M.L. 2018 Project Abstract
For the Period Ending June 30, 2022

PROJECT TITLE: Determining Risk of Toxic Alga in Minnesota Lakes
PROJECT MANAGER: Adam J. Heathcote
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

APPROPRIATION AMOUNT: $200,000
AMOUNT SPENT: $197,099
AMOUNT REMAINING: $2,901

Sound bite of Project Outcomes and Results

This project produced the first systematic survey of Minnesota's Sentinel Lakes for the toxic invasive algae *Cylindrospermopsis raciborskii* (Cylindro). Cylindro was contained to the 2 lakes where it was initially found and did not produce toxins in measurable amounts. Sediment records indicated that Cylindro has appeared in the last 10 years and has not spread statewide.

Overall Project Outcome and Results

Cylindro is a subtropical invasive species of Cyanobacteria that has been invading lakes in the Upper Midwest since the early 2000s. Cylindro is of particular concern because it is known to produce a potent liver toxin and the presence of its blooms can be difficult to identify. Cylindro was first discovered in two Minnesota lakes in 2013 and that discovery led to the design of this statewide survey of the Minnesota Sentinel Lakes to better understand the spread and invasion history of this species. Our monitoring results, based on DNA and microscopy, show that Cylindro is currently limited to the 2 lakes where it was initially found, and sediment cores indicate that it has been present in those lakes for <10 years. Additionally, even in lakes where Cylindro was present, there was no evidence of toxin production in detectable amounts, minimizing the public and wildlife health threat of this species. Thankfully, these results suggest that the threat of Cylindro invasion in Minnesota lakes is currently low, though continued monitoring for this species is important given the trend of warming lake waters across the state.

Project Results Use and Dissemination

We have shared the progress and results from this project widely over the duration of the project. This includes both articles written by our staff, shared with traditional and social media, and peer reviewed papers. These efforts are summarized below in chronological order:

- "Invisible" species of exotic algae threatens to poison Minnesota lakes, posted to SMM.org on 11/6/2018, (PDF attached in supplemental materials)
- "Conditions ripe for a record number of algae blooms", Minnesota Public Radio Climate Cast segment on 7/19/2019,

- “Why good algae go bad”, talk at the Marine Community Library by Adam Heathcote on 7/14/2019, picture of event on Twitter
- Adam Heathcote Co-chaired special session on Harmful Algal Blooms at the Minnesota Water Conference and organized a panel of experts to take questions from conference attendees in St. Paul, MN on 10/16/2019
- During the pandemic we provided information on harmful algal blooms for the public on the Science Museum website in our “Learn From Home” section, posted on 7/7/2020.
- Preliminary results from this study were presented to the Minnesota Inter-agency HABs group at their Winter Workshop on 1/25/2021. Attendees includes representatives from the Minnesota DNR, MPCA, MDH and the MVMA
- Results from this study were published in the peer-reviewed journal PLOS ONE on 3/21/2022. PDF of paper is attached in the supplemental materials.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2018 ENRTF Work Plan Final Report (Main Document)

Today's Date: August 15, 2022

Final Report

Date of Work Plan Approval: 06/05/2018

Project Completion Date: June 30, 2022

PROJECT TITLE: Determining Risk of Toxic Alga in Minnesota Lakes

Project Manager:	Adam J. Heathcote
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Location: Statewide

Total Project Budget: $200,000

Amount Spent: $197,099

Balance: $2,901

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

Appropriation Language: $200,000 the second year is from the trust fund to the Science Museum of Minnesota for the St. Croix Watershed Research Station to determine the historical distribution, abundance, and toxicity of the invasive blue-green alga, Cylindrospermopsis raciborskii, in about 20 lakes across Minnesota and inform managers and the public about the alga's spread and health risks. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

I. PROJECT STATEMENT:

Invasive microbes are easily spread, but difficult to detect, control, or reliably assess for their environmental and public health risk. Cyanobacteria (blue-green algae) are one of the most abundant and obvious microbes in lakes, and recent work suggests a shift to more toxic forms – including the invasive species, *Cylindrospermopsis raciborskii* (*Cylindro*) – with consequences including dog deaths, human illness, and reduced natural resource value. Minnesota is outside the native range of *Cylindro*, but its arrival has recently been confirmed by ENRTF supported surveys carried out by the St. Croix Watershed Research Station (SCWRS) and Minnesota Pollution Control Agency (MPCS). It is likely that recent years with warmer summer temperatures and increased nutrient pollution have provided a new niche for this species to invade.

There is little information on *Cylindro* in Minnesota and no data on the presence of toxins it produces. This information is particularly important to the State of Minnesota, because unlike other Cyanobacteria, *Cylindro* may bloom several feet below the lake surface, making it difficult to visually assess the quality and safety of waters where it is present. This project will be the first systematic survey of Minnesota for the occurrence of *Cylindro* and its cyanotoxins in Minnesota lakes – in both the water and bottom sediments. This study will determine present-day distribution and toxicity of *Cylindro*, its historic introduction and spread across the state, and develop predictive models for bloom occurrence, seasonality, toxicity, and invasion risk. These data are the critical first step in understanding and addressing the spread of any invasive species.

This project leverages current ENRTF funding for harmful algal bloom (HABs) research on the Sentinel Lakes that were selected by the DNR as a representative sample of Minnesota's lakes. It would provide an additional year of monitoring for HABs on 20 of the Sentinel Lakes during the peak bloom season and allow us to determine the historical presence of *Cylindro* through the occurrence of its toxins in those same lakes using sediment cores.

II. OVERALL PROJECT STATUS UPDATES:

First Update January 31, 2019

We surveyed all 20 target lakes in August and September of 2018, collecting water quality and algae samples described in Activity 1. Water quality samples are currently being analyzed in the SCWRS laboratory and will be completed before the beginning of the second field season in May 2019. In addition to the survey, sediment core samples were collected on 10 of the 20 lakes for Activity 2. These sediment cores have been sectioned and are currently undergoing 210-Pb dating analyses in the SCWRS laboratory. Finally, in concert with the beginning of this study, we published a new story and infographic on the toxic algae, *Cylindrospermopsis raciborskii*, which was shared through our website and social media outlets. The story can be found here: https://www.smm.org/scwrs/fieldnotes/invisible-species-exotic-algae-threatens-poison-minnesota-lakes

Second Update June 30, 2019

All water quality samples collected as part of Activity 1 in 2018 have been analyzed in the laboratory. We have also collected an additional 5 sediment cores for Activity 2 (5 remain to be collected). All new sediment cores have been sectioned and we continue to work on 210-Pb dating and other sediment analyses in the laboratory. We continue to share our work with the public, including mention of Cylindro on Minnesota Public Radio's Climate Cast (archived here: https://www.mprnews.org/episode/2019/07/19/conditions-ripe-for-a-record-number-of-algae-blooms).

Third Update January 31, 2020

All sediment cores have been collected and are in the process of being analyzed in the SCWRS laboratory. Currently we have dated and completed geochemistry (loss-on-ignition) on 5 of 10 cores and the remainder are being processed. We have begun perfecting our sediment toxin analysis for microcystin and cylindrospermopsin and have completed sediment toxin analyses on one of the ten cores. We presented information from this study as well as previous LCCMR-funded work to the public at the 2019 Minnesota Water Conference as part of a special session on Harmful Algal Blooms.

The final year and a half of this study will focus on completing all of the sediment toxin analyses and then synthesizing the results of both the water quality (Activity 1) and historical record (sediment cores; Activity 2) of Cylindro in Minnesota.

Fourth Update June 30, 2020

We have completed geochemistry (loss-on-ignition) and 210-Pb dating on all newly collected cores from this study and have used previous dating models to interpolate 210-Pb dates for sediment cores from lakes that were collected for previous ENRTF-funded research. This has produced dating models for sediment cores from all 20 Sentinel Lakes sampled as part of this process.

We planned to be well into our sediment toxin extractions at this point, however, due to the unforeseen COVID-19 pandemic, most of the Science Museum of Minnesota, including all scientific and technical staff at the SCWRS were temporarily laid off from the beginning of April through most of June. On July 27, the SCWRS laboratory began re-opening and is currently assessing the backlog of analytical work that accumulated over the shutdown. We are hopeful that this project can still be completed within its original timeline, as the bulk of the labwork had already been completed before the shutdown occurred.

Fifth Update January 31, 2021

We have primarily been working on data analysis of the water chemistry and phytoplankton samples which were collected as part of Activity 1 for this project, including preparing preliminary data for a presentation to the Minnesota Inter-Agency Panel on Harmful Algal Blooms on January 25th, 2021. Additionally, we have been working with our analytical chemistry laboratory to continue developing the sediment toxin extraction technique from our previously collected and dated sediment cores. We are still hoping to complete this work during the original project timeline, however, due to the setback of our laboratory being closed for most of the spring and summer of 2020 we requested a 1-year extension for this project in order to ensure we have enough time to complete and synthesize these results.

Project extended to June 30, 2022 by LCCMR 6/30/21 as a result of M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18, legislative extension criteria being met.

Sixth Update June 30, 2021:

We are grateful for the extension provided by the legislature to allow us to complete the final analyses on this project. During the last period we have worked primarily on preparing a synthesis of Cyanobacterial sediment DNA collected by the SCWRS' Environmental Research Fellow who was funded as a field and laboratory technician on this project. We have completed a peer-reviewed publication entitled "Diversity and distribution of sediment bacteria across an ecological and trophic gradient", which will be submitted to the journal PLOS ONE this month.

Seventh Update January 31, 2022:

We continue to work on final synthesis of the sediment toxin work as well as production of a peer-reviewed manuscript from the results of this study. On November 3rd we received positive reviews our manuscript from journal PLOS ONE and were asked to address the reviewer comments and resubmit. We revised the manuscript accordingly and resubmitted it on December 16th, 2021. Based on the initial positive response we anticipate this manuscript will be accepted for publication and are awaiting final decision from the editors.

Final Update June 30, 2022

Cylindro is a subtropical invasive species of Cyanobacteria that has been invading lakes in the Upper Midwest since the early 2000s. Cylindro is of particular concern because it is known to produce a potent liver toxin and the presence of its blooms can be difficult to identify. Cylindro was first discovered in two Minnesota lakes in 2013 and that discovery led to the design of this statewide survey of the Minnesota Sentinel Lakes to better understand the spread and invasion history of this species. Our monitoring results, based on DNA and microscopy, show that Cylindro is currently limited to the 2 lakes where it was initially found, and sediment cores indicate that it has been present in those lakes for <10 years. Additionally, even in lakes where Cylindro was present, there was no evidence of toxin production in detectable amounts, minimizing the public and wildlife health threat of this species. Thankfully, these results suggest that the threat of Cylindro invasion in Minnesota lakes is currently low, though continued monitoring for this species is important given the trend of warming lake waters across the state.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Survey Minnesota lakes for *Cylindro* and the conditions associated with its presence

Description: Current HABs monitoring by the St. Croix Watershed Research Station (SCWRS) will be enhanced by an additional year of monitoring on an expanded set of 20 Sentinel Lakes. SCWRS personnel will collect water quality, algae, and cyanotoxin samples during the peak bloom season (August-September) in 2018. Algae samples will be analyzed for the occurrence and abundance of *Cylindro* through standard microscopy techniques, and cyanotoxins will be measured using ELISA enzyme assays. All analyses will occur at the SCWRS CHARM Laboratory (Center for Harmful Algal Research in Minnesota). SCWRS personnel will develop a spatially explicit predictive model for the invasive spread and bloom risk of *Cylindro* for the major Minnesota lake regions using readily available geographical, weather, and water quality data.

ENRTF BUDGET: $72,705

Outcome	Completion Date
1. We will collect phytoplankton and water quality samples from the 20 Sentinel Lakes during peak bloom season to determine the presence and toxicity of *Cylindro*	October 2018
2. We will develop predictive criteria for the invasive spread of *Cylindro* that can be applied to Minnesota lakes based on its occurrence, abundance, and toxin production coincident with lake, water, and weather conditions	June 2021

First Update January 31, 2019

We visited all 20 study lakes in August and September of 2018 to conduct our statewide survey for the toxic algae Cylindro. We collected basic water-quality measurements and water chemistry samples as well as algal community and cyanotoxin samples to assess for the presence of this exotic species across the state. Water quality samples were collected for total and dissolved nitrogen and phosphorus, dissolved inorganic and organic carbon, and chlorophyll *a*. Water quality profiles were measured using our multi-parameter sonde (purchased with previous LCCMR funding) for water temperature, dissolved oxygen, pH, phycocyanin (cyanobacterial pigment), chlorophyll *a*, turbidity, and conductivity. Additionally, we collected a sample of the total algal community as well as samples for the toxin produced by Cylindro (cylindrospermopsin) and a toxin produced by other Cyanobacteria (microcystin).

All water quality samples are being processed by the SCWRS laboratory. All dissolved water chemistry (phosphorus, nitrogen, carbon) have been analyzed and total phosphorus and nitrogen will be completed in the next month. All algal toxin samples have been frozen and will be run in large batches to maximize efficient use of the ELISA kits. Phytoplankton community samples have been preserved and concentrated to be counted at a later date.

Second Update June 30, 2019

We have completed the water quality samples collected in the later summer and early fall of 2018. We have summarized some of the results in Figure 2. Which shows the detection of the cyanotoxins cylindrospermopsin (CYN) and microcystin (MC) across all the lakes in this study.

CYN, which is the toxin produced by Cylindrospermopsin was in very low concentrations or absent in all lakes in this study. This includes lakes with known populations of Cylindro (confirmed via microscope identification), Madison and South Center. These are promising results, which would support the hypothesis that the population of Cylindro in the Great Lakes states is not particularly toxic, but this will need to be confirmed via our sediment toxin analyses (Activity 2). Also, of note, the only lake where CYN concentrations were above our detection limit was Red Sand Lake. Cylindro has not been previously found in this lake, so we will need to check the phytoplankton sample to confirm if it is present. Alternatively, the detected CYN could have been produced by another species of Cyanobacteria.

To compare CYN levels to more commonly produced toxins we also measured MC, a toxin commonly produced by other Cyanobacteria, in all lakes. Unlike CYN, MC was detected in all but three lakes (Greenwood, Trout, White Iron) and always exceeded Minnesota's minimum drinking water standard

(0.1 ug/L). It should be noted that none of the lakes exceeded EPA recommended recreational concentrations (10.0 ug/L), and only St. James even approached that higher limit (8.5 ug/L). These results indicate the presence of toxin-producing Cyanobacteria in even some of the most protected and relatively clean Minnesota lakes (See Figure 2 top panels for total phosphorus and chlorophyll-a concentrations), although concentrations of MC are still not a concern for non-drinking use.

Third Update January 31, 2020
No activity during this period.

Fourth Update June 30, 2020
No activity during this period.

Fifth Update January 31, 2021
No activity during this period.

Sixth Update June 30, 2021:
No activity during this period.

Seventh Update January 31, 2022:
No activity during this period.

Final Update June 30, 2022

Cylindrospermopsis raciborskii (Cylindro) is an exotic species of Cyanobacteria that is native to subtropical lakes in the southeastern United States (USA). It has been documented in a growing number of states in the Midwest USA since the early 2000's and was first documented in two of Minnesota's Sentinel Lakes as part of a routine phytoplankton survey by the Minnesota Pollution Control Agency (MPCA) in 2013. The expansion of this species into temperate lakes has been hypothesized to be due to these systems becoming more hospitable to Cylindro as average summer water temperatures rise in this region.

The expansion of Cylindro in Minnesota is a concern primarily because of its ability to produce the potent hepatotoxin cylindrospermopsin (CYN), which is potentially deadly to both humans and wildlife. Additionally, unlike many other Cyanobacteria species, Cylindro often forms sub-surface blooms which are more difficult to detect and circumvent traditional public health messaging on the safety of lakes for recreation (i.e., "When in doubt, stay out"). An infographic for the public on Cylindro was created as part of this project (Figure 1).

This project represents the first systematic survey for Cylindro in Minnesota using the already established network of the Minnesota Sentinel Lakes which span all of Minnesota's ecoregions and major lake types.

Cylindro, water quality, and cyanotoxin survey of Minnesota

To detect the presence of Cylindro, we surveyed a subset of 20 of Minnesota's Sentinel Lakes in the late summer of 2018 when harmful algae blooms (HABs) are most prevalent. For each lake we visited, we collected a full suite of water chemistry data, including total phosphorus (TP), total nitrogen (TN), chlorophyll *a*, nitrate+nitrite (NOx), ammonia (NH4), soluble reactive phosphorus (SRP), dissolved inorganic carbon (DIC), and dissolved organic carbon (DOC). We also collected samples for the cyanotoxin produced by Cylindro, CYN, as well as a cyanotoxin that is commonly produced by other Cyanobacteria in Minnesota HABs, microcystin (MCY). All samples were collected from an integrated surface sample of the epilimnion (epi) and if the lake was stratified a second sample was collected from the hypolimnion (hypo). The full results from this survey are presented in Table 1.

Phytoplankton samples were collected from each lake to confirm the presence of Cylindro through microscopy, however, we opted to use a new DNA-based approach to collect a more comprehensive sample of the Cyanobacteria present in a lake. For this approach, we sequenced DNA in the surface sediments of each lake, which integrate 1-2 years of phytoplankton and benthic algae rather than a single snapshot in time. This method is also less skewed towards the more easily identifiable large colonial Cyanobacteria and gives a more comprehensive picture of the community. DNA samples were extracted from the surface sediments and sequenced at the University of Minnesota Genomic Center. Figure 5 shows the results from this work and the distribution of Cyanobacteria genera across the lakes in this study. Cylindro DNA was only detected in two samples, Madison Lake and South Center, which already had previously confirmed occurrences. Microscopic analyses of phytoplankton samples also confirmed the presence of Cylindro in these two lakes (Figure 6).

