Spatiotemporal distribution of bacterioplankton functional groups along a freshwater estuary to pelagic gradient in Lake Michigan

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Abstract

Freshwater bacteria play key roles in biogeochemical cycling and contribute significantly to biomass and energy fluxes. However, studies of Great Lakes ecosystem dynamics often omit bacteria. Here, we used high throughput sequencing to analyze how bacterial diversity and community composition (BCC) vary seasonally along the long term Muskegon estuary to pelagic research transect. Diversity was higher in the estuary than Lake Michigan, in spring compared to summer, and for particle-associated (PA) relative to free-living (FL) fractions. PA communities were distinct from, and more variable than FL communities. For both fractions, spring BCC was more similar between estuary and nearshore Lake Michigan compared to offshore waters. In summer and fall, nearshore and offshore BCC were more similar compared to estuary BCC. Most abundant taxa were inferred to be chemoorganoheterotrophs. While, as a whole, this functional group only showed habitat preference for the PA fraction, we observed phylum and class-level seasonal and spatial preferences. Chemoorganoheterotrophs that also perform bacteriorhodopsin-mediated phototrophy, such as acI *Actinobacteria* and LD12, strongly preferred FL fractions. Photoautotrophs (*Cyanobacteria*) were least abundant in spring, when mixotrophic methylotrophs were more abundant, particularly in the estuary. Organisms with chemolithotrophic capabilities, including a mixotrophic, highly abundant *Limnohabitans* (Lhab-A1) OTU, showed limited spatiotemporal patterns. One exception was *Nitrosospira*, an autotrophic ammonium oxidizer, which peaked in deep offshore waters in fall. *Nitrosopira* co-occurred with *Chloroflexi* CL500-11, which likely mineralizes nitrogen-rich organic matter in deep waters. These spatiotemporal BCC shifts suggest differences in bacterially mediated elemental cycling along estuary to pelagic gradients in Lake Michigan.

Keywords: bacterioplankton, seasonal succession, functional groups, biogeochemistry, 16S

Introduction

Bacteria play fundamental roles in freshwater community ecology and ecosystem functioning. Community dynamics are influenced by large bacterial biomass fluxes that support the higher food web via zooplankton grazing (Cole et al., 1988; Cotner and Biddanda, 2002; Scavia and Laird, 1987). Ecosystem functioning is affected as bacteria are key determinants of nutrient concentrations and fluxes by fixing carbon and nitrogen, assimilating and remineralizing dissolved and particulate organic matter, and oxidizing and reducing a range of other elements (Falkowski et al., 2008). As an example of the importance of bacteria to global elemental cycles, bacterial respiration of terrestrial carbon subsidies contributes to global net freshwater CO_2 emissions that rival net uptake by the oceans despite the relatively small footprint of freshwater systems (Cole et al., 2007; Tranvik et al., 2009).

The advent of high-throughput sequencing now allows for in-depth surveys of these highly diverse bacterial communities, which are now being performed across a variety of aquatic systems including the Great Lakes (DeLong et al., 2006; Sogin et al., 2006; Jones et al., 2009; Fisher et al., 2015; Mou et al., 2013; Newton and McLellan, 2015; Rozmarynowycz, 2014; Wilhelm et al., ~~2006~~2014; Beall et al., 2016). Yet, despite the ecological importance of bacteria, they are often not included in food web surveys and models. Case in point is the transect that runs from the Muskegon Lake drowned river mouth estuary to the pelagic Lake Michigan environment, which has been studied for over two decades (Madenjian et al., 2002; Millie et al., 2002; Pothoven and Fahnenstiel, 2014) and has been key in determining ecological impacts due to system level disturbances such as dreissenid mussel invasion (Fahnenstiel et al., 2010; Madenjian et al., 2002; Vanderploeg et al., 2010) and nutrient loadings (Dila and Biddanda,

2015; Gillett and Steinman, 2011; Marko et al., 2013; Weinke et al., 2014). However, until now, comprehensive studies of bacterial communities have been omitted from analyses of this system.

The Laurentian Great Lakes have nearly 3,000 river mouth systems, which are of increasing importance to overall system functioning since the invasion of dreissenid mussels has depleted pelagic production in most of the Great Lakes (Johengen et al., 2008; Larson et al., 2013; Turschak et al., 2014). As habitat filtering exerts strong influence on the distributions of bacterial populations (Lindström and Langenheder, 2012), the changes in resource availability existing along river mouth to pelagic gradients (Dila and Biddanda, 2015; Marko et al., 2013) are expected to drive changes in BCC (Jones et al., 2009; Kritzberg et al., 2006). Freshwater estuaries, which are adjacent to terrestrial environments, serve as a direct drainage of watersheds and receive allochthonous organic carbon and other nutrients of terrestrial origin into the system (Larson et al., 2013; Marko et al., 2013). Impact of land use decreases with distance from the shore leading to declining productivity from the estuary to the pelagic environment (Dila and Biddanda, 2015). Similarly, light penetration in the shallow nearshore environments supports the growth of benthic vegetation, while benthic primary production approaches zero in deep offshore environments. In addition to these large spatial gradients, microheterogeneity is created by the presence of particulate matter. While often omitted from BCC analyses, evidence is mounting, including from the Great Lakes (Mou et al., 2013), that strong differences exist between free-living bacterial communities that rely more on dissolved organic matter and those associated with particulate matter, which includes phytoplankton, small zooplankton and other biotic and abiotic particles (Grossart, 2010).

In addition to spatial gradients, seasonality is also expected to affect BCC, due to variation in light, temperature, nutrients and phenology (seasonal cycle) of aquatic and terrestrial

life (Ghiglione et al., 2007; Ghiglione and Murray, 2012; Kritzberg et al., 2006). Spring snow melt runoff carries allochthonous carbon and terrestrial bacterial populations into the estuary and near shore aquatic systems (Crump et al., 2003). Severe storm events in spring months also resuspend sediments and increase turbidity and water column nutrient concentrations (Johengen et al., 2008; Vanderploeg et al., 2007). In summer months, primary production reaches its peak, which in turn stimulates bacterial secondary production (Bird and Kalff, 1984; Morán et al., 2001; Obernosterer et al., 2008; Scheibner et al., 2014). Leaf litter enters streams in fall serving as resources to estuary and near shore environments (Abelho, 2001; Dila and Biddanda, 2015). In winter, organic resources synthesized in summer productive months are degraded into smaller molecules and they are utilized by a variety of bacterial heterotrophs (Ghiglione and Murray, 2012; Grzymski et al., 2012). The effect of seasonality on BCC is likely not uniform as pelagic environments may be less affected by phenology of terrestrial plants and the seasonal weather events yet are impacted more by the thermal stratification in the summer and early fall and thus creating resource variation along the depth profile (Anadón et al., 2002; Schneider et al., 2003; Turner, 2015).

