LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 64, 2019, 1347–1370

doi: 10.1002/lno.11120

Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie

Jennifer Jankowiak, Theresa Hattenrath-Lehmann,[a] Benjamin J. Kramer, Megan Ladds, Christopher J. Gobler*

School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, New York

Abstract

Although cyanobacterial harmful algal blooms (CHABs) are promoted by nutrient loading and elevated temperatures, the effects of these processes on bloom diversity are unclear. This study used traditional and next-generation sequencing approaches to assess shifts in phytoplankton, cyanobacterial (16S rRNA), and microcystin-producing (*mcy*E) communities during CHABs in western Lake Erie (Maumee and Sandusky Bays) in response to natural and experimental gradients of nitrogen (N), phosphorus (P), and temperature. CHABs were most intense near the Maumee and Sandusky Rivers and were dominated by *Microcystis* and *Planktothrix*, respectively. Sequencing of 16S amplicons revealed cryptic cyanobacterial diversity (47 genera) including high abundances of two distinct clades of *Synechococcus* in both bays and significant differences in community structure between nutrient-rich nearshore sites and less eutrophic offshore sites. Sequencing of *mcyE* genes revealed low taxonomic (n = 3) but high genetic diversity (n = 807), with toxigenic strains of *Planktothrix* being more abundant than *Microcystis* and more closely paralleling microcystin concentrations. Cyanobacterial abundance significantly increased in response to elevated N, with the greatest increases in combined high N, P, and temperature treatments that concurrently suppressed green and brown algae. N significantly increased microcystin concentrations and the relative abundance of nondiazotrophic genera such as *Planktothrix,* while diazotrophic genera such as *Dolichospermum* and *Aphanizomenon* were, in some cases, enhanced by high P and temperature. While nutrients and elevated temperatures promote CHABs, differing combinations selectively promote individual cyanobacterial genera and strains, indicating management of both N and P will be required to control all cyanobacteria in Lake Erie, particularly as lake temperatures rise.

Since the mid-1990s, Lake Erie has become increasingly prone to the occurrence of cyanobacterial harmful algae blooms (CHABs; Conroy and Culver 2005; Bridgeman et al. 2013; Steffen et al. 2014; Watson et al. 2016). This intensification has been linked to accelerated anthropogenic nutrient loading (Carpenter et al. 1998; Paerl et al. 2001; O'Neil et al. 2012) and climatic warming (Heisler et al. 2008; Paerl and Huisman 2008; Carey et al. 2012) and can result in ecosystem disruption (Chorus and Bartram 1999; Paerl et al. 2001). Further, CHABs are a growing public health concern due to their ability to produce an array of toxins, the most well-studied being the microcystins, which are produced by a variety of genera and have been implicated in animal deaths and human poisoning events (Chorus and Bartram 1999; Carmichael 2001; Carmichael and Boyer 2016). Hence, CHABs can jeopardize the sustainability of socioeconomically important freshwater resources (Fuller et al. 2002).

Lake Erie, and in particular its western basin, is the most susceptible region in the Great Lakes to CHABs (Makarewicz and Bertram 1991) as it is the smallest, shallowest, and most southern, and therefore warmest and most highly influenced by nutrient enrichment (De Pinto et al. 1986; Mortimer 1987; Davis et al. 2015; Carmichael and Boyer 2016). The western basin receives the majority of Lake Erie's nutrient load (bioavailable P and N) via the Maumee and Sandusky Rivers (Dolan and Chapra 2012; Han et al. 2012; Allinger and Reavie 2013), which deliver nonpoint runoff from highly agricultural watersheds (Ohio EPA 2010). Nutrient concentrations near these

*Correspondence: christopher.gobler@stonybrook.edu

[a]Present address:
Wadsworth Center, New York State Department of Health, Albany, New York.

Additional Supporting Information may be found in the online version of this article.

river mouths can be four-times greater than in the open waters of the western basin (Chaffin et al. 2011; Bridgeman et al. 2012) and are believed to fuel the annual late summer blooms in Maumee and Sandusky Bays (Bullerjahn et al. 2016).

Traditionally, primary production in freshwater systems has been viewed as phosphorus (P) limited (Vollenweider 1968; Edmondson 1970; Schindler 1977), with increases in P loads resulting in shifts toward cyanobacteria-dominated communities as many bloom-forming cyanobacteria (i.e., *Dolichospermum* and *Aphanizomenon*) are diazotrophic and can fix atmospheric nitrogen (N) to meet their N demands (Smith 1983; Paerl et al. 2001; Schindler et al. 2008). In Lake Erie, external P loads have been successfully used to predict the interannual intensity of CHABs (Stumpf et al. 2012; Michalak et al. 2013) and P reduction strategies implemented in the 1970s significantly reduced cyanobacteria biomass for a 20-yr period (Makarewicz and Bertram 1991; Makarewicz 1993; Stumpf et al. 2012). In the past decade, the importance of N for CHABs has been noted due to the increasing dominance of nondiazotrophic genera (Paerl and Fulton 2006; Harke et al. 2016), such as *Microcystis* and *Planktothrix,* concurrent with global increases in N loading (Vitousek et al. 1997; Galloway et al. 2004; Glibert et al. 2014). Furthermore, N availability can influence the production and congener composition of the N-rich toxin, microcystin, in *Microcystis* and *Planktothrix,* thereby impacting bloom toxicity (Davis et al. 2010; Gobler et al. 2016; Chaffin et al. 2018). This has spurred a growing emphasis on the occurrence of N limitation (Conley et al. 2009; Gobler et al. 2016), as well as N and P colimitation (Elser et al. 2007; Sterner 2008; Davis et al. 2015; Müller and Mitrovic 2015) in CHAB development, although there still remains a debate regarding the most effective and cost-efficient mitigation strategies (Paerl and Otten 2016). Additionaly, given that global temperatues are expected to rise an additional 1.5–5°C this century (Houghton et al. 2001; Paerl and Huisman 2009; O'Neil et al. 2012) and the high thermal optima of many freshwater cyanobacteria (≥ 25°C; Paerl et al. 2011; Thomas and Litchman 2016), CHABs are expected to intensify with rising surface water temperatures. Recent long-term monitoring and modeling studies have alluded to the synergistic effects of temperature elevation and eutrophication on cyanobacterial growth (Anneville et al. 2005; Elliott et al. 2006; Kosten et al. 2012), suggesting that eutrophic systems are likely to become more prone to blooms as temperatures continue to rise (Rigosi et al. 2014), supporting the need for a deeper understanding of these complex interactions.

Although numerous studies have examined the effects of nutrient availability and temperature on the growth and toxicity of common bloom-forming genera and total cyanobacteria biomass (Rinta-Kanto et al. 2009; Davis et al. 2010; Chaffin et al. 2011), there remains a limited understanding of their impacts on cyanobacteria assemblage diversity and the associated microcystin-producing communities during blooms (Rinta-Kanto and Wilhelm 2006; Steffen et al. 2012). To gain further insight into the relationships among phytoplankton community dynamics, microcystin production, and environmental drivers, we used next-generation amplicon sequencing (NGS) to evaluate changes in western Lake Erie cyanobacterial assemblages in response to natural and experimental gradients in nutrients (N and P) and temperature. Algal communities were profiled using a multitiered approach to identify variations in the composition and abundance of the total phytoplankton (cyanobacteria and green and brown algae), cyanobacterial (16S rRNA), and potential microcystin-producing (*mcyE*) communities during field surveys and experiments. The goal of the study was to identify environmental conditions associated with the dominance of specific algal and cyanobacterial communities in western Lake Erie.

Methods

Study sites and field surveys

During August 2015, large CHABs developed in Sandusky and Maumee Bays within western Lake Erie, and persisted into September (National Oceanic and Atmospheric Administration 2015*b*). Sampling transects of the blooms were conducted across each bay during mid-September 2015 aboard the RV *Erie Monitor* (The Ohio State University), spanning from the respective river mouths into the open waters of the western basin (Fig. 1a). The Sandusky transect consisted of six sites, with two sites each in the inner (western) and outer (eastern) sections of the bay divided by its hourglass shape, as well as two sites beyond Cedar Point, which separates the bay from the western basin of Lake Erie (Fig. 1a; Supporting Information Table S1). The Maumee transect consisted of five sites extending from the Maumee River to the Western Sister Islands located in the central portion of the western basin (Fig. 1a; Supporting Information Table S1). Sites were selected to represent a gradient of nutrient and temperature conditions across a range of cyanobacteria densities approximated from the 15 September 2015 MODIS satellite image (Fig. 1b; National Oceanic and Atmospheric Administration 2015*a*). Levels of cyanobacteria were confirmed on-site with a BBE Moldenke Fluoroprobe which estimates abundances of cyanobacteria, green algae, and brown algae (e.g., diatoms, dinoflagellates, raphidophytes, and haptophytes) based on differential fluorescence of photosynthetic accessory pigments (Beutler et al. 2002; Chaffin et al. 2013; Harke et al. 2015). Pigment-based class identification was affirmed with 45 cultures of diatoms, cyanobacteria, dinoflagellates, raphidophytes, green algae, and haptophytes that revealed no cross over among the three fluorescence channels for these cultures.

At each site, surface water temperature was measured using a handheld YSI sonde (model 556) and phytoplankton communities in the mixed surface layer (0.1–1 m) were assessed with a Fluoroprobe. One liter of subsurface water (~ 0.25 m) was collected from which triplicate samples were obtained for DNA-based community analysis of the cyanobacteria (16S) and microcystin-producing (*mcyE*) communities by filtering 30 mL of whole water on 0.22 μm polycarbonate filters. Filters were

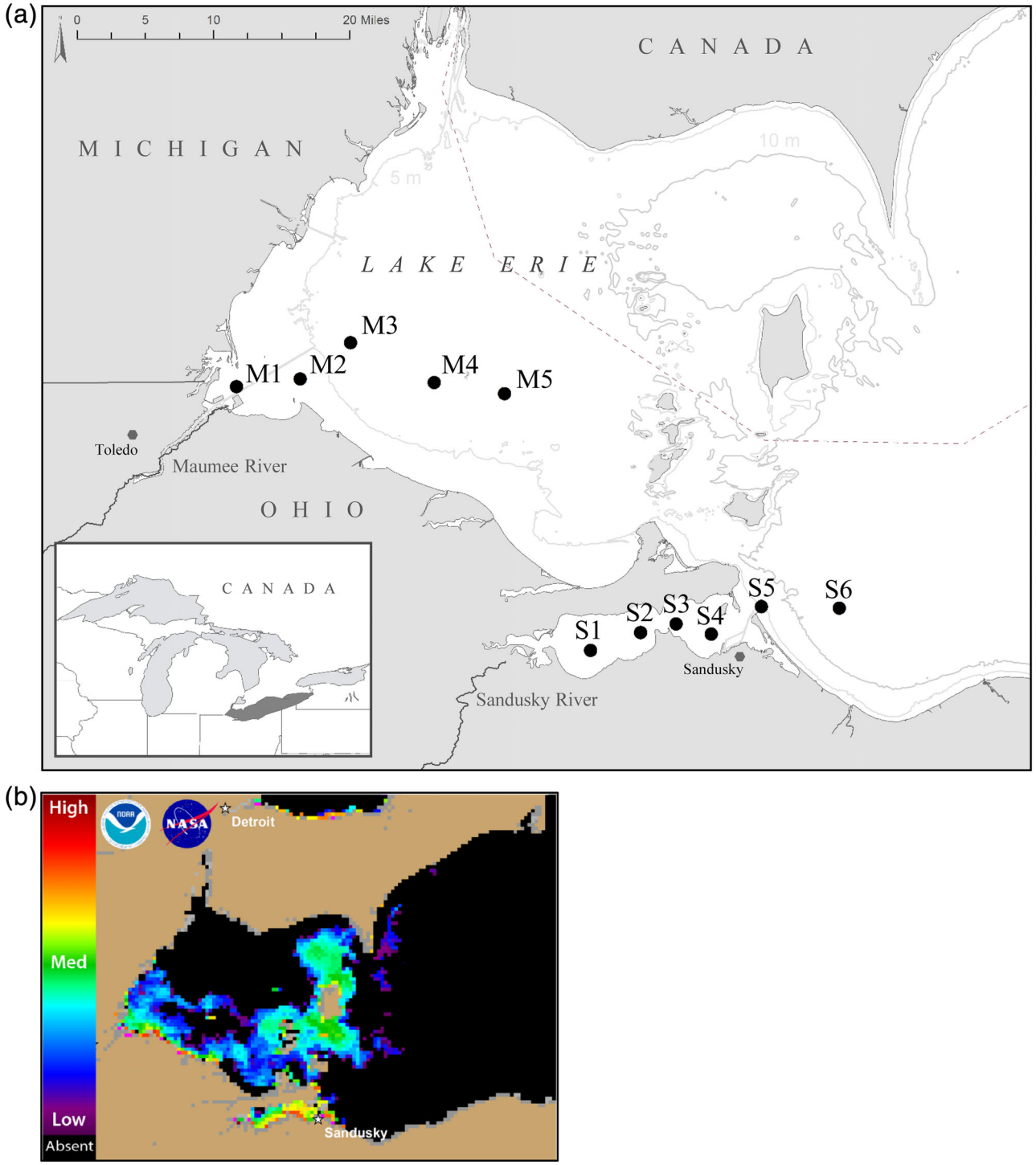

Fig. 1. Map of **(a)** the Sandusky and Maumee Bay transect sites in western Lake Erie visited on 14 and 15 September 2015, respectively. Contour lines are 5 and 10-m depth, dots indicate sampling sites, and hexagons indicate major cities. The insert shows the location of Lake Erie within the Great Lakes region. **(b)** Composite of MODIS cyanobacterial index image for 14 September 2015 from the NOAA experimental Lake Erie harmful algal bloom bulletin (https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/bulletin.html), where black represents no cyanobacteria detected and the colored pixels indicate cyanobacteria density.

immediately frozen in liquid nitrogen and stored at −80°C until DNA extraction (*see* DNA Isolation, Sequencing, and Analysis section below). Additionally, whole water samples were preserved with Lugol's iodine solution (5% v/v) for cyanobacteria identification and enumeration by light microscopy (*see* Sample Analysis section below), and subsamples were collected

and stored at −20°C until further processing for analysis of total and dissolved (combusted EMD Millipore APFB glass fiber filter) nutrients and total microcystins (whole water). Photosynthetic efficiency (F_V/F_M) of the algal assemblage was measured using the maximum quantum yield technique (*see* Sample Analysis section below for details).

Nutrient–temperature amendment experiments

During the Maumee Bay transect, an additional 60 liter of subsurface water (~ 0.25 m) was collected in acid-washed 20 liter carboys at sites M2 and M4 for experiments. Site M2, which was in Maumee Bay, hosted a cyanobacteria-dominated bloom community (Fluoroprobe-derived cyanobacteria concentrations above the 25 μg L^{-1} bloom threshold used by the New York State Department of Environmental Conservation), whereas site M4, which was within the open waters of the western basin, hosted a mixed-algal community that was more representative of a nonbloom population (< 25 μg L^{-1}; Fig. 1a). Experiments were designed to assess the effects of elevated N, P, and temperature (T), as well as their interactions, on algal community composition, abundance, and toxicity. For each experiment, two sets of 1-liter polycarbonate bottles (n = 12 per set) were filled with the surface water via laminar flow and amended in triplicate with additions of nitrate (20 μmol L^{-1} NO_3^-), phosphate (1.5 μmol L^{-1} K_2PHO_4), nitrate and phosphate (20 μmol L^{-1} NO_3^-/1.5 μmol L^{-1} K_2PHO_4), or left unamended as a control. The nutrient additions were representative of pulses of nitrate and phosphate that have been previously described in the region (Wilhelm et al. 2003; Davis et al. 2015; Harke et al. 2015) and were administered at the start of the experiment and again after 48 h to prevent depletion before the conclusion of the experiment.

