Cyanobacteria phenology and toxicity across six Minnesota temperate lakes

A Thesis
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

Leah Egan

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Christopher Filstrup and
Dr. Cody Sheik

October 2022

ACKNOWLEDGEMENTS

The work of this thesis would not have been made possible without the support, encouragement, assistance, and motivation by those listed below. I want to thank all of those who have been involved throughout this journey; you have all helped get me here today. Thank you, Chris and Cody, for all of your shared knowledge, expertise, and for providing this opportunity and experience; thank you Kaela for making early morning sampling more enjoyable and for always being there with advice; and thank you to my committee, Chris, Cody and Euan, for helping me become a better researcher and scientist. I also want to thank Elizabeth Alexson and Holly Wellard-Kelly for taxonomy training and endless hours looking under the scope together; NRRI’s Central Analytical Lab for nutrient analyses; Bridget Ulrich’s lab, including Matthew Berens and Allison Byrd, for assistance with cyanotoxin analyses; and Will Bartsch and Kristi Nixon for creating land use maps of our sample lakes. Lastly, I want to thank my family, including my husband, Ben, for being there when I doubted myself and for never letting me settle.

ABSTRACT

Cyanobacteria harmful algal blooms (cHABs) represent both chronic and emerging water quality threats in lakes globally and are the consequence of complex, interacting stressors. While we know that water temperature, nutrient loading and availability, and water column mixing conditions are important drivers of cHABs, the combination of abiotic conditions leading to bloom development, maintenance, and toxicity remain poorly understood across different lake types. To better understand relationships among cyanobacteria abundances and composition, toxin concentrations, and nutrient conditions, we monitored six temperate Minnesota lakes with differing watershed land uses and lake morphometric characteristics across a latitudinal gradient. This project combined limnological approaches, comprehensive phytoplankton community analyses using taxonomic approaches, and advanced analytical characterization of toxin molecules to determine mechanisms leading to bloom formation and toxicity. Findings show that our study lakes had differing bloom phenologies influenced by different community assemblages and nutrient limitation states. The southern lakes had contrastingly different watershed land uses, such that Peltier Lake was predominantly urbanized, and Carrie Like was highly agricultural, which led differing nutrient growth conditions. Peltier (low N:P) experienced chronic surface blooms, whereas Carrie (extremely high N:P) did not. In Peltier Lake, the dominant cyanobacteria taxa present switched midsummer from nitrogen fixers (*Dolichospermum* spp.) to non-nitrogen fixers (*Microcystis* spp.) which tracked with decreasing nitrogen to phosphorus ratios. This community shift was counterintuitive based on changes in nutrient deficient growth conditions, suggesting that other drivers were likely impacting the shift in dominant cyanobacteria genera. Random Forest Models predicted major drivers of cyanotoxins in the bloom dominated lake, Peltier, to be in-lake growth conditions including dissolved organic carbon, soluble reactive phosphorus, and total phosphorus concentrations. Cyanotoxins were surprisingly detected in all lakes, including our least productive systems such as a northernly located lake, White Iron, and we observed a gradient of microcystin congeners present among our sample lakes. Cyanotoxin production can vary at the species (strain) level, therefore, it is essential to determine abiotic drivers of cHABs for various strains in differing lake types to properly inform management and mitigation of future system specific HABs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Introduction and importance of the cyanobacteria community

1 Phytoplankton, Cyanobacteria, and Ecosystem Functioning

The commonly used, though not taxonomically accepted, term ‘algae’ broadly refers to photosynthetic organisms including both prokaryotes and eukaryotes that vary functionally, morphologically, and physiologically, yet all members play a crucial role in overall ecosystem functioning and can be used for monitoring, assessing, and predicting freshwater aquatic health (McCormick & Cairns, 1994). Specifically, phytoplankton, further defined as “plankton photoautotrophs and major producer[s] of the pelagic” (Reynolds, 2006), serves as the base of lake food webs, supplying fundamental sources of energy and elemental resources via photosynthesis to higher trophic levels. However, temporal and spatial variation, due to responding to both available resources (e.g., nutrients, light) and biotic interactions (e.g., zooplankton grazing), introduces complex interactions among phytoplankton assemblages within the pelagic zones of freshwater systems. Commonly studied phylogenetic classifications of phytoplankters include chlorophytes (green algae), chrysophytes (golden algae), bacillariophytes (diatoms), cyanophytes (blue-green algae), rhodophytes (red algae), pyrrhophytes (dinoflagellates), and cryptophytes, all of which have distinctive photosynthetic accessory pigments and provide unique functional traits to phytoplankton communities (e.g., nitrogen fixation, nutrient cycling).

Although members of the phytoplankton community, cyanobacteria are unique from algae because of their prokaryotic classification and early origin on Earth (3.5 bya). Cyanobacteria evolved oxygenic photosynthesis, during what is known as the Great Oxidation Event approximately 2.5 to 2.3 billion years ago (Soo et al., 2017). The cyanobacteria community has proven to be the fundamental backbone for ecosystem function because of their initial ability to release oxygen through the process of photosynthesis, in which photosynthetic pigments (chlorophyll-a and phycobilins) capture and convert light energy to chemical energy to fuel essential metabolic processes, growth, and reproduction (Dodson, 2004). The release of oxygen through photosynthesis resulted in Earth’s oxygenic atmosphere and contributed to the success of aquatic and terrestrial plants and animals on Earth as we know it.

Although other phytoplankters contribute to important ecosystem functions, cyanobacteria have especially stood out over recent decades due to negative consequences for public and ecosystem health. With growing concerns of severe climate change, anthropogenic eutrophication, alterations to natural hydrological systems, and increasing land use changes (Paerl et al., 2011; Tu, 2009), cyanobacteria have become an increased nuisance in both freshwater and marine systems and ultimately a danger to humans, aquatic life, and ecosystem services (Reinl et al., 2021; C. Wagner & Adrian, 2009). Though only one component of a complex phytoplankton community, cyanobacteria in freshwater systems are more commonly known for forming harmful algal blooms (HABs), defined as a rapid increase in biovolume resulting in a surface water scum, typically producing a pungent smell. These cyanobacteria harmful algal blooms (cHABs) can lead to hypoxic conditions resulting in fish kills, can produce a suite of potent cyanotoxins harmful to both humans and aquatic life, and are an increasing threat to our waterbodies’ aesthetic and recreational values (Carmichael, 1994; Ingrid Chorus, 2000; Wilhelm et al., 2006). Some suggest that cHABs are the greatest threat to human and ecological health in freshwater ecosystems (Brooks et al., 2016).

1.1 Phytoplankton Phenology and Cultural Eutrophication

Although cyanobacteria are often the predominate cause of HABs, understanding the phytoplankton community in its entirety, rather than species presence or absence of a specific taxonomic group, is essential when analyzing phytoplankton community dynamics and determining overall habitat health (Reynolds et al., 2002). Phytoplankton communities across lake types fluctuate both spatially and temporally due to dynamic chemical, physical, and biological factors, which may be influenced by latitudinal location, watershed characteristics, and lake morphometry (Katsiapi et al., 2012; Weyhenmeyer et al., 2013). Phytoplankton composition typically differs in lakes of different trophic states based on nutrient availability. Watson et al. (1997) found nutrient poor, oligotrophic systems were observed to be dominated by bacillariophytes, chrysophytes, and cryptophytes. Nutrient rich, eutrophic systems were dominated by cyanophytes with few other groups present, and hypereutrophic lakes consisted of >70% cyanophytes. This distribution of algal groups across the trophic status spectrum can be attributed to genera-specific habitat adaptations under certain conditions and interspecific competition (Reynolds, 1998). Additionally, common trends of phytoplankton community phenology across differing systems have also been observed, specifically with diatom-dominated communities early in the season shifting to chlorophytes and cyanophytes later in the season (Paerl & Otten, 2013; Sommer et al., 2012). These trends have been observed due to spring phosphorus sequestration by diatoms leading to a phosphorus-limited system (Paerl & Otten, 2016) and also correlated to differing temperature tolerances of phytoplankton groups. Maximum cyanobacteria growth rates have been associated with greater temperatures than chlorophytes, dinoflagellates, and diatoms (Paerl & Otten, 2013).

Factors influencing primary productivity in lakes in the context of eutrophication have been widely studied, including macro- and micronutrient availability, with subsequent consequences for phytoplankton biomass. The cycling of nitrogen (N), phosphorus (P), carbon, oxygen, and silica are commonly studied to understand the movement of such elements between the atmosphere-water interface and within aquatic systems, including flux to and from bottom sediments. However, N and P are two of the major elements of interest due to previous knowledge and studies showing the impact both elements have on phytoplankton biovolume and community assemblage. The positive relationship between phytoplankton biomass and P concentrations, shown by Schindler et al. (1978) in Experimental Lakes Area (ELA) 226 is well known by aquatic biologists and is fundamental in understanding algal growth dynamics. However, these experiments focused on management strategies including P-only and N+P paradigms and lacked N-only approaches. It was further explored that complex dynamics including differing habitat conditions (e.g., nutrient and light availability), interspecific resource competition, habitat adaptations, and nutrient affinity and uptake ability, may also influence the phytoplankton communities (Glibert et al., 2016; Reynolds, 1998; Watson et al., 1997). Reynolds et al. (2002) explains the approach of functional classification, in which algae are differentiated based on tolerances, sensitivities, and specialist adaptations. Therefore, if the habitat is constrained by a specific variable (i.e., N, P, light, CO_2), then it would be more likely to find organisms that are less sensitive and able to tolerate such conditions, but that does not ensure the presence of specific groups of phytoplankton. Interactions among genera also occur due to their metabolic rates and requirements, and nutrient uptake kinetics. Reynolds (1998) discusses rate-adapted (r strategist) versus affinity-adapted (k strategist) species in which organisms are restrained by biological availability of essential nutrients, such as N, P, iron, and silica. Rate-adapted species are those that can uptake necessary nutrients at a faster rate, compared to affinity-adapted species, in which those organisms are able to satisfy nutrient cellular demands at lower concentrations.

1.2 Cyanobacteria Community Dynamics

All phytoplankton groups play an important role in the freshwater plankton spectrum; however, researchers have developed a specific interest in cyanobacteria because of their increasing concern of HAB formation, toxicity, prevalence, and rapid increase in biomass in response to climate change and eutrophication. Understanding physical, chemical, and biological drivers of cHAB bloom formation, maintenance, and senescence remain highly discussed because of complex internal bloom dynamics, fluctuating environmental factors, and varying watershed characteristics. Therefore, unique combinations of biotic and abiotic factors, as well as the composition and functional traits of cyanobacteria strains, influence cyanobacteria biovolume and community assemblages across lake systems and within lake systems across time, so a monolithic approach to managing cHABs is not effective.

Previous studies have shown that diverse factors including latitude, lake type, and mixing behavior can influence cyanobacteria biovolume (Mischke, 2003; Richardson et al., 2018; Weyhenmeyer et al., 2013). Specifically, differing lake types, such as isolated versus drainage systems, allow for varying water residence times. Increased water residence time leads to poor flushing within the lake system and allows for nutrient build up over time (Cha et al., 2014; Paerl & Huisman, 2008). Likewise, scenarios that lead to stratification and reduced vertical mixing generates an environment in which warm surface waters may favor cyanobacteria species that can regulate their buoyancy

(Elliott et al., 2006; Jöhnk et al., 2008; Paerl & Huisman, 2008). Within-lake system drivers also differ from those that influence the cyanobacteria biovolume across lakes. It has been commonly reported that water temperature, total P (TP) concentrations, and the N-to-P ratio (N:P) are potential drivers of cyanobacteria biovolumes within lake systems (Cha et al., 2014; Dao et al., 2016; Dolman et al., 2012; Lu et al., 2013; Onderka, 2007; Richardson et al., 2018; Tilman et al., 1982; Watson et al., 1997). Luo et al. (2014) analyzed various environmental parameters and found that pH, temperature, and total N (TN) were most closely related to cyanobacteria community dynamics based on canonical correspondence analysis (CCA) in the Shiyan and Tiegang reservoirs in China. Therefore, previous studies indicate that drivers of cyanobacteria differ across systems and therefore may be system specific.

Although general drivers of cyanobacteria biovolume and composition have been predicted and studied, functional traits such as species-specific metabolic requirements and morphological characteristics should not be dismissed when considering cyanobacteria assemblages. Dick et al. (2021) analyzed ecological and physiological differences among *Microcystis* colonies and found that species-specific growth rates differed across temperature and light gradients, indicating spatial and temporal variation within cyanobacteria blooms due to environmental conditions. Cyanobacteria strains can also modify their morphology depending on environmental conditions. Studies have shown that temperature may influence both morphology and photosynthetic rates, specifically, higher temperatures lead to greater chain area of *Anabaena sp*. and increased photosynthesis (Giordanino et al., 2011). The importance of phenotypic plasticity is further emphasized indicating functional diversity within species due to differing tolerances and requirements. While dynamic cyanobacteria assemblages can be altered by environmental conditions, such as water chemistries, cyanobacteria can also alter abiotic conditions within lakes to further support their dominance. Shapiro (1997) analyzed the role of carbon dioxide (CO_2) and uptake rate ability across cyanobacteria dominance in lakes. Cyanobacteria caused lower concentrations of CO_2 due to photosynthetic use, indicating that not only can cyanobacteria be influenced by nutrient availability, but they can also manipulate the abiotic environment in which cyanobacteria are favored (Morales-Williams et al., 2017). Therefore, some cyanobacteria strains tend to do well under low $CO_{2,}$ high pH conditions because of carbon concentrating mechanisms (CCMs) that allow them to outcompete eukaryotic phytoplankters for limited CO_2 supplies. While often overlooked, CO_2 concentrations can shape phytoplankton community assemblages.