In addition to the presence of Cylindro DNA, our survey gave us a broader understanding of the microbial diversity of the Sentinel Lakes. This includes a significant gradient in both richness (number of species) and diversity (evenness of species) (Figure 3). Somewhat surprisingly, the highest richness and diversity in microorganisms was found in the lakes in the Cornbelt Plains and the lowest diversity in the Canadian Shield. We also characterized the diversity at the phyla level of microbial organisms in surface sediments of all 20 of the Sentinel Lakes, representing the first microbial survey of the Sentinel Lakes. For more details see Sauer *et al*. (2022) in the peer-reviewed journal PLOS ONE, included in the supplementary materials of this report.

Geographic trends in nutrient concentrations (TP), algal productivity (chl-*a*), and cyanotoxins from this survey are shown in Figure 2. The highest concentrations of TP and chl-*a* occurred in the agricultural southern portion of Minnesota (Cornbelt Plains ecoregion) and the metro area surrounding the Twin Cities. CYN, which is the toxin produced by Cylindro, was undetected in the water of all but one lake in this study. CYN was not detected in either of the lakes with known populations of Cylindro (South Center and Madison) but was detected in low concentration in Red Sand Lake. This indicates a relatively low risk of CYN toxicity in Minnesota, due to its absence or extremely low concentration even in lakes where this species is present. Unlike CYN, MCY was detected in all but three lakes (Greenwood, Trout, White Iron) and always exceeded Minnesota's minimum drinking water standard (0.1 ug/L). It should be noted that none of the lakes exceeded EPA recommended recreational concentrations (10.0 ug/L), and only St. James in southwestern Minnesota even approached that higher limit (8.5 ug/L). These results indicate the presence of toxin-producing Cyanobacteria in even some of the most protected and relatively clean Minnesota lakes in the late summer.

Predicting the spread and toxicity of Cylindro

The results of this study indicate that among the Sentinel Lakes, a representative sampling of all Minnesota Lakes, Cylindro is confined to only two lakes. This is good news in that the extent of the Cylindro invasion in Minnesota was completely unknown prior to this study. However, the isolated occurrences make it difficult to draw conclusions about what causes the spread or proliferation among Minnesota lakes in general terms. There are no obvious commonalities between the two lakes, as South Center and Madison, respectively, differ in ecoregion (Central Hardwood Forest and Cornbelt Plains), maximum depth (33 and 10 m), nutrient concentrations (21 and 89 µg/L TP), and trophic state (mesotrophic and eutrophic). Both lakes are popular for angling and recreation, but there is no obvious link between visitors of the two lakes as they are separated by nearly 200 km.

In both lakes where Cylindro was observed, no detectable concentration of the toxin CYN was found. There are two reasons that this may have occurred, 1) Cylindro was not present in high enough abundance to produce detectable toxin, and 2) the population of Cylindro in Minnesota do not possess the toxin-producing gene, as has been observed in other states in the Upper Midwest. Further genetic study of the Cylindro populations in Madison and South Center Lake could help answer this question by determining the presence of the *cyr* gene complex which is responsible for producing CYN. Although preliminary amplicon sequencing was completed as part of this study, a more detailed metagenomic approach would be required to confirm the presence of this and other genes responsible for toxin-production.

ACTIVITY 2: Using sediment cores to reconstruct the invasion history of *Cylindro* in Minnesota

Description: The exact timing and extent of the invasion of *Cylindro* in Minnesota is currently unknown. This activity would provide a statewide distribution and history of *Cylindro* invasion and spread using paleolimnological techniques. Sediment cores would be dated and analyzed for the toxin produced by *Cylindro*, cylindrospermopsin. Based on our sediment core analysis, the invasion history will be compared to known patterns of land-use, eutrophication, and climate and will be used to predict the limitations that dispersal may play in moderating the invasion front.

ENRTF BUDGET: $127,295

Outcome	**Completion Date**
1. We will collect and date sediment cores from the 20 Sentinel lakes and measure the *Cylindro* toxin (cylindrospermopsin) and the general Cyanobacteria toxin (microcystin) to determine when *Cylindro* arrived in Minnesota	February 2020
2. We will compare patterns of introduction and expansion of *Cylindro* to long-term weather data to assess the role of warming lake temperatures on range expansion vs. alternative invasion scenarios (i.e., eutrophication, human transport)	June 2021

First Update January 31, 2019

While conducting our statewide survey in Activity 1, we collected sediment cores from ten of the twenty study lakes (Trout, Greenwood, White Iron, Red Sand, Elk, South Twin, Artichoke, Shaokatan, Portage, Peltier). These cores have been sectioned in 1-2 cm intervals and are currently being analyzed by the SCWRS laboratory. Core samples are being processed for loss-on-ignition and then freeze-dried to begin the 210-Pb dating process. Once dating has been completed, samples can be selected for fossil toxin analyses. The remaining cores will be collected Feb-March (through the ice) or May-Oct (open water) of 2019 to leave ample time for all laboratory analyses to occur.

Second Update June 30, 2019
We collected five additional cores through the ice during Jan-March of 2019. These included Carlos, Cedar, Pearl, South Center, and Hill. We have completed geochemistry analysis on 14 of 15 and are in the process of optimizing our cyanotoxin extraction and measurement techniques for both CYN and MC. Once optimized, SCWRS will be the first laboratory in Minnesota to have demonstrated the capacity to accurately measure cyanotoxins in lake sediments. The remaining five sediment cores will be collected during the 2019 open-water season.

Third Update January 31, 2020

We have focused on completing 210-Pb dating and geochemistry on the ten new cores collected as part of this activity. We have completed this work on 5 of the lakes (Carrie, Belle, Hill, St. Olaf, Artichoke) and the remaining five lake cores are currently being worked on by our 210-Pb laboratory. We have processed the new cores collected for the ten lakes which were part of the LCCMR-funded Tracking and Preventing Harmful Algal Blooms project and we will apply previously determined 210-Pb dating models to those cores.

We have begun work on extracting and measuring toxins in the sediment of the cores from this study. This is a cutting edge method and we are still working at perfecting and enhancing the efficiency of the method. We have completed our first full core for the cylindrospermopsin toxin (CYN) on Hill lake and found no detections of the toxin within the sediments. We will next test the method on a lake known to contain Cylindrospermopsis (South Center Lake), with detectable toxin concentrations in lake water in previous years, and see if the toxin will also be detected in the sediments.

Fourth Update June 30, 2020

We have completed loss-on-ignition and 210-Pb dating on the remaining 5 new cores for this project (South Twin, White Iron, Greenwood, Peltier, Red Sand, Hill) and are in the process of producing interpolated dating models from the sediment cores that were dated in previous ENRTF-funded research (Portage, Elk, Trout, Shaokatan, South Center, Cedar, Pearl, Carlos, Madison, St. James) using the completed loss-on-ignition profiles from the 10 newly collected cores.

We have not progressed as planned on our cyanotoxin extractions due to the unforeseen shutdown of the Science Museum of Minnesota and SCWRS due to the COVID-19 pandemic as outlined in the overall status update for this period. We continue to work with the expectation that this project can be completed within its original timeline.

Fifth Update January 31, 2021

We continue to work on developing our cyanotoxin extraction method as this is the final analysis left to be completed for this project. We have successfully extracted the cylindrospermopsin toxin from the Hill Lake core and plan to apply this method to the remaining 19 cores in this data set. We continue to develop the microcystin extraction method.

Sixth Update June 30, 2021:
The SCWRS' Environmental Research Fellow, Hailey Saur (now a PhD student at the University of Minnesota) has completed the synthesis of her work on bacterial (including Cyanobacterial algae) DNA

collected in the sediment cores as a part of this project. Over the last 6 months she has worked with Adam Heathcote to complete her manuscript, entitled "Diversity and distribution of sediment bacteria across an ecological and trophic gradient" which sequenced DNA found in the top 2 sections of sediment cores from the MNDNR Sentinel Lakes collected and preserved as a part of this project. This included a survey of richness and diversity of the microbial community in lake sediments (Figure 3) as well as a phyla level survey of all microbial organisms present in the MNDNR Sentinel Lakes (Figure 4). This manuscript will be submitted for review to the peer-revied scientific journal PLOS ONE in September of 2021.

Seventh Update January 31, 2022:

We are working on finishing the final sediment core work required for this project which includes finalizing 210-Pb dating models and completing cylindrospermopsin and microcistin toxin extractions from the sediment cores collected in this study. In November of 2021, we received positive peer reviewer comments from the manuscript that was submitted in the previous update and were encouraged by the editor of the journal PLOS ONE to address these comments and resubmit our publication. After going through a thorough revision process the article was resubmitted in December of 2021. We are still awaiting the final decision of the editor.

Final Update June 30, 2022

Cylindro was first confirmed in Minnesota in 2013, however, there was considerable uncertainty around the timing of the invasion due to very limited phytoplankton surveys in the state and no concerted effort to look for it prior to the initiation of this study. Further, there was at least one earlier report of Cyilndro in Minnesota dating back to 1969, although it was collected by an amateur phycologist and not independently verified. To this end, we designed a paleolimnological survey of a representative sampling of 20 Minnesota lakes, including the only two lakes known to be infested by Cylindro. To look for the presence of Cylindro over the last century, we measured the occurrence of the CYN toxin in sediment, a technique that has been used to confirm the presence of Cylindro for thousands of years in Florida lakes where it is native. By measuring the occurrence of the CYN toxin in dated sediment cores, we aimed to better understand the timing of the invasion in any lakes where Cylindro was confirmed to occur.

Sediment core collection and dating

Sediment cores were collected from all 20 lakes in this study throughout the open-water and ice seasons of 2018 and 2019. Loss-on-ignition, a measure of the geochemical composition (percent organic, percent $CaCO_3$, and percent inorganic) of the sediment, was completed on all cores. New ^{210}Pb dating models were produced for ten of the lakes in this study and the remaining lakes were dated by correlating geochemical sediment markers to cores that were collected from previous MN ENRTF-funded work (M.L. 2016-186-2-04a: Tracking and Preventing Harmful Algal Blooms). Figure 7 shows an example of how this core correlation was completed on Cedar Lake (Morrison Co.) to leverage previously funded work. Age-depth profiles were successfully completed for all 20 lakes in this study and results are summarized in Table 2.

Sediment cyanotoxin extraction

In order to reconstruct the history of toxic HABs and Cylindro in Minnesota lakes, we measured the concentration of both MCY (a toxin produced by several common HABs producing Cyanobacteria) and CYN (a toxin produced by Cylindro) in dated sediment sections in all 20 lakes from this study. This work was done in collaboration with Dr. Matthew Waters at Auburn University, whose laboratory developed these techniques.

Both MCY and CYN were extracted from sediment sections into solution using methanol and then the concentration of each toxin was measured using standard ELISA kits developed by Eurofins Abraxis, Inc. This study represents the first time sediment toxins have been measured in dated sediment cores in Minnesota and is a demonstration of the viability of this method for future work.

MCY was found in the sediments of all but 2 of the lakes in this study (White Iron and Trout), both located in the Canadian Shield, demonstrating its suitability as a proxy for general Cyanobacteria toxicity across a wide variety of lakes (Figure 8). In many of the lakes, MCY was detected for 70-100+ years prior to core collection and patterns followed general trends of eutrophication (increased productivity) of lakes in Minnesota, with the largest increases seen after 1950, concurrent with widespread agricultural intensification and urbanization in the state. Concentrations ranged from 0 to >30,000 ng/g organic matter across all 20 lakes, with the highest concentrations found in the surface sediments where benthic or sedimented Cyanobacteria were likely still involved in active toxin production at the time of core collection.

CYN concentrations were much lower in the lake sediments of this study and were only detected in 40% of the lakes (Figure 9). In all cases, CYN was measured in much lower concentrations than MCY, ranging from 0 to 25 ng/g organic matter. CYN was detected in the surface sediments from both lakes where Cylindro is known to occur (Madison and South Center), however it was also seen in an additional 6 lakes where Cylindro was not detected in this study. Those lakes are spread across three ecoregions and include St. Olaf, Shaokatan, and Carrie from the Cornbelt Plains; Cedar, Peltier, from the Central Hardwood Forest; and Elk from the Northern Lakes and Forests. It is possible that Cylindro is or was present in these systems, however it was not detected via microscopy or from our DNA sequencing analysis. CYN is also known to be produced in low concentrations by the Cyanobacteria genera *Aphanizomenon* and *Dolichospermum*, both of which commonly occur in Minnesota lakes and provides an alternative explanation for the occurrence of these toxins in lakes where Cylindro was not identified.

Cylindro invasion history in Minnesota

The results from this study confirm that the invasion of Cylindro in Minnesota is both very recent and limited to a small number of lakes. No additional lakes were found to contain Cylindro among the 20 Sentinel Lakes selected for this study and evidence for its occurrence was only found in the surface sediments. The next most recent samples for Madison and South Center were dated to 2006 and 2011, respectively, and no toxins were detected at those times. Given this single data point, we cannot yet begin to analyze any trend in occurrence related to temperature or other environmental factors. Unless Cylindro is documented in more locations in subsequent years, it would be more informative to focus on these two confirmed populations to correlate intra- and inter-annual variability in abundance and toxcitiy in these two lakes with climatic and environmental variables.

Results from this study indicate that Cylindro was first detected in Minnesota shortly after its arrival in the Sentinel Lakes and that its movement across the state has been negligible. Currently, the importance of Cylindro in terms of ecological and public health is secondary to the more general issue of toxic HABs in Minnesota lakes. This finding could change, however, as Minnesota lakes continue to warm and phytoplankton monitoring projects which incorporate both microscopy and DNA sequencing will be an important tool to identify the potential spread of Cylindro in the future.

IV. DISSEMINATION:

Description: We will collaborate with our existing state agency partners at MPCA and MN DNR to provide these data in a form that will be publicly available. We will develop scientific reports and factsheets intended to inform managers and lay-persons on the spread of *Cylindro* in Minnesota and its environmental and public-health impacts. Research Station scientists will highlight this work at "Behind the Scenes" events, hosted three times a year by the Science Museum of Minnesota, which is open to all of the Museum's thousands of daily visitors. We will publicize the progress and results of this project via the Research Station's news releases and social media presence as well as through our ongoing collaboration with the University of Minnesota Extension HABs outreach efforts.

A final project report will document all findings for reference by state personnel, presentations at regional meetings will apprise stakeholders of our methods and results, and publications in peer-reviewed journals will inform the wider academic research community.

First Update January 31, 2019

In cooperation with our communications specialist, Greg Seitz, we produced a short blog on November 6, 2018, to raise awareness of Cylindro and this newly-funded ENRTF project. This blog included a newly produced infographic on Cylindro produced by our Environmental Research Fellow Hailey Sauer (Figure 1), photographs from our Summer 2019 fieldwork, and general information on the issue. This article is available at: https://www.smm.org/scwrs/fieldnotes/invisible-species-exotic-algae-threatens-poison-minnesota-lakes

Second Update June 30, 2019

We continue to engage the public using both social media and conventional media outlets. We regularly use both Twitter and Facebook to share photos regarding the ongoing Cylindo fieldwork. Additionally, Adam Heathcote was asked to participate in a special edition of Minnesota Public Radio's Climate Cast on July 19, 2019 that focused on harmful algal blooms and climate change (https://www.mprnews.org/episode/2019/07/19/conditions-ripe-for-a-record-number-of-algae-blooms). Heathcote also participated in a public event hosted by the Marine Community Library (Marine on St. Croix, MN) where he presented on harmful algal blooms in Minnesota and acknowledged the past and current support of the ENRTF on this topic (see photo at https://twitter.com/AJ_Heathcote/status/1150751280182288389).

Third Update January 31, 2020

Results from this study and previous LCCMR-funded harmful algal blooms work were show-cased at the Minnesota Water Resources Conference held on October 15-16, 2019. PI for this project, Adam

Heathcote, co-chaired a special session on Harmful Algae Blooms at the conference, which ran for the entire afternoon of October 16th. This included Heathcote giving and introductory seminar on Cyanobacteria in Minnesota as well as participating in an expert panel answering audience questions on Cyanobacteria in lakes and streams in the State.

Fourth Update June 30, 2020

Although most of the museum was shutdown for the majority of this reporting period, we did use our online social media platforms to raise public awareness about harmful algal blooms in Minnesota using Twitter and the Museum's website: https://new.smm.org/learn/media/algal-blooms

Fifth Update January 31, 2021

We provided a presentation with preliminary results from this study to the Winter HABs Inter-agency Workshop on January 25th, 2021. This workshop included agency personnel from Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Minnesota Department of Health, and the Minnesota Veterinary Medical Association and deals specifically with environmental and health concerns about harmful algal blooms.

Sixth Update June 30, 2021:

We produced a journal article that will be submitted in September 2021 for review at PLOS ONE. If accepted, a pdf of the journal article will be included in the next update.

Seventh Update January 31, 2022:

We received comments on a journal article and submitted a revised version to the journal PLOS ONE in December of 2021.