The bottles were incubated for 96 h in clear, submersible containers at 0.25 m depth in Fishery Bay, Lake Erie, near the Franz Stone Laboratory (The Ohio State University), at two temperatures. The first set of bottles (n = 12) was incubated at the ambient lake temperature, achieved with openings in the container that allowed lake water to exchange, whereas the second set of bottles (n = 12) was incubated at an elevated lake temperature (+4°C) achieved with heating wands (ViaAqua Inc.) within the container. This 4°C increase is consistent with temperature projections for the 21st century (Houghton et al. 2001) and is representative of summer heatwaves within temperate latitudes (Joehnk et al. 2008). The bottles were covered with one layer of neutral density screening to mimic in situ light conditions at ~ 0.25 m, which were measured continuously along with experimental temperatures (average 22.3 and 26.6 °C) using in situ HOBO loggers (Onset Computer Corporation). Bottles were mixed daily to promote equal phytoplankton–nutrient distribution and were inspected for floating cyanobacterial colonies prior to mixing to affirm cyanobacterial cells were healthy within the experiment vessels. At the 96 h time point, the bottles were processed in the same manner as the field samples (see above).

Sample analysis

Microscopic enumeration was used to support the pigment- and DNA-based analyses of cyanobacteria abundances. For each preserved sample, 1 mL aliquots was settled for 5 min on a gridded Sedgewick–Rafter chamber before being visually scanned on an inverted microscope at 20X and 40X. Cyanobacteria colonies and filaments were quantified and identified to the genus level, with a minimum of 200 units counted per sample. The biovolume (μm^3 L^{-1}) of each taxa was then estimated from the area per unit measured with a Nikon DS-Vi1 camera and NIS-Elements imaging software (version 3.22.11). Specifically, *Microcystis* biovolume was calculated from individual colony radii with the assumption that each colony was a sphere and that colony biovolume resembled cellular biovolume as the colonies were too dense and three dimensional to count individual cells. *Dolichospermum* biovolume was calculated by converting filament area to cells per filament using the average cell area calculated from 10 filaments per sample and then multiplying by the average cell biovolume (assuming spherical cells). *Planktothrix* and *Aphanizomenon* filament areas were converted to biovolume using the average filament width calculated from 10 filaments per sample and the assumption that each filament was a cylinder (Hillebrand et al. 1999; Harke et al. 2015). Green and brown algal relative abundances determined via Fluoroprobe analysis were also confirmed by microscopic examination (data not shown).

Nutrient samples were analyzed for nitrate, ammonium, orthophosphate, total nitrogen (TN), and total phosphorus (TP) on a Lachat Instruments autosampler (ASX-520 series) using standard wet chemistry (Valderrama 1981; Jones 1984; Parsons 2013). Recovery of standard reference material of at least 90% ± 10% was achieved for all nutrient analyses. Photosynthetic efficiency, a diagnostic measure of nutrient stress (Parkhill et al. 2001; Simis et al. 2012), was determined by the increase in fluorescence yield from dark-adapted in vivo fluorescence (F_V) to maximal DCMU-enhanced (3,4-dichlorophenyl-1,1-dimethylurea, photosynthetic inhibitor) fluorescence (F_M), measured on a Turner Designs TD-700 fluorometer (EM filter of > 665 nm and EX filter of 340–500 nm) that was blank corrected with filtered lake water. Total microcystins were extracted from whole water samples by chemical lysis using the Abraxis Quicklyse Cell Lysis Kit, then quantified using the congener-independent (Fischer et al. 2001) Abraxis Microcystin/Nodularian (ADDA) enzyme-linked immunosorbent assay kit per the manufacturer's instructions. Concentrations were measured on a SpectraMax plus 384 plate reading spectrophotometer, calculated using a logarithmic curve, and reported in microcystin LR-equivalents. This assay provided a 98.6% ± 5% average recovery of samples spiked with known concentrations of microcystin-LR standard.

DNA isolation, sequencing, and analysis

For molecular analyses, total nucleic acids were extracted from the field and experimental samples (biological triplicates) using the cetyltrimethyl ammonium bromide (CTAB) method outlined in Dempster et al. (1999). Briefly, frozen filtered samples were placed in 1 mL of CTAB lysis buffer with 4 μL beta-mercaptoethanol, heated to 50°C for 30 min and then frozen at −80°C overnight, followed by a single chloroform extraction and an isopropanol/sodium chloride precipitation. The nucleic acids were then resuspended in 25 μL of sterile nuclease free water, and double-stranded DNA was assessed for quality using a Nanodrop spectrophotometer and quantity using a Qubit fluorometer with a dsDNA BR Assay kit per the manufacturer's instructions. Samples were normalized and stored at −80°C until polymerase chain reaction (PCR) amplification and amplicon sequencing, performed at Molecular Research Labs (Shallowater). The Sandusky transect S6 sample was excluded from sequencing due to low extraction efficiency and therefore was not considered for 16S and *mcyE* analysis.

To examine the cyanobacteria community composition, the 16S rRNA gene (~ 349 bp) was amplified using the cyanobacteria-specific primer set CYA106F: 5′-CGG ACG GGT GAG TAA CGC GTG A-3′ (Nübel et al. 1997) and 530R: 5′-CCG CNG CNG CTG GCA C-3′ (Usher et al. 2014), developed to discriminate between cyanobacteria genera (Nübel et al. 1997; Castiglioni et al. 2004). To determine the composition of the microcystin-producing community, the *mcyE* gene (~ 472 bp) was amplified using the HepF 5′-TTT GGG GTT AAC TTT TTT GGG CAT AGT C-3′ and HepR 5′-AAT TCT TGA GGC TGT AAA TCG GGT TT-3′) primer set developed by Jungblut and Neilan (2006) to discriminate between toxic and nontoxic strains of microcystin-producing genera. The *mcyE* gene was chosen as it is one of several microcystin synthetase genes and encodes an aminotransferase that plays a key role in the production of microcystin (Tillett et al. 2000).

Prior to PCR amplification, an identifying barcode was placed on the forward primer for each sample and then amplification was conducted using the HotStarTaq Plus Master Mix Kit and cycling conditions as follows: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min and a final elongation step at 72°C for 5 min. The PCR products were visualized on a 2% agarose gel to determine the success of the amplification and the relative intensity of the product bands. The samples were then pooled together for each respective primer region (16S rRNA or *mcyE*) in equal proportions based on their molecular weight and DNA concentrations, and purified using calibrated Ampure XP beads. The purified products were used to prepare a DNA library following the Illumina TruSeq DNA library preparation protocol and sequenced on an Illumina MiSeq platform for paired end reads (2 × 300) following the manufacturer's guidelines.

The 16S and *mcyE* sequence data were processed using the Quantitative Insights into Microbial Ecology (QIIME) v1.9.1 pipeline (Caporaso et al. 2010). Briefly, the raw paired-ends reads were trimmed of their sample identification barcodes prior to joining using seqprep, and then the joined reads were depleted of both primers and demultiplexed into their respective samples based on the associated barcodes. Quality filtering was performed throughout this process using the default parameters in QIIME, and the resulting split library output was filtered for chimeric sequences using usearch61 (Edgar 2010; Edgar et al. 2011). The filtered sequences were then clustered into operational taxonomic units (OTUs) based on 97% (16S) or 99% (*mcyE*) sequence identity using UCLUST (Edgar 2010) and open reference clustering with their respective reference databases (see below). The resulting representative sequences were aligned using PyNAST (Caporaso et al. 2010) and taxonomically classified using BLAST (Altschul et al. 1990). To confirm taxonomic assignment, representative sequences for the most abundant OTUs were extracted and identified using the National Center for Biotechnology Information (NCBI) basic local alignment search tool (BLAST). Postprocessing in QIIME, the 16S dataset was refined to exclude all noncyanobacteria, chloroplast, and mitochondria OTUs, which were not considered for further analysis as the focus of this study was on the cyanobacteria assemblage. The 16S OTUs were reported at the genus level, whereas the *mcyE* OTUs were reported at the lowest taxonomic level identified.

The SILVA rRNA (16S SSU) release v119 database (Quast et al. 2012) was used as the reference set for OTU picking and taxonomic identification of the 16S dataset, with two taxonomic modifications conducted postprocessing. First, the informatically identified *Prochlorococcus* genus was reassigned as *Synechococcus* for several reasons. First, NCBI BLAST identifications of the "*Prochlorococcus*" OTU consensus sequences were most similar to *Synechococcus* strains (*Synechococcus sp.* MV0409 and *Synechococcus sp.* 1tu14s11) in the Cluster F/Clade VII (Loar 2009). Next, phylogenetic analysis of the QIIME identified *Prochlorococcus* and *Synechococcus* OTU representative sequences resulted in an intermixed group, further suggesting misidentification. Finally, *Prochlorococcus* has yet to be reported within freshwater systems (Ouellette et al. 2006; Takasu et al. 2015). As another distinct *Synechococcus* OTU group was already identified from our analyses, the *Prochlorococcus* OTUs were reassigned as "*Synechococcus* clade II." Second, 16S sequences that were not matched to a taxonomic assignment at the genus level were grouped into an uncultured group and consisted of sequences most similar to *Pseudanabaena, Microcystis,* and *Nodosilinea* in NCBI BLAST searches (Supporting Information Table S2). As the "uncultured" OTU was a mixed taxonomic group, caution was used when interpreting the responses of this group to environmental variables.

For the *mcyE* dataset, a custom reference database was created from available sequences (n = 507) in the NCBI nucleotide database. Briefly, BLAST search results for the *mcyE* gene were filtered to remove whole genomes (> 2000 bp, to decrease processing time) or any noncyanobacteria taxa, and

converted into taxonomy and FASTA files using the gb2qiime.py script (McCann 2014). The FASTA sequences were aligned at a 100% identity in GeneiousPro v5.6.7 (Kearse et al. 2012) using clustalW (Larkin et al. 2007) and the default settings from which duplicate sequences were removed and the remaining sequences were trimmed to the amplicon region. In QIIME the trimmed sequences were then clustered at an 80% identity using UCLUST (v1.2.22q) (Edgar 2010) and a representative sequence set was generated using the default settings. The condensed representative set was aligned using MUSCLE (Edgar 2004) to create a core alignment file that was used along with the representative sequence file during open reference OTU picking. The OTU taxonomic assignments were modified postprocessing in the case of *Oscillatoria*, which was reassigned to *Planktothrix,* based on two criteria. First, in previous studies (Harke et al. 2015), *Planktothrix* sequences have been shown to be misidentified as *Oscillatoria* by molecular pipelines due to reference database errors, and second, in samples where *Oscillatoria* was detected in high abundances by the *mcyE* sequencing, it was not detected by 16S sequencing or microscopic identification, while *Planktothrix* was detected.

For the 16S rRNA marker, the 58 in situ and experimental samples generated a total of 9,874,402 sequences with an average length of 368 bp. After joining and quality filtering a remaining 7,922,628 sequences clustered into 21,508 OTUs at a 97% sequence identity, of which 2567 were classified as cyanobacteria (Supporting Information Table S3). For the *mcyE* marker, the 58 samples generated a total of 7,050,837 sequences of ~ 413 bp length. A total of 4,172,528 sequences remained after quality filtering and joining and clustered into 868 OTUs at a 99% sequence identity (Supporting Information Table S3). Sequence data from this study has been deposited to GenBank (Accession numbers SPR8472474-SPR8472589).

Statistical analyses

All statistical analyses were performed using R software v3.2.3 (R Core Team 2013) unless otherwise noted. For the transect samples, Spearman correlations were used to compare the community abundances (Fluoroprobe biomass, biovolumes, and 16S and *mcyE* relative abundances) and biochemical metrics (F_V/F_M and microcystin concentrations) to the environmental parameters (T, nitrate, ammonium, orthophosphate, TN, and TP) to assess drivers of the biological variation. Additionally, Spearman's correlations were used to compare cyanobacteria genera abundances determined by microscopy (biovolume) and 16S rRNA sequencing (relative abundance) methods.

For the experiments, three-way analyses of variances (ANOVAs) were used to test for significant ($\alpha = 0.05$) effects of the experimental treatments (N, P, and T) on the community abundances (Fluoroprobe, biovolumes, and *mcyE* relative abundances) and biochemical metrics (F_V/F_M and microcystin concentrations). Briefly, prior to ANOVA analysis, data were assessed for normality (Shapiro–Wilk test) and equal variance (Bartlett's test) and then modeled using linear models (Gaussian error structure) which were assessed for goodness of fit through examination of qqplots and histograms of the residuals. Right skewed datasets were natural log transformed to increase normality, and the *mcyE* relative abundance datasets were arc-sin-square root transformed. Datasets that did not fit a Gaussian distribution (failed assumptions of normality and equal variance tests) were modeled using a generalized linear model assessed for goodness of fit using 1 − (residual deviance/null deviance). Specifically, fluorescence abundances were modeled with a Poisson error structure while the proportion of OTU to total sample *mcyE* reads were modeled with a binomial error structure and logit link function. The model types and transformations used are noted in the ANOVA result tables. Three-way ANOVAs (car package) were then performed on the models to determine significant main effects and interactions of the N, P, and/or temperature treatments, followed by a Tukey post hoc multiple comparison analysis.

Differential abundance analysis was used to identify significant changes in the abundances of the individual cyanobacteria genera identified by the 16S sequencing due to the experimental treatments or between the field stations, using the Phyloseq and DESeq packages (Anders and Huber 2010; McMurdie and Holmes 2013; McMurdie and Holmes 2014). Briefly, raw 16S read counts were modeled using a negative binomial distribution and parametric fitting of the dispersions, followed by Wald significance testing to calculate significant $\log_2$ fold changes in abundance ($\alpha = 0.05$). Changes in abundances were examined between bays and between the nearshore and offshore sites in the field and compared to the control in the experiments. *p* values were adjusted using the Benjamini–Hochberg procedure to correct for multiple testing.

Multivariate statistical approaches were employed to analyze differences among the 16S and *mcyE* community structures (composition/abundance) in relation to environmental and experimental variables. Specifically, hierarchical clustering analysis (average linkage algorithm) was conducted using Euclidean and Bray-Curtis dissimilarity metrics of the physio-chemical and relative abundance (16S, *mcyE*) datasets, respectively, to determine groups of field sites with similar physical conditions and community compositions. The field and experimental 16S and *mcy*E relative abundances were then analyzed with nonmetric multidimensional scaling (nMDS; vegan package) analysis using the Bray–Curtis dissimilarity metric to identify variables whose communities behaved in a similar manner. To test for significant dissimilarities in the community composition between field groups identified via clustering or experimental treatments analysis of similarity (ANOSIM; rank based) and permutational analysis of variance (PERMANOVA; distance based) tests were performed on the 16S and *mcyE* Bray–Curtis dissimilarity metrics in PAST v2.17c (Hammer et al. 2001) with 999 permutations. Similarity percentage analysis (SIMPER) was then conducted to identify taxa driving the community dissimilarity between significantly different groups. Significant differences in diversity (genus richness) between the field clusters or experimental treatments were assessed using one-way ANOVAs followed by a

Tukey post hoc analyses. Additionally, canonical correspondence analyses (CCA) were performed to identify associations between the environmental variables and the community compositions of the field samples complemented with PERMANOVA analysis (Adonis; vegan packages) to test for significant correlations between the physiochemical parameters and community variations.

