Carbon and nitrogen recycling during cyanoHABs in dreissenid-invaded and non-invaded US midwestern lakes and reservoirs

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Abstract Lakes and reservoirs play key roles in global carbon cycling, especially as a carbon sink. Enrichment of nutrients in lakes and reservoirs (eutrophication) and rising global temperatures favors the proliferation of bloom-forming cyanobacteria. Harmful blooms of cyanobacteria (cyanoHABs) alter carbon and nutrient cycling in freshwater ecosystems. Some evidence suggests the introduction or establishment of invasive mussel species (i.e., *Dreissena* spp.) also favor cyanoHAB formation through selective filter feeding, a process through which they may also impact biogeochemical processes including carbon cycling and sequestration. However, few studies have considered the combined effects of invasive mussels and cyanoHABs on carbon and nitrogen cycling in freshwater ecosystems. Here, we examined microbial

community composition and biogeochemical attributes (including carbon and nitrogen stable isotopes) in eutrophic lakes, reservoirs, and rivers in western Ohio, eastern Indiana, and northern Kentucky during the cyanobacterial bloom period of the summer of 2015. Our samples include both sites impacted by invasive mussels and those where invasive mussels have not yet been observed. Based on 16S and 18S rRNA gene sequence analysis, we found that cyanobacterial and algal communities varied across sites and were most closely related to habitat (sediment or water column sample) and site, regardless of the presence of invasive mussels or other environmental factors. However, we did find evidence that invasive mussels may influence both carbon and nitrogen cycling. While the results are based on a single time point sampling, they highlight the interactions of multiple environmental stressors in aquatic ecosystems and the critical need for more temporally intensive studies of carbon and nutrient cycling in bloom- and mussel-impacted waters.

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Introduction

Nutrient enrichment, a leading cause of eutrophication in freshwater ecosystems, leads to the proliferation of noxious, and sometimes toxic bloom-forming cyanobacteria (Fogg, 1969; Huisman et al., 2005; Paerl & Fulton, 2006). CyanoHABs negatively impact water quality by reducing visibility, creating hypoxic zones, and decreasing aquatic biodiversity (e.g., Seehausen et al., 1997; Abrantes et al., 2006; Smith & Schindler, 2009). In eutrophic lakes, genera of cyanobacteria including *Microcystis*, *Planktothrix*, *Dolichospermum* (formerly *Anabaena*), *Aphanizomenon*, *Anabaenopsis*, and *Cylindrospermopsis* exploit nutrient enrichment coupled to elevated temperatures and thus are common constituents of summer cyanoHABs. Members of these genera also produce toxins such as microcystin, cylindrospermopsin, saxitoxin, and anatoxin that can sicken humans, and kill pets, fish, birds, and other aquatic biota. The introduction of invasive mussel species (i.e., *Dreissena polymorpha*, or Zebra mussel, and *Dreissena bugensis*, or Quagga mussel) can further compound the proliferation of bloom-forming cyanobacteria through selective consumption (Vanderploeg et al., 2001) and nutrient excretion (Arnott & Vanni, 1996; Conroy et al., 2005; Sarnelle et al., 2005).

Increasing primary productivity due to the occurrence of cyanoHABs and eutrophication alters nutrient cycling (Alexander et al., 2017) and leads to an increase in carbon burial in lakes and reservoirs (Heathcote & Downing, 2012; Heathcote et al., 2015; Huang et al., 2018). Similarly, the presence of invasive mussels also alters biogeochemical cycling (Higgins & Vander Zanden, 2010; Williamson & Ozersky, 2019). Climate change, including warming temperatures and increased hydrologic variability, is expected to increase the growth and dominance of cyanoHABs (Paerl, 2017). However, it is not clear how the spread of non-native mussels will impact eutrophication,

cyanoHAB proliferation, biogeochemical cycling, and ultimately, carbon burial in lakes and reservoirs.

Non-native mussel species (i.e., Zebra and Quagga mussels) have been spreading into aquatic ecosystems across North America since their first appearance in 1988, yet much remains unknown about their long-term ecological effects (Strayer, 2009). As filter feeders, invasive non-native mussel species may improve water clarity and increase the depth of light penetration and have been linked to an increase in the productivity of toxin-producing cyanobacteria (Hecky et al., 2004), and an increase in *M. aeruginosa* biomass (Vanderploeg et al., 2002; Sarnelle et al., 2005). Mussels can also recycle nutrients contained in microbial biomass back into the water column (Kovalenko et al., 2017). All of these characteristics may assist the establishment and proliferation bloom-forming cyanobacteria. For instance, in Lake Erie, mussels exhibited selective rejection of toxin-forming *M. aeruginosa* strains during normal filter feeding, and *M. aeruginosa* was not abundant in Saginaw Bay (Lake Huron) but became abundant after mussel establishment (Vanderploeg et al., 2001). In contrast, research from a eutrophic lake in Sweden suggests zebra mussels may promote lake recovery from eutrophication through nutrient removal (Goedkoop et al., 2011). Furthermore, an increase in phosphorus loading in the presence of invasive mussels may decrease the abundance of *Microcystis* spp. (Sarnelle et al., 2012). Clearly, the effects of invasive mussels on cyanoHABs warrant further study.

Carbon in natural systems exists in many different forms including dissolved inorganic, dissolved organic, and particulate organic carbon (DIC, DOC, POC, respectively). The cycling of carbon between DIC, DOC, and POC pools is predominantly biologically mediated. Carbon can also be degraded through photochemistry (Ward & Cory, 2016). These biotic and abiotic processes impart changes in the isotope signature of carbon. The concentration and carbon isotope signal of this material integrates all of the processes that influence the movement of carbon through the water column and deposition as sediments (as well as movement of carbon from sediments back into the water column). Nitrogen isotopes in eutrophic systems can provide insight into nitrogen sources and nitrogen cycling: nitrogen can be introduced to lakes through natural processes such as in situ biological nitrogen fixation (resulting in biomass with $\delta^{15}\text{N}$

values near 0‰, the value of atmospheric air), input of allochthonous sources such as artificial fertilizer (also having $\delta^{15}\text{N}$ values near 0‰), or organic material such as plant matter and soil (contributing fixed N that tends to have $\delta^{15}\text{N}$ values that are more positive). Degradation of biomass in situ can also contribute to fixed N, including the regeneration of ammonium (Hampel et al., 2019a). Nitrogen cycling and loss via breakdown of organic material and subsequent denitrification results in residual $\delta^{15}\text{N}$ values becoming more positive (e.g., Wada et al., 1975; Mariotti et al., 1981).

In this paper, we determine community composition of planktonic and sediment microbes across a range of eutrophic lakes and reservoirs and a subset of their tributaries and use stable carbon and nitrogen isotopes to examine biogeochemical cycling in these ecosystems. We focus on select aquatic ecosystems with known mussel presence or absence that have been impacted by cyanobacterial blooms in Ohio, Indiana, and Kentucky, including portions of Lake Erie. Among these sites, Lake Erie has received considerable attention with respect to the impacts and mitigation of cyanoHABs while little to no data exist on bloom community composition or biogeochemical cycling in the others. Finally, we combine these data to develop a framework for assessing the impacts of invasive mussels on biogeochemical cycling in systems impacted by harmful cyanobacterial blooms. We acknowledge the simplification of our conceptual model and limitations of our single time point sampling. Regardless, our data suggest that, in addition to their potential in the potential proliferation of cyanoHABs, invasive mussels should also be considered in carbon cycling dynamics on short- and long-term timescales in eutrophic ecosystems.

Materials and methods

Sampling strategy

We sampled reservoirs, rivers, and Lake Erie ranging geographically from Lake Erie in the north to the Ohio River in the south and from Brookville Lake, Indiana in the west to Buckeye Lake, Ohio in the east. Sites were selected to represent ecosystems impacted by cyanoHABs only (Buckeye Lake, Grand Lake St. Marys, Kiser Lake) and by both cyanoHABs and invasive mussel species (Brookville Lake and Lake Erie). We

also sampled major rivers (e.g., Auglaize, Maumee, Sandusky, Ohio) to provide a background geochemistry signal representative of mixed groundwater and surface runoff that is feeding the lakes and reservoirs. All sites were sampled in the summer of 2015 during the eutrophically induced cyanobacterial bloom period.

Site Descriptions

Reservoirs

Four reservoirs were sampled for water column to sediment depth profiles, including three in Western Ohio (Grand Lake St. Marys, Buckeye Lake, and Kiser Lake) and one in Eastern Indiana (Brookville Lake) (Fig. 1). Grand Lake, or Grand Lake St. Marys (GLSM), is a large (58.3 km²) reservoir in central western Ohio that was created in the early nineteenth century as a feeder for the canal system that connected Lake Erie with the Ohio River, with the deepest point of a little over 3 m. Buckeye Lake is a large reservoir (12.9 km²) in central Ohio that was also created as a feeder for the canal system, converting what was a swamp/bog into a shallow lake (maximum depth of around 4.3 m, but most of the lake is 1.2 to 2.5 m deep). Kiser Lake is a smaller reservoir (1.6 km²) created when a dam was built in 1939 between moraine deposits, and has a maximum depth of ~ 3.7 m. Brookville Lake is a large reservoir (21.3 km²) in southeastern Indiana which was created as a result of the building of an earthen dam for flood control of the Whitewater River in 1974 by the US Army Corps of Engineers, with the deepest part of the lake (~ 36 m) at the base of the dam.