1.3 Nitrogen and Phosphorus Cycling

Nutrient speciation and availability have been demonstrated as major drivers of phytoplankton and cyanobacteria community structure. N and P forms are highly debated as competing predictors of the cyanobacteria community, as studies have shown that both N and P are important for algal growth (Dolman et al., 2012; Donald et al., 2011; Elser et al., 2007; Filstrup & Downing, 2017) and secondary metabolite production (Hoffman et al.; Newell et al., 2019; Wagner et al., 2021). Elser and colleagues (2007) studied the effects of co-limitation between N and P across freshwater and marine systems and showed that N and P are available in relatively close proportions in most aquatic environments, further emphasizing the importance of the N and P paradigm and a whole ecosystem approach. However, available nitrogen can be found in both organic (urea) and inorganic forms, including oxidized (nitrate, NO_3^-, and nitrite, NO_2^-) and reduced (ammonium, NH_4^+) forms. Newell and colleagues (2019) showed that an increase in total reduced nitrogen forms, from the Maumee River into the western basin of Lake Erie, was favored by cyanobacteria, specifically non-diazotrophic genera, such as *Microcystis.* Additional studies have shown an inverse relationship between both diazotrophic and non-diazotrophic cyanobacteria biovolume with NO_3^{-}:NH_3 ratios, suggesting that, in general, cyanobacteria prefer reduced forms of nitrogen (Harris et al., 2016).

N and P cycles are often studied to better understand how phytoplankters are using available forms of nutrients and how availability is affecting phytoplankton responses, such as biovolume and community composition. Both internal and external nutrient inputs are important to consider when analyzing nutrient cycling to better understand whole ecosystem mass balances, including nutrient sinks and sources. External, or allochthonous, nutrient loading is affected by overall watershed characteristics, soils composition, and surrounding land use, such as agriculture and urbanization development (J. A. Downing & McCauley, 1992; Jefferies, 2000). Internal nutrient loading, or autochthonous inputs, is derived from within the system, such as internal phosphorus recycling from sediments, and can significantly influence community assemblages (Paerl & Otten, 2016).

Due to increased developed land uses, allochthonous P loading is becoming an increased concern and commonly enters aquatic systems via runoff and groundwater contributions (Paerl & Otten, 2016). While many management strategies have focused on controlling external P loads to lakes, remobilization of legacy P from sediments (i.e., internal loading) has become a significant source of P in certain lakes (Nürnberg & LaZerte, 2016). During decomposition, P sediment burial is the primary form of recycling due to the lack of a significant gaseous state in P cycling, unlike N cycling which includes gaseous N_2. P binds to cations, such as iron (Fe) and calcium (Ca) and becomes unavailable to phytoplankton. Soluble reactive phosphorus (SRP), the most usable form of P by phytoplankters, is released from being bound to substrates during hypoxic events leading to an increase in P availability, further allowing phytoplankton growth.

N cycling is often set apart from P cycling because of the atmospheric exchange component. Unlike P, N in the form of gas (N_2) can enter aquatic systems from the atmosphere via dissolution, a form of N external loading added to the system (Loeks & Cotner, 2020). Once dissolved, N_2 can undergo N fixation (Nfix), a microbial mediated process that converts atmospheric N_2 to ammonia, a temperature and pH dependent molecule. The conversion to ammonia plays a critical role in the N cycle because of the introduction of usable N to aquatic ecosystems. Aquatic organisms can readily use ammonia as a source of reduced N for protein synthesis, ATP production, and photosynthetic pigments. Recent studies have shown that Nfix may be greater than previously anticipated and substantially contribute to usable N by cyanobacteria (Marcarelli et al., 2022; Natwora & Sheik, 2021). Certain species of cyanobacteria, such as *Dolichospermum* and *Aphanizomenon*, also contain specialized cells, called heterocytes, in which Nfix occurs. Although Nfix is metabolically expensive, drivers of Nfix, specifically when and why certain organisms may fix nitrogen, are still at question and remain poorly understood. In addition to Nfix, other external sources of inorganic and organic N can enter aquatic systems via agricultural runoff, urban fertilization, atmospheric deposition, or septic discharge (Lepori & Keck, 2012; Vitousek et al., 1997), all of which may contribute to and change the system’s natural N cycle.

1.4 Cyanotoxin Production

Cyanobacteria are an increasing concern across freshwater and marine systems because of their ability to produce harmful secondary metabolites known as cyanotoxins. Although cyanotoxins were previously associated with eutrophic systems, the presence and dominance of cyanobacteria in oligotrophic lakes (Reinl et al., 2021) provides evidence that all water bodies may be susceptible to cyanobacterial growth. Cyanotoxins are categorized into different classifications based on their target organs, including neurotoxins that target the central nervous system, hepatotoxins that target the liver, and cytotoxins that affect cellular function (Carmichael, 2001). Common neurotoxins include anatoxin-a and saxitoxins that are known to be produced by cyanobacteria genera such as *Anabaena, Oscillatoria, Aphanizomenon, Cylindrospermopsis,* and *Lyngbya.* Hepatotoxins include cylindrospermopsin, microcystins, and nodularins that can be produced by *Aphanizomenon, Cylindrospermopsis, Anabaena, Aphanocapsa, Microcystis, Nostoc, Oscillatoria,* and *Nodularia.* However, the presence of cyanotoxin production highly varies due to cyanobacteria species-strain genetic abilities and current environmental conditions (Dick et al., 2021). Previous studies have shown that N form and concentrations are likely to influence cyanotoxin production, specifically the presence of nitrates (Beversdorf et al., 2015; C. Wagner & Adrian, 2009) and ammonium (Donald et al., 2011). Other biological mechanisms may also influence toxin production such as cyanophage infection (Jiang et al., 2019; Steffen et al., 2017) and zooplankton presence and grazing (Jang et al., 2003; Lampert, 1987). However, not all cyanobacteria produce cyanotoxins, creating temporal variation across lake systems and complex competitive outcomes between toxic and non-toxic cyanobacteria strains are not well understood , making it difficult for managers to identify toxic waters properly and correctly (Van de Waal et al., 2011). As climate change continues, it is expected that nutrient loading will increase and the dominant N form available will change (Glibert et al., 2016). Because microcystins are predominantly composed of C and N, we can expect to see changes in toxin production and toxicity. Due to these concerns, it is important to fully understand what biotic drivers are leading to greater concentrations. Therefore, determining specific drivers across varying lake systems make complex bloom dynamics, phenology, and toxicity difficult to predict. These concerns and development of HABs are of rising interest to many researchers and require further comprehensive analyses of specific drivers leading to cyanobacteria bloom formation, maintenance, and toxicity.

2 Objectives and Hypotheses

Identifying both presence and quantity of phytoplankton groups and the changes in assemblages over time will allow for a better qualitative assessment of ecosystem functioning, water quality, and aquatic ecosystem health. The overall aim of my research is to analyze bloom phenology across six Minnesota lakes (varying in latitude, land uses, mixing regime, and trophic state) to address knowledge gaps between cHAB composition, cyanotoxin production and concentrations, and environmental variables to understand mechanisms driving bloom formation and maintenance.

Chapter 2:

Objective (1): Compare cyanobacteria abundances across six Minnesota sample lakes and identify cyanobacteria environmental drivers.

Hypotheses (1): Cyanobacteria abundances will:

(1) increase with higher developed and agricultural land use,

(2) be greater in southern lakes than northern lakes,

(3) be related to water column stability and therefore differ by mixing regime, and

(4) be most influenced by nitrogen and phosphorus concentrations, N:P ratios, and warmer water column temperatures.

Chapter 3:

Objective (2): Determine cyanobacteria phenology, assemblages, and community drivers.

Hypothesis (2a): Cyanobacterial blooms will begin earlier in the season and last longer into the fall in southern lakes than northern lakes; centrally located lakes will show variable responses of bloom phenology.

Hypothesis (2b): Cyanobacteria communities will differ across a latitudinal gradient, such that lakes at different latitudes will have different dominant cyanobacteria.

Hypothesis (2c): Primary drivers of cyanobacteria community assemblages will be in-lake processes including nutrient availability and composition.

Objective (3): Analyze cyanobacteria toxicity and predictors of toxin production.

Hypothesis (3): Toxin concentrations will be greatest in southern lakes than northern lakes and primary drivers of cyanotoxin concentrations with be N form and availability.

CHAPTER 2: Predictors of cyanobacteria abundances across six temperate Minnesota lakes

1 Introduction

Cyanobacteria, also known as blue-green algae, are a natural component of the phytoplankton community and provide essential ecosystem functions, including CO_2 fixation (primary production) and N_2 fixation. However, unbalanced growth conditions can lead to rapid increases in cyanobacteria biovolume (Schindler et al., 1978), also known as cyanobacteria harmful algal blooms (cHABs). cHABs are present in both marine and freshwater systems globally and have impacted human and aquatic ecosystem health, recreational and aesthetic opportunities, and regional economies, famously including Lake Taihu, China; Lake Victoria, Africa; various Laurentian Great Lakes, and most recently, the oligotrophic waters of Lake Superior. Studies have shown an increase in frequency, duration, and severity of cHABs (Ho et al., 2017; Huisman et al., 2018). For example, scientists have observed Lake Taihu, a large, shallow eutrophic lake, and noted cyanobacterial blooms occurring earlier in the growing season (Duan et al., 2009; Qin et al., 2018). Additionally studies show an unequal increase of cyanobacteria abundances within the phytoplankton community, specifically that cyanobacteria increases are outpacing those of eukaryotic algae (Reinl et al., 2021; Taranu et al., 2015). Individual lake studies have shown oxygen depletion due to cHABs and algal biomass senescence, leading to hypoxic events that threaten aquatic life and can cause fish kills (Rabalais et al., 2010). Along with increased cHAB severity and duration, cHABs also vary among watersheds differing in landscape characteristics, such as land use applications, and lake types. The Lake Victoria Basin, shared among Uganda, Kenya, and Tanzania, provides goods and services to more than 42 million people, but has experienced massive eutrophication from land use changes to satisfy food, housing, and product demands, thereby leading to increased erosion and degradation of water, soils, and wetlands (Olokotum et al., 2020).

Additionally, specific cyanobacteria genera are known to produce cyanotoxins, including neurotoxins, hepatotoxins, and cytotoxins, that are harmful to humans and other wildlife under certain environmental conditions (Carmichael & Boyer, 2016). The Toledo Water Crisis of 2014 is a notable example. Cyanobacterial blooms have been observed since 1995 in Lake Erie; however, more recently, a *Microcystis* bloom in the western basin of Lake Erie in August 2014 led to microcystin concentrations above the World Health Organization limit (1 µg L^{-1}) even after water facility treatments, leaving 400,000 citizens without immediate drinking water for over a week (Steffen et al., 2017).

cHABs are increasing in frequency across freshwater systems globally due to climate and land use change (Paerl & Huisman, 2009; but see Wilkinson et al., 2022). Specifically, rising atmospheric and water temperatures (Elliott, 2010; Jöhnk et al., 2008; Kosten et al., 2012; Paerl & Huisman, 2008), increased eutrophication and nutrient loading (O'Neil et al., 2012), and changes in storm intensity, such as increased precipitation and lake mixing events leading to the resuspension of cyanobacterial cells (Kebede & Belay, 1994), have been shown to promote cHAB formation. A diversity of drivers has been demonstrated to cause cHABs, or at least implicated in the emergence of cHABs, across broad spatial extents or within individual lakes, and can be categorized into biological, chemical, physical, and physiological mechanistic drivers of bloom formation and maintenance (Table 1). Drivers may not hold across different lake types (e.g., morphometry, size, mixing patterns) or across different ecoregions (land use, geology, hydrology), making it difficult to predict bloom formation, duration, and severity across lakes or regions.

Presence and dominance of cyanobacteria within the phytoplankton community has been shown to affect ecosystem structure and function. Filstrup et al. (2014) showed that phytoplankton richness and evenness is related to resource use efficiency (RUE, the efficiency at which an organism uses a resource), specifically phytoplankton RUE was highest when cyanobacteria dominated phytoplankton communities (i.e., low evenness), but zooplankton RUE was lowest in these same communities. Phytoplankton RUE was negatively related to community turnover, indicating the dominant cyanobacterium (i.e., *Microcystis* sp.) was able to outcompete others to remain the dominant genera once established within a lake, whereas more even communities had higher turnover rates within and across years. A more recent study by Heathcote et al. (2016) analyzed the zooplankton resource use efficiency (i.e., zooplankton biomass per unit phytoplankton biomass), and found that zooplankton biomass was limited by a decrease in edible phytoplankton. Specifically, in eutrophic and hypereutrophic lakes the mechanism correlating decreased zooplankton-to-phytoplankton ratios and increased primary production was driven by cyanobacteria dominance, and increased proportions of inedible phytoplankton.

Table 1. Biological, chemical, physical, and physiological mechanisms of cHAB formation and maintenance.

	Mechanism	Reference
Biological	Algal biomass	Downing et al., 2001
	Herbivory	Elser & Urabe, 1999
	Mussel grazing	Fisherman et al., 2010; De Stasio et al., 2014
Chemical	Nitrogen loading	Schindler, 1974
	Phosphorus loading	Paerl et al., 2014
	N:P ratio	Smith, 1983
	Nitrogen speciation	Glibert et al., 2006; Finlay et al., 2010; Newell et al., 2019
	Phosphorus remobilization	Chen et al., 2019
Physical	Surface water temperature	Paerl & Huisman, 2008; Beaulieu et al., 2013;
	pH	Lou et al., 2014
	Water column stability	Wagner & Adrian, 2009
	Light availability	Havens et al., 2003
Physiological	Cell buoyancy	Reynolds et al., 1987
	Overwintering cell	Kitchens et al., 2018

Although cHAB drivers have been well studied, recent research shows an increase in blooms and cyanobacteria abundance in oligotrophic lakes (Kosten et al., 2012; Reinl et al., 2021; Taranu et al., 2015). cHABs in Lake Erie are an annual phenomenon, however, such an occurrence in Lake Superior was unanticipated because it counters long-standing nutrient paradigms regarding cHAB formation. Lake Superior, classified as oligotrophic, is known for its pristine aesthetic and vast recreational opportunities. The first ever observed cyanobacterial bloom in Lake Superior occurred July 2012 near the Apostle Islands National Lakeshore. Subsequent blooms were also observed August 2016, 2017, and 2018 varying in location, duration, and degree of severity, which was hypothesized to be driven by tributary inflow within the western arm of Lake Superior (Sterner et al., 2020). However, the fact that cHABs have been observed in northern, oligotrophic systems suggests that our understanding of cHAB drivers may be incomplete.

