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# Tile Drainage and Anthropogenic Land Use Contribute to Harmful Algal Blooms and Microbiota Shifts in Inland Water Bodies

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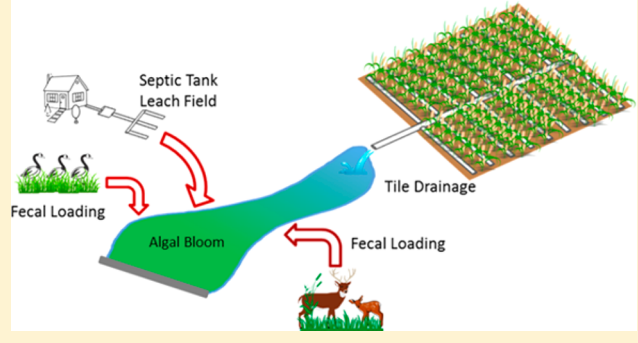
Supporting Information

**ABSTRACT:** Freshwater harmful algal blooms (HABs), driven by nutrient inputs from anthropogenic sources, pose unique risks to human and ecological health worldwide. A major nutrient contributor is agricultural land use, specifically tile drainage discharge. Small lakes and ponds are at elevated risk for HAB appearance, as they are uniquely sensitive to nutrient input. HABs introduce exposure risk to microcystin (MC), hepatotoxic and potentially carcinogenic cyanotoxins. To investigate the impact of anthropogenic land use on small lakes and ponds, 24 sites in central Ohio were sampled over a 3-month period in late summer of 2015. MC concentration, microbial community structure, and water chemistry were analyzed. Land use intensity, including tile drainage systems, was the driver of clustering in principle component analysis, ultimately contributing to nutrient deposition, a driver of HABs. Relative abundance of HAB-forming genera was correlated with elevated concentrations of nitrate and soluble reactive phosphate. One location (FC) showed MC concentrations exceeding 875 μg/L and large community shifts in ciliates (Oligohymenophorea) associated with hypoxic conditions. The prokaryotic community at FC was dominated by *Planktothrix* sp. These results demonstrate the impact of HABs in small lakes and ponds, and that prevailing issues extend beyond cyanotoxins, such as cascading impacts on other trophic levels.




## INTRODUCTION

Emerging issues related to worldwide occurrences of harmful algal blooms (HABs), which produce negative impacts on ecosystem and human health, have garnered much attention from the scientific community.[1] Eutrophication is the primary cause of HABs and is often attributed to nutrient runoff from anthropogenic sources.[2,3] Other factors such as sunlight, water temperature, and turbidity also impact HAB occurrence.[4,5]

Freshwater HABs are products of extreme proliferation of cyanobacteria, with *Microcystis* and *Planktothrix* genera being the most common in the Midwestern United States.[6] The dominant species of cyanobacteria and toxin production during HABs are determined by factors including community characteristics, seasonality, and nutrient abundance.[3,7]

HAB production of cyanotoxins has previously been linked with phosphorus and nitrogen concentrations, with nitrogen being the primary driver of toxin production.[8] Of the various cyanotoxins (microcystin, anatoxin-*a*, saxitoxin, etc.) reported in the Midwestern U.S., microcystins (MCs) are most prevalent.[9] Human exposure to MCs has been linked to symptoms ranging from skin and eye irritation, carcinogenicity, liver failure, and death in extreme circumstances.[9−13] Over 100 naturally occurring congeners of MC have been identified, each possessing unique chemical structures, dictating toxicity.[14,15] Of these congeners, MC-LR is the most common and toxic, and can persist in the environment for months at a time.[6,16]

Outcome severity and potential of exposure to cyanotoxins have been topics of many publications.[17,18] However, HABs have been least studied in small lakes and ponds, which are often overlooked by monitoring efforts due to their high numbers and sizes.[19] Currently the Ohio EPA Inland Lake Program monitors 16 lakes per year, well below the state's total lake count.[20] There are an estimated 304 million natural lakes in the world, covering 4.2 million km$^2$, while an estimated 3.2 × 10$^9$ lakes and ponds less than 1 ha in size cover an estimated 0.8 billion km$^2$.[19] Small lakes and ponds are important providers of ecosystem services such as biodiversity support,

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hosting more species per unit of area than large lakes.[19,21] Further, small lakes and ponds have irrigational and recreational uses and are often contacted by human and animals directly. Agricultural ponds comprise a large area of land worldwide, covering 77 000 $km^2$ and are increasing with growing food demand.[19]

As HABs seem to be more severe in low volume water bodies, small lakes and ponds may be more sensitive to nutrient influx from agricultural sources.[22] One major nutrient contributor is tile drainage effluent.[23] Due to decreased opportunity for nutrient adsorption into soils, subsurface transport of water from agriculture via tile drainage results in a rapid nutrient transport mechanism. This is most problematic during heavy-rains and snow melts.[24,25] Tile drainage has been shown to displace 80% more phosphorus and 43% more nitrogen in subsurface drained systems compared to managed systems, with concentrations varying with precipitation and soil composition.[23,24,26] This quality has lead us to identify tile drainage as a factor of interest for this study.[27]

