Journal of Freshwater Ecology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tjfe20

An assessment of N, P, Fe, Zn, Ni and Mo limitation on suspended nutrient diffusing substrates in nearshore areas of Lake Michigan and Lake Erie

James H. Larson, David M. Costello, Jordyn T. Stoll, Andrea S. Fitzgibbon, Sean W. Bailey & Mary Anne Evans

To cite this article: James H. Larson, David M. Costello, Jordyn T. Stoll, Andrea S. Fitzgibbon, Sean W. Bailey & Mary Anne Evans (2024) An assessment of N, P, Fe, Zn, Ni and Mo limitation on suspended nutrient diffusing substrates in nearshore areas of Lake Michigan and Lake Erie, Journal of Freshwater Ecology, 39:1, 2405748, DOI: 10.1080/02705060.2024.2405748

To link to this article: https://doi.org/10.1080/02705060.2024.2405748

Published online: 15 Oct 2024.

JOURNAL OF FRESHWATER ECOLOGY
2024, VOL. 39, NO. 1, 2405748
https://doi.org/10.1080/02705060.2024.2405748

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

An assessment of N, P, Fe, Zn, Ni and Mo limitation on suspended nutrient diffusing substrates in nearshore areas of Lake Michigan and Lake Erie

James H. Larson[a], David M. Costello[b], Jordyn T. Stoll[b,c], Andrea S. Fitzgibbon[b], Sean W. Bailey[a] and Mary Anne Evans[d]

[a]U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI, USA; [b]Department of Biological Sciences, Kent State University, Kent, OH, USA; [c]Michigan Trout Unlimited, Dewitt, MI, USA; [d]U.S. Geological Survey, Great Lakes Science Center, Ann Arbor, MI, USA

ABSTRACT

In large lakes, metal availability sometimes limits the acquisition of nutrients (nitrogen, N and phosphorus, P) in offshore waters that are relatively isolated from tributaries and sediments. We hypothesize that metals may also be important within harmful algal blooms (HABs). HABs occur where nutrient loads are elevated, but bioassays often indicate that phytoplankton in HABs are N or P limited. Nutrient limitation may be exacerbated by corresponding limitations in several metals (i.e. nickel - Ni, molybdenum - Mo, zinc - Zn, and iron - Fe) that facilitate uptake and transformation of oxidized and organic forms of nutrients, such as urea, nitrate and organic phosphorus. The cyanotoxin microcystin has been hypothesized to have a role in metal management, so metal demand may also influence the toxicity of HABs. Here, we used nutrient diffusing substrates to measure how N, P, Ni, Mo, Zn and Fe amendments influenced the growth and toxicity of periphyton. Periphyton was grown suspended in 10 nearshore sites in Lake Michigan and Lake Erie (5 with and 5 without perennial HABs). Outside of blooms, we found no evidence for metal limitation or co-limitation. However, evidence for metal co-limitation was observed in two HABs sites (Zn in Green Bay and Zn, Mo, Ni and Fe in Sandusky Bay). N, P and Zn amendments all stimulated microcystin content in Maumee Bay. These data indicate that nutrient limitation occurs even within blooms, and the availability of metals may have an influence on growth, community composition and toxicity.

ARTICLE HISTORY
Received 25 April 2024
Accepted 12 September 2024

KEYWORDS
Harmful algal blooms; microcystin; metals; nutrients; co-limitation

CONTACT James H Larson jhlarson@usgs.gov U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI, USA.

Introduction

Many elements are necessary for growth and development in algae and other taxa (Kaspari and Powers 2016), but within freshwater ecosystems two elements have received the vast majority of research interest: nitrogen (N) and phosphorus (P). This focus is due to the relative scarcity of these elements in bioavailable forms, compared to their importance in biological processes (Sterner and Elser 2002). The bioavailability of N and P have long been suspected of driving many attributes of freshwater ecosystems from organismal behavior to ecosystem structure (Schindler and Fee 1974; Sterner and Elser 2002; Heisler et al. 2008; Schindler 2012). Often this focus on just one or two nutrients has been justified by emphasizing the single-nutrient limitation paradigm (a result of Liebig's Law of the Minimum), which suggests only an increase in the single limiting nutrient (or resource) will lead to increases in growth rate. However, co-limitation occurs frequently, and may be the more common condition in aquatic ecosystems (Elser et al. 2007; Harpole et al. 2011). Harpole et al. (2011) identified several conceptual forms of co-limitation (see Box 1 in Harpole et al. 2011): 1) Simultaneous co-limitation (when two elements are both needed to increase growth), 2) Independent co-limitation (when two elements independently increase growth, and combinations may be additive [N + P effect is the N effect plus the P effect], super-additive [N + P effect is greater than the N effect plus the P effect] or sub-additive [N + P effect is less than the N effect plus the P effect]) and 3) Serial limitation (when one element increases growth, but growth is further increased by the addition of a second nutrient). Of these, serial limitation is the natural progression from the single limiting resource paradigm, but simultaneous and independent co-limitation, which do not conform with Liebig's Law of the Minimum, seem to be common (Elser et al. 2007; Harpole et al. 2011).

Within the literature on nutrient co-limitation, most of the focus has been on N and P, but other elements are essential in ways that seem likely to result in co-limiting relationships with N and P. For example, many bloom-forming cyanobacteria prefer reduced inorganic N, and may be at a competitive advantage over eukaryotic algal taxa when reduced inorganic N is available (Oliver et al. 2012; Glibert 2017). When reduced inorganic N is only available at low concentrations or absent, cyanobacteria (and other algae) activate metabolic pathways that allow them to use other forms of N such as urea, nitrate and N_2 (Oliver et al. 2012). These metabolic pathways require trace metals such as Fe, Mo and Ni (Rees and Bekheet 1982; Glass et al. 2012), thus establishing a physiological mechanism by which co-limitation could occur. Similarly, when dissolved PO_4^{3-} is not available, algae have a greater demand for Fe and Zn (or Ca in the case of some cyanobacteria) to acquire P from organic P complexes (Morel and Price 2003; Bertini et al. 2007). Because these metals effectively allow cyanobacteria to access less labile forms of N and P, metals have the potential to co-limit (with N or P) cyanobacterial growth (Figure 1). For example, if ammonium and Fe are absent, but nitrate and P are abundant, then primary producer growth may increase with the addition of ammonium, but would also increase with the addition of Fe (allowing cyanobacteria to access nitrate). Fitzgibbon and Costello (2023) observed that supplying metals seemed to partially alleviate growth limitation of biofilms in several tributary streams to Lake Superior and Lake Erie. Similarly, nitrate uptake was limited by Fe availability in Lake Superior (Ivanikova et al. 2007) and Lake Erie (Havens et al. 2012). Using the co-limitation terminology of Harpole et al. (2011), observed limitations in Lake Superior and Lake Erie would manifest as independent co-limitation.

Within large-lake ecosystems such as the North American Great Lakes, trace metal and Fe limitation have been studied where phytoplankton may become isolated from

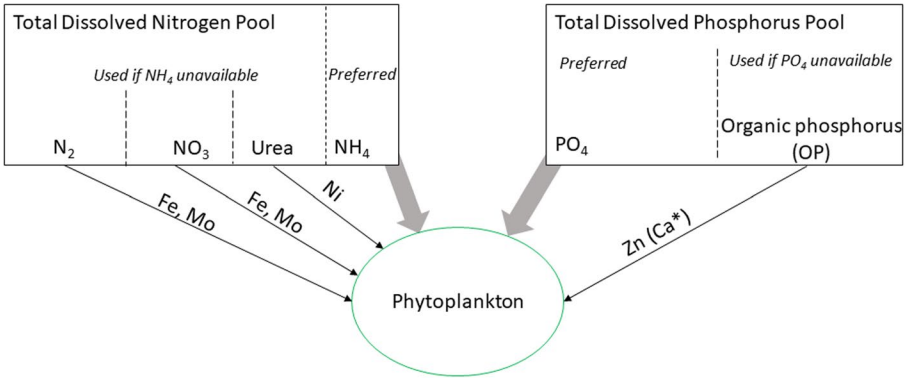

Figure 1. Conceptual model showing the hypothesized co-limitation relationships between preferred forms of nitrogen (N; NH_4) and phosphorus (P; PO_4) and the metals needed to acquire non-preferred forms of N (N_2, NO_3, urea) and P (organic P). For example, if NH_4 is absent, but NO_3 is available, then Fe and Mo would be needed to assimilate the N from NO_3. *some cyanobacteria taxa acquire OP using an enzyme that uses Ca instead of Zn.

sources of these elements (e.g. in stratified offshore waters) (Sterner et al. 2004; Ivanikova et al. 2007; North et al. 2007). Stratified offshore waters are thought to have metal limitation due to a lack of re-supply from sediments and tributary inputs. Nearshore areas, by contrast, are thought to be less likely to be limited by metals due to consistent riverine loading and periodic resuspension of sediment (Ivanikova et al. 2007; North et al. 2007).

Although nearshore areas presumably have much greater metal supply than open, stratified waters of the Great Lakes, biotic demand for nutrients and metals is often much greater as well. The open waters of many Great Lakes are relatively oligotrophic (Evans et al. 2011), whereas some nearshore areas have remained or become increasingly eutrophic and prone to harmful algal blooms (Kane et al. 2014). Whenever demand for N and P cannot be met by the supply of NH_4 and PO_4 in the water column, demand for metals will increase so that other forms of N and P can be acquired (Figure 1; Fitzgibbon and Costello 2023). Short-term bioassays on communities inside blooms often observe N or P limitation when adding NH_4 or PO_4 (Chaffin et al. 2013, 2014; Davis et al. 2015; Harke et al. 2016), indicating that these labile forms of N and P become limiting during blooms. As a result, demand for metals should also be greater as phytoplankton scavenge for any source of N or P. In smaller lakes, this increase in demand has been observed as declines of Fe concentrations within a bloom (Leung et al. 2021). Whether this increase in demand will result in metals co-limiting growth will depend on the availability of metals within the bloom. Metal co-limitation with P occurred but was uncommon in bottle experiments by Chaffin et al. (2020), which targeted mostly off-shore blooms in central Lake Erie, but Fe and boron (B) did appear to influence the taxonomic composition of the bloom and the formation of heterocytes in diazotrophic taxa. Larson et al. (2023) performed short-term bioassays targeting nearshore blooms in Green Bay (Lake Michigan) and Maumee Bay (Lake Erie), but did not observe strong growth effects from a metal treatment (including Fe, Ni, Mo, and Zn).

We hypothesize that phytoplankton demand for metals increases enough in nearshore HABs that metals become co-limiting with N and P. If this hypothesis is true, then we would expect to see periphyton communities suspended in the bloom to also experience metal and N or P co-limitation. Specifically, when less preferred forms of N and P are available, N-limited or P-limited communities will be growth-stimulated by the addition

of appropriate metals (Ni and Mo co-limiting with N, Zn co-limiting with P). Fe is an important metal for many functional roles in phytoplankton, so while we hypothesize it could also become limiting, it is equally likely to be co-limiting with N or P.

