckeye Lake (BL), William Harsha Lake (WH), or Maumee Bay (MB)

clustered together in NMDS analysis, while the FL communities from the same lakes fell outside the cluster, indicating that CA communities were more similar to each other than to their respective FL communities. Although the communities underwent some turnover between July and September, the separation between CA and FL communities within lakes and the clustering of CA communities across lakes remained consistent over time. The FL communities from the same pair of lakes also fell into a single cluster. These results support the hypothesis that cyanobacteria would provide a microhabitat

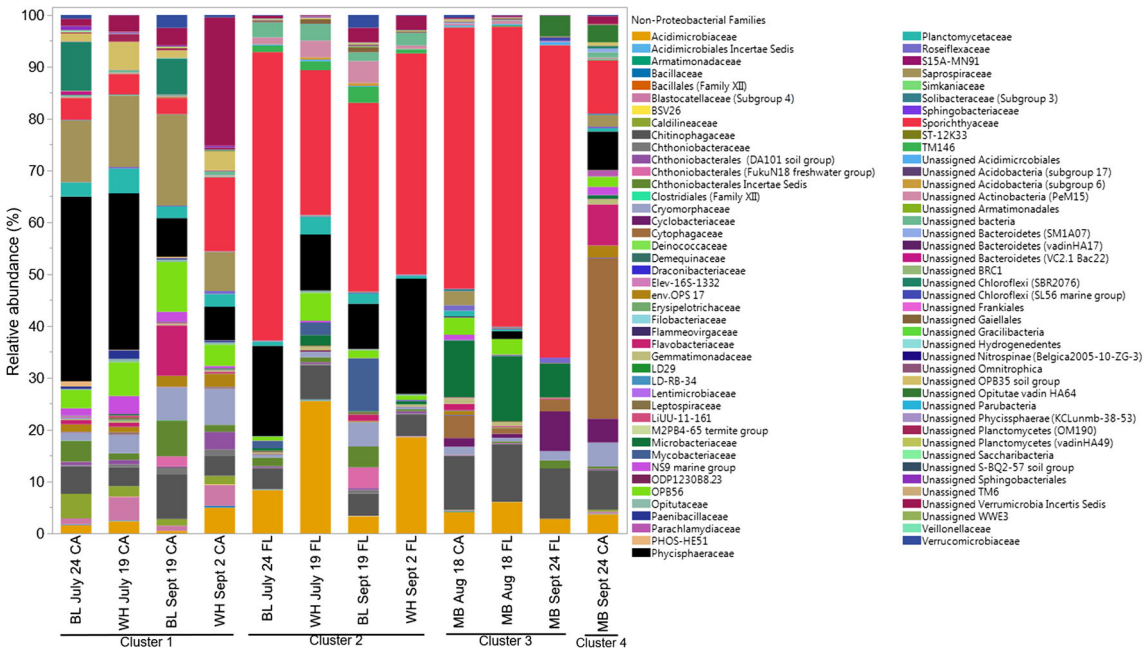

Fig. 5 Relative abundances (%) of the 98 families and family-level groups outside the *Proteobacteria* phylum that differed significantly ($\alpha < 0.05$) among the 12 sampling groups of free-living (FL) or cyanobacteria-associated (CA) communities collected from Buckeye Lake (BL), William Harsha Lake (WH), or Maumee Bay (MB)

Table 1 Physical and chemical data collected for all sampling locations and dates

Lake	Sampling date	Temperature (°C)	Dissolved oxygen (mg/L)	Conductivity (μS/cm)	Secchi depth (m)	Soluble reactive phosphorus (mg/L) Mean (SE)
William Harsha Lake	July 17	27.3	11.53	235	0.75	1.3 (0.31)
	September 2	26.7	6.93	242	1.0	1.06 (0.20)
Buckeye Lake	July 24	25.8	7.52	270	0.25	1.65 (0.47)
	September 19	19.7	7.48	290	0.5	3.78 (3.78)
Maumee Bay	August 18	24.1	13.11	408	0.25	1.42 (0.54)
	September 24	23.0	13.18	374	0.5	1.77 (0.35)

that selected for a different set of bacteria than those dominant in the water column. They did not, however, support the hypothesis that bacterial communities selected by the CA habitat type would exhibit lower alpha diversity within lakes or less compositional variation between lakes than FL communities. Furthermore, the dissimilarity between the CA community in Maumee Bay and other CA communities was equal to or greater than the dissimilarity between the FL community in Maumee Bay and other FL communities. Previous studies revealed compositional differences between CA and FL communities within lakes (Li et al. 2011; Louati et al. 2015; Niu et al. 2011; Parveen et al. 2013a, b; Shi et al. 2012) or differences among homogenized bacterial communities sampled from multiple lakes (Eiler and Bertilsson 2004), but this study is the first, to our knowledge, to distinguish between CA and FL communities and also compare them across multiple lakes.