In addition to tile drainage, nutrient runoff into watersheds occurs from nonpoint sources.[28] Nonpoint source runoff acts as a transport system for fecal matter, and may contribute to nutrient loading in watersheds.[29−31] Runoff can also transport enteric pathogens, including *Cryptosporidium*, *Campylobacter*, Shiga-toxin producing *E. coli*, and various enteric viruses.[32,33]

For the above reasons, we investigated the microbial communities and water chemistry of 24 small lakes and ponds in central OH. We focused on small water bodies proximate to agricultural lands and those receiving tile drainage effluent. Sites were chosen to represent those likely to be used for agriculture or recreation. Our goal was to determine whether human influence promoted the formation of HABs, and whether MC concentrations pose human health risks in sampled sites. Further, structures of eukaryotic and prokaryotic microbial communities were investigated. Eukaryotic and prokaryotic communities are often impacted by changing oxygen levels during bloom deaths and selective pressures exerted by HABs.[1] Therefore, findings from this study imply consequences to ecosystem and human health in HAB impacted small lake and ponds.

## METHODS

**Sample Collection.** Water samples were collected from 24 public and private small lakes and ponds (size range 0.06−50.5 ha) in Knox County, an agriculturally dense rural county in central Ohio (Figure S1 of the Supporting Information, SI). Water samples were collected at a depth of 0−20 cm using a Nasco Swing Sampler and placed in sterile 710 mL Whirl-Pak stand-up bags. Two bags were filled per site. Each site was sampled twice between June and August 2015. At the time of sample collection, water pH, conductivity, and temperature measurements were recorded using a combined field pH and conductivity meter (Hanna Instruments HI98129). Dissolved oxygen (DO) was measured using a YSI ODO Optical Meter (Yellow Springs Instruments). Water samples were transported on ice and stored at 4°C until filtration (within 24 h of collection).

**Site Characterization.** The presence or absence of septic tanks on properties housing the small lakes and ponds was determined by referencing property records at the Knox Co. Ohio Auditor's office. Land use was determined in the 100 m buffer surrounding each site using ArcGIS (version 10, ESRI, Inc.) and the 2011 National Land Cover Database.[34] We considered agricultural land and developed land use to be human land uses for the purpose of categorizing the land use of each site. We used the proportion of the area of the 100 m buffer around each site to indicate whether they were surrounded by low, medium, or high intensity land use. Sites with 0% to 30% human land use were ranked as low, 31−75% was considered moderate, and 76−100% was considered high intensity land use. If a site was in a low or medium land use setting, but was receiving water directly from drain tiles from crop fields that were more than 100 m distance (thus circumventing the function of the land buffer), then the site was considered to have high human impacts and was put into the high intensity category.

**Water Sample Processing.** Samples were vacuum filtered using sterile Microfilm V Filtration devices (Millipore Sigma) and membrane filters (Isopore TM membrane filter; 0.4 $\mu$m for water samples and 0.22 $\mu$m for DNA samples; HTTP, Millipore). Four subsamples were filtered from each water sample. Between 10 and 100 mL of water was passed through each filter, depending on the microbial concentrations. Filters were folded, placed in 2 mL sterile centrifuge tubes, and stored at −80°C. Samples for determination of MC concentrations were stored frozen in amber glass containers to prevent degradation of MC.

**Water Chemistry.** Each sample (150 mL) was filtered within 24 h of collection using a 0.4 $\mu$m pore size Isopore polycarbonate membrane, stored at −20°C, and was used to analyze water chemistry. Nutrient concentrations, including nitrate ($NO_3$−N), ammonia ($NH_3$−N), and soluble reactive phosphate ($PO_4$−P) were measured colorimetrically using EPA approved Hach (Ames, Iowa) water quality procedures and a DR890 colorimeter.

**Cyanotoxin Measurement.** Concentrations of total MC were determined using the Abraxis microcystins/nodularin (ADDA) ELISA colorimetric immunoassay kit (ABRAXIS Inc. Prod #520011). Samples were tested in duplicate. Sample dilutions were necessary to align toxin concentrations within kit detection limits (0.15 to 5 $\mu$g/L on average). A Molecular Devices SPECTRAmax PLUS 384 (Molecular Devices, Silicon Valley, CA) was used for plate reading purposes. Anatoxin-*a* (ABRAXIS Inc. Prod. #520060) and saxitoxin (ABRAXIS Inc. Prod. #52255B) concentrations were tested in sites with detectable MC levels.

**DNA Extraction.** DNA was extracted from membrane filters using two methods. Cyanobacterial DNA was extracted using a modified xanthogenate-sodium dodecyl sulfate (XS) DNA extraction method, using a modified DNeasy Blood & Tissue Kit (QIAGEN, Cat #69504).[35,36] For microbial community analysis and microbial source tracking, microbial DNA was extracted using the QIAamp DNA Stool Kit (Qiagen, Cat # 51504). Sample extraction was conducted using manufacturer instructions, followed by −80°C storage.

**PCR Inhibition Testing.** A Sketa22 assay was conducted on all samples to screen for the presence of PCR inhibition. The method previously described by Haugland et al. (2005) was used.[37] Samples exhibiting PCR inhibition were diluted and retested. This process was repeated until inhibition was within acceptable levels.