In addition to potential metal effects on growth limitation, metal availability could also be linked to cyanotoxin production. The most commonly observed class of cyanotoxins in North American lakes are the microcystins (Loftin et al. 2016). Microcystins (MCs) are cyclic heptapeptides that are potent liver toxins in mammals (Metcalf and Codd 2012; Meriluoto et al. 2016). The origin of MCs is ancient and their original function is still debated (Rantala et al. 2004; Omidi et al. 2018). One hypothesized functional role is to manage biologically important metals, either internally or externally (Utkilen and Gjølme 1995; Omidi et al. 2018). Support for this hypothesis includes evidence that Fe limitation triggers MC production (Sevilla et al. 2008), MC forms complexes with some important trace metals (Klein et al. 2013; Ceballos-Laita et al. 2017) and MC prevents the loss of photosynthetic function in experimental low Fe conditions by storing Fe (Alexova et al. 2011; Ceballos-Laita et al. 2017). Additionally, experimental evidence indicates that during N stress, cyanobacteria may upregulate their production of MCs (Ginn et al. 2010). We hypothesize that high demand for metals by cyanobacteria during blooms would lead cyanobacteria to increase their production of microcystin to assist with internally managing metals. We predict that for periphytic cyanobacteria suspended in the blooms, additions of a labile form of the limiting nutrient would reduce the metal demand, and thus reduce MC content. However, given the uncertainty about the functional role of MC, we are also interested in establishing that triggers of cyanobacterial growth are not always the same as the triggers of MC production (Zurawell et al. 2005).

Here, we used nutrient diffusing substrates (Capps et al. 2011; Costello et al. 2016; Fitzgibbon and Costello 2023) suspended in the water column to manipulate the metal and nutrient environment experienced by periphyton growing on fritted glass disks. Treatments included NH_4^+ and PO_4^{3-} in a fully factorial design, and we then added treatments that replaced NH_4^+ with Ni and Mo (to access ambient urea and nitrate) and replaced PO_4^{3-} with Zn (to access ambient organic P). Fe, which is used in a variety of metabolic processes, was included as a separate treatment, along with an Fe + N treatment. Nutrient diffusing substrates are measures of periphyton rather than phytoplankton, but suspending the substrates in the water column exposes them to similar nutrient and metal conditions as phytoplankton. The primary advantage of the nutrient diffusing substrates is that they sustain nutrient enrichment *in situ* over longer time periods (weeks) than microcosms (hours to days) (Davis et al. 2015; Larson et al. 2023), which allows us to examine the primary producer response to experimental treatments within the context of naturally varying environmental conditions with less concern for bottle effects.

Methods

Study sites

Nutrient diffusing substrates were deployed at 10 sites in Lake Michigan and Lake Erie (Table 1 and Figure 2) during late summer 2017. Sites were selected across a spectrum of eutrophication and habitat conditions. Since blooms in the Great Lakes frequently occur in nearshore areas near tributary inputs (e.g. Green Bay, Maumee Bay, Sandusky Bay), we selected sites from among areas experiencing frequent blooms and nearby nearshore areas adjacent to tributary inputs not experiencing recurring blooms. In Lake Michigan, bloom sites were selected from within the mouth of the Fox River and immediately outside the mouth of the Fox River in Green Bay (Figure 2). The Fox River drains an agriculturally

Table 1. Site locations and mean chlorophyll *a* content (±1 standard deviation), mean microcystin content per disk and mean microcystin per chlorophyll *a* in the control treatments.

Site Label	Latitude	Longitude	Deploy date	Retrieval date	Chlorophyll *a* (ug per cm^2)	Microcystin (µg per cm^2)	Cyanobacterial (proportion of cell count)
Fox River mouth	44.45296	−88.07471	8/2/2017	8/21/2017	5.51 (1.76)	0.10 (0.016)	0.67
Green Bay	44.54241	−87.99411	8/2/2017	8/21/2017	1.32 (0.78)	0.03 (0.02)	0.81
Ford 1	45.67359	−87.13923	8/2/2017	8/22/2017	5.73 (2.79)	BD	0.11
Ford 2	45.66937	−87.13384	8/2/2017	8/22/2017	3.35 (1.56)	BD	0.26
Maumee Bay 1	41.74616	−83.44367	8/1/2017	8/23/2017	16.23 (2.49)	NA	0.96
Maumee Bay 2	41.70788	−83.45702	8/1/2017	8/24/2017	14.98 (2.13)	0.003 (0.004); 1 BD	0.34
North Erie 1	41.96390	−83.16373	7/31/2017	8/23/2017	3.46 (1.62)	0.007 (0.010); 1 BD	0.35
North Erie 2	41.89411	−83.23513	7/31/2017	8/23/2017	0.34 (0.25)	0.12 (0.17); 1 BD	0.96
Sandusky Bay	41.47351	−82.80514	7/31/2017	8/24/2017	4.69 (2.99)	0.04 (0.02)	0.60
Old Woman Creek	41.38007	−82.51225	7/31/2017	8/25/2017	5.03 (1.71)	BD	0.42

BD = below the detection limit. NA = only 1 sample was collected and therefore these statistics could not be estimated.

dominated watershed and the lower Fox River and Green Bay experience annual cyanobacterial blooms of varying intensity. For comparison, we selected two sites in the nearshore adjacent to the Ford River, which drains a forested watershed with little agriculture and is a tributary on the northern edge of Green Bay (Figure 2). In previous studies, low concentrations of chlorophyll *a* and nutrients were measured in the Ford River mouth (Larson et al. 2016a). In Lake Erie, we selected two sites in the Maumee Bay as more eutrophic sites and two sites in northern Lake Erie (closer to the mouth of the Detroit River) as less eutrophic sites. Although the Detroit River supplies a large overall load of nutrients to Lake Erie, that load is delivered in a large volume of water and thus diluted to a low concentration. Nutrient concentrations and bloom intensity decrease across a gradient from the Maumee River mouth to the Detroit River mouth in Lake Erie (Larson et al. 2020; Rowland et al. 2020). We selected two other sites at the mouth of tributaries to Lake Erie (both in Ohio): a site in Sandusky Bay, which is highly productive and at the time of study was dominated by *Planktothrix agardhii* blooms (Wagner et al. 2024); and a site in the protected Old Woman Creek estuary, which drains an agricultural landscape (Herdendorf et al. 2006).

Experimental design

The nutrient diffusing substrates (NDS) were ~40 mL plastic cups filled with 2% agar, covered with a small (~4 cm^2) fritted glass disk on which primary producers grow. The agar is combined with nutrient and/or trace metals, which over the course of three weeks diffuse into the water column and fertilize the area immediately adjacent to the surface of the disk (Costello et al. 2016). Estimating the nutrient diffusion from the agar into the water immediately adjacent to the surface of the disk requires simplifying assumptions that could be inaccurate if water movement over the surface of the disk was much greater than anticipated, but we assume differences in water movement among replicates at the same site would be minimal. As in a previous study in which nutrient diffusing substrates were assembled by the Kent State University research team (Fitzgibbon and Costello 2023), metal-conscious conditions were maintained during assembly: Surfaces and containers coming into contact with materials being assembled were acid washed and triple-rinsed

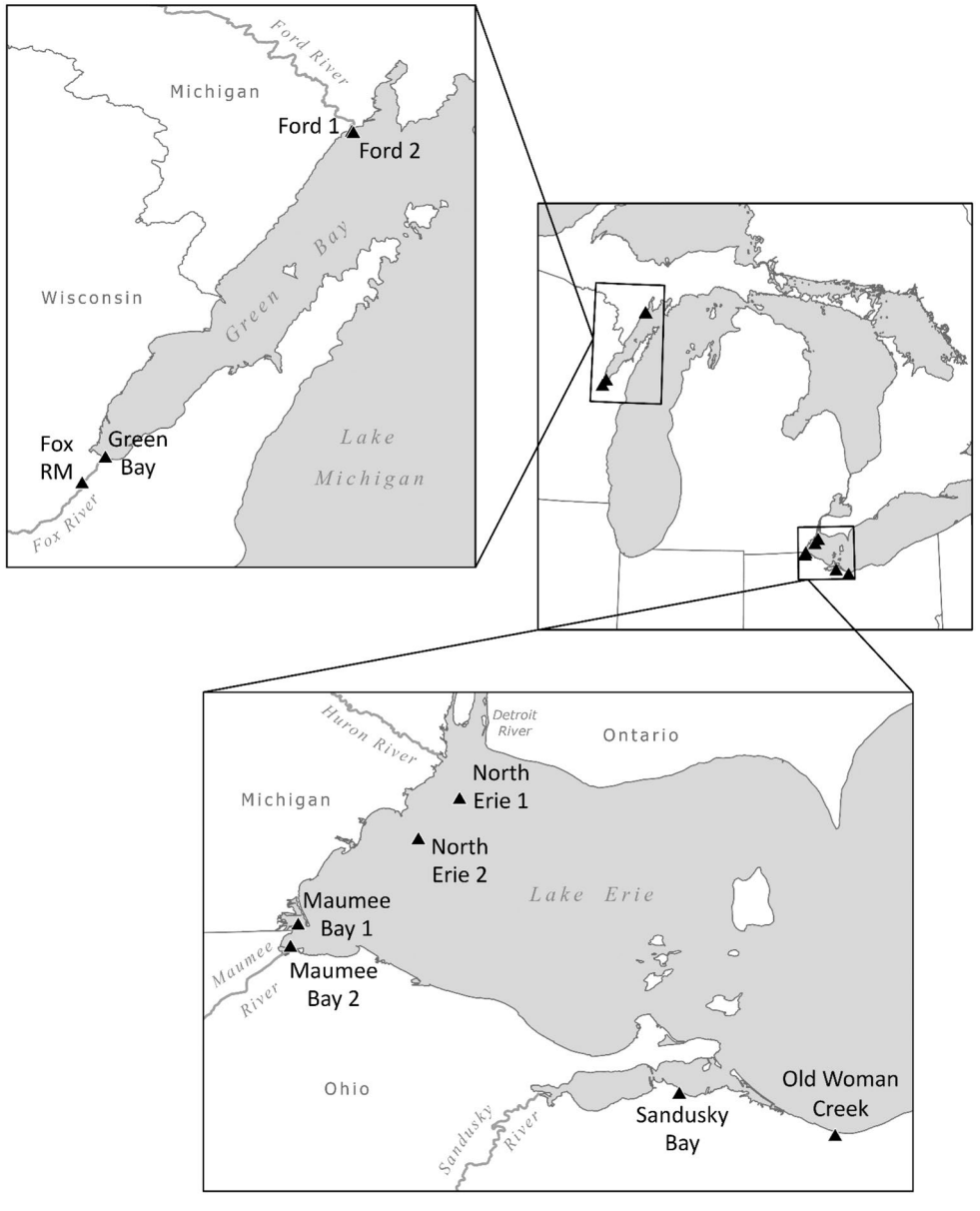

Figure 2. Locations where nutrient diffusing substrates were deployed during the summer of 2017. RM refers to river mouth. Base map from ESRI and its licensors, copyright 2022.

with purified water (Milli-Q; Millipore Sigma, Burlington, MA). However, agar metal concentrations were much higher than trace levels (80–800 µM) to maintain the low-concentration enrichment for the duration of the deployment. Trace metal-clean techniques were not used during cup preparation because trace amounts of metal contamination would be unlikely to influence the treatments. Treatments included a control with unamended agar and nine nutrient treatments of $+NH_4^-$ (N), $+PO_4^{3-}$ (P), +Zn, +Ni & Mo, +Fe, +N & P, +N & Zn, +P & Ni & Mo, and +N & Fe. Doses of all nutrients and metals were adjusted to match concentrations present in COMBO media (Kilham et al. 1998) according to diffusion equations in Costello et al. (2016). Actual concentrations in the agar

were: NH_4Cl = 268.641 moles per L; KH_2PO_4 = 9.381 moles per L; $FeCl_3$ = 737 micromoles per L; $MnCl_2$ = 582 micromoles per L; $NiCl_2$ = 77 micromoles per L; ZnSO4 = 171 micromoles per L; Na_2MoO_4 = 168 micromoles per L. One replicate of each treatment was fixed on a plastic L-bar (blocked by depth) and 4 L-bars were each screwed onto one side of a square bar (see images in Figure S1). Each bar had every treatment in equal numbers, so the differences in bar orientation should not influence the treatment effects. At the Fox Rivermouth, Sandusky Bay, Old Woman Creek, both Maumee Bay sites and Ford 1, a total of 8 replicate bars of NDS cups were deployed vertically in the water column ~1-m below a pair of surface buoys (buoys were separated by about 3 m to avoid entanglement). At the remaining sites, NDS were submerged by ~2 m (Green Bay, Ford 2) or up to ~5 m (North Erie 1, North Erie 2) to avoid creating a navigation hazard, and retrievals were facilitated by connecting a line between the two buoys that was ~7.5 m in length (i.e. similar to deployments in Larson et al. (2016b). Five replicates were sampled for chlorophyll *a* and ash-free dry mass (AFDM), two replicates were sampled for microcystin, and the remaining replicate was sampled for community composition. Half of the Maumee Bay 1 nutrient diffusing substrates were lost during deployment, and therefore one replicate was used for community composition, one for microcystin concentration and two replicates were used for chlorophyll *a*. NDS were deployed starting July 31 and retrieved by August 25, 2017 (~3 weeks; Table 1). We opted for 3-week deployments because that is in the same range as other studies (Capps et al. 2011; Costello et al. 2016; Fitzgibbon and Costello 2023), and a pre-study trial run conducted in 2015 found that 2–4 week deployments in Lake Erie did not accumulate so much biofilm mass that sloughing would occur.