Maumee Bay had highly similar FL and CA communities in August, unlike other lakes in this study and in prior studies (Li et al. 2011; Louati et al. 2015; Niu et al. 2011; Parveen et al. 2013a, b; Shi et al. 2012). The similarity of the FL and CA communities in this instance might be due to the relatively early stage of the cyanobacterial bloom. The initial sampling of each lake in this study occurred after the cyanobacterial bloom had developed thick green surface scums. This appearance coincided with the cyanobacterial biovolume of the bloom reaching a density of 1×10^7 μm^3/mL, as reported in Francy et al. (2015). The Maumee Bay cyanoHAB of 2014 reached that density at the beginning of August, whereas the blooms in Harsha Lake and Buckeye Lake had already met or exceeded that density more than a month earlier (Francy et al. 2015). It is possible that significant dissimilarities between communities of free-living and cyanobacteria-associated bacteria in other lakes were the result of processes that occurred over the course of several weeks. Thus, the CA community in Maumee Bay may have had less time to become distinct from its surrounding FL community, compared with the other CA communities in this study. By the next sampling date in September, the CA and FL communities in Maumee Bay were quite distinct, the CA community forming its own cluster while the FL community remained similar in composition to the samples collected in August. The compositional changes that made this CA community different from the other Maumee Bay communities in cluster 3 also increased its distance in the NMDS plot from the FL communities of cluster 2 and brought it closer to the CA communities of cluster 1.

Differences among lakes in physicochemical conditions presumably contributed to differences among bacterial communities. In a number of ways, such as the predominantly agricultural land use of the surrounding watersheds and the depletion of inorganic nutrients during the cyanobacterial blooms, the lakes are similar. However, other factors such as temperature, size, and conductivity differed. During the sampling period of this study, the range of water temperatures recorded in Maumee Bay was not different from that in Buckeye Lake (Table 1). However, throughout the entire summer of 2014, temperatures tended to be slightly cooler in Maumee Bay than in the other lakes (Francy et al. 2015). Lower temperatures early in the season may have slowed the development of the cyanoHAB in Maumee Bay and the differentiation of the CA community from the FL community. Another potential contributing factor is the large surface area of Maumee Bay. The cyanoHABs in the two reservoirs may have reached high biovolume densities earlier because their surface waters covered roughly one-seventh of the area of Maumee Bay. Additionally, Maumee Bay had by far the highest conductivity readings and the greatest range of conductivity readings (Table 1). Over the course of the season, the conductivity in Maumee Bay exhibited an even greater range, with a maximum of 727 μS/cm, more than double the maximum values for the other lakes (Francy et al. 2015). Although the range of variation in conductivity for these freshwater lakes is small compared to brackish waters, differences in conductivity within the range of 22–1399 μS/cm have significant effects on the structure of bacterial communities in streams (Lear et al. 2009). Dissolved oxygen was elevated in Maumee Bay, especially relative to Buckeye Lake (Table 1), but the literature shows that this difference between lakes did not persist throughout the summer (Francy et al. 2015). It is unlikely that the separation of clusters in Fig. 2 was dependent upon differences in DO. When Harsha Lake was sampled on

July 17, the DO reading was closer to the values for Maumee Bay than to those for Buckeye Lake, yet the bacterial communities, both CA and FL, clustered with the Buckeye Lake communities rather than the Maumee Bay communities (Fig. 3).

Because mucilaginous cyanobacterial offer ready access to complex organic molecules (Cottrell and Kirchman 2000; Imanishi et al. 2005; Jones et al. 1994; Kirchman 2002), it was anticipated that CA communities would be dominated by taxa that assimilate carbon and nutrients from these sources for rapid growth as is common among *Gammaproteobacteria* and *Bacteroidetes* (Cottrell and Kirchman 2000; Kirchman 2002; Newton et al. 2011), while FL communities would exhibit more evenness of OTU abundances and greater variation in the identities of dominant taxa. Contrary to expectations, the two clusters that contained FL communities, clusters 2 and 3, exhibited the greatest dominance of a single family, *Sporicthyaceae* (Table S2, Fig. 5). The greater abundance of this and other *Actinobacteria* groups in these clusters is consistent with previous studies showing that members of this phylum were predominantly free-living (Allgaier and Grossart 2006; Louati et al. 2015; Parveen et al. 2013b). However, most of the *Gammaproteobacteria* that differed significantly among clusters and two of the highest-abundance *Bacteroidetes* families, *Cyclobacteriaceae* and *Chitinophagaceae*, were affiliated with FL-dominated clusters rather than CA-only clusters. This was unexpected, given the tendency of *Gammaproteobacteria* and *Bacteroidetes* to associate with cyanobacterial particles and other large organic particles in aquatic environments (Cai et al. 2014; Cottrell and Kirchmann 2000; Crump et al. 1999; Li et al. 2011; Louati et al. 2015; Niu et al. 2011; Parveen et al. 2013a; Shi et al. 2012).