Cyanobacteria community composition varies both spatially and temporally meaning not all cHABs are the same in terms of community assemblage and toxicity potential. Although general drivers of cyanobacteria biovolume have been demonstrated at large spatial scales (e.g., Tanvir et al., 2021) or within individual lakes (e.g., Bertos-Fortis et al., 2016; David et al., 2021; Persaud et al., 2015), research suggests that cyanobacteria drivers of biovolume may vary at the genera and species level (Paerl & Otten, 2016). Functional traits, including morphological, biochemical, physiological, phenological, and behavioral characteristics, differ across cyanobacteria genera and species, and therefore express varying phenotypes. Cyanobacteria are historically durable species and have evolved different strategies (i.e., functional traits) to withstand diverse stressors, such as extreme temperature, alkalinity, and hypersaline conditions, allowing this group of phytoplankton to survive and dominate under conditions stressful to eukaryotic algae. Cyanobacteria genera vary morphologically (colonial, filamentous, single-celled) and have therefore developed physical characteristics to better adapt to changing environments. One way colonial cyanobacteria (e.g., *Microcystis*) regulate movement within the water column to improve light availability and optimize photosynthetic rates is through cell buoyancy via gas vacuoles (Reynolds et al., 1987). Walsby (1994) explained that gas vacuoles within *Microcystis* cells can withstand greater applied pressure than *Aphanizomenon* and *Oscillatoria*, allowing *Microcystis* to survive a wider range of diverse environmental conditions than other genera. Compared to other phytoplankton, cyanobacteria have higher optimal growth temperatures and can tolerate water temperatures

> 25°C (Reynolds, 2006). Cyanobacteria genera have been studied across temperature gradients; results suggest that *Microcystis* biomass increased with increasing water temperatures, specifically at 18°C compared to prior dominant genera, *Aphanizomenon,* at 15°C (Wu et al., 2016). Cell buoyancy, temperature tolerance, and other functional traits indicate that certain cyanobacteria genera may outcompete other cyanobacteria and eukaryotic algae during more favorable environmental conditions. These drivers of cyanobacteria biovolume and dominant genera are likely to differ across lakes and taxonomic groups because of these functional and physiological differences. Understanding dominant taxonomic groups present within cyanobacteria communities may allow for a greater understanding of lake-specific cyanobacterial bloom drivers.

We used an integrated multidisciplinary approach using biological, chemical, and physical parameters to analyze phytoplankton community phenology and potential drivers of cyanobacteria biovolumes across six temperate Minnesota lakes varying in land uses, latitudinal gradient, and mixing types. My primary objective of this chapter is to determine seasonal phytoplankton and cyanobacteria biovolumes within our six sample lakes during 2020 and 2021. For potential environmental drivers, we measured nutrient concentrations and physical characteristics. We also measured pigment (chlorophyll a, phycocyanin) concentrations as indicators of cHABs. I hypothesized that cyanobacteria biovolume across sample lakes will (1) increase with higher developed and agricultural land use, (2) be greater in southern lakes than northern lakes, (3) be related to water column stability and therefore differ by mixing regime, and (4) be positively affected by increasing N and P concentrations, decreasing N:P ratios, and warmer water column temperatures.

2 Materials and Methods

2.1 Study Sites

This study analyzed six Minnesota lakes across a latitudinal gradient consisting of a paired polymictic and dimictic lake at each latitude. Northern lakes were White Iron and Tait; central lakes were Portage and Hill; and southern lakes were Peltier and Carrie (Figure 1). This research builds on a previous study (Bambach, 2020) in which phytoplankton communities were analyzed across the same latitudinal gradient and pairing. Ten Mile Lake was replaced by Portage Lake due to the presence of aquatic invasive species (i.e., zebra mussels), which may have biased comparisons with previous studies and would have presented sampling challenges. These sample lakes are also a part of a broader long-term monitoring program, the Minnesota Sentinel Lakes Program, established and maintained by the Minnesota Pollution Control Agency (MPCA) and the Minnesota Department of Natural Resources (MDNR). This program focuses on efforts analyzing broad spectrum biological, chemical, and physical aspects of lake ecosystems, such as water quality, phytoplankton and zooplankton communities, aquatic plant abundances, and fisheries.

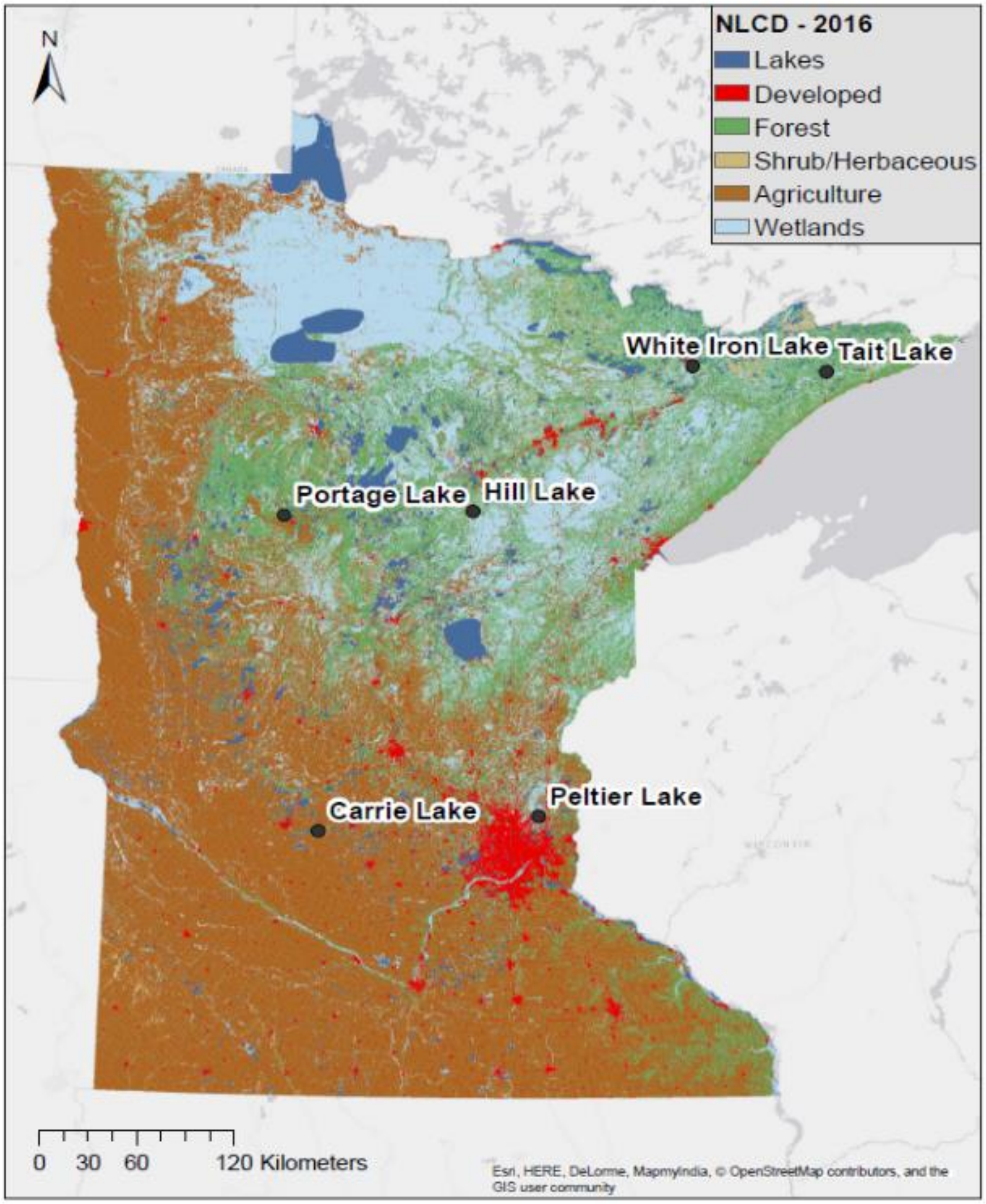


Figure 1. Minnesota land use map displaying location of six study lakes by latitudinal group. Northern lakes: White Iron, Tait; Central lakes: Portage, Hill; and Southern lakes: Carrie, Peltier.

Southern Lakes

Peltier Lake is a shallow, polymictic lake located near the Twin Cities metropolitan area and classified as hypereutrophic since the 1970s due to historically increasing P concentrations related to the 1910 dam installation (MPCA & MDNR, 2009). Peltier Lake experiences annual curly-leaf pondweed growth, an invasive and noxious aquatic plant first observed 1982. Due to the location of Peltier Lake in a highly populated county, there are significant human impacts on water quality and surrounding wildlife. Such development has led to high loading of road salts used as deicers, disturbances of fish and aquatic plants due to water-skiing, and the elimination of nesting birds on the north island. Peltier Lake was enlisted on the impaired waters list in 2002 due to annual surface blooms and severe nutrient enrichment (MPCA & MDNR 2009). In addition to the southernly latitudinal gradient location and the greatest developed watershed within this study, it is expected that Peltier Lake will have the greatest cyanobacteria biovolume across these study lakes.

Carrie Lake, located in Kandiyohi County, of southwestern Minnesota, is biologically classified as a dimictic, marl lake and is the smallest lake by surface area in this study (Table 2; MPCA & MDNR, 2012). Significant groundwater contributions lead to stable annual water levels. Due to a high agriculture region, land use application of fertilizers and pesticides may affect water quality and therefore the phytoplankton community. Peltier and Carrie provide similar meteorological and climate characteristics, however, differ in watershed and land use characteristics. Peltier Lake watershed largely consists of pastures (39%), with the highest proportion of developed land (19%) in the study. Contrastingly, Carrie Lake watershed is predominantly row-crop agriculture (65%), unlike other lake watersheds for this study (Figure 2).

Central Lakes

Portage Lake, a shallow, eutrophic, polymictic system, likely formed by glacial deposition, is in central Minnesota in Hubbard County. Although located within a watershed consisting of 56% forested land (Figure 2), the trophic state index (TSI), a biological classification system, for Portage Lake is higher compared to other lakes within the same ecoregion indicating higher nutrient availability. The lake has had previous documentation of cyanobacteria dominance among phytoplankton assemblages (Bambach, 2020).

Hill Lake is a spring fed lake formed via glacial deposition and is divided into two basins by Minnesota Highway 200. The two basins of Hill Lake act as two different systems. The North Basin (NB) is characterized as a deep, mesotrophic lake that mixes during spring and fall turn over (i.e., dimictic), while the South Basin (SB) is a much smaller and shallower eutrophic system. Although located close together, the two systems differ physically, such that the lake to watershed area ratio between the NB and SB increases greater than 14-fold (Table 2). The contributing watershed area to the SB is approximately three times larger than the NB, leading to higher external nutrient loading to the SB and episodic cyanobacteria blooms. Sampling efforts for this study focused on the NB; the deepest lake in this study (Table 2). Although not the most forested land use watershed by proportion in this study, Hill and Portage watersheds are predominantly forest land use (75% and 56% respectively; Figure 2). Surface area and watershed area are greater in Hill Lake, however, the watershed: lake area ratios are similar for both lakes (Table 2).

Northern Lakes

White Iron Lake is classified as a cold-water, dimictic system with bog-stained water (high dissolved organic carbon; DOC), attributed to the decomposition of peaty vegetation, and formed after the retreat of the last ice age, 12,000 years ago. White Iron Lake has the largest watershed: lake area ratio of our study lakes. Located within the Kawishiwi River watershed, the surrounding land use consists of 83% forested land (Figure 2), however, the south branch of the Kawishiwi River flows into White Iron Lake, draining 75% of the entire watershed into the lake.

Tait Lake, located in Cook County and the Poplar River Watershed within the Lake Superior basin, is a shallow, polymictic, mesotrophic lake. Its shallow depth and small watershed: lake area ratio (8:1, Table 2), reflects the relatively short water residence time of 1.3 years. Tait Lake's maximum depth is 4.5 m with an average depth of 2.3 m and is the shallowest lake of this study. Due to low nutrient levels and rocky substrate, Tait Lake does not experience abundant aquatic plant growth, however, there is a high diversity of plant species present. Due to their northern location and the greatest forested watershed in this study, it is hypothesized that White Iron and Tait Lakes will have the lowest cyanobacteria biovolume across these study lakes.

Table 2. Watershed, land use, and lake morphometry characteristics of six study lakes. Hill Lake: North Basin (NB), South Basin (SB). (Minnesota Sentinel Lake Assessment Reports by MPCA and MDNR).

