Trace metal limitation of biofilm growth and metabolism: potential consequences for storage of nutrients in headwater streams

David Costello
Kent State University
Progress report submitted to Ohio Water Resources Center
May 2017

Problem and Research Objectives

The increased frequency and extent of harmful algal blooms (HABs) has sparked the need for greater knowledge about the physical factors that are involved in bloom formation and toxin production. Water quality, and in particular nutrient availability, has been a major focus of researchers' attempts to understand the mechanisms of HABs. Although primary producers require at least 20 different elements (Schlesinger and Bernhardt 2013), the vast majority of what is known about nutrient limitation in aquatic ecosystems focuses on just two elements: nitrogen (N) and phosphorus (P) (Hecky and Kilham 1988, Elser et al. 2007). In freshwater lakes and large rivers, the metals required for metabolic processes (e.g., iron (Fe), zinc (Zn), molybdenum (Mo)) can be measured at concentrations known to limit growth of marine algae (e.g., Nriagu et al. 1996, Shiller 1997). This study attempts to address the unknown importance of limiting concentrations of trace metals on primary production in small streams draining into Lake Erie.

Abiotic and biotic processes in streams can retain, transform, and remove nutrients effectively (Peterson et al. 2001). Biofilms—the consortium of algae, bacteria, and fungi that cover the streambed (Lock et al. 1984)—remove N and P from the water column to fuel their growth (Arango et al. 2008, Sobota et al. 2012). Assimilation of N and P by biofilms can lessen stream nutrient loads and convert inorganic nutrients like nitrate (NO_3^-) and phosphate (PO_4^{3-}) to less bioavailable organic forms (Bronk et al. 1994, Johnson et al. 2009). Although biofilms are composed of microscopic organisms, algal-dominated mid- and low-order stream reaches can have biofilms at high biomass that drive in-stream nutrient processes and store ecologically relevant amounts of N and P (Vannote et al. 1980, Arango et al. 2008, Bernot et al. 2010). However, saturation of in-stream nutrient processing is common in streams draining urban and agricultural landscapes as biofilm growth (and related processing) is limited by resources other than N and P (Bernot and Dodds 2005, Arango et al. 2008, Johnson et al. 2009). I hypothesize that low trace metal concentrations in eutrophic streams limit biofilm growth, contribute to saturation of nutrient removal processes, and limit biofilm storage of N and P. To test this broad hypothesis, I ask two research questions:

- Q1: Are trace metals at in tributaries to Lake Erie at concentrations that may limit primary production?
- Q2: When supplemented with trace metals, does primary production and nutrient storage increase?

Methodology

Trace metal and nutrient concentrations in tributaries to Lake Erie

In summer 2016, trace metal and nutrient concentrations were measured in small to mid-order streams in the Lake Erie watershed. Smaller streams were targeted for sampling because previous studies have demonstrated that streams of this size are both more abundant on the landscape and provide the majority of nutrient processing and storage in a watershed (Vannote et al. 1980, Bernot et al. 2010). Water chemistry was monitored from spring to late summer to coincide with the periods of peak nutrient delivery and seasonal HAB formation in Lake Erie.

In collaboration with the National Center for Water Quality Research (NCWQR) at Heidelberg University, surface water samples from five Lake Erie tributaries were shipped to Kent State University for trace metal analysis. Tributaries sampled were Rock and Honey Creeks (Sandusky watershed), Lost Creek (Maumee watershed), Portage Creek, and River Raisin. Surface water samples were collected at least daily by ISCO refrigerated samplers and samplers were emptied by NCWQR staff weekly. To minimize the effects of sample holding time on metal concentrations, only the most recently collected surface water sample (i.e., <24 h since collection) was selected for metals analysis. Water samples were filtered (0.45 µm), acidified to pH <2 with HNO_3, and stored at room temperature until transport to Kent State University. Water samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) for a suite of essential trace metals (Fe, Zn, Cu, Mn, Mo, and Ni). The trace metal data was supplemented with nutrient chemistry data that NCWQR shares through the Tributary Loading Program (https://www.heidelberg.edu/academics/research-and-centers/national-center-for-water-quality-research/tributary-data-download).