**PCR Detection of *Microcystis aeruginosa* and *Planktothrix* sp.** qPCR analysis was conducted to quantify *Microcystis aeruginosa* concentrations by targeting the PC-IGS gene (total *M. aeruginosa*) and *mcy*E gene (microcystin-producing *M.*


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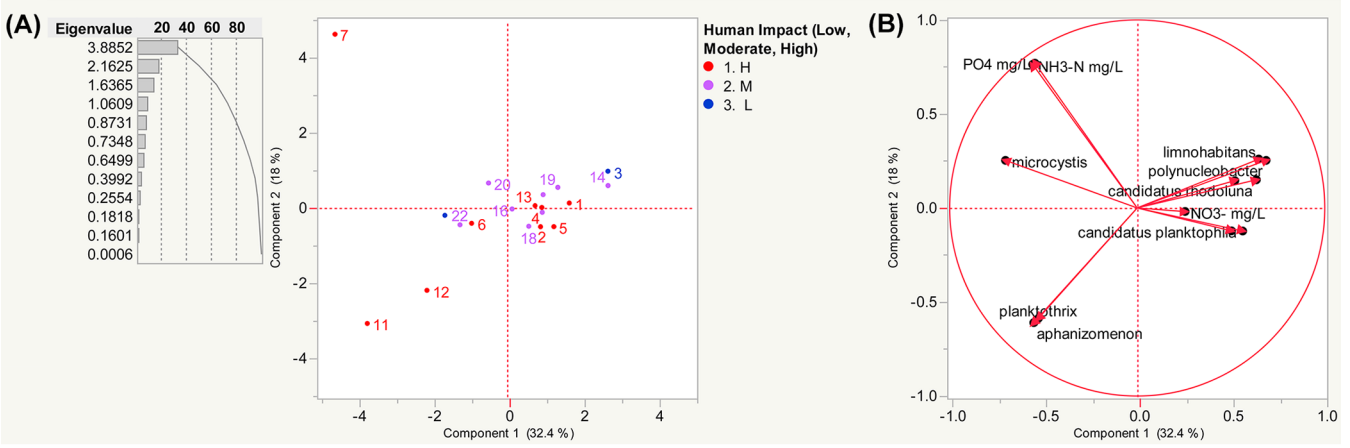


| Number | Location | Number | Location | Number | Location | Number | Location | Number | Location |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BT | 5 | CO | 12 | FC 07-28 Mean | 16 | L2 | 20 | NRN |
| 2 | BT | 6 | FC 06-18 | 13 | F2 | 18 | LE | 21 | SA |
| 3 | BJ | 7 | FC 07-07 | 14 | G2 | 18 | LE | 22 | WR |
| 4 | BD | 11 | FC 07-28 Dam | 15 | KL | 19 | NP | | |

**Figure 1.** Principal component analysis of data sets showing relationships between land use, bacterial communities, and environmental parameters in the sampled locations for the first two principal components, which explain 50.4% of the variability in the data. The first three principal components account for 64% of the variability. (A) Ponds were clustered by human impact, ranging from high to low land use. (B) Loading plot showing the relationship between the variables (vectors) of nutrient concentrations and microbial prevalence used in the PCA; the length of the variable indicates the strength of the correlation. Nutrient concentrations were correlated along the first principal component (*x*-axis) with HAB-forming cyanobacteria of the *Microcystis*, *Planktothrix*, and *Aphanizomenon* genera. Sites 8−11 were indicative of FC transects, therefore not all points were used for PC analysis. Instead a representative sample (#11) of FC on July 28th was used and a mean of the transects was included (#12).

*aeruginosa*). Both assays relied on previously established protocols (Table S2- Table S5).[7,38]

Due to the recent adoption and validation of droplet digital PCR (ddPCR) methods for cyanobacterial gene quantification, concentrations of MC-producing *Planktothrix* were quantified by targeting *mcy*E gene using previously established primers and PCR cycling conditions with a QX200 ddPCR system (Bio-Rad).[39] The ddPCR mixture (20 μL total volume) contained 2X QX200 ddPCR EvaGreen Supermix (Bio-Rad), 200 nM of each primer, DNA template, and RNase-/DNase free water. Droplets were generated using the Droplet Generator (Bio-Rad) with 70 μL of QX200 Droplet Generation Oil for EvaGreen (Bio-Rad) with 20 μL of the ddPCR PCR mixture. PCR was conducted via thermal cycler (C1000 touch thermal cycler, Bio-Rad). After PCR reaction, droplets were read using the Droplet Reader and QuantaSoft software version 1.7 (Bio-Rad).

**Microbial Source Tracking.** Potential sources of fecal bacteria were determined at each site, targeting human (HF183), bovine (Rum2Bac), canine (DG3/DG37), and avian sources (GFD). Protocols used for each assay can be found in the SI section (Tables S3−S5) and rely on previously published literature.[40−43] All MST was conducted using instrumentation identical to the *Microcystis mcy*E assay, apart from DG3/DG37 markers which were analyzed using the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems), in collaboration with Dr. Hodon Ryu (U.S. EPA, Cincinnati, OH).