GLSM, Buckeye Lake, and Kiser Lake experience CyanoHABs due to eutrophication blooms. Zebra or quagga mussels have not been reported in these sites (USGS website as of September 2015; <https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=5>). Brookville Lake experiences CyanoHABs and the presence of invasive mussel species has been reported (USGS website as of September 2015; <https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=5>). At the time of sampling, fresh fish kills were observed at Brookville Lake, GLSM, Kiser Lake, and Buckeye Lake, likely due either to toxins produced by cyanobacteria or anoxic water conditions. Lake surface areas, average depths, and maximum depths are reported in the Supplementary Online Material (Table S1).

Fig. 1 Composition of 16S rRNA gene sequences recovered from sediment and planktonic biomass in reservoirs, rivers, and lakes in Indiana, Ohio, and Kentucky. Representative OTUs for each library were binned at the Class level. Sample collection

depths are indicated when more than just the surface was collected. *Seds* sediments collected at the water–sediment interface

Lake Erie sites

Three sites were sampled along the southwestern shore of Lake Erie (Fig. 1): Bay View Park, located where the Maumee River empties into Maumee Bay on the far west end of Lake Erie, East Harbor State Park, located on the Lake Erie side of the Marblehead Peninsula which separates it from Sandusky Bay, and Sandusky Bay, a $\sim 77 \text{ km}^2$ bay located where the Sandusky River empties into the southern shore of Lake Erie. Lake Erie experiences harmful

cyanobacterial blooms due to eutrophication and invasive mussel species have been observed at all sites (USGS website as of September 2015; <https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=5>)

Rivers

The Auglaize, Maumee, and Sandusky Rivers all feed into Lake Erie. The Auglaize River joins the Maumee River in Defiance, Ohio, and the Maumee River feeds into the western Basin of Lake Erie near Toledo, Ohio.

We sampled the Maumee and Auglaize Rivers upstream from Defiance, Ohio, and the Maumee River below Defiance (Fig. 1). The Sandusky River flows north into Lake Erie at Sandusky Bay, west of Cleveland. We sampled the Sandusky River where it flows through Fremont, Ohio (Fig. 1). The Ohio River originates in the Appalachian Mountains and flows west from Pennsylvania, forming the border between West Virginia and Kentucky on the south and Ohio and Indiana on the north before emptying into the Mississippi River. The Licking River flows north from Kentucky and empties into the Ohio River near Cincinnati, Ohio. We sampled both the Ohio River and the mouth of the Licking River near Cincinnati (Fig. 1). All of the rivers sampled experience eutrophically induced cyanobacterial blooms and have had reports of invasive mussel species present (USGS website as of September 2015; <https://nas.er.usgs.gov/viewer/omap.aspx?SpeciesID=5>).

Sample collection

Water depth, temperature, conductivity, and oxidation–reduction potential (ORP) were measured in situ using a calibrated multiparameter ProDSS sonde (Yellow Springs, OH, USA). Water clarity was measured using a Secchi disk and is reported as Secchi depth. Water column samples for aqueous geochemistry, nucleic acid extraction, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determination were collected with a 3.8 l Van Dorn bottle. Approximately 60 ml of water was filtered with a sterile syringe through pre-combusted (12 h, 450°C) GF/F filters (0.3 μm pore size) (Sterlitech Corporation, Kent, WA) for each analysis. Filters were immediately frozen on dry ice and stored at -20°C until nucleic acid extraction or $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determination. Sediment samples were collected from the sediment–water interface and immediately frozen on dry ice and stored at -20°C until nucleic acid extraction or $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determination. Samples for ion chromatography analysis of major cations and anions were filtered through 25-mm-diameter, 0.22- μm polyethersulfone syringe filters (VWR International, Radnor, PA) into 15-ml centrifuge tubes that had been pre-soaked in 18.2 M Ω /cm DI water, and stored at 4°C until analysis. Samples for trace element (e.g., Fe, Mn, P) analysis (10 ml) were filtered through a 25-mm-diameter, 0.22- μm polyethersulfone syringe filters (VWR International,

Radnor, PA) into acid washed 15-ml centrifuge tubes and acidified with 200 μl of concentrated OmniTrace UltraTM concentrated nitric acid (EMD Millipore, Billerica, MA), and stored at 4°C until analysis. Samples for dissolved organic carbon (DOC) concentration and $\delta^{13}\text{C}$ analysis were filtered through 25-mm diameter, 0.22- μm polyethersulfone syringe filters into 50-ml centrifuge tubes (final volume 25–30 ml), flash frozen on dry ice, and stored at -20°C until analysis.

The Van Dorn bottle and all sampling equipment were cleaned with a 70% ethanol solution between each sampling site to minimize cross contamination. Surface sediment samples (top ~ 2 cm) were collected with a weighted scoop sampler and placed in 50-ml centrifuge tubes with no head space and kept on ice until returned to the lab where they were kept at 4°C until processed. Approximately, 20 ml of sample was flushed through the filter before the trace element sample was collected.

Nucleic acid extraction

DNA was extracted from filters or the sediments using a MoBio PowerSoil[®] DNA Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions. The concentration of DNA was determined using a Qubit dsDNA HS Assay kit (Molecular Probes, Eugene, OR, USA) and a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), respectively.

16S and 18S rRNA sequence analysis: community composition and diversity

Total DNA was submitted to the University of Minnesota Genomics Center (UMGC) where amplicons were sequenced using MiSeq Illumina 2×300 bp chemistry. Primers 515Ff and 806rB were employed to target V4 hypervariable region of bacterial and archaeal 16S SSU rRNA gene sequences (Caporaso et al., 2012; Apprill et al., 2015). Primers E572F and E1009 were employed to target the V4 hypervariable region of eukaryotic 18S SSU rRNA gene sequences (Comeau et al., 2011). Mothur (ver. 1.40.4; (Schloss et al., 2009) was used for post-sequencing processing following the MiSeq SOP (Kozich et al., 2013). Read pairs were assembled and resulting contigs with ambiguous bases were

removed. Contigs were trimmed to include only the overlapping regions and unique sequences were aligned against the Silva v132 database. Chimeras were identified and removed using UCHIME (Edgar et al., 2011) and removed. Sequences were classified using the TaxAss pipeline (<https://github.com/McMahonLab/TaxAss>; Rohwer et al., 2018) using the FreshTrain18Aug2016 database and the Silva database (v132). For bacteria and archaea, operational taxonomic units (OTUs) were assigned to all classified sequences at 97.0% using the average-neighbor algorithm. For eukarya, OTUs were assigned at 98% operational using the average-neighbor algorithm. Rarefaction was calculated within mothur and indicated the majority of predicted 16S rRNA and 18S rRNA gene diversity was sampled at this depth of sequencing (data not shown). All statistical analyses were carried out in R version 3.4.3 (R Core Team, 2018). Ordination analyses were performed using the R software packages vegan (Oksanen et al., 2019) or Phyloseq (ver. 1.16.2; McMurdie & Holmes, 2013). Differences in alpha diversity metrics were using Mann–Whitney pairwise comparison test. A hierarchical cluster analysis was applied to estimate community similarity in the 16S rRNA data using Bray–Curtis dissimilarity and Ward clustering with 1,000 resamplings. Core microbiome of 16S rRNA sequences across samples was assessed using MicrobiomeAnalyst (Dhariwal et al., 2017) at a sample prevalence of 25% and a relative sequence abundance of 5%. Results are reported at the Class and Order level. Prior to ordination, samples were rarefied to even sequencing depth of 9227 (the smallest library in the data set). Differences in 16S and 18S rRNA community compositions were visualized using non-metric multidimensional scaling (NMDS) ordination using Bray–Curtis dissimilarities. Unconstrained principal component analyses were performed on the rarefied 16S rRNA amplicon libraries using Bray–Curtis dissimilarities. A constrained ordination was performed to examine how environmental variables are associated with community composition, also using Bray–Curtis dissimilarities. Significant differences in the NMDS and constrained ordination analyses were evaluated using analysis of similarity permutational multivariate analysis of variance (PERMANOVA). The raw sequencing reads have been deposited at the NCBI Short Read Archive under BioProject ID PRJNA386694 and accession numbers

SAMN07125357 to SAMN07125385. Library designations are provided in Table S2.

qPCR

Quantitative PCR (qPCR) was used to estimate the number of archaeal and bacterial 16S rRNA gene sequences and 18S rRNA gene sequences in genomic DNA as described previously (Hamilton et al., 2013). Assays were performed in triplicate in a StepOnePlus® (Life Technologies) with the SsoFast EvaGreen Supermix qPCR kit (Bio-Rad Laboratories, Hercules, CA). As described previously, for quantification of archaeal and bacterial 16S rRNA genes, primers 344F (5′-ACGGGGYGCAGCAGGCGCGA-3′) and 915R (5′-GTGCTCCCCGCCAATTCCT-3′) and 1100F (5′-YAACGAGCGCAACCC-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were employed at an annealing temperature of 61°C or 55°C, respectively (Hamilton et al., 2013). Primers targeting the V3–V4 region of the cyanobacterial 16S rRNA gene for (Cya359F (5′-GGGGAATYTTCCGCAATGGG-3′) and Cya781R (5′-GACTACWGGGGTATC-TAATCCCWTT-3′)) (Nübel et al., 1997) were employed to quantify cyanobacterial 16S rRNA gene. Assays were performed at 64°C as described previously (Zhang et al., 2014). Primers for quantification for 18S rRNA were the same as those described above for amplicon sequencing and assays were performed at an annealing temperature of 42°C. For each set of primers, ~ 1 ng of purified genomic DNA was subjected to qPCR with the following qPCR cycling conditions: initial denaturation (95°C for 10 min) followed by 40 cycles of denaturation (95°C for 10 s), annealing (at the optimal annealing temperature for each primer as described above for 10 s), and extension (72°C for 20 s). The standard curves were constructed using 16S and 18S rRNA sequences from environmental DNA. Genes were amplified from several samples (amplification, PCR conditions, and primers followed those described above), purified using a QIAquick PCR Purification Kit (Qiagen, Carlsbad, CA, USA), and inserted into a pGEM-T easy vector (Promega, Madison, WI) and transformed into JM109 High Efficiency Competent Cells (Promega, Madison, WI). Plasmids were isolated using a Wizard® Plus SV Minipreps DNA Purification System (Promega, Madison, WI) and concentration was measured using a qubit as described above. The copy

number of each gene was normalized by the concentration of DNA and the grams of sediment (wet weight) used in the DNA extraction.