Twenty-six headwater streams (1–3rd order) in the Cuyahoga and Chagrin River watersheds (both drain to Central Basin of Lake Erie) were sampled monthly from June–August 2016. Filtered (0.45 µm) and unfiltered surface water was collected from all headwater streams within a 72-h period. Filtered samples were analyzed for nitrate (NO_3^-) by ion chromatography, soluble reactive phosphorus (SRP) by spectrophotometry (molybdate blue method), and trace metals (Fe, Zn, Cu, Mn, Mo, and Ni) by ICP-OES.

Nutrient and trace metal limitation of primary producers

Using data from the water chemistry survey of streams in the Central Basin watersheds, five streams with potential nutrient and/or trace metal limitation were identified: Brandywine Creek, Mill Creek, Breakneck Creek, Fish Creek and Cicada Creek. Nutrient and trace metal limitation was quantified using trace metal nutrient diffusing substrates (tNDS). Using this approach, the concentration of nutrient and/or trace metals are elevated in the small area around an attachment substrate (Fig. 1). If low nutrient or trace metal concentrations in the stream are limiting growth, then the biofilms growing on the substrate that supplies the limiting element should grow to a greater biomass. The tNDS were composed of a general growth agar amended with nutrients (N and P) and trace metals (Fe, Zn, Mo and Ni) and a fritted glass disk was placed on the surface of the agar to provide a substrate for biofilm attachment (Costello et al. 2016). Single element and multi-element mixtures were used

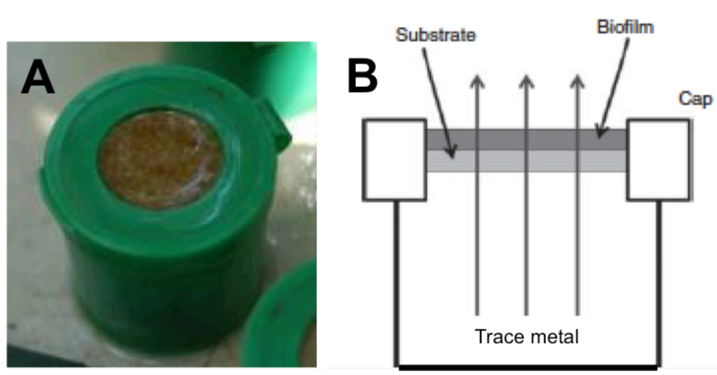

Figure 1. Photograph of a single tNDS colonized by biofilm (A) and a cross-sectional schematic of a tNDS.

to target specific mechanisms of limitation and co-limitation by nutrients and trace metals. Treatment combinations included: N only, P only, and Fe only to test for single element limitation, N-P-Fe and N-P-Fe-Mo-Ni-Zn to test for general co-limitation, Mo-P-Fe to test for co-limitation related to nitrification, Ni-P-Fe to test for co-limitation related to organic N acquisition, and N-Zn-Fe to test for co-limitation related to organic P cycling.

In September 2016, the tNDS (70 per stream) were secured to the bed of each stream with a Hobo light sensor and MiniDOT oxygen and temperature sensor to measure water column conditions. Filtered water samples were collected to measure ambient nutrient and trace metal concentrations at deployment and collection of the tNDS cups. After three weeks of incubation, the tNDS cups were collected and the fritted discs were removed from the agar and placed in a small chamber with stream water to quantify metabolism (GPP and ER) using the light-dark incubation method (Bott et al. 1997). Rates of nutrient assimilation (i.e., NO_3^- and PO_4^{3-} uptake) were also calculated during chamber incubations (Steinman and Mulholland 2006, North et al. 2007). Biofilms and glass substrates were frozen prior to measuring biofilm chlorophyll *a* and biomass. Due to a large storm event during the incubation, some individual cups and entire treatments were lost from the stream or buried; these missing data have been excluded from all analyses. For the purposes of this report, we only present the results of algal biomass (i.e., chlorophyll *a*).