In this study, we aimed to address the knowledge gap of how these existing resource gradients along river mouth to pelagic gradients in the Great Lakes impact bacterial diversity and BCC. We collected 24 water samples in 2013 along the Great Lakes Environmental Research Laboratory's (GLERL) Lake Michigan long-term research (LTR) transect near Muskegon, MI to describe free-living and particle-associated fraction bacterial communities across time (spring, summer and fall) and space (estuary, nearshore, and offshore at different depths). Bacterial communities were analyzed by high-throughput sequencing of the V4 region of the 16S rRNA gene. The Laurentian Great Lakes account for 18% of Earth's surface freshwater and are subject

to multiple stressors, such as coastal eutrophication (Dila and Biddanda, 2015; Larson et al., 2013; Steinman et al., 2008) and species invasions (Allan et al., 2013; Smith et al., 2015; Vanderploeg et al., 2010). Gathering an understanding of the current composition of Great Lakes microbial communities and the factors that affect their distribution is necessary to better understand biogeochemical cycles within these systems, and how these may be affected by environmental stressors.

Methods

Study site and sample collection. Water samples were collected on board of the R/V Laurentian as a part of NOAA GLERL's LTR ecological monitoring program near Muskegon, MI. Twenty-four sampling events occurred at offshore (M110: 43° 11' 59" N, 86° 34' 11" W) and near-shore (M15: 43° 11' 17" N, 86° 20' 38" W) stations of the NOAA's LTR transect, and adjacent Muskegon Lake (estuary of Lake Michigan) near the Grand Valley State University Annis Water Resources Institute monitoring buoy (MLB; www.gvsu.edu/wri/buoy/; 43° 14'-17"'' N, 86° 16'- 49"" W). Samples were taken during spring (April 23-24), summer (July 15-16), and fall (September 23-24) 2013 at 2-5 m below the surface and 2-5 m above the lake floor (Fig. 1, Table 1). The sites in Lake Michigan are the same sites used by Denef et al. (2016) to characterize *Chloroflexi* abundance and function. During the summer sampling, water samples were also collected from a deep–chlorophyll maximum (DCM) layer (35 m) at the M110 station. We used one 30 L Niskin sampler to collect water, which was poured through 210 µm and 20 µm mesh to remove large phyto- and zooplankton (and their associated microbial communities) into a 10 L carboy. Carboys, funnels, and mesh were bleached and MilliQ water rinsed, and rinsed twice with sample water before use. Pre-filtered water was sequentially filtered onto 3.0

137 µm polycarbonate filters and 0.22 µm polyethersulfone filter membranes (142 mm, Millipore)
138 using a Masterflex I/P peristaltic pump (Cole Parmer) between settings 11-13. Filters were
139 folded with bacterial biomass facing inwards and submersed into RNAlater (Ambion). Sample
140 filtering was limited to 10 minutes and the filtered water volumes varied between 2.8 L and 11 L
141 (Table 1). All samples were stored in RNAlater within 20 minutes of sampling. Samples were
142 stored at -20 °C on board and transferred to a -80 °C freezer within 48 h of sampling.
143 **Physical and geochemical analyses.** At each station, vertical profiles of physical
144 variables were obtained by lowering an instrument package containing a Sea-Bird CTD
145 (conductivity, temperature, depth profiler), oxygen sensor, and fluorometer, and a Biospherical
146 Instruments PAR sensor. In addition, a plankton survey system (PSS) was continuously lowered
147 and raised at ~0.25 m s^{-1} in a sinusoidal path from 1-2 m beneath the lake surface to 2-4 m above
148 the bottom as the R/V Laurentian moved at ~1.8 m s^{-1} while logging data every 0.5 second. The
149 PSS contained multiple sensors mounted on a V-fin, of which chlorophyll *a* (Wet Labs ECO
150 Fluorometer, Sea-Bird Scientific), and temperature were used to reconstruct the profiles in
151 Figure 1. The fluorometer output (volts) was converted to derived chlorophyll *a* (chl*a*)
152 concentrations by regression between fluorometer output and laboratory chlorophyll *a*
153 measurements. Temperature and derived ~~chlorophyll~~ chl*a* data from the Muskegon Lake buoy
154 were downloaded from the Muskegon Lake Buoy Observatory website (www.gvsu.edu/buoy/;
155 Prof. B. Biddanda, Annis Water Resources Institute, Grand Valley State University).
156 ~~Replicate~~ Duplicate water samples were collected and analyzed for ~~chlorophyll~~ chl*a*,
157 dissolved and particulate organic carbon (DOC, POC), particulate organic nitrogen (PON), total
158 and particulate phosphorus (TP, PP), total suspended solids (TSS) and SiO_2 according to NOAA
159 GLERL standard operating procedures (Nalepa et al., 1996). ~~Shortly~~Briefly, POC and PON were

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determined by filtering lake water through a pre-combusted 2.5 cm GF/F filter (Millipore). Filters were frozen in petri dishes immediately after filtration. Prior to analysis, filters were acidified with 3-5 drops of 10 % HCl and dried at 70 °C for 24 hours. Samples were analyzed with an Elemental Analyzer 1110 (CE Elantech). DOC samples were taken by vacuum filtering ~40 ml lake water through a combusted GF/F filter and collecting filtrate in a clean beaker, under a Kontes bell jar. The filtrate was transferred into a sterile, polypropylene, sample-rinsed, 50 ml test tube. The samples were frozen until analysis using a Shimadzu TOC 5000 high temperature combustion analyzer to determine non-purge-able organic carbon (NPOC, DOC operational definition). For total phosphorus (TP) 50 ml lake water was poured into acid-cleaned glass test tubes and sealed with Teflon-lined caps. Samples were stored cold for less than 30 days until digested. For particulate phosphorus (PP), 200 ml lake water were filtered through a 0.2 um pore size 47mm polycarbonate membrane filter (Millipore). Before digesting, 10 ml of ~~the~~ 5 % ~~by~~ persulfate solution and 50 ml ultra-pure water were added. Total and particulate phosphorus samples were digested for 35 minutes at 2 atmospheres pressure with 10 ml 5 % $K_2(SO_4)_2$ solution. Samples were analyzed on a QuAAtro® segmented flow analyzer (Seal Analytical) using the ascorbic acid - molybdenum blue method (Murphy and Riley, 1962).

DNA extraction and 16S rRNA gene sequencing. Duplicate nucleic acid extractions from different sections of the same 142 mm filter membrane were performed for each of the field samples using a modified AllPrep DNA/RNA/miRNA Universal kit protocol (Qiagen) (McCarthy et al., 2015). DNA was submitted to the Joint Genome Institute for amplicon sequencing targeting the V4 region of the 16S rRNA gene (515F/806R universal primers) (Caporaso et al., 2012). Pooled libraries were sequenced on an Illumina MiSeq sequencer, using v2 chemistry 2x250 (500 cycles) paired-end reads. RTA v1.17.28 and MCS v2.2.0 software

(Illumina, Inc.) were used to generate data. Illumina raw paired-end reads were filtered based on quality, and merged by JGI using their iTagger pipeline (Tremblay et al., 2015). This pipeline removes contaminants (PhiX control, sequencing library adapter dimers, human contaminants), trims PCR primers, trims the sequence reads based on quality, and merges paired end reads into single sequences. Raw and processed data are available on the Joint Genome Institute's genome data portal (http://genome.jgi.doe.gov/; Project IDs 1041195 and 1041198).