Aqueous geochemistry

Major cations (e.g., sodium, potassium, calcium, magnesium) and anions (e.g., chloride, sulfate, nitrate) were determined using a Dionex Liquid ion chromatograph by the STAR Lab at Ohio State University. Trace metal and phosphorus analysis was conducted via Prodigy Dual View Inductively Coupled Plasma Spectrophotometer by the STAR Lab at Ohio State University. Phosphorus represents that P detected in the 0.22 μm filtered portion of the water (detection limit = 0.52 $\mu\text{mol/l}$). Samples for P were not digested prior to analyses. Total ammonium ($\text{NH}_4(\text{T}) = \text{NH}_3 + \text{NH}_4^+$) was determined using a DR1900 portable spectrophotometer (Hach Company, Loveland, CO). Porewater samples were diluted with 18.2 $\text{M}\Omega/\text{cm}$ DI water prior to spectrophotometric analyses. Samples for dissolved inorganic carbon (DIC) concentration and $\delta^{13}\text{C}$ analysis were filtered through a 25-mm-diameter, 0.22- μm polyethersulfone syringe filters (VWR International, Radnor, PA) into pre-muffled 10-ml glass scintillation bottles (baked at 480°C for 8 h) that were aluminum-crimp-sealed with silicon-coated gray butyl rubber three-legged lyophilization septa and kept inverted and refrigerated until analysis.

Dissolved carbon

DIC concentration and $\delta^{13}\text{C}$ analysis were conducted by the Stable Isotope Facility at the University of California, Davis using a GasBench II system interfaced to a Delta V Plus isotope ratio mass spectrometer (IR-MS) (Thermo Scientific, Bremen, Germany) as previously described (Havig et al., 2017). Raw delta values converted to final using laboratory standards (lithium carbonate, $\delta^{13}\text{C} = -46.6\text{‰}$ and a deep seawater, $\delta^{13}\text{C} = +0.8\text{‰}$) calibrated against standards NBS-19 and L-SVEC.

DOC concentration and $\delta^{13}\text{C}$ analysis were conducted by the Stable Isotope Facility at the University of California, Davis via an O.I. Analytical Model 1030 TOC Analyzer (O.I. Analytical, College Station, TX) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a

GD-100 Gas Trap Interface (Graden Instruments, Ontario, Canada) for concentration and isotope ratio determination as previously described (Havig et al., 2017). Raw delta values converted to final using laboratory standards (KHP and cane sucrose) calibrated against USGS-40, USGS-41, and IAEA-600. The limit of quantification and analytical precision is 0.4‰ for $\delta^{13}\text{C}$ of DOC at DOC concentrations greater than 0.5 ppm.

Solid sample isotopes

For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determination, sediment samples and filtered water column biomass samples were treated as described previously (Schuler et al., 2017). Briefly, they were dried at 60°C for 3 days. Prior to drying, sediment samples were weighed. Dried sediment samples were weighed, homogenized with a clean mortar and pestle (pre-scoured using an 80% ethanol-silica sand slurry, then rinsed with 18.2 $\text{M}\Omega/\text{cm}$ deionized water). For organic carbon $\delta^{13}\text{C}$ determination, dried and ground sediments were decarbonated: a subset of each sediment sample was weighed, soaked in 1 M HCl for 24 h, washed with 18.2 $\text{M}\Omega/\text{cm}$ deionized water three times to remove excess HCl, dried at 60°C for 3 days, weighed, and homogenized with a clean mortar and pestle. Filtered biomass decarbonation was carried out via flushing with 1 M HCl followed by rinsing with 18.2 $\text{M}\Omega/\text{cm}$ deionized water. Sediment and filtered samples for $\delta^{15}\text{N}$ determination were not subject to decarbonation. Filters or sediments were then placed into tin boats and analyzed using a Costech Instruments Elemental Analyzer (EA) periphery connected to a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer (IR-MS) located in the Department of Geology at the University of Cincinnati as described previously (Havig et al., 2017). Linearity corrections were made using NIST Standard 2710, and $\delta^{13}\text{C}$ values were calibrated using reference standards USGS-40 and USGS-41 and checked with a laboratory standard (glycine).

All stable carbon isotope results are given in delta formation expressed as per mil (‰):

$$\delta^{13}\text{C} = \left[(R_a)_{\text{sample}} / (R_a)_{\text{standard}} - 1 \right] \times 10^3, \quad (1)$$

where R_a is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample or standard, and are reported versus the Vienna Pee Dee Belemnite (VPDB) standard. All stable nitrogen results are given

in the same delta notation as described above ($\delta^{15}\text{N}$, expressed in ‰), where R_a is the $^{15}\text{N}/^{14}\text{N}$ ratio of the sample or standard, and the results are reported versus air.

Biogeochemical model

As an exercise for comparison of the sampling sites, we estimated the fraction DIC removed as organic carbon from these systems using an equation for estimating the fraction of organic carbon removed from the global ocean system:

$$\delta^{13}\text{C}_{\text{DIC}} = \delta^{13}\text{C}_{\text{source}} + F_{\text{org}}(\Delta_{\text{C}}), \quad (2)$$

Solved for F_{org} as

$$F_{\text{org}} = (\delta^{13}\text{C}_{\text{DIC}} - \delta^{13}\text{C}_{\text{source}}) / \Delta_{\text{C}}, \quad (3)$$

where $\delta^{13}\text{C}_{\text{DIC}}$ is the $\delta^{13}\text{C}$ value of DIC, $\delta^{13}\text{C}_{\text{source}}$ is the $\delta^{13}\text{C}$ value of the source for the DIC, F_{org} is the fraction of DIC removed as organic carbon through fixation, and Δ_{C} is the fractionation factor imposed by photosynthetic fixation of DIC into organic carbon. As the $\delta^{13}\text{C}_{\text{source}}$ is approximated by either atmospheric CO_2 or the river DIC value, we incorporate both values into the equation to calculate a potential range for F_{org} . We assume that the Δ_{C} is approximated by the $\delta^{13}\text{C}$ value of seston organic carbon minus the $\delta^{13}\text{C}$ value of DIC for the surface samples (e.g., Miles, 1985; Hayashi & Rosenberry, 2002; Sophocleous, 2002; Fleckenstein et al., 2010). We also incorporate analytical uncertainty into our model by bootstrapping the distributions of $\delta^{13}\text{C}$ values from each sample type (river, lake DIC, and lake DOC). We sampled the distributions 1000 times with replacement to calculate F_{org} . All calculations were performed in R (R Core Team, 2018).

Results

Community composition, abundance, and diversity

qPCR

We quantified archaeal and bacterial 16S rRNA gene sequences, and cyanobacterial 16S rRNA gene sequences, and eukaryotic 18S rRNA gene sequences

to qualitatively estimate the contribution of these organisms to water column and sediment biomass. In general, bacterial 16S rRNA gene sequences were more abundant than archaeal 16S rRNA genes and 18S rRNA genes in all samples (Fig. S1). Archaeal 16S rRNA sequences were typically more abundant than eukaryotic 18S rRNA genes and were more abundant in sediments compared to water column samples. In contrast, cyanobacterial 16S rRNA sequences were usually detected in higher abundance in the water column samples compared to the sediments. In most samples, cyanobacterial 16S rRNA gene sequences were more abundant than 18S rRNA genes.

16S and 18S rRNA amplicon sequencing

We examined community composition in planktonic and sediment samples using 16S and 18S rRNA amplicon sequencing. Sequencing of 16S rRNA genes resulted in 773,701 sequences after quality control and removing chimeras. Clustering sequences at 97% identity resulted in 9,421 OTUs affiliated with bacteria and 1,016 OTUs affiliated with archaea. Sequencing of 18S rRNA genes resulted in 1,754,164 sequences after quality control and removing chimeras. Clustering sequences at 98% identity resulted in 16,293 OTUs. In non-metric multidimensional scaling analyses, sediment samples clustered distinctly from water column samples for both 16S and 18S rRNA analyses and not by the presence or absence of invasive mussels (Fig. S2).

At the phylum level, OTUs affiliated with cyanobacteria were abundant in the majority of the samples and archaea were much less abundant than those affiliated with bacteria (Fig. 1). In the Maumee River, and Kiser and Brookville Lake sediments, the sequences affiliated with and Beta- and Gammaproteobacteria were abundant (Fig. 1). OTUs affiliated with Actinobacteria and Alphaproteobacteria were abundant in the Ohio River and Licking River samples. In Buckeye Lake and Sandusky Bay, OTUs most closely related to Spartobacteria were abundant in addition to those affiliated with cyanobacteria. Small numbers of OTUs affiliated with Acidimicrobia, Chlorobi, Phycisphaerae, Planctomycetacia, and Saprospirae were recovered from most of the sites (Fig. 1). The most abundant archaeal OTUs were affiliated with the ammonia oxidizing archaea Nitrososphaerales, Woesearchaeia, and methanogens

including Methanobacteriales, Methanomassiliicoccales, Methanomicrobiales, and Methanosarcinales were also consistently observed (Fig. S3).