Field dissolved elemental analysis

Surface water-quality data were collected at 4 of the 10 sites as part of a related experiment. Ford 1 and Green Bay were sampled on August 2, 2017 using a pump to collect ~20 L of whole water from ~1 m below the surface. This water was shipped overnight to Kent State University, for processing as described below. On August 23, 2017, 20 L of whole water from Maumee Bay and North Erie 2 were each collected using a pump from ~1 m below the surface. This water was immediately brought back to Kent State University for processing.

At Kent State University, water was filtered through a 0.45-µm glass-fiber filter and either preserved using trace metal grade nitric acid for dissolved metal concentrations (Fe, Mo, Ni, Zn) or frozen for nutrient analysis (nitrate + nitrite, ammonium, total dissolved nitrogen, soluble reactive phosphorus and total dissolved phosphorus). Metals were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Perkin Elmer Optima 8000; PerkinElmer, Inc., Waltham, MA). The detection limits for metals were Fe = 2 µg/L, Mo = 10 µg/L, Ni = 10 µg/L, Zn = 4 µg/L. These detection limits are likely high relative to demand at many sites, although they have been sufficient in previous work (Fitzgibbon and Costello 2023). Nutrients were analyzed using a San++ (Skalar – Analytical B.V., Breda, The Netherlands) nutrient analyzer for total dissolved P (detection limit (DL) = 8 µg/L), soluble reactive P (SRP; DL = 4 µg/L), nitrate/nitrite (NO_X; DL = 5 µg/L), total dissolved N (DL = 35 µg/L), and ammonia (NH_4; DL = 5 µg/L; (Rice et al. 2012).

Chlorophyll a and AFDM

Disks for chlorophyll *a* and AFDM were removed from the NDS cups and frozen in individually labeled polyethylene bags; 20 mL of ethanol was added to each bag, and samples extracted overnight at 4 °C. Absorbance of chlorophyll *a* was measured on a

spectrometer with an acid correction for pheophytin and turbidity (Steinman et al. 2017). The disk and extractant solution were then placed in a shallow pan and ethanol was allowed to evaporate overnight at room temperature. The mass of pan and contents were weighed, combusted for 3 h at 550 °C, and reweighed to determine AFDM (difference in mass). In <1% of cases chlorophyll *a* had a slightly negative value, indicating values below our detection limit. Since this reflected a very small fraction of our data, we simply set this value equal to zero, since the bias introduced by such a small fraction is unlikely to affect inference (Zhang et al. 2004).

Community composition

Community composition samples were scraped from the disks with a stiff brush, rinsed with Milli-Q water, and preserved with Lugol's Iodine until the sample color resembled a strong black tea (Steinman and Mulholland 2006). To assess community composition, samples were homogenized, and 0.1 mL subsampled into a plankton-settling chamber with an additional 1 mL Milli-Q water added to dilute the sample enough so that cells did not overlap when settled. An Olympus IX81 inverted scope was used to count at least 400 natural units per sample, identifying each unit to the lowest practical taxonomic level (usually genus or family; Utermöhl 1931). A natural unit is a single cell or colony, with the number of cells within each colony counted or estimated.

Microcystin

To remove biofilm material for microcystin analysis, we followed methods described above, except that biofilm material was rinsed with a measured volume of Milli-Q water (usually <100 mL) into a pre-labeled 200 mL PETG bottle (1 bottle per disk) minimizing the total volume of added water. Large invertebrates (predominantly chironomids) were removed with forceps, but these occurred infrequently. Samples were then frozen in the field and kept frozen until they were returned to the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI for analysis. At UMESC, samples were put through three freeze-thaw cycles, before being filtered (0.45 GF/F filter) and two analytical replicates from each bottle were analyzed with a microcystin ELISA kit (ADDA kit, Abraxis ©, Warminster, PA). When kits failed the manufacturer-identified quality control tests, the analysis was repeated. Any samples with concentrations above 2.0 μg L^{-1} were diluted and re-analyzed so that measurements were between 0.1 and 2 μg L^{-1}. The raw absorbance data were processed using the approach described by Qian et al. (2015) to generate median estimates from each sample (with 95% credible intervals). UMESC microcystin data is available online with additional details about the methods (Larson et al. 2024b).

Statistical analysis

All analysis was done in R (R Development Core Team 2022). Simple correlation coefficients and descriptive statistics were performed using standard base R functions. Correlation coefficients were estimated using Kendall's method (τ), which is a non-parametric method that minimizes the influence of outliers and does not require distributional assumptions (Helsel 2005).

Treatment effects on chlorophyll *a* were estimated for each site using the approach developed by Harpole et al. (2011). For single addition treatments (i.e. N, P, Fe, Zn, and

Ni + Mo), natural log response ratios (*RR*) were calculated by dividing the treatment chlorophyll *a* content by the control chlorophyll *a* content, and then taking the natural log of the resulting ratio. On the log scale, $RR < 0$ indicate declines relative to the control and $RR > 0$ indicate increases relative to the control. We then estimated the interaction response ratio (*I*) for combined treatments (i.e. N + P, N + Zn, N + Fe, P + Ni + Mo) using equation 5 from Harpole et al. (2011):

$$I = \left(\left(T_{COMB} + T_{CTRL}\right) - \left(T_1 + T_2\right)\right) / T_{CTRL}$$

Where T_{COMB} is the biomass from the combined treatment, T_{CTRL} is the biomass from the control, and T_1 and T_2 are biomasses of treatments alone. As recommended, response ratios for interactions were not log-transformed because that would obscure multiplicative relationships (Harpole et al. 2011). Harpole et al. (2011) used a meta-analysis of 124 studies to show that an effect size of 1.385 (or 0.33 on the natural-log scale) was analogous to a p-value of 0.05, so we used the same effect size threshold here. For cases with an $I > 1.385$, we used the flow-chart in Harpole et al. (2011; supplemental material) to classify the type of limitation (i.e. serial, independent, simultaneous).

In addition to using the thresholds identified by Harpole et al. (2011) for response ratios, we performed a statistical analysis using just our data (Larson et al. 2024a). This consisted of a multi-factor generalized linear model (glm function in R) with depth position as a factor, along with individual amendments as factors, and interactions where we had appropriate data. Factors were P (none or amended), N (none or amended), Zn (none or amended), Ni + Mo (none or amended), Fe (none or amended) and depth (to account for variation in light availability). Multi-element amendments described in the 'Experimental design' section above were modeled as interactions. Response variables were scaled, but not otherwise transformed because residual data did not indicate that a transformation was required. We calculated a 95% confidence interval around the effect sizes for all these factors, including depth to assess whether light (depth) had a significant impact that might confound nutrient treatments. If there was a strong depth effect (depth effect interval did not overlap zero), then we considered the *RR* described above to be an unreliable estimates of treatment effects (this only happened at one site, Maumee Bay 2). Other than assessing this depth effect, we did not use this analysis for interpreting the experimental data.

Microcystin content was measured on fewer disks than chlorophyll *a* and could not be blocked by depth as effectively. However, we were interested in a preliminary estimation of elemental amendment effects on microcystin content. To accomplish this, we used a generalized linear model to model the relationship between elemental amendments and log microcystin content (per chlorophyll *a*). This model included all nutrient treatments described above, but did not include depth, as the number of predictors was too large for the available sample size. For all amendments, if the effect size's 95% confidence interval did not include zero, then we assumed that was a strong effect.

Results

Ambient and control conditions

Mean chlorophyll *a* concentration in the controls was highest in the two Maumee Bay sites and lowest in North Erie 2 and Green Bay (Table 1 and Figure 3a). Cyanobacteria (collectively) made up between 11 and 96% of the cells counted in control treatments, but there was no relationship between cyanobacterial proportion and chlorophyll *a* content in the controls (Figure 3b). Ash free dry mass (AFDM) was highest in the Maumee Bay and

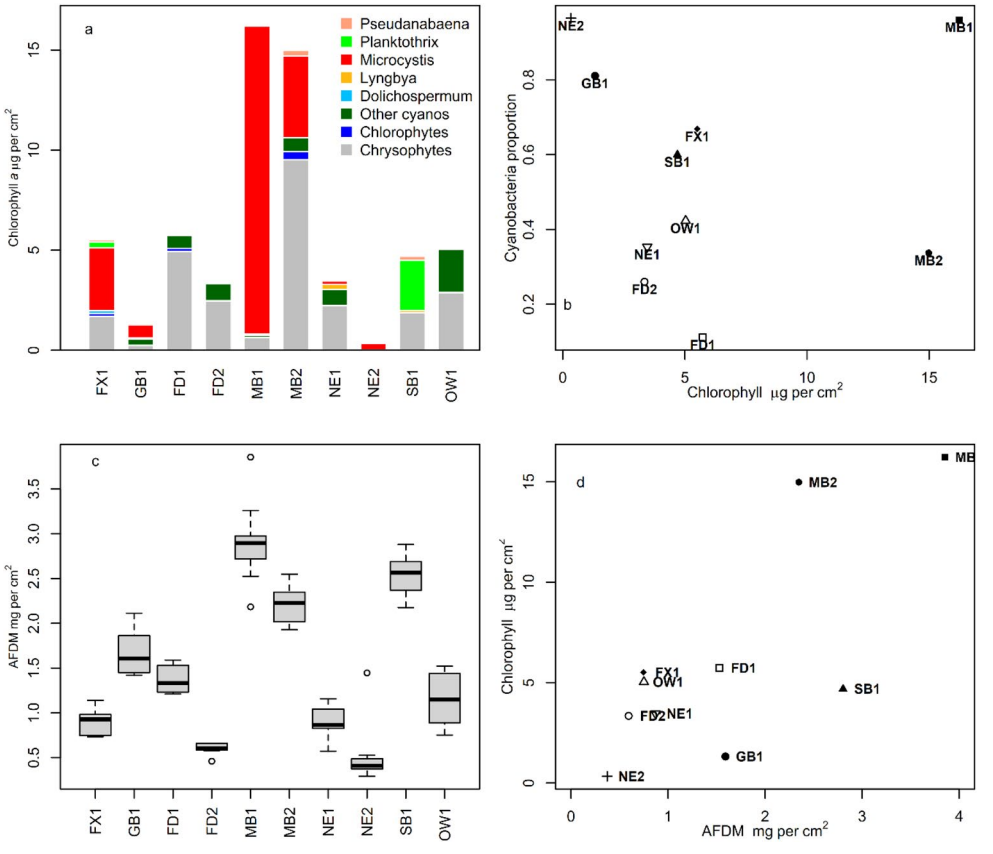

Figure 3. Mean areal chlorophyll *a* content, with divisions indicating the proportion of algal cells that are derived from different taxa (a), occurring on control substrates deployed in 2017 for two weeks in nearshore areas of Lake Michigan (LM) and Lake Erie (LE). Bi-variate plot relating the proportion of algal cells that were cyanobacterial to the chlorophyll *a* content (b). Box and whisker plot of areal ash free dry mass (AFDM) content on the control substrates (c). Bi-variate plot relating the chlorophyll *a* content to the AFDM content (d). Site labels refer to: FX1 – Fox River mouth (LM), GB1 – Green Bay (LM), FD1 – Ford 1 (LM), FD2 – Ford 2 (LM), MB1 – Maumee Bay 1 (LE), MB2 – Maumee Bay 2 (LE), NE1 – North Erie 1 (LE), NE2 – North Erie 2 (LE), SB1 – Sandusky Bay (LE), OW1 – Old Woman Creek (LE).

