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ABSTRACT

This study examined vertically-resolved patterns in microbial community structure across a freshwater trophic gradient extending 1600 km from the oligotrophic waters of Lake Superior to the eutrophic waters of Lake Erie, the most anthropogenically influenced of the Laurentian Great Lakes system. Planktonic bacterial communities clustered by Principal Coordinates Analysis (PCoA) on UniFrac distance matrices into four groups representing the epilimnion and hypolimnion of the upper Great Lakes (Lakes Superior and Huron), Lake Superior's northern bays (Nipigon and Black bays), and Lake Erie. The microbes within the upper Great Lakes hypolimnion were the most divergent of these groups with elevated abundance of *Planctomycetes* and *Chloroflexi* compared to the surface mixed layer. Statistical tests of the correlation between distance matrices identified temperature and sample depth as the most influential community structuring parameters, reflecting the strong UniFrac clustering separating mixed-layer and hypolimnetic samples. Analyzing mixed-layer samples alone showed clustering patterns were correlated with nutrient concentrations. Operational taxonomic units (OTU) which were differentially distributed among these conditions often accounted for a large portion of the reads returned. While limited in coverage of temporal variability, this study contributes a detailed description of community variability that can be related to other large freshwater systems characterized by changing trophic state.

Introduction

Anthropogenic eutrophication is a primary threat to the ecological integrity of freshwater resources (Schindler, 2006). The Laurentian Great Lakes, Earth's largest freshwater system, have experienced local shifts in productivity, and anthropogenic eutrophication remains a problem (Beeton, 1965; Dove and Chapra, 2015; Sterner et al., 2017). However, transitions in trophic state are fluid, reflecting ecosystem responses not only to direct human influence and subsequent directed management efforts (Bunnell et al., 2014), but also to events indirectly influenced by humans such as biological invasions (e.g. Hecky et al., 2004) and climate warming (e.g. Beall et al., 2016). Indeed, recent studies describe both the oligotrophication (Barbiero et al., 2012) and re-eutrophication (Kane et al., 2014; Scavia et al., 2014; Watson et al., 2016) of lakes in the Great Lakes system.

Among the Great Lakes, Lakes Superior and Erie represent extremes in trophic state (Allan et al., 2013; Dove and Chapra, 2015; Sterner, 2011) and are ecologically most divergent (Riseng et al., 2018). Lake Superior is the headwater of this system, and is oligotrophic, cold, deeply mixed and maintains a high stoichiometric imbalance of chemical nitrogen over phosphorus (Sterner et al., 2007; Sterner, 2011). It has continuous, near-saturation oxygen levels throughout the water column year-round, with oxygen extending deep (4 to >12 cm) into surface sediments (Li et al., 2012). Its watershed is home to >600,000 people. Its near-pristine condition has remained stable with long-term trends showing little variation in anthropogenic indicators such as total dissolved solids and major ions (Chapra et al., 2012). Lake Erie, in contrast, has a human-dominated watershed that is home to >12 million people. Intensive row crop agriculture in the western basin watershed, combined with lake morphometry and a short residence time (2.6 yr),

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have accelerated eutrophication, particularly of the western and central basins of the lake. The combined effects of directed management (Makarewicz and Bertram, 1991) and the unintended consequences of invasive dreissenid mussels (Nicholls and Hopkins, 1993) resulted in increased transparency in the 1980s and 1990s, but the Lake has since entered into a period of re-eutrophication (Kane et al., 2014; Scavia et al., 2014; Watson et al., 2016), characterized by blooms of pelagic (Bullerjahn et al., 2016; Michalak et al., 2013; Twiss et al., 2012) and benthic algae (Higgins et al., 2008) and the intensification of seasonal central basin hypoxia (Hawley et al., 2006; Scavia et al., 2014; Zhou et al., 2015).

Lake trophic state is an important factor influencing the diversity and composition of microbial communities (Horner-Devine et al., 2003; Jankowski et al., 2014; Newton and McLellan, 2015; Smith, 2007). Yet our knowledge of the spatial and seasonal microbial diversity in the Laurentian Great Lakes is limited. With a few exceptions (Denef et al., 2016a, 2016b; Fujimoto et al., 2016; Newton and McLellan, 2015; Olapade, 2018; Paver et al., 2018; Reed and Hicks, 2011), our knowledge of planktonic microbial diversity in the Great Lakes is largely restricted to recent next-generation sequencing studies of Lake Erie (e.g. Beall et al., 2016; Berry et al., 2017; Edgar et al., 2016; Harke et al., 2016; Mou et al., 2013a, 2013b; Salk et al., 2018; Steffen et al., 2015; Wilhelm et al., 2014), arguably the lake most influenced by human activity and eutrophication. Here, we describe the planktonic microbial diversity recorded during a 3200 km round-trip limnological survey from western Lake Superior to eastern Lake Erie (Fig. 1), spanning a scale of spatial biodiversity analysis that has been undertaken for only a few freshwater systems, all lotic (Henson et al., 2018; Kolmakova et al., 2014; Payne et al., 2017; Satinsky et al., 2015; Savio et al., 2015). The survey was conducted over a period of two weeks during the summer stratified period, and provided a unique opportunity to characterize changes in microbial communities along the trophic gradient of the largest lake system on Earth. Specifically, we sought to resolve shifts in microbial community structure between lake trophic status and with season and thermal stratification. The survey began and ended in western Lake Superior, meaning that samples for this basin were taken at two times spanning the development of thermal stratification. Additional samples from Lakes Superior and Erie, collected mainly during the stratified season, were incorporated to supplement the transect data and provide further spatial and temporal variation for the analysis.

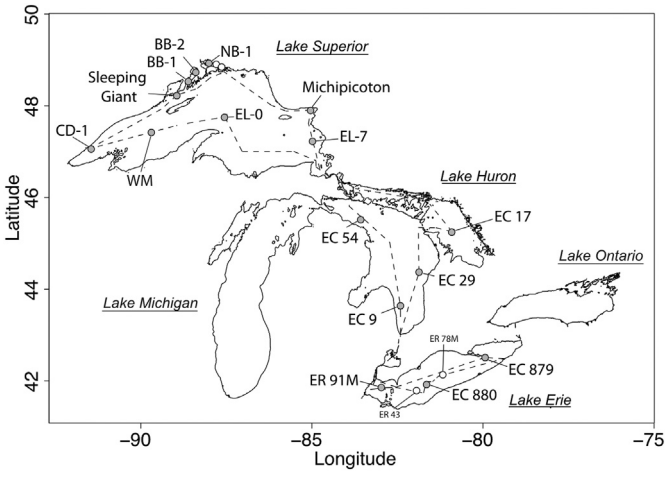

Fig. 1. Location of stations sampled in 2011 and 2012. Samples were collected in July 2011 aboard R/V *Blue Heron* 2 during a 3200 km roundtrip survey through Lakes Superior, Huron, Erie and associated bays. Dashes show actual survey track. Several sites in western Lake Superior were revisited during surveys in 2012 (Table 1). Likewise, sites in Lake Erie's central basin were revisited as part of seasonal monitoring surveys aboard R/V *Lake Guardian* and CCGS *Limnos* (Table 1).

Methods

Study sites and sampling

Samples were collected between July 14–29, 2011 aboard R/V *Blue Heron* during a 3200 km round-trip survey through Lakes Superior, Huron, and Erie (Fig. 1, Table 1). One site in western Lake Superior (WM) was visited during a survey in 2012. Likewise, additional sites in Lake Erie's central basin were visited in spring 2011 and summer 2012 as part of seasonal monitoring surveys aboard R/V *Lake Guardian* and CCGS *Limnos*, respectively. At each hydrographic station, sampling was preceded by a conductivity–temperature–depth (CTD) cast. Water was sampled from discrete depths within the epilimnion and hypolimnion of each lake (Table 1) using Niskin bottles attached to the CTD rosette and initially passed through a mesh filter of 80-μm pore size to remove zooplankton and large detritus. Depth-resolved samples were processed for total (>0.2 μm) chlorophyll (chl) *a* biomass and for dissolved (<0.2 μm) and particulate nutrients. Chl *a* biomass was measured by fluorometry following extraction in 90% (v/v) acetone at −20 °C (Welschmeyer, 1994). During the 2011 multi-lake survey, subsamples for nutrient determination were frozen in polyethylene bottles on board ship for subsequent laboratory analysis at the University of Minnesota. Nitrate was measured using a Lachat QuickChem 8500 Flow Injection Analysis System (Hach Co., Loveland, CO, USA) and a cadmium reduction column whereas measurement of total dissolved phosphate followed the ascorbic acid-molybdate method (Parsons et al., 1984). A fluorometric method was used to measure NH_4^+ as described previously (Kumar et al., 2007; Small et al., 2013).