Sequence data analysis. The 88 samples (24 samples *~~X~~ 2 replicates *~~X~~ 2 = 96, - 8 unsuccessful sequencing reactions; Table 1) were analyzed using Mothur version 1.34.3 using the MiSeq standard operating protocol (accessed on Dec 17, 2014) for sequence alignment and the generation of operational taxonomic unit (OTU, 97 % sequence similarity) table (Schloss et al., 2009). Only bacterial sequences were retained (any reads classified as chloroplast, mitochondria, eukarya, archaea, and unknown were removed, Table S1). For classification, we used a hybrid protocol using a freshwater-specific taxonomy (https://github.com/mcmahon-uw/FWMFG) and the SILVA release 119 taxonomy (Quast et al., 2013) as previously described (Schmidt et al., 2016).

Further analyses were carried out in R version 3.2.1 using phyloseq (McMurdie and Holmes, 2013), vegan (Oksanen et al., 2013) as well as R functions developed by Michelle Berry (https://github.com/DenefLab/MicrobeMiseq) ~~(Berry~~ (Berry et al, in review). Full code and input files are available at https://github.com/DenefLab/LM13_DNA. We merged replicate samples by summing read counts prior to further analysis. All figures were generated using the ggplot2 R packages (Wickham, 2009), with additional editing in Illustrator (Adobe, Inc.).

For observed richness and Simpson's evenness, the OTU table was rarefied at 70,480 reads, a subsampling that allowed inclusion of all reads of the lowest sampling depth (certain

206 samples contained up to 85% chloroplast sequences, which were removed during the mothur
207 analysis, Table S1). To test for significant differences in richness and evenness based on season,
208 station, filter fraction, or depth, we performed a Kruskall-Wallis test (kruskal.test; R Core Team.,
209 2015) along with post-hoc tests to identify significant pairwise differences (kruskalmc in
210 pgirmess R-package; Giraudoux, 2012), which were visualized using the multcompLetters
211 function (multcompView R-package; Graves et al., 2012).
212 For comparisons of community composition across space and time, we first scaled all
213 OTU read counts to the smallest merged library size (70,480 sequences) using the procedures
214 described by (McMurdie and Holmes, 2014). We calculated the (abundance-weighted, i.e.,
215 binary = FALSE) Bray-Curtis dissimilarity and the OTU presence/absence-focused Sørensen
216 dissimilarity (i.e., binary = TRUE) between samples, and visualized these distances with a
217 principal coordinates analysis (PCoA) ordination using the pcoa function (vegan). The PCoA
218 was performed on both the full datasets after scaling (16,028 OTUs), as well as after limiting the
219 OTU table to the most abundant taxa (> 0.1% on average after scaling, 139 OTUs, which
220 represented 81 +/- 12 % (standard deviation) of all sequence reads across all samples). We
221 performed a nested permutational multivariate analysis of variance (PERMANOVA; Anderson,
222 2005) using the adonis function (vegan) to test if filter fraction, season, lake, station, depth, and
223 day/night could significantly explain variation in the bacterial community composition. A
224 Kruskall-Wallis test was performed as described above to compare whether median community
225 dissimilarity was different among PA and FL fraction communities. We also performed a similar
226 test to determine if community dissimilarity between surface communities at the different
227 stations changed in function of season.

The 139 OTUs with average abundance > 0.1% across all samples were also classified to functional groups based on carbon and energy source. Functional class inferences from the taxonomic classification were made based on a literature search (papers describing isolates, genome sequences and gene expression, or substrate utilization assays combined with fluorescent *in situ* hybridization) as summarized in Table S2.

We identified significant differences in the relative abundance of these 139 OTUs between the Muskegon Lake estuary and Lake Michigan, between seasons, fractions, and depth, each time while controlling for variation in all other factors. We used the negative binomial generalized linear model framework of the DESeq function in the DESeq2 R-package (Love et al., 2014; McMurdie and Holmes, 2014). P-values were adjusted for multiple testing through the Benjamini-Hochberg false discovery rate correction (Love et al., 2014).

To explore the correlation of between-sample biological variation and geochemical and physical data available for Lake Michigan samples, we carried out a bioenv analysis (vegan), an R implementation of the multivariate statistical method devised by Clarke and Ainsworth (Clark and Ainsworth, 1993). Separate analyses were performed for all samples, the FL fraction samples only, and the PA fraction samples only. The method calculates Euclidean dissimilarity matrices for each possible combination of environmental factors (from one factor to all factors) and a Bray-Curtis dissimilarity matrix for the biological data. Then, bioenv calculates Spearman rank correlations between the biological distance matrix and each environmental distance matrix and selects the subset of environmental factors with the highest Spearman correlation. Significance levels were determined by comparing them to the distribution of maximum BIOENV correlations observed in 100 permutations obtained by randomizing row order in the biological data table.

Results

Spatiotemporal variation in environmental conditions.

The water column at the sampling stations (Fig. 1A-B) was isothermal in spring and stratified in summer and fall (Fig. 1C-F). ~~Chlorophyll~~ Chl*a* was consistently higher in the estuary than in Lake Michigan (Fig. 1C, G-I). Nearshore ~~chlorophyll~~ chl*a* was higher than at the offshore station, except in summer, when a chlorophyll maximum was observed at 35-36 m below the water surface at the M110 offshore station (Fig. 1G-I). Within Lake Michigan stations, phosphorus levels were highest at the near~~-~~shore surface in spring, and lowest in the offshore deep in summer and fall (Table 1). POC was highest at the near~~-~~shore surface in spring. In the summer and fall, POC was higher in near~~-~~ and offshore surface waters and the off~~-~~shore DCM than in off~~-~~shore deep waters (Table 1).

Bacterial richness and evenness

The analyses of bacterial community richness and evenness were performed after clustering the small subunit ribosomal RNA sequences into operational taxonomic units (OTU) at 97% sequence identity (a proxy for bacterial species) and after rarefying the data at 70,480 reads (Fig. S1). More OTUs were observed in spring compared to summer (Fig.2A: Kruskall-Wallis, $p < 0.05$), and in Muskegon Lake and the nearshore Lake Michigan site relative to the offshore Lake Michigan site (Fig. 2B). Particle-associated fraction (PA) bacterial communities were similar in richness to free-living (FL) communities (Fig. 2C) and surface and deep communities did not significantly differ in richness either (Fig 2D). Evenness was low across all samples, indicating that relative to total observed richness in each sample, a small number of

taxa predominated. Evenness was only significantly different between PA and FL fractions (Fig. 2E-H).