Sandusky Bay sites, and lowest at Ford 2 and North Lake Erie 2 (Figure 3c). Observed cyanobacteria genera included *Planktothrix*, *Microcystis*, *Pseudanabaena*, *Dolichospermum*, *Lyngbya*, *Gloeocapsa* and *Merismopedia*. Taxa known to include toxin-producing strains were observed at every site, but were observed in low proportions at sites with few cyanobacteria (e.g. Ford 1 and North Erie 1). Taxa known to include N-fixing strains were positively identified at 8 of the 10 sites, with the only exceptions being Ford 2 and Old Woman Creek. The majority of Old Woman Creek cyanobacteria were an unbranched filamentous cyanobacteria that could not be confidently identified (likely *Pseudanabaena* spp.). Biofilm chlorophyll *a* concentrations were moderately correlated with AFDM on all the disks (Kendall's $\tau = 0.41$; Figure 3d). Since our hypotheses were about the periphyton community, we limited our analysis of growth to the chlorophyll *a* data.

Green Bay and Maumee Bay both had detectable water column SRP, while North Erie and Ford had SRP below our detection limit (4 µg/L; Table S1). NO_X was much higher in the two Lake Erie sites than the two Lake Michigan sites. Ni and Mo were below our detection limits at all sites, and Zn was below our detection limit at all these sites except for Green Bay. Molar TDN:TDP ratios were >90 for all sites and the DIN:SRP ratio was

very high for Maumee Bay (341 DIN:SRP). However, DIN:SRP for Green Bay was much lower than other sites (19.4). At every site, there were sources of dissolved N or P beyond what was available in the preferred forms (NH_4 and PO_4; Table S1).

Treatment effects on chlorophyll a content

Using the threshold developed in Harpole et al. (2011) to identify biologically important treatment effects, co-limitation occurred at 3 sites (Green Bay, Sandusky Bay and Old Woman Creek; Table 2 and Figure 4). Co-limitation always involved P, and simple P limitation occurred at 3 other sites (Fox, Ford 2 and Northern Erie 1; Table 2 and Figure 4). Maumee Bay 2 was the only site where our linear model analysis indicated depth was important, and only one amendment crossed the Harpole et al. (2011) threshold at this site (Table S2). Due to the loss of samples, we did not perform this analysis on Maumee Bay 1.

Metal limitation or co-limitation occurred at two sites: Green Bay and Sandusky Bay. In Green Bay, the effect of Zn was similar to the effect of P and was consistent with our prediction that Zn could alleviate P limitation when alternative sources of P are available (Figures 1, 4b, and Table 2). The P and Zn enrichment effect was relatively modest and close to the threshold of 38.5% greater growth (Figure 4b). However, Zn and P in combination with other amendments did not always stimulate chlorophyll *a* accumulation. Although P + Ni + Mo treatments were stimulated as expected, N + P and N + Zn treatments were below the effect threshold (Figure 4b).

In Sandusky Bay, all the individual element amendments stimulated chlorophyll *a* accumulation. The strongest individual effect was P, but when P was combined with N

Table 2. Log_e response ratios [log(RR)] of chlorophyll *a* content on nutrient diffusing substrates amended with nitrogen (N), phosphorus (P), zinc (Zn), iron (Fe) or nickel plus molybdenum (Ni + Mo) after two weeks of deployment in Lake Michigan (‡) and Lake Erie nearshore areas.

Site	$\log(RR_N)$	$\log(RR_P)$	$\log(RR_{Zn})$	$\log(RR_{Ni+Mo})$	$\log(RR_{Fe})$	$I_{N:P}$	$I_{N:Zn}$	$I_{N:Fe}$	$I_{P:Ni+Mo}$	Limitation
Fox‡	−0.13	**0.76**	0.09	0.28	0.26	0.82	−0.18	−0.17	−0.51	P limitation
Green Bay‡	−0.05	**0.41**	**0.49**	0.1	−0.3	−0.34	−0.61	0.04	−0.17	Additive co-limitation P, Zn
Ford 1‡	**−0.48**	−0.04	−0.10	−0.15	0.01	1.06	0.22	0.08	0.22	Negative effect N/Simultaneous co-limitation N, P
Ford 2‡	−0.32	**0.73**	0.01	<0.01	−0.22	−0.64	0.29	0.45	−0.44	P limitation
Maumee Bay 2†	−0.23	−0.32	0.04	**−0.34**	−0.30	0.40	−0.03	0.39	0.35	No effect†
North Erie 1	−0.03	**0.93**	0.12	0.30	0.10	−0.06	−0.41	−0.05	−0.52	P limitation
North Erie 2	−0.18	**−0.54**	−0.18	<0.01	**−1.10**	1.17	0.25	1.00	0.33	Negative effect P, Fe
Old Woman Creek	**1.59**	**0.72**	0.08	0.19	0.09	0.31	−1.01	−0.50	−0.86	Additive co-limitation P, N
Sandusky Bay	**0.38**	**0.93**	**0.52**	**0.58**	**0.73**	**1.82**	−0.95	−1.21	−1.32	Super-additive co-limitation P,N; Additive co-limitation P, Ni + Mo; Fe limitation

Interaction ratio responses (*I*) are reported without log transformation to avoid log-transformation making multiplicative relationships additive. Not all possible interactions were included as experimental treatments. We used the effect size threshold developed by Harpole et al. (2011) to identify amendment effects and interaction effects (0.33 on the log_e scale, or 1.385 without log transformation), equivalent to a 38.5% increase in chlorophyll *a* concentration over the control (bold).

†Generalized linear model indicated depth effects at this site.

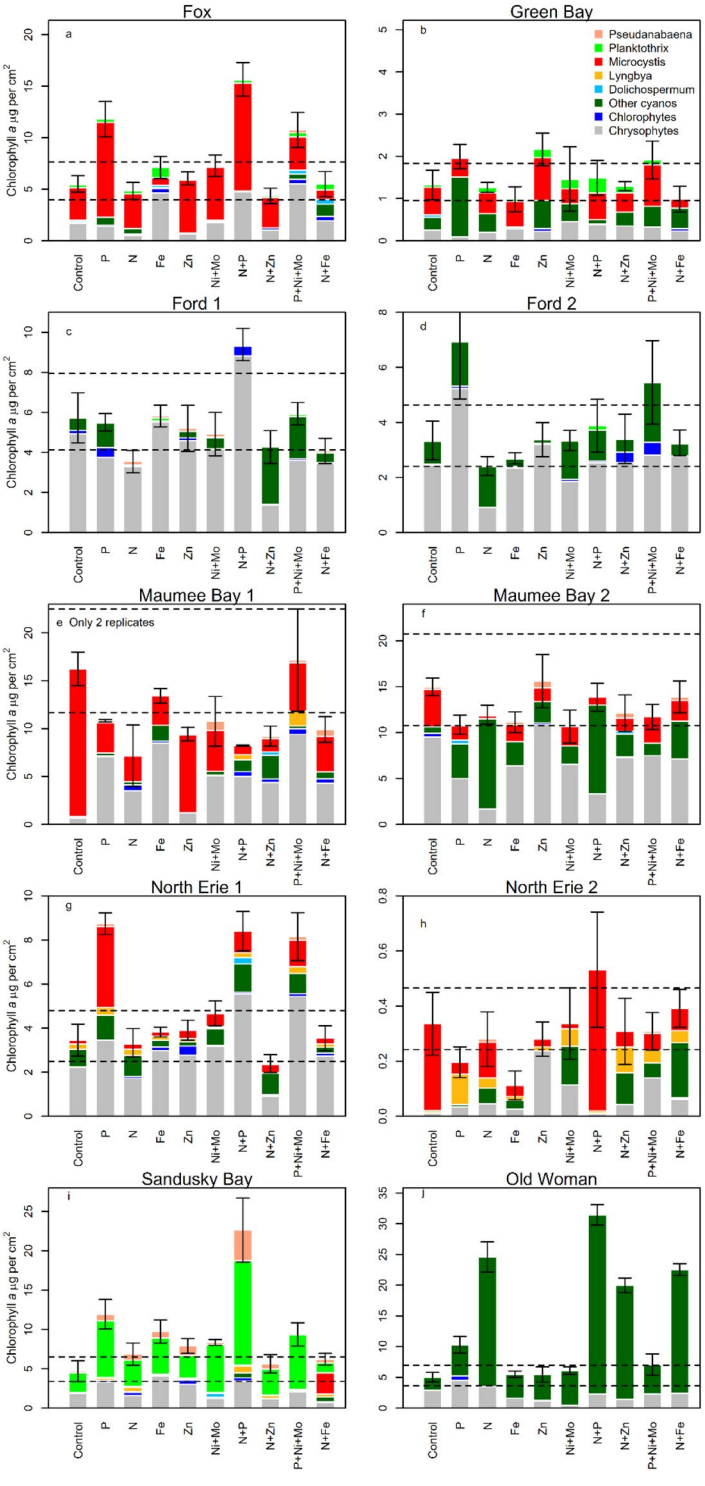

Figure 4. Mean areal chlorophyll *a* content (with standard error) on nutrient diffusing substrates deployed in near-shore areas of Lake Michigan and Lake Erie for two weeks during late summer 2017. Treatments were N – nitrogen, P – phosphorus, Fe – iron, Zn – zinc, and Ni + Mo – nickel and molybdenum. Colors indicate the relative proportion of cells belonging to different algal taxonomic groups. Dashed lines indicate the effect size thresholds developed by Harpole et al. (2011) relative to the control treatment. All treatments had 5 replicates, except for treatments from Maumee Bay 1, which had 2 replicates per treatment. Note the difference in y-axis scale across all plots.

the effect was super-additive co-limitation (Table 2 and Figure 4i). This was the only observed interaction effect between element amendments at this site (i.e. evidence of serial or simultaneous co-limitation). Metal replacements stimulated chlorophyll *a* as hypothesized (Figure 1), but combinations across N or P uptake pathways were less consistent. Chlorophyll *a* concentrations in the N + Zn and N + Fe treatments were not greater than concentrations in the controls, although P + Ni + Mo was consistent with Harpole et al. (2011) identification as additive co-limitation (Table 2 and Figure 4i).