For a subset of stations (Electronic Supplementary Material (ESM) Table S1), particulate organic C, N, and P measurements, seston passing through the 80-μm mesh was further fractionated into a picoplankton size fraction (<2 μm) and collected onto precombusted 25 mm GF/F filters, frozen, and then dried at 60 °C as described elsewhere (Sterner, 2011). Particulate organic carbon (POC) and nitrogen (PON) were determined with a 2400 Series II CHN analyzer (Perkin Elmer, Waltham, MA, USA) using acetanilide as a standard. Samples for particulate organic phosphorus (POP) were digested in a 5% potassium persulfate solution and autoclaved for 30 min. Liberated soluble reactive phosphorus was analyzed with the ascorbic acid-molybdate method (Parsons et al., 1984).

Flow cytometry

Samples were fixed in phosphate-buffered formaldehyde (final concentration 1% v/v) and flash-frozen in liquid nitrogen. Samples were kept in liquid nitrogen or at −80 °C until thawing and immediate analysis. Fixed samples were thawed and then incubated at room temperature in the dark for 15 min with SYBR Green I (Invitrogen, Life Technologies, Grand Island, NY, USA) to stain DNA (Marie et al., 1997). Flow cytometry samples were analyzed on a FACSCalibur flow cytometer (Becton-Dickinson, San Jose CA, USA). All data acquisitions were done with logarithmic signal amplification. Cytometer sample flow rates were calibrated using bead stocks of known concentration (Calibrite beads, Becton-Dickinson) and particle sizes were calibrated using beads of known diameter (Flow Cytometry Size Bead Kit, Invitrogen, Life Technologies, Grand Island, NY, USA). Bacterial heterotrophs were identified by their size using the side scatter channel (SSC) and SYBR Green fluorescence (FL1). Cell abundances were calculated from acquisition duration, the number of events, and instrument flow rate.

Leucine incorporation

Cellular incorporation of ^{3}H-leucine followed the protocol described by Kirchman (2001) and served as a proxy for bacterial production. Triplicate depth-resolved samples from each site were spiked with 4,

Table 1
Sampling sites and physico-chemical parameters from surveys.

Station	Date	Depth		Temperature	DO	Chl *a*	NH_4^+	NO_3^-	P[d]	Latitude	Longitude
		Site	Sample	(°C)	(mg/L)	(μg/L)	(μmol/L)	(μmol/L)	(μmol/L)		
Upper Great Lakes											
CD-1 (Su)[a]	7-14-2011	250	5	8.5	13.1	0.46	0.19	25.79	0.05	47.063	−91.432
			150	3.8	14.5	0.22	0.43	26.55	nd		
	7-29-2011		2	16.4	10.7	0.43	0.38	24.18	0.04		
			150	3.8	14	0.05	0.59	26.61	0.03		
EL-0 (Su)[a]	7-15-2011	165	5	7.1	13	0.29	0.11	26.71	nd	47.75	−87.5
			145	3.8	14.4	0.09	0.44	26.48	0.05		
EL-7 (Su)[a]	7-26-2011	132	124	3.8	14.3	0.14	0.51	26.85	0.04	47.167	−85.017
Michipicoten	7-26-2011	80	5	16.7	10.5	0.46	0.34	23.36	0.04	47.908	−84.007
(Su)[a]			78	4.0	14.2	0.06	0.37	26.18	0.04		
Sleeping Giant (Su)[a]	7-29-2011	272	5	13.6	11.3	0.44	0.4	22.64	nd	48.223	−88.906
			240	3.6	14.1	0.11	0.36	26.49	0.03		
EC 17 (Ge)[b]	7-24-2011	80	5	20.8	9.4	0.14	0.44	18.75	0.02	45.245	−80.864
			73	4.1	14.5	0.09	0.50	20.56	0.03		
EC 29 (Hu)[c]	7-24-2011	125	5	21.2	9.3	0.11	0.41	23.47	0.02	44.367	−81.833
			119	3.8	14.3	0.02	0.61	23.22	0.02		
EC 54 (Hu)[c]	7-17-2011	125	5	17.8	10.2	0.26	0.26	20.44	0.04	45.517	−83.417
			100	3.9	14.5	0.06	0.47	22.75	0.03		
EC 9 (Hu)[c]	7-17-2011	50	5	20.4	9.7	0.21	0.3	24.48	nd	43.633	−82.378
			48	4.8	14.3	0.58	0.50	17.59	0.04		
WM (Su)[a]	8-14-2012	192	5	6.8	12.4	0.57	nd	23.58	nd	47.313	−89.78
			90	4.2	12.6	0.11	nd	26.96	nd		
			185	3.8	12.5	0.09	nd	27.17	nd		
Upper Bays (Su)[a]											
Black-1	7-28-2011	22	2	16.5	10.1	0.28	0.30	21.11	0.10	48.5	−88.608
Black-2	7-28-2011	8	2	18.7	9.5	1.71	0.23	15.61	0.16	48.692	−88.397
Nipigon-1	7-27-2011	27	5	17.8	9.8	0.58	0.29	16.12	0.08	48.933	−88.001
Lake Erie											
ER 78M	4-12-2011	24	2	1.8	15.1	7.68	nd	2.57	0.19	41.788	−81.945
ER 43	4-12-2011	23	2	2.6	14.2	7.2	nd	2.21	0.12	42.117	−81.250
91M	7-18-2011	10	8	25.8	8.2	0.47	1.86	38.38	0.21	41.841	−82.917
EC 880	7-19-2011	25	5	24.1	8.7	0.57	0.64	7.38	nd	41.917	−81.633
			22	9.2	7.8	0.34	4.48	3.75	nd		
	7-24-2012		1	24.3	9.2	1.8	nd	1.21	nd		
			22	12.6	1.6	0.7	nd	20.99	nd		
EC 879	7-22-2011	60	5	23.5	9.1	0.52	0.58	6.66	0.11	42.507	−79.9
			25	5.9	13.6	0.91	2.76	11.00	0.11		
			60	4.6	12.3	0.02	2.77	12.15	0.02		
EC 1326	8-17-2012	25	1	nd	nd	nd	nd	nd	nd	41.733	−81.698

nd: not determined.
[a] Lake Superior.
[b] Georgian Bay.
[c] Lake Huron.
[d] Total dissolved phosphate.

5 ^{3}H-leucine ([0.75 μCi; specific activity: 54.1 Ci/mmol] MP Biomedicals, Solon, OH, USA) to a final concentration of 10 nmol/L and incubated in darkness at in situ temperature for 2 h. Trichloroacetic acid (TCA) was added to 5% (v/v) final concentration and seston collected by gentle filtration (<120 mm Hg) onto 0.2 μm polycarbonate membranes. Filters were rinsed twice with chilled TCA and then transferred to 7 mL scintillation vials. Acid-precipitable ^{3}H-leucine incorporation into protein was measured by liquid scintillation counting following the addition of Ready Safe cocktail (Beckman Coulter, Inc., Brea, CA, USA) to each vial. Background activity was determined at $t = 0$ by dispensing a sample aliquot directly into TCA prior to processing as above. Leucine incorporation rates were converted into bacterial carbon production estimates using the conversion factor (1.5 kg C/mol leucine) provided by Kirchman (2001).

High-throughput microbial community analysis

Depth-resolved total planktonic seston was concentrated by manual filtration using a 60 cc syringe connected by luer lock to a Sterivex cartridge filter (0.22 μm; EMD Millipore, Billerica, MA, USA) following which the cartridges were immediately frozen in liquid nitrogen prior to storage at −80 °C. DNA was extracted using the PowerWater Sterivex DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's instructions. Short 16S rRNA gene Illumina amplicon tag (iTag) sequencing of the V4 hypervariable region of bacterial and plastid genomes was completed at the Joint Genome Institute (JGI; Walnut Creek, CA, USA) using an Illumina MiSeq benchtop sequencer (2 × 250 bp reads) incorporating a PhiX library control according to standard JGI procedures (Tremblay et al., 2015). Primer design for universal amplification of the V4 region of 16S rDNA was based on Caporaso et al. (2011), with the forward primer (515F) unchanged but with modest variation to the reverse primers (806R) described by Tremblay et al. (2015). Resulting sequences were demultiplexed and contaminating Illumina adaptor sequences were removed using the kmer filter in BBDuk (v37.62) following Singer et al. (2016). Briefly, BBDuk was used to remove reads containing >1′N′ base, or with a quality score < 10 across the read, or length ≤ 51 bp or 33% of the full length read. Additional processing using BBMap (http://bbtools.jgi.doe.gov) mapped reads to masked human, cat, dog and mouse references, discarding hits exceeding 93% identity (Singer et al., 2016).