A number of OTUs affiliated with algae and diatoms were recovered from both the water column and sediment samples (Fig. S4). Sequences affiliated with Diatomea were particularly abundant in the Ohio and Licking River samples, the upper Maumee River samples, and Lake Erie. Sequences affiliated with Chlorophyceae were observed in all river samples, Lake Erie, Brookville Lake, Buckeye Lake, and sediments from Kiser Lake. Sequences affiliated with ciliates (Intramacronucleata) and dinoflagellates (Dinophyceae) were also recovered from the majority of the samples. Algae, including Chlorophyceae, can represent significant fractions of phytoplankton biomass during cyanobacterial blooms.

Cyanobacteria

16S rRNA gene sequences affiliated with the genus *Planktothrix* were abundant in most of the samples with several notable exceptions (Fig. 2): In Brookville Lake, OTUs affiliated with *Cylindrospermopsis* and *Planktothrix* were abundant throughout the water column. OTUs affiliated with *Microcystis* spp. were recovered from the Ohio and Licking River along with smaller numbers of sequences of *Pseudanabaena*. Water column and sediment samples from Kiser Lake were composed of cyanobacterial OTUs affiliated with *Microcystis*, *Planktothrix*, and *Cyanobium*. In samples from the Auglaize, lower Maumee, and Sandusky Rivers, the majority of cyanobacterial OTUs were most closely related to *Cyanobium* spp.

Diversity

In general, sediment communities were more diverse than planktonic communities based on alpha diversity metrics for 16S rRNA (Fig. S5A) and 18S rRNA (Fig. S6A) but none of the differences were significant. There were also no significant differences in alpha diversity metrics in samples from sites that were impacted by invasive mussels compared to those without mussels (Fig. S5B; Fig. S6B). In principle coordinate analyses of the microbial (total 16S rRNA) composition and cyanobacterial community composition, distinct patterns were observed between the two (Fig. 3). When considering the total community

composition, most sediment samples generally grouped together as did the Brookville Reservoir samples and most of the river samples. In contrast, cyanobacterial sequences in the Brookville Reservoir samples formed a distinct group from the rest of the samples. In both analyses, Lake Erie, Buckeye Lake, and Grand Lake Saint Marys samples generally clustered. This could be due to sample collection from shallow, well-mixed water columns (e.g., Sandusky Bay in Lake Erie).

Geochemistry

Lakes and reservoirs

Geochemical data indicate that lakes and reservoirs we sampled are well-mixed and impacted by abundance and activity of phototrophs in the water column (Table 1; Fig. 4). Secchi disk measurements from the water column of Grand Lake St. Marys, Buckeye Lake, Sandusky Bay, East Harbor State Park, and Bay View Boat Launch in Lake Erie, and Brookville Reservoir are consistent with increased turbidity due at least in part to cyanobacterial blooms in the water column (Table 1). In the water column of Grand Lake St. Marys, Buckeye Lake, Sandusky Bay in Lake Erie, and Brookville Reservoir, temperatures ranged from 25 to 29°C and temperature was relatively constant throughout the water column (Fig. 4). Grand Lake St. Marys, Buckeye Lake, Sandusky Bay, and Brookville Reservoir are relatively shallow, resulting in a well-mixed water column due to wind. Consistent with an active photoautotrophic population, the pH was above 8 in Grand Lake St. Marys, Buckeye Lake, the three sites in Lake Erie, and Brookville Reservoir (Fig. 4). At Grand Lake St. Marys, Buckeye Lake, the three sites in Lake Erie, and Brookville Reservoir, oxygen was above air saturation at the surface (Fig. 4). Nitrate concentration ranged from 40 to 81 µM in Grand Lake St. Marys, Brookville Reservoir, and East Harbor State Park on Lake Erie (Table 1). At all other sites, nitrate was below detection limits. NH₄(T) was only above detection limits in the pore water samples (Table 1). P (measured as total dissolved P detected in the 0.22 µm filtered portion of the water) was below detection limits (detection limit = 0.52 µmol/l) in the majority of the sites but was detected (0.6 to 3.8 µM) in samples from the Bay View Boat Launch on Lake

Fig. 2 Composition of cyanobacterial 16S rRNA gene sequences recovered from sediment and planktonic biomass in reservoirs, rivers, and lakes in Indiana, Ohio, and Kentucky. Representative OTUs for each library were binned at the Genus

level (total number of OTUs = 198). Sample collection depths are indicated (surface where not indicated). *Seds* sediments collected at the water–sediment interface

Erie, Buckeye Lake, and Grand Lake St. Marys (at 2.0 m) (Table 1).

Rivers

The geochemical attributes of the river samples were similar to water column samples. The temperature ranged from 25.4 to 28.6°C and the pH from 8.30 to 8.99 (Table 1). Dissolved oxygen was well above air saturation in the Maumee River (190.1% and 254.3%) and was also above air saturation in the Auglaize River (131.1%), the Sandusky River (103.1%), and the Licking River (103.1%). Dissolved oxygen in the Ohio River was 99.8%. No $\text{NH}_4(\text{T})$ or phosphorus was detected in any of the river samples. Nitrate concentrations ranged from 47.2 μM in the Sandusky River,

87.5 μM in the Auglaize River, and 240.3 μM in the Maumee River (Table 1). No nitrate was detected in the Maumee River sample site downstream of Defiance. In both water column and river samples, measurements of total or particulate N and P would likely have reflected substantial nutrient concentrations associated with the eutrophic status of the ecosystems. Indeed, long-term monitoring of the Maumee River suggests that while concentrations vary seasonally, soluble reactive phosphorus, total phosphorus, and total nitrogen are present throughout the year (Choquette et al., 2019).

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Table 1 Geochemical and physical analytical results for water column and sediment samples

	GPS Easting Northing		pH	Temp. (°C)	Cond. μS/cm	D.O. % Sat.	NH ₄ (T) μM	NO ₃ ⁻ μM	Biomass				DIC mM	δ ¹³ C ‰	C _{CaCO₃} %	δ ¹³ C ‰	Cl ⁻ mM	SO ₄ ²⁻ mM	Total dissolved P μM	Na mM	Ca mM	Mn mM	Fe mM
									Total N % N	δ ¹⁵ N ‰	C _{org} % C	δ ¹³ C ‰											
Grand Lake	708649 (16S)	4487570																					
0 m			9.50	25.9	378.5	134.2	bdl	64.1	13.18	-23.99	1.6	2.45	0.79	0.14	bdl	0.77	0.70	25	188				
2.0 m			9.20	25.2	378.7	82.9	bdl	40.9	12.26	-21.63	1.8	1.12	0.79	0.33	0.90	0.77	0.70	31	210				
Porewater			nd	nd	nd	nd	357	nd	nd				4.0	-2.42		0.83	0.43	bdl	nd	nd	nd	nd	
Sediments									0.67	8.18	4.14	-24.57			18.1	-4.69							
Buckeye Lake	369509 (17S)	4418855																					
0 m			9.70	28.7	311.2	169.0	bdl	bdl	8.99	-21.95	1.1	2.84	0.92	0.16	1.19	0.66	0.59	87	449				
0.4 m			8.50	28.5	349.5	7.1	bdl	bdl	bdl	-21.63	1.2	2.09	0.89	0.16	1.13	0.65	0.59	116	902				
Porewater			nd	nd	nd	nd	257	nd	nd				3.2	-7.80		0.96	0.10	3.82	0.57	0.81	15883	4609	
Sediments									0.61	6.21	4.17	-25.32			16.6	-0.75							
Kaiser Lake	248821 (16S)	4452314																					
0 m			9.22	29.2	323.9	183.7	bdl	48.9	2.48	bdl	nd	-31.54	2.3	-5.75		0.4	0.14	bdl	0.4	0.6	46	1005	
Brookville Reservoir	672947 (16S)	4374461																					
0 m			8.70	27.7	411.6	109.3	bdl	76	bdl		-30.69	2.6	-5.72	0.65	0.22	bdl	0.5	0.01	bdl	bdl			
3.5 m			8.00	25.9	413.4	38.1	bdl	79.7	bdl		-30.45	2.7	-7.68	0.64	0.22	bdl	0.5	0.01	bdl	bdl			
6.5 m			7.80	25.2	428.1	8.9	bdl	77.2	bdl		-31.17	3.3	-9.94	0.64	0.21	bdl	0.5	0.01	24	bdl			
Sediments									0.06	bdl	-27.66					36.2	-1.26						
Lake Erie																							
Sandusky Bay	347261 (17S)	4594335																					
0 m			9.40	25.1	303.8	114.5	bdl	bdl	bdl		-25.60	1.1	-4.97	0.46	0.48	bdl	0.36	0.78	44	432			
1.3 m			8.40	24.8	303.5	36.8	bdl	bdl	bdl		-26.94	1.2	-5.72	0.46	0.47	bdl	0.37	0.78	49	338			
Porewater			nd	nd	nd	nd	114	nd	bdl		-15.14	4.1	-15.14	0.76	0.72	bdl	0.05	0.16	1582	1071			
Sediments									0.53	8.02	3.21	-26.89			16.9	-3.37							
East Harbor S.P.	350059 (17S)	4601461																					
0 m			9.23	26.4	279.9	136.5	bdl	81.4	bdl		-24.52	1.5	-3.16	0.45	0.23	bdl	0.38	0.76	bdl	98			
Bay View B.L.	294081 (17S)	4619918																					
0 m			9.19	26.3	403.0	181.8	bdl	bdl	bdl		-28.43	2.4	-10.81	0.73	0.29	0.99	0.76	1.12	53	528			
River Samples																							
Auglaize River	716515 (16S)	4567748	8.41	27.5	546.0	131.1	bdl	87.5	bdl		-28.02	3.3	-9.96	0.83	0.53	bdl	0.74	1.50	58	109			
Maumee River	708129 (16S)	4572339	8.99	26.8	514.0	254.3	bdl	bdl	bdl		-25.50	2.8	-8.15	0.90	0.47	bdl	0.84	1.36	80	310			
	276184 (16S)	4602857	8.98	28.3	620.0	190.1	bdl	240.3	bdl		-31.13	2.5	-9.39	1.89	0.50	bdl	1.59	1.35	78	172			
Sandusky River	323446 (17S)	4578823	8.81	28.6	519.0	103.1	bdl	47.2	bdl		-28.51	2.3	-6.61	1.04	0.82	bdl	0.89	1.16	106	278			
Licking River	715889 (16S)	4329558	8.40	25.4	449.0	103.1	bdl	76.2		16.90	-29.16	1.7	-9.65	0.89	0.78	bdl	1.16	0.92	317	bdl			
Ohio River	715889 (16S)	4329559	8.30	25.5	460.0	99.8	bdl	60.2		18.12	-28.90	1.7	-9.56	0.93	0.83	bdl	1.08	0.92	586	bdl			