Amendments sometimes seemed to cause negative effects. Both P and Fe were associated with less chlorophyll *a* accumulation at North Erie 2 (Figure 4h). At Ford 1, the N treatment effect was negative, although when N and P were combined the chlorophyll *a* concentration was much higher than the concentration in the control, and other treatments with N were within the threshold for effects (Figure 4c). This would be classified as a negative effect using Harpole et al. (2011), but we also considered Ford 1 to have simultaneous co-limitation based on the N + P result (Figure 4c and Table 2).

Old Woman Creek was unique in having much stronger N effects (log RR= 1.59) than P effects (log RR = 0.72; Figure 4j and Table 2). The combination of N and P yielded additive growth, and metals did not appear to provide the same stimulation as N (in the case of Fe and Ni + Mo) or P (in the case of Zn; Figure 4j). Among the sites sampled here, Old Woman Creek is a much smaller waterbody, draining a small watershed relative to the Ford, Fox, Maumee and Sandusky watersheds.

Treatment effects on community composition

Because we lacked replication in the community composition data, we lacked the capacity to perform robust statistical analyses; however, some trends in the community composition were apparent. Amendments of P in the Fox River mouth corresponded with increases in the *Microcystis* spp. proportional contribution to the community (Figure 4a). Amendments that stimulated growth mostly stimulated greater cyanobacterial contribution to the communities in Sandusky Bay and Old Woman sites (Figure 4i and j). All of these are sites within the channelized portion of the river mouth for their respective tributaries (using terminology from Larson et al. 2013).

In contrast, Ford 1, Ford 2 and North Erie 1 occur in the lentic mixing zone between river (Ford River and Detroit River) and lake water. At these sites, treatments that stimulated chlorophyll *a* production did not demonstrate corresponding increases in cyanobacteria to the exclusion of other taxa. Chrysophytes (primarily diatoms) were always a large portion of these communities, and in some treatments Chlorophytes became a detectable fraction as well (Figure 4c, d, and g). North Erie 2 is the site furthest from any tributary inputs and had primarily *Microcystis* spp. in the control and in the one treatment with strong positive effects (N + P). Other North Erie 2 treatments had more variation in the community composition, including several treatments with *Lyngbya* spp., which is a typically benthic taxa that was observed in the North Erie sites more often than anywhere else (Figure 4g and h).

Treatment effects on microcystin content

Although potential toxin-producing cyanobacterial taxa were observed at every site, MC was not detected at Ford 1, Ford 2 and Old Woman Creek (Table 1). Microcystin concentrations ranged from below the detection limit to 0.11 μg MC per cm^2 (Figures 5a and 6). Microcystin concentration only weakly correlated to the fraction of the community that was cyanobacteria ($\tau = 0.13$, Figure 5b). Surprisingly, North Erie 2 had the highest areal MC content

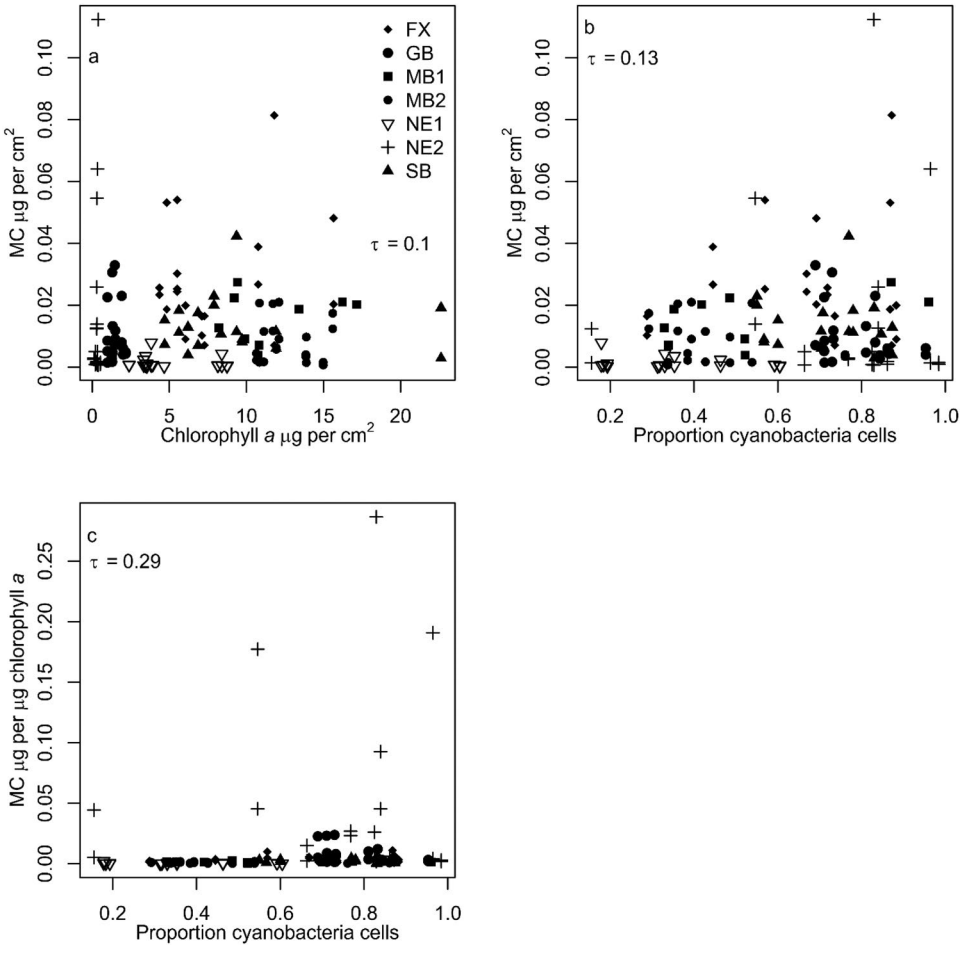

Figure 5. Microcystin (MC) content in relationship to chlorophyll and cyanobacteria in biofilms grown at different sites in lake Erie and lake Michigan during late summer 2017. Bivariate plot showing MC content per area versus chlorophyll *a* content per area (a) at sites where at least one sample had MC above the detection limit. Bivariate plot showing relationship between MC content per area and the proportional contribution of cyanobacterial cells (b), and a plot showing the relationship between MC per chlorophyll a and the proportion of cells that were cyanobacteria (c). In all cases, values below the MC detection limit are plotted as zeroes. Site labels refer to: FX – Fox river mouth, GB – Green Bay, MB1 – Maumee Bay 1, MB2 – Maumee Bay 2, NE1 – North Erie 1, NE2 – North Erie 2, SB – Sandusky Bay.

(Figure 5a), even without biomass normalization. When normalized to the chlorophyll *a* content on the disk, the highest microcystin concentration at North Erie 2 (0.287 µg MC per µg chlorophyll *a*) was an order of magnitude higher than values at any other sites (Figures 5c and 6e). Among all samples, MC content per area was weakly correlated to chlorophyll *a* ($\tau = 0.10$; Figure 5a). There was a stronger positive correlation between MC and relative contribution of cyanobacteria when standardized by chlorophyll *a* ($\tau = 0.29$; Figure 5c), and this effect persists even when the outlier North Erie 2 is removed ($\tau = 0.31$).

We often observed MC concentration had large variation among replicates within a single treatment (e.g. N treatment in the Fox River mouth, Ni + Mo treatment in Green Bay; Figure 6a and b), and identifying the effects of amendments within our sites is difficult. For example, the controls at North Erie 2 included one sample with a concentration below the detection limit, and another sample with a concentration higher than any observation at another site (Figure 6e). The North Erie sites had many non-detects, including in the control replicates, so it was not possible to model effect sizes at these

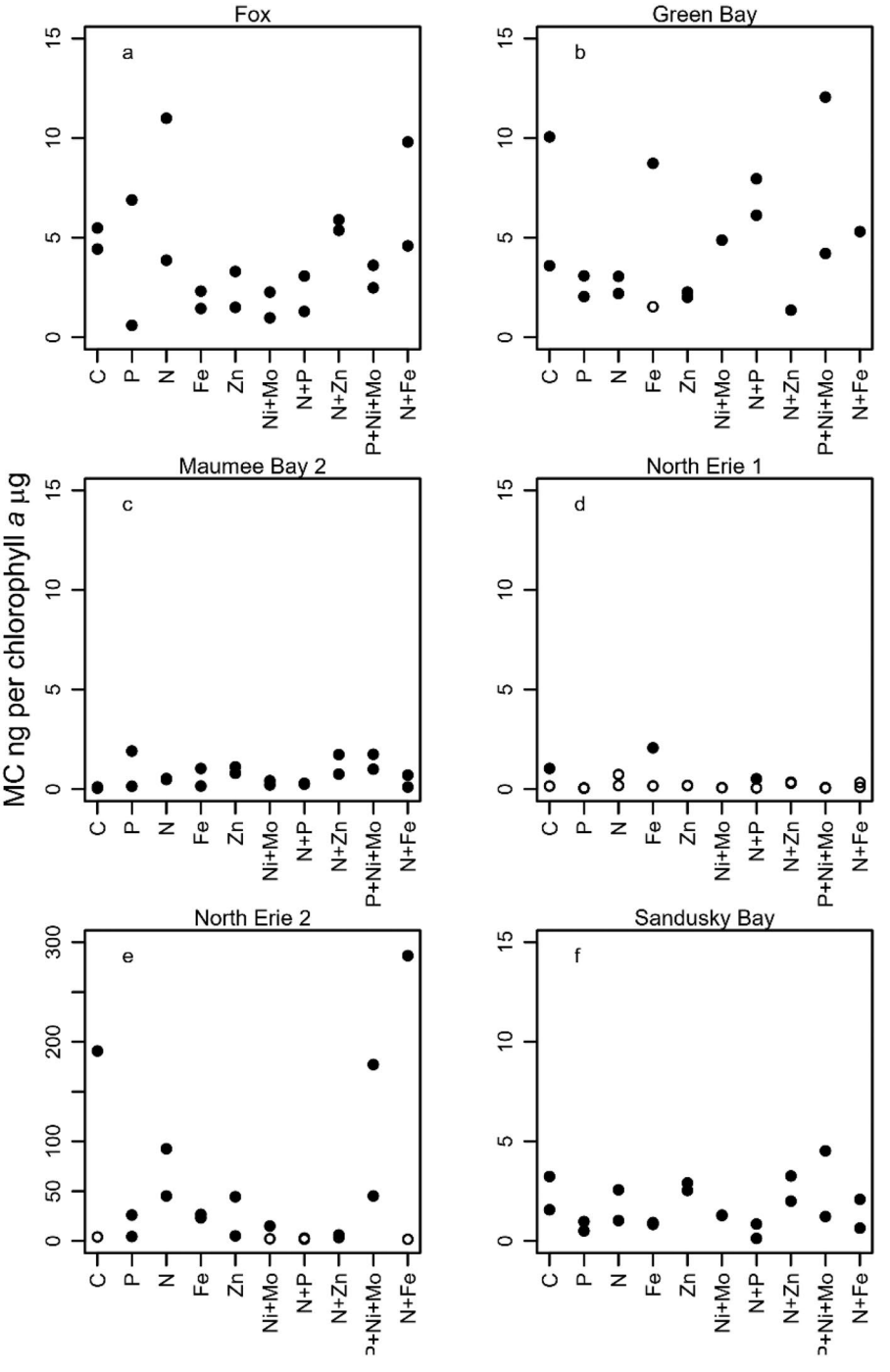

Figure 6. Microcystin (MC) content per chlorophyll *a* (ng MC per µg chlorophyll *a*) on nutrient diffusing substrates deployed for two weeks in Lake Michigan and Lake Erie nearshore locations (2017). Filled dots are samples above the detection limit, open dots are samples that fell below our detection limit.

sites. Maumee Bay 2 seemed to be light limited for growth (chlorophyll *a* accumulation), but the Maumee Bay 2 MC content seemed to be stimulated by N, P, Zn and sub-additively co-limited by N + P (Figure 6c and Table 3). In contrast to the controls, the Maumee Bay

Table 3. Standardized effect sizes (β) and 95% confidence intervals of nutrient and trace metal amendments on microcystin content per chlorophyll *a* on nutrient diffusing substrates in in Lake Michigan (‡) and Lake Erie nearshore areas.