Results

Field surveys

Physiochemical lake properties

In both the Maumee and Sandusky transects, most physiochemical parameters varied with proximity to the respective rivers, with distinct nutrient–temperature conditions observed between the nearshore and offshore sites (Fig. 2a). Surface water temperatures (19.0–21.6°C) significantly increased with distance offshore (*t*-test, $p < 0.05$; Table 1), while nutrient concentrations were typically higher nearshore. TN and TP concentrations were significantly higher at nearshore sites (M1-M2 and S1-S4; TN > 75 μM; TP > 3 μM) in both transects (*t*-test, $p < 0.01$; Table 1). Peak nitrate concentrations were three-fold greater near the mouth of the Maumee River (25.5 μM) compared to the Sandusky transect, whereas ammonium (3.47 μM) and orthophosphate (0.99 μM) concentrations were two- to three-fold higher along the Sandusky transect (Table 1). There was no significant difference between the nutrient and temperature conditions between the bays (*t*-test, $p > 0.05$).

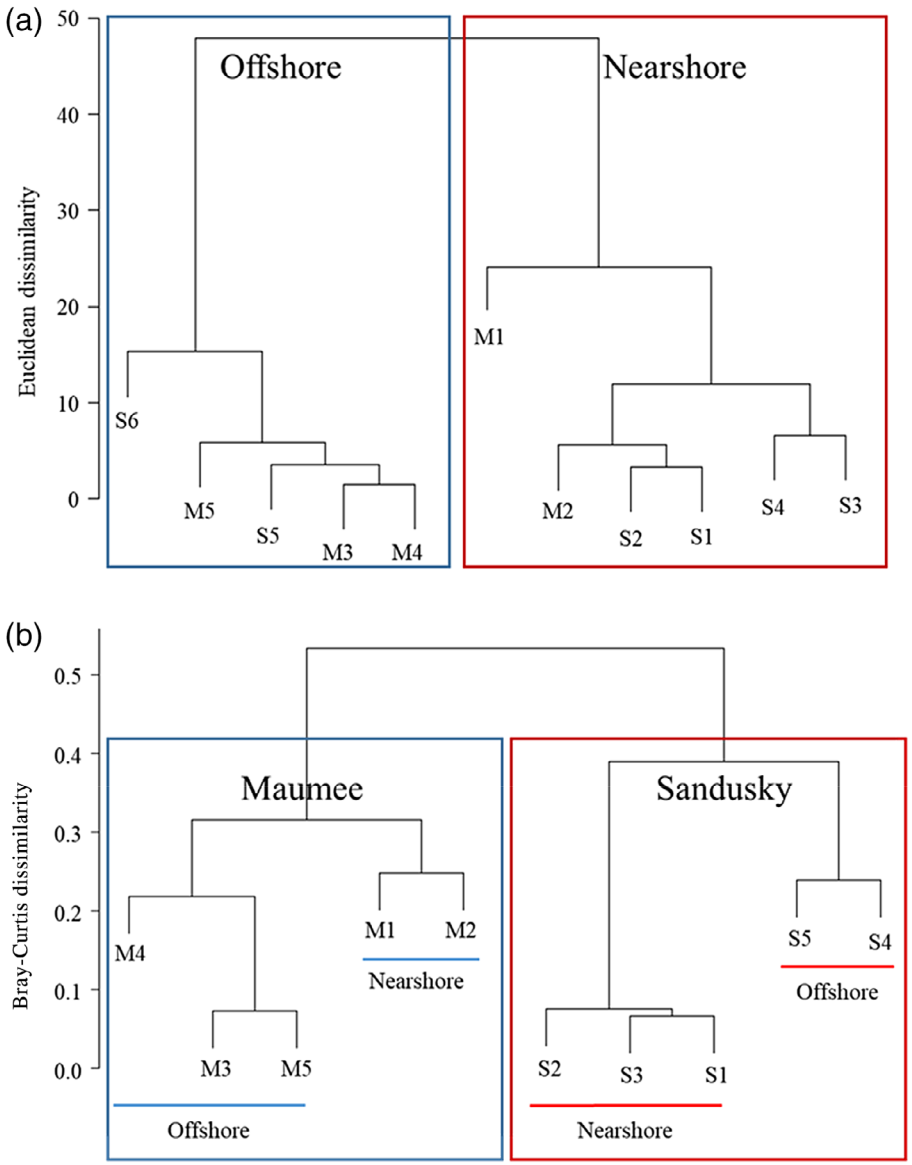

Fig. 2. Hierarchical clustering of the Maumee (M) and Sandusky (S) Bays transect sites based on **(a)** nutrient/temperature conditions using the Euclidean dissimilarity metric and **(b)** 16S rRNA relative abundance community data using the Bray–Curtis dissimilarity metric.

Table 1. In situ physiochemical conditions (water temperature, dissolved oxygen (DO) and nutrient concentrations) and biological responses (microcystin concentrations, photosynthetic efficiency (F_V/F_M) and chlorophyll *a* (Chl *a*)) for the Maumee (M) and Sandusky (S) transect sites. BDL indicates below the detection limit. Missing data are represented with a (–).

Site	Microcystin ($\mu g\ L^{-1}$)	F_V/F_M	Chl *a* ($\mu g\ L^{-1}$)	Surface temperature (°C)	Surface DO ($mg\ L^{-1}$)	Nitrate (μM)	Ammonia (μM)	Orthophosphate (μM)	TN (μM)	TP (μM)	TN : TP
Ml	0.50	0.6±0	26.5±0.4	19.78	8.66	25.5±0.3	0.87±0.06	0.33±0.02	94.5±2.4	3.4±0.3	27.4
M2	2.84	0.5±0	32±3.4	19.01	10.87	3.6±0.1	1.12±0.09	0.25±0.01	76.2±4.3	3.7±0.3	20.6
M3	1.25	0.6±0	29.1±0.7	20.73	9.25	3.6±0	0.71±0.04	BDL	42.2±1.2	1.6±0	26.3
M4	0.11	0.6±0	28.7±2.3	20.91	9.47	3.7±0	0.67±0.07	0.32±0.07	40.7±2.2	1.5±0.2	26.6
M5	0.69	0.6±0	21.4±1.2	21.03	9.42	3.9±0	0.65±0.05	0.17±0.05	36.8±0.5	1.1±0	34.3
S1	1.10	0.5±0	21.9±3.3	19.11	9.04	8.1±1	3.47±0.08	0.75±0.11	78.5±6.9	3.5±0.2	22.2
S2	2.12	0.4±0	16.7±1.1	19.34	8.42	6.6±0.1	0.9±0.04	0.64±0.07	81.5±2.6	3.7±0.2	22.3
S3	7.65	0.4±0	18.4±2.3	19.28	8.78	3.6±0.1	-	0.83±0.09	86.9±3	4.2±0	20.7
S4	2.98	0.4±0	24.5±2.6	19.06	8.72	3.6±0.1	1.32±0.12	0.99±0.21	93.5±0.5	3.7±1.1	25.0
S3	1.64	0.4±0	16±1.6	19.89	9.15	3.7±0	-	0.24±0.05	45±4.3	2.2±0.3	20.4
S6	0.30	0.4±0.1	7.5±0.2	21.60	8.61	3.8±0.1	1.71±0.36	0.24±0.02	25.9±1.5	1.1±0	24.6

Phytoplankton class dynamics

Cyanobacterial blooms were present in both the Maumee and Sandusky Bays, with similar spatial distributions of the phytoplankton classes across each transect. Cyanobacteria dominated the fluorometrically identified phytoplankton assemblages at all transect sites, comprising 40–100% of the community, with the highest abundances occurring nearshore (Fig. 3a). Along the Maumee transect, cyanobacterial biomass peaked at site M2 ($66.3 \pm 1.19\ \mu g\ L^{-1}$) and decreased to lower levels in the open waters of the western basin (M3–M4, $10.9 \pm 0.70\ \mu g\ L^{-1}$; Fig. 3a), being dominated by *Microcystis* at all sites (as a percentage of biovolume; Supporting Information Fig. S1B). Along the Sandusky transect, cyanobacteria dominated the phytoplankton assemblages throughout the inner and outer bay (S1–S4, $114 \pm 66.9\ \mu g\ L^{-1}$), peaking at site S4 ($189\ \mu g\ L^{-1}$) and decreasing into the western basin (Fig. 3a). Cyanobacterial levels were three-fold greater in the Sandusky transect compared to the Maumee transect and was composed primarily of *Planktothrix agardhii* (Supporting Information Fig. S1b). Green and brown algae were only present at sites with lower cyanobacterial levels, with the highest abundance of green algae occurring near both river mouths and brown algae only occurring offshore within the Maumee transect (Fig. 3a). ANOSIM detected a significant dissimilarity ($p < 0.05$) of the pigment-defined phytoplankton communities between transects, as the Sandusky transect phytoplankton assemblage was almost exclusively cyanobacteria (> 93%), with higher cyanobacterial biomass, compared to the Maumee transect which exhibited a more mixed phytoplankton community (Fig. 3a).

Cyanobacteria community dynamics

Both microscopy and 16S sequencing identified *Microcystis*, *Planktothrix*, *Aphanizomenon,* and *Dolichospermum* as the most abundant cyanobacteria in Maumee and Sandusky Bays with significant correlations of the relative abundances quantified via microscopy and sequencing for *Microcystis*, *Planktothrix*, and *Dolichospermum* ($p < 0.05$; Supporting Information Table S4). Amplicon sequencing of the 16S rRNA gene identified diverse cyanobacterial assemblages associated with the Maumee and Sandusky blooms (Fig. 4) that varied between bays and spatially within each transect (Fig. 2b). Along the Maumee transect, 1435 OTUs corresponding to 30 genera were identified, with a core community of 128 OTUs shared among all sites (Fig. 5). *Microcystis* was the most abundant cyanobacteria genera in Maumee Bay, comprising nearly half of the sequence reads (43.1% ± 14.0%; Fig. 3c) across all sites. *Synechococcus* clade II was also present in high abundances throughout the transect (19.8% ± 6.69%), and *Planktothrix* (10.2% ± 8.03%), *Aphanizomenon* (7.65% ± 5.88%), *Synechococcus* clade I (5.57% ± 2.47%), uncultured cyanobacteria (4.75% ± 1.45%), and *Pseudanabaena* (3.86% ± 2.21%) were other common genera across sites (Fig. 3c). Distinct cyanobacteria community structures ($p < 0.1$) were identified between the nearshore bloom sites (M1 and M2) and offshore sites (M3–M5) by clustering analysis (Fig. 2b) with an average dissimilarity of 31.6%, driven primarily by *Microcystis* (31.18%), *Aphanizomenon* (16.83%), *Planktothrix* (13.63%), and *Synechococcus* clade II (12.34%, SIMPER; Fig. 3c). The nearshore bloom communities (M1 and M2) were, on average, more diverse by genus richness than the offshore sites (M3–M5; Supporting Information Fig. S2), where *Microcystis* was significantly ($p < 0.05$) more abundant (Fig. 3c; Supporting Information Table S5A).

Along the Sandusky transect, the cyanobacterial assemblages consisted of 1936 OTUs corresponding to 36 genera of which 216 OTUs were shared between all sites (Fig. 5). The cyanobacteria communities were dominated by *Synechococcus* clade II and *Planktothrix,* which together accounted for ~ 75% of the sequence reads (Fig. 3c). *Synechococcus* clade II

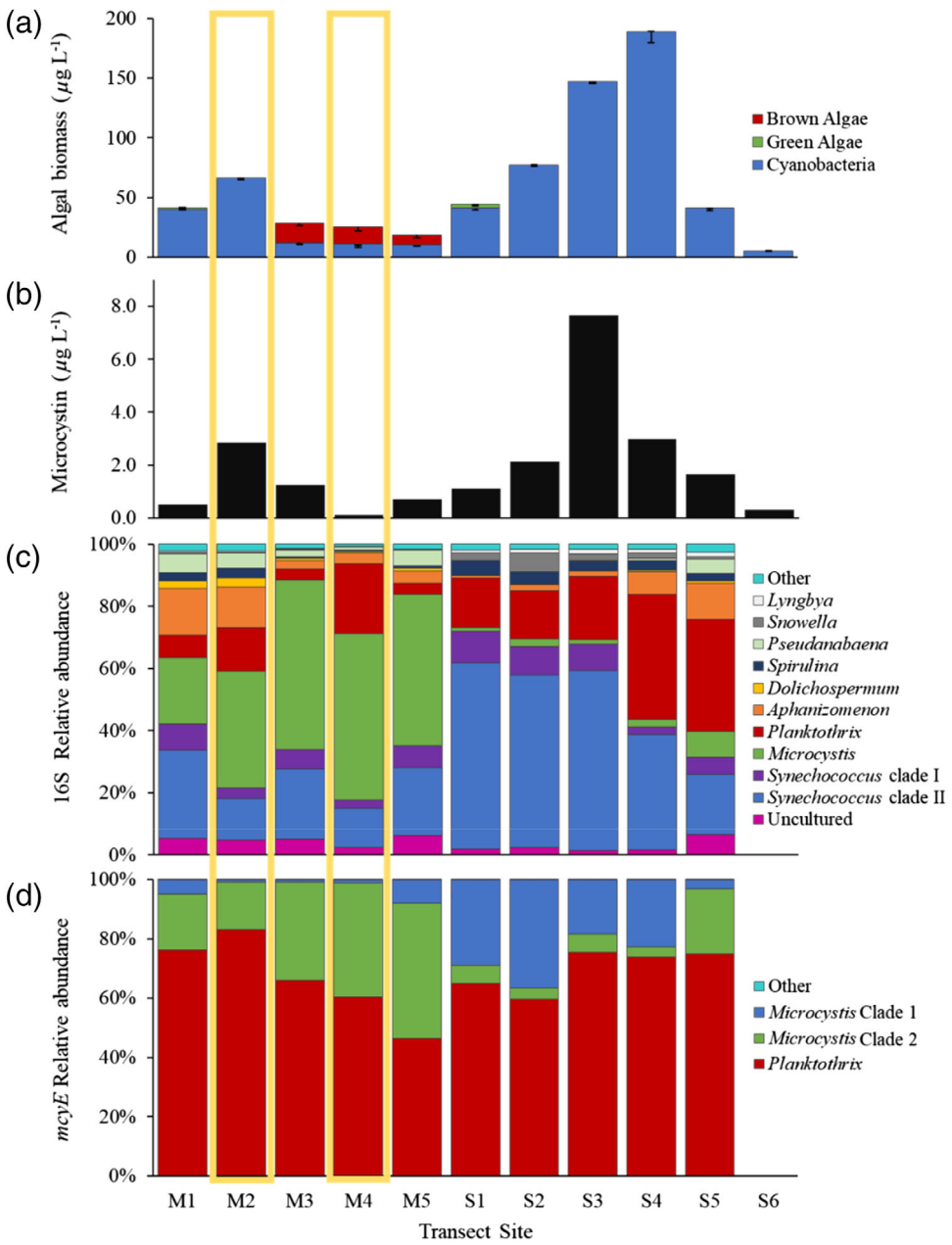

Fig. 3. Algal community and microcystin concentrations at the Maumee (M) and Sandusky (S) Bays transect sites. **(a)** Absolute abundance of the fluorometrically identified algal classes. **(b)** Total microcystin concentrations in LR-equivalents. **(c)** Relative abundance of cyanobacteria genera identified by 16S sequencing. All low-abundance genera have been condensed and listed as "Other." **(d)** Relative abundance of microcsytin-producing taxa identified by *mcyE* sequencing. Sites for which experiments were conducted are denoted with yellow boxes. Error bars are replicate standard deviations (n = 3).

(57.8% ± 2.25%) was the most abundant genera nearshore (S1–S3), where it was present at significantly higher relative abundances than in the offshore sites ($p < 0.01$; Supporting Information Table S5A; Fig. 3c); concurrently *Synechococcus* clade I was also more abundant near the river (9.30 ± 0.92; Fig. 3c). Conversely, *Planktothrix* (38.2% ± 2.88%) was more abundant offshore (S4–S5; Fig. 3c), where *Aphanizomenon* (9.39% ± 3.17%), *Microcystis* (5.34% ± 3.96%), and uncultured cyanobacteria (4.12% ± 3.45%; Fig. 3c) were also present at higher abundances. Clustering analysis of the 16S compositions indicated unique community structures between the nearshore and offshore sites ($p < 0.1$; Fig. 6a), which were 39.0% dissimilar predominantly driven by *Synechococcus* clade II (37.88%) and *Planktothrix* (26.79%; SIMPER; Fig. 3c), with the offshore communities (S4–S5) being, on average, more diverse (Supporting Information Fig. S2).