Total dissolved P measured as P detected in the 0.22 μm filtered portion of the water

Detection limits: NH₄ (T)—0.7 μM, total dissolved P—0.52 μM, Mn—20 nM, Fe—20 nM

nd not determined, *bdl* below detection limits, *Temp.* temperature, *Cond.* conductivity, *D.O.* dissolved oxygen, *% Sat.* percent saturation, *C_{org}* total organic carbon, *DIC* dissolved inorganic carbon, *C_{CaCO₃}* total carbonate

Particulate organic carbon (seston)

Particulate organic carbon (POC), or seston (> 0.3 μm filter size), ranged two orders of magnitude across sites. Grand Lake St. Marys had the highest concentration (~ 1719 μmol C/l at 2 m depth), followed by Buckeye Lake (1203 μmol C/l at 0.4 m depth) (Table 1, Fig. 5). The lowest POC concentrations were found at Sandusky Bay (46.6 μmol C/l at the surface), with Brookville Lake, Kiser Lake, and the other Lake Erie sites falling between (Table 1, Fig. 5). POC concentrations in the rivers ranged from a high of 507 μmol C/l in the upper Maumee River to a low of 32 μmol C/l in the Auglaize River (Table 1). POC δ¹³C values were the lowest at Kiser Lake (− 31.54‰ at the surface) and the highest at Buckeye Lake

(− 21.63‰ at the 0.4 m depth). River POC δ¹³C values fell between a low of − 31.13‰ for the lower Maumee River and a high of − 25.50‰ for the upper Maumee River.

Sediment organic and inorganic carbon

Surface (top ~ 2 cm) sediment samples were collected at Grand Lake St. Marys, Buckeye Lake, Brookville Lake, and Sandusky Bay. Brookville Lake sediments contained the lowest organic carbon content (0.62%) while the other sediments ranged from 3.21 to 4.17% organic carbon (Table 1). Sediment organic carbon δ¹³C values range between a low of − 27.66‰ in Brookville Lake sediments and − 24.57‰ at Grand Lake St. Marys (Table 1, Fig. 5). Brookville Lake

Fig. 4 Physical and geochemical parameters for the five lakes/reservoirs plotted versus depth in the water column. *Temp.* temperature, *Cond.* Conductivity, *D.O.* optical dissolved oxygen, *ORP* oxidation reduction potential. Note that the x-axis scales are different for temperature, conductivity, and turbidity. The light gray line in the *D.O.* plots represent values toxic for fish (< 4 mg/l), the dark gray line values toxic for invertebrates (< 1 mg/l). Spikes in turbidity and associated drops in *ORP* at the bottom of depth profiles (i.e., Grand Lake St. Marys, Brookville Lake, Sandusky Bay) may indicate interaction with anoxic sediments

sediments (with visible mussel shell fragments) had the highest carbonate concentration (36.2%), while Buckeye Lake sediments had the lowest (16.6%) (Table 1, Fig. 5). Sediment carbonate $\delta^{13}\text{C}$ values ranged from -4.69‰ (Grand Lake St. Marys sediments) to 2.09‰ (Buckeye Lake sediments).

Biomass and sediment associated nitrogen

Organic nitrogen tracked with organic carbon concentration in the lakes, reservoirs, and rivers. Seston organic nitrogen concentrations in lakes and reservoirs fell between a high of $226.2\text{ }\mu\text{mol N/l}$ at the 2 m depth of Grand Lake St. Marys and a low of $6.93\text{ }\mu\text{mol N/l}$ at the surface of Sandusky Bay (Table 1). Seston organic nitrogen concentrations in rivers fell between $55.29\text{ }\mu\text{mol N/l}$ at the upper Maumee River and $5.10\text{ }\mu\text{mol N/l}$ at the Auglaize River (Table 1). Sediment organic nitrogen concentrations fell between 2.48% at Kiser Lake and

0.06% at Brookville Lake. Lake, reservoir, and river seston organic nitrogen $\delta^{15}\text{N}$ values were all positive, ranging from 7.77‰ in the Upper Maumee River to 18.12‰ in the Ohio River. Lake and reservoir sediment $\delta^{15}\text{N}$ values were also positive, falling between 6.21‰ at Buckeye Lake and 8.18‰ at Grand Lake St. Marys (Table 1).

Discussion

We present community composition and abundance data of planktonic and sediment microbes across a range of eutrophic lakes and reservoirs on select aquatic ecosystems with known mussel presence or absence that have been impacted by cyanobacterial blooms in Ohio, Indiana, and Kentucky, including portions of Lake Erie. While Lake Erie is well-studied with respect to eutrophication, few studies have examined cyanoHABs and environmental

Fig. 5 Carbon isotope values (**A**) and percent carbon removed in lakes and reservoirs (**B**). **A** Carbon isotope values for water column and sediment samples, Secchi disk depths, and percent DIC removed as organic carbon for the five lakes/reservoirs in western Ohio and eastern Indiana, USA are shown. *HCBs* harmful blooms of cyanobacteria, *DIC* dissolved inorganic carbon, C_{org} organic carbon, *DOC* dissolved organic carbon, *GW* groundwater, *O-AT* oxic–anoxic transition (asterisk denotes depth location of the transition), *Sed./PW* sediment/porewater.

GW $\delta^{13}\text{C}$ values are the average of river DIC values. **B** Percent carbon removed in lakes and reservoirs. Bars represent analytical error. Gray line indicates average carbon removal in the global oceans. Abbreviations: *BL* Buckeye Lake*, *GLSM* Grand Lake St. Marys*, *EHSP* East Harbor State Park, *SB* Sandusky Bay, *BR* Brookville Reservoir, *KL* Kaiser Lake*, *BVBL* Bay View Boat Launch, * lakes with cyanoHABs only

stressors, including invasive mussel species, in other impacted lakes and reservoirs in the region. We combine amplicon data with stable carbon and nitrogen isotopes to examine biogeochemical cycling in these ecosystems. With these data, we develop a framework that, with higher resolution sampling and inclusion of ecosystem variability, can be used to assess the effects of invasive mussels on biogeochemical cycling, most imperatively carbon cycling and burial, in eutrophic ecosystems impacted by cyanoHABs.

Community composition

Cyanobacteria

Consistent with increased phototroph productivity and biomass during cyanoHABs, sequences affiliated with cyanobacteria were abundant in our samples (both planktonic and sediment samples, Fig. 1). However, the abundance of cyanobacterial sequences generally decreased with depth and were the lowest in sediment samples likely reflecting lowered benthic light availability (Fig. 1; Fig. S1).

Microcystis is well-studied in the context of toxic cyanobacteria blooms. *Planktothrix* spp. are also common constituents of harmful blooms—they are often low-light adapted, can tolerate a larger range of temperatures than other cyanobacteria (Halstvedt et al., 2007), and can produce cyanotoxins including microcystin and oscillapeptin J (Blom et al., 2003; Christiansen et al., 2003; Komárek and Komárková, 2004). In our study, sequences affiliated with the genus *Microcystis* were only abundant in the Ohio and Licking Rivers and Kiser Lake. In contrast, sequences affiliated with the genus *Planktothrix* were abundant in Grand Lake St. Marys, Buckeye Lake, Brookville Lake, Lake Erie, and the Maumee River (Fig. 2). Microscopy-based studies identified *Microcystis* as the dominant member of bloom biomass in Lake Erie (Bridgeman et al., 2013; Michalak et al., 2013; Steffen et al., 2014; Harke et al., 2016) while the toxin production and bloom formation in Sandusky Bay in Lake Erie have been attributed to *Planktothrix* spp. (Davis et al., 2015; Salk et al., 2018). Recent studies found that *Microcystis* spp. dominated Lake Erie cyanoHABs near the Maumee River while *Planktothrix* spp. were abundant near the Sandusky River (Rinta-Kanto & Wilhelm, 2006; Conroy et al., 2014; Jankowiak et al., 2019). Here, we recovered abundant sequences affiliated with *Planktothrix* spp. from all our sites in Lake Erie including East Harbor State Park, Bay view Boat Launch, and Sandusky Bay. However, a more diverse community of cyanobacteria including sequences affiliated with *Dolichospermum* (formerly *Anabaena*), *Microcystis*, *Pseudanabaena*, *Synechococcus*, and unclassified genera have been observed in Lake Erie blooms (Ouellette et al., 2006; Gobler et al., 2008; Davis et al., 2012; Berry et al., 2017). In Grand Lake St. Marys, seasonal cyanoHABs have been attributed to *Planktothrix* (Steffen et al., 2014) which is consistent with our data (Fig. 2). *Planktothrix* and *Cylindrospermopsis* were both abundant in Brookville Lake: the former was abundant in the sediments while sequences affiliated with *Cylindrospermopsis*, which can produce the hepatotoxin cylindrospermopsin, were abundant in the upper water column samples (0 and 3.5 m) (Fig. 2). *Cylindrospermopsis* spp. have been observed in small shallow lakes throughout North America and Lake Erie. In studies of Sandusky Bay in Lake Erie, *Cylindrospermopsis* accounted for a relatively small fraction of the biomass (typically < 2%) (Conroy et al., 2007). In this study,

water temperature, shallow depth, and adequate nutrients were linked to the occurrence of *Cylindrospermopsis*. Temperature in the upper water column of Brookeville Lake was close to 28°C and nitrate levels were the highest we observed (76–80 µM) which could help explain the abundance of *Cylindrospermopsis* at the time our samples were collected.