Final Update June 30, 2022:

We have shared the progress and results from this project widely over the duration of the project. This includes both articles written by our staff, shared with traditional and social media, and peer reviewed papers. These efforts are summarized below in chronological order:

- "Invisible" species of exotic algae threatens to poison Minnesota lakes, posted to SMM.org on 11/6/2018, (PDF attached in supplemental materials)
- "Conditions ripe for a record number of algae blooms", Minnesota Public Radio Climate Cast segment on 7/19/2019,
- "Why good algae go bad", talk at the Marine Community Library by Adam Heathcote on 7/14/2019, picture of event on Twitter
- Adam Heathcote Co-chaired special session on Harmful Algal Blooms at the Minnesota Water Conference and organized a panel of experts to take questions from conference attendees in St. Paul, MN on 10/16/2019

- During the pandemic we provided information on harmful algal blooms for the public on the Science Museum website in our "Learn From Home" section, posted on 7/7/2020.
- Preliminary results from this study were presented to the Minnesota Inter-agency HABs group at their Winter Workshop on 1/25/2021. Attendees includes representatives from the Minnesota DNR, MPCA, MDH and the MVMA
- Results from this study were published in the peer-reviewed journal PLOS ONE on 3/21/2022. PDF of paper is attached in the supplemental materials.

V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached spreadsheet

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours: 3609	Divide by 2,080 = TOTAL FTE: 1.74

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

Enter Total Estimated Personnel Hours:	Divide by 2,080 = TOTAL FTE:

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State $ To Be Applied To Project During Project Period:			
Indirect costs at 40.83% waived by the Science Museum of Minnesota (in-kind)	$ 81,660	$ 80,476	Secured
Other State $ To Be Applied To Project During Project Period:			
	$	$	
Past and Current ENRTF Appropriation:			
M.L. 2016-186-2-04a: Tracking and Preventing Harmful Algal Blooms	$ 500,000	$ 500,000	Spent
M.L. 2015-76-2-10 "Tracking and Preventing Harmful Algal Blooms"	$ 93,000	$ 93,000	Spent
Other Funding History:			
M.L. 2015-76-2-10 "Tracking and Preventing Harmful Algal Blooms"	$ 93,000	$ 93,000	Spent

M.L. 2014-226-2-3g: Watershed-Scale Monitoring of Long-Term Best-Management Practice	$ 900,000	$ 900,000	Spent

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role

VII. LONG-TERM IMPLEMENTATION AND FUNDING:

This project will provide the first baseline data on the distribution of the toxin-producing aquatic invasive species *Cylindro* through space and time. The St. Croix Watershed Research Station is currently collaborating with other research groups in Minnesota on HABs, including the St. Anthony Falls Hydraulics Laboratory, University of Minnesota Extension, and the Natural Resources Research Institute. We will continue that effort so that data collected for this and other ongoing projects will be shared collectively (including standardized protocols and inter-laboratory quality control) to provide the best possible scientific product for the people of Minnesota.

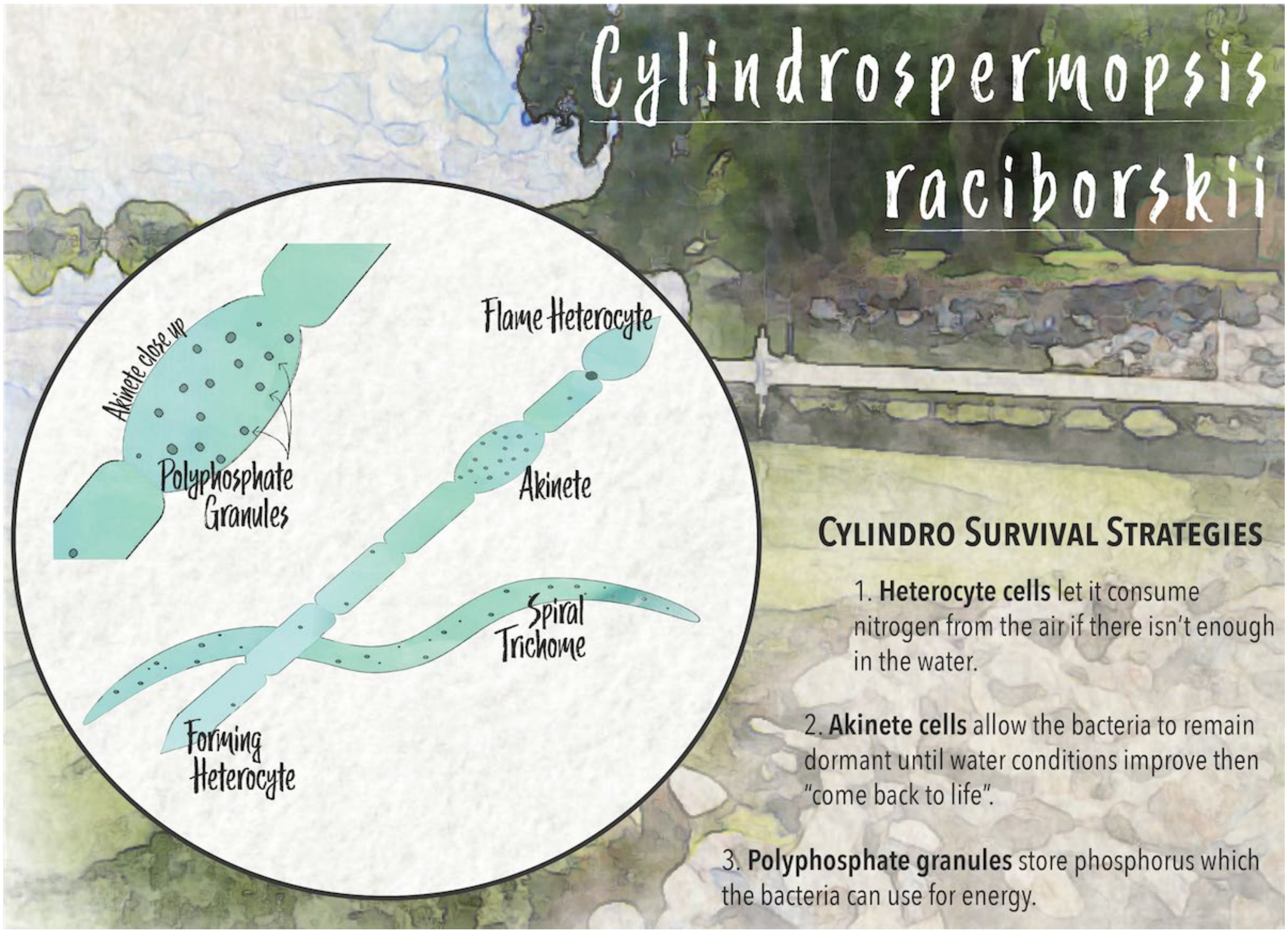
VIII. REPORTING REQUIREMENTS:

- **The project is for 4 years, will begin on 07/01/2018, and end on 06/30/2022.**
- **Periodic project status update reports will be submitted 01/31 and 06/30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2022.**

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet- N/A
D. Acquisition, Easements, and Restoration Requirements- N/A
E. Research Addendum

Figures and Tables



CYLINDRO SURVIVAL STRATEGIES

1. **Heterocyte cells** let it consume nitrogen from the air if there isn't enough in the water.
2. **Akinete cells** allow the bacteria to remain dormant until water conditions improve then "come back to life".
3. **Polyphosphate granules** store phosphorus which the bacteria can use for energy.

Figure 1. Infographic on the exotic algae *Cylindrospermopsis raciborskii* (Cylindro). This graphic was used in our blog that introduced the project and issue of this exotic algae in Minnesota. Original art was produced by SCWRS' Environmental Research Intern Hailey Sauer.

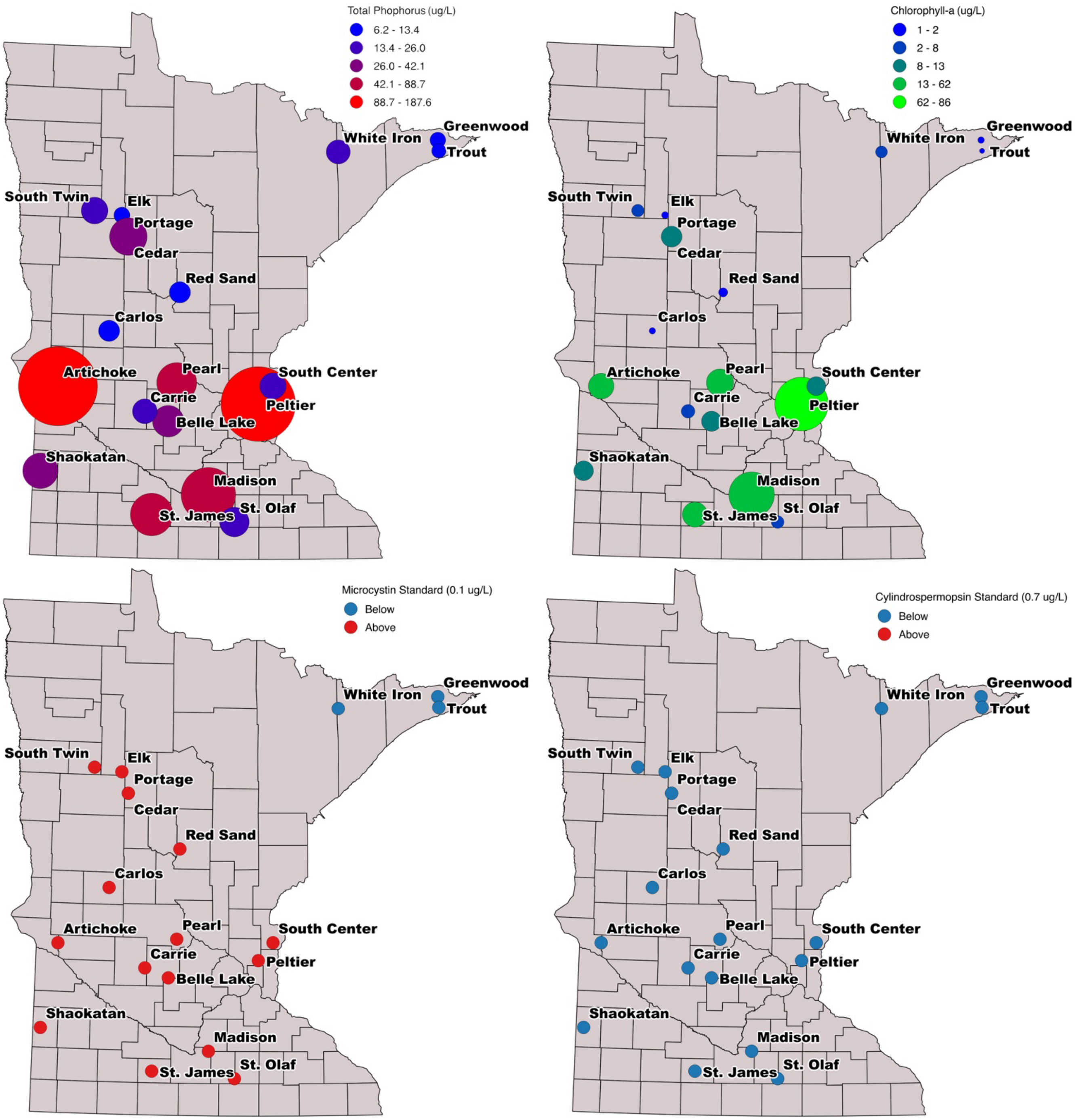


Figure 2. Map of the 20 MN DNR Sentinel Lakes included in this study and water quality parameters measured during August and September of 2018. Top left: total phosphorus concentrations which are a measure of the amount of nutrients available for algae growth; top right: chlorophyll-a concentrations which are a measure of the total biomass of algae in the lake; bottom left: detection of microcystin toxin above or below the minimum drinking standard for Minnesota (0.1 ug/L); bottom right: cylindrospermopsin toxin above or below the EPA minimum drinking standard (0.7 ug/L).

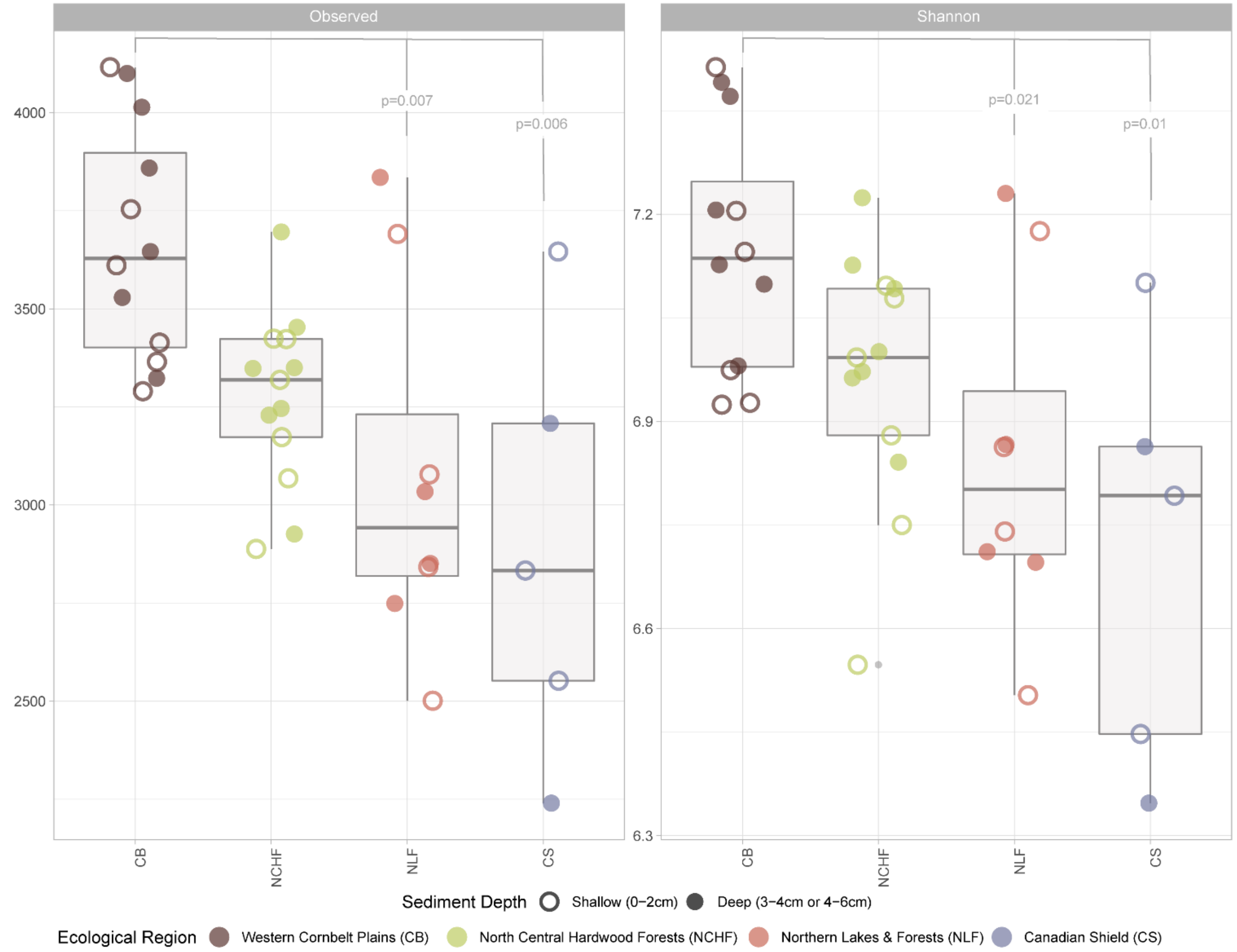


Figure 3. Microbial richness (Observed) and diversity (Shannon) for the surface (shallow, open circle) and adjacent (deep, filled circle) section of sediment cores collected from 20 MNDNR Sentinel Lakes as part of this project. Lakes are colored by their Minnesota ecoregion. Contrary to expectations, lake sediments in the traditionally more impaired Western Cornbelt Plains lakes had the highest microbial richness and diversity whereas lakes in the least impacted Canadian Shield ecoregion had the lowest.