**Microbial Community Analysis.** Barcoding analysis was conducted by MR DNA (www.mrdnalab.com, MR DNA, Shallowater, TX). For barcoding, the 16S primers were 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). 18S primers were Euk7F (AACCTGGTTGATCCTGCCAGT) and Euk570R (GCTATTGGAGCTGGAATTAC). A modified barcoded amplicon sequencing (bTEFAP) procedure was used. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, U.S.A.). Samples were sequenced using Illumina sequencing platforms according to standard protocols, as described previously.[44] The 16S sequences were obtained with Hiseq 2 × 250 bp, and the 18S sequences were obtained with Miseq 2 × 300 bp.

Q25 sequence data derived from sequencing was processed by MR DNA using a proprietary analysis pipeline. Sequences were depleted of barcodes and primers. Q25 merged sequences were clustered and operational taxonomic units were defined, clustering at 3% divergence (97% similarity), with removal of singleton sequences and chimeras and operational taxonomic units were defined.[45,46] Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes, RDPII (http://rdp.cme.msu.edu), and NCBI (www.ncbi.nlm.nih.gov), and compiled into each taxonomic level into both "counts" and "percentage" files.

**Statistical Analysis.** One-way analysis of variance (ANOVA) was used to evaluate site characteristics based on land use. Principal Components Analysis (PCA) was used to reduce data and investigate patterns of bacterial species distribution as a function of water quality and land use using the statistical package JMP 12 (SAS Institute, Inc.).

## RESULTS

**Analysis of Factors Linked to HAB-friendly Conditions.** Data were analyzed using PCA analysis to investigate



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**Table 1.** *Microcystis aeruginosa* and Cyanotoxin Concentrations of FC Samples (Jun−Aug 2015)

| date | PC-IGS (gene copy/mL) | *mcy*E (gene copy/mL) | MC ($\mu$g/L) | anatoxin-*a* ($\mu$g/L) | saxitoxin ($\mu$g/L) |
|---|---|---|---|---|---|
| June 18th | $8.00 \times 10^2$ | $5.09 \times 10^1$ | 0.17 | 0.07 | 0.01 |
| July 7th | $4.20 \times 10^5$ | $2.64 \times 10^2$ | 876.64 | 4.35 | 0.34 |
| July 28th | $2.55 \times 10^4$ | $1.15 \times 10^2$ | 1.24 | 0.06 | 0.01 |

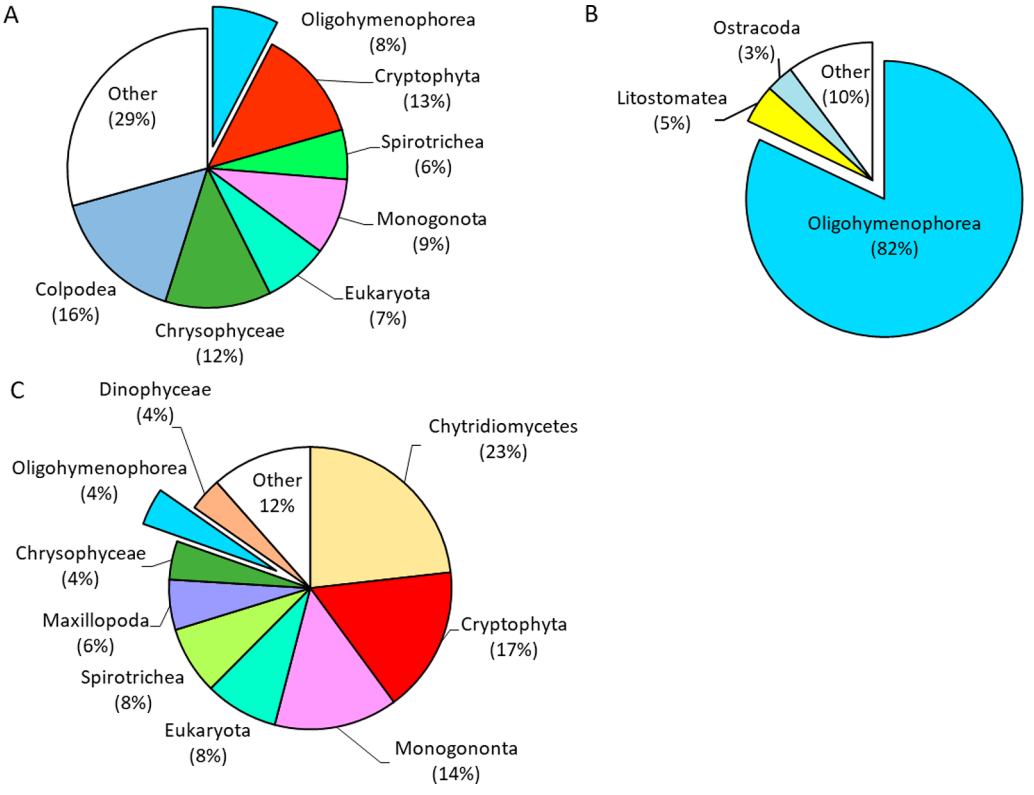


**Figure 2.** Taxonomic composition of the Eukaryotic microbial community in FC at three time-points was shown to vary drastically. Part A corresponds to the June 18th, part B corresponds to July 7th, and part C corresponds to a composite of the July 28th sampling date. The largest changes were seen in the Eukaryotic class Oligohymenophorea, a class of ciliated organisms.