Site	β_N	β_P	β_{Zn}	β_{Ni+Mo}	β_{Fe}	β_{N+P}	β_{N+Zn}	$\beta_{P+Ni+Mo}$	β_{N+Fe}	R^2
Fox‡	0.28 (-1.11, 1.67)	−0.88 (-2.28, 0.51)	−0.79 (-2.19, 0.60)	−1.20 (-2.59, 0.20)	−0.99 (-2.38, 0.40)	−0.30 (-2.27, 1.67)	0.65 (-1.32, 2.62)	1.58 (-0.39, 3.55)	1.02 (-0.95, 2.99)	0.54
Green Bay‡	−0.84 (-2.71, 1.03)	−0.87 (-2.74, 1.00)	−1.04 (-2.91, 0.83)	0.56 (-1.31, 2.43)	−0.50 (-2.37, 1.37)	1.86 (-0.78, 4.51)	1.82 (-0.82, 4.46)	0.49 (-2.16, 3.13)	1.95 (-0.70, 4.59)	0.41
Maumee Bay 2	**1.95**† **(0.20, 3.70)**	**2.00**† **(0.25, 3.75)**	**2.57**† **(0.82, 4.33)**	1.42 (-0.33, 3.18)	1.73 (-0.03, 3.48)	**−2.63**† **(-5.11, −0.15)**	−1.76 (-4.24, 0.72)	−0.51 (-2.99, 1.97)	−2.34 (-4.82, 0.14)	0.62
Sandusky Bay	−0.33 (-1.62, 0.96)	−1.16 (-2.46, 0.13)	0.19 (-1.10, 1.48)	−0.56 (-1.85, 0.73)	−0.94 (-2.23, 0.35)	−0.42 (-2.25, 1.40)	0.26 (-1.56, 2.09)	1.77 (-0.06, 3.59)	0.61 (-1.22, 2.43)	0.65

Not all interactions were measured. Effect sizes with a 95% confidence interval that did not overlap zero are highlighted in **bold**.
†Effect size does not overlap zero.

N, P and N + P treatments all had significant increases in non-*Microcystis* cyanobacteria taxa (Figure 4f). The Fox River mouth site was P limited (Table 2), but the statistical model did not clearly identify a strong effect of P on MC (Table 3). Similarly, Green Bay was P and Zn limited (Table 2), but we did not have evidence for MC effects from these amendments (Table 3 and Figure 6b). Almost every elemental amendment triggered increased chlorophyll *a* accumulation in Sandusky Bay, but overall MC content was low at this site (Figure 6), and none of the effect sizes had 95% confidence intervals that did not include zero (Table 3).

Discussion

We hypothesized that within algal blooms, preferred forms of N and P would decline and that algae and cyanobacteria would have high demand for metals associated with nutrient uptake (Figure 1, Zn for P; Ni, Mo for N). Although earlier studies have indicated that nearshore, shallow areas have adequate re-supply of metals from sediments and tributary inputs to prevent metal limitation of growth or other metabolic processes (Ivanikova et al. 2007; North et al. 2007), our hypothesis was that even this re-supply from sediments and tributary inputs would be inadequate to support blooms. All of our sites were near tributary inputs in shallow waters (relative to those discussed in earlier papers as being potentially metal limited). Consistent with the existing literature, we observed nutrient limitation but did not observe metal limitation at the 5 sites lacking dense bloom conditions. Ford, North Erie and the Old Woman Creek sites were N and P limited or co-limited but were not metal co-limited.

Of the 4 sites we sampled with high productivity and recurring algal blooms (Green Bay, Fox River mouth, Maumee Bay and Sandusky Bay), 2 had some kind of metal limitation (Green Bay and Sandusky Bay), providing evidence that at least occasionally metals can become limiting in these conditions. The cyanobacterial taxa we observed in the biofilms in this study were many of the same taxa that typically make up bloom-forming taxa in these locations, including *Microcystis* spp. in Green Bay and Maumee Bay and *Planktothrix* spp. in Sandusky Bay (De Stasio et al. 2014; Kane et al. 2014; Davis et al. 2015; Salk et al. 2018). Because *Microcystis* spp. and *Planktothrix* spp. occurred in both our NDS and the phytoplankton community, and because the NDS were suspended in the

blooms, NDS may provide a reasonable approximation of conditions experienced by the phytoplankton.

In Green Bay, P and Zn corresponded with stimulated community growth, which was consistent with the hypothesis that as highly labile forms of P become limiting, the addition of Zn could alleviate growth limitation by allowing organic P to become more available (Figure 1). We hypothesized that Zn alleviates P limitation *via* alkaline phosphatase that in many algae requires a Zn cofactor (*phoA*). However most cyanobacteria have a similar enzyme (*phoX*) which requires a Ca cofactor (Tiwari et al. 2015; Lin et al. 2018). Green Bay is a site where cyanobacterial blooms are common, and in our community composition data, the Zn treatment was mostly composed of two types of cyanobacteria (*Microcystis* spp. and *Aphanocapsa* spp.). Zn is required in many other cellular roles (Fukuda et al. 2000; Twining and Baines 2013; Jensen et al. 2019), so some other functional role may be enhanced by the Zn amendment in our experiment. Additional information on the community response to Zn amendment could help clarify whether another functional role is being enhanced.

In Sandusky Bay, all of the nutrient amendments corresponded with stimulated growth, and there were multiple forms of co-limitation. In particular, P and Fe had the largest effects. At the time of the experiments, Sandusky Bay had an unusual cyanobacterial community compared to many other areas that experience blooms in the Great Lakes, with *Planktothrix* spp. as the dominant taxa (Davis et al. 2015; Salk et al. 2018), rather than *Microcystis* spp. that are common in western Lake Erie and Green Bay (De Stasio et al. 2014; Kane et al. 2014). *Planktothrix* spp. appear to have higher demand for Fe than *Microcystis* spp. (Nagai et al. 2007). While P amendments had a larger effect in our study, N limitation is frequently observed in Sandusky Bay (Davis et al. 2015), and in our study the combination of N and P had super-additive effects on growth. Fe, Ni and Mo are important elements in the acquisition of many N species (Havens et al. 2012; Twining and Baines 2013; Salk et al. 2018), but the combination of P, Ni and Mo was not super-additive like the P and N treatment.

Logistical limitations prevented us from collecting all the data that would be ideal in this study. For example, we collected limited water column nutrient and metal concentrations. We suspect an enormous effort would be needed to acquire enough water column data to inform our understanding of nutrient limitation in suspended periphyton communities. This is partly because water column concentrations in nearshore areas near tributaries often change rapidly as water masses move as a result of seiche, wind-driven currents and tributary inputs (Larson et al. 2013). For example, the National Oceanic and Atmospheric Administration reports on buoy-collected 'continuous' data on phosphorus from the western basin of Lake Erie near Maumee Bay (Cooperative Institute for Great Lakes Research and University of Michigan; NOAA Great Lakes Environmental Research Laboratory 2019). During the time our NDS were deployed, these measured phosphorus concentrations ranged from 22.4 µg P/L to below the detection limit. Some of these changes occurred rapidly: On August 6th, a P concentration of 4.8 µg P/L was reported at 17:05, and by 20:05 the reported concentration was 22.4 µg P/L; A further 24 h after that, (17:05, August 7th) the concentration was 6.3 µg P/L, 24 h later (17:05, August 8th) the concentration was 18.5 µg P/L. These rapid shifts can be biologically important for biofilms, because algae and cyanobacteria can store nutrients when they are available in excess (Oliver et al. 2012; Glibert 2017). Even daily sampling might miss these rapid changes and thus miss important details of nutrient or metal availability in the water column. Even with a perfect dataset of water column nutrient availability, we suspect the response of the actual periphyton community would better capture the effects of these rapid shifts in P concentrations.

Our study also lacked replicated measures of some of the more sophisticated response variables (e.g. DNA-based taxonomic data, biovolumes) that Chaffin et al. (2020) suggested are needed to confirm effects in nutrient limitation bioassays. Additional studies would be needed to establish the temporal and spatial distribution of potential metal limitation within highly eutrophic conditions, both within the Great Lakes and elsewhere. However, as a preliminary experiment, our data indicate metal limitation in these areas of high demand is possible. Another recent study in stream ecosystems also found metal limitation was more common than previously assumed (Fitzgibbon and Costello 2023), although the effects in that study were not driven by eutrophic conditions.

In addition to our hypothesis regarding metals and growth in HABs, we did a preliminary analysis to assess the role of metals on microcystin (MC). We hypothesized that MC might have a functional connection to metal use or availability, and would therefore be downregulated when labile N and P are added to the system and demand for metals was reduced (Omidi et al. 2018). In the four sites where we could statistically assess microcystin concentrations (Fox, Green Bay, Maumee Bay and Sandusky Bay), we did not observe lower values in treatments with labile N or P amendments. This preliminary analysis has little power to either support or refute our predictions, because the sample size was small, and we lacked better metrics of toxin production (e.g. indicators of the up or down regulation of toxin-producing genes). Our interpretations are also limited by poor agreement between replicates, including in control treatments. Future studies with more precise measurements of microcystin concentrations and quantitative measurements of genes that produce toxins may provide more insight (e.g. *mcyE*; (Graham et al. 2020), but such work was beyond the scope of our study.

Zurawell et al. (2005) suggested that the drivers of cyanobacterial growth and toxicity can be disconnected. There was some evidence in our study to support that assessment. For example, P amendments stimulated growth in Green Bay, Fox River mouth and Sandusky Bay, but there was no corresponding change in microcystin content. Similarly, while Maumee Bay seemingly experienced light limitation for growth, N, P and Zn all increased microcystin content. In culture experiments, Zn has been shown to increase MC production (Perez and Chu 2020) and MC form complexes with Zn (Humble et al. 1997).

Beyond the experimental context, our data also provide some context to the distribution of MC in the Great Lakes. Microcystins are the most frequently observed toxins in most North American freshwater ecosystems (Loftin et al. 2016; Graham et al. 2020), and a huge variety of cyanobacterial taxa can produce MC (Rantala et al. 2004). The presumption of many studies is that the abundance of cyanobacteria can be a surrogate for the negative impacts of cyanobacteria (e.g. Stumpf et al. 2016). Although this is a reasonable initial approximation, MC content varies tremendously over space and time (Chaffin et al. 2021), and several samples in our study had relatively high proportions of cyanobacteria but low or undetectable MC content (e.g. Old Woman Creek).