Lake	Lake surface area (ac)	Watershed area (ac)	Watershed: Lake area	Dominant Land use (%)	Trophic State	Mixing Status	Max depth (m)	Mean depth (m)
Peltier	577	69,034	119:1	Pasture (39)	Hypereutrophic	Polymictic	4.9	2.1
Carrie	91	4,045	44:1	Agriculture (65)	Meso – Eutrophic	Dimictic	7.9	3.2
Portage	422	6,742	16:1	Forest (56)	Eutrophic	Polymictic	5.1	2.1
Hill (NB, SB)	907	24,721	14:1, 207:1	Forest (75)	Meso – Eutrophic	Dimictic	14.6, 7.3	7.4, 2.8
White Iron	3,429	592,626	173:1	Forest (83)	Mesotrophic	Dimictic	14	4.9
Tait	355	2,726	8:1	Forest (85)	Mesotrophic	Polymictic	4.5	2.3

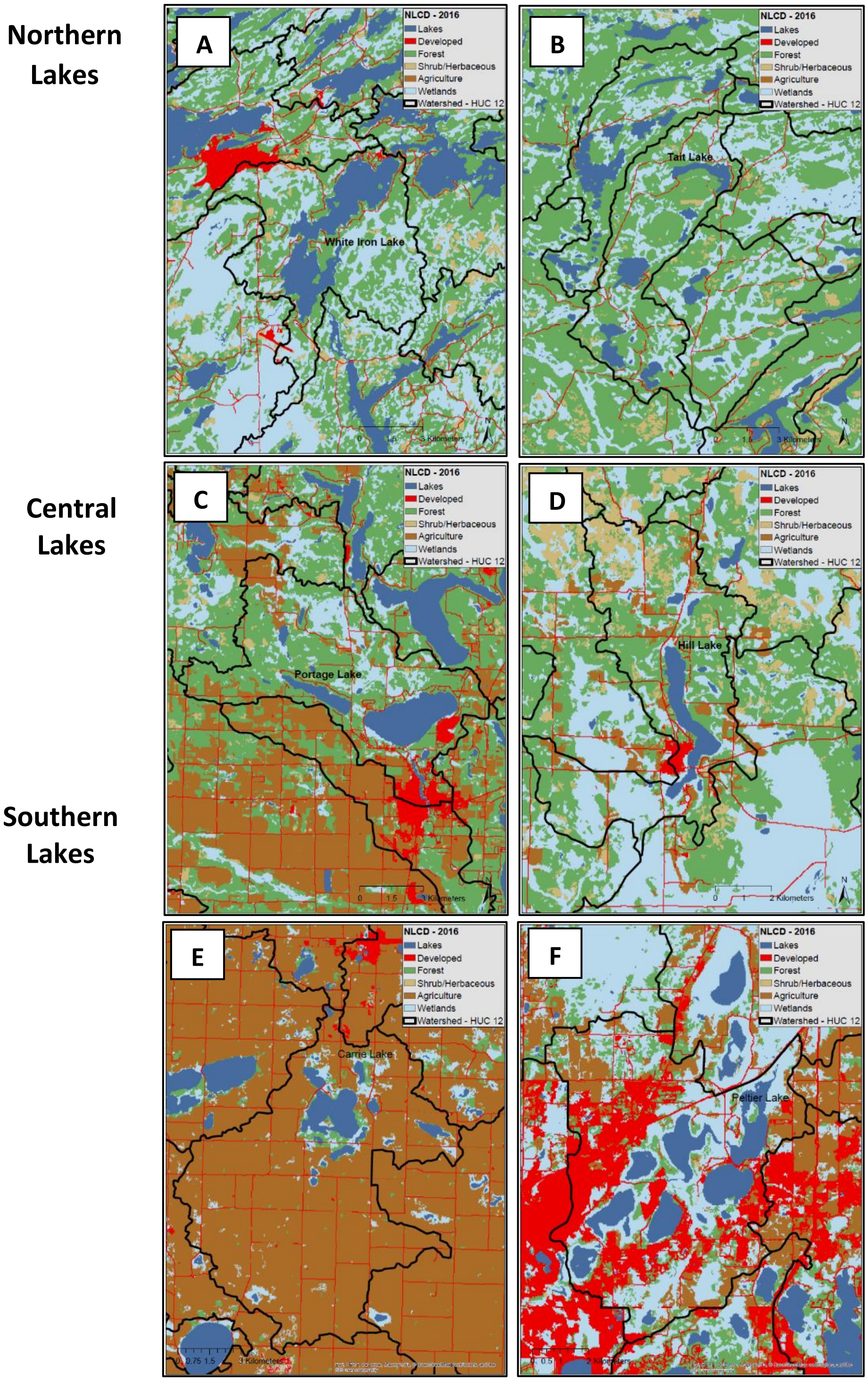


Figure 2. Watershed land use of six sample lakes. Northern lakes: (A) White Iron, (B) Tait; Central lakes: (C) Portage, (D) Hill; and Southern lakes: (E) Carrie, (F) Peltier.

2.2 Field Sampling

Sampling locations within lakes (Table 3, Figure 3) were based upon previous research (Bambach, 2020) to focus on areas where blooms previously developed. Because this project maximized temporal resolution, we sacrificed spatial coverage within sample lakes. However, due to spatially heterogeneous bloom formation, sample locations were moved within lakes based on current bloom conditions. In White Iron and Tait Lakes, sampling locations were moved to regions with higher bloom potential. In Peltier and Hill Lakes, sampling locations were switched during September 2020 due to bloom presence, as our objective was to target peak cHABs in the second project year. Subsequent sampling took place at the bloom targeted sample sites. Due to small lake size, Carrie Lake bloom conditions were relatively homogenous throughout the lake and sampling events.

Sampling occurred biweekly (May – October) in 2020 and monthly in 2021, except for the two southern lakes, Peltier and Carrie, which were sampled biweekly both years. Sampling efforts focused on Peltier and Carrie Lakes during 2021 due to contrastingly different land uses and nutrient deficiency statuses. Mixed surface water samples were taken using a two-meter integrated surface water column sampler at 0.5-m intervals to the thermocline depth (2 m maximum). Water column profile data for temperature, pH, dissolved oxygen, and conductivity were measured using a multiparameter data sonde. Water samples were collected and stored on ice in the dark and returned to the lab for further nutrient, pigment, and toxin analyses, as well as phytoplankton quantification.

Table 3. Sample location coordinates for six sample lakes.

Lake	Location name	Latitude, Longitude	Sample date
White Iron	WI 1	47.88087, -91.779	June 2020
	WI 2	47.88119, -91.7858	July 2020
	WI 3	47.84855, -91.8012	Aug – Oct 2020, May – Sep 2021
Tait	Tait 1	47.82437, -90.7007	June – July, Sep – Oct 2020, May – Sep 2021
	Tait 2	47.81541, -90.6797	Aug 2020
Portage	Portage	46.96742, -95.122	June – Sep 2020, May – Set 2021
Hill	Hill 1	47.00802, -93.5965	June – Aug 2020
	Hill 2	46.9907, -93.592	Sep 2020 May – Sep 2021
Carrie	Carrie	45.08447, -94.7857	June – Sep 2020, May – Oct 2021
Peltier	Peltier 1	45.17651, -93.0592	June – Aug 2020
	Peltier 2	45.17321, -93.0668	Sep 2020, May – Oct 2021

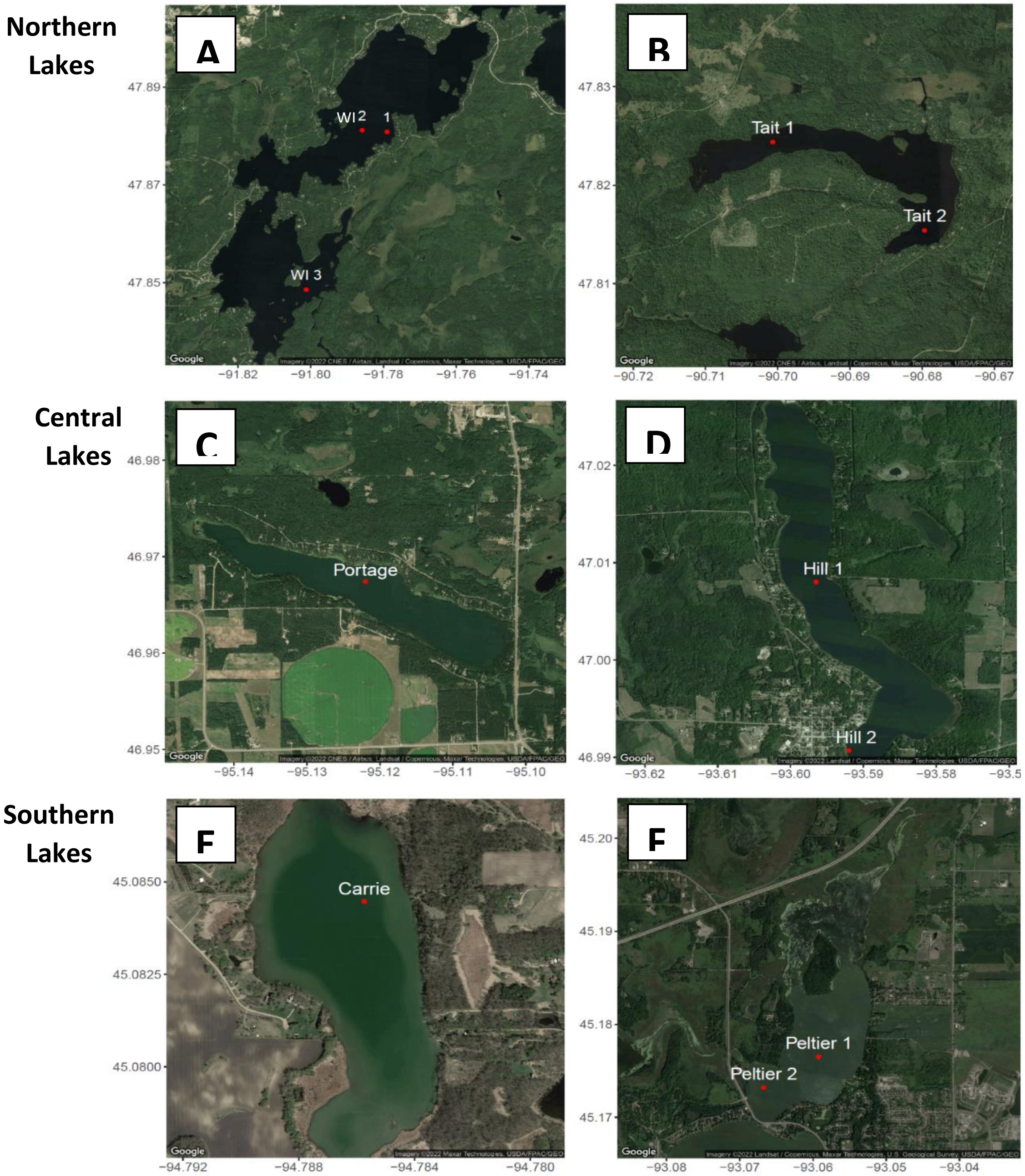


Figure 3. Sample sites at each study lake. Northern lakes: (A) White Iron, (B) Tait; Central lakes: (C) Portage, (D) Hill; and Southern lakes: (E) Carrie, (F) Peltier.

2.3 Laboratory Analyses

Nutrient and Pigment Analyses

Nutrient samples were analyzed following standard methods (APHA 2017). Analyses included total phosphorus (TP; *4500-P E*) and total nitrogen (TN; *4500 NO3-F)* following persulfate digestion (*4500-P J*), dissolved inorganic nutrients (soluble reactive phosphorus, *4500-P E*; ammonium, *4500-NH3 G*; nitrate + nitrite, *4500-NO3 F*), and dissolved organic carbon (DOC; *5310-B*). Nitrogen species were analyzed on a Lachet QuikChem 8500 Automated Flow Injection Ion Analyzer, phosphorus species were analyzed using spectrophotometry (880 nm), and carbon was analyzed on the Shimadzu TOC-L Total Organic Carbon Analyzer.

Chlorophyll-*a* was extracted in 90% acetone on filtered samples and analyzed using the acidified fluorometry method (EPA 445.0 rev. 1.2). Phycocyanin, a proxy for cyanobacteria abundance, was extracted and quantified following Kasinak et al. (2015) on filtered samples. Briefly, phycocyanin extraction was completed by adding 10 mL of 50 mM phosphate buffer and sonicating for 90 seconds. Samples were extracted at 4 °C for 2 hours and in the dark at room temperature for an additional 2 hours. Samples were then centrifuged at 4000 RPM for 20 minutes. Phycocyanin was measured using a Turner Designs Trilogy Fluorometer with the phycocyanin module. Measurements were made in RFU and later converted to phycocyanin concentrations (ug L^{-1}) using a standard curve (Kasinak et al., 2015).

Phytoplankton Quantification

Phytoplankton samples were preserved with Lugol's solution and stored until phytoplankton quantification. Samples were prepared for counting using the Utermöhl settling chamber technique (Utermöhl, 1958). The volume analyzed varied by lake due to differing algal densities. All samples were allowed to settle overnight and were counted on an inverted microscope at 400x magnification. Each slide was initially scanned at 200x for *Ceratium hirundinella*, and all cyanobacteria and other dominant genera present within the sample were noted. A minimum of 250 natural taxonomic units were counted; units were defined as colonies, filaments or individual cells if not aggregated with others. Phytoplankters were identified to genus level; however, cyanobacteria were identified to species level when possible. Cell or colonial biovolumes were calculated from linear measurements of the first 10 cells or colonies of each genus using simple geometric formulae (Hillebrand et al., 1999). Averages were taken and converted to micrometers.

Cell densities were calculated as following:

$$Equation\ 1: cell\ density\ \left(\frac{cell}{mL}\right) = \frac{[\ C\ x\ TA\ ]}{[\ S\ x\ V\ ]}$$

Where:

C = number of cells
TA = total area of chamber bottom (530.99 mm²)
S = transect area (mm²)
V = volume settled (mL)

Cell density was used to determine final biovolume for each species:

$$Equation\ 2: biovolume\ \left(\frac{mm^3}{L}\right) = \frac{(CV\ x\ CD)}{10^9}\ x\ 1000$$

Where:

CV = average cell volume (µm³)
CD = cell density (cells/ mL)

2.4 Statistical Methods

Statistical analyses were conducted using the statistical software R (version 4.0.3; R Core Team, 2020). One-way ANOVAs were used to assess significant differences ($\alpha < 0.05$) across lakes for (1) physical data (surface water temperature, average water column temperature, pH); (2) nutrient concentrations (total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), nitrates + nitrites ($NO_3^- + NO_2^-$), ammonium (NH_4^+), dissolved organic carbon (DOC)); (3) algal pigment concentrations (chlorophyll-a (Chl-a), phycocyanin (PC)); (4) phytoplankton biovolume; and (5) cyanobacteria biovolume. Nutrient, pigment, and biovolume values were $\log_{10}$ transformed before statistical analyses were conducted to correct for assumptions of normality and equal variance. We added 0.1 to all nitrate concentrations before transformation to correct for zero values (i.e., below detection limits) in the data set. TukeyHSD post hoc tests were used to determine significant differences in concentrations and biovolumes among lakes. Schmidt's Stability Index (SSI) was calculated for each sampling event using rLakeAnalyzer package (Winslow et al., 2019). Random forest models (RFMs), using the randomForest package (Liaw & Wiener, 2002), assessed predictor importance of phytoplankton biovolume across all lakes and cyanobacteria biovolume within lakes. All biovolumes were $\log_{10}$ transformed and 0.1 was added to cyanobacteria biovolumes to account for zero values (i.e., cyanobacteria not detected). Biovolume predictors were categorized into three predictor groups: watershed-level predictors, lake morphometry predictors, and in-lake growth conditions (Table 4) to determine categorical predictor importance across sample lakes.

Table 4. Categorical predictor groups of phytoplankton biovolume: watershed-level, lake morphometry, and in-lake growth conditions.