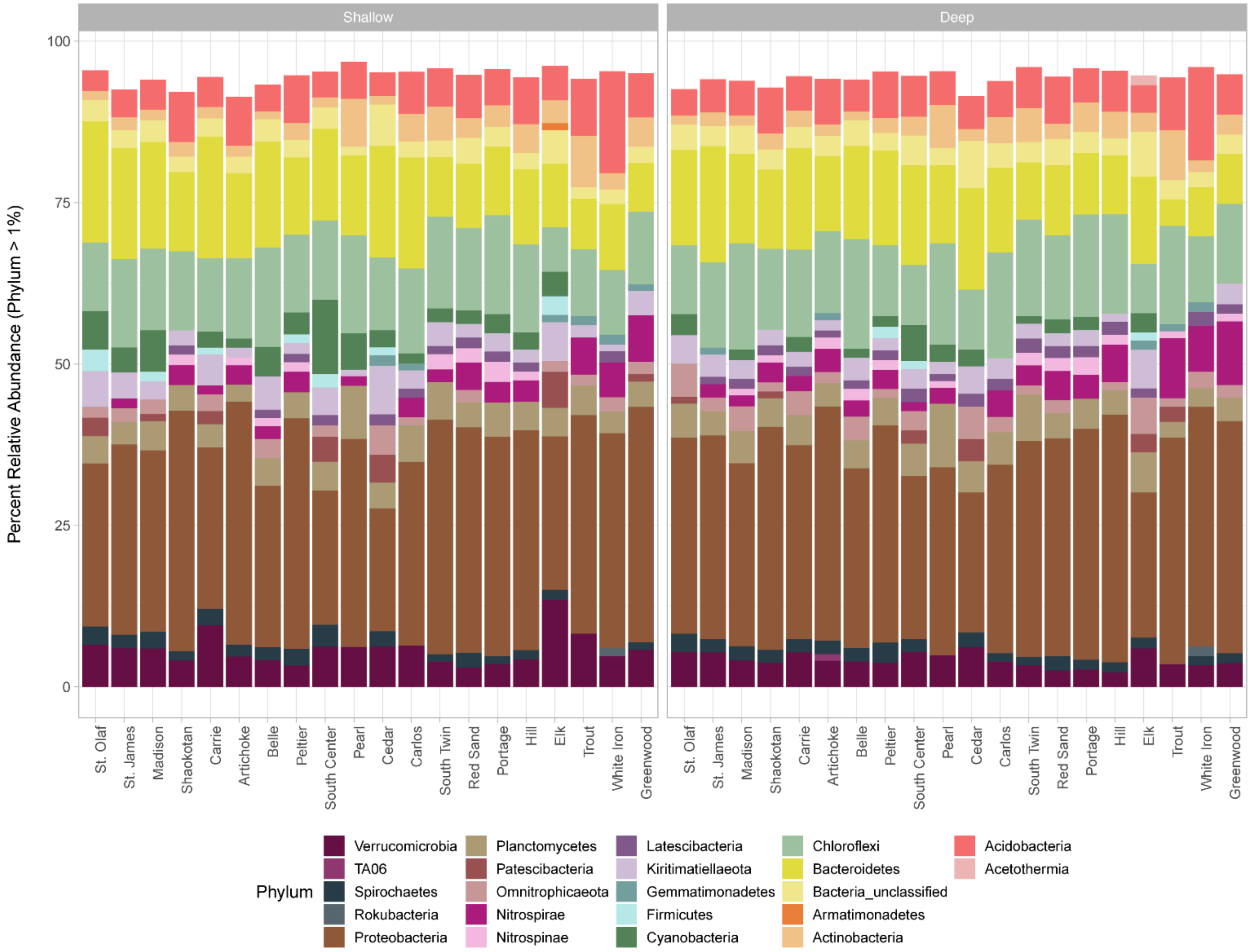


Figure 4. Relative abundance of all microbial phyla found in the surface (shallow) and deeper sediments of the 20 MN DNR Sentinel Lakes sampled as part of this project. This includes the Cyanobacteria phylum (dark green), which includes the genus *Cylindrospermopsis*. This represents the first microbial survey of lake sediments in the Sentinel Lakes.

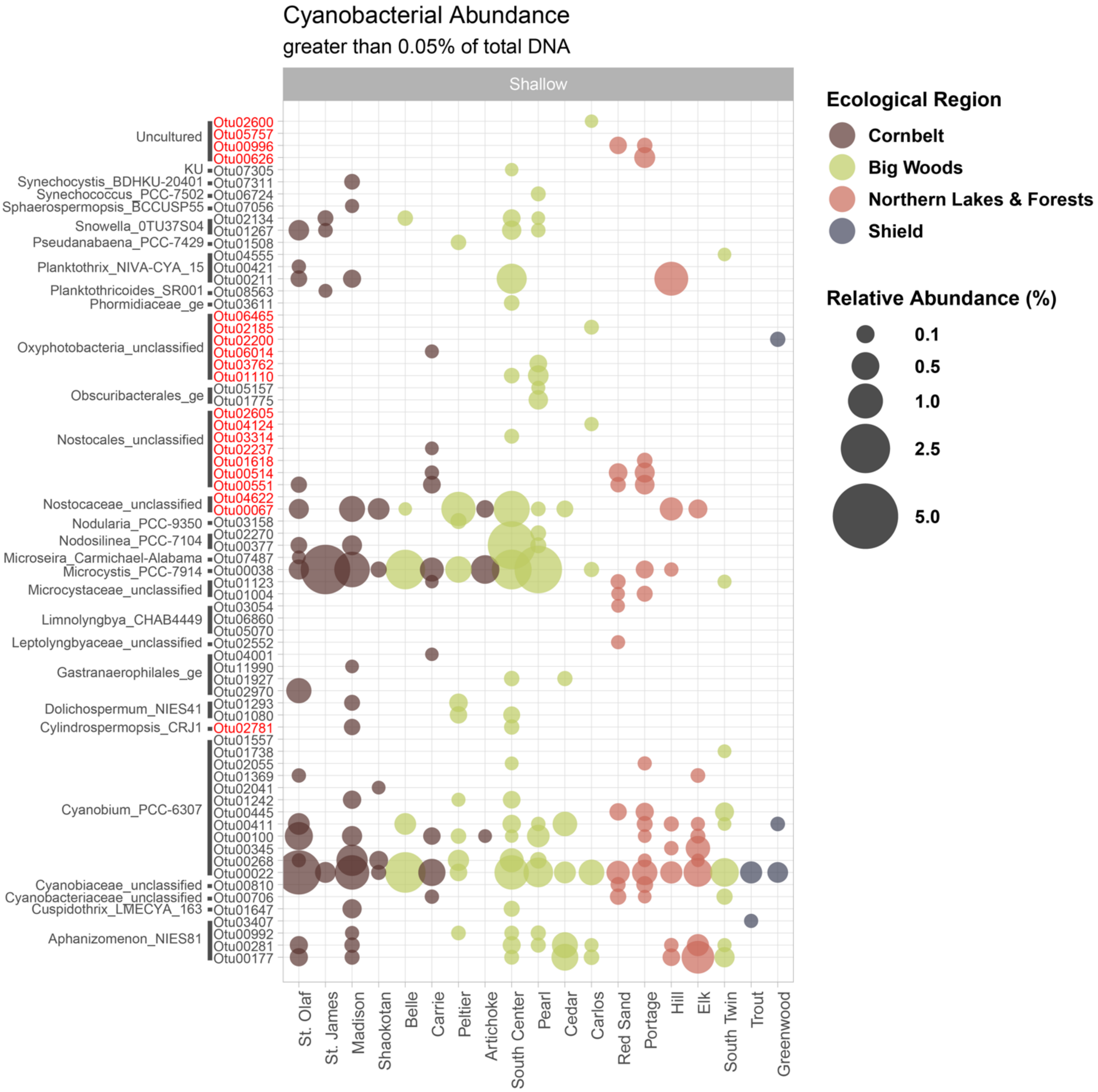


Figure 5. 16S amplicon sequencing results for Cyanobacteria DNA in surface sediments collected from the Sentinel Lakes in this study. OTUs are assigned to genera using the SILVA freshwater algae database. Species from the genus *Cylindrospermopsis* were only present in the two lakes from this study where *C. raciborskii* had been previously seen (Madison and South Center). No data from White Iron Lake are included because Cyanobacteria DNA was not present in the sample.

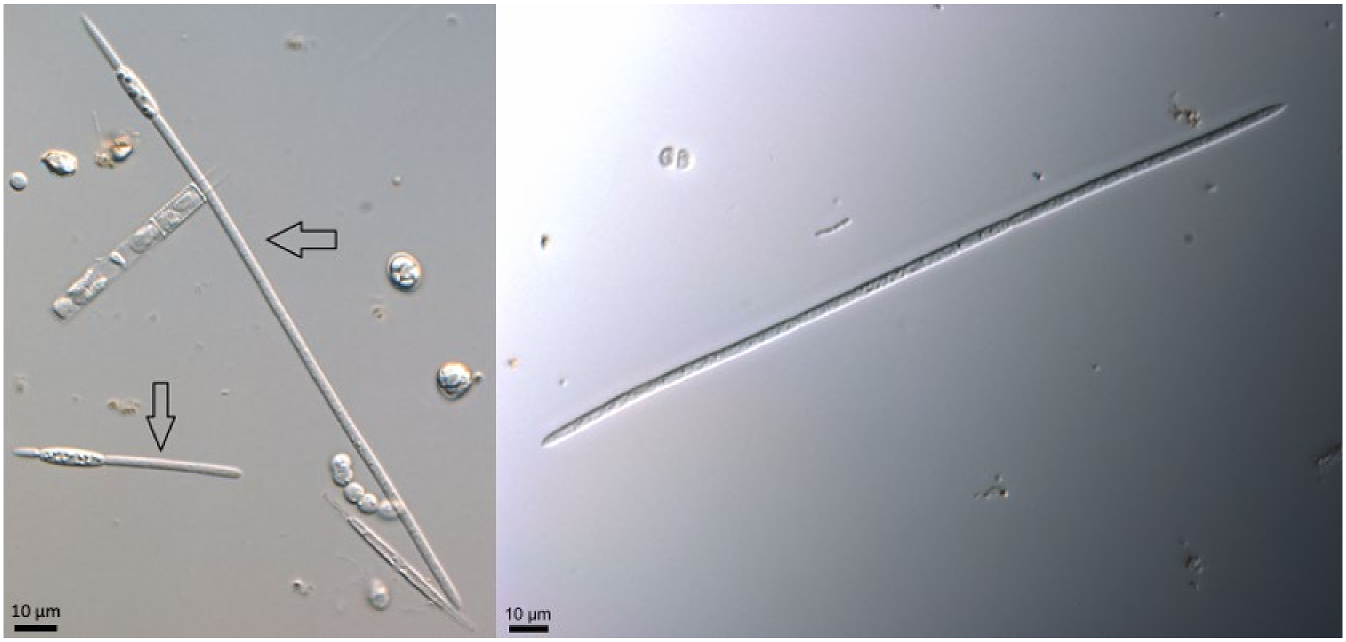


Figure 6. Light micrographs from phytoplankton samples collected from Madison Lake (left, black arrows) and South Center Lake (right) confirming the presence of *Cylindrospermopsis raciborskii* trichomes. Both samples show the typical “flame-tipped” heterocysts that are indicative of Cylindro. Akinetes are also present in the Madison lake population.

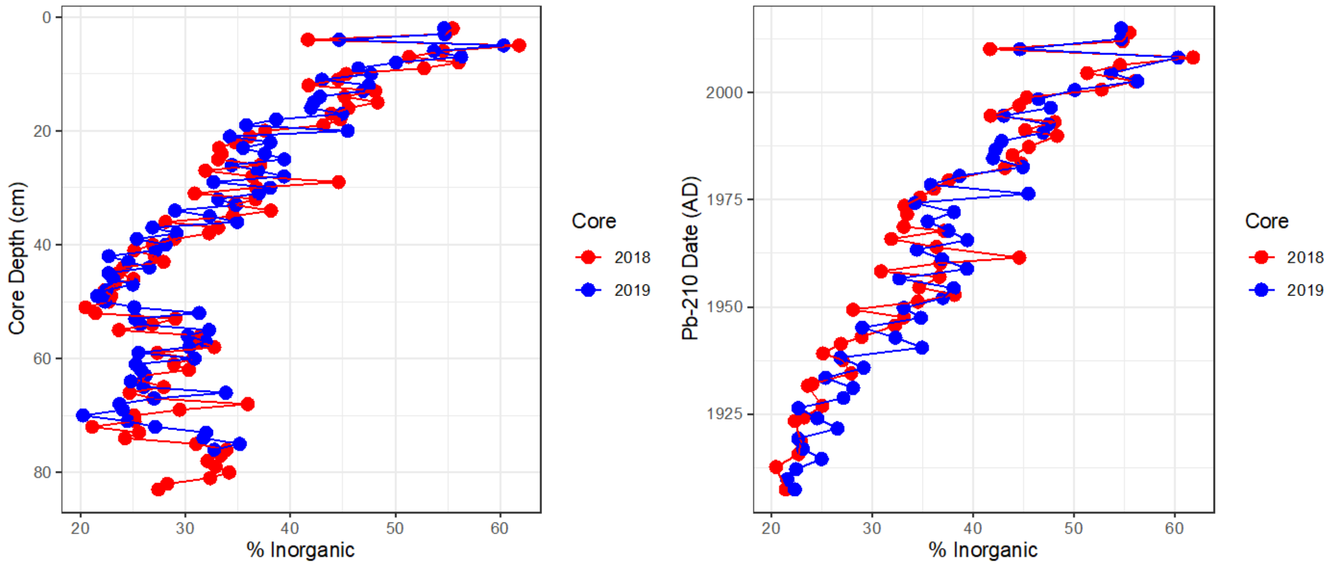


Figure 7. Example of core correlation preformed on the ten cores from this study which had been previously dated using MN ENRTF funding. The 2018 core (red) from Cedar Lake (Morrison Co.) was collected and dated using 210-Pb and the 2019 core (blue) was compared to it using common patterns across both cores in the percent inorganic matter measured via loss-on-ignition.

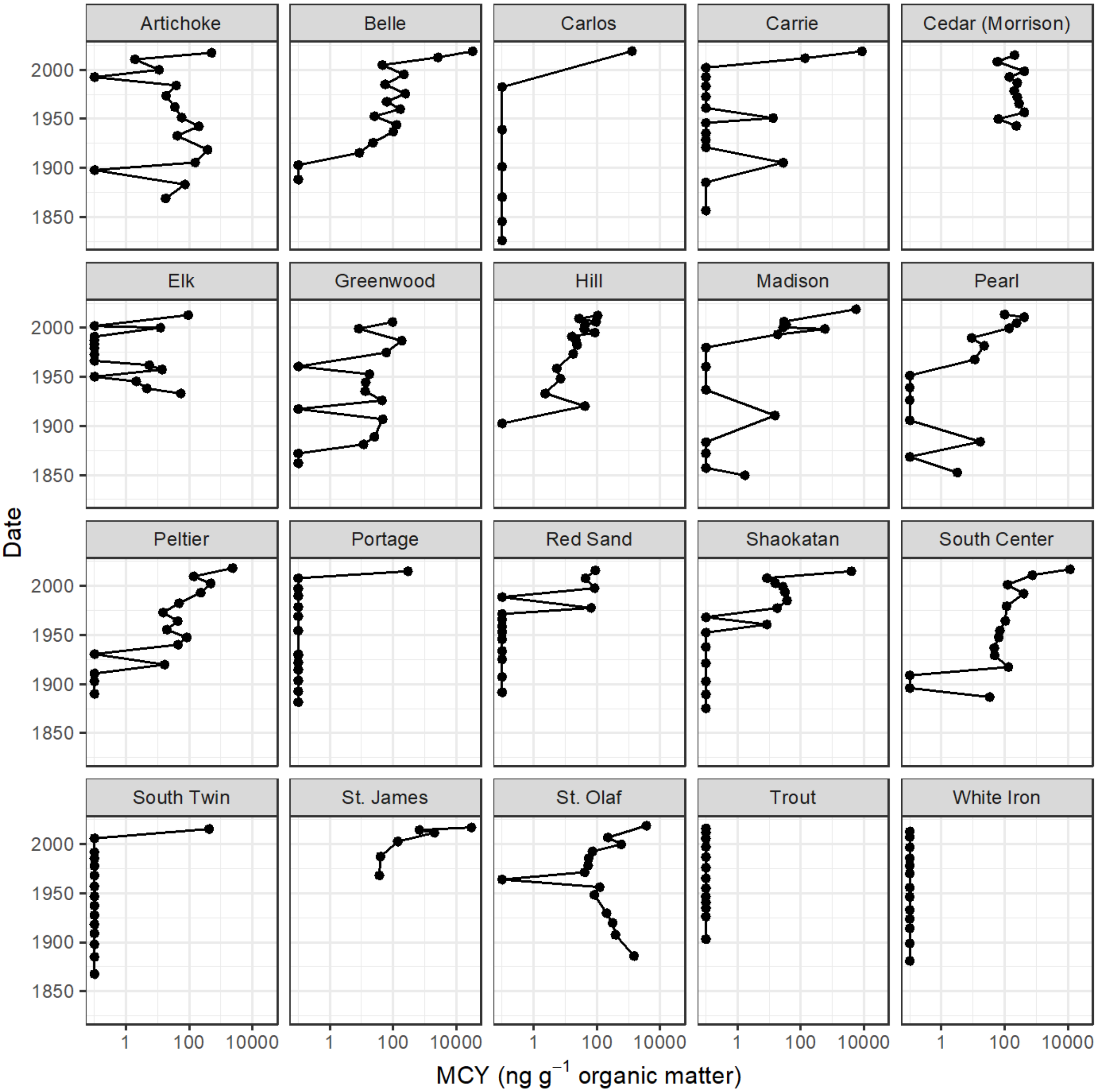


Figure 8. Concentration of the cyanotoxin Microcystin (MCY) in dated sediment cores from all lakes in this study.