Processing, clustering and classification of sequenced reads were performed as described previously (Beall et al., 2016; McKay et al., 2015). Briefly, iTags were processed in QIIME 1.8.0 (Caporaso et al., 2010a) using default settings, unless noted otherwise. OTUs were

picked using UCLUST at 97% identity (Edgar, 2010) and OTUs represented 3 or fewer times in the data set were filtered along with those shorter than 200 bp. A representative set of sequences was generated, where the most abundant sequence represented its respective OTU. Taxonomy was assigned to each representative sequence using the RDP classifier (Wang et al., 2007) with a minimum confidence of 80% for taxonomy assignment. Assignment was based on the Greengenes taxonomy (McDonald et al., 2012) and reference database version 12_10 (Werner et al., 2012).

For analysis of bacterial populations, reads that were assigned to chloroplast and mitochondrial sequences, those that were unclassified or unassignable, and those not identifiable beyond bacteria were filtered. Those reads matching 'chloroplast' were then used in subsequent analyses to examine the photosynthetic eukaryotic community. The combined total bacterial and chloroplast reads are referred to as 'total reads'. The representative sequences were aligned to the Greengenes core reference alignment (DeSantis et al., 2006) using PyNAST (Caporaso et al., 2010b) and gaps in the resulting alignment were filtered. A phylogenetic tree for all bacterial OTUs was generated from the filtered alignment using FastTree 2.1.3 (Price et al., 2010). The samples were then rarefied in QIIME.

Taxonomic analysis of the summer bacterial community was based on a total of 1.41×10^6 16S rRNA iTag sequences from 18 samples collected along the 2011 transect, together with an additional 17 samples collected mainly during the stratified season in 2011 and 2012. Output from individual sites ranged between 27,322 to 142,219 reads per sample (Electronic Supplementary Material (ESM) Fig. S2). Combined, the average sequencing depth was 73,000 reads per sample.

Statistical analysis

Alpha and beta diversity were resolved through QIIME (Caporaso et al., 2010a). Phylogenetic measures of community beta diversity, a measure of diversity between individual samples from different environments, were visualized using Principal Coordinates Analysis (PCoA) of UniFrac distance matrices (Lozupone and Knight, 2005) at a rarified depth of 20,000 reads. The significance of PCoA clustering was tested using analysis of similarities (Clarke, 1993) with 999 permutations, each cluster being tested against the remainder of samples.

The importance of environmental features on PCoA clustering was explored using QIIME's BEST analysis, an implementation of BIOENV (Clarke and Ainsworth, 1993). Distance matrices were generated for each physico-chemical parameter (QIIME), and correlations of physico-chemical measurement matrices and UniFrac plots was examined using the Mantel test with 999 permutations.

The analysis of relative abundance differences was conducted with R (v3.2.1; R Core Team, 2018) using the DESeq2 (v1.8.1) and phyloseq (v1.12.2) packages (Anders and Huber, 2010; Love et al., 2014; McMurdie and Holmes, 2013). OTUs which were significantly increased or decreased under a given condition were those identified using the negative binomial method implemented by DESeq2 as having differential abundance and an adjusted p-value of <0.01 (Wald test, local fit). Sample groupings that were compared using DESeq2 were based primarily on clustering patterns observed in unweighted UniFrac PCoA clustering. The co-occurrence of differentially abundant OTUs shared between DESeq2 analyses was examined in R using the RNeo4j (v1.2.0) package.

Sequence data deposition

Sequence data were deposited in the Sequence Read Archive of the National Center for Biotechnology Information under the accession numbers SRA211417.

Results and discussion

Physico-chemical profiles and elemental stoichiometry of size-fractionated seston along the transect

Surface mixed-layer waters of the upper Great Lakes (Superior and Huron) differed in temperature from deep waters with some evidence of modest surface water depletion of both NH_4^+ and NO_3^- (Table 1), consistent with seasonal biological uptake (Sterner et al., 2007). Within-lake spatial variability in physico-chemical parameters was substantial in Lake Erie (Table 1) and consistent with the trophic gradient existing within the lake (Ludsin et al., 2001). Lake Erie's shallow eutrophic western basin (Station ER 91M) was thoroughly mixed with elevated nitrogen concentrations compared to mixed layer water chemistry in the oligotrophic eastern basin (Station EC 879; Table 1).

Seston sampled from the surface mixed layer in the upper Great Lakes was depleted in phosphorus compared to samples from depth (Fig. 2; ESM Table S1), a pattern described previously during the summer stratified period (Sterner, 2011). For both total (<80 μm) and picoplankton (<2 μm) size fractions, molar ratios of N:P and C:P were ~two-fold higher in the epilimnion compared to the hypolimnion (ESM Table S1; paired t-test, $p < 0.05$). For the total seston fraction, median mixed layer molar ratios of N:P and C:P of 33 ($n = 6$) and 289 ($n = 6$), respectively, suggested phosphorus-depleted surface communities. While temperature can influence elemental stoichiometry and may factor into these results (Cotner et al., 2006; Martiny et al., 2013), these data are consistent with the characterization of the upper Great Lakes as phosphorus deficient, oligotrophic environments (Barbiero et al., 2012; Bunnell et al., 2014; Sterner, 2011). However, C:N:P stoichiometry of the picoplankton fraction, which comprised the free-living microbial community analyzed in this study, was not suggestive of strong phosphorus deficiency. While median mixed layer picoplankton N:P (18, $n = 6$) and C:P (161, $n = 6$) molar ratios were above Redfield stoichiometry (C:N:P = 106:16:1 by moles), these values were diagnostic of only modest phosphorus deficiency for the Great Lakes below the threshold ratios of 22 (N:P) and 258 (C:P) proposed by Guildford and Hecky (2000). Whereas cellular C and N concentrations were ~two-fold higher among total seston compared to picoplankton, cellular P increased by less than one-fold in the total fraction thus driving the change in stoichiometric ratios between these communities. This may have reflected biomolecular modifications such as phospholipid substitution (Bellinger et al., 2014) or utilization of refractory phosphonate compounds (Ilikchyan et al., 2009; Kutovaya et al., 2013) adopted by the microbial community in phosphorus-depleted environments such as Lake Superior which may lower P requirements possibly contributing to stoichiometric flexibility reported among lacustrine bacterial heterotrophs (Godwin and Cotner, 2015).

In contrast to the upper Great Lakes, Lake Erie showed little evidence of depth or seston size-related trends in N:P and C:P seston stoichiometry (Fig. 2; ESM Table S1). Furthermore, regardless of depth, samples showed no evidence of phosphorus deficiency, with median elemental ratios lower than diagnostic thresholds stated earlier for phosphorus limitation.

Water column profiles of seston C:N in the upper Great Lakes did not vary between surface mixed layer and hypolimnion for either total (paired t-test [5] = 0.56, $p = 0.599$) or picoplankton (paired t-test [5] = −0.08, $p = 0.942$) size fractions (Fig. 2; ESM Table S1). Furthermore, mixed layer seston C:N was not different between total and picoplankton size fractions (paired t-test [5] = 1.44, $p = 0.209$). Likewise in Lake Erie, there was no discernible pattern of C:N with depth (paired t-test [2] = −0.98, $p = 0.430$) nor did mixed layer seston C:N vary between total and picoplankton size fractions (paired t-test [6] = 0.27, $p = 0.796$) (Fig. 2; ESM Table S1). For all lakes, while median mixed layer C:N ratios were higher than Redfield stoichiometry (8.5 and 12.7 for the upper Great Lakes and Lake Erie, respectively), the metric was diagnostic only for moderate nitrogen deficiency (C:N: 8.3–14.6;

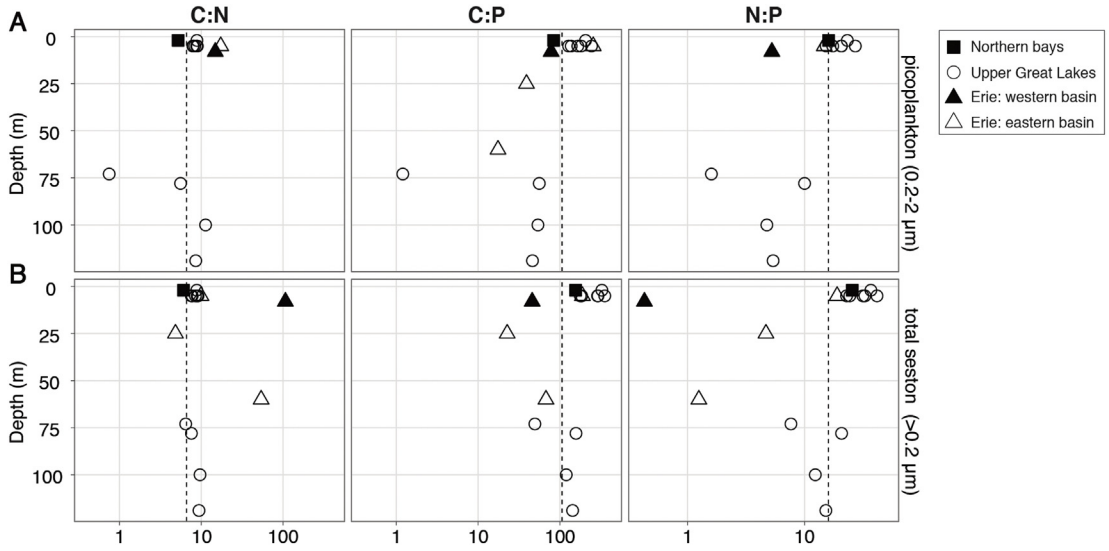

Fig. 2. Seston molar C:N:P nutrient ratios for (A) picoplankton (0.2–2 μm) and (B) total (>0.2 μm) size fractions. Redfield ratio values are indicated by the vertical dashed line.