Category	Predictor
Watershed-level	- Watershed area - Watershed: Lake area - Percent agriculture - Percent urban
Lake morphometry	- Lake area - Avg. depth - Max. depth
In-lake growth conditions	- Nutrients: TP, TN, SRP, NH4, NO3, DOC, N:P - Surface temperature - Limnological variables: avg temperature, conductivity, pH, DO - Schmidt stability - Available light - Secchi disk depth

3 Results

3.1 Physical Data

Boxplots confirmed little variance in surface water temperature and average water column pH within lakes across sample years for all six sample lakes (Appendix 1), however, interannual variability of temperature occurred within lakes (Figure 4). A peak in surface water temperatures occurred in Carrie, Hill, Peltier, and Portage Lakes in late August 2020 and then drastically declined for the remainder of the season. Water temperatures during 2021 followed anticipated patterns, with a gradual increase during early summer, mid-summer peaks, and declining into the fall for all sample lakes. Surface water temperatures (*F* = 3.312, *df* = 5, *p* = 0.009) and average water column pH (*F* = 47.587, *df* = 5, *p* < 0.001) were both significantly different among lakes, however, the only difference that occurred across surface water temperatures was that Peltier Lake was significantly higher than Tait Lake (*p* = 0.02; Table 5). Peltier Lake average water column pH was significantly higher than Carrie Lake ($p = 0.007$), White Iron ($p < 0.001$), and Tait ($p < 0.001$) Lakes (Appendix 1), likely driven by primary productivity from bloom presence, however, White Iron and Tait Lakes did not differ significantly from each other ($p = 1.0$).

Table 5. Mean, coefficient of variance (%), and ranges for surface water temperature (°C), average water column pH (<2 m), and Schmidt's Stability Index (SSI) for six sample lakes during 2020 -2021.

Lake	Surface Temp. (°C)	Avg. pH	SSI
Carrie	23.32	8.29	14.43
	14.4	2.7	9.0
	15.3 – 27.6	7.9 – 8.8	0.1 – 51.2
Hill	22.2	8.37	184.46
	14.2	3.6	57.6
	15.93 – 24.90	8.0 – 9.0	1.3-287.7
Peltier	23.88	8.75	5.96
	12.9	4.7	77.6
	16.2 – 28.7	7.8 – 9.4	0.04 – 16.6
Portage	21.99	8.42	1.32
	16.4	3.2	319.7
	14.4 – 25.3	7.9 – 9.0	-0.03 – 7.1
Tait	19.62	7.07	1.44
	17.5	6.2	99.5
	12.2 – 23.0	6.5 – 8.2	-0.03 – 3.8
White Iron	20.01	7.11	43.39
	16.6	6.6	74.3
	13.2 – 23.1	6.6 – 8.2	1.2 – 102.8

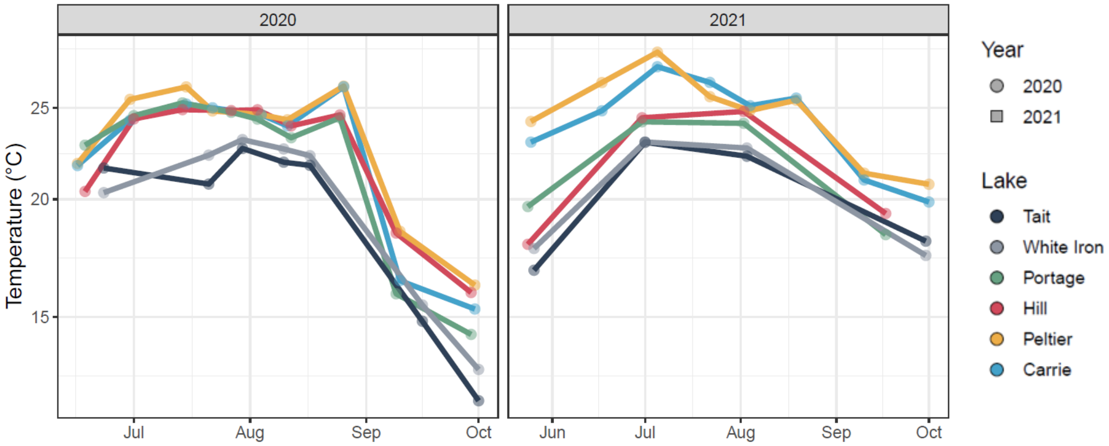


Figure 4. Surface water temperature trends for six sample lakes across two sample seasons. 7/7/20 and 9/2/20 profiles not collected for Tait and White Iron Lakes.

Schmidt's stability index (SSI) was calculated to estimate water column stability, indicative of the amount of energy required for lakes to isothermally mix. Higher SSI values indicate greater stability (i.e., during stratified conditions); lower SSI values indicate less stable water column conditions during mixing events. SSI values varied across sample lakes partially due to differing lake depths and mixing patterns. White Iron Lake (dimictic) had greater SSI values than Tait Lake (polymictic) and increased in stability early in the season, peaked during July and August, and declined during late summer into fall for both sample years. Tait Lake had opposite SSI trends between sample years with the greatest stability from May to July 2020 and decreased thereafter. During 2021, Tait Lake increased in stability throughout the duration of the sample season. However, little variability existed across sample years (Figure 5). Hill Lake (dimictic) had greater overall lake stability than Portage Lake (polymictic) remaining relatively stable until early September when stability drastically decreased (i.e., turnover) coinciding with a visible bloom on 9/9/2020 and differing sample locations. Hill Lake consistently stratified across both sample years and displayed similar patterns. Portage

Lake had lower variability during both sample years and remained relatively well mixed (Figure 5). Peltier (polymictic) and Carrie (dimictic) Lakes both exhibited decreasing water column stability throughout the summer for both sample years, however, Carrie Lake did not strongly stratify in 2020, as expected, and stability decreased throughout 2021 (Figure 5).

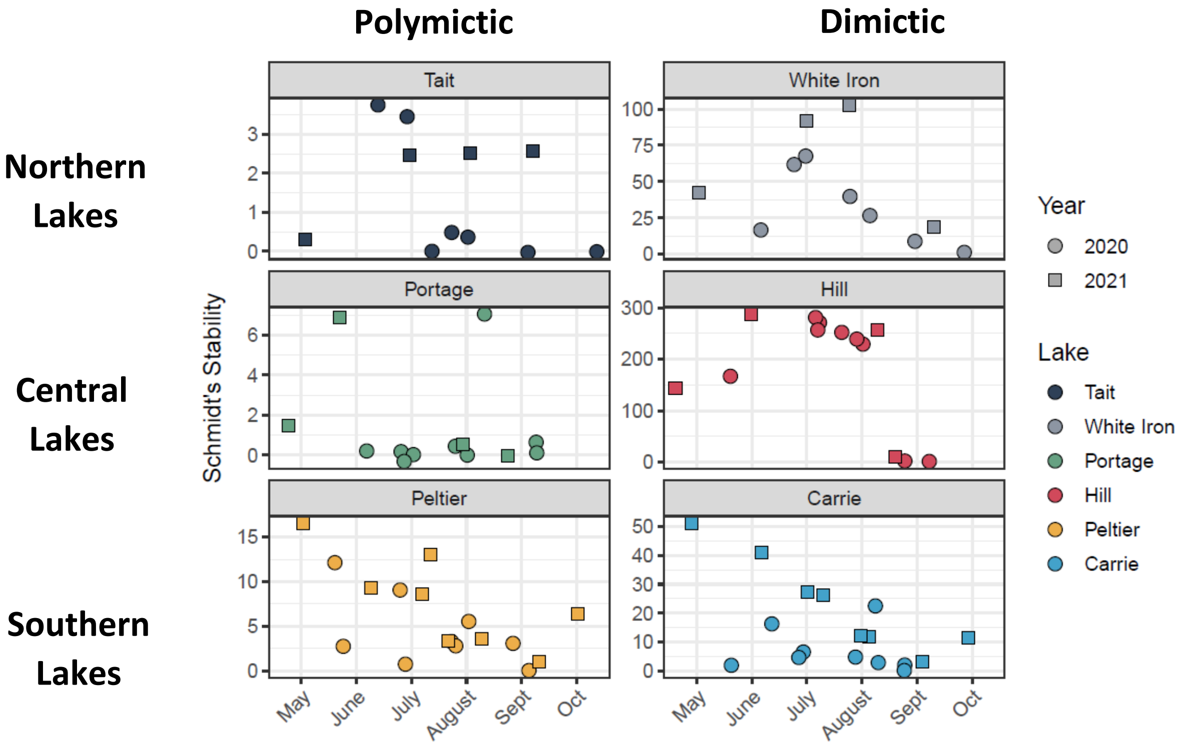


Figure 5. Schmidt's Stability Index over time in six sample lakes, polymictic (left) and dimictic (left). Y-scales differ to focus on seasonal variability.

3.2 Nutrients

Across-lake comparisons

One-way ANOVAs detected significant interactions across lakes for TP (*df* = 5, *F* = 105.9, $p < 0.001$), SRP (*df* = 5, *F* = 39.53, $p < 0.001$), TN (*df* = 5, *F* = 175, $p < 0.001$), NH_4^+ (*df* = 5, *F* = 7.256, $p < 0.001$), NO_3^- (*df* = 5, *F* = 57.68, $p < 0.001$), and DOC (*df* = 5, *F* = 76.27, $p < 0.001$).

TP concentrations were highest in Peltier Lake ($\bar{x}$ = 221 µg L^{-1}) and lowest in Tait Lake ($\bar{x}$ = 13 µg L^{-1}). Post hoc analyses showed there was no significant difference in TP among Carrie, Hill, Portage and White Iron Lakes. Peltier Lake TP was significantly higher than all other lakes ($p < 0.001$). TP concentrations in Tait Lake were significantly lower than all other lakes (Peltier, $p < 0.001$; Carrie, $p = 0.007$; Hill, $p = 0.04$; Portage, $p < 0.0001$; White Iron, $p = 0.03$). SRP values were significantly higher in Peltier Lake ($\bar{x}$ = 78 µg L^{-1}, $p < 0.001$) with remaining lakes measuring less than 7 µgL^{-1} (Table 6, Figure 6).

TN concentrations were highest in Carrie ($\bar{x}$ = 2484 µg L^{-1}) and Peltier Lakes ($\bar{x}$ = 2095 µg L^{-1}) and lowest in Tait Lake ($\bar{x}$ = 415 µg L^{-1}). There was no significant difference in TN among Portage, Hill, and White Iron Lakes. TN in Carrie Lake were significantly higher than all other lakes (Peltier, $p < 0.05$; Portage, Hill, White Iron, Tait, $p < 0.001$). TN concentrations in Tait Lake were significantly lower than all other lakes (Peltier, Carrie, Portage, $p < 0.001$; Hill, White Iron, $p = 0.001$). NH_4^+ average concentrations were highest in Carrie Lake ($\bar{x}$ = 92 µg L^{-1}); however, Peltier Lake ($\bar{x}$ = 57 µg L^{-1}) reached a greater maximum of 220 µg L^{-1} compared to 211 µg L^{-1} in Carrie Lake. Carrie Lake ammonium values were significantly higher than Hill, Tait, and White Iron ($p < 0.001$); all other lakes did not significantly differ from each other. NO_3^- concentrations were greatest in Carrie Lake ($\bar{x}$ = 1580 µg L^{-1}) and significantly higher than all other lakes ($p <$ 0.001). White Iron Lake also had significantly higher NO_3^- concentrations than Hill Lake ($p < 0.001$) and Tait Lake ($p = 0.003$; Table 6, Figure 6).

DOC concentrations varied across the six sample lakes and were greatest in White Iron Lake ($\bar{x}$ = 12.69 mg L^{-1}) and Peltier Lake ($\bar{x}$ = 12 mg L^{-1}). Peltier and White Iron lakes did not differ significantly ($p = 0.98$), but DOC concentrations were significantly higher in both lakes than all other lakes ($p < 0.001$). No significant differences occurred between Portage and Carrie ($p = 0.053$) and Tait and Hill ($p = 0.053$). DOC concentrations were significantly lower in Portage Lake ($\bar{x}$ = 4 mg L^{-1}) than Hill, Peltier, Tait, and White Iron ($p < 0.001$; Table 6, Figure 6).

Table 6. Mean, coefficient of variance (%), and ranges of nutrient concentration of six sample lakes: total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), ammonium (NH4), nitrates (NO3), dissolved organic carbon (DOC), and nitrogen: phosphorus molar ratio.

Lake	TP (µg L^{-1})	SRP (µgL^{-1})	TN (µgL^{-1})	NH4 (µgL^{-1})	NO3 (µgL^{-1})	DOC (mgL^{-1})	NP molar ratio
Carrie	22.65	2.22	2484.9	92.12	1580.65	5.82	264.88
	28.9	54.4	33.9	27.1	27.1	55.3	35.8
	9 – 34	1 – 5	1972 – 3441	12 – 211	1043 – 2751	4.2 - 18.6	140.03 – 557.8
Hill	22.46	2.12	597.8	24.08	0.58	6.97	67.27
	47.3	50.9	24.3	102.6	159.6	7.6	32.9
	12 – 46	1 – 5	422 – 984	4 – 97	0 – 2.6	5.5 - 7.8	33.8 – 114.5
Peltier	221.88	78.60	2095.4	57.71	4.42	12.34	24.51
	48.8	85.4	33.9	116.0	106.7	24.2	48.7
	64 – 458	4 – 204	838 – 3876	7 – 220	0 – 16	9.8 – 23.7	12.32 – 64.1
Portage	31.8	2.42	694.1	35	9.15	4.46	51.35
	24.0	47.9	11.3	52.6	194.2	17.6	27.2
	14 – 43	1 – 5	547 – 821	13 – 73	0 – 53	3.49 - 5.5	33.87 – 90.0
Tait	13.31	2.18	415.2	19.38	0.92	8.66	70.38
	15.4	81.2	12.3	59.5	137.4	11.9	17.3
	10 – 16	0 – 7	330 – 544	4 – 50	0 – 4	7.7 - 11.8	54.87 – 92.3
	21.00	3.32	587.2	23.54	10.92	12.69	62.87
White	16.9	33.3	8.9	55.7	115.0	6.1	12.0
Iron	16 - 30	2 - 6	476 - 681	7 - 50	0 - 44	11.8 - 14.5	50.2 – 76.6

