AN ECOLOGICAL AND BIOGEOCHEMICAL STUDY OF DISSOLVED SILICON IN HUMAN-DOMINATED FRESHWATER ECOSYSTEMS

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Submitted to the faculty of the University Graduate School in partial fulfillment of the requirements for the degree Doctor of Philosophy in the O’Neill School of Public and Environmental Affairs, Indiana University
May 2022
Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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April 22, 2022
To my grandmother, Rochelle, for seeing my dreams and making them her own.
Acknowledgements

It takes a village to raise a doctoral graduate and I am lucky to credit so many in my success. Firstly, I would like to thank my doctoral advisor, Dr. Todd V. Royer. His guidance and feedback have been integral in the completion of my dissertation research and my development as a scientist. I am especially grateful for his patience and support outside of our research, as I have developed as a person, not just a researcher, under his advising. I would also like to thank the members of my research committee: Drs. Kim Novick, Rich Phillips, and Adam Ward, whose advice and availability have propelled my dissertation research to the finish line.

The contributions of Leah Hagemeier, Lindsey Rasnake, Megan Gokey, Sarah Powers, Ursula Mahl, Dr. Shannon Speir, Dr. Matt Trentman, and Dr. Jennifer Tank, to lab analyses, field assistance, technical support, and manuscript preparation have been integral in the completion of my dissertation and jump-starting my career. Additionally, financial support from the O’Neill School of Public and Environmental Affairs, Indiana University Integrated Program in the Environment, and the Indiana University Graduate School has provided essential research and travel funding throughout my doctoral studies.

To the friends I have made during my graduate studies – Paige Becker, Molly Cain, Sander Denham, Jase Hixson, Colleen Rosales, Elena Solohin, and Emily Taylor – I thank you for your camaraderie, support, and encouragement. Through our shared meals, travels, and remote work sessions, you made the labors of doctoral work fun and rewarding. I am grateful for our shared experiences in graduate school and look forward to the shared experiences to come! And of course, to the friends I’ve made along the way – Heather Bearnes-Loza, Arizona Fox, Patrick Haggerty, Claire Huber, Katy Hunt, Michael McGrath, Myles Moore, Lucas Miller, Sydney Olund, Cameron Stewart, Brady Thompson, and Noor Vuya – thank you for being a
never-ending source of encouragement, confidence, and comfort. Most importantly, thank you for providing moments that make life colorful and exhilarating.

I have immense gratitude for my family whose pride in me has been such a positive source of encouragement throughout my life. To my parents: Farhad, Amy, and Kim; grandparents: Rochelle, Judy, Margaret, and Harry; and brothers: Cyrus, Darayus, and Devin, I appreciate you making me feel at home, no matter where I was. Thank you for setting me up for success, supporting my goals, and being there for all the important moments.

Finally, to my partner George, there are not enough kind words to express how your unwavering support and encouragement anchors my sanity, emboldens my confidence, and provides the inspiration for future pursuits. You have never doubted my abilities and only challenge me to dream bigger. I will be forever thankful for your friendship, enthusiasm, and love.
Freshwater ecosystems are critical in the biogeochemical processing of nutrients such as carbon (C), nitrogen (N), phosphorus (P), and silicon (Si). The relative proportions of these nutrients are key determinants of algal community composition and, subsequently, the water quality and trophic dynamics of aquatic systems. Human landscape modifications such as agriculture and reservoirs elevate the ratios of N and P relative to Si, leading to stoichiometric imbalance and nutrient limitation. The motivation for my research is the recognition that decreased availability of Si relative to N and P can limit diatom growth and facilitate the formation of harmful algal blooms. In this study, I seek to characterize the Si:N:P stoichiometry in human-dominated, freshwater ecosystems and fill critical knowledge gaps in our understanding of the relationship between nutrients, algal community composition, and dissolved organic matter (DOM). I monitored agricultural runoff at both the field- and watershed-scales to examine the response of Si concentrations and ratios to changing hydrology and vegetation cover and the corresponding risk of harmful algal bloom formation. Based on these findings, I sought to quantify the Si limitation of benthic diatoms, which require Si, and relate changes in algal community composition to nutrient availability. To understand Si dynamics within a reservoir, I constructed an annual Si budget for Lake Monroe, the largest reservoir in Indiana, and identified mechanisms controlling Si retention. We further analyzed the relationship between Si, algae, and DOM to determine how shifts in nutrient concentrations affect dissolved organic carbon composition. As expected, the agricultural study streams had excess N relative to Si which became increasingly
imbalanced at higher flows. I therefore hypothesized benthic diatoms would be Si-limited; however, experimental Si enrichment did not increase algal biomass or the relative proportion of diatoms, highlighting the heterogeneity of benthic communities in headwater streams. I also found Lake Monroe retained about 40% of the incoming Si which was largely mediated by diatom growth and sequestration. Furthermore, components of DOM were highly correlated with the relative abundances of both diatom and harmful algal taxa suggesting the optical properties of DOM are sensitive to algal community composition. My dissertation adds to the growing body of literature in Si biogeochemistry and addresses novel concepts such as the quantification of Si limitation in streams and the coupling between DOM and Si stoichiometry.
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Chapter 1

Introduction

Silicon (Si) is an essential nutrient in aquatic ecosystems, yet the understanding of Si transport and biogeochemical processing is lacking compared to that of nitrogen (N) and phosphorus (P), especially in freshwaters. In fact, a Scopus review of published literature with the keywords “freshwater” and either “nitrogen”, “phosphorus”, or “silicon” shows published studies focused on N or P are an order of magnitude greater than those on Si. The importance of Si in freshwater is due largely to its critical role in the production of diatoms, which tend to be the predominant form of algae in most in streams (Lowe and LaLiberte 2007; Armbrust 2009). Unlike most other algal taxa, diatoms require dissolved silica (SiO$_2$, hereafter DSi) to build frustules and, in addition to producing nearly half of the global oxygen, are an important food source for zooplankton and aquatic insects (Martin-Jezequel et al. 2000; Street-Perrott and Barker 2008; Rousseaux and Gregg 2013).

The ultimate source of DSi in the biosphere is from the chemical weathering of silicate rocks, which make up the majority of the Earth’s crust (De La Rocha and Conley 2017). The dissolution of silicate minerals consumes atmospheric carbon dioxide thus linking the biogeochemical Si cycle with the global carbon cycle (Cermeño et al. 2015). Diatoms and vegetation transform DSi into biogenic silica (BSi) to form frustules and phytoliths, respectively (Hildebrand 2008; Epstein 2009). BSi is an amorphous form of silica and can be released from organisms as amorphous silica (ASi); compared to silicate mineral weathering, BSi and ASi are bioavailable forms of Si which are readily dissolved and taken up by plants and siliceous algae (Vandevenne et al. 2013; Carey and Fulweiler 2016).
The transport and fate of DSi in freshwaters is disturbed by human landscape modifications such as agriculture and damming, which modify the biogeochemical cycling of Si through alteration of vegetative cover and increasing hydrologic residence times (Humborg et al. 2008; Struyf et al. 2010; Carey and Fulweiler 2016). Terrestrial vegetation takes up DSi from the soil to form phytoliths, a form of amorphic Si critical in plant structure and defense (Cooke and Leishman 2011; Hodson 2019). Furthermore, damming has been shown to retain Si as increased hydrologic residence times allows for increased Si uptake and settling within the reservoir (Humborg et al. 2008; Maavara et al. 2014, 2020). Long-term agricultural land use, like in the Midwestern United States, has reduced the DSi export in agricultural rivers (Clymans et al. 2011; Guntzer et al. 2012; Vandevenne et al. 2012) while increasing N and P export, ultimately changing the stoichiometry of the nutrient export from agricultural watersheds (Turner et al. 2003; Leong et al. 2014; Downing et al. 2016). Although the effect of agricultural practices on Si stoichiometry has been described for some locations, DSi data from headwater agricultural watersheds can help elucidate the mechanisms controlling Si biogeochemistry and stoichiometry in human dominated landscapes.

Stoichiometric ratios near the “Redfield ratio” of 16N:1P:20Si favor growth of diatoms over cyanobacteria in marine environments (Redfield 1963; Brzezinski 1985). Systems with ambient nutrient concentrations above 16N:20Si and 1P:20Si are considered “Si limited”; however, the Si requirements of freshwater diatoms can be up to an order of magnitude greater than marine species and there is a wide range in the stoichiometric nutrient demands between different freshwater diatoms (Conley et al. 1989). Previous studies have pointed to the role of DSi limitation relative to N and P in increased harmful algal bloom (HAB) frequency and intensity in
fresh and marine waters (Teubner and Dokulil 2002; Frings et al. 2016), yet the role of Si limitation in facilitating HABs in headwater streams and rivers has not been quantified.

Reservoirs are retaining larger proportions of DSI relative to free-flowing rivers due to the increase in water residence time and the subsequent increase in nutrient removal in reservoir sediment (Humborg et al. 2006, 2008; Harrison et al. 2012; Frings et al. 2014). In one study of the “artificial lake effect” (van Bennekom and Salomons 1981), watersheds in Finland and Sweden representing a gradient of damming showed significant reductions in DSI as the percentage of the dammed watershed area increased (Conley et al. 2000). This relationship was attributed to increased hydrologic residence time and diatom growth. Reservoirs increase light availability relative to streams, which promotes phytoplankton growth, and trap sediment, which tend to be made up of silicate minerals. Similar results were found in watersheds surrounding the Baltic Sea (Humborg et al. 2006) and the global estimate of Si retention due to dams is estimated to be 5% of the global riverine Si flux (Maavara et al. 2014). Recent reviews of the global retention of DSI through reservoirs highlight the significant fraction of DSI sequestered in lentic systems which represents an important component of the global biogeochemical cycle of Si (Harrison et al. 2012; Frings et al. 2014; Maavara et al. 2014). As a larger proportion of the world’s rivers become fragmented with dams and reservoirs, this will have significant implications for nutrient availability in downstream ecosystems, aquatic food webs, and carbon cycling.

The ratios of Si, N, and P can affect the dynamics of algal communities, including the formation of HABs by cyanobacteria, yet there have been no studies on the potential connection between nutrient stoichiometry and dissolved organic matter composition. Ratios below 1 Si: 1 N are hypothesized to shift algal communities away from diatom dominance which can alter
trophic dynamics and, ultimately, carbon sequestration. For example, Turner et al. (1998) found that primary production in the Gulf of Mexico was higher when Si:N ratios were greater than 1:1. This result bolsters the idea that diatoms, a favorable food source of zooplankton, dominate when Si:N is greater than one, but does not directly relate the abundance of diatoms to carbon quality and availability. Understanding the effect of changing Si stoichiometry on diatom productivity would link algal community dynamics to carbon quality and availability in surface waters.

**Dissertation trajectory**

The ultimate goal of my research was to identify controls on aquatic Si biogeochemistry and better understand its effects on the structure and function of human-dominated, freshwater ecosystems. In Chapter 1, I used biweekly monitoring data from stream sites and tile drains in an agricultural watershed to understand how vegetative cover and hydrology influenced both DSi concentrations and stoichiometry. I found increased vegetative cover, as winter cover crops, positively impacted the N:Si ratios; however, the nutrient imbalance between N, P, and Si was exacerbated at high flows, increasing the risk of harmful algal blooms in downstream waters. To better assess this risk, I used nutrient diffusing substrata to quantify nutrient limitation of algae in the agricultural stream studied in Chapter 1. Under conditions of increased N, P, and Si concentrations, I characterized the nature of nutrient limitation on algal biomass and community composition. While I expected diatom abundance to increase with additional Si, there was no direct effect of any nutrient treatment on algal abundance or community composition. I attributed this to the potential of selective grazing of diatoms and the flexibility in nutrient requirements of benthic phytoplankton. Furthermore, other studies of nutrient limitation in agricultural streams found little to no nutrient limitation due to high ambient concentrations. To further test the
relationship between Si and diatom abundance, I calculated a Si budget for Lake Monroe, the largest reservoir in Indiana, and found that over 40% of incoming DSi was retained in the Lake. This retention was largely driven by diatom growth as their dominance coincided with declines in epilimnetic DSi concentrations; however, shifts between diatom and non-siliceous phytoplankton dominance did not relate to changes in ambient nutrient stoichiometry, highlighting the knowledge gap in constraining the nutrient requirements of freshwater algae. The canonical “Redfield ratio” describes the stoichiometric composition of marine algae, but the requirements of freshwater algae are less understood. Finally, in Chapter 4, I characterized the optical properties of fluorescent dissolved organic matter (DOM) and related these to algal community composition and nutrient concentrations. I found strong relationships between components of DOM, nutrient concentrations, and algal biomass although our data do not show these relationships are mediated by algal productivity. Algal biovolumes were related to allochthonous, humic-like DOM despite results from direct studies of algal-derived DOM linking these substances to autochthonous, protein-like DOM. This finding highlights the complexity of DOM composition and the many environmental processes that can affect its structure and function in aquatic systems.
References


De La Rocha C, Conley DJ (2017) Silica Stories. Springer, Cham, Switzerland


Chapter 2
Silicon concentrations and stoichiometry in two agricultural watersheds: Implications for management and downstream water quality

Abstract
Agriculture alters the biogeochemical cycling of nutrients such as nitrogen (N), phosphorus (P), and silicon (Si) which contributes to the stoichiometric imbalance among these nutrients in aquatic systems. Limitation of Si relative to N and P can facilitate the growth of non-siliceous, potentially harmful, algal taxa which has severe environmental and economic impacts. Planting winter cover crops can retain N and P on the landscape, yet their effect on Si concentrations and stoichiometry is unknown. We analyzed three years of biweekly concentrations and loads of dissolved N, P, and Si from subsurface tile drains and stream water in two agricultural watersheds in northern Indiana. Intra-annual patterns in Si concentrations and stoichiometry showed that cover crop vegetation growth did not reduce in-stream Si concentrations as expected, although, compared to fallow conditions, winter cover crops increased Si:N ratios to conditions more favorable for diatom growth. To assess the risk of non-siliceous algal growth, we calculated a stoichiometric index to quantify biomass growth facilitated by excess N and P relative to Si. Index values showed a divergence between predicted algal growth and what we observed in the streams, indicating other factors influence algal community composition. The stoichiometric imbalance was more pronounced at high flows, suggesting increased risk of harmful blooms as climate change increases the frequency and intensity of precipitation in the midwestern U.S. Our data include some of the first published measurements of Si within small agricultural watersheds and provide the groundwork for understanding the role of agriculture on Si export and stoichiometry.
Introduction

Silicon (Si) is an essential nutrient in aquatic ecosystems and plays a critical role in the growth of freshwater diatoms. Diatoms are the predominant form of benthic algae in most streams (Lowe and LaLiberte 2007) and are an important food source for zooplankton and aquatic insects (Kilham 1971; Martin-Jezequel et al. 2000). Furthermore, diatoms are highly productive autotrophs, producing almost half of the total oxygen in our atmosphere and significantly affecting the global carbon cycle (Street-Perrott and Barker 2008; Smol and Stoermer 2010; Rousseaux and Gregg 2013). Unlike most other forms of algae, diatoms require dissolved silicon dioxide (SiO₂, hereafter DSi) to build frustules (silicified cell walls). Diatoms often bloom until DSi becomes depleted relative to nitrogen (N) or phosphorus (P), at which time diatoms become Si-limited and non-siliceous taxa may become dominant (Officer and Ryther 1980; Teubner and Dokulil 2002; Frings et al. 2016). Thus, stoichiometric ratios between Si, N, and P that are imbalanced relative to the requirements of diatoms can favor the growth of non-siliceous and potentially harmful taxa, such as cyanobacteria (Schelske and Stoermer 1971; Conley et al. 1993; Turner et al. 2003).

Anthropogenically-driven modifications to the landscape can disturb the transport and fate of DSi in freshwaters. In particular, long-term agricultural land use, as is found across the midwestern United States (U.S.), can modify the biogeochemical cycling of Si through alteration of vegetative cover and hydrologic flow paths (Struyf et al. 2010; Carey and Fulweiler 2016). Most terrestrial plants will incorporate Si into biomass, creating phytoliths that aid in plant defense and structure (Epstein 2009). Crops such as corn and soybeans, which are the predominant crops in the midwestern U.S., have high Si concentrations, often above 1% of their dry weight (Epstein 1994; Guntzer et al. 2012). DSi weathered from soil minerals is incorporated
into crop phytoliths, and then subject to loss from the system if grain or vegetative material is harvested and exported outside the watershed (Viaroli et al. 2013; Carey and Fulweiler 2016). Therefore, the long-term cultivation and harvest of crops can reduce Si availability in agricultural soils and the input of DSi to surface waters on century-long time scales (Struyf et al. 2010).

Hydrologic flow paths in agricultural systems often are modified to facilitate removal of water from fields and support soil conditions suitable for row-crop agriculture. In the midwestern U.S., this is accomplished through the use of subsurface tile drains, construction of drainage ditches, and the channelization of streams. Tile drains expedite the flow of water from the landscape to drainage ditches, essentially “short-circuiting” the flow of water through soil (Gentry et al. 2007; Vidon and Cuadra 2011; Williams et al. 2015). These modified hydrologic flow paths reduce rates of in-stream N and P processing and removal (Royer et al. 2006; King et al. 2015). Hydrologic modification also limits interactions between water and soil (Williams et al. 2014; King et al. 2014), which could reduce silicate weathering and the flux of DSi from tile drains to surface waters; however, we are not aware of any data characterizing DSi export from tile drains. The channelization of agricultural streams alters hydrologic residence times and reduces groundwater-surface water interactions. These highly modified agricultural streams tend to lack the geomorphic features that promote hyporheic exchange, including meanders and sediment heterogeneity (Wörman et al. 2002, Boano et al. 2014). Thus, groundwater inputs, which can have DSi concentrations 2-3x that of surface waters (Georg et al. 2009), are generally limited, potentially further altering DSi loads and downstream export of DSi.

Ultimately, row-crop agriculture reduces Si export while increasing export of N and P, thereby changing the stoichiometry of nutrient loads exported from agricultural watersheds (Turner et al. 2003; Leong et al. 2014; Downing et al. 2016). These changes in Si, N, and P
stoichiometry can contribute to the potential DSI limitation of diatoms and the increasing frequency and severity of downstream non-siliceous and often harmful blooms of algae and coastal hypoxic zones (Dupas et al. 2015; Royer 2020). The factors affecting the magnitude and temporal patterns in DSI export from small agricultural watersheds are not well described, particularly for intensively farmed regions of the midwestern U.S. (but see Viaroli et al. 2013 and Pinardi et al. 2018). The temporal patterns in stoichiometric imbalance in Si, N, and P are likewise poorly described, and this represents a knowledge gap in our understanding of how land management affects water quality in agricultural landscapes.

Agricultural conservation practices, such as winter cover crops, can reduce excess N and P export by assimilating nutrients and reducing erosion (Strock et al. 2004; Kaspar et al. 2007; Blanco-Canqui et al. 2015; Speir et al. 2021b). Most cover crops are not harvested, and their biomass (after termination) is left on the fields, increasing soil organic matter and returning assimilated nutrients, including Si, to the soil. Studies have analyzed the effects of winter cover crops on total and dissolved N and P export at the tile drain (Kaspar et al. 2012; Trentman et al. 2020; Speir et al. 2021b) and watershed scale (Daryanto et al. 2018; Hanrahan et al. 2018; Hallama et al. 2019), but the effect of cover crops on DSI is unknown. To address this knowledge gap, we monitored dissolved inorganic N (nitrate and ammonium; hereafter DIN), soluble reactive P (SRP), and DSI loss at the field and watershed-scale from two agricultural watersheds in northern Indiana twice monthly for water years 2018-2020. The main objectives of this study were to: (1) characterize the seasonal pattern in DSI concentrations and stoichiometry at the watershed and field scale, (2) quantify the field-scale effects of winter cover crops on DSI loss and nutrient stoichiometry, and (3) document temporal patterns in stoichiometric imbalances between N, P, and Si loads and the potential for the nutrient loads to facilitate downstream non-
siliceous algal blooms, including groups capable of toxin production (e.g., Cyanophycae, Dinophycae).

First, we hypothesized that seasonal patterns in DSi concentrations would relate to the growth of vegetation, including the cash crop (corn and soybeans) and the winter cover crop, as well as precipitation. Vegetative cover is a strong control on Si storage and export (Vandevenne et al. 2012; Carey and Fulweiler 2012b); therefore, we expected intra-annual variation in DSi concentrations at both the field and watershed scales. Specifically, we predicted 1a) lower tile water DSi concentrations from fields planted with cover crops, particularly during periods when cover crops were actively growing; and 1b) lower stream water DSi concentrations during the summer due to growth of the cash crop.

Second, we hypothesized cover crops would affect nutrient ratios in tile drains because cover crops affect N and P loss at the field scale. Specifically, cover crops at this study location reduce N loss (Hanrahan et al. 2018; Speir et al. 2021b; Hanrahan et al. 2021); however, the effect of cover crops on P loss to surface water is variable and depends, in part, on precipitation patterns (Trentman et al. 2020). Therefore, we predicted: 2a) tile water from cover cropped fields would have a higher Si:N molar ratio and that 2b) there would be no strong directionality in the response of Si:P molar ratios to winter cover crops.

Finally, we expected stoichiometric imbalance among N, P, and Si in these agricultural watersheds, and predicted this would: 3a) increase the potential for Si limitation of freshwater diatoms and the formation of cyanobacterial blooms in downstream waters; and 3b) the imbalance favoring cyanobacteria would be greatest in the summer and fall, when DSi concentrations are expected to be reduced relative to N and P.
Methods

Site Description

The two study watersheds, Kirkpatrick Ditch Watershed (KDW; 26.3 km²) and Shatto Ditch Watershed (SDW; 13.3 km²), both located in northern Indiana (Figure 2.1), are predominantly planted with a corn (*Zea mays* L.) and soybean (*Glycine max* L.) rotation with approximately equal amounts of each crop each year. In KDW, soils are primarily Mollisols with a silty-clay texture while the SDW has soils that are primarily Alfisols with a texture that ranges from sandy loams to loams and muck (Christopher et al. 2021). The fields in each watershed are “working lands”, managed by independent agricultural producers and are representative of typical agricultural practices in the midwestern U.S., including extensive subsurface tile drainage systems (Gökkaya et al. 2017, Trentman et al. 2020; Table 2.1) and N fertilization of corn at a rate of about 150 kg ha⁻¹ yr⁻¹, most of which occurs in the early spring. Additional fertilization with inorganic P is common in KDW, and fall manure application is occasional in both watersheds (Speir et al. 2021b).

Figure 2.1 Map of KDW (left) and SDW (right) and their respective locations within Indiana. Black points indicate tile drain sampling locations, bold lines within each watershed show perennial stream channels, and watershed outlets are denoted by stars. Shaded fields shaded indicate those planted with cover crops in the 2019 water year, the year of maximum implementation.
In both watersheds, farmers were reimbursed for costs associated with use of winter cover crops through the U.S. Department of Agriculture Regional Conservation Partnership Program. Farmer participation was voluntary and farmers made all decisions related to selection of cover crop species, timing of planting and termination, and all other management options. In KDW, cover crop planting began in 2015 and was maintained at 12-32% of the total tillable acres during our study period. In SDW, cover crop planting began in 2013 and coverage ranged from 22-68% during our study (Table 2.1). The most common cover crop species planted by producers in both KDW and SDW were annual rye-grass (*Lolium multiflorum* Lam.) and cereal rye (*Secale cereale* L.)

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Area (km²)</th>
<th>% area as row-crop</th>
<th>% Cover crop acreage (by water year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirkpatrick Ditch Watershed (KDW)</td>
<td>26.3</td>
<td>94</td>
<td>2018: 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2019: 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2020: 12</td>
</tr>
<tr>
<td>Shatto Ditch Watershed (SDW)</td>
<td>13.3</td>
<td>85</td>
<td>2018: 62</td>
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<td></td>
<td></td>
<td></td>
<td>2019: 68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2020: 22</td>
</tr>
</tbody>
</table>

Sampling design and protocol

We monitored sixteen tile drains and the watershed outlet in KDW, and only the watershed outlet for SDW, and we sampled twice monthly throughout the 2018-2020 water years (Oct 1- Sept 30). For both tile drains and stream sites, we measured instantaneous discharge and
sampled for DIN (nitrate and ammonium), SRP, and DSi. Additional results for DIN and SRP are reported in companion studies by Trentman et al. (2020, 2021) and Speir et al. (2020, 2021).

Instantaneous discharge was measured at tile drains using a timer and graduated receptacle; to ensure accurate volume and time values, discharge was measured multiple times until three consecutive measurements were all within 10%. For larger tile drains (diameter > 0.5m) or when any tile flow was > 10 L s⁻¹, we used an electromagnetic water velocity meter (March-McBirney Model 2000 Flo-Mate) and a wading rod to calculate discharge (Q) as:

\[
Q = r^2(\theta - \cos \theta \sin \theta) \times v \times 1000
\]

where \(\theta = \cos^{-1}\left(1 - \frac{d}{r}\right)\), \(r\) is the tile drain radius (m), \(d\) is the depth of water flow in the tile drain (m), and \(v\) is water velocity (m s⁻¹). Stream discharge was monitored by U.S. Geological Survey gages at the KDW (station #05524546) and SDW (station #03331224) watershed outlets.