the relationship between nutrient content and bacterial community structure in the analyzed samples (Figure 1). Results show that sites were clustered by level of anthropogenic impact into 3 categories: low (blue), moderate (gray), and high (red) anthropogenic land use. The first two principal components explain 50.4% of the variability in the data, while the first three explain 64%. Three cyanobacterial genera *Microcystis*, *Planktothrix*, and *Aphanizomenon*, were correlated with nutrient concentrations in the sampled sites. These results are in line with past findings which have shown nitrate and phosphorus to be drivers of HABs.[47]

**HAB Conditions, Toxin Concentrations, and Microbial Communities in FC.** No unique trend was observed between *M. aeruginosa mcy*E, PC-IGS, and MC concentrations in the samples gathered (Figure S4). Similarly, concentrations of *Planktothrix mcy*E gene were relatively consistent throughout the sampling sites (Figure S5). While MC content of all samples was below the Ohio guideline limit for MC-LR in recreational waters (4 $\mu$g/L), we found detectable levels of MC in 42% of samples (Figure S1), and one location (FC) which showed a maximum MC concentration of 876 $\mu$g/L on July 7th (Table 1).[48] Anatoxin-*a* (4.35 $\mu$g/L) and saxitoxin (0.34 $\mu$g/L) levels also peaked on this date. All FC samples had detectable MC levels.

**Water Chemistry.** Nutrient concentrations were highest on June 18th at the FC location. During this sampling period, concentrations of overall phosphorus (21.7 mg/L) and nitrate (30.33 mg/L) were 10-fold higher than the cumulative sampling mean. On the basis of phosphate and nitrate concentrations, FC could be classified as mesotrophic and eutrophic, respectively (Table S1).[49]

The FC location was of great interest as it received direct tile drainage effluent. On July 7th, FC experienced a spike in levels of total *Microcystis* and MC (876 $\mu$g/L) toxin levels which exceeded recreational water guidelines.[48]

**Microbial Source Tracking.** Human-associated marker (HF183) was detected in nearly all samples (Mean: 3.9 ± 1.3 log[gene/mL]; Range: 1.1−6.8 log[gene/mL]) (Figure S2). This may be attributed to use of septic tanks in the area. Goose-specific marker (GFD) was also high (mean: 3.4 ± 1.5 log[gene/mL]; range: 0−5.8 log[gene/mL]), which is consistent with our observations (geese presence and droppings) during sampling, as several sites were surrounded by grassy areas ideal for grazing by geese. While deer and dogs were sighted during sampling, canine markers (DG3/DG37) were not detected in any sample, and the ruminant marker (Rum2Bac) was detected in only one sample.

**Microbial Community Analysis.** The eukaryotic community of FC experienced substantial shifts during individual


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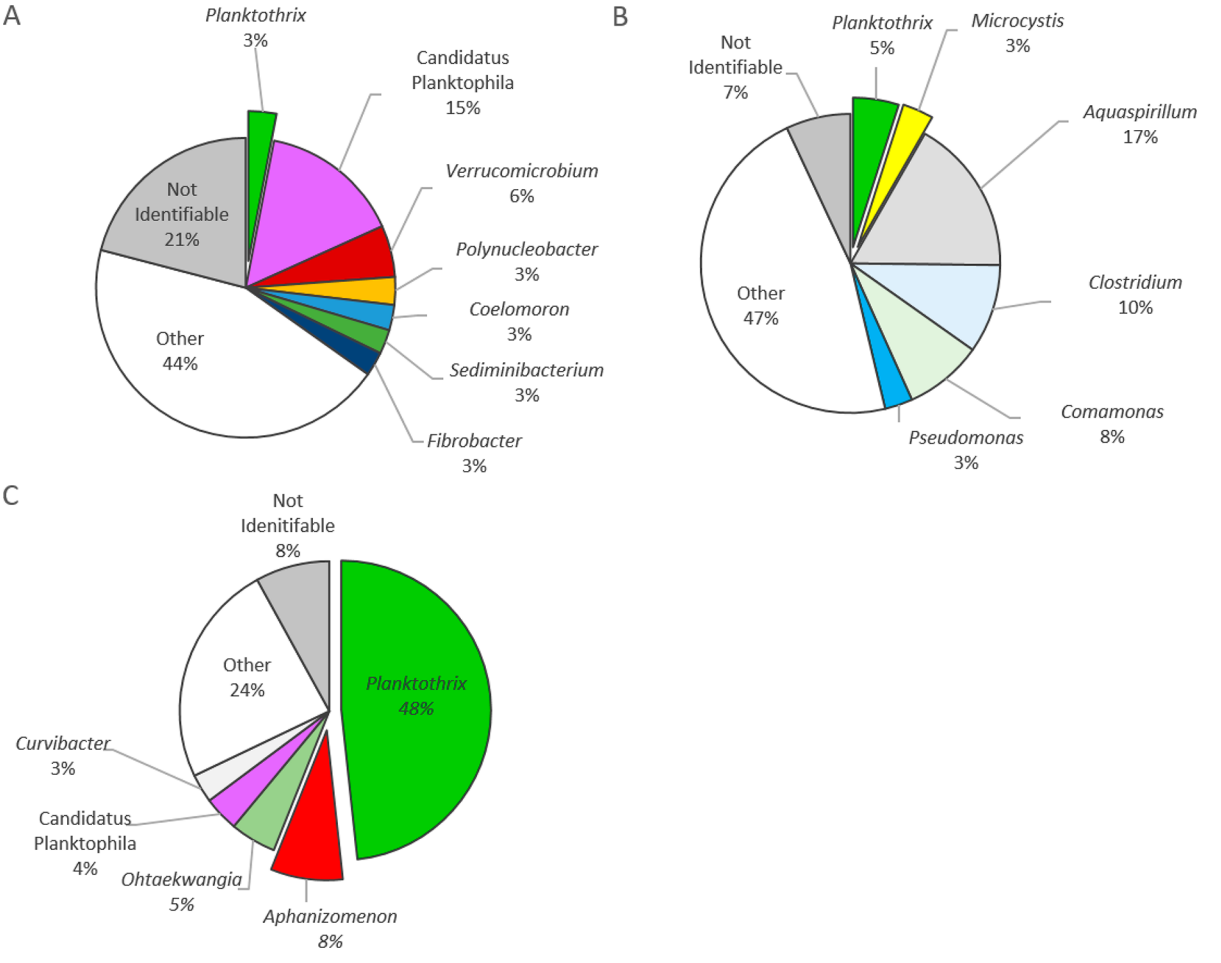