Principal Findings and Results

Trace metal and nutrient concentrations in tributaries to Lake Erie

Ambient nutrient and trace metal concentrations in tributaries to Lake Erie exhibited strong regional patterns (i.e., Western and Central Basin streams differed), but we observed potential trace metal limitation across all study streams. On average, streams draining into the Western Basin of Lake Erie had greater dissolved N and P concentrations than tributaries in the Central Basin watersheds (Table 1). However, Breakneck Creek and Mill Creek in the Cuyahoga River watershed had NO_3^- and PO_4^{3-} concentrations that approached those observed in eutrophic Western Basin streams. In all study streams, dissolved Zn, Ni, and Mo were frequently at concentrations below our detection limits, which suggests that these trace metals may be near the physiological limits of algae (Table 1). Mo concentrations were higher in the Western Basin tributaries than in the Central Basin tributaries, in which Mo was never measured above our detection limits. Fe concentrations varied greatly between and within streams (Table 1). Lost Creek (Western Basin) and Brandywine Creek (Central Basin) had the highest average Fe concentrations (Table 1), but both of those streams had Fe concentrations measured below the potential limiting concentrations at some time during the summer (Table 1). River Raisin (Western Basin) and Cicada and Breakneck Creeks (Central Basin) had average Fe concentrations that were near or below potential limiting concentrations (Table 1). The concentrations of dissolved N and P were correlated (Pearson $r = 0.34$, $p < 0.001$), but ambient trace metals were not correlated to N or P (e.g., Fe and P: Pearson $r = 0.15$, $p = 0.08$). These data from tributaries to Lake Erie confirm national trends in water quality that suggest trace metals can be measured at concentrations known to limit or co-limit (with macronutrients) primary producer growth, and suggest that there is potential trace metal limitation in both Western and Central Basin watersheds.

Table 1. Ambient nutrient chemistry of streams draining into Lake Erie. Twenty-six tributaries (1–3rd order) in the Central Basin (Cuyahoga and Chagrin River watersheds) were sampled from June–August 2016, and mean water quality is reported for the five streams in which nutrient limitation assays were completed. The range of concentrations for all 26 streams are also reported (in parentheses). Western Basin tributaries were sampled weekly from March–September 2016 (n=23–28) and values reported are means and ranges (in parentheses). Ambient trace metal concentrations that are predicted to cause growth limitation are provided for reference.

Stream	NO_3^--N (µg/L)	PO_4^{3-}-P (µg/L)	Fe (µg/L)	Zn (µg/L)	Ni (µg/L)	Mo (µg/L)	Chlorophyll *a* (µg/cm^2)
Central Basin tributaries							
Brandywine	138	17	26	< 2	< 5	< 5	65.8
Mill	587	82	17	< 2	< 5	< 5	21.6
Breakneck	3870	59	7.3	< 2	< 5	< 5	19.5
Fish	192	37	10	< 2	< 5	< 5	17.6
Cicada	87	19	1.3	< 2	< 5	< 5	9.0
26 streams	(33–3870)	(14–290)	(<1–45)	(<2–9)	(< 5)	(< 5)	
Western Basin tributaries							
Honey	2730 (260–9560)[b]	58 (12–152)[b]	29 (<1–166)	2 (<2–4)	< 5 (<5–6)	6 (<5–13)	
Lost	4220 (1520–9900)	29 (3–276)	35 (<1–167)	2 (<2–4)	< 5	5 (<5–10)	
Portage	3910 (250–9660)	91 (19–323)	19 (<1–145)	3 (<2–7)	< 5	7 (<5–15)	
Raisin	2970 (60–7900)	30 (6–66)	5.4 (<1–37)	4 (<2–23)	< 5 (<5–9)	< 5 (<5–8)	
Rock	1110 (<50–4280)	39 (9–105)	31 (<1–274)	4 (<2–41)	< 5	6 (<5–10)	
Predicted limits[a]			4.4	0.5	0.6	0.03	