Guildford and Hecky, 2000). Whereas Lake Erie has been characterized historically as a phosphorus-limited system (Guildford et al., 2005; Lean et al., 1983), evidence for seasonal nitrogen deficiency has accumulated in recent years (Chaffin et al., 2013; Davis et al., 2015; North et al., 2007; Salk et al., 2018), consistent with reduced watershed loading of nitrogen into the lake over the past two decades (Stow et al., 2015) combined with active dissimilatory sinks for nitrate (Salk et al., 2018; Small et al., 2016).

Microbial community composition and dynamics associated with lake trophic state

Whereas 16S rRNA gene tag (iTag) Illumina sequencing showed total sample reads were dominated by *Bacteria* (ESM Fig. S1), chloroplast reads accounted for 8.1% of total reads by average, with diatoms the most abundant chloroplast OTUs among eukaryotic phytoplankton at most sites in the upper Great Lakes (ESM Figs. S2, 3). This is consistent with previous studies of summer phytoplankton communities in Lakes Superior and Huron, which report a predominance of diatoms (Barbiero and Tuchman, 2001; Reavie et al., 2014). By contrast, the summer communities in Lake Erie showed fewer diatoms and higher abundances of cryptomonad OTUs. This is in stark contrast to winter (Beall et al., 2016; Wilhelm et al., 2014) and early spring (ESM Fig. S1: Stations ER 43 and ER 78 M) in Lake Erie, where diatoms overwhelmingly dominate the algal community and diatom chloroplast 16S rRNA gene reads frequently surpass those of *Bacteria*.

At a rarified sequencing depth of 20,000 reads, rarefaction curves indicated that diversity was not fully captured in all samples (ESM Fig. S3). This was likewise reflected by the Chao1 species richness estimator, which showed that 57% (±4) of all prokaryotic OTUs were observed in their highest rarefied depth (ESM Table S2). Observed OTU counts did not vary with lake (one-way ANOVA; $p = 0.20$), between surface mixed layer and deep samples (one-way ANOVA; $p = 0.25$), or by year collected (one-way ANOVA; $p = 0.47$) averaging 1402 OTUs across all sites at a depth of 20,000 reads (ESM Table S2). Whereas overall bacterial community richness was comparable to several recent studies of the Great Lakes, spatial and temporal differences in richness have been reported (Berry et al., 2017; Fujimoto et al., 2016).

Bacterial cell abundance showed only modest differences across sample locations during the 3200 km round-trip survey in 2011, yet rates of tritiated-leucine incorporation varied markedly along the transect (Fig. 4). Rates measured from Lake Superior embayments (Sleeping Giant [Thunder Bay] and Nipigon Bay) were the highest measured during the survey, yet elsewhere in Lake Superior, rates of leucine incorporation among bacterial populations in the mixed layer were two orders of magnitude lower than measured in the bays and in Lake Erie (two-tailed *t*-test [6] = −4.55, $p < 0.005$). Likewise, PCoA on UniFrac distance matrices showed clear differences in the surface mixed layer microbial community composition along the trophic gradient, both between the upper Great Lakes and Lake Erie and among the basins of Lake Erie (Fig. 5). An analysis of unweighted UniFrac distance matrices revealed that samples clustered primarily by location and depth, with sample clustering representative of Lake Superior's northern bays (Nipigon and Black bays), the upper Great Lakes surface mixed layer, the upper Great Lakes hypolimnion, and Lake Erie (Fig. 5A). Stronger dissimilarities between hypolimnetic samples from the upper Great Lakes and elsewhere were revealed by accounting for the relative abundance of each OTU (weighted UniFrac), underscoring the divergence of these communities (Fig. 5B). Mixed layer samples retained similar clustering patterns, except samples from Lake Erie, which were spread along PC2. Lake Erie's summer mixed layer samples clustered with those of Lake Superior and Lake Huron, whereas the hypolimnion of Erie's oligotrophic eastern basin and spring samples from Lake Erie during the same year did not. Several samples were outliers to this clustering pattern. With 1.5-fold higher abundance of *Verrucomicrobia* (including a single OTU of *Opitutae* contributing >10% of total reads) and *Bacteroidetes* and commensurate declines in *Planctomycetes* and *Chloroflexi* (Fig. 3), the hypolimnetic community at station EC 17 in Lake Huron's Georgian Bay clustered away from other deep-water sites in both analyses. Likewise, samples from Lake Erie's central basin hypoxic zone at station EC 880 in 2012 were separated from the remainder of samples in the unweighted analysis, where they were positioned opposite those of the upper Great Lakes mixed layer along PC2. Recent analysis of sediment microbial communities in the Great Lakes system identified samples from Lake Erie's western and central basins as highly divergent from the upper Great Lakes, a feature correlated with their higher denitrification potential (Small et al., 2016). Hypolimnetic hypoxia can influence bacterial community composition (Morrison et al., 2017; Schmidt et al., 2016) and function (Peura et al., 2015). While hypoxia was present in Lake Erie's central basin hypolimnion during our surveys in both 2011 and 2012, its severity differed markedly with the mild hypoxia encountered in 2011. By contrast, a continent-wide drought in 2012 resulted in extreme central basin hypoxia, both in terms of areal extent and intensity (Zhou et al., 2015). Indeed, hypolimnetic dissolved oxygen was severely depleted in 2012, measuring <2 mg/L for most of July and fully depleted by mid-August.