We collected water samples for nutrient analyses directly from the tile drains or stream sites using a 60-mL syringe that was rinsed with sample water before collection. We collected separate samples for DIN/SRP and DSi analysis and we filtered all DIN and SRP samples immediately upon collection using glass fiber filters (Whatman GF/F). For DSi, we used cellulose filters (0.45µm pore size; Fisherbrand) to prevent contamination from glass. We transported samples on ice, froze them until analysis, and colorimetrically analyzed all samples using a Lachat QuikChem flow injection analyzer (Hach Company). We analyzed samples for SRP using the ascorbic acid method (Murphy and Riley 1962), for nitrate and nitrite (hereafter, nitrate) using the cadmium reduction method (APHA 2017), for ammonium using the phenol-hypochlorite method (Solórzano 1969), and for DSi using the heteropoly blue method (Sultan
For all nutrient analyses, we ran a certified standard to validate the standard curve and routinely calculated the method detection limit. The dataset analyzed here includes 1,108 individual tile drain measurements and 72 outlet measurements from KDW along with 75 outlet measurements from SDW. We monitored the same tile drains every year in KDW; however, due to changes in field management, the number of tiles draining fields with and without cover crops varied each year, with cover crop tiles ranging between 14-71% of the total monitored field tile drains. We found samples that were below detection were primarily from KDW tile drains; in total less than 1% of DIN, ~10% of SRP, and no DSi samples were below detection.

Data analysis

We conducted all data and statistical analyses using R (The R Foundation for Statistical Computing, Version 4.0.5, 2021). To characterize the seasonal pattern in DSi concentrations and stoichiometry, we divided data into four seasons: autumn (October-December), winter (January-March), spring (April-June), and summer (July-September) that correspond to crop planting and harvest and distinct temperature and hydrologic conditions affecting vegetation growth and nutrient transport in midwestern agricultural systems (Williams et al. 2015; Hanrahan et al. 2018).

In order to evaluate the effect of tile drain and stream discharge on DSi concentrations, we modeled the relationship between DSi concentrations and discharge using a power-law equation, expressed as \( C = aQ^b \), where \( C \) is concentration, \( Q \) is discharge, and \( b \) is a constant representing the slope of the relationship. The sign of the slope indicates whether a solute exhibits enrichment (positive slope), dilution (negative slope), or chemostatic (zero slope) behavior with discharge (Godsey et al. 2009; Bieroza et al. 2018). The value of the slope is used
to evaluate a solute’s response to discharge, where values close to one represent a proportional change in concentration with discharge (Leong et al. 2014).

We analyzed the effects of winter cover crops on DSi concentrations, loss, and stoichiometry using data collected from fourteen unique tiles draining specific fields (field tile drains) and we removed two “county” drains which aggregate a larger drainage area across multiple fields because these drains incorporate multiple fields and cannot be classified on the basis of cover crop use. In all other analyses, we included the county drains.

Within each water year and season, we quantified the effect of winter cover crops on KDW field tile drain DSi concentrations, loss, and stoichiometry using a Hierarchical Regression Model (HRM) and pair-wise comparisons. The HRM tests for differences between cover crop and no cover crop treatments while accounting for the random effects associated with tile drain location and the fixed effects of cover crop treatment, year, and season. We then used a post-hoc pairwise test to identify which years and seasons showed a significant (p<0.05) response to cover crop planting. We conducted these tests using the lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), and emmeans (Lenth et al. 2021) packages in R.

We calculated nutrient ratios between DIN, SRP, and DSi using molar concentrations of each sample value, and we based predictions for nutrient limitation on a modified Redfield ratio for freshwater diatoms (hereafter “freshwater Redfield ratio”) of 106C:16N:1P:40Si (Redfield 1963; Brzezinski 1985). We also modeled daily loads of DIN, SRP, and DSi from each watershed outlet using Loadflex, an R package which estimates solute loads using a composite model that combines a regression model with a residuals correction for a more accurate estimation of nutrient loads (Appling et al. 2015); we then used estimated loads to calculate daily yields of nutrients based on the area of each watershed.
We used estimated daily yields to calculate the Indicator of Freshwater Eutrophication Potential (IFEP) which predicts the potential growth of non-siliceous algae based on the amount of N or P in excess relative to the N:P:Si stoichiometric demand of diatoms expressed in the freshwater Redfield ratio (Garnier et al. 2010; Dupas et al. 2015). The IFEP is expressed as kg C area\(^{-1}\) time\(^{-1}\) and is calculated as follows:

\[
\text{N- IFEP} = \left( \frac{\text{DIN yield}}{14 \times 16} - \frac{\text{DSi yield}}{28 \times 40} \right) \times 106 \times 12
\]

\[
\text{P- IFEP} = \left( \frac{\text{SRP yield}}{31} - \frac{\text{DSi yield}}{28 \times 40} \right) \times 106 \times 12
\]

Positive IFEP values represent potential DSi limitation of diatoms, and thus the potential for growth of non-siliceous taxa, including cyanobacteria. Conversely, negative values indicate DSi is abundant relative to N and P, and thus conditions are favorable for diatom growth. When comparing N- and P-IFEP values, the nutrient (N or P) with the lower IFEP value will likely limit non-siliceous algal growth (Dupas et al. 2015). Using one-sample t-tests (\(\bar{x}=0, \alpha=0.05\)), we assessed whether monthly averages of daily IFEP values were significantly greater than zero, indicating the stoichiometry of the nutrient loads favored non-siliceous, and possibly harmful, algal growth.

Results
Seasonality of DSi concentrations and stoichiometry

In KDW, DSi concentrations in both field and county tile drains averaged 9.2 mg SiO\(_2\) L\(^{-1}\) for the entire period of record (n=613, SE=0.06; Table 2.2), with a distinct seasonal pattern that corresponded with the growing season. Concentrations were generally highest in late summer
through early fall, declined steadily over the fall to the lowest observed concentrations in winter, then increased in late spring (Figure 2a). Average DSi concentrations were significantly different across all seasons in KDW tile drains, and also between fall and winter at the SDW outlet (Tukey Honestly Significant Differences, p<0.05; Figure S2.1a, c). There were no seasonal differences in DSi concentrations for the KDW outlet (one-way ANOVA; Figure S2.1b). At the KDW watershed outlet, DSi concentrations averaged 7.2 mg SiO₂ L⁻¹ (n=64, SE=0.39) and similar values were observed in the SDW outlet, averaging 7.4 mg SiO₂ L⁻¹ over the period of record (n=53, SE=0.39; Table 2.3). There was more inter- and intra-annual variation in DSi concentrations at the outlets than in the tile drains (Figure 2.2b-c), suggesting in-stream processes influenced DSi concentrations between the field- and watershed-scales. Across the two watersheds, yields of DSi ranged from 1454 to 4969 kg km⁻² yr⁻¹ and the variation was strongly correlated to total annual runoff (Pearson’s r = 0.89, p<0.05; Table 2.3).

**Table 2.2** Median and maximum instantaneous tile discharge and mean DSi concentrations for all KDW field tile drains and county tile drains between water years 2018-2020.

<table>
<thead>
<tr>
<th>Water year</th>
<th>Number of samples</th>
<th>KDW field tile drains</th>
<th>KDW county drains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median instant. Q (L s⁻¹)</td>
<td>Maximum instant. Q (L s⁻¹)</td>
</tr>
<tr>
<td>2018</td>
<td>311</td>
<td>0.4</td>
<td>9.0</td>
</tr>
<tr>
<td>2019</td>
<td>273</td>
<td>0.4</td>
<td>10.5</td>
</tr>
<tr>
<td>2020</td>
<td>356</td>
<td>0.4</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Figure 2.2 (a) Average DSi concentrations (in mg SiO$_2$ L$^{-1}$) of bi-weekly KDW tile drain measurements throughout the water year. Averages were calculated using the arithmetic mean of all tile drain sample DSi for any given water year day between October 2017-September 2020. Error bars represent the standard error of the mean. (b-c) KDW and SDW outlet DSi concentrations measured from bi-weekly samples collected between October 2017-September 2020. Samples are plotted by the water year day on which they were collected. For all plots, symbol shape and color correspond to the water year.
Table 2.3 Total annual runoff, mean DSi concentrations, and DSi yields from KDW and SDW outlets between water years 2018-2020.

<table>
<thead>
<tr>
<th>Water Year</th>
<th>Runoff (mm)</th>
<th>Mean DSi conc. (mg SiO\textsubscript{2} L\textsuperscript{-1})</th>
<th>Yield (kg SiO\textsubscript{2} km\textsuperscript{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirkpatrick Ditch Watershed (KDW)</td>
<td>2018</td>
<td>334</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>487</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>2020</td>
<td>313</td>
<td>6.2</td>
</tr>
<tr>
<td>Shatto Ditch Watershed (SDW)</td>
<td>2018</td>
<td>594</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>579</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>2020</td>
<td>372</td>
<td>4.1</td>
</tr>
</tbody>
</table>

At KDW, we used DSi concentrations from tile drains and watershed outlets, as well as discharge measurements, to model C-Q relationships at the field and watershed scales. For tile drains, the modeled DSi-Q relationship had a near-zero slope of 0.005 (power-law fit, p<0.001; Figure 2.3a) which suggested chemostatic behavior at the field-scale. For the watershed outlets, DSi and discharge had a slightly positive slope of 0.114 (r\textsuperscript{2}=0.14, p<0.001; Figure 2.3b), suggesting DSi was transport-limited at the watershed scale (i.e., DSi concentrations increased with increasing discharge). Mean DSi concentration from the tile drains was significantly higher than at the outlet (Wilcoxon Sign-Rank test, p<0.001), indicating the potential for dilution of DSi inputs from the watershed.
Figure 2.3 (a) All KDW tile drains (field and county) measured DSi concentrations (in mg SiO$_2$ L$^{-1}$) plotted against measured instantaneous tile drain discharge. (b) Measured DSi concentrations plotted against mean daily discharge from both KDW and SDW outlets. Data is plotted on logarithmic axes and modeled with power-law fits ($p<0.001$).
In KDW, we modeled the relationship between DSi loads from tile drains and the watershed outlet using measured DSi concentrations and discharge. Outlet DSi loads were significantly higher than the total tile input (Wilcoxon Sign-Rank test, p<0.001), indicating sources other than tile drains contributed to the watershed export of DSi. There was no linear relationship between tile drain and outlet DSi concentrations, and nearly all points were below the 1:1 line (Figure 2.4a); however, there was a significant linear relationship between DSi loads from the outlet and the total input from all tile drains (p<0.001, n=55, R²=0.40; Figure 2.4b).
We calculated molar ratios between DSi:DIN and DSi:SRP concentrations (hereafter Si:N and Si:P, respectively) for all KDW tiles and analyzed variation through time and between seasons. The average Si:N in all KDW tiles over the monitoring period was 0.40 (n=524, SE=0.02) while Si:P averaged 576 (n=526, SE=0.002). For Si:N, nearly all sample means were lower than the freshwater Redfield ratio, whereas most Si:P values were higher than the Redfield ratio, indicating the potential for limitation by Si relative to N, but abundant Si relative to P throughout the year (Figure 2.5).

**Figure 2.4** (a) Daily outlet DSi concentrations plotted against mean daily tile drain DSi concentrations in KDW and (b) daily outlet DSi loads plotted against total daily tile inputs summed from all KDW tile drains (field and county). The solid lines indicate the 1:1 ratio between outlet and tile DSi concentrations and loads.
There was little intra-annual variation in Si:P; however, Si:N ratios decreased from fall through spring and increased in the summer (Figure 2.5). Neither Si:N nor Si:P ratios showed a significant linear relationship with instantaneous tile drain discharge; however we found variation in both Si:N and Si:P was more closely linked with variation in DIN (linear regression; p<0.001, n=524, $R^2 = 0.89$) and SRP concentrations (linear regression; p<0.001, n=466, $R^2 = 0.96$), rather than DSi concentrations, indicating variation in stoichiometric ratios was primarily a function of variation in N and P rather than DSi (Figure S2.2).
Effect of winter cover crops on DSi concentrations, loads, and stoichiometry

We used nutrient concentration and discharge data from KDW tile drains to assess the effects of winter cover crops on DSi concentrations, loads, and stoichiometry. For each water year, tile drains were classified as either “cover crop” or “no cover crop” based on the presence or absence of a winter cover crop during the fallow period (October – April). Neither the water

**Figure 2.5** (a) Molar DSi:DIN ratios and (b) molar DSi:SRP ratios from all KDW tile drains. Each point represents the mean of all tile drain samples from individual sampling events throughout the 2018-2020 years. Error bars extend to one standard deviation from the mean. Dashed lines denote the “freshwater Redfield ratio” of 16N: 1P: 40Si (Dupas et al. 2015). Data below the dashed line suggest Si limitation relative to either N or P according to the demands of freshwater diatoms – conversely values above the line indicate Si in excess of either N or P.

Effect of winter cover crops on DSi concentrations, loads, and stoichiometry

We used nutrient concentration and discharge data from KDW tile drains to assess the effects of winter cover crops on DSi concentrations, loads, and stoichiometry. For each water year, tile drains were classified as either “cover crop” or “no cover crop” based on the presence or absence of a winter cover crop during the fallow period (October – April). Neither the water
year nor season had significant differences in DSi concentrations or loads between cover crop and no cover crop conditions (HRM, α=0.05); however, Si:N molar ratios were significantly higher in tiles draining cover crop fields during autumn and winter (HRM, p<0.05; Figure 2.6a), showing cover crops can influence Si:N ratios by reducing N loss from fields (as reported at these sites by Speir et al. 2021b). There was no difference in Si:P molar ratios between treatments during any season (Figure 2.6b).

![Figure 2.6](image)

**Figure 2.6 (a)** Molar DSi:DIN ratios and (b) molar DSi:SRP ratios from KDW field tile drains. Each box represents sample DSi:DIN and DSi:SRP molar ratios grouped into seasons. Within each plot, each box corresponds to the 25th and 75th percentiles, the solid line within each box denotes the median value, hinges extend to ±1.5 interquartile range, and points indicate outliers in each group. Asterisks below box plots indicate seasonal differences in molar DSi:SIN between cover crop and no cover crop treatments (HRM, p<0.05). Dashed lines denote the “freshwater Redfield ratio” of 16 N: 1 P: 40 Si (Dupas et al. 2015).

**Indicator of Freshwater Eutrophication Potential**

We calculated daily N- and P-IFEP values using daily nutrient yields from both KDW and SDW stream outlets; there was a distinct intra-annual pattern in IFEP values indicating
seasonal changes in the yields of DSi relative to N and P and the subsequent potential for non-siliceous algal growth. Daily N-IFEP values grouped by month were above zero in all months of the year, although values were highest during the winter and spring relative to late summer and fall conditions in both KDW and SDW (Figure 2.7a). Conversely, P-IFEP values showed the opposite response, with values significantly less than zero in all months of the year and lower in the winter and spring relative to summer and fall (Figure 2.7b). P-IFEP values were consistently lower than N-IFEP values, indicating potential P limitation relative to N according to the freshwater Redfield ratio.

For both N-IFEP and P-IFEP, we modeled relationships with discharge using total daily discharge values recorded by the USGS gage in each watershed. On a daily timescale, N-IFEP values increased exponentially with total daily discharge while P-IFEP values decreased exponentially relative to total daily discharge (power-law fit; p<0.001 for both N- and P-IFEP). The exponential relationships between IFEP values and discharge indicate a disproportionate increase in the stoichiometric imbalance between N, P, and Si with increasing flow. Mean values from both watersheds across all water years were 39.2 and -7.7 for N- and P-IFEP, respectively, and at the maximum recorded discharge (10^6 m^3 day^-1), N-IFEP values were approximately 18-fold greater than the mean (Figure 2.8a). In contrast, while the P-IFEP values also responded to changes in discharge, the values never exceeded zero across the range of observed discharge (Figure 2.8b).
Figure 2.7 (a) Daily N-IFEP values and (b) daily P-IFEP values from KDW outlet (white boxes) and SDW outlet (gray boxes). Each box represents sample daily IFEP values grouped into months and plots follow those described in Fig 5. For the N-IFEP, the median value was above zero for all months (one sample t-test, p<0.001, except for KDW in February p<0.05) while the P-IFEP medians were below zero for all months (one sample t-test, p<0.001). Dashed lines at zero denote stoichiometric balance between N, P, and Si according to IFEP equations described in the text. Positive values indicate non-siliceous algal biomass growth while negative values indicate diatom growth.
Figure 2.8 (a) Daily N-IFEP values and (b) daily P-IFEP values from KDW and SDW outlets plotted against total daily discharge. Note that only the x-axis is plotted on a logarithmic scale.
Discussion

Annual pattern of DSi concentrations at the field- and watershed-scale

Contrary to our predictions, DSi concentrations increased in tile drains and watershed outlets during the summer growing season, indicating crop DSi demand did not draw down concentrations as expected. This may be a result of crop growth which facilitates mineral Si dissolution in soils and increased soil temperatures during the growing season (Drever 1994). Crops incorporate this Si into phytoliths, thereby increasing stores of biogenic Si (Van Cappellen 2003; Cornelis et al. 2010; Struyf and Conley 2012). Experimental studies have quantified the rate of phytolith dissolution to be an order of magnitude greater than Si mineral weathering, indicating biogenic Si may contribute significantly to the total bioavailable Si pool (Alexandre et al. 1997; Fraysse et al. 2009). Thus, crop growth facilitates increased silicate mineral weathering and production of phytoliths, and both processes may have contributed to the increase in DSi concentrations observed during the cash crop growing season.

We observed a chemostatic relationship between discharge and DSi concentrations in tile drains, whereas stream DSi concentrations exhibited transport limited behavior. The difference in DSi response to discharge between field- and watershed-scale measurements might reflect the various biogeochemical processes affecting DSi from soil to tile drain and within streams. The dissolution rate of DSi from soils may have reached equilibrium with DSi export through tile drains at the field-scale (Maher 2010) while biotic processes and landscape inputs were integrated at the watershed-scale, resulting in a chemodynamic relationship between DSi concentrations and discharge. For example, as discharge increased, connectivity between the landscape and the stream channel expanded and likely mobilized DSi from new source areas within the watershed, thereby increasing stream water DSi concentrations with increasing
discharge. Furthermore, the strong linear relationship between total DSi inputs from tile drains (both field and county drains) and the DSi loads at the KDW watershed outlet indicate total DSi loads at the watershed-scale were proportional to the sum of all tile inputs; however, stream DSi loads were significantly higher than tile loads suggesting other sources of DSi contributed to loads at the outlet, such as groundwater or overland flow paths.

Higher DSi loads at the watershed scale relative to the field scale can be an indicator of long-term decline in DSi availability within a watershed (Conley et al. 2008; Struyf et al. 2010). While DSi yields from both KDW and SDW were comparable to those measured in forested watersheds of New England (Carey and Fulweiler 2012a), intensive agriculture has been shown to reduce the export of DSi over long timescales (Struyf et al. 2010; Keller et al. 2012), and KDW (and fields in this region of the Midwest more generally) has been in continuous cultivation for >100 years. Long-term decline in DSi availability due to agricultural land use has global impacts on the biogeochemical processing of Si, ecology of aquatic systems, and sequestration of atmospheric carbon dioxide (De La Rocha 2003; Beusen et al. 2009; Frings et al. 2016).

The annual pattern in DSi concentrations in tile drains and watershed outlets is similar to the pattern observed in forested and urban streams in New England (Carey and Fulweiler 2013) as well as agricultural rivers in Poland (Humborg et al. 2006), France (Abbott et al. 2018), and Italy (Viaroli et al. 2013). However, crop production and harvesting has undoubtedly altered the terrestrial Si cycle and significantly affected DSi inputs to aquatic systems (Van Cappellen 2003; Tubana et al. 2016). The mechanisms influencing seasonal variation in our study systems likely included temperature, soil mineral dissolution rates, stream discharge, and uptake by diatoms. Higher temperatures during the summer can contribute to increased dissolution rates and
chemical weathering of silicate minerals in the soil. Conversely, DSi concentrations declined in the winter possibly as a result of increased demand for DSi by diatoms, which can outcompete other algal groups for nutrients at lower temperatures (MacIntyre et al. 2004; Anderson et al. 2008).

**Impact of winter cover crops on DSi concentrations in tile water and soil**

We also predicted cover crop growth would reduce DSi concentrations, but there was no difference in tile drain DSi between cover crop and no cover crop treatments in any season or water year. However, an analysis of water extractable DSi in soil from KDW showed significantly lower DSi concentrations from soils planted with winter cover crops compared with soils without winter cover crops (Hagemeier, unpublished data), suggesting the potential for uptake of DSi by cover crops. Termination of cover crop biomass in late spring should return Si to the soil, as cover crops are not harvested and the plant biomass remains in the fields. The biogenic Si contained within cover crop biomass is more soluble than mineral silicates; therefore, cover crops increase the bioavailability and transport of Si from soils to stream.

Although we have some evidence that cover crops affected DSi in soil, there was no difference in tile drain DSi concentrations. Similar to the time lags observed between nitrate mitigation practices and surface water nitrate reductions (Grimvall et al. 2000; Rabalais 2002; Meals et al. 2010), differences in soil DSi concentrations might take many years of continued cover crop planting to be expressed in tile drain outflow. The lack of response between implemented conservation practices and field-scale observations highlights the “hydrological time lag” from field to stream and the importance of evaluating conservation practices over multi-year or decade long time-scales (Fenton et al. 2011).
Global crop uptake is estimated to be between 210-224 million Mg Si annually (Matichenkov and Bocharnikova 2001), which is of the same order of magnitude as the Si exported to the oceans from rivers globally (Meunier et al. 2008) and heavily contributes to the “desilication” of agricultural soils (Haynes 2014, 2017a). Our results suggest the use of winter cover crops might enhance Si storage in soils which, over the long term, could increase the availability of biogenic Si to adjacent waters and decrease the risk of harmful algal blooms (Cornelis and Delvaux 2016; Haynes 2017b). It is well established that cover crops reduce loss of N (Hanrahan et al. 2018; Speir et al. 2021b) and P (Trentman et al. 2020) through tile drains at this site. Continuous and widespread use of cover crops might therefore facilitate a combination of increased Si availability and reduced inputs of N and P to freshwater systems—a situation that would shift stoichiometric ratios towards conditions more favorable to diatoms rather than cyanobacteria (Dupas et al. 2015, Royer 2020). In fact, at KDW, cover crops significantly increased Si:N during the fall and winter, the cover crop growing season, suggesting cover crops can affect stoichiometric ratios in addition to mass loss of nutrients.

*Seasonality of nutrient limitation and eutrophication potential*

Stoichiometric ratios between N, P, and Si consistently indicated abundant N relative to Si whereas P availability was limited relative to both N and Si; however, the seasonal patterns did not align with observations of in-stream algal communities. For example, low Si:N molar ratios indicate conditions favorable for growth of non-siliceous algae, yet we did not observe cyanobacterial blooms at any period during the 3-year study (L.R. Sethna, *personal observation*). Instead, during times of decreased Si:N, we observed diatom dominance and when Si:N increased in the summer months we observed blooms of filamentous, green algae. The deviation between theorized nutrient limitation and observed algal composition is not unexpected as
stoichiometric ratios are but one driver of the seasonal shifts in algal community composition (e.g., Stevenson 1997). It is also possible that a bloom of diatoms preceded the nutrient sampling and contributed to low Si:N ratios in the stream water; however, explicit studies of nutrient limitation and uptake are necessary to quantify the role of diatoms and ambient nutrient stoichiometry.

Interestingly, in the upper Mississippi River system, stoichiometric ratios indicate Si-limitation relative to N and, at times, P while N is limited relative to P in both the mainstem of the river as well as its major tributaries (Carey et al. 2019). The change from P-limiting conditions in the headwater streams of KDW and SDW to N-limitation in the larger Mississippi River subbasins might explain why cyanobacterial blooms are rarely observed in headwater, agricultural streams while becoming increasingly common on larger rivers such as the Ohio (ORSANCO 2016) and Maumee (McKay et al. 2018) rivers.

Our data clearly show a disproportionate change in both N- and P-IFEP values with increasing discharge. At high flows, N-IFEP values exponentially increased while P-IFEP values exponentially decreased. The differences in response to high flows highlights the different mechanisms mobilizing N, P, and Si typical of agricultural systems across the Midwest, leading to the increased imbalance between these three nutrients at high flows. For example, N is rapidly mobilized in the late winter and spring, during high flows (Royer et al. 2006; David et al. 2010; Speir et al. 2021a), contributing to the exponential increase in the imbalance between N and Si. Conversely, in tile-drained landscapes P can be diluted at high flows, which explains why P-IFEP values exponentially decreased with discharge in our study watersheds. Finally, DSi concentrations have been shown to exhibit a chemostatic relationship with discharge, within
larger Mississippi River subbasins (Leong et al. 2014) and in smaller, headwater streams (Godsey et al. 2009).