[a] Potential limiting concentrations are based on cellular quotas from marine algae (Moore et al. 2013) and measured inorganic carbon concentrations (130 mg/L). [b] NO_3^- and PO_4^{3-} data from Western Basin tributaries is from the Heidelberg University Tributary Loading Program.

Nutrient and trace metal limitation of primary producers

Nutrient additions performed in five streams in the Cuyahoga River watershed demonstrated that trace metals may be limiting algal growth, nutrient limitation differed between streams, and the differential response of algae to nutrient amendments was related to ambient nutrient concentrations. Single element additions (Fig. 2) had less of an effect on stimulating algal biomass when compared to multi-element treatments (Fig. 3). This supports cross-ecosystem studies that found co-limitation of primary producer growth is more common

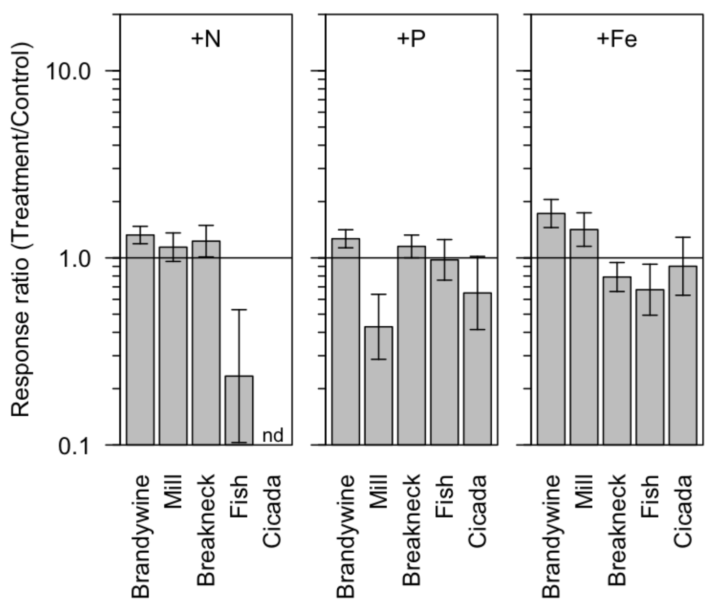

Figure 2. Response of primary producer biomass in five streams to single element additions of essential nutrients (N, P, or Fe). Response ratios >1 indicate greater biomass with nutrient amendment relative to controls and ratios <1 had lower biomass on nutrient-amended treatment relative to controls. Error bars indicate standard errors. nd = no data.

that limitation by a single nutrient (Francoeur 2001, Elser et al. 2007). N and P only stimulated algal biomass in Brandywine Creek only, and Fe stimulated growth in Brandywine and Mill Creeks (Fig. 2). This suggests that although single nutrient limitation is less common than multi-element co-limitation, it is just as likely for a trace metal alone to be limiting as a macronutrient like N or P.