Microcystins have also not been commonly measured in Great Lakes biofilms, and we found substantial variation in the MC content across sites where we detected MC. Chaffin et al. (2021) found a 5.3 fold variation in MC to chlorophyll *a* ratios in surface water samples in Lake Erie, whereas we observed a 2,680 fold difference in MC per µg chlorophyll *a* just among samples with detectable MC concentration (0.0001 µg MC per µg chl *a* in a Maumee Bay 2 sample and 0.268 µg MC per µg chl *a* in a Northern Lake Erie 2 sample). The very high values at North Erie 2 are partially a result of low chlorophyll *a* concentration that was near our detection limit. However, even the uncorrected areal MC content was high 0.11 µg MC per cm^2, which is within concentrations previously observed in rivers (maximum of 0.47 µg MC per cm^2; Fetscher et al. 2015). North Erie 2 rarely

experiences blooms that can be observed with satellite data (Larson et al. 2020), so the presence of toxic biofilms in this region is a reminder that water column cyanobacteria are not the only taxa that could potentially create harmful conditions. Further study could help understand the contributions of biofilms to water column MC.

Conclusions

Community co-limitation of major nutrients N and P occurred in 4 of the 9 sites (44%) in this study, which is similar to earlier studies that found 38% and 54% of sites had co-limitation of some type (Elser et al. 2007; Harpole et al. 2011). Those studies focused exclusively on N and P, whereas our study included two sites where co-limitation occurred with metals (as defined by Harpole et al. 2011). We hypothesized that these metals would alleviate N and P limitation as metals are needed co-factors for enzymes that allow algae to access other forms of N and P (McKay et al. 2001). Our NDS results are consistent with our hypothesis at some sites (Green Bay and Sandusky Bay) and indicate metals have the potential to influence cyanobacterial growth and toxicity within blooms.

Of the metals studied here, Fe is the one that has most often been identified as either growth limiting, limiting for nutrient acquisition, or demonstrably depleted during bloom events, and that is needed at higher concentrations for photosynthesis than other micronutrients (Ivanikova et al. 2007; Havens et al. 2012; Leung et al. 2021). To our knowledge, Fe limitation has not previously been studied in Sandusky Bay, but Fe is often limiting in aquatic ecosytems globally (Tagliabue et al. 2017). Fe availability varies substantially among different ecosystems due to variation in its solubility and speciation under different conditions (Schlesinger and Bernhardt 2013; Kappler et al. 2021). For all these reasons, further study would be needed to understand physiological responses of HABs taxa to Fe within blooms.

One of the major concerns with the common HABs-forming taxa in freshwaters is their ability to produce toxins. Although N and P availability are useful predictors for identifying when and where blooms are more likely to form, predicting the timing and severity of toxin production has been more challenging (Taranu et al. 2017). The most commonly observed cyanotoxin, microcystin, has an ancient evolutionary origin, an uncertain functional role, many congeners, and is produced by many taxa (Rantala et al. 2004; Omidi et al. 2018). However, not all HABs include microcystin-producing taxa, and not all taxa that can produce toxins do so all the time (Zurawell et al. 2005; Omidi et al. 2018). During a bloom, environmental conditions that trigger shifts in community composition from non-toxic to toxic taxa, or conditions that trigger toxin production, remain difficult to identify. Our preliminary study did not identify a clear relationship between microcystin and metals. Additional laboratory and *in situ* studies could help understand the environmental conditions that trigger toxin production within blooms to improve predictions about toxicity.

Acknowledgments

Enrika Hlavacek created Figure 1. Devin Edge, Samantha Foltz and Maya Agata assisted with field work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was partially funded by the U.S. Geological Survey Ecosystem Mission Area and the Great Lakes Restoration Initiative. DMC and JTS were partially supported by an award from NSF (Division of Environmental Biology #1943182).

Data availability

The data used in this manuscript are available in Larson et al. (2024a).

References

Alexova R, Fujii M, Birch D, Cheng J, Waite TD, Ferrari BC, Neilan BA. 2011. Iron uptake and toxin synthesis in the bloom-forming Microcystis aeruginosa under iron limitation. Environ Microbiol. 13(4):1064–1077. doi: 10.1111/j.1462-2920.2010.02412.x.

Bertini I, Gray HB, Stiefel EI, Valentine JS. 2007. Biological inorganic chemistry: structure and reactivity. Sausalito (CA): University Science Books.

Capps KA, Booth MT, Collins SM, Davison MA, Moslemi JM, El-Sabaawi RW, Simonis JL, Flecker AS. 2011. Nutrient diffusing substrata: a field comparison of commonly used methods to assess nutrient limitation. J N Am Benthol Soc. 30(2):522–532. doi: 10.1899/10-146.1.

Ceballos-Laita L, Marcuello C, Lostao A, Calvo-Begueria L, Velazquez-Campoy A, Bes MT, Fillat MF, Peleato ML. 2017. Microcystin-LR binds iron, and iron promotes self-assembly. Environ Sci Technol. 51(9):4841–4850. doi: 10.1021/acs.est.6b05939.

Chaffin JD, Bratton JF, Verhamme EM, Bair HB, Beecher AA, Binding CE, Birbeck JA, Bridgeman TB, Chang X, Crossman J, et al. 2021. The Lake Erie HABs Grab: a binational collaboration to characterize the western basin cyanobacterial harmful algal blooms at an unprecedented high-resolution spatial scale. Harmful Algae. 108:102080. doi: 10.1016/j.hal.2021.102080.

Chaffin JD, Bridgeman TB, Bade DL, Mobilian CN. 2014. Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years. J Great Lakes Res. 40(3):524–531. doi: 10.1016/j.jglr.2014.04.009.

Chaffin JD, Bridgeman TB, Bade DL. 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. Adv Microbiol. 03(06):16–26. doi: 10.4236/aim.2013.36A003.

Chaffin JD, Stanislawczyk K, Kane DD, Lambrix MM. 2020. Nutrient addition effects on chlorophyll a, phytoplankton biomass, and heterocyte formation in Lake Erie's central basin during 2014–2017: insights into diazotrophic blooms in high nitrogen water. Freshwater Biol. 65(12):2154–2168. doi: 10.1111/fwb.13610.

Cooperative Institute for Great Lakes Research, University of Michigan; NOAA Great Lakes Environmental Research Laboratory. 2019. Physical, chemical, and biological water quality monitoring data to support detection of Harmful Algal Blooms (HABs) in western Lake Erie, collected by the Great Lakes Environmental Research Laboratory and the Cooperative Institute for Great Lakes Research since 2012. [indicate subset used]. NOAA National Centers for Environmental Information. Dataset. doi: 10.25921/11da-3x54.

Costello DM, Rosi-Marshall EJ, Shaw LE, Grace MR, Kelly JJ. 2016. A novel method to assess effects of chemical stressors on natural biofilm structure and function. Freshwater Biol. 61(12):2129–2140. doi: 10.1111/fwb.12641.

Davis TW, Bullerjahn GS, Tuttle T, McKay RM, Watson SB. 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during planktothrix blooms in Sandusky Bay, Lake Erie. Environ Sci Technol. 49(12):7197–7207. doi: 10.1021/acs.est.5b00799.

De Stasio BT, Schrimpf MB, Cornwell BH. 2014. Phytoplankton communities in Green Bay, Lake Michigan after invasion by dreissenid mussels: increased dominance by cyanobacteria. Diversity. 6(4):681–704. doi: 10.3390/d6040681.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett. 10(12):1135–1142. doi: 10.1111/j.1461-0248.2007.01113.x.

Evans MA, Fahnenstiel G, Scavia D. 2011. Incidental oligotrophication of North American Great Lakes. Environ Sci Technol. 45(8):3297–3303. doi: 10.1021/es103892w.

Fetscher AE, Howard MDA, Stancheva R, Kudela RM, Stein ED, Sutula MA, Busse LB, Sheath RG. 2015. Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae. 49:105–116. doi: 10.1016/j.hal.2015.09.002.

Fitzgibbon AS, Costello DM. 2023. Trace metal–macronutrient colimitation of algal biofilms in streams with differing ambient inorganic nutrients. Freshwater Sci. 42(3):285–295. doi: 10.1086/726684.
Fukuda R, Sohrin Y, Saotome N, Fukuda H, Nagata T, Koike I. 2000. East—west gradient in ectoenzyme activities in the subarctic Pacific: possible regulation by zinc. Limnol Oceanogr. 45(4):930–939. doi: 10.4319/lo.2000.45.4.0930.
Ginn HP, Pearson LA, Neilan BA. 2010. NtcA from Microcystis aeruginosa PCC 7806 is autoregulatory and binds to the microcystin promoter. Appl Environ Microbiol. 76(13):4362–4368. doi: 10.1128/AEM.01862-09.
Glass JB, Axler RP, Chandra S, Goldman CR. 2012. Molybdenum limitation of microbial nitrogen assimilation in aquatic ecosystems and pure cultures. Front Microbiol. 3:331. doi: 10.3389/fmicb.2012.00331.
Glibert PM. 2017. Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. Mar Pollut Bull. 124(2):591–606. doi: 10.1016/j.marpolbul.2017.04.027.
Graham JL, Dubrovsky NM, Foster GM, King LR, Loftin KA, Rosen BH, Stelzer EA. 2020. Cyanotoxin occurrence in large rivers of the United States. Inland Waters. 10(1):109–117. doi: 10.1080/20442041.2019.1700749.
Harke MJ, Davis TW, Watson SB, Gobler CJ. 2016. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. Environ Sci Technol. 50(2):604–615. doi: 10.1021/acs.est.5b03931.
Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, et al. 2011. Nutrient co-limitation of primary producer communities. Ecol Lett. 14(9):852–862. doi: 10.1111/j.1461-0248.2011.01651.x.
Havens SM, Hassler CS, North RL, Guildford SJ, Silsbe G, Wilhelm SW, Twiss MR. 2012. Iron plays a role in nitrate drawdown by phytoplankton in Lake Erie surface waters as observed in lake-wide assessments. Can J Fish Aquat Sci. 69(2):369–381. doi: 10.1139/f2011-157.
Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, et al. 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae. 8(1):3–13. doi: 10.1016/j.hal.2008.08.006.
Helsel DR. 2005. Nondetects and data analysis. Hoboken (NJ): John Wiley & Sons, Inc.
Herdendorf CE, Klarer DM, Herdendorf RC. 2006. The ecology of Old Woman Creek, Ohio: an estuarine and watershed profile. 2nd ed. Columbus (OH): Ohio Department of Natural Resources, Division of Wildlife.
Humble AV, Gadd GM, Codd GA. 1997. Binding of copper and zinc to three cyanobacterial microcystins quantified by differential pulse polarography. Water Res. 31(7):1679–1686. doi: 10.1016/S0043-1354(97)00033-X.
Ivanikova NV, McKay RML, Bullerjahn GS, Sterner RW. 2007. Nitrate utilization by phytoplankton in Lake Superior is impaired by low nutrient (P, Fe) availability and seasonal light limitation - a cyanobacterial bioreporter study. J Phycol. 43(3):475–484. doi: 10.1111/j.1529-8817.2007.00348.x.
Jensen EL, Clement R, Kosta A, Maberly SC, Gontero B. 2019. A new widespread subclass of carbonic anhydrase in marine phytoplankton. ISME J. 13(8):2094–2106. doi: 10.1038/s41396-019-0426-8.
Kane DD, Conroy JD, Peter Richards R, Baker DB, Culver DA. 2014. Re-eutrophication of Lake Erie: correlations between tributary nutrient loads and phytoplankton biomass. J Great Lakes Res. 40(3):496–501. doi: 10.1016/j.jglr.2014.04.004.
Kappler A, Bryce C, Mansor M, Lueder U, Byrne JM, Swanner ED. 2021. An evolving view on biogeochemical cycling of iron. Nat Rev Microbiol. 19(6):360–374. doi: 10.1038/s41579-020-00502-7.
Kaspari M, Powers JS. 2016. Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. Am Nat. 188(S1): S62–S73. doi: 10.1086/687576.
Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L. 1998. COMBO : a defined freshwater culture medium for algae and zooplankton. Hydrobiologia. 377(1–3):147–159. doi: 10.1023/A:1003231628456.
Klein AR, Baldwin DS, Silvester E. 2013. Proton and iron binding by the cyanobacterial toxin microcystin-LR. Environ Sci Technol. 47(10):5178–5184. doi: 10.1021/es400464e.
Larson JH, Bailey SW, Costello DM, Stoll JT. 2024a. Data associated with nutrient diffusing substrate experiments conducted in Lake Michigan and Lake Erie (2017). Reston, VA: U.S. Geological Survey. doi: 10.5066/P9EM043D.
Larson JH, Bailey SW, Milde AS. 2024b. Estimates of microcystin concentration and content using an enzyme-linked immunosorbent assay on samples collected from experiments on cyanobacteria in the Great Lakes and field data from the Mississippi River. Reston, VA: U.S. Geological Survey. doi: 10.5066/P96KS2Y2.
Larson JH, Frost PC, Vallazza JM, Nelson JC, Richardson WB. 2016a. Do rivermouths alter nutrient and seston delivery to the nearshore? Freshwater Biol. 61(11):1935–1949. doi: 10.1111/fwb.12827.
Larson JH, Hlavacek E, DeJager N, Evans MA, Wynne T. 2020. Preliminary analysis to estimate the spatial distribution of benefits of P load reduction: identifying the spatial influence of phosphorus loading from the Maumee River (USA) in western Lake Erie. Ecol Evol. 10(9):3968–3976. doi: 10.1002/ece3.6160.