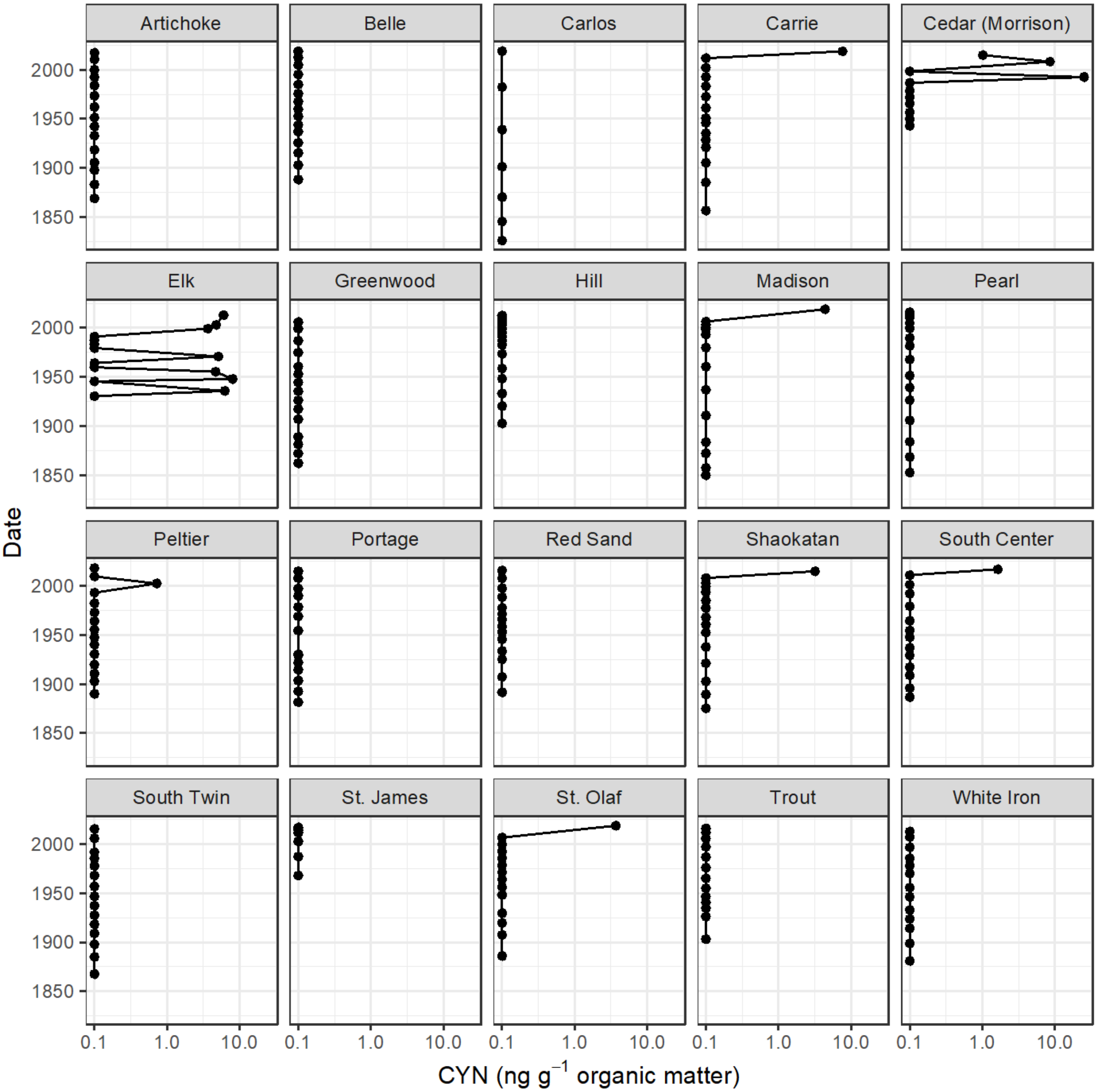


Figure 9. Concentrations of the cyanotoxin cylindrospermopsin (CYN) in dated sediment cores from all lakes in this study.

Table 1. Late summer 2018 water quality and cyanotoxin results from the 20 Sentinel Lakes included in this study. <MDL indicates the result was less than the detection limit.

Lake	Sample	TP (µg/L)	TN (mg/L)	Chl-a (µg/L)	NOx (mg/L)	NH4 (mg/L)	SRP (µg/L)	DIC (mg/L)	DOC (mg/L)	MCY (µg/L)	CYN (µg/L)
Cedar	Epi	12.9	0.6	1.5	<MDL	0.01	<MDL	32.1	6.3	0.1	<MDL
	Hypo	355.9	2.2	0.7	0.00	1.31	311.0	49.5	6.4	0.2	<MDL
Carlos	Epi	13.3	0.6	1.2	<MDL	<MDL	<MDL	34.4	7.0	0.3	<MDL
	Hypo	9.9	0.7	0.3	0.07	0.05	<MDL	38.5	6.5	0.4	<MDL
Pearl	Epi	47.8	0.9	22.1	<MDL	<MDL	<MDL	33.2	5.7	3.6	<MDL
Red Sand	Epi	13.4	0.7	2.5	<MDL	0.01	40.4	10.7	8.2	0.4	0.1
Portage	Epi	42.1	1.0	13.0	<MDL	<MDL	43.2	28.5	6.2	2.8	<MDL
South Twin	Epi	21.3	0.7	4.6	<MDL	<MDL	22.0	27.9	7.2	0.7	<MDL
Elk	Epi	7.7	0.5	1.4	<MDL	<MDL	34.9	33.1	6.7	0.2	<MDL
	Hypo	57.9	1.1	1.6	0.01	0.54	32.7	40.6	6.6	0.2	<MDL
Hill	Epi	18.0	0.7	7.6	<MDL	<MDL	8.1	29.9	9.3	0.3	<MDL
	Hypo	112.7	0.8	1.2	<MDL	0.22	69.6	37.6	8.2	0.3	<MDL
Peltier	Epi	166.7	2.3	85.9	<MDL	<MDL	5.0	26.7	14.2	0.9	<MDL
South Center	Epi	20.9	1.1	10.6	<MDL	<MDL	<MDL	11.1	9.6	0.7	<MDL
	Hypo	293.5	2.0	0.9	0.01	0.91	167.5	16.2	10.6	0.4	<MDL
White Iron	Epi	17.3	0.5	4.2	0.03	0.01	<MDL	4.9	14.3	<MDL	<MDL
	Hypo	22.3	0.7	0.7	0.13	0.02	6.0	6.2	14.7	<MDL	<MDL
Trout	Epi	6.2	0.1	0.8	<MDL	0.01	<MDL	4.3	4.0	<MDL	<MDL
	Hypo	10.2	0.3	0.9	0.04	0.01	<MDL	4.3	3.5	<MDL	<MDL
Greenwood	Epi	7.4	0.2	1.3	<MDL	0.01	<MDL	2.3	5.2	<MDL	<MDL
	Hypo	9.9	0.3	0.6	0.02	0.00	<MDL	2.5	4.6	<MDL	<MDL
Belle	Epi	29.8	1.0	12.1	<MDL	0.00	<MDL	31.5	10.0	3.9	<MDL
Carrie	Epi	18.3	2.3	5.5	1.20	0.31	<MDL	47.8	7.8	1.6	<MDL
Artichoke	Epi	187.6	1.6	19.5	0.01	0.03	114.6	46.2	15.1	0.3	<MDL
Shaokatan	Epi	37.8	1.0	11.6	<MDL	<MDL	<MDL	38.3	9.1	0.2	<MDL
St. James	Epi	54.1	1.4	18.8	0.07	0.02	<MDL	35.5	9.0	8.8	<MDL
Madison	Epi	88.7	1.6	61.9	<MDL	<MDL	3.1	31.0	10.0	0.9	<MDL
St. Olaf	Epi	26.0	0.7	4.8	<MDL	<MDL	<MDL	23.7	7.0	0.3	<MDL
	Hypo	460.4	6.5	20.3	<MDL	4.23	183.2	46.2	10.7	2.1	<MDL

Table 2. Summary of core-dating results for the 20 lakes from this study.

Lake	Dating Method	Oldest Age (AD)	Depth of Oldest Age (cm)
Artichoke	210-Pb	1845	66
Belle	210-Pb	1859	120
Carlos	Correlation	1836	36
Carrie	210-Pb	1833	150
Cedar	Correlation	1907	50
Elk	Correlation	1853	55
Greenwood	210-Pb	1852	21
Hill	210-Pb	1869	35
Madison	Correlation	1842	186
Pearl	Correlation	1836	41
Peltier	210-Pb	1860	188
Portage	Correlation	1865	49
Red Sand	210-Pb	1859	48
Shaokatan	Correlation	1860	44
South Center	Correlation	1887	92
South Twin	210-Pb	1731	46
St. James	Correlation	1972	14
St. Olaf	210-Pb	1824	168
Trout	Correlation	1831	20
White Iron	210-Pb	1804	43

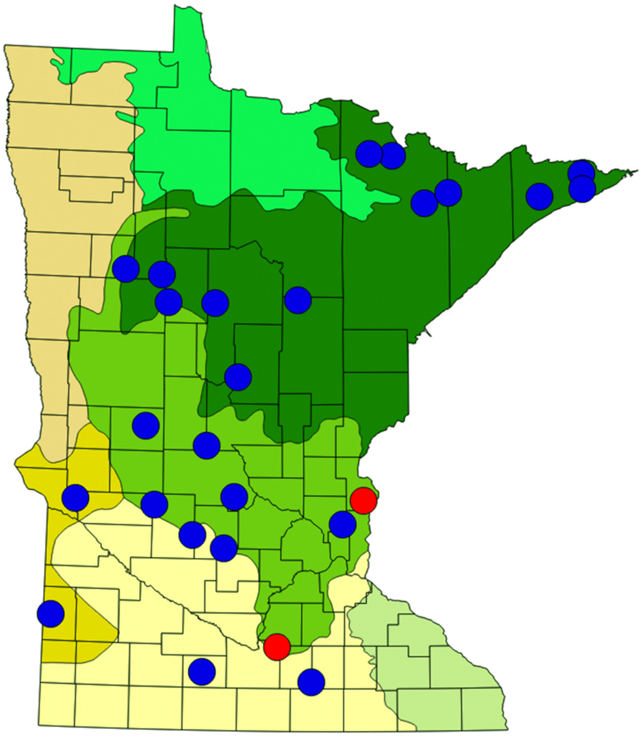
Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet-Final

Project Title: Determining Minnesota's risk of a toxic algal invader
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f
Project Manager: Adam Heathcote
Organization: Science Museum of Minnesota
College/Department/Division: St. Croix Watershed Research Station
M.L. 2018 ENRTF Appropriation: $200,000
Project Length and Completion Date: 4 years, June 20, 2022
Date of Report: 8/15/2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	TOTAL SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$115,800	$115,800	$0
Heathcote, Associate Scientist: Phyto & Cylindro toxins; 33% FTE for 2 yrs; Salary=71%, Benefits=29% ($56,300 over 2 years); this is a grant-funded position			
Edlund, Senior Scientist: Phyto & Cylindro toxins; 33% FTE for 1.5 yrs; Salary=71%, Benefits=29% ($48,400 over 1.5 years); this is a grant-funded position			
Field and Laboratory Technician: Field work and lab analyses; 25% FTE for 1 yr; Salary=71%, Benefits=29% ($11,100 over 1 years); this is a temporary position			
Professional/Technical/Service Contracts			
Equipment/Tools/Supplies			
Field and laboratory supplies: including bottles, reagents, calibration solutions, core tubes, sample cups	$4,002	$4,002	$0
Cyanotoxin ELISA kits: Cylindrospermopsin (22 @ $600 = $13,200) Microcystin (22 @ $600 = $13,200)	$26,400	$26,400	$0
Capital Expenditures Over $5,000			
Printing			
Travel expenses in Minnesota			
Field travel to 20 lakes for monitoring Hotel: 2 persons for 8 nights @ $76.50 = $1,224 Per diem: 2 persons for 8 days @ $36 = $576 Mileage & gas: 3,530 miles @ $0.545 = $1,924	$3,724	$3,724	$0
Field travel to 20 lakes for paleolimnology Hotel: 2 persons for 12 nights @ $76.50 = $1,836 Per diem: 2 persons for 12 days @ $36 = $864 Mileage & gas: 3,530 miles @ $0.545 = $1,924	$4,624	$1,723	$2,901
Other			
Lab analysis of water samples: TN/TP: 20 @ $36 DIN/SRP: 20 @ $36 DOC: 20 @ $20 DIC: 20 @ $15 Fe: 20 @ $36 SO4: 20 @ $36 CYN toxin: 20 @ $50 MC-LR toxin: 20 @ $44	$5,460	$5,460	$0
Lab analysis of sediment samples: 210-Pb (dating): 10 @ $2,250 ($22,500) loss-on-ignition: 20 @ $800 ($16,000)	$38,500	$38,500	$0
QA/QC of ELISA cylindrospermopsin sediment samples via HPLC (inter-lab comparison via University of Minnesota or competitive bid) ($1490)	$1,490	$1,490	$0
COLUMN TOTAL	**$200,000**	**$197,099**	**$2,901**

Cylindro: Minnesota's microscopic lake invader

What is the range, abundance, and toxicity of *Cylindro*?



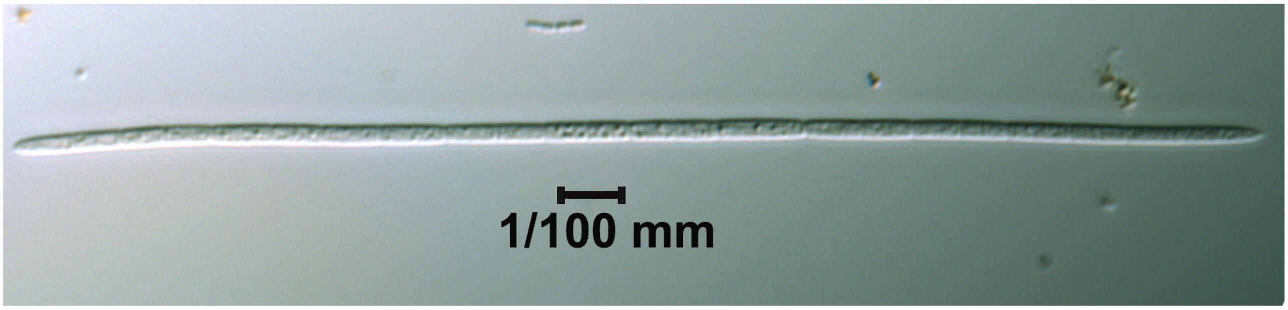
What is *Cylindro*?

a toxin-producing blue-green alga that has invaded MN lakes

Where has it been found?

Cylindro found in two lakes in 2013 and 2014 (red dots). Blue dots show other lakes to be surveyed by this project

CYN Toxin: Hepatotoxin produced by *Cylindro* is harmful to humans and pets



Cylindrospermopsis raciborskii: It may not look like much, but *Cylindro* (above) is an exotic species known to produce four different toxins. This specimen was seen by Research Station scientists in a Chisago County lake in 2015

PLOS ONE

RESEARCH ARTICLE

Diversity and distribution of sediment bacteria across an ecological and trophic gradient

Hailey M. Sauer[1,2], **Trinity L. Hamilton**[1,3]*, **Rika E. Anderson**[4], **Charles E. Umbanhowar, Jr.**[5], **Adam J. Heathcote**[2]

1 Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, United States of America, **2** St. Croix Watershed Research Station, Science Museum of Minnesota, Marine on St. Croix, Minnesota, United States of America, **3** The Biotechnology Institute, University of Minnesota, St. Paul, Minnesota, United States of America, **4** Biology Department, Carleton College, Northfield, Minnesota, United States of America, **5** Department of Biology and Environmental Studies, St. Olaf College, Northfield, Minnesota, United States of America

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Citation: Sauer HM, Hamilton TL, Anderson RE, Umbanhowar CE, Jr., Heathcote AJ (2022) Diversity and distribution of sediment bacteria across an ecological and trophic gradient. PLoS ONE 17(3): e0258079. https://doi.org/10.1371/journal.pone.0258079

Editor: Clara Mendoza-Lera, Brandenburgische Technische Universitat Cottbus-Senftenberg, GERMANY

Received: September 16, 2021

Accepted: March 4, 2022

Published: March 21, 2022

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0258079

Data Availability Statement: All relevant data are within the paper and its Supporting information

Abstract

The microbial communities of lake sediments have the potential to serve as valuable bioindicators and integrators of watershed land-use and water quality; however, the relative sensitivity of these communities to physio-chemical and geographical parameters must be demonstrated at taxonomic resolutions that are feasible by current sequencing and bioinformatic approaches. The geologically diverse and lake-rich state of Minnesota (USA) is uniquely situated to address this potential because of its variability in ecological region, lake type, and watershed land-use. In this study, we selected twenty lakes with varying physio-chemical properties across four ecological regions of Minnesota. Our objectives were to (i) evaluate the diversity and composition of the bacterial community at the sediment-water interface and (ii) determine how lake location and watershed land-use impact aqueous chemistry and influence bacterial community structure. Our 16S rRNA amplicon data from lake sediment cores, at two depth intervals, data indicate that sediment communities are more likely to cluster by ecological region rather than any individual lake properties (*e.g.*, trophic status, total phosphorous concentration, lake depth). However, composition is tied to a given lake, wherein samples from the same core were more alike than samples collected at similar depths across lakes. Our results illustrate the diversity within lake sediment microbial communities and provide insight into relationships between taxonomy, physicochemical, and geographic properties of north temperate lakes.

Introduction

A community of microorganisms living together in a particular environment or habitat are referred to as a microbiome. In the past decade, studies into the microbiomes of human organs, plants, soils, waters, and even space station astronauts have enhanced our

files. All 16S rRNA amplicon data are available from the SRA database at BioProject PRJNA763898.