According to the U.S. Global Change Research Program (2018), the Midwest is expected to experience more frequent and intense precipitation events, leading to increased stream flow and nutrient export from agricultural landscapes (Rabalais et al. 2010; Raymond et al. 2012; Michalak et al. 2013; Grimm et al. 2013). Specifically, precipitation is expected to increase in the winter and spring, which are periods of high nutrient loss, thereby exacerbating nutrient loading and eutrophication in inland and coastal waters (Sinha et al. 2017; Bowling et al. 2020; Hamlet et al. 2020; Cherkauer et al. 2021). Given the varying response in N, P, and Si to high flow events, we expect a corresponding increase in nutrient imbalances which contribute to the cultural eutrophication of surface waters, particularly the proliferation of cyanobacterial blooms (Glibert and Burford 2017; Le Moal et al. 2021). Future work in these study sites could include an analysis of particulate Si, including amorphous and biogenic Si, in order to better quantify the transport and biogeochemical cycling of total Si within the watersheds. Further study on how warming and changing precipitation might affect mineralization of phytoliths in soils and, by extension, DSi inputs to freshwaters would also be valuable.

Studies on the transport and cycling of Si in aquatic systems are vastly underrepresented in the field of nutrient biogeochemistry, despite its importance in diatom productivity and the growth of many terrestrial and aquatic plants. Billions of dollars are directed to mitigating N and P export, while little attention has been paid to the role of Si in the determination of algal community composition and, more broadly, ecosystem function. This paper provides the groundwork for understanding the role of tile drainage and row-crop agriculture on Si export and
stoichiometry and can further inform land management decisions that prioritize water quality and ecosystem function.

Acknowledgements
This research was funded by grants from the US Department of Agriculture, Regional Conservation Partnership Program; the Walton Family Foundation; and the Indiana Soybean Alliance. We thank the Jasper County and Kosciusko County Soil and Water Conservation Districts for help implementing the project, and the farmers and landowners in KDW and SDW for access to sampling sites. Numerous students contributed to field sampling and laboratory analyses including Nina Jung, Victoria Anderson, and Laura Gerber. We acknowledge the Myaamia (Miami), Kaskaskia, Bodwéwadmik (Potawatomi), Peoria, and Kiikaapoi (Kickapoo) People, Indigenous communities native to the KDW and SDW region. Acknowledging the history of these lands is simply a first step in identifying land stewardship and research practices that better connect the land to the people who rely on it.
References


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Supplemental Information

**Figure S2.1** Monthly DSi concentrations for (a) field and county KDW tile drains, (b) the KDW watershed outlet, and (c) the SDW watershed outlet. Each box represents the 25th-75th percentile in sampled DSi concentrations. The solid line within each box denotes the median value, whiskers extend to ±1.5 interquartile range, and points indicate outliers in each group. Letters above each box indicate significant differences between seasons (Tukey HSD, p<0.05).
Fig S2.2 Molar ratios Si:N (top) and Si:P (bottom) plotted against the molar concentrations of their constituents. Significant relationships between Si:N and molar N; and Si:P and molar P indicate stoichiometric ratios are sensitive to variation in N and P concentrations, respectively, rather than Si.
Chapter 3
Assessing silicon limitation of benthic algae in an agricultural headwater stream

Abstract
The availability of dissolved silicon (DSi) relative to nitrogen (N) and phosphorus (P) influences the relative proportion of diatoms to non-silicious, potentially harmful, algal taxa. DSi limitation of diatoms is hypothesized to be a significant factor leading to the formation of cyanobacterial blooms, especially in eutrophic systems with abundant N and P. However, a significant knowledge gap exists regarding the potential for DSi limitation in streams. We conducted four nutrient diffusing substrata (NDS) experiments, one in each season, to assess nutrient limitation of periphyton communities in a headwater, agricultural stream in northern Indiana. The experiments included DSi, N, and P alone, as well as in combination. We quantified responses in chlorophyll-α and community composition, measured as the relative abundance of diatoms versus non-siliceous taxa. Ambient nutrient stoichiometry of the study streams indicated abundant N relative to P and DSi, and we therefore expected algal communities to respond to increased P and DSi treatments. Unexpectedly, neither P nor DSi treatments promoted increased responses in chlorophyll-α relative to the control. We attribute this to potential preferential grazing of diatoms, which were proportionately more abundant on treatments amended with DSi, by herbivorous invertebrates. Differences in chlorophyll-α content between diatoms and non-siliceous algal taxa could also be a factor. Our results highlight the importance of DSi in promoting benthic algal communities that serve as a quality food resource for invertebrates, particularly in nutrient-rich streams. Consideration of DSi and N:P:Si stoichiometry could provide a stronger ecological perspective to management strategies targeting the prevention of cyanobacterial blooms.
Introduction

Periphyton, the diverse assemblage of microorganisms growing on hard surfaces in aquatic ecosystems, are a fundamental component of headwater streams. The periphyton play an important role in primary productivity, nutrient retention and cycling, and biotic interactions (Pringle and Triska 2007; Larned 2010). Diversity within the periphyton community arises from variation among species in nutrient requirements and preferences in light, flow, and substratum (Wetzel 1983; O’Brien and Wehr 2010; Taylor et al. 2020). The growth and composition of periphyton communities is regulated by a number of environmental factors, and numerous studies have quantified the effects of pollutants, grazing, and nutrient availability on periphyton using various descriptive and experimental approaches (Flecker et al. 2002; Tank et al. 2006; Rosi-Marshall et al. 2013).

While a number of factors influence algal productivity and community composition, the availability of inorganic nitrogen (N) or phosphorus (P) is frequently reported as a primary control on abundance, typically measured as the mass of chlorophyll-\(a\) (chl-\(a\)) per unit area (Dodds et al. 1997; Biggs 2000; Bennett et al. 2021). Studies using nutrient diffusing substrata (NDS; Capps et al. 2011), commonly find that stream periphyton communities are co-limited by nitrogen (N) and phosphorus (P) (Tilman et al. 1982; Francoeur 2001). Nutrient limitation of periphyton is thus a consequence of insufficient availability of N and P, either alone or in combination. However, unlike other periphyton taxa, diatoms require silicon (Si) in addition to N and P, and the growth and abundance of diatoms potentially can be limited by insufficient dissolved Si. In lakes and large rivers, planktonic diatoms often are limited seasonally by dissolved silicon (DSi; e.g., Kilham 1971; Koch et al. 2004). Despite the importance of Si to diatoms, very few studies have examined DSi as a factor controlling the abundance and
composition of stream periphyton. We are aware of only one study of stream periphyton that included Si treatments as part of \textit{in situ} substratum experiments: Pringle et al. (1986) tested nutrient limitation of benthic algae in a tropical Costa Rican stream and found no significant increase in chl-\textit{a} on +N, +P, or +Si treatments after 14 days of incubation, despite ambient nutrient stoichiometry suggesting potential N-limitation. The average ambient concentration of DSi in the Costa Rican stream was 14 mg L\textsuperscript{-1} (as Si), well above a concentration that could be limiting (Pringle et al. 1986). In a study of benthic algae in the littoral zone of Lake Michigan, Carrick and Lowe (1988) reported that diatoms became Si-limited after 14 days of N and P enrichment, indicating Si limitation can emerge as a consequence of nutrient loading (\textit{sensu} Schelske and Stoermer 1971).

Agriculture tends to reduce DSi concentrations in streams via changes in vegetation cover and modified hydrologic flow paths (Struyf et al. 2010; Struyf and Conley 2012; Carey and Fulweiler 2016) while also contributing large amounts of N and P. This dual effect of agriculture can thus alter the relative proportions of Si, N, and P in agricultural streams in ways that favor non-siliceous algae (Conley et al. 1993; Blann et al. 2009). Although the effects of agriculture on nutrient concentrations in streams have been widely studied, the potential for silica limitation in agricultural streams has not been investigated. Diatoms have been shown to outcompete other taxa for available N and P when their Si requirements are met; therefore, the availability of Si should promote a phytoplankton community that is abundant in diatoms (Kilham 1971, 1986; Tilman et al. 1982; Hecky and Kilham 1988). We hypothesized that silica limitation of diatoms is possible in nutrient-rich agricultural streams and that it would reduce the relative abundance of diatoms, but not necessarily the standing stock of periphyton because non-siliceous algae could
compensate for the loss in diatom production. Because of this, identifying Si limitation requires examining responses in both abundance (chl-α) and community composition.

In this study, we conducted four NDS experiments, one in each season, in a small, agricultural watershed in northwestern Indiana to quantify the nutrient limitation of periphyton growth. Specifically, we aimed to characterize how periphyton biomass and the relative abundance of diatoms responded to enrichment with Si, either alone or in combination with N and P. We quantified the abundance of periphyton, measured as chl-α, and the algal community, measured as the relative abundance of diatoms compared to non-siliceous taxa. We expected algal periphyton to be co-limited by both P and Si, as our previous work in this stream identified both nutrients to be limiting relative to N according to the freshwater Redfield ratio (Sethna et al. 2022). Diatoms are the primary user of Si in aquatic systems (Tréguer et al. 1995; Martin-Jezequel et al. 2000); therefore, if Si was limiting, we expected to observe a greater proportion of diatoms on treatments amended with Si. If P was limiting, we predicted a greater proportion of non-siliceous taxa relative to diatoms on treatments amended with P; however, on treatments with both Si and P, we expected relative diatom abundance to be greater than non-siliceous taxa. We conducted this study in a nutrient-rich agricultural stream with limited shading from riparian vegetation – conditions that should facilitate detection of Si limitation should it occur. Nonetheless, it is possible that factors such as light, temperature, or selective grazing could obscure a clear signal of Si limitation. Consequently, the results presented here are considered an initial investigation, rather than a definitive assessment, on the potential for Si to influence the abundance and composition of stream periphyton.
Materials and Methods

Study area

Kirkpatrick Ditch (KD) is a small headwater stream that drains a 26.3 km² watershed of predominantly row-crop agriculture in northwestern Indiana (Fig. 3.1). Approximately 94% of the watershed is in a corn-soybean rotation and thus is representative of headwater, agricultural watersheds across the intensively farmed regions of the midwestern U.S. We conducted NDS experiments in January, May, July, and November 2020 and in each case allowed the diffusers to incubate in the stream for two-weeks. The NDS were deployed at 10 stations throughout the stream network. The stations had variable widths ranging from 2 m to 10 m and a range in canopy cover with variable shading from riparian vegetation. Substrate varied from pebble-cobble dominated to gravel-sand dominated between sampling sites.

Invertebrate grazers observed
in the stream included mayfly and caddisfly larvae in the genus *Heptagenia* and *Helicopsyche*, respectively (A. Pruitt, unpublished data).

*Nutrient Diffusing Substrata*

We constructed NDS using 60-mL plastic centrifuge tubes filled with an agar solution and one of seven nutrient treatments according to methods detailed in Tank et al. (2006). Nutrient treatments included a control (agar solution only), 0.5 M Na₂SiO₃·H₂O (+Si), 0.5 M NaNO₃ (+N), 0.5 M NaPO₄ (+P), 0.5 M NaNO₃ and 0.5 M Na₂SiO₃·H₂O (+N+Si), 0.5 M NaPO₄ and 0.5 M Na₂SiO₃·H₂O (+P+Si), or 0.5 M NaNO₃ and 0.5 M NaPO₄ and 0.5 M Na₂SiO₃·H₂O (+N+P+Si). Each treatment was then topped with a porous ceramic disk (Elemental Microanalysis Consumables, SKU C4505). NDS were then grouped into “units” consisting of two replicates of each treatment for a total of fourteen NDS per unit. Individual NDS were attached to PVC pipes using zip ties following a random block design to eliminate carry-over effect between treatments. Ten units were deployed during each experiment and were anchored to the stream bottom using steel rebar (Fig. 3.1a-b). Prior to each NDS incubation, water velocity was measured to ensure at least 0.1 m s⁻¹ across the diffusers. After the 14-day incubation period, NDS were retrieved from the streams and transported on ice, in the dark to the laboratory. Within each unit and treatment, one replicate was reserved for chlorophyll-α (chl-α) analysis and the other for algal identification.

*Ambient nutrient concentration*

At the time of deployment and retrieval at each station, samples were collected for ammonium, nitrate, soluble reactive phosphorus (SRP), and dissolved silicon (DSi). Ammonium and nitrate concentrations were summed and are reported here as DIN. Samples were collected
using a 60-mL syringe that was rinsed with stream water, and then filtered in the field using 0.6 μm glass fiber filters (Whatman GF/F) for all but DSi for which we used cellulose filters (0.45 μm; Fisherbrand). All nutrient samples were analyzed colorimetrically using a Lachat Quikchem 8500 (Hach Inc., Loveland, CO). Samples were analyzed for nitrate using the cadmium reduction method (APHA 2017), for ammonium using the phenol-hypochlorite method (Solórzano 1969), for SRP using the ascorbic acid method (Murphy and Riley 1962), and for DSi using the heteropoly blue method (Sultan 2014). For all nutrient analyses, we ran a certified standard to validate the standard curve and routinely calculated the method detection limit, which averaged 0.2 mg N L⁻¹ for nitrate, 9.3 μg N L⁻¹ for ammonium, 4.0 μg P L⁻¹ for SRP, and 0.04 mg SiO₂ L⁻¹ for DSi. If samples were below detection, a value of one-half the calculated detection limit was used for statistical analyses. Of all nutrient samples analyzed during the study, 18% of nitrate samples, 43% of ammonium samples, and 14% of SRP samples were below detection. No DSi samples were below the detection limit.

Chlorophyll-a analysis

For each experiment, NDS disks reserved for chl-a were kept in the dark from the time of retrieval until analysis. Samples were stored in dark film canisters, frozen, and analyzed within 28 days post-incubation. We used a hot ethanol extraction method described by Sartory and Grobbelaar (1984) as it enabled extraction from the ceramic disks without the need for grinding. We added 95% ethanol to each film canister and placed them in a 79°C hot water bath for 5 minutes. Samples were left to cool for 24 hours before the solution was pipetted to dark tubes and centrifuged for 10 minutes at 600 g. The supernatant solution was then analyzed for absorbance at 665 and 750 nm using an Aqualog spectrometer (Horiba Instruments, Inc., Irvine, CA). Each sample was analyzed twice, once without acid and again with the addition of 10 μL of
0.1 N HCl (to a 1 mL aliquot of sample) to convert all chlorophyll to phaeophytin. Chl-$a$ was determined according to the following equation:

\[
\text{(1) Chlorophyll} - a \ (\text{mg m}^{-2}) = 28.78(B_0 - B_1) \frac{v}{A \times i}
\]

where $B_0$ is the difference between absorption at 665 nm and absorption at 750 nm before acid addition, $B_1$ is the same as $B_0$ but after acidification, $v$ is volume of extractant used (4 mL), $A$ is the area sampled (2 cm$^2$), $i$ is the path length of the spectrometer cuvette (1 cm), and 28.78 is the absorbance coefficient for chl-$a$ in ethanol.

**Algal community composition**

A subset of three algal samples were analyzed for community composition for each of the four experiments. NDS disks used for algal analysis were rinsed with 60 mL of pressurized, deionized water and stored in dark bottles prior to quantification with a FlowCam 5000 (Yokogawa Fluid Imaging Technology, Inc.). We used 20x magnification which had a flow rate of 0.04 mL min$^{-1}$ and an efficiency of 13.9% (ratio of the sample volume imaged to the total sample volume). The FlowCam imaged at least ten thousand particles per sample which were then classified as either siliceous algae, non-siliceous algae, or unidentified using the Virtual Spreadsheet (version 4.0.27) Auto Classification. The Auto Classification function was optimized according to the methods described by Camoying and Yniguez (2016), including the organization of classification libraries and statistical filters that could more accurately filter images into their respective groups. However, as a check, each sample classification was manually verified to ensure particles were grouped correctly. For the siliceous and non-siliceous algal groups, data were exported as a concentration (particles mL$^{-1}$). The percentage of the community comprised of diatoms versus non-siliceous algal taxa was calculated by dividing the
concentration of each group by the total particle concentration of the sample and multiplying by one hundred.

**Statistical analyses**

In order to quantify the magnitude of the response to nutrient enrichment, we calculated response ratios for chl-\(a\) and diatom relative abundance within each unit as follows:

\[
\text{(2) Chl} – a \text{ response ratio} = \ln \left( \frac{\text{Chl} – a_{\text{treatment}}}{\text{Chl} – a_{\text{control}}} \right)
\]

\[
\text{(3) Diatom response ratio} = \ln \left( \frac{\text{Diatom abundance}_{\text{treatment}}}{\text{Diatom abundance}_{\text{control}}} \right)
\]

Response ratios were natural log transformed to normalize the data and reduce statistical biases (Elser et al. 2007; Isles 2020). The calculation of response ratios also normalized the effect of the treatments relative to the control at each station to account for variation among stations in light, flow, grazing, or other factors. We tested the effect of each treatment in each month using two-sided t-tests to detect significant deviation from zero; positive response ratios indicate a positive response from the treatment relative to the control for both chl-\(a\) and diatom data. We also used one-way ANOVA to test for differences among treatments within each month. If significant differences existed among treatments, we used Tukey’s Honestly Significant Difference (HSD) test to determine which nutrient treatments were different from others within each month. All data and statistical analyses were performed using R (version 4.0.5; R Project for Statistical Computing).
Results

Stream site conditions

Stream concentrations for DIN, SRP, and DSi ranged between 0.01-19.35 mg L$^{-1}$, 1.39-345.20 μg L$^{-1}$, and 3.43-18.10 mg L$^{-1}$, respectively, during the incubation periods (Fig. 3.2). Stream temperature ranged between 5.2 and 25.6 °C and discharge ranged between 0.3 and 125 L s$^{-1}$. Summary data for temperature, turbidity, flow, and nutrient data in each season appear in Table 3.1. The ambient nutrient stoichiometry of the streams varied between seasons, although consistently indicated abundant N relative to P and Si, according to the freshwater Redfield ratio of 16N:1P:40Si. Freshwater diatoms have highly variable Si requirements, and the freshwater Redfield ratio is a conservative estimate of diatom nutrient requirements (Conley et al. 1989). Average molar ratios for Si:N, Si:P, and N:P were 11, 285, and 1056, respectively. Average molar ratios by month are presented in Table 3.1 and expectations for the limiting nutrient for both diatoms and non-siliceous algal taxa are in Table 3.2. Diatoms were expected to be Si limited in months where both the Si:N and Si:P ratio were below 2.5 and 40, respectively. Non-siliceous algae were expected to be P-limited when the N:P ratio was below 16. The N:P ratio

![Figure 3.2 Mean of biweekly measurements of DIN, SRP, and DSi from all sampling sites in 2020. Dates in which diffuser experiments were carried out are colored in red.](image)
indicates P limitation relative to N; therefore, we expected the nutrient limitation of diatoms to correspond with Si availability relative to P. Based on the range in ambient Si:P ratios during the experimental periods, diatoms were expected to be primarily P limited relative to Si, and secondarily Si limited relative to N (Table 3.2). Non-siliceous algae were expected to be P limited in all months. Regardless of the ambient Si:N:P, relative diatom abundance was always above 50% of the total cells in each month (Table 3.2).

Chlorophyll-a Analyses

There was a wide range in chl-a values depending on the season and treatment (Fig. 3.3). Based on the responses to the control, the average mass of chl-a varied between 31, 51, 222, and 39 between January, May, July, and November, respectively. There were significant responses in +N treatment in January, +Si and +N+Si treatments in July, and +P treatment in November (p<0.05, two-sided t-tests); however, only the +N response had a positive response ratio, while the other treatments appeared to inhibit chl-a (Fig. 3.4). Analysis of variance of the ln-transformed response ratio indicates there were only differences between the +N and +Si treatments, and between the +N and +N+Si treatments in January (Tukey Honestly Significant Differences, p<0.05).
Table 3.1 Summary statistics describing temperature, turbidity, discharge, nutrients, and molar nutrient ratios during each NDS incubation. For temperature, turbidity, and discharge, the range is listed for each variable and the mean value is reported in parentheses. For nutrient concentrations and ratios, the values listed are the means of all sampling sites for the deployment and retrieval dates, respectively.

<table>
<thead>
<tr>
<th></th>
<th>January</th>
<th>May</th>
<th>July</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temp. (°C)</strong></td>
<td>5.2-7.9 (6.2)</td>
<td>16.7-21.3 (18.4)</td>
<td>22.7-25.1 (23.8)</td>
<td>3.0-8.1 (5.5)</td>
</tr>
<tr>
<td><strong>Turb. (NTU)</strong></td>
<td>1.3-15.5 (5.1)</td>
<td>2.9-16.1 (7.3)</td>
<td>4.0-24.9 (12.7)</td>
<td>1.2-12.9 (5.5)</td>
</tr>
<tr>
<td><strong>Discharge (L s⁻¹)</strong></td>
<td>30-120 (80)</td>
<td>50-130 (70)</td>
<td>1.6-14.1 (6.6)</td>
<td>0.3-15.7 (6.0)</td>
</tr>
<tr>
<td><strong>DIN (mg L⁻¹)</strong></td>
<td>7.7, 10.5</td>
<td>16.4, 11.7</td>
<td>3.2, 1.9</td>
<td>1.7, 2.3</td>
</tr>
<tr>
<td><strong>SRP (µg L⁻¹)</strong></td>
<td>2.0*, 17.8</td>
<td>62.9, 7.1</td>
<td>16.6, 13.7</td>
<td>34.7, 21.6</td>
</tr>
<tr>
<td><strong>DSi (mg L⁻¹)</strong></td>
<td>6.3, 7.5</td>
<td>11.1, 8.1</td>
<td>6.3, 5.1</td>
<td>11.7, 9.1</td>
</tr>
<tr>
<td><strong>Molar Si:N</strong></td>
<td>0.2, 0.2</td>
<td>0.2, 0.2</td>
<td>0.5, 3.0</td>
<td>30.4, 8.8</td>
</tr>
<tr>
<td><strong>Molar Si:P</strong></td>
<td>1615.5, 231.9</td>
<td>105.1, 615.8</td>
<td>279.9, 321.1</td>
<td>264.3, 358.5</td>
</tr>
<tr>
<td><strong>Molar N:P</strong></td>
<td>8482.9, 1346.3</td>
<td>679.1, 3882.7</td>
<td>771.1, 677.8</td>
<td>128.4, 704.3</td>
</tr>
</tbody>
</table>
Table 3.2 Expected limited nutrient for diatoms and non, siliceous algal taxa based on the freshwater Redfield ratio of 16 N: 1 P: 40 Si as well as the relative abundance of diatoms observed. The observed diatom abundance is based on the algal abundance averaged across each month and treatment (including the control).

<table>
<thead>
<tr>
<th>Month</th>
<th>Diatoms</th>
<th>Non, siliceous algae</th>
<th>Diatom abundance (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>P, Si</td>
<td>P</td>
<td>55</td>
</tr>
<tr>
<td>May</td>
<td>P, Si</td>
<td>P</td>
<td>58</td>
</tr>
<tr>
<td>July</td>
<td>P, Si</td>
<td>P</td>
<td>63</td>
</tr>
<tr>
<td>November</td>
<td>P</td>
<td>P</td>
<td>51</td>
</tr>
</tbody>
</table>
Figure 3.3 Mean chl-a abundance for the control and each nutrient treatment by season. Error bars indicate the standard error around the mean, n=10. Note the changes in scale among the plots.
Figure 3.4 Boxplots of chlorophyll-a response ratios in each month. For each treatment, boxes represent data within the 25th and 75th percentiles, solid lines indicate the median value, whiskers extend to ±1.5 times the interquartile range, n=10. Each point represents samples that fall outside this range. The dashed line at zero indicates a response ratio value in which the treatment response is equal to the control.

Concurrent responses in algal community composition and chlorophyll-a

Algal taxa were divided into two main categories: diatoms and non-siliceous taxa. The dominant diatoms included the genera *Meridion*, *Encyonema*, *Aulacoseira*, *Diatoma*, and *Stauroforma* and made up over 95% of all identified diatom cells. Identification of non-siliceous algae was limited.
to the level of broad taxonomic groups including chlorophytes, chrysophytes, and cyanobacteria. The percentage of diatoms ranged widely among sampled units (Fig. 3.5); however, none of the diatom response ratios in any month differed significantly from zero, indicating that increased nutrient availability did not affect the relative proportion of diatoms (two-sided t-tests; Fig. 3.6). Furthermore, treatments were not significantly different from one another in any month (one-way ANOVA, $\alpha=0.05$).