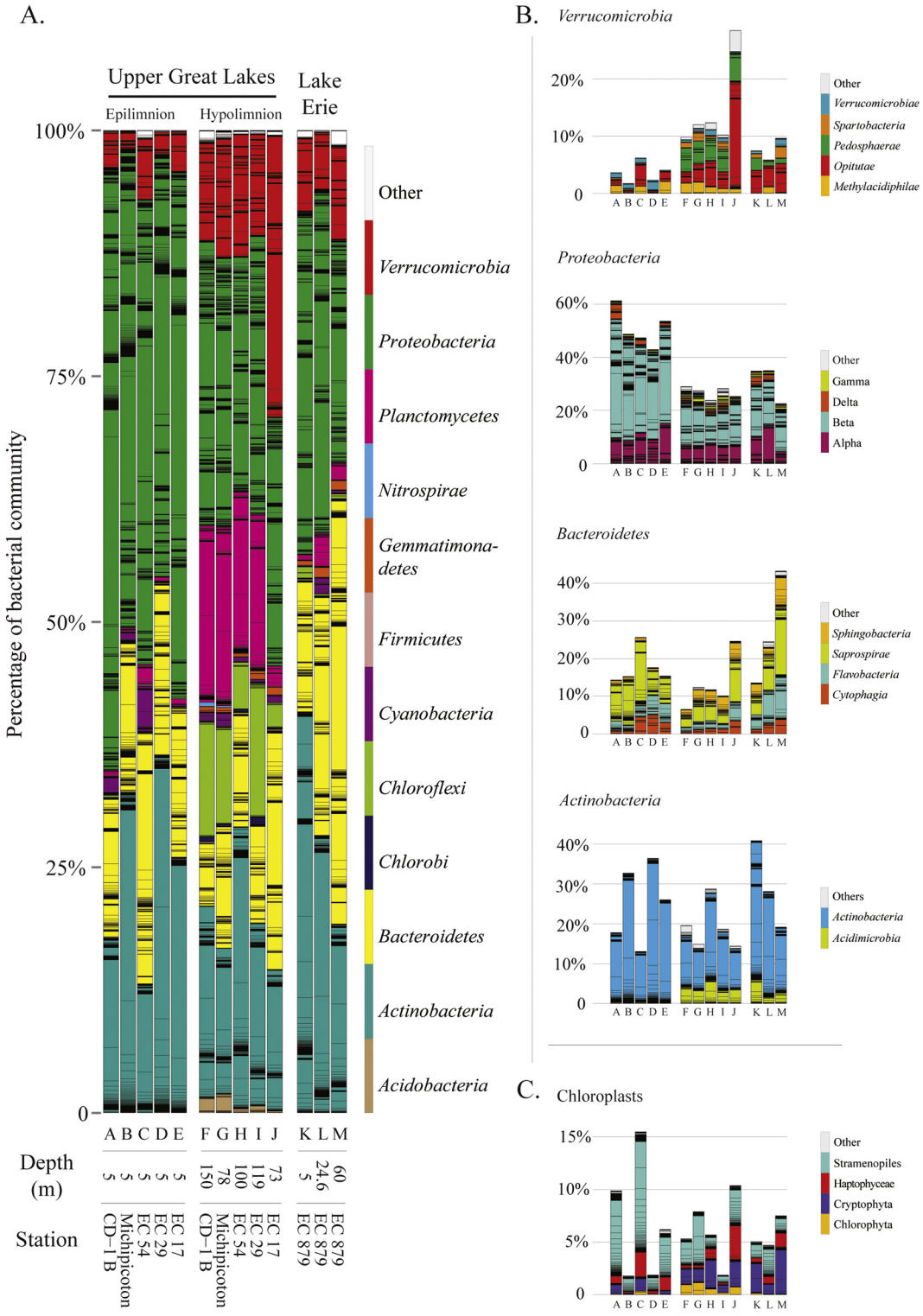

Fig. 3. 16S sequence abundance expressed as percent of the bacterial community for paired depth-resolved samples collected during the July 2011 multi-lake survey. A) Taxonomic breakdown of the bacterial communities by phylum. Each phylum is color coded, and individual OTUs are separated by black lines. B) Bacterial classes within the dominant phyla. Each class is color coded, and individual OTUs are separated by black lines. Letters correspond to sites identified in panel A. C) Chloroplast OTU abundances as a percent of total chloroplast and bacterial reads.

Environmental factors recorded during the survey were compared with observed clustering patterns of 2011 survey samples using Mantel (Table 2) and BEST (BIO-ENV; ESM Table S3) analyses. These analyses identified temperature and sample depth as the most influential parameters structuring the community in weighted analyses, reflecting the strong UniFrac clustering between mixed-layer and hypolimnetic samples (Fig. 5). Due to the strong influence of deep-water samples on the overall analysis, a separate analysis was completed with mixed-layer samples alone to elucidate the factors influencing microbial populations along the Great Lakes trophic gradient more clearly. Both analyses showed that clustering patterns were correlated with nutrient concentrations, either directly or as related through seston elemental

Table 2
Correlations between phylogenetic dissimilarity and physico-chemical parameters for both weighted and unweighted UniFrac distance matrices using the Mantel test with 999 permutations. Analysis was performed for a set containing mixed layer samples alone, as well as a set containing all samples from the 2011 multi-lake survey. For each column, the four values with the highest significant correlation have been highlighted. DIC: dissolved inorganic carbon; DO: dissolved oxygen; z: sample depth; z_{max}: maximum station depth.

	Mixed layer				All					
	Unweighted		Weighted		Unweighted		Weighted			
	r statistic	*p*-Value	r statistic	*p*-Value	r statistic	*p*-Value	r statistic	*p*-Value		
Chl-*a*	0.11	0.659	0.01	0.957	0.03	0.815	0.02	0.944	Physico-chemical parameters	
DIC	0.32	**0.024**	0.33	**0.021**	0.14	0.079	0.10	0.187		
DO	0.15	0.462	0.21	0.297	0.50	**0.001**	0.49	**0.002**		
NH_4^+	0.54	**0.004**	0.61	**0.002**	0.40	**0.015**	0.33	**0.003**		
NO_3^-	0.51	**0.007**	0.50	**0.016**	0.31	**0.02**	0.20	0.119		
z	0.25	0.142	0.36	**0.043**	0.29	**0.027**	0.56	**0.001**		
z_{max}	0.09	0.579	0.03	0.886	**−0.04**	0.764	0.01	0.93		
Temp	0.30	0.116	0.35	0.088	0.58	**0.001**	0.55	**0.001**		
C:N	0.43	**0.021**	0.30	0.082	0.03	0.846	0.14	0.237	Picoplankton	Size-fractionated particulate matter
C:P	0.45	**0.013**	0.23	0.052	0.20	**0.039**	0.14	0.127		
N:P	0.48	**0.005**	0.24	**0.043**	0.31	**0.002**	0.11	0.202		
POC	0.34	**0.019**	0.02	0.918	0.08	0.445	**−0.01**	0.963		
PON	0.17	0.349	0.02	0.939	0.08	0.509	0.00	0.999		
PP	0.66	**0.003**	0.44	**0.019**	0.29	**0.036**	0.10	0.459		
C:N	0.49	**0.019**	0.55	0.076	0.40	0.05	0.30	0.063	Total seston	
C:P	0.45	**0.007**	0.39	**0.029**	0.37	**0.004**	0.25	**0.031**		
N:P	0.52	**0.005**	0.44	**0.031**	0.46	**0.001**	0.28	**0.018**		
POC	0.36	**0.016**	0.11	0.524	0.04	0.78	**−0.02**	0.884		
PON	0.33	0.084	0.27	0.207	0.02	0.863	**−0.03**	0.847		
PP	0.77	**0.001**	0.56	**0.005**	0.37	**0.012**	0.19	0.148		

stoichiometry (Table 2, ESM Table S3). The Mantel test indicated that both NO_3^- and NH_4^+ concentrations were significant parameters influencing microbial community clustering patterns (Table 2). Likewise, the molar ratios of total seston N:P and C:N were significant for UniFrac clustering suggesting a strong relationship between bacterial community composition and the nutrient status of phytoplankton which were most likely the main factors driving the stoichiometry of the total seston fraction. BIO-ENV returned similar results, where total seston particulate phosphorus and NH_4^+ correlated best for unweighted and weighted Unifrac, respectively (ESM Table S3). These results are consistent with recent characterization of sediment microbial communities in the Great Lakes (Small et al., 2016) as well as with previous studies which recognize the importance of lake trophic state (Jankowski et al., 2014), primary production (Horner-Devine et al., 2003; Kolmonen et al., 2011) and productivity indicators such as organic carbon (Llirós et al., 2014; Schiaffino et al., 2015) in shaping bacterial community composition. Whereas a core pelagic bacterial community exists across trophic gradients, taxa augmentation in eutrophic systems increases bacterial richness, driving important differences in community structure (Newton and McLellan, 2015).

Mixed layer communities in the Great Lakes consisted of phyla typical of freshwater lakes (Newton et al., 2011; Zwart et al., 2002), represented primarily by *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Figs. 3, 6, ESM Fig. S3). To identify the individual OTUs responsible for the observed UniFrac clustering patterns, differential abundance was determined using the DESeq2 package. This study compared sets of samples as indicated by unweighted UniFrac clustering patterns and included the oxic hypolimnia of upper Great Lakes Superior and Huron, their respective mixed layer samples, samples collected from Lake Erie, and from Lake Superior's northern bays (Fig. 5A).

Comparatively few of the 12,964 OTUs observed in this study were differentially distributed, reinforcing previous observations that the majority of OTUs in freshwater lakes do not show differential abundance between aquatic habitats (Schmidt et al., 2016). Comparisons of upper Great Lakes (including mixed layer, hypolimnion and northern bays) sample sets with Lake Erie returned a total of 383 OTUs with significantly increased abundance when compared with Lake Erie (Fig. 7A). Of these, only 18 were found to be shared between UniFrac groupings, and none were represented in all three sets. While comparatively few OTUs were distributed differentially, those that were, sometimes accounted for a large portion of the reads returned. The upper Great Lakes surface mixed layer yielded 96 OTUs that were present in higher abundance than in Lake Erie, which together averaged >20% of reads (Figs. 7A, 8B). Of those 96 OTUs, the majority of reads belonged to the phenotypically-diverse *Comamonadaceae* ([BetI-A], *Betaproteobacteria*), which accounted for 17% of the community (ESM Table S4). Conversely, 515 OTUs were significantly decreased in the upper Great Lakes surface mixed layer, with 85 OTUs more abundant in Lake Erie (Fig. 7B). These 85 OTUs accounted for an average of 10% of Lake Erie communities (Fig. 8C) with *Actinobacteria* (ACK-M1[acI-A], *Microbacteriaceae*, and C111 [acIV]) and *Cyanobacteria* being most highly represented (ESM Table S5) consistent with studies showing dominance by the same actinobacterial clades in eutrophic Lake Taihu (Tang et al., 2017) and niche partitioning among the acI lineage in response to cyanobacterial blooms in Lake Erie's western basin (Berry et al., 2017). Lake Erie was most similar to the northern bays of Lake Superior, with only 23 differential OTUs exhibiting decreased abundance in Lake Erie (Fig. 7A) where they accounted for <5% of reads (Fig. 8B, ESM Table S6). These habitats fall within the designated 'nearshore waters of the Great Lakes' (Edsall and Charlton, 1997), with a shallow bathymetry and predisposition to eutrophication. They also show signs of impairment by human activity with Nipigon Bay listed as one of 43 Great Lakes Areas of Concern (Kelso and Cullis, 1996) due to a legacy of industrial activity and collapsed fish stocks in both northern bays attributed to commercial harvest (Schneider and Leach, 1977; Wilson et al., 2007). Among impairments to Lake Erie, filamentous and colonial cyanophytes (*Dolichospermum* spp., *Planktothrix* spp., *Microcystis* spp.) form massive surface blooms in the western and central basins of the lake that recur

each summer (Bullerjahn et al., 2016; Michalak et al., 2013; Salk et al., 2018) and were evident during our survey, although perhaps underrepresented due to pre-screening of water through 80 μm mesh prior to sample concentration. In addition to these large-sized cyanobacteria, measurements made by flow cytometry revealed mean picocyanobacterial (likely *Synechococcus* spp.) abundances (total phycoerythrin- and phycocyanin-rich cells) of 1.8×10^5 cells/mL (± 0.7) in Lake Erie, an order of magnitude higher than in the upper Great Lakes (0.19×10^5 cells/mL [±0.06] in Lake Superior, and 0.36×10^5 cells/mL [±0.2] in Lake Huron) and consistent with a recent report demonstrating abundant picocyanobacteria among the more familiar filamentous- and colonial bloom forming cyanobacteria in Lake Erie (Berry et al., 2017).