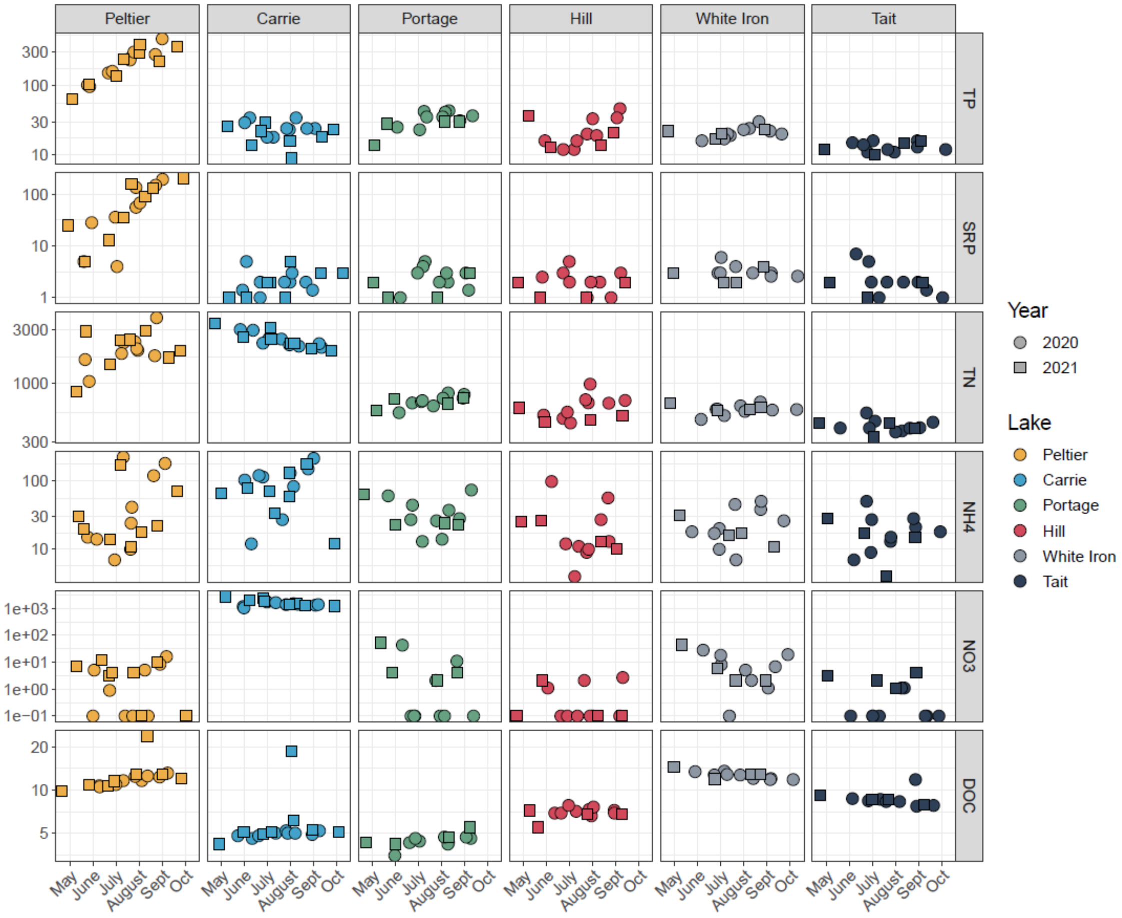


Figure 6. Nutrient trends of TP, SRP, TN, NH4, NO3 (µg L^{-1}) and DOC (mg L^{-1}) across six sample lakes for 2020 and 2021.

Nitrogen to phosphorus ratios

Total nitrogen-to-total phosphorus molar ratios (N:P) indicated differences in nutrient deficient growth conditions across study lakes (Figure 7). Guildford and Hecky (2000) described balanced growth versus nutrient deficient growth as N deficiency when N:P < 20, P deficiency when N:P > 50, and balanced nutrient availability when ratios fall between 20 and 50. The northern and central lakes (White Iron, Tait, Portage, Hill) with predominantly forested land cover had N:P ratios ranging from 37.04 (Hill) to 90.03 (Portage). White Iron and Tait lakes were classified as having P-deficient growth during all sampling events across both years (Figure 7). The central lakes, Portage and Hill, both showed opposite deficiency trends between years. Portage Lake predominantly consisted of balanced growth during 2020, except on 7/1/2020 and 9/29/2020 when P-deficient, and was predominantly P-deficient during 2021, except on 8/2/2021 when balanced. Hill Lake began as P-deficient during early summer, until 8/25/2020 when N:P decreased due to increasing TP (Figure 6) and shifted to a balanced nutrient regime for the remainder of the summer. The opposite effect was seen in 2021 when the lake was classified as balanced and shifted to P-deficient on 6/30/2021 due to a decrease in TP (Figure 6). However, the two southern lakes, Peltier and Carrie, with more developed land uses showed contrastingly different nutrient deficiency statuses. Carrie Lake was always P-deficient due to significantly higher TN concentrations ($p < 0.05$; Figure 6). Peltier Lake, on the other hand, had increasing TP and SRP concentrations throughout both sample years (Figure 6). A transition from balanced nutrient conditions early in the season to N-deficiently later in the season was observed during both sample years (8/11/2020, 8/4/2021), with the exception on 7/5/2021 when nutrient status was P-deficient due to an increase in TN (Figure 6 & 7).

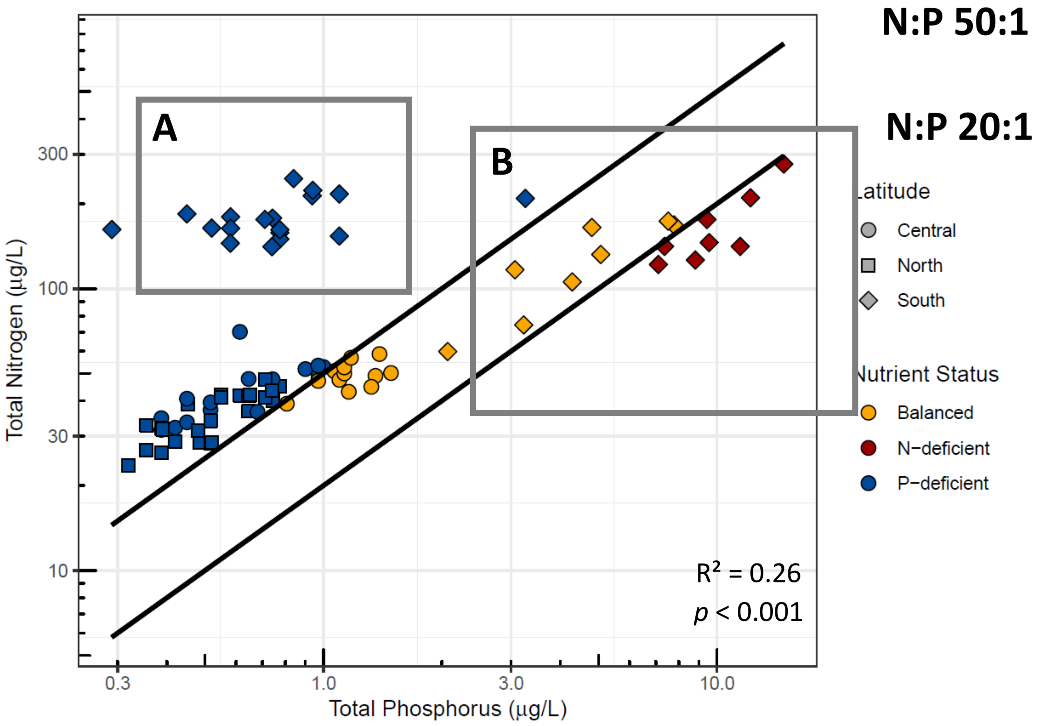


Figure 7. Total nitrogen versus total phosphorus concentrations across six study lakes. Symbol colors represent growth status based on total nitrogen-to-total phosphorus (TN:TP) molar ratios, indicating balanced growth (TN:TP = 20-50), N-deficient (TN:TP < 20), and P-deficient (TN:TP > 50) conditions (Guildford & Hecky 2000). Bounding boxes highlight the two southern Minnesota lakes: (A) Carrie and (B) Peltier.

3.3 Phytoplankton Pigments

Chlorophyll-*a* (chl-a) and phycocyanin (PC) pigments were measured as bulk indicators of phytoplankton and cyanobacteria abundances, respectively (Appendix 2). Sample lakes significantly differed in chl-a (*df* = 5, *F* = 38.65, $p < 0.001$) and PC (*df* = 5, *F* = 21.27, $p < 0.001$) concentrations.

Chl-*a* concentrations were significantly higher in Peltier Lake ($\bar{x}$ = 165 µg L^{-1}) than all other lakes ($p < 0.001$), and significantly lower in Tait Lake ($\bar{x}$ = 1.7 µg L^{-1}) than Carrie, Portage, and Hill lakes ($p < 0.001$). White Iron Lake ($\bar{x}$ = 4.5 µg L^{-1}) was also significantly lower than Carrie Lake

($\bar{x} = 12$ µg L^{-1}, $p = 0.006$; Table 7). PC concentrations were not significantly different among Carrie, Hill, Portage, and White Iron lakes. As for chl-a, PC concentrations in Peltier Lake ($\bar{x} = 556$ µg L^{-1}) were significantly higher than all other lakes ($p < 0.001$). Tait Lake ($\bar{x} = 1.7$ µg L^{-1}) was significantly lower than Carrie Lake ($\bar{x} = 12$ µg L^{-1}, $p = 0.022$; Table 7). All other lakes did not significantly differ.

Table 7. Mean, coefficient of variance (%), and ranges of chlorophyll-*a* and phycocyanin concentrations for six sample lakes during 2020-2021.

Lake	Chl-*a* (µgL^{-1})	PC (µgL^{-1})
Carrie	12.07	19.57
	61.7	116.3
	3 – 32.52	0.9 – 80.14
Hill	10.74	21.48
	83.2	112.2
	1.69 – 34.15	0.91 – 69.57
Peltier	165.32	556.82
	92.6	108.0
	10.29 – 550.64	0.81 – 2702.42
Portage	8.95	12.26
	61.2	98.5
	1 – 23.47	0.86 – 37.26
Tait	1.69	2.08
	31.8	50.0
	0.44 – 2.74	0.55 – 3.86
White Iron	4.45	13.66
	84.6	107.1
	0.25 – 15.26	1.17 – 53.87

Chl-a and PC were analyzed across time for both sample years to evaluate general phytoplankton and cyanobacteria biovolume trends and community dominance (Figure 8). PC and chl-a were significantly correlated ($p < 0.001$, $R^2 = 0.26$), although there was a lot of unexplained variance in the relationship. PC concentrations were greater than chl-a concentrations for most of the 2020 sample season across all lakes, indicating that cyanobacteria were a substantial component of phytoplankton communities. However, the revese trend was observed during 2021 in which chl-a concentrations were greater than PC concentrations across all lakes, indicating that cyanobacteria were a smaller component of a largely eukaryotic phytoplankton community. PC concentrations increased throughout the year in Carrie, Hill, Portage, Tait, and White Iron lakes during both years, indicating that cyanobacteria were becoming a larger portion of the community. Peltier Lake consistently had PC concentrations greater than or equal to chl-a concentrations indicating cyanobacteria dominance during both sample years.

Interannual variability of pigment concentrations within lakes was considered. Visualization of boxplots confirmed that average chl-a concentrations were significantly higher in Peltier Lake during 2021 ($\bar{x}$ = 262 µgL^{-1}) than 2020 ($\bar{x}$ = 97 µgL^{-1}; t = -2.73, *df* = 8.06, *p* = 0.03). No significant differences occurred within other sample lakes for chl-a and PC (Appendix 2).

The PC:Chl-a ratio was calculated for all lakes and analyzed across time (Figure 9). A greater PC: chl-a ratio indicates increasing proportions of cyanobacteria to total phytoplankton abundance (Figure 9). The PC: chl-a ratios were greater in 2020 than 2021 in all lakes. In general, PC: chl-a ratios surpassed a ratio of 1 before or during July 2020. However, in 2021, the PC: chl-a ratios did not surpass a ratio of 1 for most lakes until mid-July or later. Therefore, PC: chl-a ratios were higher earlier in 2020 than 2021 indicating that the cyanobacteria started developing earlier in 2020 than 2021 in sample lakes and were sustained for longer periods.

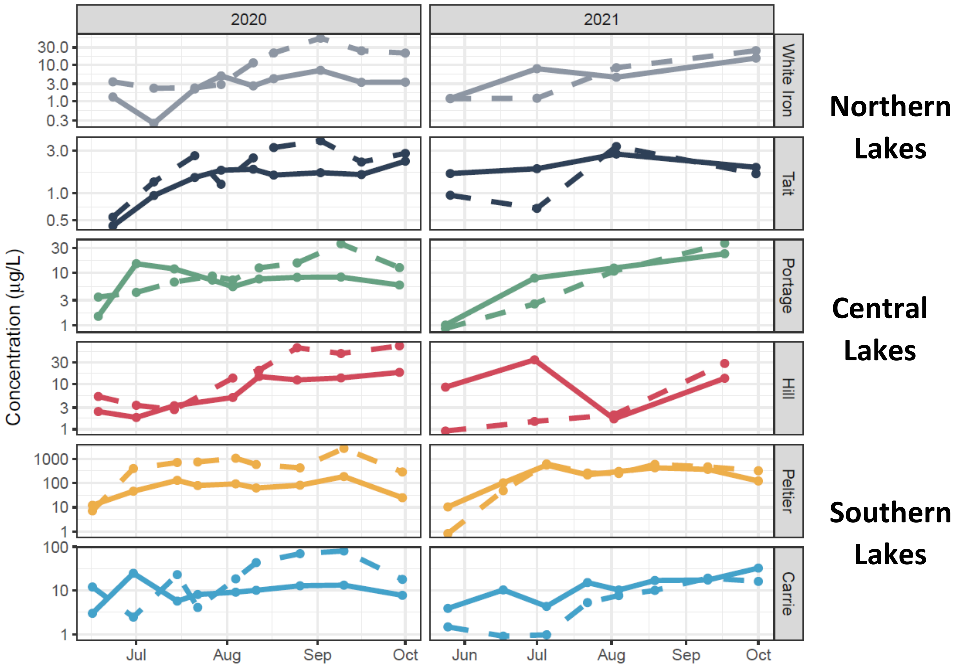


Figure 8. Chlorophyll-*a* (solid line) and phycocyanin (dashed line) trends for six sample lakes across two sample seasons. Y-scales differ to focus on seasonal variability.