**Figure 3.5** Effect of each nutrient treatment on the proportion of diatoms, calculated as the percent of diatom cells. Each bar represents the value for each replicate sample.
Figure 3.6 Response ratios for the relative abundance of diatoms across each treatment and month. The dashed line at zero indicates a response ratio for which the treatment value is equal to the control. Boxplots are described in figure 3.3, n=3.
The diatom response ratio was not different among treatments; however, comparisons between +N and +N+Si response ratios as well as +P and +P+Si indicated that, for over 50% of samples, Si additions in combination with N and P elicited a greater response in diatom abundance than N or P alone thus supporting our expectation of secondary Si limitation (Fig. 3.7). Increasing the number of replicate samples analyzed for algal community composition would improve our understanding of the effects of Si additions on diatom abundance. While chl-\(a\) and community composition alone did not provide evidence for the Si limitation of diatoms, comparing the response ratios shows that 43% of the samples that demonstrated an inhibited chl-\(a\) response also had an increased proportion of diatoms relative to the control (Fig. 3.8). Furthermore, 79% of those samples were treatments that included Si. This indicates that Si enrichment shifted periphyton communities towards greater diatom abundance without a response in chl-\(a\).

Figure 3.7 Comparison of the diatom abundance response ratios between +N and +N+Si treatments (left) and +P and +P+Si treatments (right). Response ratios greater than zero (horizontal and vertical dashed lines) indicate a greater proportion of diatoms on the treatments relative to the control. Samples above the 1:1 line represent a response to Si beyond N or P. Points are colored by season.
Discussion

Based on the ambient nutrient stoichiometry for the majority of the sampling period, N was in excess of both P and Si, while Si was in excess of P according to the freshwater Redfield ratio. We therefore expected treatments enriched with P and Si to stimulate increased algal growth; however, we found only the +N treatment in January to significantly increase chl-a values relative to the control. The independence of chl-a from nutrient availability is not surprising as other abiotic factors, such as light, temperature, and hydrology, have been found to influence benthic periphyton abundance in nutrient-rich, agricultural streams (Munn et al. 1989, 2010; Figueroa-Nieves et al. 2006; Black et al. 2011). In fact, previous studies have found nutrient concentrations to be a poor predictor of algal biomass, likely as a result of the high concentration of nutrients in agricultural systems (Morgan et al. 2006; Royer et al. 2008; Maret et al. 2010). The range in nutrient concentrations in our study system is similar to other agricultural streams in the midwestern U.S. and, as Si and P enrichment never promoted biomass growth and N enrichment promoted growth only in January, our experiments indicate that the

Figure 3.8 A comparison between chl-a response ratios and diatom response ratios for (A) treatments amended with Si, and (B) treatments without Si. Data that fall within the yellow shaded region represent samples that had a negative chl-a response with a positive response in the relative abundance of diatoms. Samples are colored by season.
ambient nutrient concentrations meet the nutrient demands of the algal periphyton in Kirkpatrick Ditch and increased nutrient availability does not alter the community composition. These findings are consistent with other enrichment studies in agricultural watersheds where little to no N-, P-, or co-limitation was observed (Chessman et al. 1992; Reisinger et al. 2016).

Pringle et al. (1986) also found no effect of Si enrichment, either alone or together with N and P, on the total chl-α accrual on artificial substrata in a Costa Rican stream. This likely was because ambient Si concentrations never fell below 23.5 mg L⁻¹, more than twice the global riverine DSi concentration (Tréguer et al. 1995) and orders of magnitude above the 0.1 mg L⁻¹ threshold for diatom dominance estimated by Egge and Aksnes (1992). Furthermore, the study by Egge and Aksnes (1992) showed diatom dominance above this threshold regardless of other environmental conditions such as N and P concentrations, temperature, and light. This may explain why Si limitation was not observed in either Pringle et al. (1986) or the present study, as ambient DSi concentrations in both cases were always above 0.1 mg L⁻¹. Finally, only diatoms and some taxa of Chrysophytes require Si for growth; therefore, Si availability does not limit overall benthic algal biomass and the response in chl-α because other phytoplankton species, such as green algae and cyanobacteria, will continue to grow in the absence of Si (Hecky and Kilham 1988).

We attribute the declines in chl-α seen on +P and +Si treatments to increases in the proportion of diatoms colonizing the disks which, as higher quality food sources, tend to be preferentially grazed by invertebrates and have a lower chl-α content than green algae (Porter et al. 2008; Kasprzak et al. 2008; Guo et al. 2016, 2018). This aligns with our comparison between chl-α and diatom response ratios as a majority of the treatments promoting diatom growth did not increase the chl-α content relative to the control. We also found that, in most instances, there was
an increase in diatom abundance under +N+Si and +P+Si treatments relative to +N and +P, alone. These results suggest that increased Si availability could promote the growth of diatoms over non-siliceous taxa, although additional data on selective grazing in combination with Si enrichment could help elucidate these interactions. Furthermore, the inclusion of other algal pigments such as carotenoids and other chlorophylls in future studies could help clarify differential responses among algal groups (Leavitt et al. 1989; Młodzińska 2009).

Other factors confounding the results of our study include: (1) the flexibility in the nutrient demands of freshwater periphyton, (2) variation in light availability, temperature, and turbidity between the incubation sites, and (3) the heterogeneity in nutrient concentrations at sites within the watershed. We predicted nutrient limitation of P and Si based on the freshwater Redfield ratio, which presumes the nutrient requirements for algae are relatively constant (Redfield 1963); however, more recent work suggests algae can adjust their biomass composition to match the ambient stoichiometry of their environment (Urabe et al. 2003; Persson et al. 2010). While we did not quantify the stoichiometric composition of the benthic algae that grew on the diffusers, we hypothesize that the relative uniformity of biomass and diatom abundance among treatments could have resulted, at least in part, from the flexible stoichiometry of the periphyton community.

Past studies of nutrient limitation in agricultural streams have found temperature and turbidity to be better predictors of algal biomass than nutrient concentrations. For example, Johnson et al. (2009) found that chl-α in agricultural watersheds across nine ecoregions was rarely nutrient limited, in fact, percent canopy cover and photosynthetically active radiation were important drivers of the magnitude of nutrient limitation. Furthermore, periphytic chl-α in agricultural streams in Illinois demonstrated light limitation due to increased turbidity (Figueroa-
Nieves et al. 2006). At Kirkpatrick Ditch, there was variation in canopy cover, which created a gradient of light availability in the water column that potentially contributed to the heterogeneity in chl-\(a\) responses. This means that variation in light, substrate, and temperature across sites and seasons was a stronger driver on the variation in chl-\(a\) rather than nutrient availability.

In this study, chl-\(a\) peaked in July; the mass of chl-\(a\) on the control treatment was an order of magnitude greater in July than the other months which is likely due to increased temperature and light availability. Given the amount of algal growth in July, we expected to observe the Si limitation of diatoms; however, response to the +Si and +N+Si treatments were lower relative to the control suggesting these treatments supported a community prone to selective grazing (i.e., more diatom-rich) or a community that did not respond with increased chl-\(a\) (but possibly in other algal pigments). It is also possible that seasonal Si limitation did not occur in this agricultural stream due to high DIN, SRP, and DSi concentrations. Seasonal fluctuations in algal community structure in Kirkpatrick Ditch might be attributable to light availability, which can be a larger influence on algal production compared to nutrient limitation when irradiance is low and ambient nutrient concentrations are high (Von Schiller et al. 2007; Hill et al. 2009).

Finally, the biogeochemical variability between sites within our watershed likely contribute to the heterogeneity in our results. Within our study watershed, nutrient concentrations were variable between sites within sampling dates, especially among DIN and SRP concentrations (Table 2). This agrees with the spatiotemporal variation in water chemistry reported in other headwater catchments, such as within the Hubbard brook Experimental Forest (Zimmer et al. 2013). Furthermore, the spatial heterogeneity of benthic periphyton communities,
both in composition and function, likely contributes to the variability in nutrient demands and uptake (Kotliar and Wiens 1990; Clapcott and Barmuta 2010).

Ultimately, our study aimed to characterize the seasonal variation in nutrient limitation in agricultural, headwater streams with a particular focus on the role of Si limitation in facilitating the growth of non-siliceous algae. Our results suggest other environmental variables, such as light and temperature, drove the seasonal shifts in algal biomass and community composition in our study system despite ambient nutrient stoichiometry indicating Si and P limitation according to the freshwater Redfield ratio. Future work can include measurements of metabolism, increased taxonomic resolution in algal identification, and other metrics of functional and compositional changes related to nutrient availability. This paper is among the first experiments testing Si limitation in agricultural, headwater streams and can inform future studies on the role of Si in facilitating harmful algal blooms in these streams as well as downstream waters.

Acknowledgements
This research was funded in part by a grant from the Indiana University Office of Sustainability and the Integrated Program in the Environment. I would like to thank Laura Gerber, Leah Baumann, Abagael Pruitt, and Ursula Mahl for their help with the experiment and monitoring of the field sites. I would also like to thank Dr. Virginia Card for allowing me access to her lab at Metropolitan State University and the use of her Flow Cam.
References


Chapter 4

Dynamics of dissolved silicon in a large drinking water reservoir and its tributaries

Abstract

The transport and fate of nutrients is disturbed by river damming, which alters biogeochemical cycling within reservoirs and decreases the total flux of nutrients downstream. Reservoirs alter the cycling and transport of nutrients in different ways; for example, silicon (Si) is retained in larger proportions relative to nitrogen (N) and phosphorus (P). The sequestration of Si is driven by the growth of diatoms, which, over the long term, can reduce dissolved Si (DSi) availability and push phytoplankton communities to be dominated by non-siliceous, potentially harmful taxa, such as cyanobacteria. Lake Monroe is the largest reservoir in Indiana and provides drinking water for more than 140,000 people in Monroe, Brown, and Lawrence counties; therefore, water quality and ecosystem function have large implications for public health and local economies. In order to quantify Si mass retention in Lake Monroe, we sampled Si inputs from tributaries and outputs from the dam and drinking water withdrawals from April 2020-March 2021 to calculate an annual Si budget. We also measured in-lake dissolved Si, N, and P concentrations and phytoplankton community composition between May and October 2020 to better understand the relationship between ambient nutrient stoichiometry and the abundance and distribution of phytoplankton. We found that Lake Monroe retained over 40% of its annual Si inputs over the monitoring period and that retention was driven by diatom uptake and sequestration. Surprisingly, nutrient stoichiometry appeared not to affect phytoplankton community composition, suggesting DSi concentration alone is the largest control on diatom growth and abundance. As the construction of large dams continues globally, it is important to quantify how the biogeochemical cycling and transport of DSi is changing due to reservoirs and the corresponding implications for coastal and receiving waters.
Introduction

The importance of silicon (Si) to inland and coastal ecosystems is well described, particularly in the context of eutrophication and harmful algal blooms (e.g., Officer and Ryther 1980, Conley et al. 1993, Billen and Garnier 2007). The silica depletion hypothesis posits that nutrient loading, particularly of phosphorus (P), can increase diatom production in lakes and the subsequent sequestration of biogenic Si in lake sediments (Schelske and Stoermer 1971; Schelske et al. 1986). Over periods of years to decades, increased sequestration of biogenic Si disrupts the steady-state processes of dissolved Si (DSi) uptake and biogenic Si dissolution, thereby reducing DSi availability in the epilimnion of stratified lakes. Eventually this results in nutrient conditions that limit diatom production and favor blooms non-siliceous taxa, such as cyanobacteria. The process of long-term depletion of Si is distinct from the annual utilization of DSi by diatoms that temporarily reduces DSi concentrations (Conway et al. 1977; Schelske et al. 1986), though both phenomena result from the coupled cycling of nitrogen (N), P, and Si by phytoplankton.

Damming of rivers alters the quantity, timing, and stoichiometry of nutrient delivery to downstream water bodies which affects phytoplankton productivity and, subsequently, food web dynamics, carbon sequestration, and water quality (Smith 2003; Paerl et al. 2006; Conley et al. 2009; Poff and Schmidt 2016). Previous work has quantified global Si retention by river damming, which significantly alters the export of Si to downstream ecosystems and the subsequent stoichiometry of available nutrients (Beusen et al. 2009, Laruelle et al. 2009, Maavara et al. 2014). Additionally, reservoirs can alter the limiting nutrient in freshwater and marine systems, including potentially shifting diatoms from N- or P- limited to Si-limited (Paerl
et al. 2006; Howarth and Marino 2006), yet few studies have examined reservoir Si budgets and the role of Si stoichiometry in phytoplankton community succession.

The load of DSi transported by the Mississippi River to the northern Gulf of Mexico declined by approximately 50% during the middle of the 20th century (Turner et al. 1998; Turner and Rabalais 2004). One proposed mechanism for the decline in DSi loads in the Mississippi River is sequestration of biogenic Si in nutrient-rich reservoirs on streams and rivers within the Mississippi River basin. Indeed, mass balance studies have shown reservoirs to be retention structures for Si (Maavara et al. 2014, 2015a); however, there are very few published Si mass balance studies for lakes or reservoirs within the Mississippi River basin. Triplett et al. (2008) reported annual Si retention for two natural riverine lakes in Minnesota (Lakes Pepin and St. Croix), with higher retention occurring in eutrophic Lake Pepin. At present, the role of constructed reservoirs on DSi fate and transport in the Mississippi basin is largely unquantified.

Here we detail a one-year DSi budget for Lake Monroe, a reservoir in southern Indiana and within the Mississippi River basin, using inputs from major tributaries and precipitation, outflow from the dam, drinking water withdrawal, and in-lake processing and storage. Our study had three main objectives: (1) use a mass-balance approach to determine whether, and to what degree, Lake Monroe retained DSi, (2) characterize the mechanisms driving DSi retention, and (3) calculate the relative retention of DSi and compare it with published values across a gradient of reservoir trophic status. We hypothesized Lake Monroe would show net retention of DSi during the sampling period; the main driver of this retention was expected to be uptake by diatoms, which can effectively sequesters DSi through sedimentation. Diatoms are the most significant user of DSi in freshwater systems (Wetzel 2001; Thamatrakoln and Kustka 2009); therefore, the concentration of DSi in the epilimnion should correspond to the relative proportion
of diatoms versus non-siliceous phytoplankton. If DSi concentrations decreased with increasing diatom abundance, this would indicate that the growth of diatoms influenced DSi concentrations and, subsequently, DSi availability. Finally, calculating the relative retention of DSi ($R_{DSi}$) in Lake Monroe quantified the proportion of DSi inputs retained in the reservoir and allowed for a comparison with other reservoirs and natural impoundments. As the rate of dam construction continues to increase globally (Zarfl et al. 2015), it is critical to quantify DSi retention in reservoirs, and this study provides estimates of retention within the Mississippi River basin where the load of DSi can strongly influence coastal food webs and ecological processes (Turner and Rabalais 2004; Turner et al. 2007).

### Methods

**Site Description**

Lake Monroe is the largest lake in Indiana, with a surface area of approximately 44 km$^2$ at normal pool and a maximum depth of 17 m. The average volume and residence time calculated during the study period were 0.25 km$^3$ and 186 days, respectively, based on the daily volume and outflow from the dam measured by the U.S. Army Corps of Engineers. The lake is an impoundment on Salt Creek and was constructed in 1965 for the primary purpose of flood control and flow regulation during dry periods. The lake is primarily fed by the North, Middle, and South Forks of Salt Creek (Figure 4.1), with a watershed of approximately 230 km$^2$ consisting of more than 86% forested land and about 12% agricultural land. Soils in the watershed are poorly to well drained and are all classified as “highly erodible lands” given the steep slopes within the watershed (Jones et al. 1997). Today, Lake Monroe provides drinking water for more than 140,000 people, with withdrawals totaling 0.02 km$^3$ between April 2020 –
March 2021 (City of Bloomington Utilities). Lake Monroe is Meso-Eutrophic according to the Carlson Trophic State Index (Carlson 1977).

![Map of the Lake Monroe Watershed. Lake sampling sites are numbered 1-5 within each point.](image)

**Figure 4.1** Map of the Lake Monroe Watershed. Lake sampling sites are numbered 1-5 within each point.

**Sampling regime**

We monitored four tributaries and the dam outflow monthly for one calendar year between April 2020 and March 2021, collecting samples for DSi analysis and measuring instantaneous discharge. Each of the four tributaries drained sub-watersheds that, combined, account for about 55% of the Lake Monroe watershed area (Table 4.1; Figure 4.1). Stream water samples were filtered in the field using 0.45 µm cellulose filters (Fisherbrand), transported on
ice, and frozen until analysis. Instantaneous discharge \( Q \) was measured using an electromagnetic water velocity probe (March-McBirney Model 2000 FloMate) as described in Hauer and Lamberti (2006).

**Table 4.1** Lake Monroe tributary and outlet sampling sites. Each site was sampled monthly to construct a DSi budget for the watershed.

<table>
<thead>
<tr>
<th>River</th>
<th>Flow</th>
<th>USGS gage number</th>
<th>Drainage area (km(^2))</th>
<th>Fraction of watershed (%)</th>
<th>% Forest</th>
<th>% Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crooked Creek</td>
<td>Inlet</td>
<td>--</td>
<td>7</td>
<td>0.6</td>
<td>97</td>
<td>0</td>
</tr>
<tr>
<td>N. Fork Salt Creek</td>
<td>Inlet</td>
<td>03371650</td>
<td>276</td>
<td>25</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>M. Fork Salt Creek</td>
<td>Inlet</td>
<td>--</td>
<td>99</td>
<td>9</td>
<td>84</td>
<td>8</td>
</tr>
<tr>
<td>S. Fork Salt Creek</td>
<td>Inlet</td>
<td>03371600</td>
<td>230</td>
<td>21</td>
<td>76</td>
<td>17</td>
</tr>
<tr>
<td>Salt Creek</td>
<td>Outlet</td>
<td>03372500</td>
<td>1095</td>
<td>100</td>
<td>86</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 4.2** Lake Monroe sampling site locations and average depth, range in surface temperature, and mean DSi concentrations between May-October 2020. Sites are numbered relative to their position along the reservoir continuum.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Coordinates</th>
<th>Average Depth (m)</th>
<th>Surface temp. range (°C)</th>
<th>Mean DSi (mg SiO(_2) L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>39.0725, -86.4054</td>
<td>8.4</td>
<td>14.4 - 29.0</td>
<td>8.02</td>
</tr>
<tr>
<td>Site 2</td>
<td>39.0578, -86.4417</td>
<td>--</td>
<td>14.9 – 29.7</td>
<td>7.79</td>
</tr>
<tr>
<td>Site 3</td>
<td>39.0308, -86.4727</td>
<td>6.5</td>
<td>15.2 - 29.2</td>
<td>8.15</td>
</tr>
<tr>
<td>Site 4</td>
<td>39.0079, -86.4862</td>
<td>--</td>
<td>14.7 - 29.7</td>
<td>8.09</td>
</tr>
<tr>
<td>Site 5</td>
<td>39.0089, -86.5163</td>
<td>15.2</td>
<td>15.5 - 29.7</td>
<td>9.28</td>
</tr>
</tbody>
</table>

To characterize nutrient concentrations and phytoplankton community composition, we sampled five sites along a longitudinal transect through the lake monthly between late May and late October 2020 (Figure 4.1; Table 4.2). From the three main sampling locations (Sites 1, 3, and 5), we collected epilimnetic nutrient samples using a 2 m integrated sampler and, during periods of stratification, hypolimnetic samples about 1 m above the lake bed using a Van Dorn sampler. Lake DSi samples were filtered and stored in the same way as tributary nutrient
samples, and we took additional, unfiltered samples for total nitrogen (TN) and total phosphorus (TP) analysis. We also measured photosynthetically active radiation at the surface and collected temperature profiles at 1 m intervals to determine thermal stratification and metalimnion depth. At two midpoint sites between the main sites (Sites 2 and 4), we collected epilimnetic temperature and DSi samples. Lastly, we collected depth profiles of DSi concentration at 2 m intervals at Site 5, the deepest site located approximately 500 m upstream of the dam.

Phytoplankton samples were collected from the epilimnion of the three main sites, stored in dark plastic, and preserved with glutaraldehyde. Samples were imaged and identified to the genus level by PhycoTech, Inc. using the Imaging FlowCytobot (McLane Research Laboratories, Inc.). In this analysis, we grouped phytoplankton into “diatom”, “cyano”, “HAB”, and “chlorophyte” functional groups. The “diatom” functional group included Chrysophytes and Bacillariophyta; the “cyano” functional group was dominated by *Aphanocapsa, Aphanothece, Planktolyngbya, Pseudanabaena,* and *Merismopedia*; the “HAB” functional group included the toxin-producing cyanobacteria such as *Microcystis, Aphanizomenon, Planktothrix, Raphidiopsis,* and *Woronichinia*; and the “chlorophyte” group was dominated by the genera *Desmodesmus, Scenedesmus,* and *Micractinium.* Identified taxa that did not fall into these functional groups were grouped into an “other” category which included *Cryptomonas, Euglena, Ceratium,* and unclassified cells. Biovolume of the phytoplankton was estimated for each individually imaged cell by the imaging software (Moberg and Sosik 2012). Relative biovolume was calculated as the total biovolume in each division and functional group divided by the total phytoplankton biovolume in the sample.

All tributary and lake DSi samples were analyzed using the heteropoly blue method (Sultan 2014) on a Lachat QuikChem flow injection analyzer (Model 8500; Hach Company;
Samples were analyzed for TN and TP colorimetrically following an alkaline persulfate digestion (APHA 2017) using an Alpkem FLOW Solution Autoanalyzer (Model 3570; OI Analytical; College Station, TX). For all nutrient analyses, we ran a certified standard to validate the standard curve and routinely calculated the method detection limit. Samples below the detection limit included four TN samples from Crooked Creek and one TP sample from the epilimnion of Site 5. No DSi samples were below detection. Samples below detection were set to one half of the measured detection limit for purposes of statistical analysis.

**DSi budget calculations**

We quantified DSi inputs and outputs for Lake Monroe to construct an annual DSi mass balance from April 2020 through March 2021. Tributary DSi loads and atmospheric wet deposition were considered watershed inputs while the dam outflow and drinking water withdrawal were considered outputs. Total DSi retained in Lake Monroe during the sampling period was therefore calculated as the difference between total watershed inputs and outputs. The relative retention of DSi ($R_{DSi}$) was calculated as:

$$R_{DSi} = \frac{DSi_{in} - DSi_{out}}{DSi_{in}}$$

where $DSi_{in}$ and $DSi_{out}$ are the total fluxes of DSi in and out of Lake Monroe, respectively (Maavara et al. 2014). We then compared the $R_{DSi}$ of Lake Monroe to the global dataset of reservoirs compiled by Maavara et al. (2014).

Daily loads in the tributaries and outflows were modeled using Loadflex, a package in R that allows for the simple, linear interpolation of solute loads between sampling events (Appling et al. 2015). Loads were interpolated between sampling events since DSi concentrations
exhibited a chemostatic relationship with discharge at all sampling sites. We used U.S. Geological Survey (USGS) continuous, daily discharge data (gage number 03371650), monthly DSi concentrations, and a simple linear interpolation model to estimate total daily DSi loads from the North Fork Salt Creek. These daily loads were then scaled to the other sampled watersheds based on the size of each sub-watershed relative to the size of the USGS gaged watershed. Similarly, we scaled loads from the sub-watersheds to represent the DSi loads from the 45% of the watershed not captured by the tributary sampling sites. Atmospheric deposition was calculated by multiplying the annual volume of precipitation over the lake by the average DSi concentration in precipitation (both rain and snow) collected during three distinct events during the monitoring period. Outflow DSi loads were modeled using the same Loadflex methods, but discharge data were provided by the U.S. ACOE. The City of Bloomington drinking water plant is located near Site 2 and monitors daily withdrawals. We estimated the total mass of DSi removed with drinking water using the average DSi concentration at Site 2 measured between April and October of 2020 multiplied by the total volume of water withdrawn.

Statistical analysis

All analyses and statistical tests were carried out in R (The R Foundation for Statistical Computer, Version 4.0.5, 2021). We hypothesized that uptake by diatoms was the main driver of DSi retention in the lake, therefore, we expected a declining trend in DSi concentrations along the downstream gradient of the reservoir, higher DSi concentrations in the hypolimnion relative to the epilimnion, and a relationship between DSi concentrations and the relative abundance of diatoms that reflected uptake and sedimentation. We used simple linear regression to model the spatial trend in DSi concentrations as well as a one-way analysis of variance (ANOVA) to test for differences in DSi concentrations between lake sampling sites for each sampling date. We
also used t-tests to detect significant differences in DSi concentrations between the epilimnion and hypolimnion. The relationship between DSi concentrations and the abundance of diatoms relative to the total phytoplankton community was modeled using simple linear regression. Prior to running each statistical analysis, we tested all data to ensure the assumptions for each test were met, including normality and heteroskedasticity.