The dynamics of the composition of cyanobacteria throughout bloom formation are not captured by our single time point sampling and remain largely enigmatic across the span of eutrophic systems: cyanoHABs can display spatial and temporal dynamics and changes in water geochemistry can occur on short timescales (Woodhouse et al., 2015; Harke et al., 2016). For instance, Berry et al. (2017) note that the cyanobacterial community composition in Lake Erie blooms fluctuates over time but that *Microcystis* and *Synechococcus* sequences were the most abundant in the bloom. Other studies note *Planktothrix* spp. are abundant at the beginning and end of cyanobacterial blooms in eutrophic lakes (Lampert & Sommer, 1997). In contrast to previous studies, the majority of cyanobacterial OTUs we recovered from the Auglaize, lower Maumee, and Sandusky Rivers were most closely related to *Cyanobium* spp. Previous studies found abundant *Microcystis* in the Maumee and Sandusky Rivers (Conroy et al., 2014) and *Planktothrix* in the Maumee River (Kutovaya et al., 2012; McKay et al., 2018). We also observed a small number of algae and diatoms in our water column samples. The abundance of these eukaryotes also likely depends on seasonality and the influx of nutrients and increase in temperature that lead to the formation of harmful blooms. For instance, in Lake Harsha (a eutrophic reservoir in southwestern Ohio), algae were more abundant prior to and following the bloom of cyanobacteria (Chen et al., 2017). Thus, our observations of abundant *Microcystis* in the Ohio and Licking Rivers, *Cylindrospermopsis* in Brookeville Lake, *Cyanobium* in the Auglaize, lower Maumee, and Sandusky Rivers, and *Planktothrix* in most lakes and reservoirs, could reflect sampling time (e.g., September and August, respectively).

Potential biogeochemical cycling in blooms assemblages

Our data highlight a diverse community of aerobic heterotrophic bacteria implicated in C, N, and P

cycling and underscore the need for systematic studies of spatial and temporal dynamics in harmful blooms beyond the dominant primary producer component. Microbiota that were consistently observed in the “core microbiome” include members of the Proteobacteria, Acidobacteria, Acidimicrobia, Planctomycetacia, and Spartobacteria. OTUs affiliated with these lineages were most closely related to aerobic, heterotrophic taxa including Chthoniobacterales within the Spartobacteria (Figs. 1, 6). OTUs affiliated with *Accumulibacter* (within the subclass Betaproteobacteria) and Actinomycetales (Actinobacteria) including *Tetrasphaera* spp. were recovered from all lake samples (water column and sediments) (Figs. 1, 6). Microbes within these lineages are polyP-accumulating organisms and have been implicated in enhanced biological phosphorus removal (McMahon & Read, 2013). Planctomycetacia are often observed in close association with phototrophs in aquatic habitats, including in eutrophic Lake Taihu (Cai et al., 2013) and have been implicated in degradation of phototroph biomass (Pizzetti et al., 2011a, b). Heterotrophic bacteria in blooms assemblages have also been indicated in toxin degradation including members of the *Caulobacteriales* (Alphaproteobacteria) and *Xanthomonadales* (Gammaproteobacteria), and *Burkholderiales* (subclass Betaproteobacteria) (Mou et al., 2013). Sequences affiliated with these lineages were recovered from the majority of our samples (Figs. 1, 6). OTUs affiliated with ammonia oxidizing archaea Nitrososphaerales were abundant in most samples including samples from the water column and sediments (Fig. S3). The functional gene for NH_4^+ oxidation, *amoA*, was detected in sediments of hypereutrophic Lake Taihu, particularly archaeal *amoA* (Hampel et al., 2019b). Ammonia oxidation could compete with bloom-forming cyanobacteria for ammonia and/or consume ammonia from cyanoHAB biomass. Both scenarios would contribute to nitrogen depletion consistent with the low levels of ammonia measured in our sites. OTUs affiliated with the Woesearchaeia were also abundant, particularly in water column samples as well as the Licking and Ohio River samples. Smaller numbers of OTUs affiliated with methanogens including Methanobacteriales, Methanomassiliicoccales, Methanomicrobiales, and Methanosarcinales were also consistently observed (Fig. S3). Methanogenesis is an important process in lake sediments for the degradation of organic matter

and biomass from cyanoHABs can serve as a labile carbon source for methanogens (Schwarz et al., 2008; Duc et al., 2010; West et al., 2012; Grasset et al., 2018).

Environmental variables and community diversity

We employed principal coordinate analysis and constrained coordination analyses to examine relationships between community diversity and environmental variables including the presence and absence of invasive mussel species. Unique clustering of planktonic 16S and 18S rRNA sequences suggests site factors other than mussel presence/absence are important to determining these microbial communities. In principle component analyses of the microbial (total 16S rRNA) composition and cyanobacterial community composition, a significant relationship was observed between community composition for the total community composition and the presence of mussels (Fig. 3A, *P* value 0.042). When only considering the cyanobacteria, a significant relationship was not observed (Fig. 3B, *P* 0.065). In constrained coordination analyses of community composition and environmental variables, we observed distinct patterns in community composition and environmental variables between the complete microbial

Fig. 6 Core microbiome of 16S rRNA sequences at the Class (A) and Order (B) level recovered from reservoirs, rivers, and lakes in Ohio, Kentucky, and Indiana

community and the cyanobacterial fraction (Fig. 7). When considering the entire microbial (16S rRNA) community, dissolved oxygen, chloride, calcium, and DIC concentration were correlated with the majority of the water column samples from Lake Erie, Buckeye Lake, and Grand Lake Saint Marys as well as the Auglaize, Maumee, and Sandusky River samples. Sediment samples from Lake Erie, Buckeye Lake, and Grand Lake Saint Marys were associated with elevated Mn, Fe, ammonium, organic carbon, and phosphorus concentrations (Fig. 7). No environmental variables were associated with the Brookeville Reservoir samples or the Ohio and Licking River samples. When considering only the cyanobacterial fraction of the microbial community, variables including Fe, Mn, DIC, P, ammonium, and organic carbon were correlated with samples sediment and water column samples from Lake Erie, Buckeye Lake, and Grand Lake Saint Marys while as the Auglaize, Maumee, and Sandusky River samples were correlated with sulfate, temperature, Na, conductivity, and ^{13}C DIC (Fig. 7). Nitrate was associated with Kaiser Lake samples and the Ohio and Licking River samples. These data suggest that environmental variables affect community composition in different ways.

In general, trends in species abundance, diversity, or evenness were stronger between sampling location or depth than between ecosystems with or without invasive mussels, suggesting invasive mussel species may not be greatly impacting microbial community compositions in these sites at the time of sampling. Unique clustering of planktonic 16S and 18S rRNA sequences suggests site factors other than mussel presence/absence are important to determining these microbial communities (Fig. 3). Similar to the community composition data, these observations are confounded by temporal and spatial variability in blooms and require higher resolution sampling (i.e., Berry et al., 2017) at more bloom-impacted sites. Thus, strategies to alleviate the toxicity and proliferation of cyanoHABs should carefully consider the bloom dynamics of the entire microbial community. While mussels may assist in proliferation of bloom-forming cyanobacteria, this effect is not detected in the composition of cyanobacteria in our study which are peak bloom. Finally, identifying biotic interactions between cyanobacteria and water column heterotrophs and how these dictate bloom characteristics including

proliferation, toxicity, and longevity warrants further study.