**Figure 3.** Prokaryotic microbial community structure of FC during the sampling period, as determined by 16S and 18S Barcoding analysis. (A) June 18, 2015; (B) July 7, 2015; and (C) composite result from the four transect samples on July 28, 2015.

sampling periods. During the June 18th sampling date (Figure 2A), the eukaryotic community was made up of various eukaryotic classes, with the highest proportion being Colpodea (16%) and Chrysophyceae (12%). A substantial shift in the eukaryotic community occurred on July 7th, as Oligohymenophorea (82%) became dominant (Figure 2B). July 28th saw a diminished proportion of Oligohymenophorea (4%), but the appearance and dominance of new classes such as Chytridiomycetes (23%) and Cryptophyta (17%) (Figure 2C).

The prokaryotic community composition also fluctuated, although to a lesser extent. June 18th, the notable cyanobacterial genus was *Planktothrix* (Figure 3A). On July 7th, a similar percentage of *Planktothrix* (5%) was noted, and was accompanied by *Microcystis* (3%) (Figure 3B). At this point, the community shifted from one dominated by *Candidatus* Planktophilla to one dominated by *Aquaspirillum* (18%), *Clostridium* (10%), and *Comamona* (9%). On July 28th, the Prokaryotic community was dominated by *Planktothrix* (52%) and *Aphanizomenon* (8%) (Figure 3C). A change in community structure was seen over time.

FC, which showed high levels of MC, anatoxin-*a*, and saxitoxin, (Table 1) was chosen for further examination. Four transects of FC were sampled on July 28, 2015, and the distribution of MC and microbial community were investigated (Figure 4). The highest concentration of MC (3.95 μg/L) was observed near the dammed portion of the lake. Microbial community analysis showed that the Eukaryota were dominated by Cryptophyta near FC's inlet (38.7%) and dammed portion (21.2%), while Chrysophyceae (36.7%, 26.9%) dominated the middle portions. The prokaryotic community was dominated by *Planktothrix* throughout all four transects, which accounted for 36.0% to 57.7% of the Prokaryotic community. The dammed portion of the lake also contained a significant community of *Aphanizomenon* (22%).

## DISCUSSION

The objective of the study was to evaluate the influence of anthropogenic activity on HAB-related water quality of small water bodies. These lakes are overlooked by state and local agencies in terms of water quality monitoring, yet are some of the most numerous bodies of water worldwide, providing vital services to humans and ecosystems.[19,20] Our study found that 42% of sampled lakes had detectable levels of MC.

Our study design also tested multiple variables linked to HABs and water quality in 24 unique sites. First, sources of fecal contamination in the sites were analyzed using MST methodologies. Results show that intrusion of fecal bacteria originate from two main contributors: avian, specifically geese, and human sources. This trend holds true to on-site observations, where geese were often seen grazing near the sampled sites. Further, in Ohio, over 250 000 septic tanks discharge waste without meeting water quality requirements and are a likely source of human fecal contamination.[50] Fecal bacteria associated with canine source were not detected, while