Despite similar physiochemical conditions, distinct cyanobacterial assemblages were associated with the Maumee and

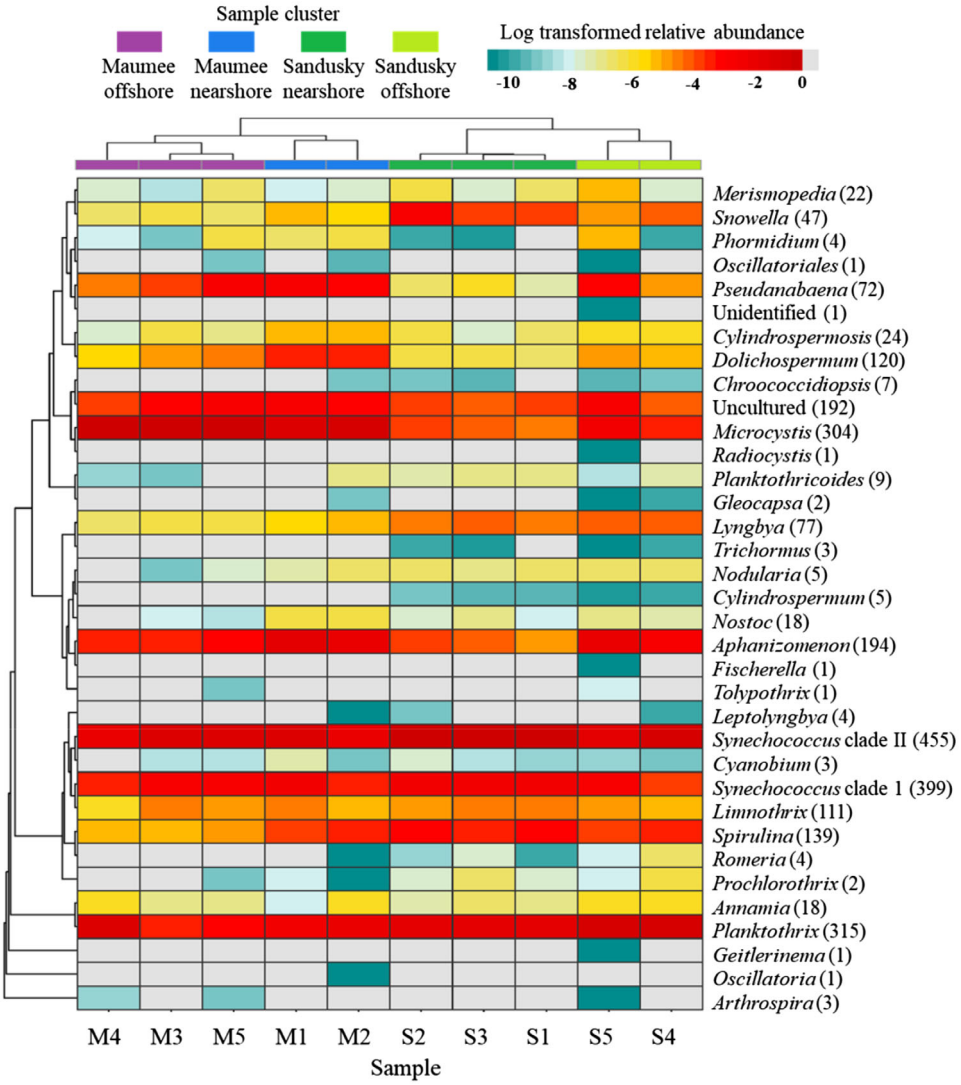

Fig. 4. Heatmap of 16S relative abundances of cyanobacteria detected in the Maumee (M) and Sandusky (T) transects. The abundances have been natural log transformed to better visualize changes among the low-abundance taxa. Gray boxes indicate not present. The sites (columns) are clustered by 16S community Bray–Curtis dissimilarity, and the genera (rows) are clustered by phylogenetic relationship. Numbers next to the taxa name indicate number of OTUs identified for that genera.

Sandusky blooms (Fig. 6a). Among individual genera, *Microcystis* ($p < 0.001$), *Dolichospermum* ($p < 0.001$), and *Pseudanabaena* ($p = 0.02$) were significantly more abundant in Maumee Bay, whereas several low-abundance genera (e.g., *Spirulina* [$p = 0.05$], *Snowella* [$p < 0.001$], and *Lyngbya* [$p < 0.001$]) were significantly more abundant in Sandusky Bay (Supporting Information Table S5A). Furthermore, the 16S community compositions differed significantly between transects (ANOSIM and PERMANOVA; $p < 0.01$) with an average dissimilarity of 53.4% driven by *Microcystis* (37.4%), *Synechococcus* clade II (25.6%), and *Planktothrix* (15.6%; SIMPER; Fig. 3c). On average, the Sandusky cyanobacterial communities (28 genera) were more diverse than the Maumee communities (22 genera) by genus richness (Supporting Information Fig. S2). Together, the measured environmental variables explained 77% of the cyanobacteria community composition variability between the transect sites (Fig. 6b), with significant correlations between the 16S dissimilarities and surface temperature, orthophosphate, TN, and TP ($p < 0.05$; Adonis). Generally, both the Maumee and Sandusky Bays nearshore communities were associated with higher nutrient conditions (TN and TP), with a stronger correlation between the Maumee communities and nitrate concentrations, and between the Sandusky communities and orthophosphate (Fig. 6b), whereas the Maumee Bay offshore communities were correlated with surface water temperatures (Fig. 6b).

Microcystin levels and the microcystin-producing assemblage

In both transects, microcystin concentrations generally paralleled and were significantly correlated with cyanobacterial

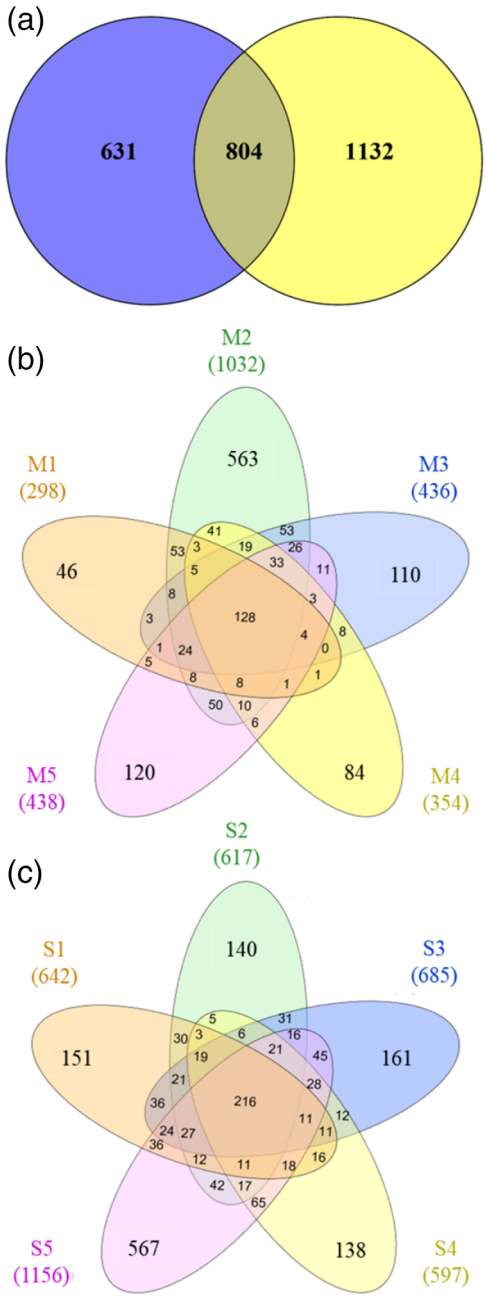

Fig. 5. Venn diagram showing the number of unique and shared (overlapped regions) OTUs identified by 16S sequencing during the field survey between the Maumee (M) and Sandusky (S) transects **(a)** and between sites within the Maumee **(b)** and Sandusky **(c)** transects. Parenthesis indicates total number of OTUs per location.

biomass across all sites ($n = 10$, $p < 0.001$; Table 1; Fig. 3). Along the Maumee transect, microcystin concentrations averaged 1.08 ± 1.07 μg L^{-1}, reaching a maximum concentration of 2.84 μg L^{-1} at site M2 (Table 1), where cyanobacterial biomass was highest (Fig. 3). Microcystin concentrations were, on aver age, two-fold higher in the Sandusky transect (2.60 ± 2.62 μg L^{-1}) peaking at 7.65 μg L^{-1} at site S3 (Table 1; Fig. 3).

The potential microcystin-producing (*mcyE* gene containing) communities in both systems exhibited a low community diversity (Supporting Information Fig. S3), with only 486 genotypes corresponding to three cyanobacteria taxa—one *Planktothrix* strain and two *Microcystis* clades (Fig. 3d). The first *Microcystis* clade consisted of 81 OTUs most similar to *Microcystis* PCC 7005 (100% identity), *Microcystis* UTEX LB 2664 (100% identity), and *Microcystis* PCC 7806SL (99% identity) in NCBI BLAST search. The second *Microcystis* clade consisted of 500 OTUs most similar to *Microcystis* NIES-102 (99% identity) and *Microcystis* NIES-843 (99% identity). *Planktothrix* dominated the microcystin-producing community in both transects with a mean relative abundance of $64.9\% \pm 13.7\%$ in Maumee Bay and $68.0\% \pm 6.72\%$ in Sandusky Bay (Fig. 3d). Of the *Microcystis* clades identified, clade 2 was more abundant in the Maumee transect, with a relative abundance ranging from 15.6% to 44.6%, while clade 1 was more abundant along the Sandusky transect, with an average relative abundance ranging from 3.11% to 35.9% (Fig. 3d). Microcystin concentrations were most strongly correlated with the *Planktothrix mcyE* relative abundances and *Planktothrix* cell abundances (by biovolume and filament counts) in Sandusky Bay ($p < 0.05$ for all; Supporting Information Table S6).

Nutrient–temperature amendment experiments

Maumee Bay experiment

After 96 h, the total phytoplankton community in the control differed from that of the initial bloom community, with a decrease in cyanobacterial biomass to 23 μg L^{-1} and an increase in green algae to 8.05 μg L^{-1} (Fig. 7a). Compared to the control, green algal biomass was significantly lower in all +N treatments ($p < 0.001$; Supporting Information Table S7A and Fig. S4; Fig. 7a), whereas cyanobacterial biomass significantly increased by three- to four-fold in all +N treatments ($p < 0.001$; Supporting Information Table S7A and Fig. S4; Fig. 7a). Within the +N treatments, there was a significant, synergistic +N + T interaction ($p < 0.001$) on cyanobacterial abundance resulting in the greatest cyanobacterial biomass, whereas there was a significant, antagonistic interaction of +N + P at ambient temperature with cyanobacteria levels lower than the +N only treatment ($p < 0.001$, Supporting Information Table S7A and Fig. S4). Higher temperatures also yielded a significant increase in cyanobacterial biomass ($p < 0.001$; Supporting Information Table S7A) among all nutrient treatments (Supporting Information Fig. S4), with a synergistic interaction of T and P yielding levels higher than the individual treatments ($p < 0.05$; Supporting Information Table S7A and Fig. S4). Concurrent with the increase in cyanobacterial biomass, photosynthetic efficiency (F_V/F_M; Table 2) was significantly higher in the N and T treatments compared to the control after 96 h ($p < 0.05$; Supporting Information Table S7C).

The cyanobacteria community structure (16S) was only significantly altered by N (PERMANOVA, $p < 0.01$), with an average community dissimilarity of 29.9% (SIMPER) between

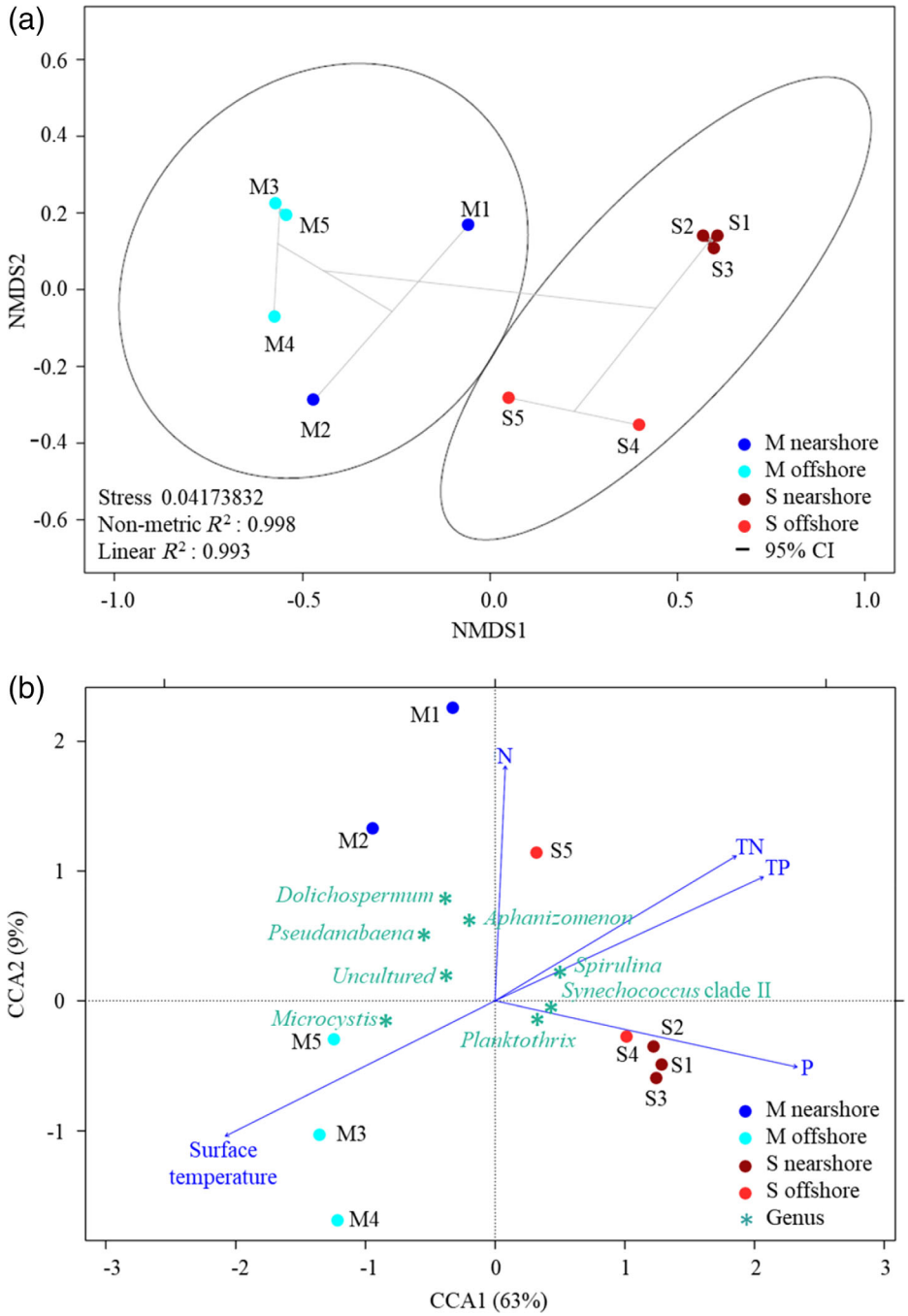

Fig. 6. Multivariate analysis of the Maumee (M) and Sandusky (S) Bays transect cyanobacteria communities using the Bray–Curtis dissimilarity metric. **(a)** nMDS analysis of the transect sites by 16S community composition overlaid with hierarchical clustering and 95% confidence intervals (CI) delineating significantly different community compositions. **(b)** CCA analysis showing the strength (length) and correlation (direction) of the environmental variables of interest (bi-plot arrows) to the 16S community clusters among sites along with ordination of most abundant genera.

treatments with and without N, driven by variations in the relative abundance of *Planktothrix* (42.0%), *Microcystis* (20.1%), *Synechococcus* clade II (13.6%), and *Aphanizomenon* (11.4%; Fig. 7c). The relative abundance of seven genera were significantly altered by N ($p < 0.05$; Supporting Information Table S5B), with an increase in *Planktothrix* and *Nodularia* and decrease in *Microcystis, Aphanizomenon, Dolichospermum, Limnothrix, and Chroococcidiopsis* among the +N treatments (Supporting Information Table S5B; Fig. 7c). Elevated P caused a significant increase in the relative abundance of *Aphanizomenon* and *Dolichospermum* ($p < 0.05$; Supporting Information Table S5B; Fig. 7c) and decrease in *Chroococcidiopsis*, while the relative abundance

of *Dolichospermum* and *Chroococcidiopsis* significantly increased due to elevated temperature ($p < 0.05$; Supporting Information Table S5B).