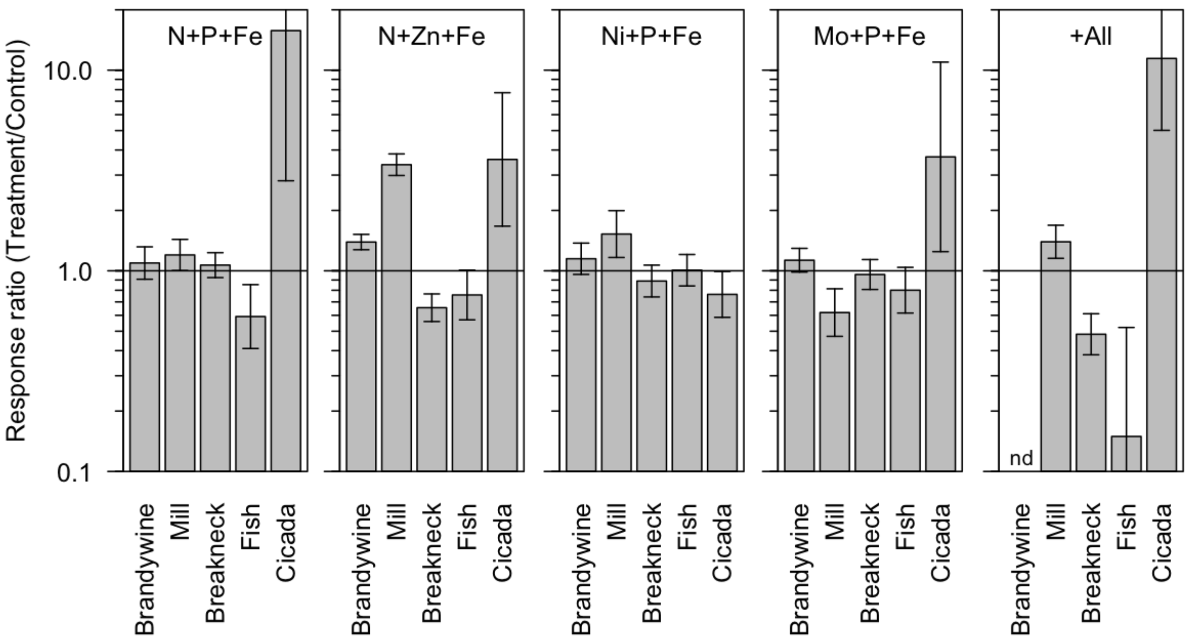

Figure 3. Response of primary producer biomass in five streams to multi-element additions of potentially limiting nutrients. Response ratios >1 indicate greater biomass with nutrient amendment relative to controls and ratios <1 had lower biomass on nutrient-amended treatment relative to controls. Error bars indicate standard errors. nd = no data.

For the multi-element treatments, there was evidence that three of our five student streams (Brandywine, Mill, and Cicada Creeks) all may have been co-limited by nutrients and trace metals (Fig. 3). Biofilms in Brandywine Creek exhibited stimulated growth when provided with N, Fe, and Zn together (Fig. 3 N+Zn+Fe). Zn is needed in the enzyme alkaline phosphatase, and if Zn stimulates growth, then biofilms in Brandywine may have been limited by their ability to access organic P. Biofilms in Mill Creek were co-limited by nutrients and trace metals, and growth was stimulated by Zn and Ni (Fig. 3 N+Zn+Fe and Ni+P+Fe). This also suggests greater growth when alleviating limitation to enzymes responsible for using organic P (Zn in alkaline phosphatase) and organic N (Ni in urease). Most striking was Cicada Creek, which did not respond to any of the single element additions but showed a ten-fold increase in biomass when provided with a mixture of N, P, and trace metals. Inorganic N and P in combination with trace metals caused the greatest stimulation of biomass (Fig. 3 N+P+Fe and +All), but the Ni+Zn+Fe and Mo+P+Fe treatments also caused a large increase in algal biomass. This suggests that biofilms in Cicada Creek can use organic P (Fig. 3 N+Zn+Fe) and N_2 via nitrification (Fig. 3 Mo+P+Fe) as alternative nutrient sources when the appropriate trace metal is supplied. Results from both Breakneck Creek and Fish Creek indicated that these biofilms were likely not limited or co-limited by nutrients or trace metals and thus growth was limited by other factors (e.g., light, disturbance). All together, the magnitude of response from the Zn amended treatments suggests that Zn-P co-limitation is common even in relatively nutrient rich streams. These data highlight the importance that P availability and P recycling play in driving primary production.