Spatiotemporal differences in abundance-weighted community composition.

When taking the relative abundance of taxa into account (Bray-Curtis dissimilarity), filter fraction and season were the strongest explanatory factors for differences in community composition (Fig. 3A, Table 2). Additional variation was explained by sampling station, primarily Muskegon Lake vs. Lake Michigan stations (Table 3). Depth influenced community composition less, and no significant differences were found between samples taken during the day and night. The ~~patterns of~~ community composition patterns ~~differences~~ remained the same whether all 16,028 OTUs or only the 139 OTUs with relative abundance > 0.1% were included (Fig. S2A).

When comparing surface communities (deep samples excluded from comparison, as depth varied across the transect), the difference in community composition between spring estuary and nearshore samples was smaller than between estuary and offshore samples (Fig. 4; $p < 0.05$, Kruskall-Wallis). In summer and fall, the difference in community composition between the estuary and both Lake Michigan stations was significantly larger than between the nearshore and offshore station (Fig. 4).

In addition to being the strongest correlating factor with abundance-weighted community dissimilarity, the dissimilarity among PA communities (community turnover) was also significantly higher than among FL communities (Fig. 5A).

Bioenv analysis found no significant correlation between geochemical and physical parameters and biological data when both fractions were included, but did find different sets of

297 factors resulting in the highest correlation between environmental and biological dissimilarities
298 between samples (Table 3). When taking the best single environmental parameter correlating
299 with FL biological dissimilarities and testing the correlation with PA biological dissimilarities
300 and vice versa, correlation coefficients decreased (log(chl*a*) vs PA: r = 0.38; T vs FL: r = 0.45).
301
302 *Difference in OTU presence between samples.*
303 When only considering the presence or absence of taxa (Sørensen dissimilarity), season
304 and lake were the strongest explanatory factors for differences in community composition (Fig.
305 3B, Table 2). Filter fraction and depth explained additional, but less variation (Table 3). Mainly,
306 it became apparent that many OTUs were uniquely present in spring communities and that in
307 summer and fall, Muskegon Lake harbored many unique OTUs compared to both Lake Michigan
308 stations. While the patterns shift more than for the abundance-weighted PCoA, this analysis
309 remained qualitatively similar whether all 16,028 OTUs or only the 139 OTUs with relative
310 abundance > 0.1% were included (Fig. 3B, S2B).
311
312 *Functional groups and taxa driving spatiotemporal gradients in BCC.*
313 Instead of focusing strictly on taxonomic classifications, we classified the 139 OTUs with
314 average relative abundance > 0.1 % into functional groups using a literature search based on the
315 assigned taxonomy. Functional groups were delineated based on carbon source (autotroph,
316 heterotroph, mixotroph) and energy source (chemoorganotroph (organic energy source, including
317 one carbon compounds (methylotroph)), chemolithotroph (inorganic energy source), phototroph
318 (light, harvested with ~~chlorophyll~~ chl*a*, bacteriochlorophyll, or bacteriorhodopsin) (Table S2).

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We first performed a DESeq analysis to identify OTUs with significantly different relative abundance between the fractions, which indicated partitioning of functional groups between the FL and PA fractions (Fig. 5B). For instance, we noted strong overrepresentation of autotrophic phototrophs (*Cyanobacteria*) and chemoorganoheterotrophs in the PA fraction, while chemoorganoheterotrophs that were inferred to complemented their energy generation with bacteriorhodopsin-mediated phototrophy were overrepresented in the FL fraction. The latter pattern was driven in large part by the strong preference of *Actinobacteria* for the FL fraction (Fig. S3). Due to these large differences between the fractions at the functional group level, further analyses of differential abundance (Muskegon Lake estuary vs. Lake Michigan, between seasons, and between surface and deep) were performed for each fraction separately (Fig. 6).

In line with the correlation values in the PERMANOVA analysis (Table 2), season and lake comparisons resulted in more OTUs with significantly different relative abundance and with larger effect sizes ($\log_2$ fold ratio, X-axes in Fig. 6) than comparisons between surface and deep communities. In line with the large dissimilarity of spring relative to summer and fall communities, more differentially abundant OTUs and larger effect sizes were observed for summer to spring than fall to summer comparisons (Fig. 6). Specific taxa were annotated on Ffigure 6 based on (1) their overall relative abundance, (2) differential abundance patterns along the spatiotemporal gradient, and/or (3) differential abundances observed in a recent study along an estuary to pelagic gradient near Milwaukee, on the other side of Lake Michigan (Fig. 1A).

The relative abundance of photoautotrophs was higher in summer and fall than in spring, and different taxa predominated in Muskegon Lake and Lake Michigan, though *Synechococcus* was the most abundant photoautotroph in both systems (Fig. 6). Only in the FL fraction did we observe taxa with higher relative abundance in the surface compared to the deep (note, deep was

~10 m below the surface at the nearshore location, and ~110 m at the offshore location, though *Cyanobacteria* were found at all depths throughout the system (Fig. 7, OTU3)).

Most of the 139 most abundant OTUs were chemoorganoheterotrophs and this functional group also contributed most of the differentially abundant OTUs, though for most comparisons there were a similar number of OTUs differentially abundant in both conditions (Fig. 6). Several *Planctomyces* OTUs had higher relative abundance in the estuary, though none were closely related to species able to perform the anaerobic oxidation of ammonium to N_2 (Table S2). Different clades of *Verrucomicrobia* showed different patterns, with an OPB35 soil group OTU reaching higher relative abundances in the estuary, while a *Verrucomicrobiaceae* OTU reached higher relative abundances in Lake Michigan (Fig. 6, Fig. 7). Several of the most abundant chemoorganoheterotrophs reached their highest relative abundance in summer, including the most abundant *Bacteroidetes* taxon in the PA fraction (Aquir tribe, Fig. 6, Fig. 7).

Methylotrophs, which are chemoorganotrophs that oxidize one-carbon molecules as a source of energy (and potentially carbon), generally had higher relative abundance in the estuary than Lake Michigan. This was particularly true for mixotrophic methylotrophs (Fig. 6, Fig. 7), which also reached higher relative abundance in spring and fall compared to summer. The patterns varied between stations and fractions depending on the OTU, and generally we observed higher relative abundance of methylotrophs at the offshore location in Lake MI compared to the nearshore station (Fig. 7). The sole autotrophic methylotroph, LD19, gradually increased in relative abundance at both Lake Michigan stations from spring to fall (Fig. 6, 7).