**Results**

*Annual DSi budget*

The primary source of DSi to Lake Monroe was tributary inputs, which varied through time and between sites. Between all sites and sampling dates, DSi concentrations in the tributaries ranged between 3.6 and 13.3 mg L\(^{-1}\) while discharge ranged over multiple orders of magnitude (Table 4.3). Due to the inherent uncertainty associated with stream discharge measurements, we applied 10% error to each value measured at the U.S. Geologic Survey gaging stations (Harmel et al. 2006). Annual input from the monitored tributaries and unmonitored watershed were estimated to be 2328 (+233) Mg SiO\(_2\) and 1692 (+169) Mg SiO\(_2\), respectively. The total annual DSi input from precipitation onto the lake was approximately 7 Mg SiO\(_2\), which represents 0.1% of the 4,027 (+402) Mg of total DSi inputs to the lake (Figure 4.2).

**Table 4.3** Lake Monroe tributary and outlet mean DSi concentrations, measured instantaneous discharge, and calculated instantaneous loads during the sampling period (April 2020-March 2021).

<table>
<thead>
<tr>
<th>River</th>
<th>Mean DSi (mg SiO(_2) L(^{-1}))</th>
<th>Mean Q (m(^3) s(^{-1}))</th>
<th>Min. Q (m(^3) s(^{-1}))</th>
<th>Max. Q (m(^3) s(^{-1}))</th>
<th>Annual DSi Load (Mg SiO(_2))</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crooked Creek</td>
<td>9.6</td>
<td>0.1</td>
<td>0.0</td>
<td>0.3</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>N. Fork Salt Creek</td>
<td>7.4</td>
<td>4.1</td>
<td>0.01</td>
<td>36.1</td>
<td>1049</td>
<td>12</td>
</tr>
<tr>
<td>M. Fork Salt Creek</td>
<td>9.1</td>
<td>0.7</td>
<td>0.0</td>
<td>5.5</td>
<td>376</td>
<td>12</td>
</tr>
<tr>
<td>S. Fork Salt Creek</td>
<td>8.9</td>
<td>1.0</td>
<td>0.01</td>
<td>5.9</td>
<td>876</td>
<td>12</td>
</tr>
<tr>
<td>Salt Creek</td>
<td>7.0</td>
<td>11.8</td>
<td>1.5</td>
<td>55.8</td>
<td>2181</td>
<td>12</td>
</tr>
</tbody>
</table>
Outflows from Lake Monroe include the dam outlet and drinking water withdrawals; DSi concentrations from the outlet fluctuated between 8-10 mg L\(^{-1}\) between April and October 2020 before declining through the winter and early spring of 2021. The total output of DSi from the dam was 2181 (±218) Mg SiO\(_2\) over the period of record. The drinking water withdrawals totaled 0.02 km\(^3\) from April 2020-May 2021 and we calculated the total mass of DSi removed with drinking water to be 203 Mg SiO\(_2\). Monthly DSi loads from tributaries and outflow showed intra-annual variation in DSi retention within the lake; loads in the outflow exceeded the total incoming load for most months in 2020 but were much less than the total input during the winter and early spring of 2021 (Figure 4.3). Based on total average input and output estimates, the annual retention of DSi was 1643 (±184) Mg SiO\(_2\) and the average \(R_{DSi}\) was 0.41, meaning that during the study period, DSi outflow was equivalent to 41% of the DSi inputs.

Figure 4.2 Annual DSi budget for the Lake Monroe watershed. Inputs include DSi loads from monitored and unmonitored tributaries as well as precipitation over the lake. The outputs from the watershed include the dam outflow and drinking water withdrawals. The ranges in tributary inputs and dam outflow represent the 10% error we applied to discharge measurements. The difference between the inputs and outputs, between 1459-1827 Mg SiO\(_2\), represents the net retention of DSi in the reservoir.
In-lake measurements of DSi from both the epilimnion and hypolimnion showed variation in DSi storage longitudinally through the lake. Total DSi storage at Sites 1 and 3 remained relatively constant over the period of record, however; DSi storage at Site 5 showed an order of magnitude decrease from May to July before increasing from August through October (Figure 4.4). All sampling basins were thermally stratified for the majority of the sampling period, limiting mixing between the epilimnion and hypolimnion; therefore, changes in DSi concentrations were likely a result of diatom uptake in the epilimnion and dissolution of biogenic Si in the hypolimnion.
Figure 4.4 Mass of DSi (as Mg SiO$_2$) in the epilimnion and hypolimnion contained within Sites 1, 3, and 5 during the monitoring period. Boundaries between each of the main sites were marked by the midpoint sites, Sites 2 and 4. The volume of water within each site was calculated using the lake surface area and the measured depth at the sampling location. Dotted lines in each plot indicate the period of thermal stratification at each site.

Variation in DSi concentrations in Lake Monroe

Contrary to our predictions, epilimnetic DSi concentrations did not significantly decline along the longitudinal gradient of the lake. In fact, epilimnetic DSi concentrations were not significantly different among any of the epilimnion sampling sites during the monitoring period. However, there were differences between epilimnion and hypolimnion concentrations that indicated changes in DSi storage through time within the lake (Figure 4.5). Specifically, when the majority of the basins were stratified, DSi concentrations in the hypolimnion were significantly greater than epilimnion DSi, except in June (t-test, \( p < 0.01 \); Figure S4.1).
The dominant phytoplankton functional group varied throughout the monitoring period; however, non-siliceous phytoplankton (cyanos and HABs) dominated most sites on each sampling date (Figure 4.6). The “cyanos” functional group dominated the phytoplankton community throughout most of the monitoring period; however, the HAB functional group dominated the community in August and diatoms made up the largest portion of algal biovolume at Sites 3 and 5 in July. As we expected, DSi concentrations were significantly related (simple linear regression, $p<0.001$) to relative diatom biovolume at each sampled site throughout the monitoring period (Figure 4.7) suggesting diatoms, as the most significant user of DSi, reduced
epilimnetic DSi concentrations. This is especially pronounced when comparing changes in relative diatom biovolume simultaneously with DSi concentrations through time (Figure 4.8). When diatoms peaked in June and October, there was a corresponding decline in DSi concentrations. While there was a significant relationship between diatom biovolume and DSi concentrations, there was no effect on phytoplankton community composition with changes in nutrient stoichiometry (Figure 4.9). Interestingly, HAB biovolume was above 20% mostly when the ambient N:P ratio was above 16:1, indicating N-limitation relative to P was favorable to harmful cyanobacteria while P-limiting conditions coincided with increased diatom abundance (Figure 4.10).

![Figure 4.6](image)

**Figure 4.6** Relative biovolume (as % of the total) of the major phytoplankton functional groups found in Lake Monroe.
Figure 4.7 Relative diatom biovolume (as a percent of total phytoplankton biovolume) compared to DSi concentrations in the epilimnion of the Upper, Center, and Lower basins. The solid lines represent the linear regression model that best fits the data.
Figure 4.8  (Top) The relative biovolume of diatoms, non-harmful cyanobacteria (cyanos), and the harmful cyanobacteria (HAB) functional groups. The HAB functional group consists of several genera of cyanobacteria known to produce toxins during blooms. (Bottom) The epilimnetic DSI concentration during the period of record. Each point represents the mean value of each site for each sampling event. Error bars extend to one standard deviation around the mean and in some cases are too small to be visible.
Figure 4.9 Relative biovolume of diatoms and HAB functional groups in all basins over the period of record. Vertical dashed lines indicate the Redfield ratio of 16 N: 1 P: 20 Si. Brown circles represent diatom biovolume relative to all algal taxa while green diamonds represent HABs.

Figure 4.10 A three-way array including DSi concentrations, N:P, and either (A) relative diatom biovolume or (B) relative HAB biovolume. Relative biovolume is represented by the size and color of each point. The vertical, dashed line denotes the 16N:1P Redfield ratio; samples to the left of the line were collected during N-limiting conditions while samples to the right of the line were collected during P-limiting conditions.

Discussion

Lake Monroe was a net sink of DSi

Based on our measurements over the course of one year, Lake Monroe retained about 41% of the total DSi inputs. Compared with other reservoirs around the world, the R_{DSi} of Lake
Monroe is in the top 25th percentile but is similar to the median value of reservoirs with similar residence times (Harrison et al. 2012, Maavara et al. 2014). Globally, the average R_{DSi} is 0.13, with values above 0.3 generally associated with water residence times greater than 5 years (Maavara et al. 2014; 2015a). The average water residence time of Lake Monroe during the sampling period was about 0.5 years, indicating the DSi retention observed in Lake Monroe during our monitoring period was higher than expected based on the relationship between water residence times and DSi retention described by Maavara et al. (2014); however, this is not unexpected since this relationship in Maavara et al. (2014) was established using budgets for just 20 reservoirs from a global population of more than 75,000 reservoirs with a surface area >0.1 km² (Lehner et al. 2011). The water retention time in Lake Monroe during the sample period was similar to the median value of historic water volume and dam outflow, indicating the DSi retention we measured likely is representative of annual Si retention during the last decade (Table S4.1). The significant DSi retention observed in Lake Monroe provides further evidence of the impact of reservoirs on nutrient delivery to downstream waters and, ultimately, coastal ecosystems. In the midwestern U.S., where precipitation is predicted to increase with climate change, we expect increased loads of DSi to the Mississippi River; although high flow events will likely disproportionately increase N loads, reducing the availability of DSi relative to N in receiving waters (Leong et al. 2014; Sinha et al. 2017; Royer 2020; Hamlet et al. 2020).

The Si retention within Lake Monroe has significant implications for the transport of DSi to the Mississippi River and, ultimately, the Gulf of Mexico. Two natural impoundments in the Mississippi River basin in Minnesota, Lakes Saint Croix and Pepin, had R_{DSi} values of 0.04 and -0.11, respectively, with the lower of the two corresponding to Lake Pepin, which had an order of magnitude greater Si inputs and was also more eutrophic relative to Lake Saint Croix (Triplett et
al. 2008; Maavara et al. 2014). All three lakes are located on higher order streams within the Mississippi River basin and DSi retention will continue via uptake and sedimentation before water reaches the Gulf of Mexico. The study by Triplett et al. (2008) is the only other published study of DSi retention within the Mississippi River basin, yet continued study of Si transport and availability in river networks is necessary to identify nutrient management practices that alleviate cultural eutrophication and hypoxia in the Gulf of Mexico. These studies are especially critical in other large rivers such as the Yangtze River in China and the Amazon River in Brazil, where many large dams are currently under construction, or planned for in the near future (Yang et al. 2011; Zarfl et al. 2015; Anderson et al. 2018; Flecker et al. 2022). Increasing Si retention in these basins, coupled with increased anthropogenic loads of N and P, will contribute to more frequent and intense Si limitation in coastal systems which has severe implications for phytoplankton productivity, water quality, and carbon cycling (Maavara et al. 2020, Garnier et al. 2010).

Contrary to our predictions, epilimnetic DSi concentrations did not decrease along the lake continuum suggesting watershed inputs maintained relatively constant Si concentrations. There were, however, significant differences in DSi concentrations between the epilimnion and hypolimnion, indicating that the mechanism likely facilitating retention is the growth and sequestration of diatoms. Diatom growth reduces DSi concentrations during blooms and sink after death or consumption, effectively sequestering the biogenic Si in lake sediments. As biogenic Si is more soluble than crystalline Si (Cornelis et al. 2011), some diatom frustules are recycled to DSi, thereby increasing the DSi concentration in the hypolimnion. The storage and dissolution of diatom frustules in the hypolimnion explains why hypolimnetic DSi concentrations were higher than epilimnetic DSi. Further examination of the biogeochemical
cycling of DSi between the water column and lake sediments is necessary to determine the proportion of Si that is sequestered and Si that is dissolved and recycled at turnover.

Reservoirs are retaining larger proportions of DSi relative to free-flowing rivers due to the increase in water residence time and the subsequent increase in nutrient removal in reservoir sediment (Humborg et al. 2006, 2008; Harrison et al. 2012; Frings et al. 2014). In one study of the “artificial lake effect” (van Bennekom and Salomons 1981), watersheds in Finland and Sweden representing a gradient of damming showed significant reductions in DSi as the percentage of the dammed watershed area increased (Conley et al. 2000). This relationship was attributed to increased hydrologic residence time and diatom growth; reservoirs trap sediment, which tend to be made up of silicate minerals, and increase light availability which promotes phytoplankton growth. Similar results were found in watersheds surrounding the Baltic Sea (Humborg et al. 2006) and the global estimate of Si retention due to reservoirs is estimated to be 5% of the global riverine Si flux (Maavara et al. 2014). As a larger proportion of the world’s rivers become fragmented with dams and reservoirs, this will have significant implications for nutrient availability in downstream ecosystems, aquatic food webs, and carbon cycling.

*Silica Utilization in Lake Monroe*

The inverse relationship between the relative proportion of diatoms and DSi concentrations indicates that DSi availability was influenced by diatom productivity. Elevated DSi concentrations created conditions suitable for diatom growth and their dominance depleted the available DSi in the lake, thus creating conditions more favorable for the growth of non-siliceous phytoplankton groups. The relationship between DSi concentrations and diatom biovolume likely differed between sites due to the fact that Site 1, as the most “upstream” site was most strongly influenced by riverine inputs, while conditions at Sites 3 and 5 were
influenced by in-lake processes. Interestingly, the minimum DSi concentration in Lake Monroe was 5.09 mg SiO₂ L⁻¹, well above the limiting concentration of 0.1 mg SiO₂ L⁻¹ reported by Schelske and Stoermer (1971). In fact, Si:N and Si:P molar ratios indicated abundant DSi availability relative to both N and P, despite the dominance of non-siliceous phytoplankton taxa throughout most of the monitoring period. Our monthly sampling frequency was likely not of fine enough resolution to capture the minimum DSi concentration at the peak of the diatom bloom; however, our data clearly show a shift from diatom to HAB dominance as DSi concentrations decline, and a shift from HAB to diatom dominance once DSi concentrations rose above 8 mg SiO₂ L⁻¹. This study is a clear example of annual Si utilization by diatoms, but quantitative studies of nutrient limitation are necessary to determine how N and P availability affect Si uptake and patterns in phytoplankton succession. As we only collected DSi data over the course of one calendar year, we do not have sufficient evidence to determine whether the occasional reduction in DSi concentrations due to diatom growth will cause long-term DSi depletion in Lake Monroe.

There was a strong relationship between diatom biovolume and DSi concentrations, but no relationship between biovolume and stoichiometry, temperature, or photosynthetically active radiation. This suggests DSi concentrations had the strongest control on diatom growth (explaining 65% of the variance), despite previous work connecting decreasing Si:N and Si:P ratios with increasing occurrences of HABs and eutrophication (Officer and Ryther 1980; Justić et al. 1995; Turner et al. 1998; Teubner and Dokulil 2002). Reservoirs have been shown to preferentially retain TN and TP over Si thereby increasing the relative Si availability to downstream waters (Maavara et al. 2020b). In other words, while reservoirs can induce DSi depletion and subsequent reductions in riverine DSi flux, they can reduce TN and TP to an even
greater extent, thus increasing Si:N and Si:P ratios to those favoring siliceous phytoplankton taxa in downstream waters (Humborg et al. 2000, 2008). However, in human-dominated regions reservoirs cannot buffer riverine stoichiometry against the ever increasing anthropogenic inputs of N and P relative to Si, which results in a declining trend of Si:N and Si:P in coastal receiving waters (Maavara et al. 2020a).

**Implications and direction for future work**

Reservoirs have played a vital role in human civilization through flood protection, maintenance of water supplies, support for transportation and navigation, and as a source of renewable power generation (Lehner et al. 2011). At the same time, reservoirs have negative environmental impacts including declines in biodiversity, contributing significant quantities of greenhouse gas emissions to the atmosphere, and altering biogeochemical processing along the river continuum (Deemer et al. 2016; Grill et al. 2019; Maavara et al. 2020b). Quantification of N, P, and Si retention in reservoirs is lacking in published literature, limiting our ability to accurately model changes in nutrient delivery to coastal systems (Maavara et al. 2014; 2015b; Akbarzadeh et al. 2019). As dam construction continues to impede free-flowing rivers, an improved understanding of biogeochemical nutrient cycling is necessary for accurate environmental assessments and water quality projections (Friedl and Wüest 2002; Maranger et al. 2018).

Our study is the first report of DSi dynamics in Lake Monroe and provides further evidence of DSi retention in reservoirs. Recent reviews of the global retention of DSi through reservoirs highlight the significant fraction of DSi sequestered in lentic systems which represents an important component of the global biogeochemical cycle of Si (Harrison et al. 2012; Frings et al. 2014; Maavara et al. 2014). Longer term analyses of DSi retention within Lake Monroe and
other similar reservoirs would allow for better characterization of DSi dynamics under a range of hydrologic conditions and an examination of trends in retention through time, including explicit testing of the silica depletion hypothesis.

Acknowledgements
This work was supported by funds from the Indiana Department of Environmental Management Section 319 Nonpoint Source Management Program, City of Bloomington Utilities Service Board, and Indiana University O’Neill School of Public and Environmental Affairs. I would like to thank Sarah Powers, Lindsey Rasnake, Megan Gokey, and the many other members of the Limnology Lab at Indiana University for their support in data collection and sample analysis.
References


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**Figure S4.1** Epilimnion versus hypolimnion DSi concentrations during the sampling period. Data are grouped by depth and sampling month. Each box represents the 25th to 75th percentiles, the horizontal line indicates the median value, whiskers extend to ±1.5 Interquartile Range, and points indicate outliers in each group.
Table S4.1 Average annual volume, dam outflow, and water residence time for Lake Monroe between 2012 and 2022. Mean values for volume and outflow are based on daily measurements taken by the Army Corps of Engineers. Water residence time is calculated using the annual average of lake volume and dam outflow.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean volume (km$^3$)</th>
<th>Mean Dam Outflow (m$^3$ s$^{-1}$)</th>
<th>Water Residence Time (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>0.21</td>
<td>3.4</td>
<td>1.9</td>
</tr>
<tr>
<td>2013</td>
<td>0.24</td>
<td>12.5</td>
<td>0.6</td>
</tr>
<tr>
<td>2014</td>
<td>0.25</td>
<td>18.3</td>
<td>0.4</td>
</tr>
<tr>
<td>2015</td>
<td>0.27</td>
<td>17.1</td>
<td>0.5</td>
</tr>
<tr>
<td>2016</td>
<td>0.26</td>
<td>21.4</td>
<td>0.4</td>
</tr>
<tr>
<td>2017</td>
<td>0.25</td>
<td>12.7</td>
<td>0.6</td>
</tr>
<tr>
<td>2018</td>
<td>0.27</td>
<td>18.9</td>
<td>0.4</td>
</tr>
<tr>
<td>2019</td>
<td>0.33</td>
<td>26.5</td>
<td>0.4</td>
</tr>
<tr>
<td>2020</td>
<td>0.26</td>
<td>18.4</td>
<td>0.5</td>
</tr>
<tr>
<td>2021</td>
<td>0.25</td>
<td>19.5</td>
<td>0.4</td>
</tr>
<tr>
<td>2022</td>
<td>0.30</td>
<td>28.1</td>
<td>0.3</td>
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</tbody>
</table>
Chapter 5

Optical properties of dissolved organic matter reveal coupling between carbon quality, nutrient availability, and algal community composition in a mesotrophic reservoir

Abstract

The composition and concentration of dissolved organic matter (DOM) influence biotic communities, nutrient dynamics, and light regimes in aquatic systems. Demand for DOM by microorganisms influences the biogeochemical cycling of inorganic nutrients, but the potential for DOM quality to be coupled to nutrient ratios and, by extension, phytoplankton community composition has not been well documented. The stoichiometric ratios of dissolved nutrients can influence the types and abundances of phytoplankton taxa and, in lakes, this includes the concentration of dissolved silicon, a required nutrient for diatoms but not for other major phytoplankton groups. Here we analyze data collected monthly from a large reservoir in south-central Indiana between May and October 2020. Epilimnetic samples were collected from five points along the longitudinal gradient of the reservoir and analyzed for concentration of dissolved organic carbon, dissolved inorganic nitrogen, phosphorus, and silicon; phytoplankton community composition; and the chemical nature of the DOM using optical properties and excitation emission matrices (EEMs). Dissolved silicon concentrations in the epilimnion ranged between 5 and 13 mg SiO₂ L⁻¹ and corresponded closely with relative diatom biovolume. A large increase in diatom relative abundance from less than 10% to over 40% occurred in late July and resulted in a decline in dissolved silicon from nearly 9 mg L⁻¹ to 6.5 mg L⁻¹. This was followed by an increase in cyanobacteria from less than 1% to over 60% between late July and late August, with a corresponding increase in silicon concentrations to nearly 8 mg L⁻¹. Other shifts in phytoplankton community composition occurred throughout the monitoring period, but there was no relationship between relative biomass of major phytoplankton groups and nutrient
stoichiometry. Dissolved organic carbon (DOC) concentrations ranged from 2 to nearly 5 mg L⁻¹, increasing from late May through September before declining slightly in late October. We modeled the composition of DOM using EEM Parallel Factor Analysis, which identified six individual DOM components; five of which were related to humic compounds while the sixth related to protein-like, phytoplankton-derived, DOM. The variation in the relative abundance of DOM components signaled changes in carbon inputs and processing throughout the monitoring period, which we were able to relate to DOC concentrations and variation in phytoplankton biovolume. This study is one of the first to relate nutrient stoichiometry, phytoplankton community, and DOM composition and reveals the potential coupling between ambient nutrient stoichiometry and DOM composition and quality.

Introduction

Dissolved organic matter (DOM) includes a diverse set of organic molecules whose heterogeneity contribute to various effects on aquatic ecology such as light attenuation, biogeochemical nutrient cycling, and ecosystem productivity (Fellman et al. 2008, 2011; Cory and Kling 2018). The source and composition of DOM plays a role in its bioavailability and lability (Findlay and Sinsabaugh 2003; Bianchi and Canuel 2011); sources of DOM in surface waters include humic compounds from soils and terrestrial vegetation and proteins from aquatic primary productivity (Spencer et al. 2007; Nebbioso and Piccolo 2013; Massicotte et al. 2017). Demand for DOM by microorganisms influences the biogeochemical cycling of inorganic nutrients and, in turn, the density and distribution of the microbial community influences the concentration and composition of DOM (Kaplan and Bott 1989; Conan et al. 2007; Tank et al. 2010). Furthermore, aquatic, or autochthonous, organic matter is considered generally to be more labile than terrestrial, or allochthonous, organic matter thus facilitating an essential feedback
Reservoirs play an important role in DOM transformations along the aquatic continuum. Firstly, reservoirs have reduced canopy cover and increased hydrologic residence time, relative to rivers, promoting increased photodegradation and microbial breakdown of organic compounds (Vähätalo et al. 2005; von Wachenfeldt and Tranvik 2008; Koehler et al. 2012). Furthermore, reservoirs support increased phytoplankton DOM production thereby increasing the proportion of autochthonous DOM relative to allochthonous inputs (de Moura et al. 2021). Secondly, hydrologic variability throughout the year alters the sources and processing of DOM within reservoirs (Oliver et al. 2016; Bao et al. 2019). For example, significant differences in DOM composition were found in the Three Gorges Dam in China between periods of storage and drainage. During the drainage period, defined as the first six months of the year over which the water level in the dam decreased, there was a larger proportion of autochthonous DOM relative to the storage period, defined as the latter half of the year when the water level increased (He et al. 2020).

The characterization of DOM in aquatic ecosystems has implications in understanding carbon cycling and transport, the role of organisms in DOM transformations, and the spatial and temporal changes in DOM within aquatic systems. The composition of DOM determines its reactivity in the environment, bioavailability in aquatic food webs, and cycling and transport to downstream waters (Bernhardt and Likens 2002; Johnson et al. 2012; Oviedo-Vargas et al. 2013). While the couplings between DOM and algal productivity as well as the relationship between nutrient availability and algal community composition are well known, direct linkages between nutrient concentrations and the abundance and composition of DOM have not been
described. Nutrient availability affects the abundance and diversity of algal species which in turn can influence the concentration and chemical composition of DOM (Hong et al. 2012); therefore, we expect a coupled response in nutrient concentrations and DOM that is mediated by the density and distribution of phytoplankton.