Resampling in 2012 allowed a better characterization of *Cyanobacteria* populations that were in relative low abundance at the time of sampling (mid-July) in 2011. Surface waters collected from station WM in Lake Superior August 2012 contained a higher abundance of *Cyanobacteria* than in 2011, accounting for 24% of bacterial reads (Fig. 6), with most reads represented by a single OTU attributed to *Synechococcus* spp. LSI and LSII clades. These clades represent novel ecotypes described originally from Lake Superior (Ivanikova et al., 2007), which exhibit restricted geographic distribution (Callieri et al., 2013). Likewise, cyanobacterial abundance in surface waters at station EC 880 (Lake Erie central basin) was higher in summer 2012 than in July 2011, with cyanobacteria totaling 13% of total bacterial reads attributed to *Synechococcus*, including the previously mentioned LSI and LS II clades.

Depth-resolved microbial community shifts

Other studies of seasonally-stratified lakes have reported depth-resolved profiles which reveal contrasting bacterial communities between the discrete water masses of the epilimnion and hypolimnion (Garcia et al., 2013; Kurilkina et al., 2016; Schmidt et al., 2016). Lake layer was the most important determinant of bacterial community composition in a recent study of 11 north temperate lakes varying in trophic state as characterized by total phosphorus (Schmidt et al., 2016). In the present study, PCoA identified some of the strongest phylogenetic dissimilarities among depth-resolved communities in the upper Great Lakes (Fig. 5). Unlike epilimnetic waters along the Great Lakes transect where bacterial abundance often showed one-fold or more variance, cell numbers were remarkably stable in hypolimnetic waters (Fig. 4). Likewise, microbial communities were stable across hypolimnia of lakes Superior and Huron, with the exception of Georgian Bay (Figs. 3, 5). Nearly 250 OTUs were identified as differentially abundant at depth when compared with the surface mixed layer of the upper Great Lakes and Lake Erie (Figs. 7A, B, 8B, C). Most notable was the abundance of *Planctomycetes* and *Chloroflexi* in hypolimnetic samples of the upper Great Lakes, where they increased from an average of 4% in surface waters to 28% of the deep-water community in paired samples (Fig. 3). Three OTUs representing the classes *Phycisphaerae* (CL500-3) and *Planctomycetia* of the *Planctomycetes* dominated the reads in these samples, as well as a single OTU belonging to the *Chloroflexi* lineage H39 (CL500-11), which was the most abundant read in the hypolimnion, where it averaged ~10% of bacterial reads.

Planctomycetes are known for their role in nitrogen removal from wastewater via anammox (Strous et al., 1999), but far less is known about the environmental niche they occupy in natural systems (Fuerst, 1995). Their abundance in lakes has generally been perceived as low; nevertheless they have been recorded at abundances of >10% of the microbial community (Gade et al., 2004), often partitioned to cold, oxygenated hypolimnetic waters (Okazaki et al., 2017) and enriched in particle-associated fractions (>3 μm; Schmidt et al., 2016). Novel *Planctomycetes* active in anammox are also reported from sediment of Lake Superior where they contribute to sediment N_2 production (Crowe et al., 2017). In the present study, highest abundance of *Planctomycetes* was observed near Michipicoten Island in eastern Lake Superior where they accounted for 18% of the hypolimnetic community reads. Their differential abundance in hypolimnetic waters of the upper Great Lakes may reflect the lower N:P stoichiometric ratios of these waters compared to their respective surface mixed layers (Fig. 2). *Planctomycetes* are reported to be poor competitors for P, which may help explain their absence from the oligotrophic surface waters of these lakes (Pollet et al., 2014).

The dominant *Chloroflexi* OTU clustered with representatives from the CL500-11 clade (data not shown), a sister clade of the deep-ocean SAR202 clade, and has been found in abundance in oxygenic hypolimnetic waters of Crater Lake, USA (Urbach et al., 2001), multiple

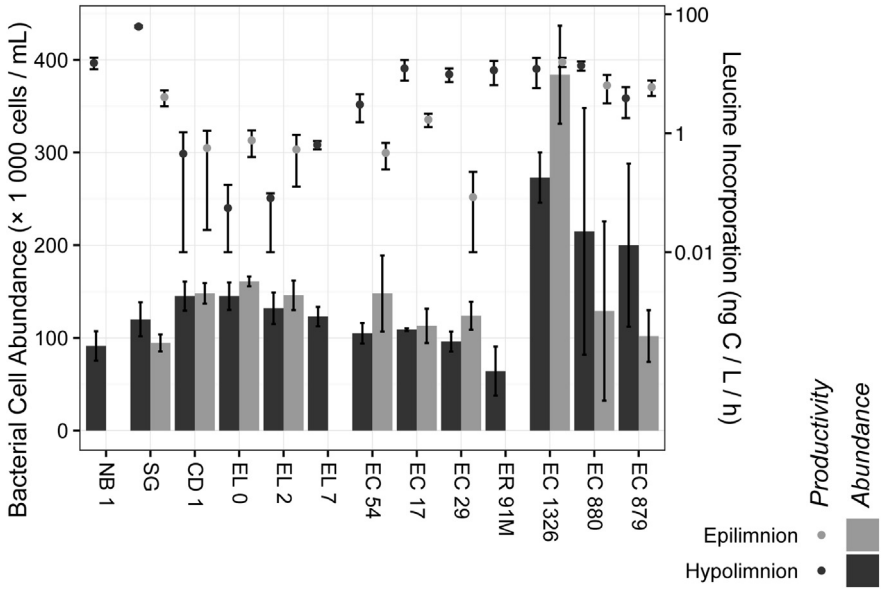

Fig. 4. Depth-resolved abundance and leucine incorporation by bacterial heterotrophs along the 3200 km round trip Great Lakes survey. Bacterial abundances are in a linear scale, whereas rates of leucine incorporation are on a base-10 logarithmic scale. Error bars show ±1 standard deviation, with small or negative lower bounds for productivity rescaled to 0.01 ng C/L/h. A Levene's test for equality of variance (using the car package, version 2.1-2 in R; R Core Team, 2018) on the sample means from epilimnion and hypolimnion across the lakes did not identify a significant difference in the variance in bacterial abundances between epilimnion and hypolimnion ($F(1,18) = 3.7745$, $p = 0.068$).

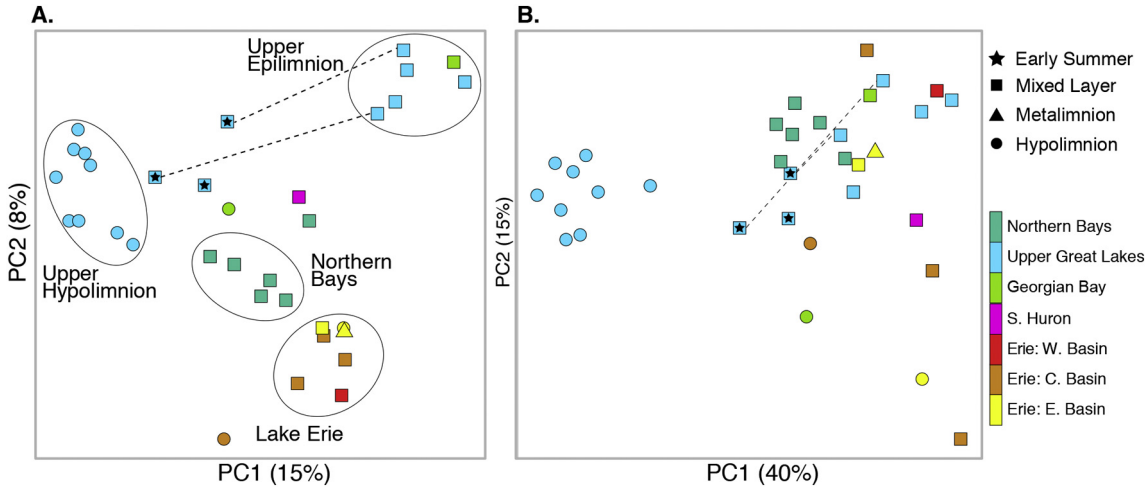

Fig. 5. Phylogenetic measures of community beta diversity were visualized using PCoA of UniFrac distance matrices representing: (A) dissimilarity between samples when each observed taxon is held equally (unweighted UniFrac) as well as (B) sample dissimilarity when taxon abundance is taken into account (weighted UniFrac). Dotted lines indicate temporal community shifts in paired epilimnetic samples from June and early July (stars) to the communities present in mid-July and August.