A variety of chemoorganoheterotrophs that complement their energy generation with phototrophy using either bacteriorhodopsin or bacteriochlorophyll were present among the 139 most abundant OTUs. While many of them, particularly *Actinobacteria* acI lineages, had similar

relative abundance throughout space and time, some specialization between the estuary and Lake Michigan and across time and depth was observed (Fig. 6). LD12, a sister clade to one of the most abundant marine bacterial clades (SAR11), had higher relative abundance in Lake Michigan than Muskegon Lake, though this difference was not statistically significant as in fall relative abundances were similarly high across all stations. *Chloroflexi* CL500-11 became increasingly abundant in deep offshore waters (Fig. 6,7). This increase coincided with an increase of the sole OTU that we could confidently assign as a chemolithoautotroph, *Nitrosospira*, which is an autotrophic ammonium oxidizer.

Finally, a series of OTUs were assigned to the most versatile functional groups, which combined all three types of energy generation. The most abundant one, a *Limnohabitans* OTU (tribe Lhab-A1), showed limited differential abundance across space and time (Fig. 6). However, other OTUs with the same taxonomic assignment showed more station-specific dynamics, such as OTU105979, which was only found in spring in the estuary, when it contributed 10% of all sequences (Fig. 7). We also observed this divergence of spatiotemporal dynamics among related *Polynucleobacter* OTUs (Fig. 7).

Discussion

The Muskegon transect of Lake Michigan is one of the most extensively and longest studied areas of the Laurentian Great Lakes. Most studies have focused on fish and eukaryotic plankton community composition along this estuary to pelagic gradient (Fahnenstiel et al., 2010; Gillett and Steinman, 2011; Madenjian et al., 2002). Despite the importance of bacterial populations in biogeochemical cycling and potential importance in food web interactions with higher trophic levels (Scavia and Laird, 1987), this study is the first report on the drivers of

bacterial diversity and community composition along this transect. Not unexpectedly, seasonal changes in environmental conditions are a main driver of both species richness and differences in bacterial compositions, with more diverse and distinct communities, based on both species presence/absence and relative abundance of shared taxa, existing in the highly productive estuary compared to the Lake Michigan communities. Our study also highlights the distinct bacterial communities associated with particulate matter relative to free-living bacteria, which were also observed in previous studies in western Lake Erie (Mou et al. 2013) as well as smaller freshwater lake and marine systems (Allgaier and Grossart, 2006; Bižić - Ionescu et al., 2014; Rösel and Grossart, 2012; Schmidt et al., 2016). Particle-associated (PA) fraction bacterial populations are particularly interesting along the Muskegon transect as they are more diverse, highly distinct in phylogenetic and functional group composition, and more variable over time and space than free-living (FL) communities. As the PA fraction likely contains more eukaryotic cells relative to bacterial cells than the FL fraction, as evidenced by the higher fraction of chloroplast sequences removed (Table S1), it is possible that interactions with eukaryotic phytoplankton and microzooplankton drive these patterns. Future integration with data gathered in parallel to our efforts will allow us to explore this hypothesis.

More broadly, despite the fact that the Laurentian Great Lakes contain approximately one fifth of the world's surface freshwater, high-throughput sequencing to survey bacterial community composition has only recently been applied to the Great Lakes. Currently multiple surveys are being undertaken, with first results highlighting divergent communities across time, depth, and space in the pelagic environment of the Great Lakes (Fisher et al., 2015; Mou et al., 2013; Newton and McLellan, 2015; Rozmarynowycz, 2014; Wilhelm et al., ~~2006~~2014; Beall et al., 2016). Our study, together with recently published studies on a similar transect near

411 Milwaukee (Fisher et al., 2015; Newton and McLellan, 2015) add insights into the bacterial
412 communities in the more productive regions of the lakes, specifically nearshore and estuary
413 regions. A better understanding of compositional differences between these regions of the Great
414 Lakes is a stepping stone towards studies that investigate links between compositional changes
415 and shifts in bacterially mediated functions (Nemergut et al., 2014). Better understanding of
416 drivers of compositional and functional changes in nearshore and estuary regions in particular is
417 of interest as these regions have become of increased importance in sustaining Great Lakes food
418 webs since the invasion of dreissenid mussels (Vanderploeg et al., 2010).
419 Our observations of diversity and BCC trends along the Muskegon transect partially
420 corroborate the studies on the Milwaukee transect (Fisher et al., 2015; Newton and McLellan,
421 2015),. They extend these first studies by (1) resolving the different dynamics in the FL and PA
422 fractions, (2) including seasonal and depth profile analyses, and (3) focusing on geochemically
423 important functional groups. The distinct composition of bacterial communities in the estuary
424 and pelagic environments are likely due to the difference in organic matter concentrations and
425 compositions along estuary to pelagic gradients (Dila and Biddanda, 2015; Marko et al., 2013).
426 Previous experimental studies have shown that resource differences lead to differences in
427 bacterial community compositions (Jones et al., 2009; Kritzberg et al., 2006). The estuary is also
428 marked by substantially higher water temperatures during summer and fall (Fig. 1C-F), which is
429 another known factor shaping bacterial community composition (Hall et al., 2008; Kosten et al.,
430 2012; Scheibner et al., 2014), and which our bioenv analysis identified among the subset of
431 highest correlating environmental factors with community composition in both FL and PA
432 fractions. In addition, nutrient levels differ between oligotrophic Lake Michigan and meso- to
433 eutrophic Muskegon Lake, which can affect bacterial community composition and diversity

(Jankowski et al., 2014; Lauro et al., 2009; Schmidt et al., 2016; Yannarell et al., 2003). The above factors, as well as increased mass effects from the Muskegon River and urban runoff are likely driving factors for the BCC differences along this spatial gradient as suggested for the Milwaukee transect study (Newton and McLellan, 2015).

While these general trends of richness and community composition are comparable to the Milwaukee results, a more detailed analysis of the taxa that show preference between Lake Michigan and the estuary does reveal several differences. Whereas the acI-A lineage was shown not to show differential representation between the Milwaukee estuary and Lake Michigan, subgroups of this lineage do show strong preferences between the Muskegon estuary and Lake Michigan in our study. Similarly, we showed that specific OTUs within the acI-B lineage either show no or strong preference for Lake Michigan, whereas the acI-B lineage taken as a whole was indicated to show preference for Lake Michigan by Newton (Newton and McLellan, 2015). It has to be noted that a more resolved analysis by Newton (oligotyping) did reveal similar divergences in habitat specialization. LD12, which showed the strongest preference of all taxonomic groups in the Milwaukee study (a 12-fold preference for Lake Michigan), did not show a significant preference in our case. In spring and summer, LD12 was indeed almost exclusively found in Lake Michigan (representing up to 25% of all sequencing reads in offshore surface waters), yet in fall levels at all stations were similar (~ 10 % at the surface and ~ 5 % in the deep). Our study thus shows that seasonality has strong effects on habitat preference patterns across this estuary to pelagic gradient. Similar to the conclusion from Newton and McLellan's oligotyping work, which resolves sequence differences down to the single nucleotide level, our work shows that even at the level of OTUs (which bundles sequences up to 3 % divergent from each other) that were assigned the same or very similar taxonomy (e.g., Pnec, Lhab), highly

457 distinct patterns in spatial and temporal relative abundance dynamics can be seen. Whether these
458 closely related taxa carry out similar functions or may contribute differently to bacterially
459 mediated processes remains to be determined.