The microcystin-producing (*mcyE*-containing) community structure was significantly altered by the +N + P treatments ($p < 0.05$; PERMANOVA; Fig. 7d), with an average community dissimilarity of 19.3% compared to the non + N + P treatments. Within the +N + P treatments, *Planktothrix,* which remained the dominant strain across all treatments (75.4% ± 9.48%; Fig. 7d), significantly increased in relative abundance ($p < 0.01$; Supporting Information Table S7B and Fig. S4), whereas the *Microcystis* clade 2 significantly decreased in relative abundance ($p < 0.05$;

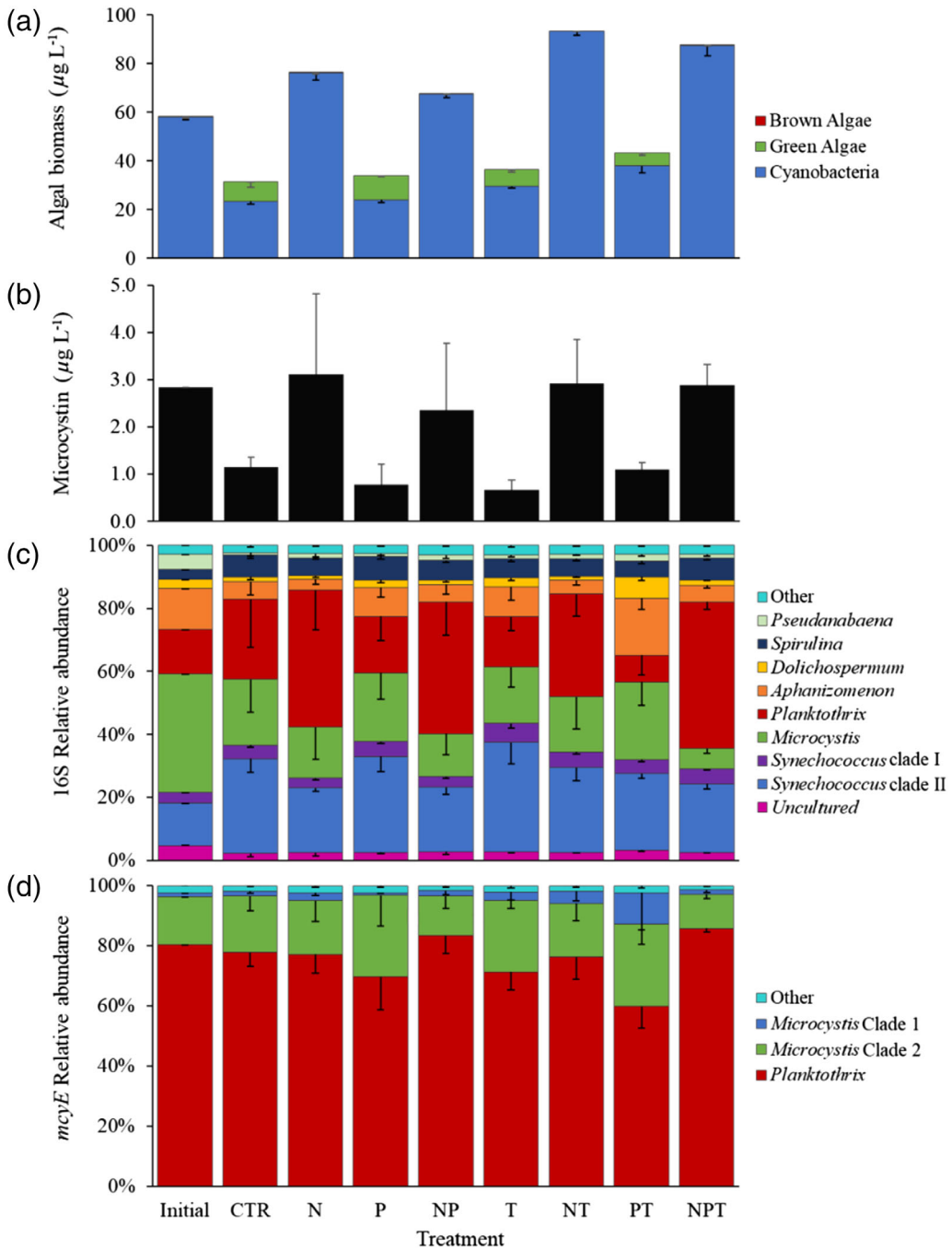

Fig. 7. Algal community composition and microcystin content in the Maumee Bay experiment (M2) per treatment (CTR, control; N, nitrogen; P, phosphorus; and T, temperature). **(a)** Absolute abundance of the fluorometrically identified algal classes. **(b)** Total microcystin concentrations. **(c)** Relative abundance of cyanobacteria genera identified by 16S sequencing. All low-abundance genera have been condensed into and "other" group. **(d)** Relative abundance of microcystin producing taxa identified by *mcyE* sequencing.

Table 2. Response of the biological processes (F_V/F_M, Chl *a*, and microcystin concentration) to the nitrogen (N), phosphorus (P), and temperature (T) treatments in **(A)** the Maumee Bay experiment and **(B)** the Western Basin experiment.

Treatment	F_V/F_M	Chl *a* (μ L^{-1})	Microcystin (μg L^{-1})
A			
Initial	0.52±0.02	35.9±1.49	2.84±0.00
Control	0.46±0.03	23.1±1.79	1.14±0.22
N	0.49±0.03	40.5±8.39	3.11±1.70
P	0.43±0.01	26.5±2.18	0.77±0.44
NP	0.48±0.01	43.5±2.93	2.35±1.42
T	0.47±0.04	23.7±2.07	0.66±0.22
NT	0.50±0.02	37.0±3.63	2.92±0.93
PT	0.47±0.02	31.5±1.28	1.09±0.15
NPT	0.63±0.13	39.4±2.76	2.89±0.43
B			
Initial	0.60±0.04	33.9±1.46	0.11±0.00
Control	0.50±0.04	21.5±0.54	0.37±0.21
N	0.52±0.03	39.3±2.33	0.66±0.53
P	0.50±0.08	23.4±1.03	0.35±0.28
NP	0.53±0.02	40.2±1.83	1.08±1.35
T	0.40±0.02	20.6±0.51	0.22±0.07
NT	0.51±0.05	32.5±2.04	0.68±0.44
PT	0.37±0.16	21.5±0.93	0.30±0.17
NPT	0.55±0.12	48.8±3.58	5.64±6.69

Supporting Information Table S7B and Fig. S4). Additionally, the relative abundance of *Microcystis* clade 1 significantly increased under elevated temperature ($p < 0.05$; Supporting Information Table S7B). Microcystin concentrations significantly increased in response to +N ($p < 0.001$; Supporting Information Table S7C; Fig. 7b) and were significantly correlated with the relative abundance (16S and *mcyE*) of *Planktothrix* across all experimental bottles ($p < 0.05$, Spearmans Correlation).

Western basin experiment

The non-bloom total phytoplankton community structure was more stable than the bloom community in Maumee Bay over the 96-h incubation, with a slight increase in brown algal biomass (23.3 ± 2.05 μg L^{-1}) and decrease in cyanobacterial biomass (7.21 ± 0.72 μg L^{-1}) in the control relative to initial conditions (Fig. 8a). Green algal abundance significantly increased in the +N + P treatment compared to the control ($p < 0.01$; Supporting Information Table S7A; Fig. 8a) with a synergistic interaction occurring in the +N + P + T treatment, resulting in significantly higher abundances than in all other treatments ($p < 0.001$; Supporting Information Fig. S4). Cyanobacterial abundance significantly increased in all +N treatments compared to the control ($p < 0.001$; Supporting Information Table S7A; Fig. 8a), with significant interactions within the +N + P and +N + T treatments ($p < 0.001$; Supporting Information Fig. S4), and the highest abundances occurring in the +N + P + T treatment (Fig. 8a). Conversely, brown algal abundance was significantly lower in the +N and +T treatments ($p < 0.001$; Supporting Information Table S7A, Fig. 8A), as well as the +N + P + T treatment compared to the control ($p < 0.001$; Supporting Information Fig. S4). On average, the photosynthetic efficiency of the phytoplankton community was significantly higher under +N conditions ($p < 0.05$, Supporting Information Table S7C).

After 96 h, within the cyanobacteria assemblage, N enrichment resulted in a significant decrease in the relative abundance (16S) of *Synechococcus* clade I ($p < 0.05$) and the elevated temperature conditions resulted in a significant decrease in relative abundance (16S) of *Microcystis* ($p < 0.05$; Supporting Information Table S5C; Fig. 8c). Among the microcystin-producing cyanobacteria, *Microcystis* clade 2 became the most abundant taxa in all treatments after 96 h (Fig. 8d) but had significantly lower relative abundances in the +N treatments ($p < 0.05$; Supporting Information Table S7B and Fig. S4). *Planktothrix* became more abundant in the +N, +P, and +T treatments (Fig. 8c); however, abundances were not significantly different in any individual treatments compared to the control (Supporting Information Fig. S4). Overall, the microcystin-producing community structure was significantly altered under the +N + P treatment ($p < 0.05$; PERMANOVA). Microcystin concentrations significantly increased in the N enrichment treatments ($p < 0.05$; Supporting Information Table S7C; Fig. 8b), with the highest concentration (5.64 ± 6.69 μg L^{-1}) in the +N + P + T treatment (Table 2; Fig. 8b).

Discussion

Nutrient and temperature controls on cyanobacteria abundance and phytoplankton community dynamics

The 2015 CHAB bloom in Lake Erie studied here was one of the most severe to date (National Oceanic and Atmospheric Administration 2017) and formed following a period of high rains associated with the record discharges from the Maumee River during June and July (National Oceanic and Atmospheric Administration 2015*b*). Findings indicated that cyanobacterial abundance in September 2015 was strongly influenced by nutrient availability, with peak cyanobacterial abundances present under high nutrient regimes. Across the Maumee and Sandusky transects, cyanobacterial abundance was significantly correlated with TN and TP ($p < 0.05$; Supporting Information Table S6), with the highest concentrations at the nearshore sites and decreasing with distance offshore, associated with reduced influence of the river discharges. Although this correlation is in part due to cyanobacterial cells contributing to these pools as particulate N and P, the individual nutrient species followed similar trends. These similar cyanobacteria–nutrient spatial distributions in both bays support the hypothesis that nutrient inputs from the

Maumee and Sandusky tributaries are important stimuli for cyanobacteria growth in this system and help fuel the late-summer bloom in Lake Erie's western basin (Millie et al. 2009; Michalak et al. 2013).

Experiments further indicated the influence of nutrient enrichment on cyanobacterial abundance, as N additions were the primary driving factor of cyanobacteria growth. During the Maumee Bay and western basin experiments, N enrichment significantly increased cyanobacterial abundance three- and two-fold higher compared to the controls, respectively, with concurrently increased photosynthetic efficiency of the phytoplankton communities. Together, these findings suggest that the phytoplankton community throughout western Lake Erie was N limited (Parkhill et al. 2001; Simis et al. 2012), which has been previously described during late-summer blooms in this region (Chaffin et al. 2013). In contrast, P enrichment alone did not alter cyanobacterial abundance in either experiment. This response may be partially attributed to the ability of *Microcystis,* which was a primary component of the Maumee Bay communities, to persist under low P conditions due to efficient P sequestering strategies (i.e., luxury uptake, high-affinity P transporters, and alkaline phosphatases; Wilhelm et al. 2003, Gobler et al. 2016, Harke et al. 2016). Spatial differences were observed, however, between Maumee Bay and the open waters of the western basin that had lower total P levels and the greatest increase in cyanobacteria abundance with the dual addition of N and P, suggesting cyanobacteria in the open waters of the western basin may be under dual N and P limitation. These results affirm the traditional paradigm that excess P is an important factor promoting the intensity of cyanobacteria blooms in western Lake Erie (Stumpf et al. 2012; Michalak et al. 2013), but indicates that N availability is an important driver for nondiazotrophic genera (*Microcystis, Planktothrix*), which dominate western Lake Erie cyanobacteria communities in late summer (Conley et al. 2009; Paerl and Huisman 2009; Gobler et al. 2016).

Cyanobacteria abundance was also found to be significantly enhanced by elevated temperature, with the combined increase in temperature and N yielding the highest cyanobacteria concentrations in both experiments, suggesting that although temperature is not the primary driver of cyanobacteria proliferation, it may additively enhance the impact of eutrophication. This finding has important ecological implications as it suggests that eutrophied freshwater systems will become more impaired with the projected increases in global temperatures (Paerl and Huisman 2009). Given that temperatures are expected to rise through this century regardless of global carbon measures (Edenhofer et al. 2014), restrictions to nutrient loads will be needed to mitigate cyanobacteria proliferation (Conley et al. 2009; Paerl and Huisman 2009; Michalak et al. 2013).

In both the field surveys and experiments, phytoplankton diversity as defined by pigment-based classes was inversely correlated with cyanobacterial abundance with reduced levels under high nutrient–temperature. Specifically, in the transects, green and brown algae were only detected at sites with lower cyanobacteria abundances, with green algae present at the more nutrient-rich sites near the Maumee and Sandusky Rivers (M1, S1) and brown algae in the nutrient-limited sites offshore (M3–M5), consistent with phytoplankton distributions previously reported for Lake Erie (Ghadouani and Smith 2005; Millie et al. 2009). Similarly, in the experiments, green and brown algae were not present (Maumee Bay experiment) or present at low abundances (Western basin experiment) in the N-enrichment treatments and reduced (to a lesser degree) by elevated temperature, conditions which significantly increased cyanobacteria abundance. However, in the western basin experiment, where cyanobacteria were less abundant, green algae abundance significantly increased under +N + P conditions and there was a smaller reduction in brown algae over time in the +P treatments indicative of N and/or P limitation among these communities. Together, these finding suggest the green and brown algae may benefit from nutrient additions but are outcompeted by cyanobacteria (Dokulil and Teubner 2000; Wilhelm et al. 2003; Paerl and Paul 2012), particularly in combination with warmer temperatures (Rigosi et al. 2014). Beyond nutrient competition (Elliott et al. 2006), these patterns may also be associated with allelopathic inhibition by cyanobacteria (Sukenik et al. 2002; LeBlanc et al. 2005; Chia et al. 2018), which can become more intense under nutrient-limited conditions (Chia et al. 2018) such as those described here.