Another unusual feature of the communities examined in this study was the importance of *Deltaproteobacteria*. *Bacteriovoracaceae* (*Bdellovibrionales*) were abundant in all three lakes, and this family and *Bdellovibrionaceae*, also a member of order *Bdellovibrionales*, were especially abundant in clusters 1 and 2. Several *Deltaproteobacteria* phylotypes in the order *Myxococcales* were detected in cluster 1 at abundances that were significantly higher than in the other clusters. *Myxococcales* are more characteristic of soils and sediments than of surface waters (Basak et al. 2015; Kim et al. 2016; Kou et al. 2016; Zlatković 2017). These bacteria may have entered the lakes along with sediment from surrounding agricultural lands. Both *Bdellovibrionales* and *Myxococcales* are highly motile predators that attack and lyse other Gram-negative bacteria (Cai et al. 2014; Davidov and Jurkevitch 2004; Rotem et al. 2014; Velicer et al. 2014), including cyanobacteria (Caiola and Pellegrini 1984; Maruyama et al. 2003). Prior studies of lakes undergoing cyanoHABs reported *Deltaproteobacteria* as present but not abundant (Cai et al. 2014; Eiler and Bertilsson 2004; Li et al. 2011; Louati et al. 2015; Niu et al. 2011). The high abundance of cell-lysing predatory bacteria in these lakes may also explain how large numbers of *Gammaproteobacteria* and *Bacteroidetes* were able to access high molecular weight organic compounds without associating with intact cyanobacteria. Widespread cell lysis would release into the water cellular products that would otherwise be exuded gradually, thus eliminating the need for bacteria that metabolize these compounds to associate directly with cyanobacterial cells. *Flavobacteriaceae* have been known to become highly abundant among free-living bacteria when high molecular weight organic compounds occurred at high concentration in open water (Kirchman 2002), a condition which could follow from high abundances of bacterial predators.

Conclusion

Results from this study support the hypothesis that the kind of microhabitat created by dense cyanoHABs selected for similarly structured assemblages of bacteria across multiple lakes, and that the selective pressures of this habitat type were different from those in the water column. Insights gained from this study further understanding of the ways in which cyanoHABs shape aquatic microbial communities. Cyanobacteria-associated communities from three lakes were significantly different from free-living communities in taxonomic composition. The 12 bacterial communities sampled fell into four clusters based on compositional similarity, with one cluster consisting of CA communities from Harsha Lake and Buckeye Lake and a second cluster consisting of the FL communities from these two lakes. Cross-lake similarities in the composition of CA communities or FL communities can be attributed to the similar sizes and physicochemical properties of these lakes as well as to similarities of the microhabitat types. Samples from Maumee Bay formed their own distinct clusters, one consisting of CA and FL samples collected shortly after the bloom reached its maximum density by cyanobacterial biovolume as well as FL samples from a later date, while the CA samples from a later stage of the bloom clustered separately. As the bloom progressed in Maumee Bay, the FL community remained similar to the earlier samples, while the composition of the CA community became significantly different. The hypothesis that the CA habitat type would select for a less diverse set of bacteria was not supported, as the CA communities overall had neither significantly lower measures of alpha diversity nor greater compositional similarity among lakes than did the FL communities. This is the first study to separate bacterial communities physically associated with mucilaginous cyanobacterial colonies or filaments from free-living bacteria and to compare the diversity and composition of the two types of communities across multiple lakes. Further studies are needed to explore the processes that differentiate CA from FL communities and the time scale on which these processes occur.

Acknowledgements We thank Christopher Blackwood and Xiaozhen Mou for comments on the methods and Alescia Roberto for technical assistance.

Funding Funding sources were an Integrative Graduate Education and Research Traineeship grant from the National Science Foundation (0903560) and an Art and Margaret Herrick Memorial Award from Kent State University.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals No human or animal participants were involved in this study.

Informed consent Informed consent rules were not applicable to this research because no human participants were involved.

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