460 The compositions of PA bacterial communities are more variable across seasons and
461 spatial gradients than FL communities, which is similar to previous reports in small inland lakes
462 (Rösel and Grossart, 2012; Schmidt et al., 2016). Particles in aquatic environments are
463 heterogeneous in nature by being comprised of eukaryotic phytoplankton, small zooplankton,
464 excretes of zooplankton, detritus, and other organic particulate matter including allochthonous
465 materials from rivers (Anadón et al., 2002; Turner, 2015). The concentration and the
466 composition of particles are known to vary across time and locations (Turner, 2015). Substrates
467 for free-living organisms are primarily different types of dissolved organic matter (DOM), and
468 their composition and concentration also changes through time and locations (Crump et al.,
469 2003; Reche et al., 1998), which likely contributes to the spatiotemporal patterns in free-living
470 fractions. Our data do~~es~~ not allow us to identify the cause for this pattern, though correlation
471 with ~~chlorophyll~~ chl*a* levels, which only varied moderately across space and time in Lake
472 Michigan is supportive of the role of algal-derived organic matter as a shaping force. In contrast,
473 the best subset of environmental factors correlating with PA community turnover included total
474 suspended solids, implicating particulate matter concentrations and possibly its composition in
475 Lake Michigan. The Milwaukee transect BCC combined both size fractions in one and thus their
476 patterns were driven by the more numerically dominant FL fraction bacterial populations. Hence,
477 they observed similar patterns as we observed for the FL fraction (i.e., several abundant
478 populations occurring throughout the transect) (Newton and McLellan, 2015).

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While the abundance of bacterial populations associated with particles are typically small relative to FL bacteria (Azam et al., 1983; Bižić‐Ionescu et al., 2014; Ghiglione et al., 2007), PA bacterial populations can be metabolically highly active and thus play significant roles in decomposition and remineralization of particulate organic matter (Ghiglione et al., 2007; Grossart et al., 2007). The higher variability among PA than FL bacterial composition, differences in phylogenetic and functional group composition of FL and PA fractions, and differences between the environmental factors that correlate with changes in community composition in the fractions suggest different drivers of community assembly between FL and PA fractions. Further work is needed to link differences in process levels across the system to community turnover in these fractions and identify which communities underpin changes in ecosystem functioning across the transect and over time.

While depth overall is less of a driver of community dissimilarity, very distinct taxa were found at the offshore station in particular. Of particular interest is the high relative abundance of *Chloroflexi* CL500-11 (OTU21), the genomic analysis of which has recently suggested its importance in the remineralization of nitrogen-rich DOM (Denef et al., 2016), and the co-occurrence of an autotrophic ammonia oxidizer (*Nitrosospira*, OTU119). Considering the gradually increasing levels of nitrate in the upper Great Lakes (Dove and Chapra, 2015), interactions between the abundant *Chloroflexi* and *Nitrosospira* will be of interest for future studies. Recent studies in the Great Lakes have documented higher levels of ammonium oxidation in the hypolimnion compared to the epilimnion (Small et al., 2013), which is in line with our observed absence of ammonium oxidizers in the epilimnion. In addition, ammonium-oxidizing archaea (AOA) were more prevalent in the hypolimnion of Lake Superior, while ammonium oxidizing bacteria (AOB), particularly *Nitrosospira*, predominated in Lake Erie

(Mukherjee et al., 2016). As we focused on the bacterial communities in this study, we cannot determine whether AOA are prevalent in Lake Michigan, but the same AOB taxa predominating in Lake Erie appear to be present in Lake Michigan.

In our study, the highest bacterial diversity was observed in spring compared to summer when productivity (assessed based on system-wide ~~chlorophyll~~ chl*a* concentrations) peaks. Recent studies by others have found a negative relationship between productivity and bacterial diversity, where winter bacterial populations under ice were more diverse than in summer (Ghiglione and Murray, 2012; Grzymski et al., 2012). The explanation provided for this phenomenon was that resource diversity may be high in winter while resources are relatively homogeneous in summer productive months. This hypothesis of low resource diversity in summer productive month was supported by a recent study (Becker et al., 2014). In contrast, we found that along the transect, the highest diversity was observed in the productive estuary relative to the nearshore and nutrient scarce offshore environment in summer and fall. The fact that the estuary was not just productive but also had high resource diversity by receiving allochthonous organic matter may explain this contradictory result. The estuary also has the highest stochastic mass effects as it experiences a high influx of bacteria from terrestrial origins.

Previous process level measurements have indicated that the Muskegon Lake estuary and near shore of Lake Michigan serve as carbon sinks while the offshore pelagic environment serves as carbon source with respiration exceeding gross production in the surface waters during summer (Weinke et al., 2014). Dominance of bacterial heterotrophs in pelagic surface communities in summer and increased presence of bacterial primary producers in the estuary and nearshore surface waters are in line with these findings. Our study adds insight into the potential bacterial roles in carbon flux when the deeper parts of the water column are taken into account.

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First, while ~~chlorophyll~~ chl*a* levels were low throughout the water column at the offshore station in spring, a chlorophyll maximum formed in summer (~35 m below surface) and thus total water column primary production could exceed respiration if this zone is taken into account. Second, when other seasons are considered, a previous study suggested net carbon emissions from Muskegon Lake in winter months (Ogdahl et al., 2010). Indeed, few cyanobacterial sequences were identified in spring, though several OTUs were classified as taxa with known mixotrophic carbon source usage, which may affect the carbon flux. As our analyse~~i~~s were restricted to the bacterial community, no information was gained on eukaryotic phytoplankton, which based on ~~chlorophyll~~ chl*a* levels in spring were likely abundantly present. Previous studies identified nearshore diatom and cryptophyte blooms in spring (Millie et al., 2002), which would explain the high levels of ~~chlorophyll~~ chl*a* despite cyanobacterial absence in our data.