Associated additional research

In addition to the proposed research, an unfunded project was completed in the Central Basin tributaries. By gaining site access and visiting the 26 tributaries repeatedly through the summer, we could leverage this effort into a related undergraduate project (see Citations). In 18 of the 26 tributaries that spanned a rural–urban gradient, we placed organic substrates that differed in nitrogen content (cotton and silk strips representing low and high N, respectively) into the stream to measure rates of decomposition and microbial community composition. We found that rates of decomposition were correlated to ambient nitrate concentrations, which suggests that N availability plays an important role in decomposition. The taxonomic diversity of the microbial community was similar on the different organic materials but the composition of the community differed between substrates. These data suggest that nitrogen content in substrates and stream water is a critical driver of microbial decomposition in these diverse streams.

Finding Significance

This study addresses crucial knowledge gaps about how trace metals may limit algal production. The data show that limitation of primary production by trace metals can occur as frequently as limitation by macronutrients like N and P. Importantly, limitation by trace metals was observed in both nutrient-replete eutrophic streams and nutrient-poor oligotrophic streams. Excess nutrient inputs (mostly P) to Lake Erie have been linked to recurring seasonal planktonic HABs, and a massive amount of resources are being devoted to reducing P loading into the lake (IJC 2014). Small streams can be very efficient at slowing nutrient transport to downstream ecosystems by storing nutrients in biomass and potentially removing N and P through burial and nutrient transformations (Peterson et al. 2001, Bernot et al. 2010). This data show that trace metals (especially Zn) may be a pathway for promoting algal growth in streams, which can increase nutrient removal rates and ultimately reduce or delaying the export of macronutrients to Lake Erie. Given the extent of nutrient sources that drive HABs, management efforts that consider trace metals may be an important tool for addressing nutrient load reduction goals.

References

Arango, C. P., J. L. Tank, L. T. Johnson, and S. K. Hamilton. 2008. Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams of varying land use. *Limnology and Oceanography* 53:2558–2572.

Bernot, M. J., and W. K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems* 8:442–453.

Bernot, M. J., D. J. Sobota, R. O. Hall, P. J. Mulholland, W. K. Dodds, J. R. Webster, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, S. V Gregory, N. B. Grimm, S. K. Hamilton, S. L. Johnson, W. H. McDowell, J. L. Meyer, B. J. Peterson, G. C. Poole, H. M. Valett, C. P. Arango, J. J. Beaulieu, A. J. Burgin, C. L. Crenshaw, A. M. Helton, L. T. Johnson, J. Merriam, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, S. M. Thomas, and K. Wilson. 2010. Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology* 55:1874–1890.

Bott, T. L., J. T. Brock, A. Baattrup-Pederson, P. A. Chambers, W. K. Dodds, K. T. Himbeault, J. R. Lawrence, D. Planas, E. Snyder, and G. M. Wolfaardt. 1997. An evaluation of techniques for measuring periphyton metabolism in chambers. *Canadian Journal of*