deepwater lakes in Japan (Mehrshad et al., 2018; Okazaki et al., 2013, 2017), Lake Zurich (Mehrshad et al., 2018), a Czech reservoir (Mehrshad et al., 2018) and Lake Michigan, part of the Laurentian Great Lakes system (Denef et al., 2016b; Fujimoto et al., 2016). Likewise, this group has been detected in samples collected from Lakes Malawi, Onega, and Winnipeg (Rozmarynowycz, 2014), reinforcing a global distribution of this clade. The ecophysiology of *Chloroflexi* is unclear; however based on a recent metagenomic analysis, members of this clade likely occupy an important role in the mineralization of nitrogen-rich dissolved organic matter exported to the hypolimnion (Denef et al., 2016b). Notably, *Chloroflexi* were not exclusive to hypolimnetic populations but were also moderately abundant in the mixed layers of Lake Superior's northern bays, where they accounted for >5% of sequence reads in Black Bay (ESM Fig. S2). Likewise, the CL500-11 clade of *Chloroflexi* were abundant in ice-covered surface waters of northern Lakes Michigan and Huron (Rozmarynowycz, 2014) and were recently reported abundant in the epilimnion of Lake Zurich during winter where rhodopsin-based photoheterotrophy may contribute to their success (Mehrshad et al., 2018). At the time of sampling, the northern bays had surface mixed layer temperatures above 16 °C, similar to pelagic Lake Superior (Table 1) and thus ruling out temperature as the primary factor driving the distribution of the *Chloroflexi*. Among the few environmental parameters shared by the northern bays of Lake Superior and the pelagic hypolimnion of the Great Lakes is a reduced light climate, which in the bays, is attributed to resuspension of bottom sediments by wave action (Schertzer et al., 1978). Consistent with this, transmissometry readings at the two Black Bay stations ranged from 45 to 60% compared to >90% at pelagic sites. Inhabiting such low light environments reduces the need to allocate cellular resources required to respond to light-induced oxidative stress (Denef et al., 2016b). A separate group of freshwater *Chloroflexi*, cluster SL56, was recently reported from the epilimnion of a large reservoir in the Czech Republic where they co-locate with a low light-adapted cyanobacterium, *Planktothrix rubescens* (Mehrshad et al., 2018).

A single OTU of a chemolithoautotroph, the nitrite-oxidizing bacterium *Nitrospira*, was likewise differentially abundant, present only in the hypolimnion of samples from Lake Superior (Figs. 3, 6). In-lake nitrification has been implicated in the century-long accumulation of nitrate in Lake Superior (Sterner et al., 2007) and our results support the vertical distribution of *Nitrospira* previously reported for Lake Superior using CARD-FISH (Mukherjee et al., 2016; Small et al., 2013). These data reflect prior studies showing the exclusion of nitrification activity from the Lake Superior epilimnion, attributed previously to the photosensitivity of ammonia oxidizers that support a nitrite oxidizing community (French et al., 2012; Merbt et al., 2012). The results also follow the pattern of *Nitrospira* distribution recently reported along a nearshore to pelagic gradient of Lake Michigan (Fujimoto et al., 2016). Further, the apparent absence of these bacteria from hypolimnetic waters of Lake Erie (Figs. 3, 6) is consistent with a reduced role for this taxa in water column nitrification in Lake Erie with *Nitrobacter* serving as the dominant nitrite-oxidizing group (Mukherjee et al., 2016).

Historically, the onset of stratification in Lake Superior, the deepest and most northerly of the Laurentian Great Lakes, has occurred later in summer (Chapra and Dobson, 1981). However, a recent decline in ice cover across the Great Lakes has been attributed to climate warming (Wang et al., 2012), and over the past 3 decades the onset of stratification across Lake Superior has been occurring increasingly earlier at a rate of approximately one-half day per year which has advanced stratification to late June-early July (Austin and Colman, 2007). Coincident with the early stages of thermal stratification of the lake, our survey provided an opportunity to address the establishment of depth-resolved bacterial communities. Evidence for a temporal change in microbial community structure was provided by sampling western Lake Superior station CD-1 on both outbound- and return legs of the survey (Fig. 1). The surface mixed layer warmed from 8.5 °C to 16.4 °C during this period which reflected increased stability of the thermocline (Table 1). The re-occupation of this station 15 days following initial occupation was preceded by a 5-day period of relative calm in western Lake Superior, as recorded by National Data Buoy Center surface buoy 45,006 deployed in the western arm of the lake (ESM Fig. S4). In both weighted and unweighted UniFrac PCoA, a community shift was observed in the surface waters of the upper Great Lakes with the strengthening of thermal stratification (Fig. 5). In the early stages of stratification during the outbound leg of the survey, the read composition of surface waters was more similar to post-stratification hypolimnetic communities later in July (Fig. 5). Underlying this community shift was a sharp increase in OTUs identified as differentially abundant in the epilimnion of the upper Great Lakes. These OTUs, which accounted for 18% of the microbial community before the start of the strong stratification, increased to 48% on the return trip. The largest gains in read abundance were observed for *Betaproteobacteria*, consistent with high growth rates associated with this class in other freshwater systems (Newton et al., 2011). Among *Betaproteobacteria*, phototrophic purple non-sulfur bacteria *Rhodoferax* showed particularly high post-stratification abundance in the surface mixed layer (ESM Fig. S2). Conversely, OTUs dominated by *Acidomicrobia*, *Chloroflexi* and *Planctomycetes* that were significantly abundant in the hypolimnion accounted for 28% of the surface water community during weak stratification and decreased to 3% when the

site was revisited two weeks later following strengthening of stratification. Likewise, high abundances of deep-water OTUs were observed in June the following year, where they accounted for 30% and 46% of the surface water communities at western Lake Superior stations CD-1 and WM, respectively (not shown). Resampling station WM in August 2012 (Fig. 6) revealed that hypolimnion-associated OTUs again declined