In this study, we used nutrient concentrations, algal community composition, and DOM fluorescence data to examine the potential for nutrients and algae to influence the composition and quality of organic matter. The ultimate goal of our study was to characterize the changes in the optical properties of DOM in response to shifts in phytoplankton community composition and nutrient dynamics in a large midwestern reservoir. We expected both spatial and temporal variability in DOM composition, as DOM is highly reactive and past studies have shown changes in DOM fluorescence along the downstream gradient of waterbodies (Sinsabaugh and Findlay 2002; Stedmon et al. 2006; Hounshell et al. 2021). Furthermore, variation in seasonal light availability and primary productivity rates will affect DOM composition through time (Wetzel 2001; Maavara et al. 2021). We hypothesized that the relationship between nutrient concentrations, including Si, and the composition of DOM would be mediated through the density and composition of the phytoplankton community. Therefore, we expected DOM optical properties to change through time in response changes in the relative abundance of phytoplankton taxa (Figure 5.1). In particular, we expected the utilization of dissolved Si by diatoms would facilitate a shift to cyanobacterial dominance (Kilham 1971, Conley et al. 1993), which in turn would drive a change in the quality of the DOM, as measured by optical properties. Linking N, P, and Si availability with DOM composition is a novel concept and is pertinent to studying the effects of harmful algal blooms (HABs) on freshwater trophic dynamics and carbon cycling.
Methods

Site description and sampling regime

Lake Monroe is the largest reservoir in Indiana, providing drinking water for more than 140,000 people in Monroe, Brown, and Lawrence counties. The reservoir was constructed in 1965 has a surface area of approximately 43.5 km², with more than 86% of the watershed forested and about 12% in agricultural use (Figure 5.2). We sampled five points along the reservoir monthly between May and October 2020 for dissolved nutrient concentrations – including nitrate (NO$_3^-$-N), soluble reactive phosphorus (SRP), silicon (DSi), and organic carbon (DOC) – dissolved organic matter, chlorophyll-$a$ (chl-$a$), and phytoplankton community...
composition. The Army Corps of Engineers (ACOE) monitors dam outflow, total lake volume, and estimates total inflow from the watershed (Figure 5.2).

Samples were collected from the epilimnion for nutrients, DOC, and DOM using a 2 m integrated sampler at each site. Every sample was filtered in the field using 0.45 µm membrane filters (Fisherbrand). Nutrients samples were stored in 60-mL high-density polyethylene Nalgene bottles. Samples for DOC were collected in dark Nalgene bottles and were acidified in the field with 1 M hydrochloric acid to a pH of 2. Samples collected for DOM optical analysis were filtered using membrane filters rinsed with ultra-pure, deionized water and stored in dark glass bottles. We transported samples on ice, in the dark, and froze nutrients and DOC samples until analysis. Samples were analyzed for NO$_3^-$ and SRP using an Alpkem™ FLOW Solution Autoanalyzer (Model 3570; OI Analytical; College Station, TX) using the cadmium reduction method (APHA 2017) and the ascorbic acid method (Murphy and Riley 1962), respectively. We analyzed DSi samples using a Lachat QuikChem™ Analyzer (Hach Company; Loveland, CO) and the heteropoly blue method (Sultan 2014). Samples for DOC were analyzed using high-temperature oxidation (Suzuki et al. 1992) on a Shimadzu Total Organic Carbon Analyzer (Shimadzu; Kyoto, Japan). Optical DOM samples were kept on ice and analyzed using an Aqualog™ scanning fluorometer (Horiba Instruments; Irvine, CA) on the same day as sample collection. Quality control checks for nutrient and DOC concentrations were conducted using an external standard to validate the standard curve and by calculating the method detection limit for each analytical run. The method detection limits were 0.008 mg N L$^{-1}$ for NO$_3^-$, 0.002 mg L$^{-1}$ for SRP, 0.05 mg SiO$_2$ L$^{-1}$ for DSi, and 0.5 mg L$^{-1}$ for DOC. If samples were below detections, we set the concentration to one-half the calculated detection limit. Of all samples analyzed in the
study, only 35% of NO$_3^-$ samples were below detection.

![Map of Lake Monroe and its surrounding watershed](image1)

**Figure 5.2** (Left) Map of Lake Monroe and its surrounding watershed. Sampling points are denoted by red triangles and numbers correspond to the site numbers in the text. (Right) Total water inflows and outflows from Lake Monroe, as recorded by the Army Corps of Engineers from May through October 2020.

Chlorophyll-$a$ samples were collected on glass-fiber filters and analyzed according to EPA method 446.0 (Arar, 1997). Samples were extracted in 90% acetone solution overnight, ground, and analyzed using an Evolution 220 UV-Vis spectrophotometer (Fisher Scientific; Waltham, MA). Phytoplankton samples were collected from the epilimnion of Sites 1, 3, and 5. Phytoplankton were enumerated and identified to the division level by PhycoTech, Inc. (St. Joseph, MI) using the Imaging FlowCytobot (McLane Research Laboratories; Falmouth, MA). Here we present phytoplankton biovolume of the main functional groups – non-harmful cyanobacteria, chlorophytes, diatoms, and toxin-producing cyanobacteria (cyanoHAB). Non-harmful cyanobacteria include the taxa that do not produce toxins; in our study, we identified
Aphanocapsa, Aphanothece, Chroococcus, Merismophedia, Planktolyngbya, and Romeria. The chlorophyte functional group contained a broad variety of green algae but the most abundant taxa were Chlorophytes, Micractinium, Botryoccus, and Peridinales. The diatom functional group included silica-using algal taxa, the Bacillariophyta as well as Chrysophytes; the most abundant taxa were Aulacoseira and Dinobryon. Finally, the cyanohAB functional group included the toxin-producing cyanobacteria such as Microcystis, Aphanizomenon, Planktothrix, Raphidiopsis, and Woronchina. Biovolume of the phytoplankton was estimated for each individually imaged cell by the imaging software (Moberg and Sosik 2012). Relative biovolume was calculated as the total biovolume in each functional group divided by the total algal biovolume in the sample. For all phytoplankton functional groups, cell density (as cells per mL) was strongly correlated with biovolume (Pearson’s correlation, r=0.93, p<0.001); therefore, we used biovolume as the metric of algal abundance as it better quantified total phytoplankton growth.

Optical properties of dissolved organic matter

We used fluorescence spectra to construct excitation-emission matrices (EEMs) for each DOM sample. The integration time was set as 1.5 seconds, which covers two orders of magnitude in fluorescence intensity. Excitation wavelengths were scanned from 230 nm to 800 nm at 5 nm increments and emission wavelengths were scanned from 240 nm to 830 nm at 2 nm increments. We did not dilute any samples as absorbance values at 255 nm were always <2.0 (Kothawala et al. 2013). Every sample was then corrected for instrument-specific influences (Stedmon and Bro 2008), absorbance baseline drift, and inner-filter effects (Kothawala et al. 2013). We also blank-corrected each sample excitation-emission matrix (EEM) using fluorescence spectra from ultra-pure, dionized water samples obtained on the same day as each sample analysis (Stedmon et al. 2003). While the blank correction partially removed Rayleigh
and Ramen scattering, we removed additional scatter to ensure the remaining peaks accurately represented the DOM data. Detailed protocols for fluorescence analysis are included in Appendix A.

Fluorescence and absorbance data were used to calculate the slope ratio (SR), specific ultraviolet absorbance (SUVA), biological index (BIX), fluorescence index (FI), and peaks A, B, C, and T. These values are indicative of the source and quality of fluorescent DOM (Gabor et al. 2014). The SR is related to the molecular weight of DOM and is indicative of photodegradation or microbial alteration (Helms et al. 2008). SUVA$_{254}$, specific absorbance at 254 nm, indicates the bulk aromaticity of the DOM (Weishaar et al. 2003). BIX is an indicator of the sourcing of DOM, larger values (>1) correspond with recently produced DOM with autochthonous, biological sources (Huguet et al. 2009). The FI is an indicator of DOM precursor material; values ~1.8 indicate microbial sources and values ~1.2 indicate terrestrial sources (McKnight et al. 2001; Cory and McKnight 2005). DOM peaks are indicative of either “humic-like” or “protein-like” classes of organic matter and are defined in Table 5.1 (Coble 1996; Gabor et al. 2014).
**Table 5.1** Explanation of DOM indices and peaks, including its calculation and what fractions of DOM each optical property represents.

<table>
<thead>
<tr>
<th>Index or Peak</th>
<th>Parameters</th>
<th>Properties</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope Ratio</td>
<td>Ratio of slope of absorbance spectra within 275-295 nm to the slope of absorbance spectra within 350-400 nm. These two spectral ranges correspond to the absorbance values that shift dramatically during photochemical alteration of DOM</td>
<td>Related to the molecular weight of DOM; particularly of isolates of fulvic acids</td>
<td>Helms et al. 2008; Limnol. Oceanogr. 53(3)</td>
</tr>
<tr>
<td>SUVA$_{254}$</td>
<td>Absorbance at 254 nm divided by the DOC concentration</td>
<td>Bulk aromaticity of DOM</td>
<td>Weishaar et al. 2003; ES&amp;T 37(20)</td>
</tr>
<tr>
<td>Biological Index (BIX)</td>
<td>Ratio between Peak M and Peak C regions of fluorescence measured at an excitation wavelength of 310 nm</td>
<td>Indicates autochthonous, recently produced DOM</td>
<td>Huguet et al. 2009; Organic Geochem. 40(6)</td>
</tr>
<tr>
<td>Fluorescence Index (FI)</td>
<td>Ratio between fluorescence values at emission wavelengths 470 nm/520 nm at excitation wavelength 370 nm</td>
<td>Indicates the proportion of DOM attributed to microbial or terrestrial precursor material</td>
<td>McKnight et al. 2001; Cory and McKnight 2005</td>
</tr>
<tr>
<td>Peak A</td>
<td>Fluorescence peak at the excitation wavelength of 260 nm and the emission wavelength between 380-460 nm that results in the maximum fluorescence value</td>
<td>Humic-like</td>
<td>Coble 1996; Marine Chemistry 51(4)</td>
</tr>
<tr>
<td>Peak B</td>
<td>Fluorescence peak at the excitation wavelength of 275 nm and the emission wavelength of 310 nm</td>
<td>Protein-like (tyrosine)</td>
<td>Coble 1996; Marine Chemistry 51(4)</td>
</tr>
<tr>
<td>Peak C</td>
<td>Fluorescence peak at the excitation wavelength of 350 nm and the emission wavelength between 420-480 nm that results in the maximum fluorescence value</td>
<td>Humic-like</td>
<td>Coble 1996; Marine Chemistry 51(4)</td>
</tr>
<tr>
<td>Peak T</td>
<td>Fluorescence peak at the excitation wavelength of 275 nm and the emission wavelength of 340 nm</td>
<td>Protein-like (tryptophan)</td>
<td>Coble 1996; Marine Chemistry 51(4)</td>
</tr>
</tbody>
</table>
**PARAFAC modeling**

Parallel factor (PARAFAC) analysis was conducted using the staRdom package in R (Pucher et al. 2019) in order to reduce the corrected EEMs into discrete components (Stedmon and Bro 2008; Murphy et al. 2013). EEM data was constrained to positive fluorescence values (removing any false negative values) and normalized to account for the correlation between components. A series of PARAFAC models was calculated by varying the number of components in order to maximize the model variance while minimizing the residual error. For each PARAFAC model, we examined the randomness of the residuals and the spectral loadings of each component to ensure the final model fit the correct number of components to our dataset. We also explored the relative leverage each sample contributed to the PARAFAC model and excluded one sample that disproportionately affected the calculation of the model components. We validated the final model using split-half analysis, random initialization, Tucker’s Congruency coefficients, and comparisons with other published PARAFAC components in the OpenFluor database (Murphy et al. 2014). Code for PARAFAC modeling are included in Appendix B.

**Statistical analyses**

We used one-way ANOVA to detect significant differences in SR, SUVA$_{254}$, BIX, FI, and peaks A, B, C, and T between sampling locations and dates to understand spatial and temporal changes in the optical properties of DOM. If significant differences were detected, we used Tukey’s Honestly Significant Differences test to determine which sites and months were different from others. In order to relate changes in DOM optical properties to nutrient concentrations, stoichiometry, and algal community composition, we first used simple linear regression to model the relationships between indices, peaks, and algal community data. We also
used principal component analysis to reduce the dimensionality of the optical dataset which allowed us to relate changes in nutrient concentrations and algal community composition with their loadings on the first principal component. Finally, we used PARAFAC modeling to identify unique components within the DOM dataset and analyzed the components’ relationships with nutrients and algae using principal component analysis. All statistical tests were completed in R.

**Results**

*Variation in nutrient concentrations and phytoplankton*

Across the period of record, NO$_3^-$, SRP, DOC, and DSi concentrations ranged between 0.004-0.054 mg N L$^{-1}$, 0.002-0.019 mg L$^{-1}$, 2.5-4.0 mg L$^{-1}$, and 6.5-9.0 mg L$^{-1}$, respectively. Monthly concentrations for NO$_3^-$, SRP, and DSi fluctuated throughout May-October, but DOC concentrations increased from May-September before declining in October (Figure 5.3). Average chl-$_a$ for all sites during the sampling period was 10 μg L$^{-1}$ and ranged between 2-30 μg L$^{-1}$. Chl-$_a$ concentrations were significantly higher from August-October

![Image](image-url)

**Figure 5.3** Mean concentration of NO$_3^-$ (as N), SRP (as P), DSi as (SiO$_2$), and DOC (as C) for each sampling site during the monitoring period. For NO$_3^-$ and SRP, n=3; for DSi and DOC, n=5. Error bars extend to the standard error of the mean.
relative to May-July (t-test; p<0.01) which coincided with increased growth of non-harmful cyanobacteria, cyanoHABs, and diatoms (Figure 5.4).

**Figure 5.4** Monthly average chl-a (top) and average biovolume of main functional groups from Sites 1, 3, and 5 (bottom).

*Phytoplankton biomass related to protein fraction of DOM and bioavailability*

We used simple linear regression to relate changes in the protein fraction of DOM (peaks B and T) to algal community composition and chl-a (Fig. 5.4). For both peaks B and T, there was a significant linear relationship (p<0.05) between all algal functional groups, except cyanoHABs
(Figure 5.5). This suggests increasing phytoplankton biomass, especially from specific taxa, has a positive relationship with the protein fraction of DOM. We also modeled the relationship between BIX and phytoplankton functional groups as BIX values are indicative of the amount of recently produced organic matter (Figure 5.6). Unlike peaks B and T, there was no relationship between BIX and individual phytoplankton function groups although BIX had a positive, linear relationship with chl-\(a\) (\(p=0.02, r^2=0.17\)). Both BIX and chl-\(a\) generally increased through time, indicating greater phytoplankton production as the summer growing season progressed.

**Figure 5.5** Biovolume of non-harmful cyanobacteria, diatoms, chlorophytes, and cyanoHAB functional groups as well as chl-\(a\) against peak B (top) and peak T (bottom). Points are colored by sampling month. Relationships between each of the variables and either Peak B or Peak T were modeled using simple linear regression; significant relationships (\(p<0.05\)) are shown by the solid black line.
Biovolume of non-harmful cyanobacteria, diatoms, chlorophytes, and cyanoHAB functional groups as well as chl-$a$ against the biological index (BIX). Points are colored by sampling month. Relationships between each variable and BIX were modeled using simple linear regression; the significant relationship between chl-$a$ and BIX ($p=0.02$) is shown by the solid black line.

Optical properties of DOM change through time

There were significant differences across months for all calculated indices and peaks (one-way ANOVA, $p<0.001$) although not all months were distinctly different from one another (Figure 5.7). Peak A, peak C, slope ratio (SR), and SUVA$_{254}$, were variable across the monitoring period while peak B, peak T, and BIX, generally increased through time. The FI stayed relatively constant across the monitoring period, and with values falling within the continuum between microbial and terrestrially derived DOM. Variation in SUVA$_{254}$ is indicative of the reactivity of humic DOM components, which is especially relevant to the formation of disinfection byproducts during the water treatment process (Weishaar et al. 2003). The SR value, which is related to the molecular weight of DOM compounds and can indicate variation in photobleaching and microbial alteration of DOM (Helms et al. 2008), decreased between May and July but increased through the rest of the monitoring period. Peaks B and T correspond with protein-like, aquatic DOM while peaks A and C correspond with humic-like, terrestrial DOM (Coble 1996). Increases in peaks B and T throughout the monitoring period suggest an increase in aquatic primary productivity, which was reinforced by the increasing BIX values (Parlanti et
Changes in peaks A and C likely reflect changes in terrestrial inputs of DOM to Lake Monroe or rates of biodegradation.

**Figure 5.7** Monthly boxplots of key DOM optical properties. Values for slope ratio (SR), biologic index (BIX) and fluorescence index (FI) are unitless as each metric is a ratio of fluorescence intensities at various wavelengths. Intensity values for each peak are in Ramen Units (RU). Boxes correspond to the 25th and 75th percentiles, the solid line within each box denotes the median, and whiskers extend to ±1.5 times the interquartile range. Letters above each box indicate which months are significantly different from others within each variable.
In order to better relate these optical properties to nutrient availability and algal community data, we used principal component analysis to simplify the variables into two components. The first principal component explained ~58% of the total variance in the dataset and showed negative loadings for parameters associated with protein-like (peaks B and T) and humic-like (peak C) moieties. The second principal component was largely associated with SUVA$_{254}$ and explained ~25% of the variation among samples. Samples grouped largely by month, providing further evidence of changes in DOM composition through time (Figure 5.8).

![Figure 5.8](image)

**Figure 5.8** Principal component analysis of DOM optical properties. Samples are colored by sampling month.

The first principal component (PC1) had significant linear relationships with DOC ($p<0.01$, $r^2=0.51$) and DSi concentrations ($p=0.03$, $r^2=0.18$); however, there was no relationship with the other nutrients (Figure 5.9) or ratios between Si, N, and P (Figure S5.1). Of the algal
functional groups, PC1 had a significant linear relationship with diatoms (p=0.01, r²=0.37) and chlorophytes (p<0.01, r²=0.46) as well as chl-a (p<0.01, r²=0.51). The relationship between the optical properties of DOM and the concentration of DOC suggests that increases in concentration are associated with changes in the source of DOM which could be an indicator of changes in the production rates of different types of organic matter through time. Although DOM optical properties were only related to two of the main algal functional groups, as well as chl-a, these relationships suggest that algal biomass significantly affects DOM composition.

Figure 5.9 Nutrient concentrations, functional group biovolume, and chl-a values plotted against the loadings of the first principal component (PC1). The color of the point corresponds to the sampling month. Relationships between the variables and PC1 were modeled using simple linear regression; significant relationships (p<0.05) are shown by the solid black line.
PARAFAC model components

The PARAFAC analysis validated a six-component model (numbered C1-C6) that describe 99.5% of the variation in the EEMs. Comparisons between the components in our PARAFAC model with other published spectra for DOM samples are shown in Table 5.2. Other studies have identified components similar to our modeled C1-C5 as humic-like; C1, C4, and C5 are all associated with terrestrial humic material (Søndergaard et al. 2003; Stedmon and Markager 2005; Murphy et al. 2011). While C2 and C3 were also identified as humic-like compounds, C2 was similar to soil fulvic peaks and C3 was associated with Peak A (Stedmon et al. 2003; Søndergaard et al. 2003; Wünsch et al. 2015). In this study, only C6 was identified as tryptophan-like which is representative of protein-like, autochthonous DOM. In our study, the fluorescence intensity (F_max) of each component varied through time and generally increased from May through October (Figure 5.10). The fluorescence of C1, C5, and C6 decreased slightly in the early months of our sampling period, while C2, C3, and C4 remain relatively constant between May and July.

Table 5.2 Explanation of the six fluorophore components identified by our PARAFAC model.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Ex. peak (nm)</th>
<th>Em. peak (nm)</th>
<th>F_max range (RU)</th>
<th>Matches in OpenFluor database</th>
<th>Description</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>345</td>
<td>433.8</td>
<td>0.06-0.21</td>
<td>4</td>
<td>Terrestrial humic material; Peak C</td>
<td>Stedmon and Markager 2005</td>
</tr>
<tr>
<td>2</td>
<td>305</td>
<td>373.9</td>
<td>0.04-0.22</td>
<td>2</td>
<td>Humic-like; Soil fulvic peak</td>
<td>Søndergaard et al. 2003</td>
</tr>
<tr>
<td>3</td>
<td>255</td>
<td>440.7</td>
<td>0.02-0.15</td>
<td>33</td>
<td>UV humic-like; Peak A</td>
<td>Stedmon et al. 2003</td>
</tr>
<tr>
<td>4</td>
<td>290</td>
<td>491.8</td>
<td>0.02-0.08</td>
<td>3</td>
<td>Terrestrial humic material</td>
<td>Søndergaard et al. 2003</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>406.1</td>
<td>0-0.12</td>
<td>4</td>
<td>Terrestrial humic material</td>
<td>Wünsch et al. 2015</td>
</tr>
<tr>
<td>6</td>
<td>265</td>
<td>328.3</td>
<td>0-0.09</td>
<td>32</td>
<td>Protein, tryptophan-like</td>
<td>Murphy et al. 2011</td>
</tr>
</tbody>
</table>
Figure 5.10 Average monthly $F_{\text{max}}$ between sampling sites for each modeled PARAFAC component. Error bars extend to the standard error around the mean. $F_{\text{max}}$ refers to the maximum fluorescence intensity in Ramen Units (RU).

To examine the relationship between the PARAFAC model components, nutrient concentrations, and algae community composition, we reduced the dimensionality of the dataset using principal component analysis (Figure 5.11). We did not include nutrient ratios in the PCA.
as both Si:N and Si:P were significantly correlated with NO$_3^-$-N (p<0.01, Pearson’s r = -0.75) and SRP concentrations (p<0.01, Pearson’s r = -0.68), respectively. The first principal component (PC1) explained 49% of the variance and was strongly influenced by the PARAFAC intensities of DOM components C1 and C4 as well as NO$_3^-$-N concentrations. The second principal component (PC2) explained 17% of the variance and was positively correlated with DSi concentrations and negatively correlated with C3 and diatom biovolume; thus, PC2 largely explained the inverse relationship between DSi availability and diatom abundance. Additionally, C3 was positively correlated with diatom biovolume, suggesting the concentration of DSi had a significant effect on the relative intensity of C3, mediated by the abundance of diatoms (Figure 5.12). Components C1, C2, C4, and C5 were positively correlated with DOC concentrations, indicating that the source(s) of these components were likely responsible for the increase in DOC concentration. Interestingly, chlorophyte biovolume and chl-$\alpha$ concentrations were strongly related even though chlorophytes were not the dominant algal taxa in any month during the sampling period.
Figure 5.11 Principal component analysis including the six PARAFAC components identified in this study, biovolume of the major algal functional groups, and concentrations of DOC, NO₃⁻, SRP, and DSi.

Figure 5.12 Relationship between DSi concentrations and the relative intensity of DOM component C3 modeled using simple linear regression. Points are colored by sampling month.
Discussion

DOM composition through time

As expected, the composition of DOM changed through time, as evidenced by changes in fluorescence peaks and indices as well as PARAFAC components. There was no relationship between DOM composition and sampling site, indicating that seasonal factors were largely responsible for DOM composition and quality. We expected DOM composition to vary along the downstream gradient of the reservoir due to changes in terrestrial inputs and biogeochemical processing; however, the data suggest factors that are relatively constant throughout the lake, such as temperature and photodegradation rates, were likely the major controls DOM composition. Nutrients and phytoplankton community composition also showed more variation between sampling dates than between sites, further evidence that biogeochemical processes in the epilimnion of Lake Monroe were influenced by temporal variables acting across the lake as a whole.

Increases in Peaks B and T through time indicated increases in the protein fraction of DOM, which aligns with the increased algal productivity during the monitoring period. Protein fractions of DOM are more bioavailable than humic-like DOM; therefore, increases in peaks B and T relative to peaks A and C indicate an increase in the bioavailability of DOM between late May and October. Further evidence of increased autochthonous DOM production with time was seen with increasing BIX values, which correspond to recently produced, aquatic DOM (Huguet et al. 2009; Gabor et al. 2014). The fluorescence index (FI) was relatively constant through time, indicating that the relative contribution of microbial versus terrestrial sources to the DOM pool did not vary with changes in nutrients, algal biomass, or riverine inputs. Additional data on
heterotrophic metabolism could elucidate the impact of increasing DOM bioavailability on
trophic dynamics throughout the growing season.

The maximum fluorescence intensity ($F_{\text{max}}$) of each DOM component generally increased
during the sampling period, indicating the increase in DOC concentrations can be attributed to
increases in each DOM component. Variation in $F_{\text{max}}$ values correspond to changes in the inputs,
production, and processing of DOM; however, we observed steady increases in each DOM
component between July and September that did not correspond with the pattern in water inflows
recorded for the Lake (Figure 5.1). For example, the peak in July for C3 was recorded before the
increase in water flux in late July indicating the increase in C3 was likely related to in-lake DOM
production and processing, such as the large diatom bloom that occurred in July. Similarly, the
declines in C1 and C5 between May and July might indicate biodegradation or photolysis was
occurring at those times (Wetzel et al. 1995; Sharpless and Blough 2014). Variation in C6 was
similar to the temporal variation in chl-$a$, which aligns with our interpretation of C6 representing
protein-like, algal DOM (Murphy et al. 2011). The ranges in $F_{\text{max}}$ values between components
also signals the relative abundance of each component; C1-C3 were much higher than C4-C6
indicating these humic compounds made up the majority of the DOM. The relative proportions
of various DOM components as well as their processing and fate in aquatic ecosystems has
tremendous implications for the global carbon cycle as lakes are hotspots for both the outgassing
and sequestration of carbon (Cole et al. 2007; Tranvik et al. 2009; Raymond et al. 2013). In fact,
the total carbon dioxide emission from inland waters is estimated to be of similar magnitude to
terrestrial net ecosystem productivity globally, and the sequestration of carbon in lake sediments
is greater than that of the ocean (Downing et al. 2006, 2008). Further quantification of the
sequestration and removal of organic matter in reservoirs, especially in relation to specific DOM
compounds, will help us understand how the global rise in reservoir construction contributes to greenhouse gas emissions and disrupts the global carbon cycle (Jones et al. 2003; Garnier and Billen 2007; Wang et al. 2007; Maavara et al. 2017, 2020).