Larson JH, Loftin KA, Stelzer EA, Costello DM, Bailey SW, Evans MA, Givens CE, Fogarty LR. 2023. Role of trace metal co-limitation in cyanobacterial blooms of Maumee Bay (Lake Erie) and Green Bay (Lake Michigan). J Freshwater Ecol. 38(1):2222747. doi: 10.1080/02705060.2023.2222747.

Larson JH, Richardson WB, Evans MA, Schaeffer JS, Wynne TT, Bartsch MR, Bartsch LA, Nelson JC, Vallazza JM. 2016b. Measuring spatial variation in secondary production and food quality using a common consumer approach in Lake Erie. Ecol Appl. 26(3):873–885. doi: 10.1890/15-0440.

Larson JH, Trebitz AS, Steinman AD, Wiley MJ, Mazur MC, Pebbles V, Braun HA, Seelbach PW. 2013. Great Lakes rivermouth ecosystems: scientific synthesis and management implications. J Great Lakes Res. 39(3):513–524. doi: 10.1016/j.jglr.2013.06.002.

Leung T, Wilkinson GM, Swanner ED. 2021. Iron availability allows sustained cyanobacterial blooms: a dual-lake case study. Inland Waters. 11(3):417–429. doi: 10.1080/20442041.2021.1904762.

Lin W, Zhao D, Luo J. 2018. Distribution of alkaline phosphatase genes in cyanobacteria and the role of alkaline phosphatase on the acquisition of phosphorus from dissolved organic phosphorus for cyanobacterial growth. J Appl Phycol. 30(2):839–850. doi: 10.1007/s10811-017-1267-3.

Loftin KA, Graham JL, Hilborn ED, Lehmann SC, Meyer MT, Dietze JE, Griffith CB. 2016. Cyanotoxins in inland lakes of the United States: occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. Harmful Algae. 56:77–90. doi: 10.1016/j.hal.2016.04.001.

McKay RML, Twiss MR, Nalewajko C. 2001. Trace metal constraints on carbon, nitrogen and phosphorus acquisition and assimilation by phytoplankton. In: Rai LC, editor. Algal adaptation to environmental stresses. Berlin: Springer; p. 111–134.

Meriluoto JL, Spoof L, Codd GA, editors. 2016. Handbook of cyanobacterial monitoring and cyanotoxin analysis. Chichester, UK: John Wiley & Sons, Ltd.

Metcalf JS, Codd GA. 2012. Cyanotoxins. In: Whitton BA, editor. Ecology of cyanobacteria II. 2nd ed. Dordrecht: Springer Netherlands; p. 651–675.

Morel FMM, Price NM. 2003. The biogeochemical cycles of trace metals in the oceans. Science. 300(5621):944–947. doi: 10.1126/science.1083545.

Nagai T, Imai A, Matsushige K, Fukushima T. 2007. Growth characteristics and growth modeling of Microcystis aeruginosa and Planktothrix agardhii under iron limitation. Limnology. 8(3):261–270. doi: 10.1007/s10201-007-0217-1.

North RL, Guildford SJ, Smith REH, Havens SM, Twiss MR. 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. Limnol Oceanogr. 52(1):315–328. doi: 10.4319/lo.2007.52.1.0315.

Oliver RL, Hamilton DP, Brookes JD, Ganf GG. 2012. Physiology, blooms and prediction of planktonic cyanobacteria. In: Whitten BA, editor. Ecology of cyanobacteria II. Dordrecht: Springer Netherlands; p. 155–194.

Omidi A, Esterhuizen-Londt M, Pflugmacher S. 2018. Still challenging: the ecological function of the cyanobacterial toxin microcystin–what we know so far. Toxin Rev. 37(2):87–105. doi: 10.1080/15569543.2017.1326059.

Perez JL, Chu T. 2020. Effect of zinc on Microcystis aeruginosa UTEX LB 2385 and its toxin production. Toxins (Basel). 12(2):92. doi: 10.3390/toxins12020092.

Qian SS, Chaffin JD, Dufour MR, Sherman JJ, Golnick PC, Collier CD, Nummer SA, Margida MG. 2015. Quantifying and reducing uncertainty in estimated microcystin concentrations from the ELISA method. Environ Sci Technol. 49(24):14221–14229. doi: 10.1021/acs.est.5b03029.

R Development Core Team. 2022. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rantala A, Fewer DP, Hisbergues M, Rouhiainen L, Vaitomaa J, Börner T, Sivonen K. 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. Proc Natl Acad Sci USA. 101(2):568–573. doi: 10.1073/pnas.0304489101.

Rees TAV, Bekheet IA. 1982. The role of nickel in urea assimilation by algae. Planta. 156(5):385–387. doi: 10.1007/BF00393307.

Rice EW, Baird RB, Eaton AD, Clesceri LS. 2012. Standard methods for the examination of water and wastewater. 22nd ed. Washington (DC): American Public Health Association.

Rowland FE, Stow CA, Johengen TH, Burtner AM, Palladino D, Gossiaux DC, Davis TW, Johnson LT, Ruberg S. 2020. Recent patterns in Lake Erie phosphorus and chlorophyll a concentrations in response to changing loads. Environ Sci Technol. 54(2):835–841. doi: 10.1021/acs.est.9b05326.

Salk KR, Bullerjahn GS, McKay RML, Chaffin JD, Ostrom NE. 2018. Nitrogen cycling in Sandusky Bay, Lake Erie: oscillations between strong and weak export and implications for harmful algal blooms. Biogeosciences. 15(9):2891–2907. doi: 10.5194/bg-15-2891-2018.

Schindler DW, Fee EJ. 1974. Experimental lakes area : whole-lake experiments in eutrophication. J Fish Res Board Can. 31(5):937–953. doi: 10.1139/f74-110.

Schindler DW. 2012. The dilemma of controlling cultural eutrophication of lakes. Proc Biol Sci. 279(1746):4322–4333. doi: 10.1098/rspb.2012.1032.
Schlesinger WH, Bernhardt ES. 2013. Biogeochemistry: an analysis of global change. 3rd ed. Waltham (MA): Academic Press.
Sevilla E, Martin-Luna B, Vela L, Bes MT, Fillat MF, Peleato ML. 2008. Iron availability affects mcyD expression and microcystin-LR synthesis in Microcystis aeruginosa PCC7806. Environ Microbiol. 10(10):2476–2483. doi: 10.1111/j.1462-2920.2008.01663.x.
Steinman AD, Lamberti GA, Leavitt PR, Uzarski DG. 2017. Biomass and pigments of benthic algae. In: Hauer R, Lamberti GA, editors. Methods in stream ecology. Vol. 1. US & UK: Academic Press; p. 223–241.
Steinman AD, Mulholland PJ. 2006. Phosphorus limitation uptake, and turnover in benthic stream algae. In: Hauer FR, Lamberti GA, editors. Methods in stream ecology. 2nd ed. Burlington (MA): Academic Press; p. 187–212.
Sterner RW, Elser JJ. 2002. Ecological stoichiometry. Princeton (NJ): Princeton University Press.
Sterner RW, Smutka TM, McKay RML, Xiaoming Q, Brown ET, Sherrell RM. 2004. Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. Limnol Oceanogr. 49(2):495–507. doi: 10.4319/lo.2004.49.2.0495.
Stumpf RP, Johnson LT, Wynne TT, Baker DB. 2016. Forecasting annual cyanobacterial bloom biomass to inform management decisions in Lake Erie. J Great Lakes Res. 42(6):1174–1183. doi: 10.1016/j.jglr.2016.08.006.
Tagliabue A, Bowie AR, Boyd PW, Buck KN, Johnson KS, Saito MA. 2017. The integral role of iron in ocean biogeochemistry. Nature. 543(7643):51–59. doi: 10.1038/nature21058.
Taranu ZE, Gregory-Eaves I, Steele RJ, Beaulieu M, Legendre P. 2017. Predicting microcystin concentrations in lakes and reservoirs at a continental scale: a new framework for modelling an important health risk factor. Global Ecol Biogeogr. 26(6):625–637. doi: 10.1111/geb.12569.
Tiwari B, Singh S, Kaushik MS, Mishra AK. 2015. Regulation of organophosphate metabolism in cyanobacteria. A review. Microbiology. 84(3):291–302. doi: 10.1134/S0026261715030200.
Twining BS, Baines SB. 2013. The trace metal composition of marine phytoplankton. Ann Rev Mar Sci. 5(1):191–215. doi: 10.1146/annurev-marine-121211-172322.
Utermöhl H. 1931. Neue Wege in der quantitativen Erfassung des Plankton.(Mit besonderer Berücksichtigung des Ultraplanktons.) Mit 4 Abbildungen im Text Verh Int Ver Theor Angew Limnol. 5(2):567–596.
Utkilen H, Gjølme N. 1995. Iron-stimulated toxin production in Microcystis aeruginosa. Appl Environ Microbiol. 61(2):797–800. doi: 10.1128/aem.61.2.797-800.1995.
Wagner RS, Neudeck MJ, Heath AE, Barker KB, Brown KM, Buchholz S, Ward CS, Bullerjahn GS. 2024. The recent disappearance of a persistent Planktothrix bloom: characterization of a regime shift in the phytoplankton of Sandusky Bay (USA). Harmful Algae. 136:102656. doi: 10.1016/j.hal.2024.102656.
Zhang Z, Lennox WC, Panu US. 2004. Effect of percent non-detects on estimation bias in censored distributions. J Hydrol. 297(1–4):74–94. doi: 10.1016/j.jhydrol.2004.04.017.
Zurawell RW, Chen H, Burke JM, Prepas EE. 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J Toxicol Environ Health B Crit Rev. 8(1):1–37. doi: 10.1080/10937400590889412.