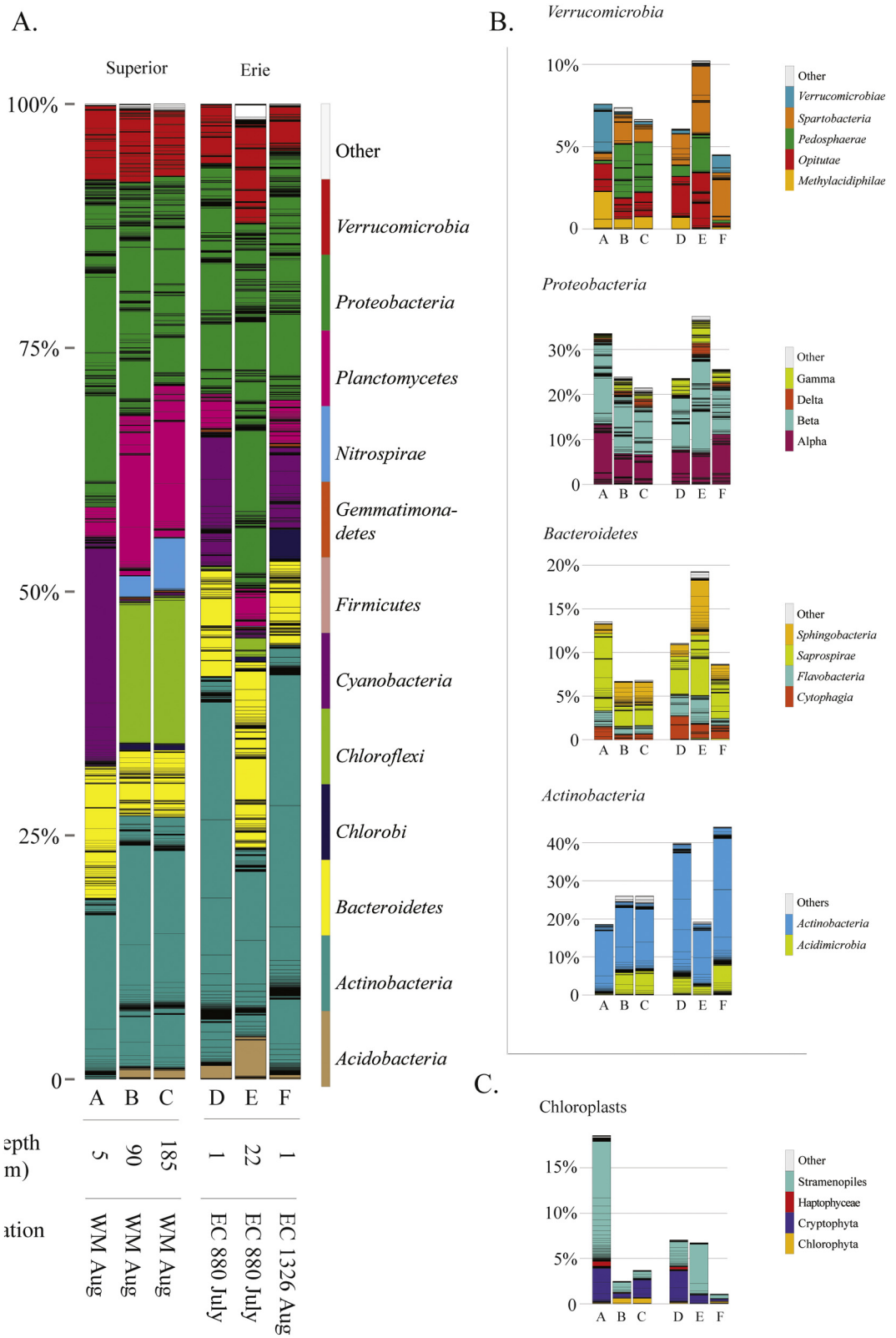

Fig. 6. 16S sequence abundance expressed as percent of the bacterial community for additional paired depth-resolved samples collected during 2012 from stratified locations in Lake Superior (WM) and Lake Erie's central basin (EC 880, EC 1326). A) Taxonomic breakdown of the bacterial communities by phylum. Each phylum is color coded, and individual OTUs are separated by black lines. B) Bacterial classes within the dominant phyla. Each class is color coded, and individual OTUs are separated by black lines. Letters correspond to sites identified in panel A. C) Chloroplast OTU abundances as a percent of total chloroplast and bacterial reads.

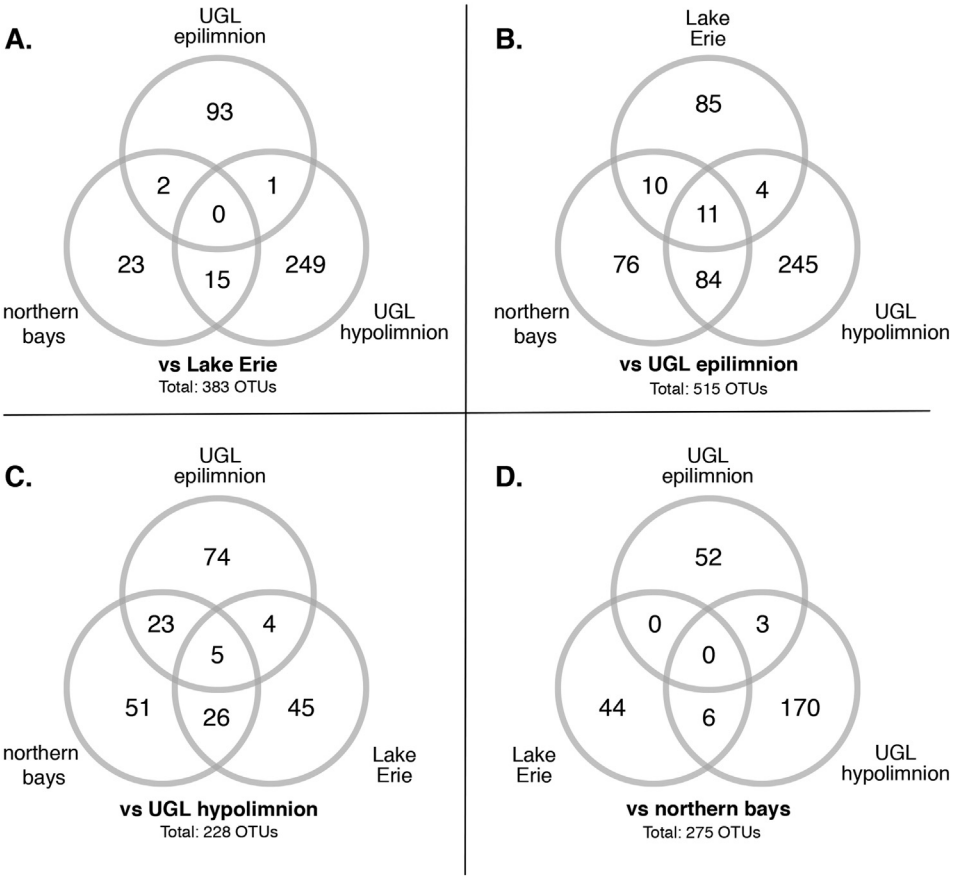

Fig. 7. Venn diagrams of differentially distributed OTUs. This figure summarizes the results of 12 DESeq2 analyses that compared sample groupings from Lake Erie, the northern bays, the upper Great Lakes epilimnion, and the upper Great Lakes hypolimnion. Each diagram summarizes the results of three analyses comparing the indicated sample groupings with the one listed beneath. Each circle indicates the number of OTUs that had significantly increased abundance ($p < 0.01$) in a single analysis, with overlapping regions indicating the number of OTUs which were differentially abundant in two or more analyses. UGL; upper Great Lakes.

to 3% of the epilimnion microbial reads, mirroring those seen at station CD-1 the previous year. Under conditions of weak stratification as experienced during early summer in Lake Superior, variability between bacterial communities in epilimnion and hypolimnion is sharply reduced (Shade et al., 2010). As stratification strengthens, depth-resolved communities diverge. This divergence is often rapid, as highlighted by an artificial mixing study of a small, stratified north-temperate lake where bacterial community composition in the epilimnion recovered to the pre-disturbance state within 7 days after mixing was stopped (Shade et al., 2012).

Conclusions

This study highlights the dynamic nature of microbial communities present in the Laurentian Great Lakes, where community shifts were associated with lake trophic status as well as with season and thermal

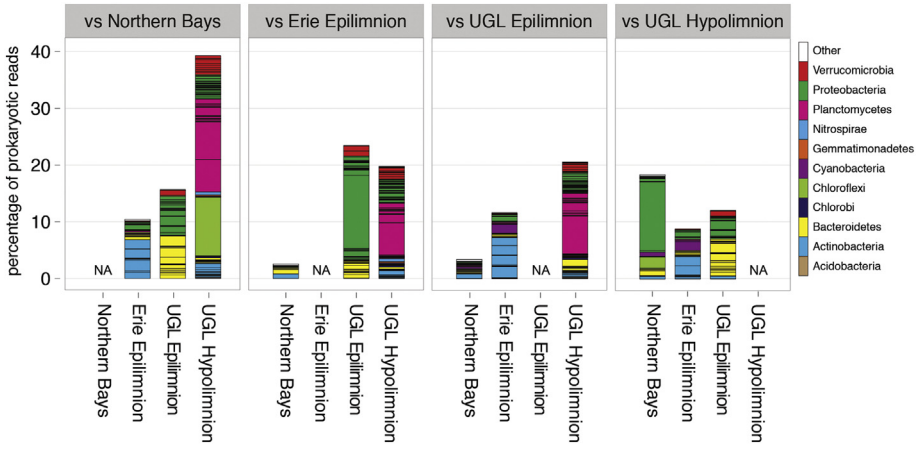

Fig. 8. Stacked bar plots of differentially distributed OTUs. Each phylum is color coded, and individual OTUs are separated by black lines. Each bar plot illustrates the results of a DESeq2 analysis comparing the condition listed below with the one above. Each set of boxplots corresponds with a Venn diagram (Fig. 7) and illustrates OTUs which, between the three analyses, were differentially abundant only for the given condition. UGL; upper Great Lakes.

stratification. Microbial community patterns in some respects fell in line with expectations based on trophic gradients across the Great Lakes, but some unexpected patterns, such as the occurrence of a typical deep-water *Chloroflexi* in the mixed layer of Lake Superior's northern bays, should be investigated further. Although the differentially abundant OTUs identified in this study shed light on taxa associated with geography and trophic status, they represent only a snapshot of the microbial communities present in these environments. While the level of taxonomic classification presented in this study is coarse (Family level), the fact remains that taxonomy assignment of freshwater bacteria remains limited by inadequate phylogenies incorporated into commonly used databases. Recent advances such as applying a custom freshwater database (TaxAss; Rohwer et al., 2018) are beginning to address these challenges and will be useful in future studies on these Great Lakes communities to identify community variability over longer periods of time, genetic connectivity between lakes and functional heterogeneity across these same gradients.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2019.01.004.

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