**Algal community composition and the protein fraction of DOM**

There was a positive relationship between algal biomass and both peaks B and T, which was expected as the production of protein-like DOM fractions are associated with algal growth. Similarly, there was a corresponding increase in chl-\(a\) with BIX, which was expected as both are indicators of autochthonous organic matter production. Past studies have found a strong relationship between protein-like fluorescence and biodegradable organic matter (Stedmon and Markager 2005; Hounshell et al. 2021); thus, our results suggest that concomitant increases in chl-\(a\), peak B, peak T, and BIX relate to increases in the proportion of organic matter produced by phytoplankton and is available for microbial degradation. Interestingly, there was no relationship between either peaks B or T and cyanoHAB biomass despite other studies reporting protein-like components associated with cyanoHAB DOM (Bittar et al. 2015b; Patriarca et al. 2021). One possible explanation for this is the relatively rapid UV-degradation of cyanoHAB DOM which, in laboratory experiments, degraded by up to 91% in the first 0.5 days of UV exposure (Bittar et al. 2015a). The photodegradation of phytoplankton DOM can result in absolute fluorescence loss as well as transformations to less bioavailable, humic substances (Bittar et al. 2015b). Experiments comparing the photodegradation rates of DOM derived from other phytoplankton taxa as well as more frequent sampling could help us understand the effect of phytoplankton community composition on the protein fraction of DOM.
Correlation between nutrients, algae, and DOM components

In Chapter 4, we found DSi to be the strongest control on diatom abundance; we also know diatoms have a higher protein content relative to other algal taxa (Brett et al. 2000). Therefore, we expected DSi to influence the protein fraction of DOM, mediated through diatom productivity. Interestingly, diatoms were strongly correlated with C3; however, C1-C5 were identified as humic-like DOM. This result was contrary to our expectations as direct studies of algal-derived DOM found these substances to be primarily made up of aromatic amino acids (Nguyen et al. 2005; Henderson et al. 2008). It is possible that the sorption of N and P to terrestrial DOM compounds made these nutrients more bioavailable for phytoplankton growth (Stedmon et al 2006; Moran et al. 2000; Mulholland 2003); thereby linking increased humic DOM with increased phytoplankton production. Another possible explanation for the significant relationship between humic-like organic matter and algal biovolume is the high biodegradation rate of protein-like material which can rapidly transform to high molecular weight aromatic material, such as fulvic and humic acids (Hansen et al. 2016; Massicotte et al. 2017).

Furthermore, our analysis of fluorescent DOM includes only a subset of DOM compounds; therefore, including other spectrometric analyses could identify additional DOM components linked with phytoplankton community composition. Additional data on the removal rates of algal-derived DOM by heterotrophic bacteria could help reveal relationships between primary productivity and the abundance of humic-like DOM (Attermeyer et al. 2014; Bittar et al. 2015b). Ultimately, our study showed that phytoplankton biovolume influenced DOM composition; therefore, changes in the phytoplankton community can directly affect the quantity and quality of DOM and changes in nutrient availability can indirectly affect DOM by causing shifts in the dominant phytoplankton groups.
The DOM component indicating tryptophan-like substances (C6) was strongly related to humic-like components C1, C2, C4, and C5, suggesting processes, such as photodegradation, might affect the abundance of both protein-like and humic-like components of DOM in similar ways. We also observed significant relationships between peaks B and T (which represent the protein-like fraction of DOM) and specific algal taxa biovolume, suggesting simple peak picking complimented our PARAFAC component modeling. Our monthly sampling frequency is likely the largest limitation of our study, as more samples during phytoplankton growth could have captured the production of protein-like DOM rather than the byproducts of their biodegradation (Nieto-Cid et al. 2006; Guillemette and del Giorgio 2011). Furthermore, more frequent sampling might capture the relationship between diatom productivity and the abundance of protein-like DOM components, thus linking DSi concentrations with the quality and composition of DOM.

**Implications with climate change and eutrophication**

Lake Monroe is the source of drinking water for over 140,000 people in south-central Indiana, therefore, the concentration and composition of DOM have serious implications for the formation of disinfection by-products (DBPs) during the water treatment process. Managing reactive DOM compounds that serve as precursor material for DBP is the most efficient and cost-effective way to mitigate DBP exposure (Richardson et al. 2007); however, increasing levels of DOM in freshwaters with climate change may require some water treatment plants to modify their treatment methods (Worrall et al. 2003; Evans et al. 2005; Andersson et al. 2020). While the DBP levels in Lake Monroe currently meet federal and state standards, the increasing trend in DOC concentrations and DOM components over our sample period indicate the risk of DBP formation may be higher in the late summer and fall (City of Bloomington Utilities 2021). Characterization of the DOM components involved in the formation of DBPs is critical as
climate change continues to drive increases in temperature and precipitation, thereby increasing the flux of terrestrial organic matter as well as the production of aquatic organic matter (Cole et al. 2007; Tranvik et al. 2009; Williamson et al. 2009; Gonsior et al. 2019).

Climate change and eutrophication are contributing to increased temperature, dissolved carbon dioxide concentrations, and nutrient inputs to aquatic systems (Seitzinger et al. 2010; Hasler et al. 2016; Asmala et al. 2018). These factors lead to increased phytoplankton productivity which alters trophic dynamics and carbon cycling (Schindler 2006; Lee et al. 2019; Wurtsbaugh et al. 2019). With ongoing environmental change, we can expect to see concurrent shifts in the phytoplankton community, DOM composition, and water quality that negatively affect the structure and function of aquatic ecosystems (Thornton 2014; Cavicchioli et al. 2019). Further characterization of DOM compositional changes as a result of nutrient enrichment and the formation of cyanoHABs is essential to our understanding of aquatic food webs and the global carbon cycle (Paerl and Otten 2013).

Acknowledgements
This research was funded in part by grants from the Indiana Department of Environmental Management Section 319 Nonpoint Source Management Program, the Indiana University Office of Sustainability, the Indiana University Integrated Program in the Environment, and the Consortium of Universities for the Advancement of Hydrologic Sciences, Inc. I would also like to thank Megan Gokey, as her assistance with sample analysis and organization was instrumental in the completion of this research chapter.
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Supplemental Information

Figure S5.1 Molar ratios of Si:N, Si:P, and N:P plotted against the loadings of the first principal component (PC1). The color of the point corresponds to the sampling month. Relationships between each variable and PC1 were modeled using simple linear regression; however, no model was significant (p>0.05)
Human modifications to the landscape, such as with agriculture and damming, have significantly altered the inputs and biogeochemical cycling of nutrients in aquatic systems. Since the Industrial Revolution, over 38% of Earth’s land surface has been converted to agriculture making it the largest use of land globally (Ellis and Ramankutty 2008; Foley et al. 2011; Ramankutty et al. 2018). Similarly, to support growing human civilizations, at least 2.8 million dams have been constructed, leaving only about 37% of rivers longer than 1,000 kilometers free-flowing from their headwaters to their mouths (Lehner et al. 2011; Grill et al. 2019). Both agriculture and damming impact nutrient delivery to receiving waters as well as the biogeochemical processes that affect the structure and function of freshwaters. For example, agriculture increases the inputs of nitrogen (N) and phosphorus (P) to surface waters due to fertilizer and manure applications while decreasing the input of silicon (Si) due to changes in vegetation and crop harvest (Clymans et al. 2011; Struyf and Conley 2012; Vandevenne et al. 2012; Carey and Fulweiler 2016). Reservoirs impede the flow of water along the land-ocean continuum, increasing residence time and enhancing nutrient transformations; however, the biogeochemical cycles of N, P, and Si are altered in different ways, thereby altering the stoichiometry of nutrient export to receiving waters (Friedl and Wüest 2002; Van Cappellen and Maavara 2016; Maavara et al. 2020). Ultimately, anthropogenic landscape modifications create a stoichiometric imbalance between N, P, and Si which influences the abundance and composition of phytoplankton communities leading to water quality impairments, changes in trophic dynamics, and alterations to the global carbon cycle (Wurtsbaugh et al. 2019).
To determine the effects of changing vegetative cover and hydrology on Si concentrations and stoichiometry, I monitored tile drains and stream sites in a small, agricultural watershed in northwestern Indiana biweekly for three water years. The main goal of the study was to understand the effect of increased vegetation cover during the fallow period on Si loads and nutrient stoichiometry. I found that while the use of winter cover crops increased the ratio of N:Si to conditions more favorable for diatom growth at the field scale, high flow events exacerbated the stoichiometric imbalance between N, P, and Si at the watershed scale. With climate change, precipitation and high flow events are predicted to increase in the Midwestern United States, thus nutrient loads from agricultural watersheds will continue to contribute to eutrophication and harmful algal bloom formation in receiving waters (Sinha et al. 2017; Bowling et al. 2020; Cherkauer et al. 2021). Therefore, identifying and promoting land management practices that lead to more balanced nutrient export from agricultural watersheds could enhanced efforts to mitigate water quality impairments.

In my next chapter, I conducted nutrient enrichment experiments to assess the nature of nutrient limitation in an agricultural stream. I used nutrient diffusing substrata to increase the availability of N, P, and Si to periphyton and measured the response in chlorophyll-\(a\) and algal community composition. Given the ambient nutrient concentrations in the stream (see Chapter 2), I expected the abundance of benthic diatoms to increase on treatments amended with Si; however, there was no response in either chlorophyll-\(a\) or algal community composition. The results from this study highlight the flexibility in the nutrient demands of freshwater diatoms and suggest other factors, such as substrate type, light availability, and temperature, largely control the seasonal succession and abundance of benthic algae (Schiller et al. 2007; Johnson et al. 2009; Sanderson et al. 2009).
In Chapter 4, I sought to further understand the relationship between Si availability and diatom abundance by constructing a Si budget for Lake Monroe, the largest reservoir in Indiana. Reservoirs have been shown to retain Si as the transformation from a river to a lake ecosystem increases water residence time, thereby promoting increased phytoplankton growth and sedimentation (Humborg et al. 2006; Harrison et al. 2012; Maavara et al. 2014). I measured inputs and outputs of Si over one calendar year to calculate the amount of Si retained within Lake Monroe and determined the primary mechanism of retention was uptake by diatoms. Diatoms are the most significant user of Si in aquatic systems, and I observed clear patterns of Si utilization with increases in diatom abundance (Hildebrand 2008; Thamatrakoln and Kustka 2009). My results support the notion presented by Turner and Rabalais (1991) that retention of Si in reservoir systems within the Mississippi River basin contributes to the imbalance between Si, N, and P at the river mouth and contributes to the formation of the hypoxic zone in the Gulf of Mexico.

Based on the relationship between Si concentrations and diatom abundance, I hypothesized nutrient concentrations would influence the concentration and composition of dissolved organic matter (DOM). The source and composition of DOM is critical to the structure and function of aquatic systems (Findlay and Sinsabaugh 2003). For example, terrestrially derived DOM generally is less bioavailable to microorganisms than aquatic DOM; therefore, the relative abundance of these compounds affects trophic dynamics and carbon cycling (Guillemette and del Giorgio 2011; Bai et al. 2017). My analysis of the optical properties of DOM showed that algal biomass drove the increase in the protein-fraction of DOM, as expected; however, I did not see strong relationships between nutrient concentrations and DOM composition despite the established connection between nutrients and algae as well as between
algae and DOM (del Giorgio and Davis 2003; Thornton 2014). The complexity of DOM compounds and the biogeochemical processes that affect them contribute to the confounding nature of the coupled relationship between nutrient availability, algal community abundance, and DOM composition.

Recommendations for future work include the analysis of particulate Si in order to better quantify the biogeochemical cycling and availability of total Si in freshwater systems. Past studies have shown the potential of diatomaceous Si and plant phytoliths to be recycled into DSi at faster rates relative to mineral weathering, thus serving as an important source of Si in aquatic systems (Cornelis et al. 2011; Haynes 2014; Tubana et al. 2016). To better understand the role of Si in the formation of harmful algal blooms, more frequent sampling of nutrient concentrations and the phytoplankton community would allow for a better characterization of Si utilization and, over long timescales, Si depletion in lakes and reservoirs (Schelske et al. 1986; Conley et al. 1993). My study of the coupled relationship between nutrients, algae, and DOM composition could be strengthened by an exploration of the taxa-level photodegradation rates of algogenic organic matter to better understand how algal abundance affects the lability of DOM (Bittar et al. 2015; Patriarca et al. 2021). Furthermore, finer temporal resolution for field sampling might have captured coupled relationships between nutrient concentrations, phytoplankton community, and DOM composition that we did not observe at the monthly scale.

My dissertation research contributes to the growing body of literature on the coupled biogeochemical cycles of C, N, P, and Si in freshwaters and the subsequent effect on aquatic ecology. Globally, billions of dollars are directed annually to mitigating N and P pollution, while little attention has been paid to the role of Si in the determination of algal community composition and, more broadly, ecosystem function. Each of the preceding chapters emphasizes
the importance of Si in streams and lakes and lays the groundwork for future research on the role of Si in nutrient biogeochemistry, phytoplankton dynamics, and the global carbon cycle. As human land use practices and climate change continue to degrade aquatic ecosystems, it is imperative to explore management strategies that focus on the quality and integrity of freshwaters.

Climate change is already altering the frequency and intensity of precipitation across the agricultural Midwest; therefore, managing for Si, N, and P in concert is especially important because changing hydrology will alter the export of these nutrients in different ways. My study supports the use of winter cover crops as one strategy for increasing Si:N in agricultural streams, thereby improving conditions for freshwater diatoms and reducing the potential for non-siliceous algal growth in downstream waters. Additionally, controlling N and P inputs to reservoirs will aid in maintaining stoichiometric ratios that deter harmful algal blooms, particularly during periods when DSi concentrations are low and diatoms are potentially Si-limited. The research presented in my dissertation highlights the importance of considering Si in land management decisions that prioritize ecosystem function, carbon cycling, and water quality.
References


Wurtsbaugh WA, Paerl HW, Dodds WK (2019) Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. WIREs Water 6:. https://doi.org/10.1002/wat2.1373
Appendix A

Royer Lab Aqualog Protocol for FDOM Analysis

Section 1: Aqualog and samples warm-up:

1. Quartz cuvettes (no frosted sides) should be rinsed (inside and outside) 20-30 times with milli-Q water
2. Always handle the cuvettes while wearing gloves or with kimwipes. Change your gloves frequently throughout the analysis to avoid contaminating the cuvette surface
3. Turn on the Aqualog thirty minutes before running samples, using the switch located on the left side, toward the back
4. Remove samples from the refrigerator and allow them to warm to room temperature
5. Open the Aqualog software located on the desktop of the computer

Section 2: Raman Scattering Area Unit - Everyday Tests to make sure lamp is operating correctly

1. Insert the Starna Standard Raman water sample
   a. The Raman water is located in the cardboard box behind the Aqualog.
   b. Remove the cuvette sample holder inside the Aqualog and replace with the Raman water sample in attached holder so that the edge of the cuvette holder is in the back left hand corner.
2. Close sample chamber lid.
3. Click on the Raman Scattering Area Unit button (blue RU button) located on the menu bar.
4. In the experiment setup window, adjust the following:
   NOTE: It is very important that the Increment and CCD gain settings match the settings that will be used for your samples for correct normalization. Integration time can be adjusted later.
   a. Integration (s): 10 (from 30s so it doesn’t overload the detectors)
   b. Increment (nm): 4 pixels
   c. CCD gain
      i. Low – for really concentrated, high FDOM samples
      ii. Medium – for most samples
      iii. High – for really dilute, oceanic, low FDOM samples.
   d. Data Identifier: RS\textit{date} (e.g., RS20Apr22)
5. Click Run.
6. If this is the first test of the day, click Browse and select a location to save the Project data in when the Project Name window appears.
   a. Start a new folder for the day’s sample analyses within your project folder.
7. Should look like this:

8. When the test completes, record the sample conditions (integration time, increment, and CCD gain) along with peak wavelength and peak area for 1 s integration time:
   a. The peak wavelength should be 397 ± 1 nm.
      i. This lamp usually has a peak wavelength of 397.5 nm (see screen shot above).
         ii. If peak wavelength is out of this range, allow for lamp to warm up for ~10 more minutes. If spectrum is not peak shaped, lamp may be dead.
   b. Click on the RSU Adjust tab and record the RSU Adjust Area (at the bottom of the spectrum) for 1 s integration time (it should automatically be set for 1 s, no need to change anything).

Section 3: Raman (Normalization) Area for Sample Blank
**Skip this step if using a blank solution other than ultra-pure water**
Tests to make sure cuvette and blank are clean.
1. Rerun the Raman Scattering Area test using a cuvette filled with blank solution. **Make sure to use a quartz cuvette with no frosted sides, as glass and acrylic cuvettes can impair UV fluorescence and cause filtering effects.

2. Insert the cuvette containing the blank solution into the sample compartment.
   a. Make sure to insert the cuvette the same way each time (e.g., the “Fisherbrand” logo on the cuvette always facing left).

3. Close the lid of the sample chamber.

4. Click on the Raman Scattering Area Unit button located on the menu bar.

5. In the experiment setup window, adjust the following:
   a. Integration (s): 10
   b. Increment (nm): 4 pixels (or same as for Raman Scattering)
   c. CCD gain: Medium (or same as for Raman Scattering)
   d. Data Identifier: RSB\text{date} (e.g., RSB20Apr22)
   e. **NOTE**: The Increment and CCD gain settings need to match the settings that will be used for your samples. Integration time can be adjusted later.

6. Click Run

7. When the test completes, record values or export the datasheet
   a. To export: File>Export>ASCII>Select project folder>Save as type .csv
   b. The peak wavelength should be 397 ± 1 nm.
   c. Click on the RSU Adjust tab and record the RSU Adjust Area for 1 s integration time.

8. Compare the RSU Adjust area for your blank with the Raman Scattering Adjust area; these two values should be close to one another. If the two values differ by more than ~1%, clean the cuvette and fill with fresh ultrapure water and rerun test if using this value to normalize your data.

**Section 4: Running a Sample Blank**

1. Collect a sample blank
   **NOTE**: All blanks need to be collected prior to running samples. Run blanks for all integration times that will likely be needed for the day prior to starting sample analyses.

2. Press the “Aqualog Main Experiment Menu” button (blue button with H2O) on the menu bar in the Aqualog’s main window or select Collect > Aqualog Main Experiment Menu.

3. Select 3D

4. Choose Emission CCD + Absorbance in the Aqualog Experiment Type window and click Next.

5. In the Aqualog Experiment Setup widow, click Load to select a saved method.

6. Check to make sure the Wavelength Settings are appropriate for the desired method and match settings to be used for the samples:
   a. Integration time: **1.5 s** works for most samples (covers 2 orders of magnitude in fluorescence intensity).
      i. For more concentrated samples reduce integration time to 0.1-0.5 s.
      ii. For more dilute samples increase integration time to 2-5 s or longer, 8 s for oceanic samples.
   b. Excitation Wavelength: High = 800 nm, Low 230 nm, increment 5 nm
   c. Emission Coverage: This cannot be changed.
d. Increment: Needs to match the settings used for Ramen Scattering
e. CCD gain: Needs to match the settings used for Ramen Scattering
f. Data Identifier: SBdate/time (e.g., SB20Apr22x02s for sample blank with integration time of 0.2s. Decimal and special characters cannot be used when naming files.
g. Select Blank Only.
   i. Choose the location to save the blank file by clicking the ‘…’. This is typically the folder for the day in your project and personal folder. The blank file should be named the same as the data identifier and end in ‘.blank’.

7. Ensure cuvette with blank sample is in the sample compartment.
8. Click run.
9. The Intermediate Display window will appear and show an absorbance spectrum followed by the EEM as it is collected.
10. When scan is complete, the emission spectrum window appears. Make sure that your blank looks clean and free of unexpected peaks. Clean cuvette and rerun blank if needed.
11. A clean blank should be free of extra, stray peaks other than the Raman and Rayleigh scattering peaks:
Very short integration times (e.g., 0.5 s) may be slightly nosier at the lower excitation wavelengths:

Example EEM of pure water with the Raman and Rayleigh scattering peaks labeled.
http://dellwindowsreinstallationguide.com/steadystatefluorescencespectroscopybasics/
Section 5: Performing an Integration Test and collecting new blank

*Used to determine the optimum integration time for your sample*

1. Press the “Aqualog Main Experiment Menu” button (blue button with H2O) on the menu bar in the Aqualog’s main window or select Collect > Aqualog Main Experiment Menu.
2. Select 3D
3. Choose Emission CCD + Absorbance in the Aqualog Experiment Type window and click Next.
4. Save the file in your project folder (e.g., “IntTest20Apr22.xml”)
5. Keep wavelength settings the same as your blank file, but change the integration time
   a. Integration time: 0.1s
   b. Excitation wavelength: High=800nm, Low=230nm, Increment=5nm
   c. Emission coverage: This cannot be changed
   d. Increment: 4 pixels (or match settings used for Ramen Scattering)
   e. CCD gain: Medium (or match settings used for Ramen Scattering)
   f. Data identifier: IntTest20Apr22
6. In the Blank/Sample set up, select “Sample and Blank” and “Blank from file”
   a. Click “…” and select the blank file you ran in Section 4
7. Fill the cuvette with one of your samples
8. Click “Run”
9. Check the results of the test:
   a. On the “Abs Spectra Graphs” tab, ensure absorbance at 254 nm is <0.6
      i. If abs>0.6, you will need to dilute your sample and run another test

12. To run additional sample blanks, repeat steps 5-12 for each blank.
ii. If dilution is necessary, note this dilution factor as you will need to apply it to the EEM correction
b. On the “Sample-Blank” tab, find the maximum value at excitation wavelength 250nm, determine the integration time as follows:

<table>
<thead>
<tr>
<th>Signal Intensity (counts per second)</th>
<th>Integration time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 80</td>
<td>5</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>4</td>
</tr>
<tr>
<td>100 to 999</td>
<td>2 to 3</td>
</tr>
<tr>
<td>1000 to 5000</td>
<td>1.5 to 2</td>
</tr>
<tr>
<td>5001 to 50,000</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 50,000</td>
<td>0.1</td>
</tr>
</tbody>
</table>

c. Determine the emission increment for your samples on the “Abs Spectrum Sample” tab
i. If absorbance at 254 nm < 0.1, then use the larger emission increment of 3.28 nm
ii. If absorbance at 254 nm > 0.1, then use the smaller emission increment of 1.64 nm
iii. In general, if a sample needs > 4 second integration time, use the larger emission increment.

10. Recollect a sample blank following the steps in Section 4, but with corrected integration time and emission increment

Section 6: Single or Explorative Experiment (no post-processing will be performed on samples ran this way).

Running a Sample:
1. Insert the water sample into the sample compartment.
2. Make sure the sample is the correct position (marks on the cuvette toward the left).
3. Press the Experiment Menu button (blue button with H₂O) on the menu bar in the Aqualog’s main window or select Collect > Aqualog Main Experiment Menu.
4. Select 3D to run Excitation Emission Matrix plus Absorbance
5. Choose Emission CCD + Absorbance in the Aqualog Experiment Type window and click Next.
6. In the Aqualog Experiment Setup widow, click Load to select a save method.
7. Check to make sure the Wavelength Settings are appropriate for the desired method and match settings to be used for the samples:
   a. Integration time: Use time indicated by test results in Section 5
      i. 1.5 s works for most samples (covers 2 orders of magnitude in fluorescence intensity).
   b. Excitation Wavelength: High = 800 nm, Low 230 nm, increment 5 nm
   c. Emission Coverage: This cannot be changed.
   d. Increment: Use increment indicated by test results from Section 5
i. Most samples work with 2.1 nm (4 pixel) increment
  e. CCD gain: Typically medium, unless your samples are really dilute (high) or concentrated (low)
  f. Data Identifier: *datetime* (e.g., s20Apr22x02s for sample with integration time of 0.2s). Decimal and special characters cannot be used when naming files.
8. In the Blank/Sample Set Up box, select Sample and Blank.
9. Select Blank from File, and select the blank file generated with the corrected integration time (see section 5).
10. Ensure cuvette with sample is in the sample compartment.
11. Click run, sample will run immediately.
12. For each additional sample, click the “Previous Experiment Setup” button (/button) to load the same settings for the previous sample
   a. Rename the sample and be sure to save it to a new location within the project folder
   b. Repeat for each sample in the set with the same blank file

**Section 7: Save Your Results and Shutdown Aqualog**
1. Save everything to My Documents > Jobin Yvon > Data > YourProjectFolder (make a new one if it doesn’t exist)
2. Make sure your data has been properly backed-up on a flash drive and transferred to the lab-drive (the Aqualog computer is not connected to the network, you need to manually transfer the files)
3. When finished close the Aqualog program and click Yes when asked to save changes.
4. Shut down computer.
5. Shut off Aqualog using switch on the back left-hand side.
6. **NOTE:** Do not leave any samples in the Aqualog at the end of the analysis. Samples that evaporate in the chamber will damage the instrument.
Appendix B

Compiled R scripts for excitation emission matrix (EEM) correction, peak picking and index calculation, and Parallel Factor Analysis (PARAFAC).