Funding: Funding for this project was provided by a grant to AJH from the Minnesota Environment and Natural Resources Trust fund as recommended by Legislative-Citizen Commission on Minnesota Resources (LCCMR). https://www.lccmr.leg.mn/ HMS and TLH were also supported by NSF grant #1948058. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

understanding of how microbiota protect us from pathogens, increase agricultural production, and ultimately cycle nutrients in and throughout the natural environment [1–4]. Microbiomes are connected to the biogeochemical cycling of nutrients at both local and global levels, and as a result, disturbances or variations in their community composition can result in gains or losses of functional attributes, changes in nutrient availability, and shifts in ecosystem adaptability [5–7]. Understanding the selective pressures on microbiome community composition can provide insight into an environment's ability to support higher trophic levels and respond to anthropogenic change. Freshwater lakes are an ideal system to explore these insights as they provide a variety of regulating and cultural services, which hinge on the composition of their microbiome [8].

Despite their relatively small surface area, lakes contribute disproportionately to biogeochemical cycles, including the essential macronutrients carbon, nitrogen, and phosphorus [9–13]. Nutrients in lakes are recycled through several biotic and abiotic processes, but ultimately a significant proportion end up in sediments, where bacterial abundance and diversity typically exceeds that of the water column [14–16]. In the sediments, bacteria and archaea degrade organic matter—consuming oxygen and proceeding with anaerobic respiration processes. These respiratory processes occur along a redox gradient, eventually leading to the transformation of nitrogen, iron, and sulfur compounds. The complementary metabolisms of sediment microbiomes make sediments a global biogeochemical hotspot, one in which there has been a concerted effort to understand the environmental factors that regulate composition and function [17–20].

Chemical and physical characteristics of the lake such as salinity, pH, temperature, and nutrient concentrations select for specific bacteria, a process commonly referred to as species sorting [21]. The physicochemical characteristics of the system are partly based on the external inputs of both organic matter and nutrients, such as phosphorus and nitrogen, from the surrounding watershed [22]. Different land uses (e.g., agricultural, urban, forested, etc.) in the watershed strongly influence the amount and types of terrestrial organic matter and nutrients that enter the water, and therefore land use may subsequently affect community composition [23]. While there have been several studies that address the effects that local environmental factors have on microbiome species selection (e.g., eutrophic reservoirs, alpine lakes), few have examined the effects of the land-use of the watershed on bacterial community assembly [22– 30].

In this study we selected twenty lakes with varying physio-chemical properties across four ecological regions with varying land use in Minnesota (U.S.A.) (Fig 1). We sought to (i) evaluate the diversity and spatial variation of the bacterial community at the sediment-water interface and (ii) determine how lake location and watershed land-use impact aqueous chemistry and influence bacterial community structure. We hypothesize that community composition of lake sediments will appear homogeneous across ecological regions and land use at higher taxonomic levels; however, we hypothesize increased structure by eco-region at lower taxonomic levels. To test this, we compare the alpha and beta diversity of bacteria across taxonomic scales (Phylum to Order) and ecological regions, and we highlight important regional and local factors that influence community composition.

Materials & methods

Site description

For this study, we selected twenty lakes within Minnesota's Sentinel Lakes in a Changing Environment (SLICE) program. SLICE is a collaborative research initiative providing long-term data on a representative sub-sampling of Minnesota's lakes that span the diverse geographic,

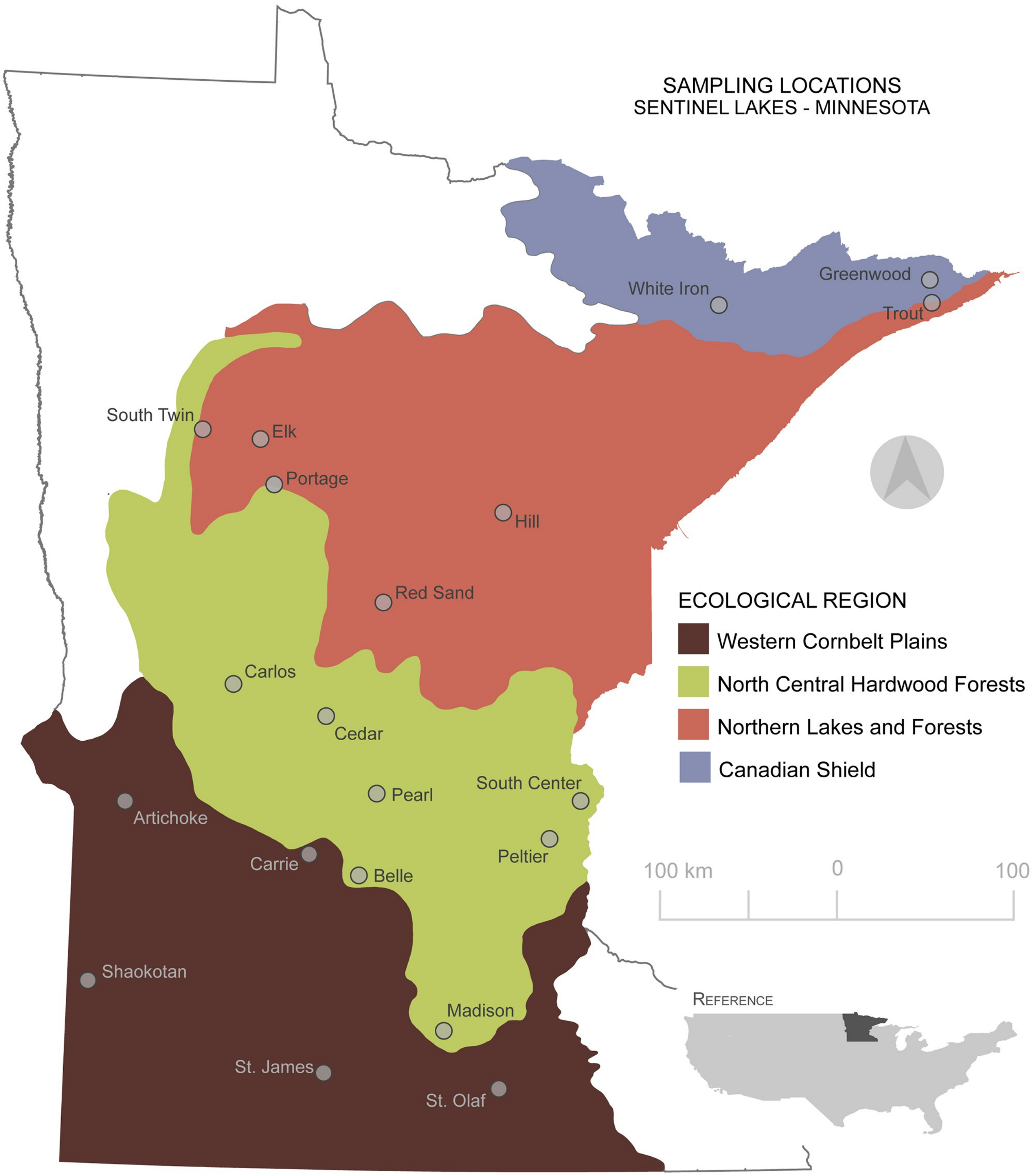

Fig 1. Map of sampling locations. Location of the twenty study lakes that were cored across the state of Minnesota (U.S.A.) between the summer of 2018 and 2019, shaded by ecoregion. Map was created using QGIS and data were made available by the MN Geospatial Commons (public domain) https://gisdata.mn.gov/.

https://doi.org/10.1371/journal.pone.0258079.g001 land-use, and climatic gradients present in Minnesota (Fig 1). The lakes span four of the seven Environmental Protection Agency/Commission for Environmental Cooperation's (level III) ecological regions. These regions can be characterized by their underlying geology, soils, vegetation, and land use (S1 Table). This is the first comprehensive sediment bacterial survey of these lakes.

Water sample collection & analysis

From each site we collected water profile measures for temperature, pH, conductivity, turbidity, and dissolved oxygen using a YSI XO2 multi-parameter sonde (YSI, Inc.). We also collected an integrated (0-2m) epilimnetic water sample, and a hypolimnetic (maximum lake depth–1m) water sample when thermal stratification was present. All samples were stored on ice in the field and at 4˚C or -20˚C in the laboratory, depending on methodology, until processed. Samples for soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), and dissolved inorganic carbon (DIC) were filtered, processed, and analyzed within 36 hours of sampling using standard methods for SRP (4500-P) on a SmartChem 170 (Unity Scientific, Inc.) and DIC/DOC Method 5310-C using a Torch Combustion TOC Analyzer (Teledyne Tekmar, Inc.) [31]. Samples for total nitrogen (TN) and total phosphorus were frozen and analyzed using standard methods for TN (4500-N), and TP (4500-P). Samples for ammonia (NH_3) and nitrate (NO_3) were filtered and frozen prior to analysis following methods NH_3 (4500-NH_3) and NO_3 (4500-NO3). All TP, TN, NH3, NO3 samples were analyzed within six months of sampling on a SmartChem 170 (Unity Scientific, Inc.) discrete analyzer (APHA 2012). Additionally, we filtered, froze, and analyzed samples for chlorophyll-a concentrations via fluorometry following the EPA method 445.0 [32]. We provided a complete summary of aqueous chemistry results, including sampling dates, in the S2 Table.

Sediment sample collection & DNA isolation

We collected sediment cores from July 2018 through June 2019 using a rod-driven piston corer with a 7cm diameter polycarbonate tube [33]. We determined coring locations (i.e., flat areas near the deepest basin) using publicly available bathymetric maps (https://www.dnr.state.mn.us/lakefind/index.html), avoiding steep-sided "holes" where sediment-focusing may be high. After sediment core retrieval, we stabilized core tops in the field using a gelling agent (e.g., Zorbitrol) and returned intact cores to the laboratory where we stored them vertically at 4˚C for no more than seven days until processing. In cases where the upper sediments were extremely flocculent, we immediately sectioned the upper most sections (~0–30 cm) in the field to prevent mixing during transport.

We vertically extruded the cores in the lab in 1 to 2 cm intervals, depending on lake productivity, and took subsamples from two intervals for DNA analysis. The subsamples collected were from the 0-2cm (hereafter referred to as shallow) and either the 3-4cm or 4-6cm interval (hereafter referred to as deep). Subsamples were frozen under nitrogen for up to three months before DNA was extracted (S3 Table). We extracted DNA from 0.25g of wet sediment from each subsample using a PowerSoil DNA Isolation Kit (Qiagen, Inc.) following the manufacturer's protocols. We performed negative controls by carrying out extractions on blanks, using only reagents without sample. We determined final bulk DNA concentrations using a Qubit™ dsDNA HS Assay kit (Molecular Probes, Eugene, OR, USA) and Qubit™ Fluorometer (Invitrogen, Carlsbad, CA, USA). The detection limit for the Qubit™ dsDNA HS Assay Kit is 10 pg/μL. All samples that yielded detectable amounts of DNA were sent for sequencing (S3 Table). Despite not detecting DNA in our negative controls, these were submitted for sequencing where they failed to pass quality control performed by the University of Minnesota Genomic Center (UMGC) and no sequencing information was obtained.

Nucleic acid preparation, amplification, and sequencing

We submitted the DNA samples to the UMGC where they performed library preparation for Illumina high-throughput sequencing using a Nextera XT workflow and a 2x300 bp chemistry. The workflow utilizes transposome-based shearing which fragments the DNA and adds adapter sequences in one step. The DNA was amplified and dual-indexed with adapter sequences through PCR, using primers 515F (`5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA-3′`) and 806R (`5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3′`) to target the V4 hypervariable region of bacterial 16S SSU rRNA gene sequences. The amplicon library preparation methods created and employed by the UMGC have been shown to be more quantitatively accurate and qualitatively complete—detecting taxonomic groups that often go undetected with existing methods [34]. The indexed samples were then sequenced once using an Illumina MiSeq at the UMGC. A total of 3.29 million *(3,290,170)* raw reads were obtained from 40 samples.

Data processing

We conducted post-sequence processing in Mothur (v1.43.0) following the MiSeq SOP [35, 36]. Briefly, we merged forward and reverse reads, and screened, trimmed and removed ambiguous bases. We then aligned the reads to references in the SILVA database (v.132), and identified and removed chimeras using vsearch (v2.13.3) [37, 38]. Finally, given the nature of the study (i.e., broad scale patterns of diversity), we classified the sequences as operational taxonomic units (OTUs) using a 97% similarity threshold and assigned taxonomy using the SILVA database [39, 40].

Community analysis & statistics

Unless otherwise stated, we conducted all statistical analyses in R (v4.0.0) [41, 42]. We loaded both the environmental and community data into R using Phyloseq (v1.32.0) [43] and removed any reads classified as mitochondrial or chloroplast. Our final dataset after all post-processing contained 2,181,132 reads assigned to 53,854 taxa across 40 (two sediment depths/lake) samples.

Alpha diversity. We removed all singletons (OTUs observed only once across all 40 samples) from the data before calculating alpha diversity statistics. Given the observed correlation of richness based on sample read depth across sequencing batches (S1 Fig), we chose to rarefy the data to 90% the read depth of the lowest samples (15,771 reads; S2 Fig and S3 Table). Our final dataset for alpha diversity included 630,840 read counts of 25,563 taxa across 40 samples. We calculated alpha diversity measures using the Phyloseq package in R (S3 Fig and S4 Table) [43]. We compared the richness (observed number of OTUs) and evenness (Shannon) of the samples based on sample depth (shallow n = 20, deep n = 19) using a Wilcox test, and trophic status (hypereutrophic n = 4, eutrophic n = 16, mesotrophic n = 16, and oligotrophic n = 3) and ecological status (Western Cornbelt Plains n = 12, North Central Hardwood Forests n = 14, Northern Lakes & Forests n = 8, Canadian Shield n = 5) using a Kruskal-Wallis test with a Dunn Post Hoc test and Bonferroni correction. In all tests, one outlying sample (Trout, Deep) was removed due to uncharacteristically low diversity. Finally, we assessed the predictive capabilities of the environmental parameters, collected at the time of sampling (S2 Table), on the alpha diversity of the sample using multiple regression and determined the significance and variance partitioned by each regressor using the relaimpo (v.2.2.3) and vegan (v.2.5–6) packages in R [44, 45]. We selected the final models based on AIC scores for both richness (observed) and evenness (Shannon).

Beta diversity. Prior to beta diversity analysis we filtered the samples by removing any OTU that did not have 2 or more counts and occur in at least 10% of the samples. Post filtering, the average number of reads per sample was reduced to 47,605, the minimum read depth was 15,150, and the maximum read depth was 99,561. Since OTU data have a strong positive skew, we attempted to diminish the effects using a variance stabilizing transformation (VST) [46]. Log-like transformations, like VST, have been shown to transform count data to near-normal distributions and produce larger eigengap values, ultimately leading to more consistent correlation estimates which influence downstream analyses [47]. After filtering and transformation, the final dataset for beta diversity analysis included 5,512 taxa across 40 samples.

We visualized the sample dissimilarity using principal component analysis (PCA) and the ordinate function in Phyloseq [43]. After ordination, we further analyzed the distribution of taxa based on the ecological regions using permutational analysis of variance (PERMANOVA) and the "adonis" function in vegan [45]. We used a Bray-Curtis dissimilarity to test for group differences and assessed dispersion within groups using permutations and vegan's "betadisp" and "permutest" functions. Prior to creating the dissimilarity matrix, we converted negative VST values to zero because negative values after transformation likely represent zero counts or very few counts and for the distances and hypothesis in future tests these values would be negligible. We performed a cluster analysis using Ward's (D2) method and the same dissimilarity matrix generated for the PERMANOVA analysis.

Results and discussion

Alpha diversity

We used alpha diversity metrics to summarize the structure of the bacterial communities in terms of the number of OTUs (richness) and the distribution of their abundances (evenness) for all samples. We then compared the observed diversity (a measure of richness) and Shannon diversity (a measure of evenness) across sampling locations and ecological regions (S4 Fig). Sample richness varied from ~2000–4000 OTUs and sample evenness varied from 5.5 to 7.5. Sediments, both shallow and deep, from Carrie Lake were the most diverse in terms of richness (4116 OTUs and 4100 OTUs; shallow and deep respectively) and evenness (7.41, 7.39; shallow and deep respectively). The least diverse shallow sample in terms of richness was Pearl Lake (2371 OTUs) and evenness was Greenwood Lake (6.44). Trout Lake was the least diverse deep sample in both the total number of OTUs (552 observed) and Shannon diversity (5.49). There was no significant difference within lake diversity between shallow and deep lake sediments across all samples (Wilcoxon test $p > 0.05$); however, the deeper interval sample was more diverse in both richness and evenness in a majority of lakes. The exception to that pattern were the samples from lakes in the Canadian Shield (CS) where all of the shallow interval samples were more diverse.