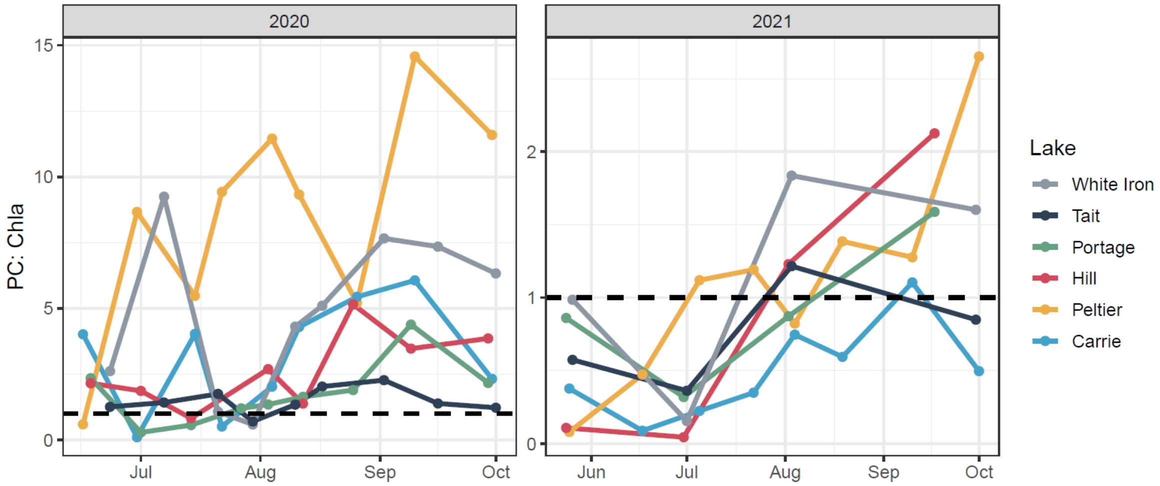


Figure 9. Phycocyanin (PC) to chlorophyll-*a* (Chla) ratio across time. PC:Chla ratio < 1 (above dashed line) indicates cyanobacteria-dominated communities.

3.4 Phytoplankton and Cyanobacteria Biovolumes

Biovolume Comparisons

Consistent with chl-a patterns, phytoplankton biovolume differed across sample lakes ($F = 30.331$, $df = 5$, $p < 0.001$). Peltier Lake, in southern Minnesota, had significantly higher biovolume than all other lakes ($p < 0.001$; Appendix 3). Carrie Lake also had significantly higher phytoplankton biovolume than all other lakes (Portage, $p = 0.013$; Tait, $p < 0.001$; White Iron, $p < 0.001$), except Hill Lake ($p = 0.26$) and Peltier. Lastly, Tait Lake had significantly lower phytoplankton biovolume than Hill Lake ($p = 0.004$). Phytoplankton biovolume across White Iron, Tait and Portage lakes did not differ significantly from each other ($p > 0.11$; Table 8). Cyanobacteria biovolume also differed across sample lakes ($F = 15.427$, $df = 5$, $p < 0.001$). Peltier Lake ($\bar{x} = 14$ mm^3 L^{-1}) had significantly higher cyanobacteria biovolume than all other lakes ($p < 0.001$). Carrie Lake ($\bar{x} = 1$ mm^3 L^{-1}, $p < 0.001$) and Hill Lake ($\bar{x} = 1$ mm^3 L^{-1}, $p = 0.016$) had significantly higher average cyanobacteria biovolume than Tait Lake ($\bar{x} = 0.05$ mm^3 L^{-1}). Average cyanobacteria biovolume in Portage, Tait, and White Iron did not significantly differ ($p > 0.11$; Table 8).

There was little variation in phytoplankton and cyanobacteria biovolumes between sample years, expect for Carrie Lake (Appendix 3). Average phytoplankton biovolume was significantly higher in 2020 ($\bar{x} = 8.45$ mm^3 L^{-1}) than 2021 ($\bar{x} = 3.01$ mm^3 L^{-1}). Average cyanobacteria biovolumes did not differ between years in Carrie Lake.

Table 8. Mean, coefficient of variance (%), and ranges of phytoplankton and cyanobacteria biovolumes (mm^3 L^{-1}) across both sample years.

Lake	Phytoplankton	Cyanobacteria
Carrie	5.99	1.22
	55.2	94.5
	1.01 – 13.78	0.05 – 4.49
Hill	4.63	1.20
	135.3	124.4
	0.69 – 25.37	0.004 – 5.44
Peltier	20.14	14.08
	50.5	75.6
	4.82 – 39.09	0.007 – 37.83
Portage	2.21	0.32
	155.9	108.0
	0.40 – 3.71	0.004 – 1.06
Tait	1.04	0.05
	40.0	90.0
	0.16 – 1.58	0.005 – 0.19
White Iron	1.93	0.86
	76.7	150.0
	0.16 – 6.11	0.003 – 4.96

3.5 Predictors of Phytoplankton and Cyanobacteria Biovolumes

Watershed-level, lake morphometry and in-lake growth condition predictors (Table 4) were assessed using random forest models (RFM) to determine predictor importance of phytoplankton and cyanobacteria biovolumes across sample lakes. Cyanobacteria biovolume was also assessed at the individual lake level to determine if lake-specific predictors differed from overall cyanobacteria biovolume predictors and among sample lakes.

Predictor variable importance was similar between phytoplankton and cyanobacteria biovolumes. TP and TN were the most important predictors for both response variables. Predictors of total phytoplankton biovolume explained 68% of total variance. In-lake growth conditions, such as nutrient availability (TP, TN, N:P), played the most important role in determining total phytoplankton biovolume, however, limnological variables (average conductivity) and watershed characteristics (% agriculture) were also included in the top five most important predictors (Figure 10A). The top predictors of phytoplankton biovolume, in decreasing order, are TP, TN, average conductivity, N:P, and % agriculture. The top predictors of cyanobacteria biovolume were TP, TN, secchi depth, % urban, average pH, average conductivity, N:P, NH4, maximum depth, and average depth, with 68% variance explained among the 21 predictor variables. However, the top five predictors are categorized as in-lake growth conditions (TP, TN, secchi depth, average pH) and watershed-level (% urban) predictors (Figure 10A). Peltier Lake, classified as hypereutrophic and which exhibited visible blooms throughout the duration of sampling, had differing cyanobacteria in-lake predictors than other sample lakes (White Iron, Tait, Portage, Hill and Carrie (Figure 11); Hill Lake experienced a surface bloom on 9/9/2020), and may have strongly influenced results of RFMs. To reduce the influence of this single lake on results, we further conducted RFM analyses to determine phytoplankton and cyanobacteria biovolume predictors of lakes excluding Peltier (Figure 10B). TN became the most important predictor for both phytoplankton and cyanobacteria biovolumes. Nitrates and average conductivity increased in importance, and TP and % urban decreased in importance for phytoplankton biovolume when considering these lakes. Explained variance also decreased, when excluding Peltier Lake, from 68% to 44% for phytoplankton biovolume and from 68% to 37% for cyanobacteria biovolume.

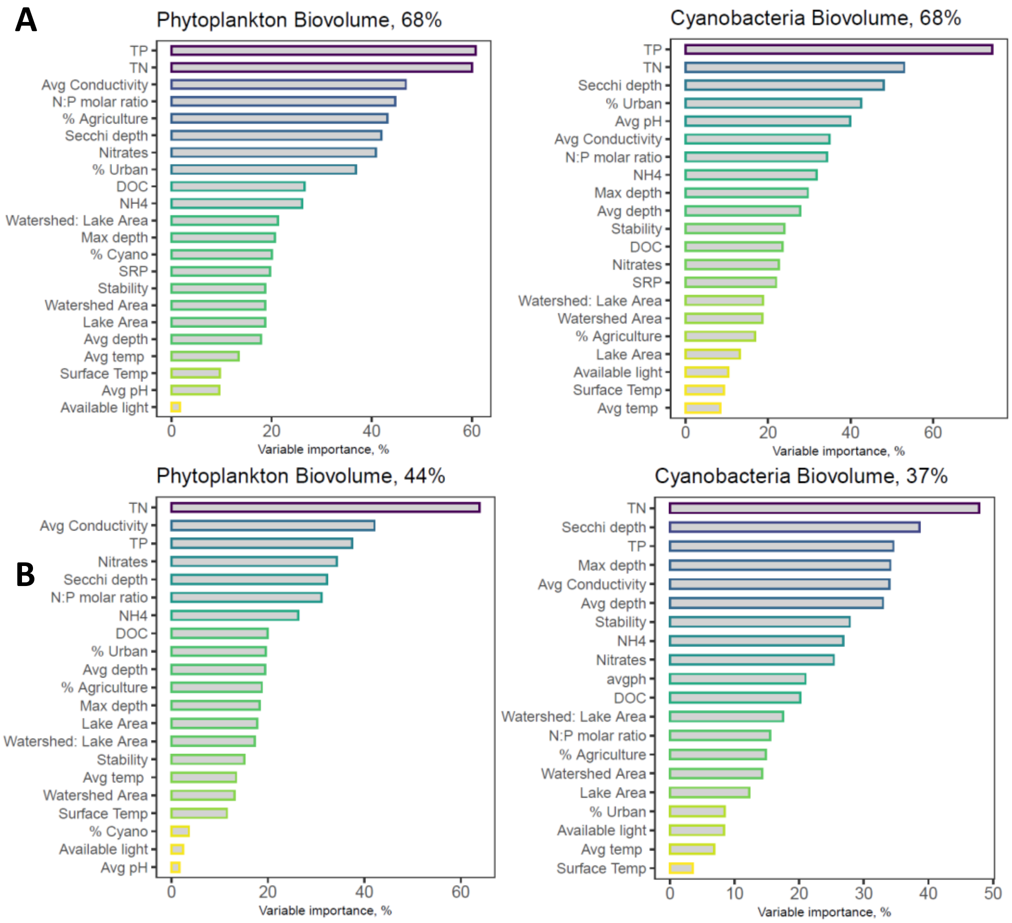


Figure 10. Random Forest model predictor importance of log_{10} transformed phytoplankton and cyanobacteria biovolumes across (A) six sample lakes and (B) five lakes, excluding Peltier.

Only in-lake growth conditions were considered when analyzing cyanobacteria biovolume within sample lakes because watershed and lake morphometry predictors remained constant across sampling events. Predictors of in-lake growth conditions differed across our six sample lakes. Carrie Lake had the greatest explained variance (44%) when considering the predictor variables in Table 4, followed by Hill (41%), Peltier (38%), White Iron (38%), Portage (25%), and Tait (17%) lakes (Figure 11). All lakes had a form of nitrogen (TN, NH_4^-, nitrates) present within the top three predictor variables. Average pH was determined as the most important predictor in Peltier Lake. Spearman rank correlation results show a significant positive relationship between average pH and cyanobacteria biovolume (p = 0.002, S = 236, ρ = 0.71). Average pH was not present among top five predictors Tait, Portage, or Hill lakes (Figure 11).

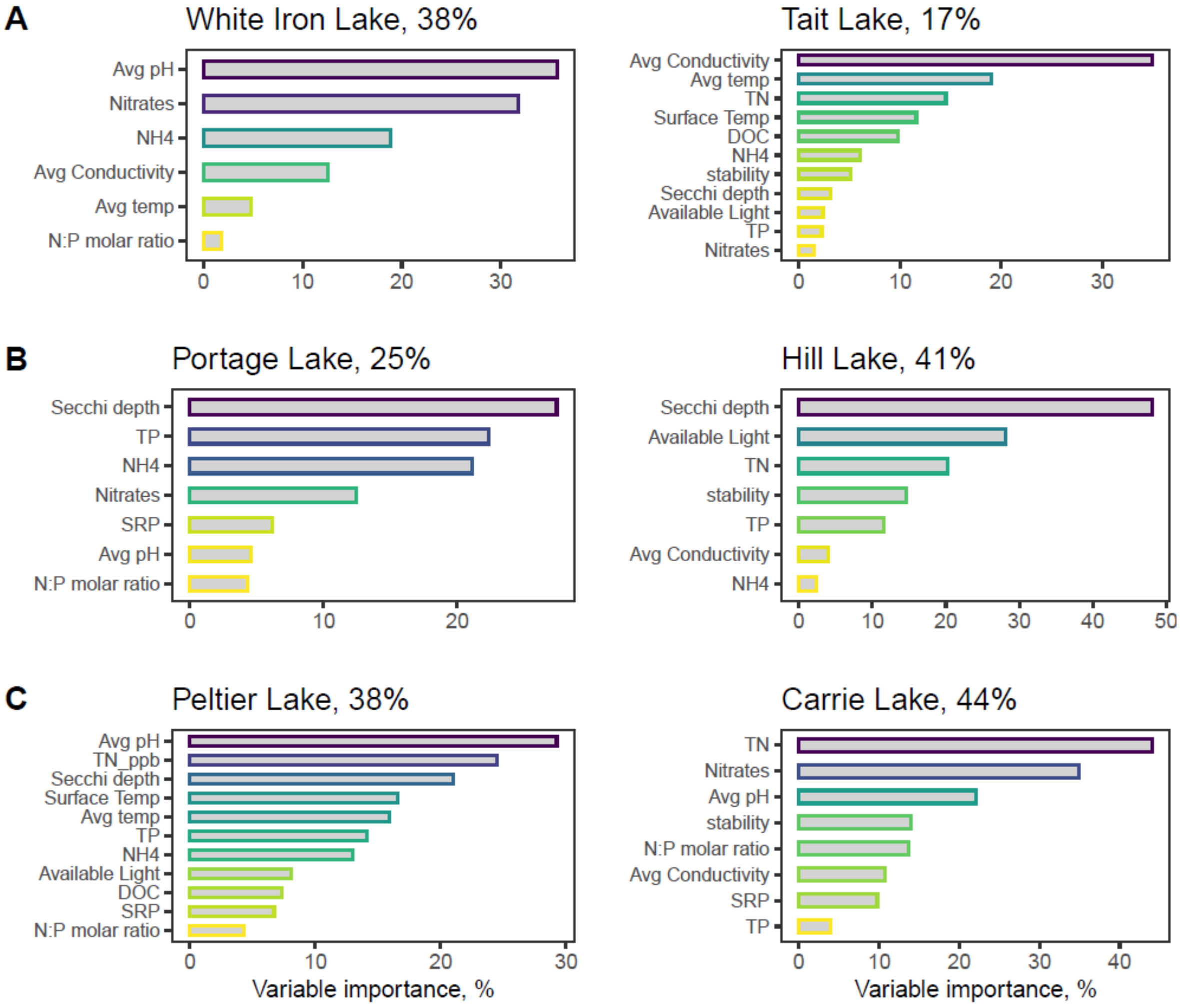


Figure 11. Random forest model of in-lake predictor importance of $\log_{10}$ transformed cyanobacteria biovolume. A) Northern lakes: White Iron and Tait, B) Central lakes: Portage and Hill, C) Southern lakes: Peltier and Carrie.