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The spatiotemporal shifts in bacterial community composition, and of the inferred functional groups the differentially represented OTUs belong to, suggest that bacteria-driven biogeochemical processes differ between FL and PA fraction communities, seasons, depth, and along the estuary to pelagic gradient. Yet, the delineated functional groups were very broad, and interpretations of the impact of the observed differences in bacterial community composition on overall biogeochemical cycling are limited by the limited correlation between phylogeny and functional traits, such as the ability to degrade specific classes of organic matter, among bacteria (Martiny et al., 2015). Future studies that focus on genome reconstruction and analysis of *in situ* gene expression patterns of important Great Lakes bacterial populations, as recently performed for CL500-11 *Chloroflexi* (Denef et al., 2016) and ongoing work focused on substrate uptake assays and obtaining bacterial isolates (Salcher et al., 2013; Salcher et al., 2015), will provide much needed insights into functional repercussions of the observed differences in community

548 composition. Another limitation of this study is that the observed spatiotemporal trends in
549 bacterial communities cannot be explained solely by heterogeneity in geochemical and physical
550 factors. Predation by nanoflagellates (Callieri et al., 2002; Christaki et al., 2001; Duarte et al.,
551 2005; Šimek and Chrzanowski, 1992) and population control by parasitic viruses (Brum et al.,
552 2015; Payet and Suttle, 2008; Pearce et al., 2007) also plays a critical role in shaping bacterial
553 communities, but predation was not assessed in this study.
554
555 **Conclusion**
556 Human activities continue to affect the Great Lakes basin, mainly through eutrophication
557 of estuaries and nearshore regions and introduction of invasive mussels that have deplete
558 resources in mid depth offshore regions. We know very little about how these disturbances affect
559 Great Lakes bacterial communities and their impact on ecosystem processes. While no
560 comparable pre-invasion data are available, dreissenid mussels have likely altered bacterial
561 communities directly through selective filter feeding (Cotner et al., 1995; Findlay et al., 1998;
562 Frischer et al., 2000; Vanderploeg et al., 2001) and indirectly by affecting concentrations and
563 compositions of primary producers, organic matter, and consumers (Higgins and Zanden, 2010).
564 Global climate change can alter the timing and depth of the thermocline (King et al., 1997) and
565 the introduction of new invasive species such as Asian Carp (Cuddington et al., 2014; Jerde et
566 al., 2013) will continue to alter Great Lakes bacterial communities. Our study has outlined how
567 functional groups are partitioned across small (PA vs. FL) and larger scale (spatial and temporal)
568 gradients along one of the best-monitored research transects in the Great Lakes. Integrating these
569 insights with data on other trophic levels obtained simultaneously with our data will help
570 increase understanding of the role of these bacterial communities in ecosystem function.

Together with efforts elsewhere in the Great Lakes, we can now establish an understanding of the current baseline of bacterial community composition and functioning, and through experimentation and continued monitoring we can infer how these communities and the ecosystem services they provide may change in the future.

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Tables and Figures

Table 1. Summary of samples and environmental data. At each sampling event, a particle-associated (PA) and free-living (FL) fraction was collected (Su.PA.M110.D.N failed to amplify) and two replicate extractions and sequencing libraries were generated (* only one replicate available). Geochemical data originated from laboratory analyses, temperature (T) was determined using a CTD cast, and dissolved oxygen (DO) and photo-active radiation (PAR) derived from the plankton survey system data. Sample names: Sp = Spring, Su = Summer, Fa = Fall; MLB = Muskegon Lake Buoy, M15 = 15 m depth station Lake Michigan, M110 = 110 m depth station Lake Michigan; S = Surface, D = Deep, M= chlorophyll maximum; D = day, N = night. Environmental data: Chl = ~~chlorophyll~~ chl*a*, TP = total phosphorus, PP = particulate phosphorus, POC = particulate organic carbon, DOC = dissolved organic carbon, PON = particulate organic nitrogen, TSS = total suspended solids. Nutrients were only analyzed once per station (so day and night samples for microbial community analysis have the same nutrient values). ** Summer and fall geochemistry was determined on samples taken at 90 and 80 m depth, respectively.

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Sampling event	Date	Time	Depth (m)	Volume (L)	T (°C)	DO (mg/L)	Chl (µg/L)	TP (µg/L)	PP (µg/L)	POC (mg/L)	DOC (mg/L)	PON (mg/L)	SiO_2 (mg/L)	TSS (mg/L)	PAR (W/m2)
Sp.FL/PA.MLB.S.N	4/24	2:09 AM	5	2.8	-	-	-	-	-	-	-	-	-	-	-
Sp.FL/PA.M15.S.N	4/24	1:10 AM	5	4	5.59	15.00	5.67	27.82	22.30	0.59	3.66	0.08	2.53	7.30	0.00
Sp.FL/PA.M15.S.D	4/23	10:40 AM	5	7.5	4.82	15.00	5.67	27.82	22.30	0.59	3.66	0.08	2.53	7.30	25.78
Sp.FL/PA.M15.D.D	4/23	1:30 PM	10	9.5	4.82	15.00	-	-	-	-	-	-	-	-	1.29
Sp.FL/PA*.M110.S.N	4/23	10:00 PM	5	7.5	2.71	13.50	0.68	3.42	1.80	0.09	2.22	0.01	1.82	0.12	0.00
Sp.FL/PA.M110.S.D	4/23	5:45 PM	5	8.5	2.81	13.50	0.68	3.42	1.80	0.09	2.22	0.01	1.82	0.12	15.99
Sp.FL*/PA.M110.D.D	4/23	6:36 PM	108	9	3.17	15.00	-	-	-	-	-	-	-	-	0.00
Su.FL*/PA.MLB.S.D	7/15	6:15 PM	1	10	27.99	8.64	-	-	-	-	-	-	-	-	1042.60
Su.FL*/PA.MLB.D.D	7/15	6:55 PM	8	8.5	17.56	-0.42	-	-	-	-	-	-	-	-	0.04
Su.FL/PA.M15.S.N	7/15	9:25 PM	5	10	16.99	9.24	2.38	5.86	3.90	0.38	6.13	0.05	1.12	1.12	85.08

Su.FL/PA.M15.S.D	7/16	2:35 PM	5	10	16.06	7.34	2.38	5.86	3.90	0.38	6.13	0.05	1.12	1.12	834.83
Su.FL/PA.M15.D.N	7/15	10:00 PM	15	10	9.13	10.19	-	-	-	-	-	-	-	-	9.62
Su.FL/PA.M110.S.N	7/16	3:25 AM	5	10	21.91	8.19	1.03	5.08	3.08	0.21	2.52	0.03	1.25	0.56	0.00
Su.FL/PA.M110.S.D	7/16	8:41 AM	5	10	20.88	7.67	1.03	5.08	3.08	0.21	2.52	0.03	1.25	0.56	134.83
Su.FL/PA.M110.M.D	7/16	9:20 AM	36	10	5.65	11.40	2.96	5.16	2.68	0.19	2.14	0.03	1.82	0.58	4.04
Su.FL.M110.D.N	7/16	4:03 AM	110**	10	4.28	11.34	0.24	2.99	1.41	0.05	2.11	0.01	1.85	0.10	0.00
Fa.FL*/PA.MLB.S.N	9/23	7:45 PM	2	10	18.61	-	-	-	-	-	-	-	-	-	2.70
Fa.FL*/PA.MLB.D.N	9/23	8:10 PM	8	10	17.08	-	-	-	-	-	-	-	-	-	0.00
Fa.FL/PA.M15.S.N	9/23	9:18 PM	5	11	13.01	14.50	1.33	4.15	2.93	0.26	2.07	0.03	1.46	0.53	0.00
Fa.FL/PA.M15.S.D	9/24	1:30 PM	5	9	14.32	14.50	1.33	4.15	2.93	0.26	2.07	0.03	1.46	0.53	417.72
Fa.FL/PA.M15.D.N	9/23	10:00 PM	15	10	12.00	14.50	-	-	-	-	-	-	-	-	-
Fa.FL/PA.M110.S.N	9/24	3:04 AM	5	10	15.00	13.50	1.17	4.77	2.55	0.38	2.11	0.05	1.18	0.58	0.00
Fa.FL/PA.M110.S.D	9/24	9:28 AM	5	10.5	17.33	13.50	1.17	4.77	2.55	0.38	2.11	0.05	1.18	0.58	19.83
Fa.FL/PA.M110.D.N	9/24	4:02 AM	108**	10.5	4.27	15.00	0.16	2.55	0.88	0.07	1.85	0.01	1.94	0.09	0.00