Limitations of DNA-based analyses

Previous studies have observed an increase in cyanobacterial biomass with nutrient input (Lürling et al., 2017). However, estimating the cyanobacterial portion of the biomass is difficult: algae and cyanobacteria (and some anoxygenic phototrophs) use chlorophyll *a* and its abundance is not necessarily correlated with cell number. Direct cell counts or cell sorting are time consuming and the latter is expensive. Some bloom-forming cyanobacteria (i.e., *Microcystis* spp., *Planktothrix* spp.) employ gas vacuoles to move vertically in the water column. Bloom-forming cyanobacteria also range in morphology including examples of coccoid cells of *Microcystis* which can form aggregates or colonies (Holt et al., 1994) or filaments (i.e., *Planktothrix* spp.). These characteristics hinder cell counts and biovolume estimates. For instance, highly variable annual biovolume of *Microcystis* was observed in a multi-year study in Lake Erie and large *Microcystis* colonies rely on abundance estimates from colony measurements rather than direct cell counts (Bridgeman et al., 2013). Based on relative abundance in our amplicon sequencing data, cyanobacteria accounted for $< \sim 10\%$ of the sequences recovered from the sediments (Fig. 1). In the water column, sequences affiliated with cyanobacteria ranged from ~ 10 to 55% of the total sequences recovered. In contrast, qPCR assays indicate cyanobacteria comprise 0.04 to 2.5% of the total sediment microbial community (all 16S rRNA gene sequences). In the water column, gene copies of cyanobacterial 16S rRNA ranged from 2.2% of the total community to 73% (Fig. S1). The amplicon data likely overestimate the relative abundance of cyanobacteria because the number of Archaea are underestimated with the primer set we employed. Similarly, the cyanobacterial 16S rRNA primers can amplify 16S rRNA genes from other bacteria but do not seem to amplify chloroplast 16S rRNA gene sequences (T.L. Hamilton, unpublished). And, our data do not account for variation in the number of rRNA gene sequences per genome (Acinas et al., 2004). However, the amplicon data provide species-specific information in a single reaction where a similar qPCR method would require multiple primer

Fig. 7 Constrained ordination of environmental variables and the community composition for the total microbial community (16S rRNA, CAP—All) and for the cyanobacterial fraction only (CAP—Cyanobacteria). The ordination axes were constrained to linear combinations of environmental variables and then the environmental scores were plotted onto the ordination (bottom panel). Labels are removed in the bottom panel for clarity.

sets and assays and conversion of qPCR results to cell counts is not straightforward (Rinta-Kanto et al., 2005). A combined approach employing quantification of *Microcystis*-specific 16S rRNA genes and *mcyD*, a microcystin toxin synthetase gene, can facilitate determining the abundance of toxic *Microcystis* as has been demonstrated in Lake Erie (Rinta-Kanto et al., 2005). However, this method is limited by primer sets specific for each bloom-forming and toxin-producing taxa. A detailed framework for relating amplicon data to biomass abundance could prove useful for identifying and quantifying specific bloom-forming cyanobacteria. Regardless, our data are

Cyano copies: abundance of the cyanobacterial 16S rRNA genes determined by qPCR; *Seds* sediment samples. Geochemical results are reported in Table 1. Abbreviations: *AR* Auglaize River, *BR* Brookville Reservoir, *BL* Buckeye Lake, *BVBL* Bay View Boat Launch, *EHSP* East Harbor State Park, *GLSM* Grand Lake St. Marys, *KL* Kaiser Lake, *LR* Licking River, *MR* Maumee River, *OR* Ohio River, *SB* Sandusky Bay

consistent with previous studies that have observed an increase in cyanobacterial biomass with nutrient input (Lürling et al., 2017): our data indicate cyanobacteria were abundant—particularly in the water column of the bloom-impacted lakes and reservoirs in this study. The decrease in cyanobacterial abundance in the sediments might indicate some consumption of biomass in the water column (and sediments). Further studies are required to determine sedimentation rates of bloom biomass and the potential for remineralization of biomass throughout bloom progression.

Stable isotopes—carbon and nitrogen cycling

Cyanobacteria are typically a large fraction of total biomass in eutrophic lakes (Van Dam et al., 2018) and were abundant in our samples (Figs. 1, 2; Fig. S1). As a result, they likely influence carbon and other biogeochemical cycles. Here, we examined carbon and nitrogen isotope systematics in reservoirs impacted by cyanoHABs (Buckeye Lake and Grand Lake St. Marys) where, as of 2015, there had been no reports of invasive mussel species recovered (USGS website; Ohio DNR) and lakes impacted by both cyanoHABs and invasive mussel species (Brookville Lake and Sandusky Bay in Lake Erie). Buckeye Lake and Grand Lake St. Marys are reservoirs which experience cyanoHABs annually due to anthropogenic input of P and fixed N from surrounding urban and agricultural land use. Cyanotoxins have been reported, with average microcystin concentration levels well above 1 µg/l (Wolf & Klaiber, 2016). Brookville Lake and Sandusky Bay in Lake Erie also experience cyanoHABs due at least in part to anthropogenic input of P and fixed N and invasive mussel species have been documented in Brookville Lake and Sandusky Bay (i.e., *Dreissena* spp., USGS; Ohio DNR).

Carbon isotopes

Seston carbon concentrations in the water column of both Grand Lake St. Marys and Buckeye Lake were the highest of all sites sampled, exceeding 1000 µmol C/l, consistent with Secchi disk depths of 18 and 12 cm, respectively (Figs. 4, 5; Table 1). DIC, seston, DOC, and sediment organic C $\delta^{13}\text{C}$ values were more positive in Grand Lake St. Marys and Buckeye Lake relative to sites with invasive mussel species (discussed below). We hypothesize that this heavier C signal is due to lower discrimination against isotopically heavy C from the DIC pool due to high productivity by bloom-driven photosynthesis. Seston C concentrations at Brookville Lake and Sandusky Bay were lower than Grand Lake St. Marys and Buckeye Lake, with associated Secchi disk depths of 110 cm and 24 cm, respectively (Figs. 4, 5). The more negative DIC and seston $\delta^{13}\text{C}$ values at Brookville Lake and Sandusky Bay likely reflect consumption of biomass by heterotrophs, including the diverse heterotroph microbes recovered in our amplicon studies and mussels, and subsequent respiration of

isotopically light CO_2 because the $\delta^{13}\text{C}$ values with increasing proportion of CO_2 from respiration (Schleser & Jayasekera, 1985).

Nitrogen isotopes

In general, more positive $\delta^{15}\text{N}$ values are associated with nitrogen loss via denitrification which is expected to be the dominant biologically mediated nitrogen removal pathway in freshwater ecosystems (Seitzinger et al., 2006). Denitrifiers are taxonomically diverse and include examples of bacteria, archaea, and eukaryotes and potential denitrifiers are represented in our data set. In our samples, all $\delta^{15}\text{N}$ values were positive—the isotopically lightest $\delta^{15}\text{N}$ values were found at Buckeye Lake, with a sediment $\delta^{15}\text{N}$ value of 6.21‰ while lakes and reservoirs impacted by invasive mussels had more positive $\delta^{15}\text{N}$ values (Table 1). For instance, Grand Lake St. Marys had the most positive values for seston (13.18‰ for the surface) and sediments (8.18‰). Buckeye Lake could receive greater input of lighter fixed N from either artificial fertilizer or via biological nitrogen fixation in the water column contributing N with $\delta^{15}\text{N}$ values closer to ~ 0. The $\delta^{15}\text{N}$ values for seston recovered from the Licking River and the Ohio River were the most positive in this study (16.90‰ and 18.12‰, respectively), suggesting denitrification is even more prevalent in the impacted rivers sampled, through input of fixed nitrogen from human waste (via discharge from wastewater treatment plants) would also have extremely positive $\delta^{15}\text{N}$ values (Cabana & Rasmussen, 1996). Implications from recent ammonium uptake experiments (e.g., Hampel et al., 2019a) and the nitrogen stable isotope results presented here suggest coupling multiple techniques for tracing nitrogen cycling in lacustrine environments might be an important next step for better understanding nitrogen cycling and availability as influences on cyanoHABs. This is supported by previous work: even in the presence of available fixed N, competition by specific portions of the microbial community can render fixed N unavailable to the rest of the community, and impact community composition and function (e.g., Hamilton et al., 2014).

Dissolved P and fixed N (e.g., NO_3^- and $\text{NH}_4(\text{T})$) were typically near or below detection limits in the lakes and reservoirs sampled during periods of cyanoHABs for this study (Table 1). In these

instances, the impact of cyanobacterial blooms (and a key competition strategy of bloom-forming cyanobacteria) is the proliferation of populations in the water column that effectively capitalizes on nutrient enrichment. Indeed, depletion of bioavailable nitrogen has been observed in Sandusky Bay during late summer coincident with cyanoHABs (Chaffin et al., 2018; Salk et al., 2018) as well as increasing rates of ammonium uptake (Hampel et al., 2019a). In previous studies of Lake Erie, *Planktothrix* spp. persisted in N-limited bays and tributaries (Conroy et al., 2007; Kutovaya et al., 2012; Davis et al., 2015) likely due the ability of these populations to scavenge fixed N (Conroy et al., 2007). The abundance of *Planktothrix* spp. in our sample sites coupled to low levels of fixed nitrogen suggest our samples were collected at a time that coincided with enough fixed nitrogen available for *Planktothrix* spp. to outcompete other bloom-forming cyanobacteria. It should be noted that there are strains of *Planktothrix* that have the capacity for nitrogen fixation (Pancrace et al., 2017). These observations coupled to our 16S rRNA amplicon data indicating diverse populations of heterotrophic bacteria coincident with cyanoHABs suggest complex competitive anabolic and catabolic trophic interactions in cyanoHABs.

During a bloom period, bloom-forming cyanobacteria can outcompete non-bloom-forming microorganisms for fixed N as well as P, maintaining high biomass loads in the water column and effectively removing P and fixed N from the water column (Table 1). Thus, while a lake may be designated eutrophic due to loading from anthropogenically driven input of P and fixed N and may have measurable and excessive levels of dissolved P and fixed N during periods of the year, cyanoHABs can act to reduce dissolved P and fixed N in the water column through sequestration into bloom biomass. Measurements of total or particulate N and P would likely have reflected substantial nutrient concentrations associated with the eutrophic status of the ecosystems. Management strategies for mitigating cyanoHABs include more efficient removal of fixed N and reduction of N + P loading (Paerl et al., 2011; Hampel et al., 2019b); however, varying combinations of nutrients, including N and P, along with elevated temperatures can promote different taxa of bloom-forming cyanobacteria (Jankowiak et al., 2019). Regardless, our data indicate a higher removal of fixed N in

systems impacted by cyanoHABs and invasive mussels, suggesting a beneficial role for mussels in terms of N.