Spatial dynamics of the Lake Erie cyanobacteria community structure

Although similar nutrient, temperature, and fluorometric community trends were observed in the Maumee and Sandusky transects, statistically distinct cyanobacteria communities were found within each system. Of the 2567 cyanobacteria genotypes identified, 25% of the OTUs were unique to Maumee Bay, whereas 44% were unique to Sandusky Bay (Fig. 5), with more diverse communities in Sandusky Bay as well. Community dissimilarity was driven by different bloom-forming genera within each system, as the Maumee Bay 16S communities were dominated by *Microcystis* (43%), a well-documented pattern in Lake Erie's western basin since the mid-1990s (Brittain et al. 2000; Wilhelm et al. 2003; Millie et al. 2009; Harke et al. 2016), whereas Sandusky Bay communities were dominated by *Planktothrix*, which is less studied but increasingly reported in recent years (Rinta-Kanto and Wilhelm 2006; Conroy et al. 2007; Davis et al. 2015). This difference may be, in part, associated with the differing orthophosphate levels between the bays, which were lower in the Maumee transect, and *Microcystis*' tolerability of low P conditions (Harke et al. 2012). Under P-limited conditions, *Microcystis* has been found to up-regulate several P-scavenging genes allowing it to persist in low P conditions (Harke et al. 2012), whereas *Planktothrix* has been observed to respond more conservatively, down-regulating alkaline phosphatase, photosynthesis, and cell division genes (Harke et al. 2015) and up-regulating fatty acid degradation

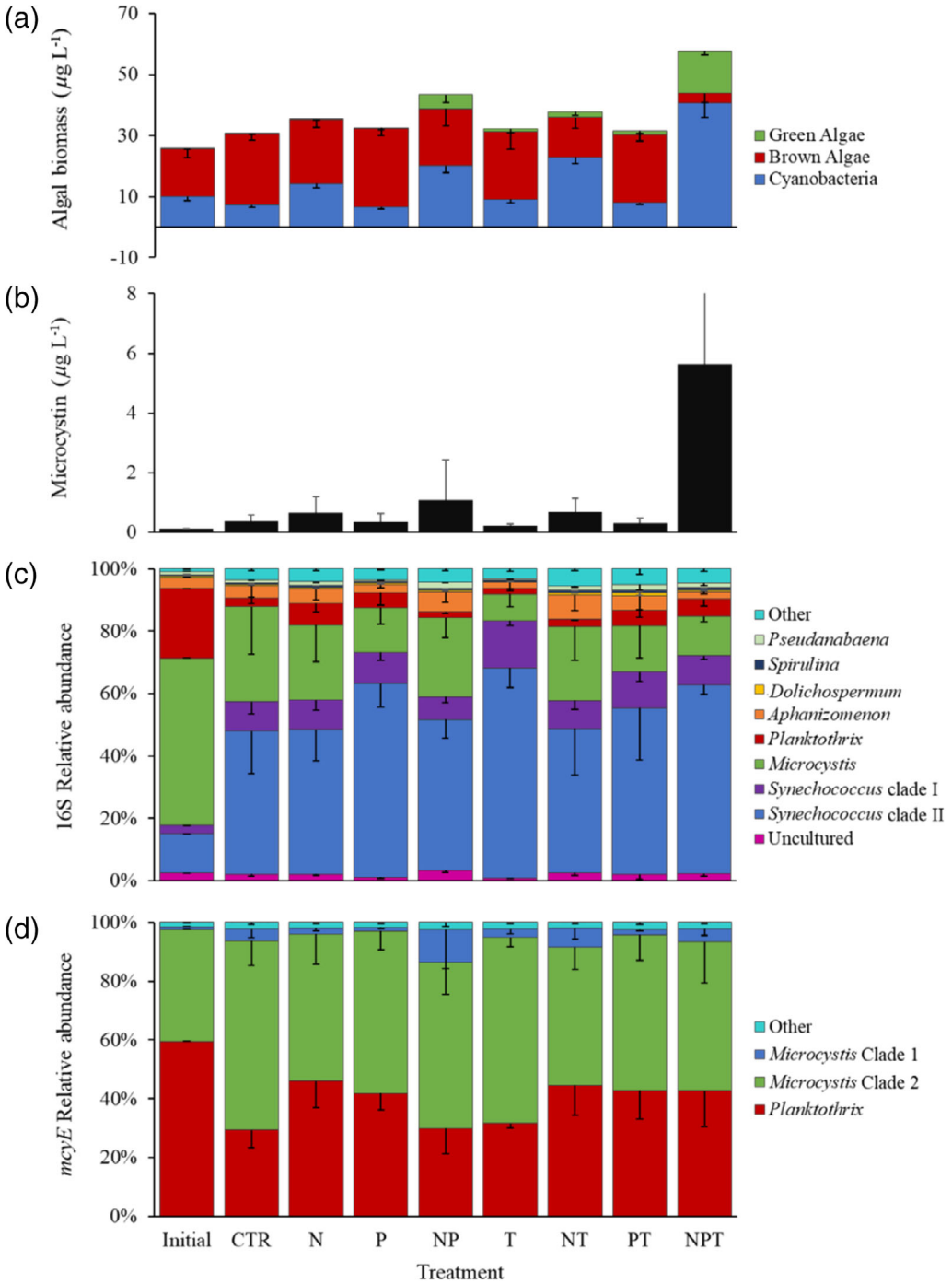

Fig. 8. Algal community composition and microcystin content in the Western Basin (M4) experiment (CTR, control; N, nitrogen; P, phosphorus; and T, temperature). **(a)** Absolute abundance of the fluorometrically identified algal classes. **(b)** Total microcystin concentrations. **(c)** Relative abundance of cyanobacteria genera identified by 16S sequencing. All low-abundance genera have been condensed into and "other" group. **(d)** Relative abundance of microcystin producing taxa identified by *mcyE* sequencing.

genes as an energy-sparing mechanism (Kaczmarzyk and Fulda 2010). Furthermore, *Planktothrix* has often been reported to dominate phytoplankton communities in temperate systems which are N limited but P rich (Donald et al. 2013; Steffen et al. 2014; Andersson et al. 2015), supporting their higher demand for P. However, these differences may also be related to physical differences between the bays. *Microcystis* is more likely to benefit in deeper, stable water conditions, such as Maumee Bay, due to its ability to regulate its bouyancy (Dokulil and Teubner 2000), allowing it to remain within surface waters

to prevent against light limitation as well as migrate to deeper nutrient-rich waters. In contrast *Planktothrix*, which does not have this ability, is often favored in turbid, shallow waters (Davis et al. 2015; Berger 2017), such as Sandusky Bay. Factors such as light or carbon limitation (Poste et al. 2013; Bullerjahn et al. 2016) may further explain the variation exhibited in the cyanobacteria community composition during this study, as the parameters examined only accounted for 77% of the variability (Fig. 6). Noteably, however, *Planktothrix* was also relatively abundant in the Maumee transect, accounting for around 10% of the community, and has been recorded in high abundances (up to 90% of the cyanobacteria community) in the western basin of Lake Erie in recent years (Millie et al. 2009), indicating it is an important component of CHABs across the western basin of Lake Erie.

Although *Planktothrix* and *Microcystis* are nondiazotrophic cyanobacteria, both dominated under the relatively low dissolved inorganic nitrogen (DIN) conditions observed in this study (~ 4 μM), despite N-stress among these populations displayed during experiments. In the Maumee Bay experiment, the relative abundance of *Planktothrix* was significantly increased by N enrichment, a finding consistent with prior studies of *Planktothrix* communities from Sandusky Bay (Davis et al. 2015). *Microcystis*, conversely, did not increase in relative abundance in the N-enrichment treatments but remained a large portion of the cyanobacteria community. However, given that total cyanobacteria abundance increased two- to three-fold during experiments, the absolute abundance of *Microcystis* likely increased during N additions as well. Free-living or epiphytic N-fixing heterotrophic bacteria may play an important role in providing N to these populations (Worm and Søndergaard 1998; Davis et al. 2010), thereby supporting their persistence under N-Limited conditions, although there remains a limited understanding of their importance in Lake Erie (Mou et al. 2013). Such bacteria have been found to be imbedded within *Microcystis* colonies (Song et al. 2017), which may help explain the muted response of *Microcystis* to N additions compared to *Planktothrix*. Additionally, it has been proposed that the presence of co-occurring diazotrophic taxa may help relieve N stress of these populations, as N fixation can "leak" N-rich amino acids and ammonium (Ohlendieck et al. 2000; Wetzel 2001). Consistent with this theory, in both transects, sites with peak cyanobacteria abundance exhibited higher abundances of diazotrophic genera (*Aphanizomenon* and *Dolichospermum*), a pattern previously described for Sandusky Bay (Davis et al. 2015). However, due to their relatively low abundance, the quantitative importance of dizaotrophs in this system remains unclear.

Beyond the toxic, bloom-forming genera detected during this study (i.e., *Microcystis* and *Planktothrix*), *Synechococcus* was a major component of the Lake Erie cyanobacterial communities, accounting for 25–75% of 16S sequences in both bays. This is an important finding as *Synechococcus* has often been overlooked in prior microscopy-based Lake Erie studies (Hopkins and Lea 1982; Makarewicz 1993; Postius and Ernst 1999), in part due to its small size (< 2.0 μm) and nondistinct morphology. Molecular methods have, however, revealed that *Synechococcus* can be abundant in freshwater systems (Wilhelm et al. 2006) and even dominant among picoplankton (Weisse 1993; Padisák et al. 1997). Our findings support those of Ouellette et al. (2006) and Wilhelm et al. (2006), which found *Synechococcus*-dominated 16S rRNA clone libraries across Lake Erie and suggests that although *Synechococcus* is likely a minor contributor to cyanobacterial biomass due to its small size, it likely plays an important role in Lake Erie's ecosystem and food web functioning due to its high relative abundance (Fahnenstiel et al. 1998; Postius and Ernst 1999; Gobler et al. 2008). For example, Wilhelm et al. (2006) found *Synechococcus* may contribute toward the development of hypoxic zones in Lake Erie's central basin where it dominates the prokaryotic assemblage during seasonal rapid draw-down of dissolved oxygen. The decreased and unchanged relative abundance of *Synechococcus* (clade I and II) in response to N in the Maumee Bay and western basin experiments, respectively, despite the increase in total cyanobacteria abundance indicates that, unlike bloom-forming cyanobacteria, the niche of *Synechococcus* is defined by low available N levels.

Nitrogen availability, bloom toxicity, and the potential microcystin-producing community

This study provided insight regarding the toxicity of Lake Erie CHABs during the late summer by characterizing potential microcystin-producing taxa present, their associations with microcystin concentrations, and the effect of nutrients and temperature on both. Although several known microcystin-producing genera were detected during this study via microscopy and 16S sequencing (e.g., *Dolichospermum*, *Planktothrix*, and *Microcystis*), all 868 *mcyE* genotypes mapped exclusively to *Planktothrix* and *Microcystis,* a finding consistent with prior investigations of the microcystin-producing communities in Lake Erie (Rinta-Kanto and Wilhelm 2006) and elsewhere (Hotto et al. 2008; Ye et al. 2009). There was a significant correlation between microcystin levels and *Planktothrix* filament abundance and biovolume in Sandusky Bay ($p < 0.05$ for both; Supporting Information Table S6). Additionally, microcystin concentrations closely paralleled toxigenic (*mcyE*-containing) *Planktothrix* abundances across western Lake Erie, with peak microcystin concentrations coinciding with peak *Planktothrix mcyE* relative abundances in both transects, suggesting *Planktothrix* was an important contributor to microcystin production in both Maumee and Sandusky Bays. This finding was supported by experimental findings as microcystin concentrations more closely paralleled changes in the relative abundance of *Planktothrix* than the *Microcystis* clades within the *mcyE* containing community. The relative abundance of the *Planktothrix mcyE* strain was generally higher in the +N treatments in which microcystin concentrations significantly increased ($p < 0.05$). Although there was not a significant increase in the relative

abundance of the *Planktothrix mcyE* strain in the +N treatments, cyanobacteria biomass significantly increased in the +N treatment, indicating that the absolute abundance of *Planktothrix* likely increased in parallel with microcystin concentrations. Further, microcystin concentrations were significantly correlated with the relative abundance (16S and *mcyE*) of *Planktothrix* across all experimental bottles in the western basin experiment. While *Planktothrix* has been previously identified as the primary microcystin producer in Sandusky Bay (Rinta-Kanto and Wilhelm 2006; Davis et al. 2015), *Microcystis* has typically been described as the primary microcystin producer in Maumee Bay and has been a primary focus of CHAB studies conducted in the western basin of Lake Erie (Rinta-Kanto et al. 2005; Davis et al. 2014; Harke et al. 2016). Supporting our findings, however, an examination of microcystin-producing communities in Maumee Bay by Rinta-Kanto and Wilhelm (2006) found that although all toxic genotypes detected mapped to *Microcystis*, a subgroup contained two additional amino acids which were similar residues to known *Planktothrix* sequences, suggesting that toxigenic *Planktothrix* was present but not detected in Maumee Bay due to prior methodological constraints (i.e., use of clone libraries). Additionally, Kutovaya et al. (2012) found that *Planktothrix* was an important component of the microcystin-producing community in the Maumee River and hypothesized this population may seed Maumee Bay. Collectively, these findings may have important implications for bloom toxicity as *Planktothrix* and *Microcystis* produce different microcystin variants that vary in potency and *Planktothrix* has been shown to have higher microcystin production rates than *Microcystis* in the field (Christiansen et al. 2003). This suggests *Planktothrix*, along with *Microcystis,* should be a focus for management strategies focused on bloom toxicity in Lake Erie. It should be noted, however, that while the *mcyE* gene plays a major role in microcystin synthesis, it is one of several genes necessary for production and, therefore, its detection does not absolutely confirm the presence of potentially toxigenic *Planktothrix* strains. Furthermore, this study only examined the microcystin producing community at one point during the bloom and thus may not be representative of the *mcyE* containing community at other times.

Several lines of evidences emphasized the importance of N in controlling microcystin levels in Lake Erie, with little to no effect of P and temperature. In both experiments, N additions yielded significantly higher microcystin concentrations (Supporting Information Table S7), and microcystin concentrations were significantly correlated with TN in Sandusky Bay (Supporting Information Table S6). These responses are similar to those observed in field studies by Davis et al. (2015) and Donald et al. (2011) that found a greater increase in microcystin concentrations in response to N than P additions and supports the hypothesis that N availability controls microcystin cellular quotas as microcystins are N-rich molecules (Harke and Gobler 2013; Horst et al. 2014; Van de Waal et al. 2014; Harke and Gobler 2015). This direct control of N availability on microcystin production is consistent with molecular analyses that have indicated the microcystin biosynthesis gene cluster is regulated by a N uptake transcription promoter (*NtcA*; Ginn et al. 2010, Neilan et al. 2013) and that the *mcy* genes are upregulated in response to N additions (Harke et al. 2016). Increases in microcystin concentrations in response to N enrichment may also be related to an increase in the abundance of toxic strains, which have been found to be selectivity promoted over non-toxic strains under N enriched conditions in *Microcystis* (Vézie et al. 2002; Davis et al. 2009). In the field, the relative abundance of *mcyE*-containing *Planktothrix* was significantly correlated with the ammonium levels ($p < 0.05$). Further, in the experiments, N increased the levels of cyanobacteria and microcystin but significantly decreased the relative abundance of *Microcystis* clade 1, indicating that the other dominant *mcyE*-strain, *Planktothrix*, had a higher absolute abundant in the presence of excess N and contributed to the higher microcystin levels in the treatment. All of these findings indicate that N loading plays an important role in CHAB toxicity and that N management is necessary to control bloom toxicity as well as cyanobacterial biomass.