Formatting Aqualog .dat files

# for use with simple EEM analysis in staRdom package # Can only convert one folder/ Aqualog run at a time
options(scipen = 999)

convert_aqualog <- function()
{
  library(tcltk)
  main_folder<"/Users/prime/gdrive/uf_aqualog/uf_aqualog/20190408/20190408_Group1"
  library(utils)
  destination_folder<-choose.dir()
  x <- "yes"
  while (x == "yes") {
    main_folder <- choose.dir()
    cat("\nchoose name for project")
    proj_name <- readLines(n = 1)
    abs_files <- list.files(main_folder,pattern = "*ABS.dat")

    dir.create(paste(destination_folder,"/abs/", proj_name,sep = ")
    dir.create(paste(destination_folder,"/eem/", proj_name,sep = "")
    abs_cor_folder <- paste(destination_folder,"/abs/", proj_name, sep = "")
    eem_cor_folder <- paste(destination_folder,"/eem/", proj_name, sep = "")

    for (i in 1:length(abs_files)) {
      abs_temp <- read.table(paste(main_folder,"/", abs_files[i], sep = ""), sep = "\t")
      abs_temp <- subset(abs_temp,!is.na(V2))
      write.table(abs_temp,paste(abs_cor_folder,"/",gsub("ABS.dat",".csv",abs_files[i],fixed = TRUE), sep = ""), row.names = FALSE, col.names = FALSE, sep = "")
    }
  }
}

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sem_files <- list.files(main_folder, pattern = "*SEM.dat")
for (i in 1:length(sem_files)) {
  abs_temp <- read.table(paste(main_folder,"/", sem_files[i], sep = ""),
              sep = "\t")
  abs_col <- ncol(abs_temp) abs_temp2 <- abs_temp[,2:abs_col]
  abs_temp2 <- abs_temp2[,order(abs_temp2[1,])] abs_temp3 <-
  cbind.data.frame(abs_temp[,1], abs_temp2)
  write.table(abs_temp3,paste(eem_cor_folder,"/",gsub("SEM.dat",".csv",
      sem_files[i], fixed = TRUE), sep = ""), row.names = FALSE, col.names =
      FALSE, sep = ",")
}

bem_files <- list.files(main_folder, pattern = "*BEM.dat")
bem_temp <- read.table(paste(main_folder,"/", bem_files[1],sep = ""),
              sep = "\t")
bem_col <- ncol(bem_temp) bem_temp2 <- bem_temp[,2:bem_col]
  bem_temp2 <- bem_temp2[,order(bem_temp2[1,])] bem_temp3 <-
  cbind.data.frame(bem_temp[,1], bem_temp2)
  write.table(bem_temp3,paste(eem_cor_folder,"/",gsub("BEM.dat","_blank.
      csv", bem_files[i], fixed = TRUE), sep = ""), row.names = FALSE, col.names =
      FALSE, sep = ",")

#print("\nCorrect Another Folder?") x <- readLines(n = 1)
print(x)
}

convert_aqualog()
Correcting EEMs for analysis

# load packages
library(dplyr)
library(tidyr)
library(staRdom)

# Set the directory with your sample files.
sample_dir = "W:/Lab/Lab-troyer/Lienne/Lake Monroe/Lake Monroe DOM/EEM output/ EEMsDat"

# path of adsorbance data as directory or single file, sub folders are not read:
absorbance_dir = "W:/Lab/Lab-troyer/Lienne/Lake Monroe/Lake Monroe DOM/EEM output/AbsDat"

# Path length of absorbance measurement in cm that was used in absorbance measurement.
absorbance_path = 1

### Spectral correction of EEMs ###
# Some instruments, but not all need a spectral correction to compensate for specific deviations in the measurements. A vector for emission and excitation is used each. EEMs are cut to match the wavelength range of the vectors if used. Please provide paths to csv tables containing wavelengths in the first column and correction values in the second.
# Emission correction vector
Emcor = "W:/Lab/Lab-troyer/Lienne/Lake Monroe/Lake Monroe DOM/MCorrect_RoyerAqualog.csv"

# Excitation correction vector
Excor = "W:/Lab/Lab-troyer/Lienne/Lake Monroe/Lake Monroe DOM/XCorrect_RoyerAqualog.csv"

spectral_cor = TRUE

### Normalizing absorbance data to baseline ###
# Absorbance data can be corrected by subtracting a baseline value from each sample.
# In high wavelength ranges (default 680-700 nm), the absorbance is assumed to be 0.

# The average value of that range (or any other range) is subtracted from the whole spectrum.

# abs_norm can be set TRUE to use the default range, you can specify the desired range by a vector of length 2 and you can set it FALSE to skip this correction.

abs_norm = TRUE

#### Cut data to certain range ####

# Set a vector with range of wavelengths to be plotted and saved. Peak picking is done before range reduction.

# Emission wavelength:
em_range = c(300, 500) # e.g. c(300,500), c(0,Inf) to use everything

# Excitation wavelength:
ex_range = c(300, 500) # e.g. c(300,500), c(0,Inf) to use everything

# Cut all samples to fit largest range available in all samples
cut_range_to_smallest = TRUE

#### Blank correction ####

# A blank sample is subtracted from each sample.

blank_correction = TRUE

#### Inner filter effect correction ####

# Inner filter effects are corrected. Absorbance data is needed. File or column designations of the absorbance data have to resemble file names of the EEM data.

ife_correction = TRUE

#### Remove scattering and interpolate missing data ####

# Scattering is removed from the EEM spectra.

remove_scatter <- c(TRUE, TRUE, TRUE, TRUE)

# logical values, ordered by raman1, raman2, rayleigh1, rayleigh2
# Set the width of removed scatter slot (usually 10 to 20).

# If you can still see traces of scattering after interpolation, this value # should be increased. You can specify a vector containing # separate widths # for each scatter c(15,16,16,14), ordered by # raman1, raman2, rayleigh1, rayleigh2.

remove_scatter_width = c(15, 20, 15, 18)

# state whether removed scattering should be interpolated

interpolation <- TRUE

#### Raman normalization ####

# State whether a Raman normalisation should be performed

# Either "blank" if a blank is present in each (sub)folder of the EEM data. # Blank samples have to be in the same (sub)folder as the EEM samples. So # different blanks are used for different subsets. The file names of the # blanks have to contain nano, miliq, milliq, mq or blank (cases are ignored). # Other samples must not contain these words in their names respectively!

# Normalization is then calculated with this blank, the raman area as a # number or "meta" if the raman areas should be taken from the meta data table. raman_normalisation = "blank"

#### Smooth data for peak picking ####

# Moving window size in nm for smoothing data along excitation wavelengths. # Data must be interpolated if you want to use smoothing.

# This is used for peak picking but not saved.

smooth = 4

#### Read EEM data ####

# recursive = TRUE means that subdirectories are included

eem_list = eem_read(sample_dir, recursive = TRUE, import_function = fluorometer)

#### Read absorbance data ####

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if(absorbance_dir != FALSE){
    abs_data <- absorbance_read(absorbance_dir, verbose = FALSE)
    if(abs_norm != FALSE){
        if(abs_norm == TRUE) abs_norm <- c(680, 700) abs_data <-
        abs_blcor(abs_data, abs_norm)
    }
}

#### Data check ####
#the data is checked for several often occurring problems. Most of
#them are related to how you organized your data.
#if the script stops here please have a look at the error messages and
#correct your data.
eem_checkdata(eem_list, abs_data, error = FALSE)
#absorbance wavelength range is smaller than emission wavelength range
eem_list <- eem_range(eem_list, ex = c(250, 450), em = c(250, 600))

#### Data correction ####

#### Spectral correction ####
if(spectral_cor){
    Excor <- data.table::fread(Excor) Emcor <-
    data.table::fread(Emcor)
    # adjust range of EEMs to cover correction vectors
    eem_list <- eem_range(eem_list, ex = range(Excor[,1]), em =
    range(Emcor[,1]))
    eem_list <- eem_spectral_cor(eem_list, Excor, Emcor)
}

#### Blank subtraction ####
if(blank_correction) eem_list <- eem_list %>% eem_remove_blank()

#### Inner filter effects correction ####
if(absorbance_dir != FALSE){
if(absorbance_path == "meta") cuvl <- meta[col_cuv_len] else cuvl <- absorbance_path

if(ife_correction) eem_list <- eem_list %>%
  eem_ife_correction(abs_data, cuvl)

### Raman normalisation ###
if(raman_normalisation != FALSE){
  if(raman_normalisation == "meta") ram_data <- meta[col_raman_area] else ram_data <- raman_normalisation
  eem_list <- eem_list %>% eem_raman_normalisation2(ram_data)
}

### Remove blank samples from sample list ###
# Blanks used for correction are not needed anymore from this step
eem_list <- eem_list %>%
  eem_extract(c("nano", "miliq", "milliq", "mq", "blank"), ignore_case = TRUE)

abs_data <- abs_data %>%
  select(-matches("nano|miliq|milliq|mq|blank", ignore.case=TRUE))

### Remove scattering ###
# Raman and Rayleigh scattering
eem_list <- eem_rem_scat(eem_list, remove_scatter, remove_scatter_width)

### Interpolate scattering ###
# Removing scattering left NAs. They will be interpolated.
if(interpolation) eem_list <- eem_list %>% eem_interp(type = TRUE, extend = FALSE)

### Smooth data for peak picking only ###
#~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~#
if(sMOOTH != FALSE & interpolation) eem4peaks <- eem_list %>%
eem_smooth(n=smooth) else eem4peaks <- eem_list

Peak picking and index calculation

#### Peak picking ####
indices_peaks <- eem4peaks %>% eem_biological_index() %>%
full_join(eem4peaks %>% eem_coble_peaks(), by="sample") %>%
full_join(eem4peaks %>% eem_fluorescence_index(), by="sample") %>%
full_join(eem4peaks %>% eem_humification_index(scale=TRUE),
by="sample")

#### Slope parameter calculation ####
if(absorbance_dir != FALSE){
indices_peaks <- indices_peaks %>%
full_join(abs_parms(abs_data,cuvl,p=TRUE), by="sample")
}

#### Plots of EEM spectra ####
if(overview | output_overview_png) {
    ov_plots <- eem_list %>%
    eem_overview_plot(spp = overview_number, contour = contour)
}

if(overview){
    lapply(ov_plots,print) %>% invisible()
}

PARAFAC modeling

#The following steps outlined in PARAFAC vignette: <https://cran.r-
project.org/
web/packages/staRdom/vignettes/PARAFAC_analysis_of_EEM.html#data-
preparation- and-correction>

library(staRdom)
library(dplyr)
library(tidyr)
library(ggplot2)

# detect cores for parallel processing
cores = detectCores(logical = FALSE)

# import EEM data from correction file
# these EEMs have been spectrally corrected and interpolated!
# plot EEMs to make sure scatter is removed
eem_overview_plot(eem_list_done, spp=6, contour = TRUE)

# explore EEMs to determine number of components that optimizes PARAFAC model
# minimum and maximum of numbers of components
dim_min = 3
dim_max = 9
nstart = 50 # number of similar models from which best is chosen
maxit = 10000 # maximum number of iterations in PARAFAC analysis
ctol = 10^-9 # tolerance in PARAFAC analysis

# calculating PARAFAC models, one for each number of components
pfl = eem_parafac(eem_list_done, comps=seq(dim_min,dim_max),
                  normalise=FALSE, const=c("uncons", "uncons", "uncons"),
                  maxit=maxit, nstart=nstart, ctol=ctol, cores=cores)

# same model but using non-negative constraints; common assumption
# because fluorescence cannot be negative
pfln = eem_parafac(eem_list_done, comps=seq(dim_min,dim_max),
                  normalise=FALSE, const=c("nonneg", "nonneg", "nonneg"),
                  maxit=10000, nstart=nstart, ctol=ctol, cores=cores) # with only 5000
iterations, 11/25 models converged; increased to 10000 iterations to
improve 7 components model
# rescale B and C modes to a maximum fluorescence of 1 for each component
```
pf1 = lapply(pf1, eempf_rescaleBC, newscale="Fmax")
pf1n = lapply(pf1n, eempf_rescaleBC, newscale="Fmax")
```

# plot the created model components
```
eempf_compare(pf1n, contour=TRUE)
```

# model 5 (7 component model)

# check correlation between components
```
eempf_cortable(pf1n[[5]], normalisation = FALSE)
eempf_corplot(pf1n[[5]], progress = FALSE, normalisation = FALSE)
```

# components should not be correlated, but is typical for DOM in natural waters

# normalize EEMs to account for this:
```
const = c("nonneg", "nonneg", "nonneg"),
maxit = maxit, nstart = nstart, ctol = ctol, cores = cores)
# rescale B and C modes
```

```
pf2n = lapply(pf2n, eempf_rescaleBC, newscale = "Fmax")
```

# explore normalized plots
```
eempf_compare(pf2n, contour = TRUE)
```

# calculate leverage
```
cpl = eempf_leverage(pf2n[[5]])
```

# plot leverage (nice plot)
```
eempf_leverage_plot(cpl, qlabel=0.1)
```

# indicates outlier samples, add them to "exclude" df
```
exclude = eempf_leverage_ident(cpl, qlabel=0.1)
```

# exclude outliers if necessary. if so, restart PARAFAC analysis
```
eem_list_ex = eem_exclude(eem_list_done, exclude)
```

# exclude Upper_13July20 sample
# recalculate PARAFAC models

```r
pf3n = eem_parafac(eem_list_ex, comps=seq(dim_min, dim_max), normalise = TRUE, const = c("nonneg", "nonneg", "nonneg"),
maxit = maxit, nstart = nstart, ctol = ctol, cores = cores) pf3n = lapply(pf3n, eempf_rescaleBC, newscale = "Fmax")
```

# plot the created model components
```r
eempf_compare(pf3n, contour=TRUE)[[1]]
```

# continue with model 5, 7 components OR model 4, 6 components? # ended up choosing model 4 to minimize residuals

# examine residuals
```r
residuals = eempf_residuals(pf3n[[5]], eem_list_ex, cores=cores)
leverage = eempf_leverage(pf3n[[5]])
metrics = eempf_residuals_metrics(residuals, leverage)
```

# plot residual metrics
```r
lapply(names(metrics), function(name){
metrics[[name]] %>%
mutate(mode = name, element = !!sym(name))
}) %>%
bind_rows() %>%
pivot_longer(cols = RSS:LEV, names_to = "metric", values_to = "value")
```

# interpret these?

# Quality parameter integrating the model fit, the core consistency and the split-half analysis
eempf_corcondia(pf3n[[4]], eem_list_ex) eempf_eemqual(pf3n[[4]], eem_list_ex, sh, cores=cores)

# recalculate model with increased accuracy
# only need to recalculate 6 component model (comps=6); will speed up analysis time
# We use strictly_converging = TRUE here to derive a meaningful number of truly converging models
pf4n = eem_parafac(eem_list_ex, comps = 6, normalise = TRUE,
const = c("nonneg", "nonneg", "nonneg"),
maxit = maxit, nstart = nstart, ctol = ctol, output = "all",
cores = cores,
strictly_converging = TRUE)

# Plot the resulting components and loadings
eempf_comp_load_plot(pf4n[[1]], contour = TRUE)

# split-half analysis
# The split-half analysis is intended to show the stability of your model
# set random to T to split data randomly (i.e. not according to sample date or location) and make conditions more "stable"
# this command takes a long time to run
sh = splithalf(eem_list_ex, 6, normalise=TRUE, rand=TRUE, cores=cores,
nstart=nstart, strictly_converging=TRUE, maxit=maxit,
ctol=ctol)

# Plot the results from the split-half analysis. Your model is stable if the graphs of all components look similar.
splithalf_plot(sh)
# Tucker's Congruency Coefficients is a value for the similarity of the splits (and different loadings in general)

# 1 would be perfect similarity

splithalf_tcc(sh)

# compare results to models listed on openfluor.org

eempf_openfluor(pf4n[[1]], file = "Sethna_MonroeDOMmodel_openfluors.txt")

# report of PARAFAC analysis

eempf_report(pf4n[[1]], export="MonroeDOMparafac_report.html", eem_list=eem_list_ex, shmodel=sh, performance=TRUE)

# export the model

write.csv(eempf_export(pf4n[[1]]), file="MonroeDOMparafac_model.csv")

model = eempf_export(pf4n[[1]])

# separate model outputs into Fmax, ex loadings, and em loadings

Fmax = model[1:29,1:7]

# plot components of the model as em/ex loadings

eempf_plot_comps(pf4n,type=2,contour=T)+

scale_color_manual(values=c("lightblue","darkblue"))+
theme(legend.position="bottom")
PARAFAC model results

Components

Loadings
<table>
<thead>
<tr>
<th>Sample</th>
<th>Comp.1</th>
<th>Comp.2</th>
<th>Comp.3</th>
<th>Comp.4</th>
<th>Comp.5</th>
<th>Comp.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center_28Aug20</td>
<td>0.0380737</td>
<td>0.0445523</td>
<td>0.0271745</td>
<td>0.0374928</td>
<td>0.0378284</td>
<td>0.0356522</td>
</tr>
<tr>
<td>Lower_28Aug20</td>
<td>0.0339227</td>
<td>0.0393309</td>
<td>0.0242172</td>
<td>0.0321171</td>
<td>0.0270397</td>
<td>0.0346531</td>
</tr>
<tr>
<td>Site1a_28Aug20</td>
<td>0.0389292</td>
<td>0.0456896</td>
<td>0.027968</td>
<td>0.0388229</td>
<td>0.0326943</td>
<td>0.0408654</td>
</tr>
<tr>
<td>Site2a_28Aug20</td>
<td>0.0362427</td>
<td>0.0425891</td>
<td>0.0255646</td>
<td>0.0356154</td>
<td>0.029103</td>
<td>0.0401282</td>
</tr>
<tr>
<td>Upper_28Aug20</td>
<td>0.0376048</td>
<td>0.0431938</td>
<td>0.0267887</td>
<td>0.0377636</td>
<td>0.0311575</td>
<td>0.0392678</td>
</tr>
<tr>
<td>Center_13July20</td>
<td>0.0267254</td>
<td>0.0223019</td>
<td>0.0757929</td>
<td>0.030798</td>
<td>0.0030764</td>
<td>0</td>
</tr>
<tr>
<td>Lower_13July20</td>
<td>0.0211695</td>
<td>0.027048</td>
<td>0.065612</td>
<td>0.0266286</td>
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</tr>
<tr>
<td>Site1a_13July20</td>
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<td>0.0740412</td>
<td>0.0280412</td>
<td>0.0197326</td>
<td>0.0268058</td>
</tr>
<tr>
<td>Site2a_13July20</td>
<td>0.0207392</td>
<td>0.02772</td>
<td>0.0672396</td>
<td>0.0257316</td>
<td>0.0138459</td>
<td>0.026636</td>
</tr>
<tr>
<td>Center_25Jun20</td>
<td>0.02194</td>
<td>0.0206852</td>
<td>0.0130866</td>
<td>0.0231245</td>
<td>0.0254986</td>
<td>0.0090789</td>
</tr>
<tr>
<td>Lower_25Jun20</td>
<td>0.0223382</td>
<td>0.0168214</td>
<td>0.0132838</td>
<td>0.0226666</td>
<td>0.0193724</td>
<td>0.00966</td>
</tr>
<tr>
<td>Site1a_25Jun20</td>
<td>0.0237637</td>
<td>0.0192782</td>
<td>0.0150499</td>
<td>0.0241528</td>
<td>0.0240934</td>
<td>0.002435</td>
</tr>
<tr>
<td>Site2a_25Jun20</td>
<td>0.0217858</td>
<td>0.0167631</td>
<td>0.0137341</td>
<td>0.0219368</td>
<td>0.0195175</td>
<td>0.0033385</td>
</tr>
<tr>
<td>Upper_25Jun20</td>
<td>0.0318677</td>
<td>0.021939</td>
<td>0.0199354</td>
<td>0.0314991</td>
<td>0.0202525</td>
<td>0.0005886</td>
</tr>
<tr>
<td>Center_26May20</td>
<td>0.0278695</td>
<td>0.014705</td>
<td>0.0159253</td>
<td>0.0266801</td>
<td>0.0126137</td>
<td>0</td>
</tr>
<tr>
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Model performance

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<th>Core Consistency</th>
<th>Split-half</th>
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Split-half analysis

![Graph showing loading against wavelength for different subsets (AB, AC, AD, BC, BD, CC)]
Lienne R. Sethna
Curriculum Vitae
lsethna@iu.edu

**Education:**
Indiana University, Bloomington
O’Neill School of Public and Environmental Affairs
Ph.D. Environmental Science, 2022
   Individualized Minor: Translational Ecology
Dissertation: An ecological and biogeochemical study of dissolved silicon in human-dominated freshwater ecosystems
Advisor: Todd V. Royer

The Ohio State University, Columbus
B.S. Earth Sciences, 2016

**Publications:**


**Publications in Prep:**
**Teaching:**
Indiana University:
- Spring 2021: Introduction to Water Resources; Instructor of Record; SPEA-E260
The Ohio State University
- Spring 2016: Geology of National Parks; Teaching Assistant; ES-1105
- Fall 2015: Planet Earth; Lab Instructor; ES-1100

**Awards and Grants:**
- 2021 Fulbright U.S. Student Program Alternate; Project title: “Identifying nutrient requirements of positive algae to predict and mitigate harmful blooms”
- 2020 CUAHSI Instrumentation Discovery Travel Grant; Project title: “Examining the optical properties of dissolved organic matter to investigate coupling between carbon quality, nutrient availability, and algal community composition”; Amount: $1000
- 2020 Indiana University Sustainability Research Development Grant; Project title: “Agricultural conservation practices, nutrient ratios, and the prevention of harmful algal blooms”; Amount: $9,540
- 2020 Society for Freshwater Science Endowment Award for Research; Project title: “Role of winter cover crops on silica concentrations and stoichiometry”; Amount: $1000
- 2019 Indiana University Bloomington Provost’s Travel Award for Women in Science; Amount: $1000
- 2019 Indiana University Graduate and Professional Student Government Travel Award; Amount: $1000
- 2019 Cary Institute for Ecosystem Studies: Fundamentals of Ecosystem Ecology Course Award; $1000
- 2018 Society for Freshwater Science Endowment Award: Mulholland Award for Biogeochemical Research; Project title: “Responses of N, P, and Si ratios to hydrologic and land use changes”; Amount: $1000

**Workshops and courses:**
- 2022 Parallel Factor Analysis for Dissolved Organic Matter fluorescence; Chalmers University Virtual Course
- 2021 Indiana Department of Environmental Management BATHTUB Model Training; Virtual workshop
- 2020 University Science Teaching Course, Indiana University; Bloomington, IN
- 2019 Woodstoich 4 Stoichiometry Workshop. Flathead Lake Biological Station, University of Montana.
- 2019 Diatoms of North America Taxonomy Workshop. Society for Freshwater Science workshop; Salt Lake City, UT.
- 2017 Using in-situ water quality sensors: Lagrangian and Eulerian applications. CUAHSI training workshop; Gainesville, FL.
Select Conference Presentations:


2020 “Winter cover crops may reduce harmful algal bloom frequency and intensity in agricultural watersheds” International Association for Great Lakes Research Virtual Conference.


2018 “Responses of silica stoichiometry to hydrologic and vegetation changes” Society for Freshwater Science Annual Meeting. Detroit, Michigan.

2018 “Responses of Lake Erie phytoplankton communities to phosphorus, nitrogen, and silica loading from the Maumee River” Ohio State University Lake Erie – Inland Waters Annual Research Review. Columbus, Ohio.

Lectures and seminars:


2020 “Addressing climate change misinformation”. Indiana University Environment and People course (virtual lecture). April 2020

2020 “Communicating climate change” Indiana University Environment and People course (virtual lecture). April 2020


2019 “Biogeochemistry and environmental cycling of silica in freshwaters” Indiana University Limnology course. Bloomington, IN. Nov. 2019

Select Co-authored presentations:


Service:

American Geophysical Union Thriving Earth Exchange
  Community Science Fellow (July 2021 – present)

Society for Freshwater Science
  Board of Directors, Student Resources Committee representative (2021-2022)
  Student Resources Committee Chair (2020-2021)
  Live Auction Committee Chair (2019-2020)
  Workshop Committee Chair (2018-2019)

Advocates for Science at Indiana University (2019-present)

Indiana University Jim Holland Research Initiative in STEM Education
  Limnology and Hydrology lab (2018-2019)