Potential impacts on carbon sequestration

Cyanobacteria can use bicarbonate in addition to CO_2 , via the enzyme carbonic anhydrase which catalyzes the conversion of HCO_3^- to CO_2 and OH^- (thus affecting pH). However, CO_2 is the preferred substrate for photoautotrophic Cyanobacteria. During blooms, cyanobacterial primary productivity decreases $p\text{CO}_2$, increasing pH (pH values > 8.5, see Fig. 4) and shifting speciation from CO_2 to HCO_3^- or CO_3^{2-} . To overcome CO_2 limitation, Cyanobacteria employ efficient carbon concentrating mechanisms which enable them to consume HCO_3^- or CO_3^{2-} (Van Dam et al., 2018). Here, we assume photosynthesis from water column algal and cyanobacterial carbon fixation is the dominating factor imparting the $\delta^{13}\text{C}$ values of organic carbon. We acknowledge that this is a simplification as biotic and abiotic factors including the carbon concentrating reaction, growth rate, photochemical degradation, and $p\text{CO}_2$ can influence $\delta^{13}\text{C}$ values of organic carbon in eutrophic lakes (Morales-Williams et al., 2017; Van Dam et al., 2018). However, given the abundance of cyanobacteria during cyanoHABs including in our data set (Fig. 1; Fig. S1), it is reasonable to assume that photosynthesis in the water column is the major factor imparting the $\delta^{13}\text{C}$ values of organic carbon. Indeed, in Lake Taihu, $p\text{CO}_2$ was below equilibrium with the atmosphere at the peak of the cyanoHAB (Van Dam et al., 2018).

Earth-system modelers have long recognized that carbon isotope values of organic carbon, inorganic carbon, primary system inputs, and estimates of carbon fractionation during carbon fixation can be used to estimate the fraction of inorganic carbon buried as organic carbon (e.g., Hayes et al., 1999). While a likely over-simplification for lakes, it is useful to use this relationship for a first-order comparison between lakes, and for placing short-term lake carbon sequestration in the context of ocean carbon sequestration. Using Eq. 3 (see methods) and the assumptions discussed above, the fraction of DIC removed from the surface and buried as organic carbon in the sediments at Grand Lake St. Marys and Buckeye might be 50–300% over that of the ~ 20% assumed modern average global value for the world's oceans

(Fig. 5; Hayes et al., 1999; Schrag et al., 2013). This is consistent with recent work suggesting lakes and reservoirs sequester up to five times as much carbon as the world's oceans (Dean & Gorham, 1998; Cole et al., 2007; Downing et al., 2008; Tranvik et al., 2009). For lakes impacted by cyanoHABs and invasive mussels, the fraction of DIC removed could be much lower (Fig. 8). Combining these data with surface sediment carbon (presented in the Supporting Information), our data suggest shallow eutrophic lakes or reservoirs impacted by blooms are efficient at removing organic C from the water column, and thus potentially efficient at sequestering C. While the mechanisms of carbon fixation and organic carbon production are effectively the same for lakes/reservoirs and the ocean, the time scales for burial of organic carbon into sediments and sequestration of that carbon away from the carbon cycle for lakes/reservoirs are much shorter than that of the ocean. Reservoirs and lakes are effective transient bodies on continental surfaces with lifespans measured in hundreds to tens of thousands of years. Burial of organic carbon in sediments on ocean crust can sequester organic carbon for millions to hundreds of millions of years, and burial of organic carbon in ocean sediments deposited on continental crust can sequester organic carbon for millions to billions of years. However, these differences are effectively moot when considering human time scales in relation to anthropogenic global climate change.

Conceptual model

Our data suggest that the presence or absence of invasive mussel species is not correlative to the composition of cyanobacteria in cyanoHABs. However, we acknowledge that our single time point samples do not capture temporal or seasonal variability in bloom community structure. Regardless, we examined sites with and without invasive mussel species that experience cyanoHABs annually to build a framework to assess how the presence of invasive mussels may affect carbon sequestration in eutrophic systems. We constructed a conceptual model for the movement and sequestration of carbon in the different types of lakes and reservoirs sampled in this study (Fig. 9, see SOM for description of the model). Using this framework, our data suggest lakes and reservoirs that have experienced eutrophication that drives cyanoHABs such as Buckeye Lake and Grand Lake

Fig. 8 Percent carbon removed in lakes and reservoirs. Boxplots represent analytical error. Gray line indicates average carbon removal in the global oceans. Abbreviations: *BL* Buckeye Lake*, *GLSM* Grand Lake St. Marys*, *EHSP* East Harbor State Park, *SB* Sandusky Bay, *BR* Brookville Reservoir, *KL* Kaiser Lake*, *BVBL* Bay View Boat Launch, * lakes with cyanoHABs only

St. Marys (GLSM) have potential for efficient sequestration of both C_{org} and C_{carb} (see SOM for further discussion). However, the C_{org} sediment concentrations at those two lakes (4.14 to 4.17%; Table 1) indicate an approximate three-fold reduction in C_{org} burial compared to an average of surface sediments in 20 lakes across Minnesota (Dean, 1999). Furthermore, the C_{carb} sediment concentrations (1.99% at Buckeye Lake and 2.17% at GLSM; Table 1) indicate less carbonate burial compared to an average of 3.13% C as $CaCO_3$ (Dean, 1999). The data reported by Dean (1999) were for lakes associated with glacial till (similar to those in Ohio), but only 25% of the lakes had bedrock with carbonate rocks, suggesting there may be differences in the geochemistry of water delivered to the other Minnesota lakes. Lakes and reservoirs with cyanoHABs which have also been colonized by invasive mussel species (such as Brookville Lake and Sandusky Bay, Lake Erie) appeared to exhibit a reduced capacity for carbon sequestration compared to the cyanoHAB only sites. Reduced burial of organic carbon is likely linked directly to consumption and respiration/metabolic breakdown of organic matter from the water column by mussels. Burial of inorganic carbon may be influenced by several factors. First, reduced cyanobacterial biomass may be related to decreased rates of photosynthetically driven calcite production due to

decreased nucleation sites (fewer cells in the water column). Second, increased uptake of calcium by mussels for shell formation may lead to less calcium available for calcite precipitation. Finally, increased growth of mussels may lead to increased production of CO_2 in the water column (due to mussel metabolism) generating carbonic acid which will react with calcite crystals in the water column to dissolve it.

Fig. 9 Conceptual model for the flow of carbon through lakes/reservoirs in western Ohio and eastern Indiana, USA. *CyanoHABs* eutrophication-induced cyanobacterial blooms, *DIC* dissolved inorganic carbon, *GW* groundwater. Water column shading indicates density of suspended biomass, with darker shading representing higher density. Arrow widths represent qualitative differences between representative models. Lake/reservoir cross sections are not to scale. **A** Carbon flow through lakes/reservoirs that do not experience *cyanoHABs* and do not have invasive mussel species present. **B** Carbon flow through lakes/reservoirs that experience *cyanoHABs*, but do not have invasive mussel species present. **C** Carbon flow through lakes/reservoirs that experience *cyanoHABs* and have invasive mussel species present

Limitations of this study

The presence or absence of invasive mussel species was not correlative to the composition of cyanobacteria in *cyanoHABs* in our single time point samples; however, we acknowledge our samples do not capture temporal or seasonal variability in bloom community structure. The treatment of invasive mussel species was simplified to yes/no, assuming presence equates complete colonization of the water body in question and negating potential influence of relative mussel population. We also assume mussels contribute to heterotrophic activity in the ecosystems where there are present. In addition, our sample sites—13 sites across lakes, rivers, and reservoirs—vary in numerous ways including depth, volume, residence time, internal and external nutrient loading, the duration, frequency, and severity of *cyanoHABs*, food web structure, mussel density, and invasion history. Finally, our model simplifies carbon cycling (we ignore retention time, sedimentation rates, morphometry, catchment sizes, etc.). Indeed, the combination of environmental stressors including, invasive mussels, that lead to *cyanoHAB* proliferation and altered carbon cycling and burial require further testing with a more comprehensive treatment of lakes and reservoirs.

Conclusions and outlook

Analyses of physicochemistry and community composition of eutrophic lake/reservoir and river sites in western Ohio, eastern Indiana, and northern Kentucky indicate that invasive mussel species do not necessarily translate into large differences in microbial community composition during *cyanoHABs*. However, we did observe large changes in the carbon stable isotope compositions of water and sediment carbon pools between systems impacted by *cyanoHABs* versus both *cyanoHABs* and invasive mussels. From our assessment, we predict a decrease in carbon sequestration potential with increasing occurrence of invasive mussel species in eutrophic ecosystems. Future studies including deep sediment cores could constrain the relationship between surface sediment burial to deeper burial and sequestration of C in these systems as they progress from natural to anthropogenically influenced/eutrophic and/or colonized by invasive mussel species. There is also a paucity of data on the abundance

of invasive mussels, both spatially and temporally, in impacted water. Our 16S rRNA amplicon studies bolster other studies that suggest complex trophic level interactions in the water column that may affect bloom severity and longevity based on competition for N and P. The effects of these interactions on bloom toxicity are largely uncharacterized. In summary, our data highlight potential long-term effects of eutrophication and in particular, harmful cyanobacterial blooms and invasive mussels, on carbon burial which warrants further study.

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