Table 2: Nested PERMANOVA analysis of bacterial community dissimilarity. R^2 and p values between parentheses for PERMANOVA analysis performed on the data after removal of taxa with average relative abundances < 0.1% (139 OTUs retained). * Sp = spring, Su = summer, Fa = fall; ** ML = Muskegon Lake, LM = Lake Michigan; *** S = surface, M = chlorophyll maximum, D = deep.

Factor	Fraction	Season*	Lake**	Station	Depth***	Diel	Residuals
Values	*PA, FL*	*Sp, Su, Fa*	*ML, LM*	*MLB, M15, M110*	*S, M, D*	*Day, Night*	-
Bray-Curtis (n = 47)	0.28 (0.001)	0.2 (0.001)	0.08 (0.001)	0.03 (0.007)	0.05 (0.006)	0.01 (0.445)	0.36
Sørensen (n = 47)	0.11 (0.001)	0.31 (0.001)	0.20 (0.001)	0.06 (0.001)	0.06 (0.002)	0.01 (0.214)	0.25

Table 3: Summary of BIOENV analyses. Spearman correlations of between-sample physical and geochemical differences (Euclidean distance) and community composition differences

(Bray-Curtis dissimilarity, 139 OTUs > 0.1% average abundance). The best combination of parameters is shown for analyses using all samples (PA and FL), and separate analyses per fraction (PA, FL). P-values supporting whether the correlation coefficient were significantly different from zero were determined using a random permutation test. The boldface type parameter is the strongest single correlating factor (correlation coefficient and p-value between parentheses). Analyses only included samples for which data was available for all environmental factors (see Table 1).

Biological data	Environmental factors	Best match	R	P value
PA and FL	T, DO, log(Chla), log(TP), POC, PON, log(DOC), SiO2, log(TSS), log(PAR)	T, **DO**, log(Chla), log(DOC)	0.14 (0.12)	0.26 (0.38)
PA		**T**, log(TSS)	0.77 (0.62)	<0.01 (<0.01)
FL		T, **log(Chla)**, SiO_2	0.76 (0.64)	<0.01 (<0.01)

Figure 1: Sampling sites and sonde data. (A-B) Map (© Google) of Great Lakes region with detail of the Muskegon Lake freshwater estuary and the NOAA GLERL Lake Michigan transect. (C) Water temperature and derived ~~chlorophyll~~ chl*a* obtained from the Muskegon Lake Buoy, which was deployed from May 15 until November 2, 2013. Summer and Fall sampling dates are highlighted in red. (D-F) Lake Michigan water temperature profiles and (G-I) derived ~~chlorophyll~~ chl*a* at the times of sampling, as measured using the plankton survey system data. Red and black circles indicate the sampling stations on the profiles.

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Figure 2: Observed bacterial richness and Simpson's evenness. (A-D) Observed richness and (E-H) Simpson's evenness comparisons based on (A,E) season, (B,F) sampling station, (C,G) filter fractions (FL = free-living, PA = particle-associated), and (D,H) sampling depth (See table 1 for specific depths). Individual sample data was plotted within boxplots and colored by season. Letter(s) below each boxplot identify sample groups within each plot that have significantly different median richness or evenness, as determined by pairwise post-hoc testing ($p < 0.05$) of the Kruskall-Wallis test results. All libraries were rarefied to the smallest library size after merging of replicates (70,480 sequences).

Figure 3: Principal coordinates analysis (PCoA). Ordinations of the first two coordinates based on (A) Bray-Curtis and (B) Sørensen dissimilarity between bacterial communities. The analysis only included the 139 OTUs with average relative abundance > 0.1 %. Percentages next to each coordinate label indicate % of total sample variation represented by the coordinate.

Figure 4: Dissimilarities between surface communities along the transect over time. Boxplots represent the Bray-Curtis dissimilarities between samples when considering the 139 OTUs > 0.1 % average relative abundance. Within each season, dissimilarities were determined between samples obtained from surface water at the estuary (MLB), nearshore (M15) and offshore (M110) station. Comparisons were made within each fraction, not between. Letter(s) below boxplots differentiate sample groups that differ significantly in dissimilarity (i.e., between station comparisons with significantly different medians do not share any letters), as determined by pairwise post-hoc testing ($p < 0.05$) of the Kruskall-Wallis test results.

Figure 5. Dissimilarities among and between PA and FL fraction communities. (A) Boxplots represent the Bray-Curtis dissimilarities between samples when considering the 139 OTUs > 0.1 % average relative abundance. Dissimilarities were determined within the FL fraction and within the PA fraction (X-axis). Data points within each boxplot were colored to allow evaluation of matching comparisons for the PA and FL fractions. The comparisons from left to right are listed in the legend from top to bottom, from more similar (same season and station, to more dissimilar (different season, different station). The medians of FL and PA dissimilarities significantly differed (Kruskall-Wallis test, $p < 0.05$). (B) Differential abundance of OTUs categorized by functional group based on carbon and energy source. Energy source: O = chemoorganotroph, P = phototroph (BR = bacteriorhodopsin, C = ~~chlorophyll~~ chl*a*, BC = bacteriochlorophyll), L = chemolithotroph (S = sulphur, N = NH_4^+).

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Figure 6: Differential abundance of OTUs categorized by functional group based on carbon and energy source. OTUs with average relative abundance across all samples > 0.1 % were included for analysis of differences in relative abundance using DEseq. Comparison were made separately for (A, C, E, G) FL and (B, D, F, H) PA communities between (A,B) lake, (C-F) seasons, and (G,H) depth while controlling for variation of all other factors. See Table S2 for details on functional group classification. Energy source: O = chemoorganotroph, P = phototroph (BR = bacteriorhodopsin, CHL = ~~chlorophyll~~ chl*a*, BCHL = bacteriochlorophyll), L = chemolithotroph (S = sulphur, N = NH_4^+). Numbers next to X-axis category labels indicate number of OTUs that were significantly ($p < 0.01$) more abundant in that category.

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Figure 7: Line plots of select OTUs. Relative abundance of OTUs across space and time. Plot background color reflects energy source.