While all samples were highly diverse when compared to the bacterial diversity of the overlying water column or the number diatom species found in the sediments (Observed Richness > 2250 OTUs; Shannon 5.5–7.5), there were differences in the levels of richness and evenness when comparing samples across the ecological region (Fig 2 and S5 Fig) [48–50]. Shannon diversity (evenness) levels were statistically different across the ecological regions (Kruskal Wallis $p = 0.008$). Samples from lakes in the Western Cornbelt Plains (CB) were more diverse than both the Northern Lakes and Forests (NLF) (Dunn's test $p = 0.0206$) and CS (Dunn's test $p = 0.0099$) samples. Observed diversity (richness) was also statistically significant across the ecological regions (Kruskal Wallis $p = 0.003$). Again, there were statistical

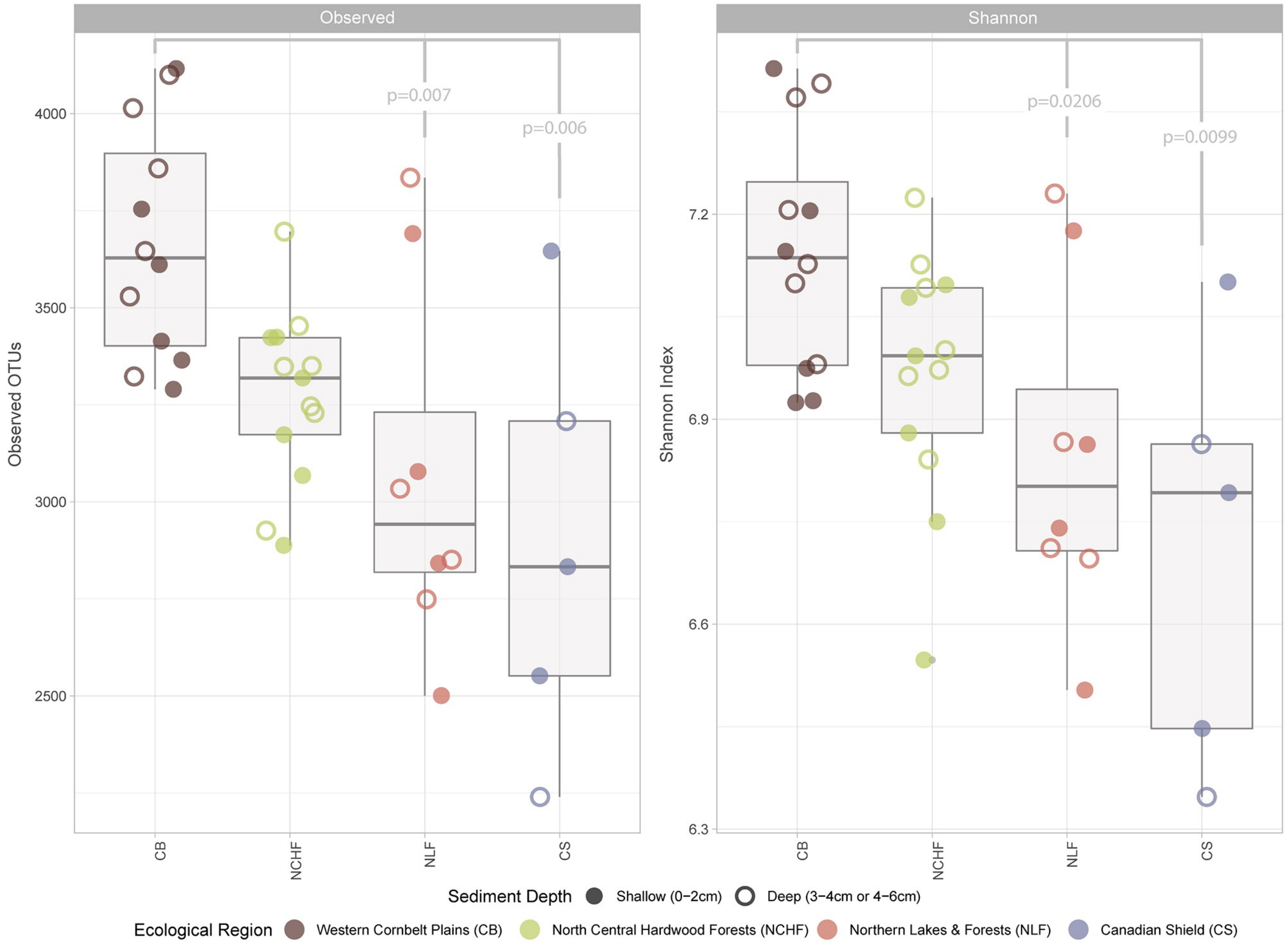

Fig 2. Bacterial alpha diversity by ecological region. Box plots show mean alpha level diversity of the observed Operational Taxonomic Units (OTUs) and Shannon indices of the four distinct ecological regions present within the study area: Western Cornbelt Plains (CB), North Central Hardwood Forests (NCHF), Northern Lakes and Forests (NLF), and Canadian Shield (CS). Open (shallow) and closed (deep) circles indicate unique samples and color indicates the ecological region. One sample was removed from both plots for due to uncharacteristically low diversity. Significance between regions was calculated nonparametrically using a Kruskal Wallis H test followed by a Dunn post hoc test with a Bonferroni correction. Reported p values indicate significant differences in Observed and Shannon diversity (respectively) across ecological regions, specifically the diversity of CB lake sediments when compared to NLF (p = 0.007 & p = 0.0206) and CS (p = 0.006 & p = 0.0099) sediments.

https://doi.org/10.1371/journal.pone.0258079.g002

differences in the diversity levels of the CB and NLF (Dunn's test p = 0.007) and CS (Dunn's test p = 0.006) wherein the CB samples had greater richness.

The lakes in the CB are highly impacted by agricultural activity and have cultivated or pastureland comprising approximately 50–90% of their total watershed. This land use contrasts with other ecological regions like the NLF (2–24% agriculture) and the CS (<3% agriculture) where species richness was statistically less rich. Agricultural runoff and drainage can carry a variety of contaminants (e.g., herbicides, pesticides) which can stimulate the growth of certain bacteria (e.g., *Planktothrix*) [7, 51]. The selecting pressure of land-use on microbial taxa and food webs varies within and across ecosystems from highly selective to uninformative, and often land use is described as having indirect effects on microbial composition and diversity (i.e., differing land covers lead to differing nutrient loads in runoff) [22, 52, 53]. Ultimately, our data confirm patterns observed by others: that eco-regional or eco-zone concepts can affect alpha diversity and species richness [53]. We explored the eco-regional-diversity relationship further by examining within phyla richness across the four ecoregions (S5 Table). From this we found 15 phyla which were statistically different in terms of richness in one ecoregion. Several of these phyla (e.g., Bacteroidetes, Spirochaetes and Lentisphaerae) exhibited patterns of richness across ecological regions like that seen in the entire microbiome (i.e., a decreasing richness from CB to CS) while only one phylum, Armatimonadetes, showed an opposite richness pattern. Other phyla like Epsilonbacteraeota and Modulibacteria were more diverse in the CB, while Kiritimatiellaeota were more diverse in CS samples. The identification of specific phyla that display varying richness depending on the broader ecological region of their environment may provide insight into the more nuanced indirect effects that geography plays in microbial assembly. For example, a shift in Bacteroidetes richness across these ecological regions may be indicative of the changing land use and subsequent nutrient regimes which could lead to increased algal biomass within the lake (as discussed below). However, given the low resolution of 16S rRNA gene sequencing (particularly partial gene amplicons) and an inability to confidently determine unique species and potential functional differences we could not specifically address the mechanisms that lead to increased richness across ecological regions.

Because lakes in these ecological regions also tend to vary based on their trophic status, we compare the differences in alpha diversity based on proxies for lake productivity. Using previously reported (yearly average values) of chlorophyll-a and total phosphorous (Chl-a, TP), and Secchi depth provided by the MN Department of Natural Resources, we classified the lakes as hypereutrophic, eutrophic, mesotrophic, and oligotrophic (S1 Table). We found that both Shannon diversity and observed OTUs were greater in hypereutrophic systems compared to oligotrophic systems, and eutrophic systems were also statistically richer than oligotrophic systems (S6 Fig). Since most of the lakes were classified as eutrophic or mesotrophic and the values of TP and Chl-a vary seasonally depending on sampling times, we further assessed the effects of local water chemistry, measured at time of sampling, on alpha diversity. Using an exhaustive search with AIC selection criteria we modeled Shannon and Observed diversity using all aqueous chemistry measures, lake latitude, depth, trophic status, ecoregion, and land cover use in the watershed. The lake's latitude, temperature, and specific conductivity as well as the concentrations of total phosphorus (TP), total nitrogen (TN), Chlorophyll-a best predicted Shannon diversity (adj $R^2 = 0.586$). Whereas the lake's latitude as well as the concentrations of TP, TN, DOC, specific conductivity, pH, turbidity and ecological region best predicted observed diversity (adj $R^2 = 0.7229$).

The results of our alpha diversity analysis indicate that sediments in more eutrophic systems, like those of the CB, are more diverse. This is in contrast to unimodal diversity-productivity relationships seen among other freshwater communities such as phytoplankton, zooplankton, and fish but similar to other studies on freshwater bacterial communities along a trophic gradient [54–58]. Previous work addressing the diversity-productivity relationship of bacterial communities highlights the importance of rare or dormant taxa, in that as trophic status increases the diversity of rare/dormant taxa increases [59]. In our study we deemed rare taxa at the phylum level as those not comprising more than 1% relative abundance of the sample. We then compared the richness of these phyla individually across ecological region and trophic status (S4 Table). From this, we found that no rare taxa (at the phylum level) exhibited a statistically significant (Kruskal Wallis $p<0.01$) increase in richness due to increased trophic status. Nevertheless, three common phyla (Chloroflexi, Spirochaetes, and TA06) did increase as a function of trophic status. Among these three phyla only Chloroflexi, which play an important role in the degradation of labile carbon and secretion of organic acids in subsurface sediments, have previously been shown to increase in abundance and diversity with eutrophication in aquatic environments [60, 61]. By examining the trends in richness across trophic status, like ecological region, we may begin to uncover indicator taxa for nutrient pollution and eutrophication; however, coarser species and function relationships need to be considered.

Beta diversity

To further distinguish trends in the data based on ecoregion and other environmental measures, we examined beta diversity (diversity between samples). Using our variance stabilized data, we conducted a principal component analysis (PCA) to explore the differences in community composition of samples across sites and sediment depths. The first two components explained ~30% of the variation in the samples (Fig 3). In addition to PCA we performed a cluster analysis to determine which samples were most similar (Fig 4). Using both approaches, we concluded that there was a clear distinction between community composition based on ecological region and lake depth.

In terms of ecological region, samples from the CS were the first to cluster out. The second cluster consists of six samples from three lakes that are best characterized as deep (max depth

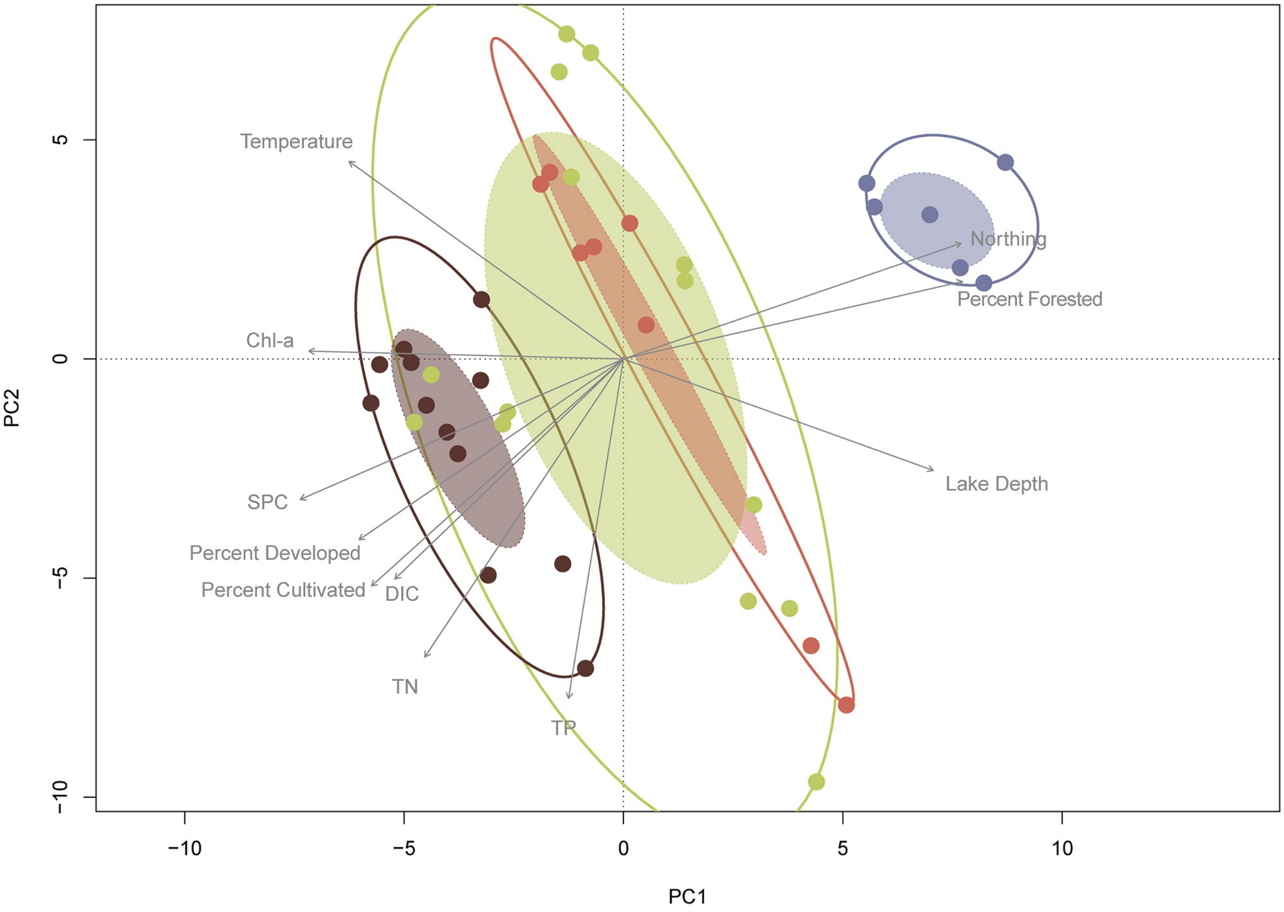

Fig 3. Beta-diversity: Principal component analysis of samples. Principal component analysis (PCA) of surface sediment microbiome samples where color represents ecological region. Components one and two explained 31.4% of the total variance. Environmental variables were fit using linear regressions where each component was plotted as a function of an environmental vector and those with p<0.01 were plotted. Solid line ellipses are the outer sample bounds for each region and the shaded ellipses are the standard error of the weighted centroids for the data. Abbreviations: Dissolved Inorganic Carbon (DIC), Total Nitrogen (TN), Total Phosphorus (TP), Chlorophyll-a (Chl-a), Specific Conductance (SPC).

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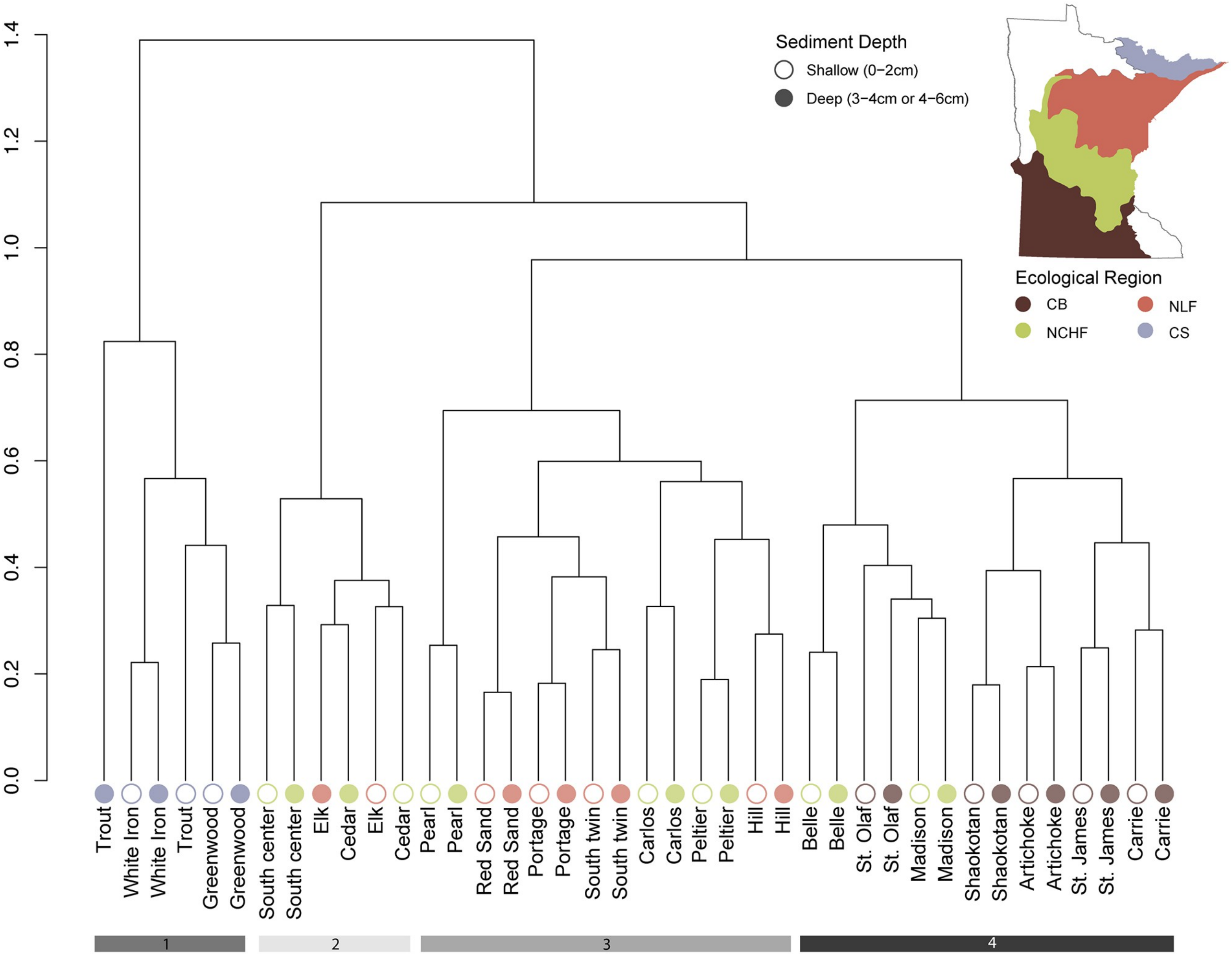

Fig 4. Beta-diversity: Hierarchical clustering of samples. Hierarchical clustering analysis of sediment bacterial communities using Ward's D linkages. Clusters reflect the dissimilarities (Bray-Curtis) between variance stabilized 16S rRNA OTUs within each sediment sample where shape indicates depth of samples and color ecological region. Bars along the bottom highlight the first four clusters. These clusters highlight differences in ecological region and depth, where bars 1 & 4 respectively separate Canadian Shield (CS) and Western Cornbelt Plains (CB) samples and bar 2 clusters samples from lakes ~20m or deeper. Bar 3 represents the remaining samples from the Northern Lakes and Forests (NLF) and Northcentral Hardwood Forest (NCHF) regions. Map was created using QGIS and data were made available by the MN Geospatial Commons (public domain) https://gisdata.mn.gov/.