NGS as a tool for cyanobacteria community studies

Overall, NGS proved to be a powerful tool for examination of the cyanobacterial assemblages during this study, allowing for a more detailed assessment of cyanobacteria community diversity and composition. While microscopy facilitated the identification of the six larger cyanobacteria genera, NGS detected over 2500 genetically unique OTUs at a 97% identity, corresponding to 46 genera, across Lake Erie's western basin. This allowed for identification of both rare (e.g., *Chroococcidiopsis*) and difficult to identify taxa, such as *Synechococcus*, including the differentiation of two sequentially distinct clades of *Synechococcus,* that in some cases responded differently to environmental drivers. There are also, of course, some caveats to consider regarding amplicon sequencing data. Read counts can provide an inaccurate measure of cell abundances, as multiple copies of a gene may be present in a genome and copy numbers can differ between species and within species (Acinas et al. 2004). This has been shown to occur with the 16S rRNA gene in several common cyanobacteria, including as *Microcystis* (Kaneko et al. 2007) and *Synechococcus* (Fogel et al. 1999). Additionally, PCR biases can arise due to the inability of primer sets to uniformly amplify all taxa (Polz and Cavanaugh 1998; Farris and Olson 2007), even with the use of "universal" primers and normalization of amplified DNA, as was the case in this study. Finally, taxonomic identification of sequence data can be limited by the robustness of reference databases (Shendure and Ji 2008), and therefore genes that are not highly sequenced, such as the *mcyE* gene used in this study, can result in limited taxonomic identification. This accounts for the discrepancy between the low taxonomic but high genetic diversity identified in the microcystin-producing communities and suggests that other genera may be present within this community but

are not yet distinguishable, which should be improved with further sequencing studies. Despite these potential pitfalls, there were statistically significant correlations between the microscopic (biovolumes) and 16S relative abundances of the most abundant cyanobacterial genera during this study (*Microcystis, Planktothrix,* and *Dolichospermum*), indicating the robustness of the two approaches. Given that 40 of the 46 genera detected via NGS were not observed microscopically, correlations among the three other, less abundant genera observed microscopically were not necessarily expected.

Conclusion

This study demonstrated that N was the most influential environmental factor on cyanobacteria blooms in Lake Erie in late summer, promoting the abundance of cyanobacteria, microcystin concentrations, and causing the greatest changes in cyanobacteria community structure. Given that the combined N, P, and T treatment yielded the highest levels of cyanobacteria in both experiments and that P was an important factor promoting several diazotrophic genera cyanobacteria, dual management of N and P will be important for mitigation of CHABs in Lake Erie, especially in the future as summer lake temperatures rise. More studies using NGS to characterize bloom communities and their responses to environmental drivers are needed in freshwater systems to better understand the onset and persistence of cyanobacteria blooms.

References

Acinas, S. G., L. A. Marcelino, V. Klepac-Ceraj, and M. F. Polz. 2004. Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. J. Bacteriol. **186**: 2629–2635. doi:10.1128/JB.186.9.2629-2635.2004

Allinger, L. E., and E. D. Reavie. 2013. The ecological history of Lake Erie as recorded by the phytoplankton community. J. Great Lakes Res. **39**: 365–382. doi:10.1016/j.jglr.2013.06.014

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. JMOBAK **215**: 403–410. doi:10.1016/S0022-2836(05)80360-2

Anders, S., and W. Huber. 2010. Differential expression analysis for sequence count data. Genome Biol. **11**: R106. doi:10.1186/gb-2010-11-10-r106

Andersson, A., H. Höglander, C. Karlsson, and S. Huseby. 2015. Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea. Estuar. Coast. Shelf Sci. **164**: 161–171. doi:10.1016/j.ecss.2015.07.013

Anneville, O., S. Gammeter, and D. Straile. 2005. Phosphorus decrease and climate variability: Mediators of synchrony in phytoplankton changes among European peri-alpine lakes. Freshw. Biol. **50**: 1731–1746. doi:10.1111/j.1365-2427.2005.01429.x

Berger, C. 2017. Occurrence of *Oscillatoria agardhii* Gom. In some shallow eutrophic lakes. Int. Assoc. Theor. Appl. Limnol.: Negotiations **19**: 2689–2697. doi:10.1080/03680770.1974.11896365

Beutler, M., K. H. Wiltshire, B. Meyer, C. Moldaenke, C. Lüring, M. Meyerhöfer, U.-P. Hansen, and H. Dau. 2002. A fluorometric method for the differentiation of algal populations in vivo and in situ. Photosynth. Res. **72**: 39–53. doi:10.1023/A:1016026607048

Bridgeman, T. B., J. D. Chaffin, D. D. Kane, J. D. Conroy, S. E. Panek, and P. M. Armenio. 2012. From river to lake: Phosphorus partitioning and algal community compositional changes in Western Lake Erie. J. Great Lakes Res. **38**: 90–97. doi:10.1016/j.jglr.2011.09.010

Bridgeman, T. B., J. D. Chaffin, and J. E. Filbrun. 2013. A novel method for tracking western Lake Erie *Microcystis* blooms, 2002–2011. J. Great Lakes Res. **39**: 83–89. doi:10.1016/j.jglr.2012.11.004

Brittain, S. M., J. Wang, L. Babcock-Jackson, W. W. Carmichael, K. L. Rinehart, and D. A. Culver. 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. J. Great Lakes Res. **26**: 241–249. doi:10.1016/S0380-1330(00)70690-3

Bullerjahn, G. S., and others. 2016. Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. Harmful Algae **54**: 223–238. doi:10.1016/j.hal.2016.01.003

Caporaso, J. G., and others. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods **7**: 335. doi:10.1038/nmeth.f.303

Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton, and J. D. Brookes. 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Res. **46**: 1394–1407. doi:10.1016/j.watres.2011.12.016

Carmichael, W. W. 2001. Health effects of toxin-producing cyanobacteria:"The CyanoHABs". Hum. Ecol. Risk Assess. Int. J. **7**: 1393–1407. doi:10.1080/20018091095087

Carmichael, W. W., and G. L. Boyer. 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. Harmful Algae **54**: 194–212. doi:10.1016/j.hal.2016.02.002

Carpenter, S. R., J. J. Cole, T. E. Essington, J. R. Hodgson, J. N. Houser, J. F. Kitchell, and M. L. Pace. 1998. Evaluating alternative explanations in ecosystem experiments. Ecosystems **1**: 335–344. doi:10.1007/s100219900

Castiglioni, B., and others. 2004. Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. Appl. Environ. Microbiol. **70**: 7161–7172. doi:10.1128/AEM.70.12.7161-7172.2004

Chaffin, J. D., T. B. Bridgeman, S. A. Heckathorn, and S. Mishra. 2011. Assessment of *Microcystis* growth rate

potential and nutrient status across a trophic gradient in western Lake Erie. J. Great Lakes Res. **37**: 92–100. doi:10.1016/j.jglr.2010.11.016

Chaffin, J. D., T. B. Bridgeman, and D. L. Bade. 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. Adv. Microbiol. **3**: 16–26. doi:10.4236/aim.2013.36A003

Chaffin, J. D., T. W. Davis, D. J. Smith, M. M. Baer, and G. J. Dick. 2018. Interactions between nitrogen form, loading rate, and light intensity on *Microcystis* and *Planktothrix* growth and microcystin production. Harmful Algae **73**: 84–97. doi:10.1016/j.hal.2018.02.001

Chia, M. A., J. G. Jankowiak, B. J. Kramer, J. A. Goleski, I.-S. Huang, P. V. Zimba, and C. J. Gobler. 2018. Succession and toxicity of *Microcystis* and *Anabaena* (*Dolichospermum*) blooms are controlled by nutrient-dependent allelopathic interactions. Harmful Algae **74**: 67–77. doi:10.1016/j.hal.2018.03.002

Chorus, I., and J. Bartram. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management, 1st ed. CRC Press.

Christiansen, G., J. Fastner, M. Erhard, T. Börner, and E. Dittmann. 2003. Microcystin biosynthesis in *Planktothrix*: Genes, evolution, and manipulation. J. Bacteriol. **185**: 564–572. doi:10.1128/JB.185.2.564-572.2003

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling eutrophication: Nitrogen and phosphorus. Science **123**: 1014–1015.

Conroy, J. D., and D. A. Culver. 2005. Do dreissenid mussels affect Lake Erie ecosystem stability processes? Am. Midland Naturalist **153**: 20–32. doi:10.1674/0003-0031(2005)153[0020:DDMALE]2.0.CO;2

Conroy, J. D., E. L. Quinlan, D. D. Kane, and D. A. Culver. 2007. Cylindrospermopsis in Lake Erie: Testing its association with other cyanobacterial genera and major limnological parameters. J. Great Lakes Res. **33**: 519–535. doi:10.3394/0380-1330(2007)33[519:CILETI]2.0.CO;2

Davis, T. W., D. L. Berry, G. L. Boyer, and C. J. Gobler. 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae **8**: 715–725. doi:10.1016/j.hal.2009.02.004

Davis, T. W., G. S. Bullerjahn, T. Tuttle, R. M. McKay, and S. B. Watson. 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. **49**: 7197–7207. doi:10.1021/acs.est.5b00799

Davis, T. W., S. B. Watson, M. J. Rozmarynowycz, J. J. Ciborowski, R. M. McKay, and G. S. Bullerjahn. 2014. Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. PLoS One **9**: e106093. doi:10.1371/journal.pone.0106093

Davis, T. W., M. Harke, M. A. Marcoval, J. A. Goleski, C. Orano-Dawson, D. L. Berry, and C. J. Gobler. 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. Aquat. Microb. Ecol. **61**: 149–162. doi:10.3354/ame01445

Dempster, E., K. Pryor, D. Francis, J. Young, and H. Rogers. 1999. Rapid DNA extraction from ferns for PCR-based analyses. Biotechniques **27**: 66–68. doi:10.2144/99271bm13

De Pinto, J. V., T. C. Young, and L. M. McIlroy. 1986. Great Lakes water quality improvement. Environ. Sci. Technol. **20**: 752–759. doi:10.1021/es00150a001

Dokulil, M. T., and K. Teubner. 2000. Cyanobacterial dominance in lakes. Hydrobiologia **438**: 1–12. doi:10.1023/A:100415581

Dolan, D. M., and S. C. Chapra. 2012. Great Lakes total phosphorus revisited: 1. Loading analysis and update (1994–2008). J. Great Lakes Res. **38**: 730–740. doi:10.1016/j.jglr.2012.10.001

Donald, D. B., M. J. Bogard, K. Finlay, and P. R. Leavitt. 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. **56**: 2161–2175. doi:10.4319/lo.2011.56.6.2161

Donald, D. B., M. J. Bogard, K. Finlay, L. Bunting, and P. R. Leavitt. 2013. Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. PloS One **8**: e53277. doi:10.1371/journal.pone.0053277

Edenhofer, O., and others. 2014. Summary for policymakers: Climate change 2014. IPCC.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32**: 1792–1797. doi:10.1093/nar/gkh340

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics **26**: 2460–2461. doi:10.1093/bioinformatics/btq461

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics **27**: 2194–2200. doi:10.1093/bioinformatics/btr381

Edmondson, W. 1970. Phosphorus, nitrogen, and algae in Lake Washington after diversion of sewage. Science **169**: 690–691. doi:10.1126/science.169.3946.690

Elliott, J., I. Jones, and S. Thackeray. 2006. Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia **559**: 401–411. doi:10.1007/s10750-005-1233-y

Elser, J. J., and others. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10**: 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Fahnenstiel, G. L., A. E. Krause, M. J. McCormick, H. J. Carrick, and C. L. Schelske. 1998. The structure of the planktonic

food-web in the St. Lawrence Great Lakes. J. Great Lakes Res. **24**: 531–554. doi:10.1016/S0380-1330(98)70843-3

Farris, M., and J. Olson. 2007. Detection of Actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett. Appl. Microbiol. **45**: 376–381. doi:10.1111/j.1472-765X.2007.02198.x

Fischer, W. J., and others. 2001. Congener-independent immunoassay for microcystins and nodularins. Environ. Sci. Technol. **35**: 4849–4856.

Fogel, G., C. Collins, J. Li, and C. Brunk. 1999. Prokaryotic genome size and SSU rDNA copy number: Estimation of microbial relative abundance from a mixed population. Microb. Ecol. **38**: 93–113. doi:10.1007/s002489900

Fuller, K., H. Shear, and J. Wittig. 2002. The Great Lakes: An environmental atlas and resource book. US EPA/The Government of Canada.

Galloway, J. N., and others. 2004. Nitrogen cycles: Past, present, and future. Biogeochemistry **70**: 153–226. doi:10.1007/s10533-004-0370-0

Ghadouani, A., and R. E. Smith. 2005. Phytoplankton distribution in Lake Erie as assessed by a new in situ spectrofluorometric technique. J. Great Lakes Res. **31**: 154–167. doi:10.1016/S0380-1330(05)70311-7

Ginn, H., L. Pearson, and B. Neilan. 2010. NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. Appl. Environ. Microbiol. **76**: 4362–4368. doi:10.1128/AEM.01862-09

Glibert, P. M., R. Maranger, D. J. Sobota, and L. Bouwman. 2014. The Haber Bosch–harmful algal bloom (HB–HAB) link. Environ. Res. Lett. **9**: 105001. doi:10.1088/1748-9326/9/10/105001

Gobler, C. J., T. W. Davis, S. N. Deonarine, M. A. Saxton, P. J. Lavrentyev, F. J. Jochem, and S. W. Wilhelm. 2008. Grazing and virus-induced mortality of microbial populations before and during the onset of annual hypoxia in Lake Erie. Aquat. Microb. Ecol. **51**: 117–128. doi:10.3354/ame01180

Gobler, C. J., J. M. Burkholder, T. W. Davis, M. J. Harke, T. Johengen, C. A. Stow, and D. B. Van de Waal. 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. Harmful Algae **54**: 87–97. doi:10.1016/j.hal.2016.01.010

Hammer, Ø., D. Harper, and P. Ryan. 2001. PAST-palaeontological statistics. Available from www.uv.es/~pardomv/pe/2001_1/past/pastprog/past.pdf. [Accessed May 7, 2018].

Han, H., J. D. Allan, and N. S. Bosch. 2012. Historical pattern of phosphorus loading to Lake Erie watersheds. J. Great Lakes Res. **38**: 289–298. doi:10.1016/j.jglr.2012.03.004

Harke, M. J., D. L. Berry, J. W. Ammerman, and C. J. Gobler. 2012. Molecular response of the bloom-forming cyanobacterium, *Microcystis aeruginosa*, to phosphorus limitation. Microb. Ecol. **63**: 188–198. doi:10.1007/s00248-011-9894-8

Harke, M. J., and C. J. Gobler. 2013. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. PLoS One **8**: e69834. doi:10.1371/journal.pone.0069834

Harke, M. J., T. W. Davis, S. B. Watson, and C. J. Gobler. 2015. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. Environ. Sci. Technol. **50**: 604–615. doi:10.1021/acs.est.5b03931

Harke, M. J., and C. J. Gobler. 2015. Daily transcriptome changes reveal the role of nitrogen in controlling microcystin synthesis and nutrient transport in the toxic cyanobacterium, *Microcystis aeruginosa*. BMC Genomics **16**: 1068. doi:10.1186/s12864-015-2275-9

Harke, M. J., M. M. Steffen, C. J. Gobler, T. G. Otten, S. W. Wilhelm, S. A. Wood, and H. W. Paerl. 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. Harmful Algae **54**: 4–20. doi:10.1016/j.hal.2015.12.007

Heisler, J., and others. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae **8**: 3–13. doi:10.1016/j.hal.2008.08.006

Hillebrand, H., C. D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. **35**: 403–424. doi:10.1046/j.1529-8817.1999.3520403.x

Hopkins, G., and C. Lea. 1982. A ten—Year study of phytoplankton biomass and composition in the Nanticoke region of long Point Bay, Lake Erie. J. Great Lakes Res. **8**: 428–438. doi:10.1016/S0380-1330(82)71984-7

Horst, G. P., O. Sarnelle, J. D. White, S. K. Hamilton, R. B. Kaul, and J. D. Bressie. 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. **54**: 188–198. doi:10.1016/j.watres.2014.01.063

Hotto, A. M., M. F. Satchwell, D. L. Berry, C. J. Gobler, and G. L. Boyer. 2008. Spatial and temporal diversity of microcystins and microcystin-producing genotypes in Oneida Lake, NY. Harmful Algae **7**: 671–681. doi:10.1016/j.hal.2008.02.001

Houghton, J. T., and others. 2001. Climate change 2001: The scientific basis. The Press Syndicate of the University of Cambridge.