Fisheries and Aquatic Sciences 725:715–725.
Bronk, D. A., P. M. Glibert, and B. B. Ward. 1994. Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science* 265:1843–1846.
Costello, D. M., E. J. Rosi-Marshall, L. E. Shaw, M. R. Grace, and J. J. Kelly. 2016. A novel method to assess effects of chemical stressors on natural biofilm structure and function. Freshwater Biology 61:2129–2140.
Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10:1135–1142.
Francoeur, S. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society* 20:358–368.
Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnology and Oceanography* 33:796–822.
[IJC] International Joint Commisssion. 2014. A balanced diet for Lake Erie: Reducing phosphorus loadings and harmful algal blooms.
Johnson, L. T., J. L. Tank, and W. K. Dodds. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. *Canadian Journal of Fisheries and Aquatic Sciences* 66:1081–1094.
Lock, M. A., R. R. Wallace, J. W. Costerton, R. M. Ventuilo, and S. E. Chariton. 1984. River epilithon: toward a structural-functional model. *Oikos* 1:10–22.
Moore, C. M., M. M. Mills, K. R. Arrigo, I. Berman-Frank, L. Bopp, P. W. Boyd, E. D. Galbraith, R. J. Geider, C. Guieu, S. L. Jaccard, T. D. Jickells, J. La Roche, T. M. Lenton, N. M. Mahowald, E. Maranon, I. Marinov, J. K. Moore, T. Nakatsuka, A. Oschlies, M. A. Saito, T. F. Thingstad, A. Tsuda, and O. Ulloa. 2013. Processes and patterns of oceanic nutrient limitation. *Nature Geoscience* 6:701–710.
North, R. L., S. J. Guildford, R. E. H. Smith, S. M. Havens, and M. R. Twiss. 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnology and Oceanography* 52:315–328.
Nriagu, J. O., G. Lawson, H. K. T. Wong, and V. Cheam. 1996. Dissolved trace metals in Lakes Superior, Erie, and Ontario. *Environmental Science and Technology* 30:178–187.
Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Martí, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. *Science* 292:86–90.
Schlesinger, W. H., and E. S. Bernhardt. 2013. Biogeochemistry: An analysis of global change. 2nd edition. Academic Press, Boston, MA, USA.
Shiller, A. M. 1997. Dissolved trace elements in the Mississippi River: Seasonal, interannual, and decadal variability. *Geochimica et Cosmochimica Acta* 61:4321–4330.
Sobota, D. J., S. L. Johnson, S. V. Gregory, and L. R. Ashkenas. 2012. A stable isotope tracer study of the influences of adjacent land use and riparian condition on fates of nitrate in streams. *Ecosystems* 15:1–17.
Steinman, A. D., and P. J. Mulholland. 2006. Phosphorus limitation, uptake, and turnover in benthic stream algae. Pages 187–212Methods in Stream Ecology. 2nd edition.
Vannote, R. L., G. W. Minshall, K. W. Cummins, K. W. Sedell Jr., and C. E. Cushing. 1980. River continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130–137.

2. Citations

No citations to date, but two presentations planned for June 2017:

- Costello, D, and A Fitzgibbon. Beyond N and P: Trace metal limitation of algal production. *Annual Meeting of the Society for Freshwater Science*, June 2017, Raleigh, NC.
- Risteca, P., A. Fitzgibbon, D. Costello, S. Tiegs, and J. Kelly. Composition and function of microbial communities colonizing organic substrates along a rural to urban gradient. *Annual Meeting of the Society for Freshwater Science*, June 2017, Raleigh, NC.

3. Students Supported

Andrea Fitzgibbon, Kent State University, PhD student, Fall 2016

4. Profession Placement of Graduates

None

5. Awards or Achievements

None

6. Additional Funding for this Project

No additional funds directly related to this project; however, one funded project is related and will be used for comparison, and one pending proposal has used the data presented here to support research questions and methods.

Costello, D. and A. Fitzgibbon. 2016–2017. Primary production in Huron Mountain streams: Trace metal use and spatial variability. *Huron Mountain Wildlife Foundation*, $3358.

- Funding does not directly support this project, but the OWRC project and the HMWF project use similar methods. Ultimately, we hope to compare the results from Ohio streams to results from the relatively pristine streams in the Upper Peninsula of Michigan.

Costello, D. 2018–2020. Understanding the role of trace metals in harmful algae growth, composition, and toxin production in the Great Lakes. *EPA STAR Early career: Freshwater harmful algal blooms* (EPA-G2017-STAR-A2). Pending programmatic review, $319,956.

- Proposal received a “favorable” rating based on scientific and technical merit and is now under programmatic review.