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> ~20m) meso-eutrophic systems. After depth, the CB samples cluster and the remaining cluster consists of samples from both the NLF and NCHF. Because the most predominant clusters were based on ecological region, we used a non-parametric multivariate analysis of variance (PERMANOVA) with the four regions as our independent variable and tested for differences in community dissimilarities at the OTU level using the same Bray-Curtis dissimilarity matrix as in the cluster analysis. The results of the PERMANOVA indicate there was an ecoregional difference in composition. To ensure these results reflected distinct groups and not over dispersion within groups, we used vegan's "betadisp" function to test for homogeneity of variance. The resulting insignificant values led us to conclude that community composition is a function of ecoregion and sample sites within these regions are not over dispersed.

Because ecoregion was only a consistent explanatory variable for the more geographically distant ecological regions (CB, CS), we wanted to determine if any of the local physicochemical or watershed land-use factors were also driving community composition. We passively fit these factors to the PCA, treating them as dependent variables explained by the scores from the ordination. Each variable was analyzed separately and added to the plot where the direction of the arrow indicates the gradient direction, and the length indicates the strength of the correlation. After correcting the p-values for multiple comparisons (i.e., Bonferroni), the concentrations for DIC, TP, TN, and Chl-a and lake depth, temperature, specific conductance, and latitude were the most significant local physicochemical factors differentiating the samples. The four land uses include pastured, cultivated, developed, and forested of which only developed, forested, and cultivated lands were significant sorting factors. Forested land use was correlated in the direction of the CS whereas cultivated and urban land-uses were correlated in the opposite direction. Beyond land use, the concentration of DIC and SPC was negatively correlated with samples from lakes in the CS. Finally, the cluster of deep lakes was best explained by a combination of lake depth, temperature, and TP. These are likely related, as the greater depths of these lakes can lead to stronger and prolonged periods of thermal stratification wherein temperatures are around 4˚C and redox conditions of the sediment change to release phosphorus bound to reducible forms of iron.

The results from our study indicate that beta diversity among lake sediment microbiomes is determined by a combination of land use and productivity. These results are consistent with previous studies examining inter-lake microbiome variability across a variety of spatial scales [22, 26, 53, 58, 62]. More specifically, our work parallels findings that nitrogen is a selective variable for community composition and that it covaries with urban and agricultural land use [52]. Beyond nutrients, lake depth is commonly identified as a partitioning factor for microbial communities, as it was in our system [53]. DOC concentrations have also been strongly associated with beta diversity of microbial communities; however, our data do not reflect this trend, potentially highlighting the importance of other micronutrients and abiotic factors for explaining community variation from lake to lake [63]. Importantly, while our findings suggest a combination of productivity measures and land use are drivers of lake sediment microbiome structure, the abiotic measures used to assess these relationships were taken from water column measurements. While surface sediments are suitable for estimating site diversity, the specific vertical abiotic properties of sediments may provide deeper context to the microbiome as a whole [20, 26, 64].

Community composition

From the 40 samples we recovered 55 unique phyla—22 of which were dominant (~90% of the total sample relative abundance) (S7 and S8 Figs). In all samples Proteobacteria were the most abundant, comprising approximately 5–20% of the total population of a given sample. Proteobacteria are often the most abundant phylum in sediment and soil ecosystems given their diverse metabolisms and role in the degradation of organic matter [65–67]. At the class level, samples were predominated largely by Deltaproteobacteria and Gammaproteobacteria and there was no clear pattern in their distribution across ecological or trophic gradients. One exception was the presence and/or high abundance of the order MBNT15 (class Deltaproteobacteria) in CS sediments (Fig 5). While there is little known about the ecological significance of MBNT15, these organisms are obligate anaerobes commonly found in stable sediments and known to reduce nitrate; as such, their presence correlates negatively with rates of nitrogen cycling [68, 69]. Additionally, they have been found to be minor constituents in sediments

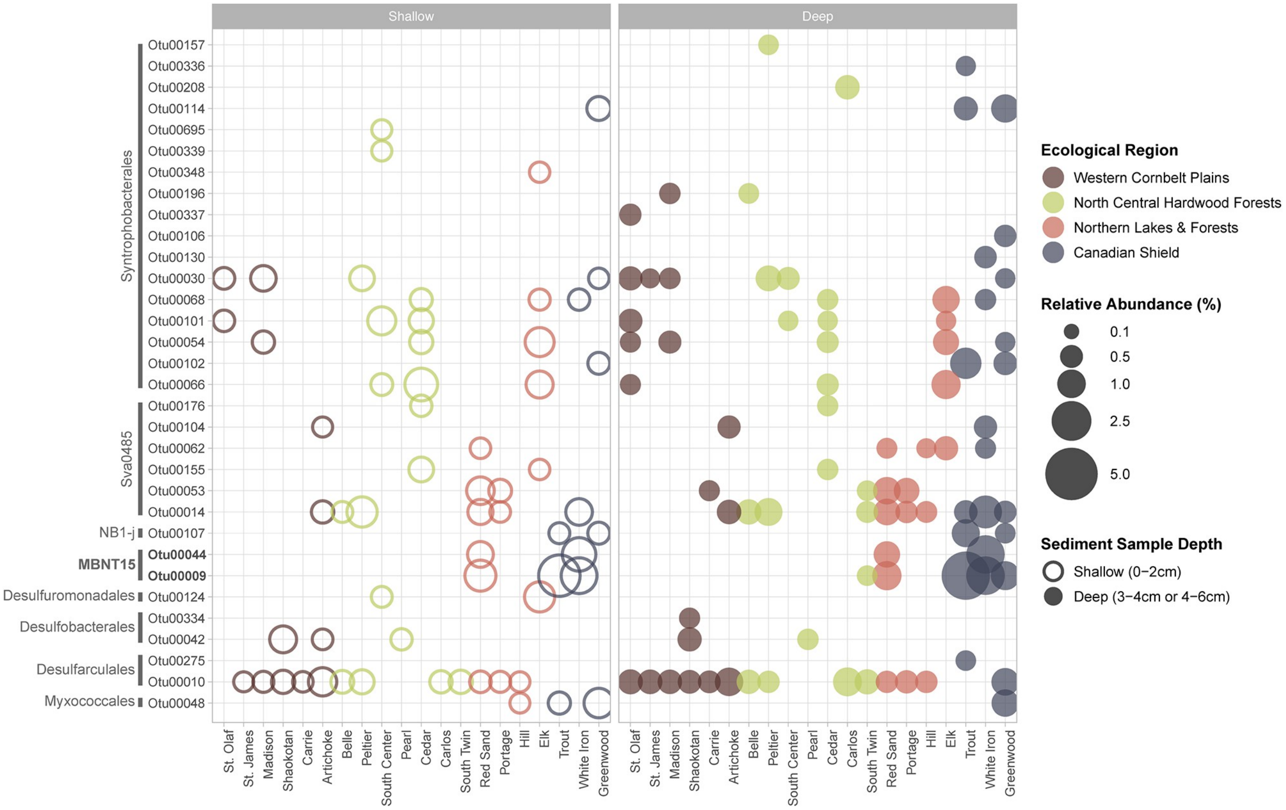

Fig 5. Deltaproteobacteria abundance across samples. Abundance comparison of sediment deltaproteobacterial communities where shape indicates depth of samples, color ecological region, and size the relative abundance in percent. Bars along the left group the OTUs by order. OTUs were selected if they comprised >0.05% of the total relative abundance of the sample. The OTUs bolded are mentioned in the text.

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overburdened with organic matter [70]. Given the physicochemical properties of lakes in the CS region (low DOC, low DOM, and TN) our observations of greater MBNT15 abundance in the CS are not unexpected. Additionally, the presence of orders Syntrophobacterales and Desulfobacterales in several of our shallow (0-2cm) samples indicate active sulfur cycling and the depletion of oxygen in the surface sediment, as the genera within these orders are strict anaerobes [71].

Beyond Proteobacteria, other phyla like Acidobacteria, Actinobacteria, Bacteroidetes, Latescibacteria, Nitrospirae, and Spirochaetes exhibit shifts in abundance based on ecological region (S5 Fig). Latescibacteria, for example, show a subtle increase in abundance moving from the CB to the CS across the NLF and NCHF. The presence of Latescibacteria in all systems is likely due to their ability to degrade several different polymers (proteins, lipids, polysaccharides, fatty acids) and their presence in freshwater sediments supports their proposed role in algal detritus turnover [72]. The increase in abundance as a function of ecoregion may be related to the number of stratified lakes within the CS region, especially given the increase in abundance with sediment depth for most lakes.

Other phyla like Acidobacteria and Bacteroidetes display greater changes in abundance across ecological regions (S5 Fig). For example, Acidobacteria comprise <1% of the total population in CB sediments; however, in CS sediments they can make up as much as 6% of the total population. Acidobacteria, like Proteobacteria, are well distributed across environments and metabolically diverse [73]. In soil systems these bacteria have been shown to partition based on regional land use. Notably, the order subgroup 6 (phylum Acidobacteria) has previously been observed more frequently in pastured regions compared to forested region; however, in our sediment samples these trends do not exist [74]. Subgroup 6 predominates the Acidobacteria population, is found throughout all geographic regions, and is more abundant in White Iron lake—which has one of the most heavily forested watersheds (83%). Bacteroidetes populations exhibit an opposite trend to Acidobacteria—decreased abundance in the CS sediments compared to the CB. Bacteroidetes species specialize in the degradation of organic polymers (e.g., *Bacteroides* in the gut microbiome) [75]. Given their proclivity for polymers over monomers, members of Bacteroidetes are often more abundant in aquatic systems during and shortly after algal blooms [76, 77]. Our data confirm the relationship between Bacteroidetes and productivity, as lakes in the CB region receive excess nutrient loads and often experience algal blooms during the open water season.

In addition to shifts in composition across ecological regions, there were certain phyla that were not observed at our filtering level (OTUs comprising $>0.5\%$ of the total abundance within a sample). Most notably were the absence of Cyanobacteria in CS lake sediments. Land use models have previously been used to partially estimate total cyanobacterial biomass and examine cyanobacterial community structure and genes related to the production of the cyanotoxin microcystin [78, 79]. Our data show similar findings to these previous works in which the populations of toxin-forming members like those found in the order Nostocales (S9 Fig) were more abundant in heavily agriculture and urban settings where they are well adapted to handle higher nutrient levels in the water [80].

Caveats and conclusion

The data obtained during this study provide unique insight into the structure, diversity, and distribution of sediment bacteria across both a trophic and ecological region gradient. A primary caveat is the limited explanation of variance (~30%). While we were able to explain a similar amount of the variability to that observed in other studies, there is still a large amount unexplained [21, 52, 81]. This could be in part due to measurements that were not collected. For example, specific parameters like pH and redox potential at the sediment water interface within each lake would provide greater context for the sediment microbiome structure. Our analysis was also limited to abiotic factors as explanatory variables of alpha and beta diversity for bacterial communities. However, biotic interactions exert a selective force on community structure through a variety of control methods (e.g., grazing, phage infection) [82]. Moreover, the horizontal structuring of sediment communities as well as the overall food web dynamics especially given the differing productivity-diversity relationships could be considered in future studies.

In summary, we examined the lake sediment bacterial communities of 20 lakes to determine the influence of land-use and large-scale land classifications on community structure and diversity. We observed that ecological region with more agricultural land use and greater eutrophication exhibited higher diversity. Likewise, we found that toxin-forming community members were more abundant in heavily agriculture and urban settings. While the ability to connect changes in taxonomic composition using physio-chemical and geographical patterns is possible for some organisms, the limited resolution of short read 16S rRNA data prevent us from detecting specific taxa differences across ecological or trophic gradients.

Many land managers have access to land use maps, and remote sensing is improving our means of evaluating land use in poorly accessible parts of the world. Our results, along with future studies, offer opportunities to connect land use with sediment microbial structure and ultimately to understand lakes' abilities to adapt to anthropogenic changes.

Supporting information

S1 Fig. Batch effect on richness. Observed richness (total number of OTUs) based on the total number of reads recovered per sample where color indicates the sequencing batch. There was a statistically significant Pearson's correlation between the number of total reads and the observed richness; $p < 0.001$ and $R2 = 0.63$.
(TIF)

S2 Fig. Rarefaction curves. Rarefaction curves for all forty samples in the dataset. Where each curve indicates a different sample and the vertical line is the sampling depth of 15,771 reads.
(TIF)

S3 Fig. Alpha diversity measures by sample. Alpha diversity measures across samples, where shape indicates depth of sample and color indicates ecological regions. All measures were calculated using Phyloseq and exhibit similar patterns in diversity; decreasing diversity across a northeasterly transect.
(TIF)

S4 Fig. Bacterial alpha diversity across samples. Observed diversity, a measure of richness, and Shannon diversity, measure of evenness, for all samples where shape indicates the sediment depth, color indicates the ecological region, and sites are ordered based on ecological region then latitude. One sample (Trout, Deep—CS), with lower diversity, was removed for visualization.
(TIF)

S5 Fig. Bacterial alpha diversity across ecological region and depth. Boxplots show mean alpha level diversity of the Observed Operational Taxonomic Units (OTUs) and Shannon indices for the four ecological regions within the study area: Western Cornbelt Plains (CB), North Central Hardwood Forests (NCHF), Northern Lakes and Forests (NLF), and Canadian Shield (CS). Samples are faceted by their sediment depth where Shallow is 0-2cm deep and Deep is 3–4 or 4-6cm deep. One deep, CS sample was removed from alpha diversity metric both plots for due to uncharacteristically low diversity.
(TIF)

S6 Fig. Bacterial alpha diversity by trophic status. Box plots show mean alpha level diversity of the observed Operational Taxonomic Units (OTUs) and Shannon indices of the four trophic status classifications within the study area: Oligotrophic, Mesotrophic, Eutrophic, and Hypereutrophic. Each point represents a given sample where shape indicates depth of sample and color indicates the trophic status. One sample with extremely low richness and diversity was removed from both plots for visualization. Significance between regions was calculated nonparametrically using a Kruskal Wallis H test followed by a Dunn post hoc test with a Bonferroni correction. Reported p-values indicate significant differences in Observed and Shannon diversity, respectively, across trophic status, specifically the diversity of Oligotrophic lake sediments when compared to Eutrophic ($p = 0.0224$) and Hypereutrophic ($p = 0.0038$ & $p = 0.013$) sediments.
(TIF)

S7 Fig. Bacterial relative abundance. Bar plots of phyla that comprise >1% of the total relative abundance of a given sample. Samples are sorted along the X axis by ecological region.
(TIF)

S8 Fig. Bacterial abundance at the phylum level across ecological regions. Box plots show mean relative abundance for the phyla across ecological region. Each point is a sample.

Abbreviations: Western Cornbelt Plains (CB), North Central Hardwood Forests (NCHF), Northern Lakes and Forests (NLF), and Canadian Shield (CS).
(TIF)

S9 Fig. Cyanobacterial abundance across samples. Abundance comparison of sediment cyanobacterial communities where shape indicates depth of samples, color ecological region, and size the relative abundance in percent. Bars along the left group the OTUs by order. OTUs were selected if they comprised >0.01% of the total relative abundance of the sample.
(TIF)

S1 Table. Description of ecological regions.
(XLSX)

S2 Table. Aqueous chemistry data.
(XLSX)

S3 Table. Sediment extraction, DNA quality, and read depth data.
(XLSX)

S4 Table. Alpha diversity measures by sample. Values for all alpha diversity measure calculated by sample.
(XLSX)

S5 Table. Kruskal Wallis P-values for phyla richness across ecological regions and trophic status. Table of Kruskal Wallis p-values for individual phyla within the alpha diversity dataset (rarefied). P-values for both the significance based on ecological region and trophic status are reported and bolded values are significant at $p = 0.001$. Dunn post hoc test with Bonferroni correction p-values are reported for those KW $p<0.001$. Phyla below the bolded line are phyla that comprise of <1% of the total relative abundance of a given sample (see S4 Fig).
(XLSX)

Acknowledgments

The authors acknowledge the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported within this paper. Michele Natarajan, Erin Mortenson, and Alaina Fedie of the St. Croix Watershed Research Station for their coordinated laboratory analyses.

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