Joehnk, K. D., J. Huisman, J. Sharples, B. Sommeijer, P. M. Visser, and J. M. Stroom. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Chang. Biol. **14**: 495–512. doi:10.1111/j.1365-2486.2007.01510.x

Jones, M. N. 1984. Nitrate reduction by shaking with cadmium: Alternative to cadmium columns. Water Res. **18**: 643–646. doi:10.1016/0043-1354(84)90215-X

Jungblut, A.-D., and B. A. Neilan. 2006. Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. Arch. Microbiol. **185**: 107–114. doi:10.1007/s00203-005-0073-5

Kaczmarzyk, D., and M. Fulda. 2010. Fatty acid activation in cyanobacteria mediated by acyl-acyl carrier protein synthetase enables fatty acid recycling. Plant Physiol. **152**: 1598–1610. doi:10.1104/pp.109.148007

Kaneko, T., and others. 2007. Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. DNA Res. **14**: 247–256. doi:10.1093/dnares/dsm026

Kearse, M., and others. 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**: 1647–1649. doi:10.1093/bioinformatics/bts199

Kosten, S., and others. 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Glob. Chang. Biol. **18**: 118–126. doi:10.1111/j.1365-2486.2011.02488.x

Kutovaya, O. A., R. M. L. McKay, B. F. N. Beall, S. W. Wilhelm, D. D. Kane, J. D. Chaffin, T. B. Bridgeman, and G. S. Bullerjahn. 2012. Evidence against fluvial seeding of recurrent toxic blooms of *Microcystis* spp. in Lake Erie's western basin. Harmful Algae **15**: 71–77. doi:10.1016/j.hal.2011.11.007

Larkin, M. A., and others. 2007. Clustal W and Clustal X version 2.0. Bioinformatics **23**: 2947–2948. doi:10.1093/bioinformatics/btm404

LeBlanc, S., F. R. Pick, and R. Aranda-Rodriguez. 2005. Allelopathic effects of the toxic cyanobacterium *Microcystis aeruginosa* on duckweed, *Lemna gibba* L. Environ. Toxicol. **20**: 67–73. doi:10.1002/tox.20079

Loar, S. N. 2009. Seasonal variation in Lake Erie picoplankton. Knoxville: University of Tennessee.

Makarewicz, J. C. 1993. Phytoplankton biomass and species composition in Lake Erie, 1970 to 1987. J. Great Lakes Res. **19**: 258–274. doi:10.1016/S0380-1330(93)71216-2

Makarewicz, J. C., and P. Bertram. 1991. Evidence for the restoration of the Lake Erie ecosystem. Bioscience **41**: 216–223. doi:10.2307/1311411

McCann, M. 2014. gb2qiime.py. MBARI.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PloS One **8**: e61217. doi:10.1371/journal.pone.0061217

McMurdie, P. J., and S. Holmes. 2014. Waste not, want not: Why rarefying microbiome data is inadmissible. PLoS Comput. Biol. **10**: e1003531. doi:10.1371/journal.pcbi.1003531

Michalak, A. M., and others. 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. USA **110**: 6448–6452. doi:10.1073/pnas.1216006110

Millie, D. F., G. L. Fahnenstiel, J. D. Bressie, R. J. Pigg, R. R. Rediske, D. M. Klarer, P. A. Tester, and R. W. Litaker. 2009. Late-summer phytoplankton in western Lake Erie (Laurentian Great Lakes): Bloom distributions, toxicity, and environmental influences. Aquat. Ecol. **43**: 915–934. doi:10.1007/s10452-009-9238-7

Mortimer, C. 1987. Fifty years of physical investigations and related limnological studies on Lake Erie, 1928–1977. J. Great Lakes Res. **13**: 407–435. doi:10.1016/S0380-1330(87)71664-5

Mou, X., J. Jacob, X. Lu, S. Robbins, S. Sun, and J. D. Ortiz. 2013. Diversity and distribution of free-living and particle-associated bacterioplankton in Sandusky Bay and adjacent waters of Lake Erie Western Basin. J. Great Lakes Res. **39**: 352–357. doi:10.1016/j.jglr.2013.03.014

Müller, S., and S. M. Mitrovic. 2015. Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: Progressing from the phosphorus limitation paradigm. Hydrobiologia **744**: 255–269. doi:10.1007/s10750-014-2082-3

National Oceanic and Atmospheric Administration. 2015*a*. Experimental Lake Erie harmful algal bloom bulletin. Bulletin 19. NOAA.

National Oceanic and Atmospheric Administration. 2015*b*. Experimental Lake Erie harmful algal bloom bulletin. Bulletin 27. NOAA.

National Oceanic and Atmospheric Administration. 2017. Experimental Lake Erie harmful algal bloom bulletin. Seasonal assesment. NOAA.

Neilan, B. A., L. A. Pearson, J. Muenchhoff, M. C. Moffitt, and E. Dittmann. 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ. Microbiol. **15**: 1239–1253. doi:10.1111/j.1462-2920.2012.02729.x

Nübel, U., F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. **63**: 3327–3332.

Ohio EPA. 2010. Ohio Lake Erie phosphorus task force final report. Ohio EPA OH Task Force.

Ohlendieck, U., A. Stuhr, and H. Siegmund. 2000. Nitrogen fixation by diazotrophic cyanobacteria in the Baltic Sea and transfer of the newly fixed nitrogen to picoplankton organisms. J. Mar. Syst. **25**: 213–219. doi:10.1016/S0924-7963(00)00016-6

O'Neil, J., T. Davis, M. Burford, and C. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae **14**: 313–334. doi:10.1016/j.hal.2011.10.027

Ouellette, A. J., S. M. Handy, and S. W. Wilhelm. 2006. Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. Microb. Ecol. **51**: 154–165. doi:10.1007/s00248-004-0146-z

Padisák, J., L. Krienitz, R. Koschel, and J. Nedoma. 1997. Deep-layer autotrophic picoplankton maximum in the oligotrophic Lake Stechlin, Germany: origin, activity, development and erosion. Eur. J. Phycol. **32**: 403–416.

Paerl, H. W., R. S. Fulton, P. H. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci. World J. **1**: 76–113. doi:10.1100/tsw.2001.16

Paerl, H. W., and R. Fulton. 2006. Ecology of harmful cyanobacteria, p. 95–109. *In* Ecology of harmful algae. Springer.

Paerl, H. W., and J. Huisman. 2008. Blooms like it hot. Science **320**: 57–58. doi:10.1126/science.1156721

Paerl, H. W., and J. Huisman. 2009. Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. **1**: 27–37. doi:10.1111/j.1758-2229.2008.00004.x

Paerl, H. W., N. S. Hall, and E. S. Calandrino. 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Sci. Total Environ. **409**: 1739–1745. doi:10.1016/j.scitotenv.2011.02.001

Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Res. **46**: 1349–1363. doi:10.1016/j.watres.2011.08.002

Paerl, H. W., and T. G. Otten. 2016. Duelling 'CyanoHABs': Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. Environ. Microbiol. **18**: 316–324. doi:10.1111/1462-2920.13035

Parkhill, J. P., G. Maillet, and J. J. Cullen. 2001. Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J. Phycol. **37**: 517–529. doi:10.1046/j.1529-8817.2001.037004517.x

Parsons, T. R. 2013. A manual of chemical & biological methods for seawater analysis. Elsevier.

Polz, M. F., and C. M. Cavanaugh. 1998. Bias in template-to-product ratios in multitemplate PCR. Appl. Environ. Microbiol. **64**: 3724–3730.

Poste, A., R. Hecky, and S. Guildford. 2013. Phosphorus enrichment and carbon depletion contribute to high *Microcystis* biomass and microcystin concentrations in Ugandan lakes. Limnol. Oceanogr. **58**: 1075–1088. doi:10.4319/lo.2013.58.3.1075

Postius, C., and A. Ernst. 1999. Mechanisms of dominance: Coexistence of picocyanobacterial genotypes in a freshwater ecosystem. Arch. Microbiol. **172**: 69–75. doi:10.1007/s002030050742

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2012. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. **41**: D590–D596. doi:10.1093/nar/gks1219

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rigosi, A., C. C. Carey, B. W. Ibelings, and J. D. Brookes. 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnol. Oceanogr. **59**: 99–114. doi:10.4319/lo.2014.59.1.0099

Rinta-Kanto, J., A. Ouellette, G. Boyer, M. Twiss, T. Bridgeman, and S. Wilhelm. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environ. Sci. Technol. **39**: 4198–4205. doi:10.1021/es048249u

Rinta-Kanto, J. M., and S. W. Wilhelm. 2006. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. Appl. Environ. Microbiol. **72**: 5083–5085. doi:10.1128/AEM.00312-06

Rinta-Kanto, J. M., E. A. Konopko, J. M. DeBruyn, R. A. Bourbonniere, G. L. Boyer, and S. W. Wilhelm. 2009. Lake Erie *Microcystis*: Relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. Harmful Algae **8**: 665–673. doi:10.1016/j.hal.2008.12.004

Schindler, D. 1977. Evolution of phosphorus limitation in lakes. Science **195**: 260–262. doi:10.1126/science.195.4275.260

Schindler, D. W., and others. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proc. Natl. Acad. Sci. USA **105**: 11254–11258. doi:10.1073/pnas.0805108105

Shendure, J., and H. Ji. 2008. Next-generation DNA sequencing. Nat. Biotechnol. **26**: 1135–1145. doi:10.1038/nbt1486

Simis, S. G., Y. Huot, M. Babin, J. Seppälä, and L. Metsamaa. 2012. Optimization of variable fluorescence measurements of phytoplankton communities with cyanobacteria. Photosynth. Res. **112**: 13–30. doi:10.1007/s11120-012-9729-6

Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science **221**: 669–671. doi:10.1126/science.221.4611.669

Song, H., J. Xu, M. Lavoie, X. Fan, G. Liu, L. Sun, Z. Fu, and H. Qian. 2017. Biological and chemical factors driving the temporal distribution of cyanobacteria and heterotrophic bacteria in a eutrophic lake (West Lake, China). Appl. Microbiol. Biotechnol. **101**: 1685–1696. doi:10.1007/s00253-016-7968-8

Steffen, M. M., Z. Li, T. C. Effler, L. J. Hauser, G. L. Boyer, and S. W. Wilhelm. 2012. Comparative metagenomics of toxic freshwater cyanobacteria bloom communities on two continents. PloS One **7**: e44002. doi:10.1371/journal.pone.0044002

Steffen, M. M., B. S. Belisle, S. B. Watson, G. L. Boyer, and S. W. Wilhelm. 2014. Status, causes and controls of cyanobacterial blooms in Lake Erie. J. Great Lakes Res. **40**: 215–225. doi:10.1016/j.jglr.2013.12.012

Sterner, R. W. 2008. On the phosphorus limitation paradigm for lakes. Int. Rev. Hydrobiol. **93**: 433–445. doi:10.1002/iroh.200811068

Stumpf, R. P., T. T. Wynne, D. B. Baker, and G. L. Fahnenstiel. 2012. Interannual variability of cyanobacterial blooms in Lake Erie. PloS One **7**: e42444. doi:10.1371/journal.pone.0042444

Sukenik, A., R. Eshkol, A. Livne, O. Hadas, M. Rom, D. Tchernov, A. Vardi, and A. Kaplan. 2002. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp.(cyanobacteria): A novel allelopathic mechanism. Limnol. Oceanogr. **47**: 1656–1663. doi:10.4319/lo.2002.47.6.1656

Takasu, H., M. Ushio, E. L. LeClair, and S.-i. Nakano. 2015. High contribution of *Synechococcus* to phytoplankton biomass in the aphotic hypolimnion in a deep freshwater lake (Lake Biwa, Japan). Aquat. Microb. Ecol. **75**: 69–79. doi: 10.3354/ame01749

Thomas, M. K., and E. Litchman. 2016. Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia **763**: 357–369. doi: 10.1007/s10750-015-2390-2

Tillett, D., E. Dittmann, M. Erhard, H. von Döhren, T. Börner, and B. A. Neilan. 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: An integrated peptide–polyketide synthetase system. Chem. Biol. **7**: 753–764. doi:10.1016/S1074-5521(00)00021-1

Usher, K., A. Kaksonen, and I. MacLeod. 2014. Marine rust tubercles harbour iron corroding archaea and sulphate reducing bacteria. Corros. Sci. **83**: 189–197. doi:10.1016/j.corsci.2014.02.014

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. **10**: 109–122. doi:10.1016/0304-4203(81)90027-X

Van de Waal, D. B., V. H. Smith, S. A. Declerck, E. Stam, and J. J. Elser. 2014. Stoichiometric regulation of phytoplankton toxins. Ecol. Lett. **17**: 736–742. doi:10.1111/ele.12280

Vézie, C., J. Rapala, J. Vaitomaa, J. Seitsonen, and K. Sivonen. 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. Microb. Ecol. **43**: 443–454. doi: 10.1007/s00248-001-0041-9

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. **7**: 737–750. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Vollenweider, R. A. 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors. Organisation for Economic Cooperation and Development.

Watson, S. B., and others. 2016. The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. Harmful Algae **56**: 44–66. doi:10.1016/j.hal.2016.04.010

Weisse, T. 1993. Dynamics of autotrophic picoplankton in marine and freshwater ecosystems, p. 327–370. Advances in microbial ecology. Springer.

Wetzel, R. G. 2001. Limnology: Lake and river ecosystems. Gulf Professional Publishing.

Wilhelm, S. W., G. S. Bullerjahn, M. L. Eldridge, J. M. Rinta-Kanto, L. Poorvin, and R. A. Bourbonniere. 2006. Seasonal hypoxia and the genetic diversity of prokaryote populations in the central basin hypolimnion of Lake Erie: Evidence for abundant cyanobacteria and photosynthesis. J. Great Lakes Res. **32**: 657–671. doi:10.3394/0380-1330(2006)32[657:SHATGD]2.0.CO;2

Wilhelm, S. W., and others. 2003. Effect of phosphorus amendments on present day plankton communities in pelagic Lake Erie. Aquat. Microb. Ecol. **32**: 275–285. doi: 10.3354/ame032275

Worm, J., and M. Søndergaard. 1998. Dynamics of heterotrophic bacteria attached to *Microcystis* spp. (cyanobacteria). Aquat. Microb. Ecol. **14**: 19–28. doi:10.3354/ame014019

Ye, W., X. Liu, J. Tan, D. Li, and H. Yang. 2009. Diversity and dynamics of microcystin—producing cyanobacteria in China's third largest Lake, Lake Taihu. Harmful Algae **8**: 637–644. doi:10.1016/j.hal.2008.10.010

Acknowledgments

We would like to thank Justin Chaffin, the captains of the research vessel RV *Erie Monitor* and the support staff at the Ohio State University Franz Theodore Stone Laboratory for their assistance and use of their facility during this study. We would also like to thank Jennifer Goleski for analytical assistance. This work was supported by the Simons Foundation and funding from the New York State Department of Environmental Conservation Harmful Algal Bloom Surveillance Program.

Conflict of Interest

None declared

Submitted 24 August 2018
Revised 17 November 2018
Accepted 02 January 2019

Associate editor: Maren Voss