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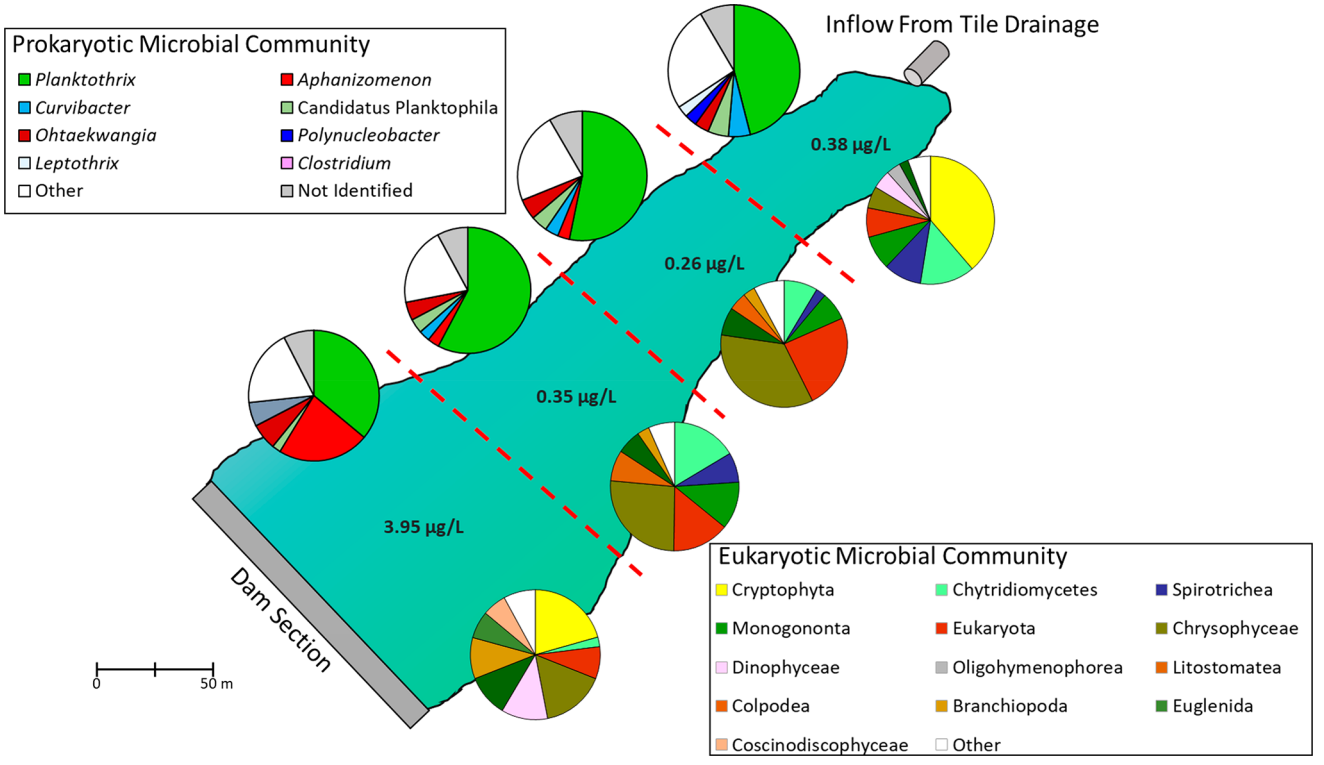


**Figure 4.** MC concentration and microbial communities of FC by transect. Samples are representative of the total microbial community in each transect, as determined by 16S/18S sequencing. The Prokaryotic community was largely dominated by the cyanobacterial genus *Planktothrix*, while the dammed transect of the lake also included a large proportion of *Aphanizomenon* (22.7%).

one site (BD) showed presence of ruminant-specific fecal bacteria. Intrusions of such fecal wastes were previously shown to increase nitrogen loads in Lake Erie beaches, leading to increased concentrations of MC synthase genes.[30] Although incomplete, our data show seven sites with septic tanks in use, and five without.

Water chemistry data (Table S1), showed that lakes impacted by tile drainage or animal presence had higher nutrient content than nonimpacted lakes. Results suggest that areas with high nutrient runoff are more likely to experience HAB development. This finding goes in line with past studies citing tile drainage, and animal wastes as sources of nutrient loading.[25,27,51]

To better understand the influence of anthropogenic impacts on water quality, a cluster analysis was conducted. Sites were clustered based on human land use: the most developed land was classified as "high" and the least developed was termed "low". Our results show that human land use impacts concentrations of HAB-driving nutrients: ammonia, nitrate, and phosphate (Table S1).[47] This relationship was also seen within the small lakes and ponds' cyanobacterial community structure, as nutrient content was correlated with higher proportions of cyanobacterial cells within the small lakes and ponds (Figure 1). The first three components of the PCA explain 64% of the variability in the data, and indicate that the concentrations of ammonia and phosphate were most important in explaining species composition.

These results indicate that anthropogenic influence increases the deposition of nutrients in small lakes and ponds, shifting microbial community structures and establishing conditions more favorable to cyanobacteria (i.e., *Planktothrix*, *Microcystis*, and *Aphanizomenon*). These shifts are exacerbated by the deposition of nutrient-rich waters near dams, a trend also noted by Pearce et al. (2017).[52] Nutrient-rich conditions drive cyanobacterial proliferation and MC production, shifting small lake and pond microbial communities and allowing cyanobacteria to dominate.[8,52]

In all, 10 of the 24 sampled sites showed detectable levels of MC (Figure S1). While MC concentrations currently do not seem to be of concern in all sampled sites, it is important to note that repeated sampling was limited and that further study should use more robust longitudinal sampling. Among the sampled sites, only one site showed total MC concentrations exceeding Ohio EPA guidelines on two of three sampling dates: FC. Therefore, FC was studied more closely for the duration of the sampling period.

While other small lakes and ponds contained tile drainage systems in the studied buffer area, FC was the only one to receive direct tile drainage effluent from nearby agricultural fields. Surrounding FC is an area with large trees and dense vegetation, minimizing surface runoff intrusion. Therefore, FC is a unique system which can be used as a model to study direct impacts of tile drainage on small water bodies. This type of relationship is not readily seen in larger watershed studies as they often involve large areas with high volumes of surface runoff and confounders such as industrial and wastewater effluent.[53] While several studies have linked the influence of tile drainage to the elevated nutrient concentrations in large water bodies, a direct link between tile drainage and HABs is difficult to establish.[24,25,54] Further, the impact of tile drainage may be elevated in small lakes and ponds, as highly concentrated nutrients are introduced into a smaller volume of water, making small lakes and ponds more sensitive to nutrient influx.

FC was the site noted as having the largest blooms among those sampled, and had detectable MC levels at every sampling


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point. The maximal MC level reached 876 μg/L MC in early July, exceeding the State of Ohio recreational water guideline of 4 μg/L for MC, while saxitoxin and anatoxin-*a* were also detected (Table 1).[48] It is important to note that MC concentrations determined by ELISA do not account for MC congeners and are more vulnerable to concentration variations due to the need for dilutions in samples over 5 μg/L total MC. Further analysis should utilize more precise methods, such as LC-MS/MS, which are able to identify MC congeners individually and are more accurate at varying MC concentrations. Analysis reveals that the presence of toxin producing *Microcystis* genes was relatively low during this event, indicating that the cyanobacteria responsible for MC production is most likely outside of the *Microcystis* genus (Figure S3). In addition, MC synthesis gene abundance does not necessarily represent the relative expression of the targeted genes, a relationship which should be explored in the future. Ingestion of water with such MC concentrations could cause human and animal illness, and be especially worrisome in seldom monitored sites.[55] Further, elevated nitrate levels (30.33 mg/L), were detected on June 18th, and were likely responsible for elevated MC concentrations on July seventh.[8]

Due to the unique hydrodynamic nature, nutrient influx source, and high level of toxin detected in FC, the July 28th sampling was separated into 4 transects. Levels of MC were lowest near the tile drainage inlet, and highest, near the constructed dam (Figure 4). The nature of this MC concentration distribution is most likely due to water flow created by the inlet and the stagnation of water created by the dam. On July 7th, the cyanobacterial community of FC was dominated by *Planktothrix*, with a lesser, yet substantial *Microcystis* community. A shift in the community was seen on July 28th, as the community was largely dominated by *Planktothrix* (Figure 3).

Results show nutrient content of FC on June 18th was 10-fold greater than the cumulative sampling mean (Table S1), reaching mesotrophic levels of phosphorus (21.7 mg/L) and eutrophic levels of nitrogen (30.33 mg/L). Such nutrient concentrations indicate that bloom-friendly conditions were highest in June, but returned to low concentrations during July 7th. It is likely that nutrient concentrations were depleted by cyanobacteria, and dissipated following bloom death. Lysis of cyanobacteria has been shown to release intracellular MC into waters producing high MC concentrations, such as those seen in early July, which would be accompanied by relatively low cyanobacterial DNA concentrations.[56] Further, the emergence of *Planktothrix* as the dominant Prokaryotic genus on July 28th could be attributed to competitive pressures exerted by cyanobacteria, establishing favorable conditions after dissipation of the July 7th bloom.

We noted a sizable shift from the initial (June 18th) proportion of the eukaryote Oligohymenophorea (8%) to 82% during the suspected bloom (July 7th) (Figure 2). On July 28th, proportions of Oligohymenophorea reverted to June 18th levels, hinting that selective pressure from bloom-forming cyanobacteria may have influenced the prokaryotic community. Oligohymenophorea have previously been linked with hypoxic conditions in freshwater environments.[57] While we did not directly measure oxygen concentration in sites, based on the evidence presented, we hypothesize that Oligohymenophorea, a class of ciliated, predatory, eukaryotes are migrating to sites of algal blooms, to prey upon HAB associated microbes and take advantage of potentially hypoxic conditions.[58] This finding should be further investigated, as HAB-driven shifts in microbial community structure, particularly in eukaryotic species, are poorly understood and could have cascading impacts on small lake and pond food chains.

Our findings indicate that HAB conditions in FC are of potential concern. Due to the site's status as a popular lake for fishing and shoreline gatherings, we recognize the possibility of human and animal exposure exists. If MC levels seen during the July 7th sampling date occur in the future, without a proper monitoring system, exposures could result in human and animal illness. Further studies should focus on microbial community shifts seen during HABs and monitoring methods for small lakes and ponds used for recreation, irrigation, or drinking water.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03269.

Map of sampling locations (S1), results of microbial source tracking (S2), quantification of *M. aeruginosa* genes in FC (S3), quantification of MC concentration and *M. aeruginosa* genes across all sites analyses (S4), and A water chemistry table (Table S1) (PDF)

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### Notes

The authors declare no competing financial interest.

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