4 Discussion

Our results indicate that cyanobacteria biovolumes differed across lakes due to combinations of watershed characteristics, lake morphometry, and most importantly, in-lake processes and consequently require lake-specific models to accurately predict in-lake drivers of cyanobacteria biovolume. Our hypothesis that greater cyanobacteria biovolume would occur in higher developed land use (Peltier) was supported, however, results indicate significantly lower cyanobacteria biovolume in the agriculture-dominated watershed (Carrie; Table 8). Additionally, our hypothesis that southern lakes (Peltier and Carrie) would have greater cyanobacteria biovolume than northern lakes (White Iron and Tait) was supported (Table 8). Our third hypothesis, that cyanobacteria biovolume would be affected by mixing type and water column stability, was only supported for the southern and central lakes, and not supported for the northern lakes (Tables 2, 8). Lastly, our hypothesis that cyanobacteria biovolume would be positively related to TN and TP concentrations was supported, but the relationship with warmer water temperatures was not.

Watershed land use has been shown to influence lake productivity (Ewing et al., 2020), which becomes an increasing concern due to greater developed land uses. Thus, cyanobacteria biovolume was assessed across land use and latitudinal gradients. Land use application studies have shown that urban development leads to lower N:P ratios than agricultural land uses because the source of nutrient highly impacts the N:P ratio (J. A. Downing & McCauley, 1992). Our observations of Peltier and Carrie lakes were supported by this finding (Table 6). Peltier Lake, located in southern Minnesota and having the greatest proportion of developed land use in this study, had the greatest cyanobacteria biovolume across all lakes. Long-term monitoring shows that Peltier Lake experiences annual invasive curly-leaf pondweed growth and mid-season senescence, which may be partially fueling internal nutrient loading due to corresponding increases in TP concentrations (MPCA & MDNR, 2009). Additionally, the lack of stratification due to the shallow nature of Peltier Lake likely allows resuspension of released phosphorus, specifically SRP, from sediments. We observed increased levels of SRP concentrations within Peltier Lake (Figure 6), indicating N-deficient growth conditions, which was further supported by low N:P ratios (Figure 7). The combination of these in-lake characteristics and biological processes are likely contributing to the severe algal blooms present during the duration of spring to fall in Peltier Lake.

Carrie Lake watershed, also located in southern Minnesota and consisting of 65% agricultural land use, displayed significantly higher TN, NO_3^-, and NH_4^+ concentrations than all other sample lakes (Figure 6). We hypothesized that Carrie Lake cyanobacteria biovolume would be greatest due to surrounding agricultural land use, however, results indicate a factor constraining cyanobacteria biovolume. Previous monitoring by MPCA and MDNR (2012) predicted that high calcium carbonate concentrations in Carrie Lake may contribute to reduced light availability necessary for algal growth and calcium in calcium carbonate binding with and precipitating phosphorus from the water column, leading to lower P concentrations readily available to phytoplankton. Although calcium carbonate can precipitate during high productivity due to increases in pH, the N:P regime imbalances are not likely due to calcium carbonate presence. Based on TP concentrations, Carrie Lake is classified as mesotrophic to eutrophic (Table 6). Filstrup & Downing (2017) showed a high nutrient low chlorophyll-a (HNLC) regime in which chlorophyll-a was negatively influenced by TN, especially consisting of high NO_3^-, in eutrophic lakes than in oligotrophic lakes. These findings suggest that the low cyanobacteria biovolume in Carrie Lake may have been stressed by high reactive oxygen species (ROS) production due to high NO_3^- concentrations (Figure 6; Filstrup & Downing, 2017). This nutrient regime of high TP and even greater nitrate concentrations indicate that nutrient imbalances are driving algal biomass rather than calcium carbonate concentrations. In general, when analyzing the contrastingly different nutrient deficiencies in the southern lakes (Peltier and Carrie), it can be hypothesized that excessive TP concentrations lead to greater cyanobacteria biovolume, and excessive TN concentrations constrain cyanobacteria biovolume.

To address our third hypothesis, that cyanobacteria biovolume would differ between mixing statuses, we calculated Schmidt's stability index (SSI) for each sampling event and compared across time within lakes. It was hypothesized that dimictic lakes would result in greater cyanobacteria biovolumes due to more stable water column conditions that are likely to favor cyanobacteria (C. Wagner & Adrian, 2009). Average SSI values were higher in dimictic lakes (Carrie, Hill, and White Iron) when compared to corresponding polymictic lakes (Peltier, Portage, and Tait; Table 5), however, SSI did not correlate to bloom formation. There was no significant difference in cyanobacteria biovolumes in the northern lakes, White Iron and Tait, which indicates that differing mixing status and lake stability regimes did not affect cyanobacteria biovolume at the northern latitude (Table 8). The central lakes, Portage and Hill, exhibited expected trends because Hill Lake (dimictic) had greater cyanobacteria biovolume than Portage Lake (polymictic; Table 8). Hill Lake experienced a surface bloom on 9/9/2020 corresponding to a drastic decrease in SSI. This decrease in stability did not follow expected trends because high stability is advantageous to buoyancy regulating cyanobacteria. This decrease in stability likely indicated that fall turnover led to increased nutrient availability for cyanobacteria uptake, and other abiotic conditions (e.g., temperature) were still conducive to bloom formation. Additionally, because a bloom was present on 9/9/20, locations were moved to sample bloom conditions. The new sample site was shallower compared to the original sampling location. A similar turnover event occurred in 2021 however no surface bloom was observed during sampling. Peltier (polymictic) and Carrie (dimictic) lakes exhibited decreasing SSI trends over time indicating less stable water columns forming through the duration of summer. Although similar SSI trends occurred, Peltier Lake averaged higher cyanobacteria biovolume than Carrie Lake and exhibited visible blooms. SSI values for Peltier and Carrie were relatively low compared to Hill and White Iron, possibly leading to differences in anticipated relationships. Peltier and Carrie lakes, and Portage and Hill lakes differed in cyanobacteria biovolume, however, opposite trends based on mixing type were observed across the latitudinal gradient (i.e. Peltier (polymictic) had greater biovolume than Carrie; Hill (dimictic) had greater biovolume than Portage). However, studies have shown cyanobacteria blooms are influenced by numerous factors (Beaulieu et al., 2013), therefore, relationships with a single variable may not be as predicted and may not hold true for individual lakes, implying multivariate approaches are necessary.

Previous research has shown the importance of lake morphometry, watershed land use, and abiotic conditions on cHAB formation and maintenance (Arbuckle & Downing, 2001; Ewing et al., 2020; Rose et al., 2019). RFMs were used to assess watershed-level, lake morphometry, and in-lake growth condition influences on cyanobacteria biovolume across all sample lakes and at the lake-specific level. When considering all lakes, the top predictor of cyanobacteria biovolume was TP, as supported by previous literature (Beaulieu et al., 2013; Gobler et al., 2016). However, when Peltier was removed from the model and remaining lakes were analyzed, TN became the most important predictor (Figure 10), indicating that Peltier Lake, due to significantly higher TP concentrations and cyanobacteria biovolume, may be dominating the RFM when analyzing all six lakes. Our research, therefore, further emphasizes the importance of managing for both N and P, rather than traditional P-only approaches, to better understand the complex interaction between cyanobacteria and nutrient availability across differing lake systems.

Further lake-specific models were analyzed and showed important predictors of cyanobacteria biovolume differed across sample lakes. Although our first predictive models including all sample lakes were good at predicting cyanobacteria biovolumes across large spatial scales (Figure 10; 68% explained variance), which supported current nutrient paradigms regarding the importance of N and P on phytoplankton abundance and composition, they did not however consider temporal dynamics, such as seasonality of bloom drivers, and were not suited to predict bloom dynamic in individual lakes. Our models for individual lakes and previous research forecasts for individual lakes, such as Lake Erie (Stumpf et al., 2016), have shown some success, however, these models cannot be directly applied to other lakes due to reasons previously mentioned. Therefore, these results suggest individual-lake models are necessary to accurately forecast in-lake cHAB predictors (Figure 11). Although some models do well at predicting over all cyanobacteria biomass response at large scales, these models will not fully explain mechanisms leading to bloom phenology in individual lakes and likely will not predict bloom toxicity. Our inclusive model of all six lakes predicted greater explained variance than models of the individual lake scale (Figures 10, 11). However, changing climate conditions may also be changing main drivers leading to cyanobacteria development and expansion into areas where they were previously absent (Lake Superior; Sterner et al., 2020), therefore, this indicates that older models and paradigms may not apply anymore. RFM results demonstrated that pH was the most important predictor of cyanobacteria biovolume in Peltier Lake and White Iron Lake, however, it is hypothesized that the high pH in Peltier Lake is driven by biological productivity. Due to significant levels of algal biovolume within the lake, it is likely that CO_2 is being removed from the system via photosynthesis and leading to more alkaline water conditions (Shapiro, 1997). The high pH in White Iron Lake is not likely biologically influenced due to lower algal biovolume observed, therefore it is hypothesized that high pH is driven by terrestrial inputs. Interestingly, TP was not within the top five important predictors in Peltier Lake (Figure 11), likely because it was consistently high over time, therefore it is not anticipated to explain variance in cyanobacteria biovolume. On the other hand, the top three predictors in Carrie Lake include TN and nitrates and are likely due to the significantly higher TN concentrations due to agricultural runoff (Figure 11).

Several limitations existed within this study and indicated further research necessary to expand our hypotheses. Chapter 3 discusses cyanobacteria community assemblages, bloom phenology, and cyanotoxin production to better understand community dynamics, such as how cyanobacteria physiology is impacted by cHABs in these lakes. Having a more in-depth understanding of species-specific functional traits will improve understanding of lake-specific drivers. However, overall, our study design lacked large spatial variation due to the feasibility of a latitudinal gradient sampling regime. Biweekly monitoring allowed for temporal variation from spring to fall, however, we were still limited by the number of observations. Continuous real-time water quality sensors would allow future studies to detect and observe immediate, minor fluctuations in nutrient changes that may not have been captured with biweekly sampling. Nutrient concentrations and forms are constantly influenced by algal biomass and in-lake growth conditions (nitrogen fixation, denitrification, sedimentation), which makes it difficult to accurately measure useable forms of essential nutrients.

5 Conclusions

Our findings showed that cyanobacteria biovolumes were (a) greatest in the southern lake with the greatest developed land use (Peltier Lake), (b) influenced by mixing type in the central and southern lakes (however showing opposite biovolume and mixing status trends), and (c) were likely constrained under high TN concentrations in agricultural dominated land use (Carrie Lake). The variation among nutrient concentrations and forms, lake morphometric characteristics, and watershed contributions suggests that lake-specific drivers of cyanobacteria biovolume and formation vary across latitude, mixing type, land uses. Our study emphasizes the necessity of lake-specific management plans to mitigate and prevent future blooms, and to understand lake-specific nutrient regimes influencing cyanobacteria. Future management plans should be developed at the individual lake level with an understanding of cyanobacteria species composition and functional traits to be most effective at bloom reduction.

Chapter 3: Cyanobacteria bloom phenology and toxin production across six temperate Minnesota lakes

1 Introduction

Cyanobacteria harmful algal blooms (cHABs) are a common phenomenon across eutrophic aquatic systems (Huisman & Hulot, 2005) and are increasingly causing concern among public health agencies due to potential cyanotoxin production (Carmichael, 2001), which can be harmful to both humans and aquatic life. While natural resource management agencies have been concerned about excess phytoplankton biomass for decades, increased dominance by cyanobacteria strains, as well as potentially toxic waters, have become an emerging management priority in many regions (Brooks et al., 2016; Donald et al., 2011). However, cyanobacteria community assemblages are temporally and spatially heterogeneous, making the development and senescence of cyanobacteria communities and toxin presence or absence difficult to predict across systems due to differing watershed, lake morphometric, and nutrient dynamic characteristics. Therefore, understanding bloom phenology (cyanobacteria bloom formation, maintenance, and senescence) across lake systems and ecological gradients may help researchers better identify drivers of cHABs composed of different cyanobacteria strains and their resulting toxicity potential.

Drivers of cyanobacteria community dominance have been found to differ across genera and species taxonomic groups (Reynolds, 1998) and include environmental and biological factors such as nitrogen form and availability (Glibert et al., 2016; Newell et al., 2019; Wagner et al., 2021), resource uptake ability (Holland et al., 2012; Huisman & Hulot, 2005; Reynolds, 1998), water temperature (Paerl & Otten, 2016), and colony-forming abilities to increase buoyancy (Reynolds et al., 1987) and decrease grazing rates (Lampert, 1987). Nutrient concentrations and speciation have been predicted as major drivers of cyanobacteria community structure. Reynolds (1998) classified major cyanobacteria into dominant genera present at varying energy (light) and nutrient availability, including trophic statuses: eutrophic= *Microcystis*, *Pseudanabaena*, and *Limnothrix*; mesotrophic= *Aphanizomenon*, *Dolichospermum*, and *Comphosphaeria*; and oligotrophic= *Merismopedia*. In addition to cyanobacteria nutrient regime classification, some species have adapted to extreme light conditions, such as *Prochlorococcus* which can withstand low-light environments (Bibby et al., 2003).

However, when nutrient conditions are deficient, specifically in N, cyanobacteria may provide a unique service to the phytoplankton community called nitrogen fixation (N-fix), a metabolic process in which atmospheric N_2 is converted (or ‘fixed’) to ammonia, a readily usable form by other phtyoplankters. Common diazotrohpic (N-fixing) cyanobacteria genera include *Dolichospermum*, *Aphanizomenon*, *Anabaena,* and *Oscillatoria*. It has been suggested that cyanobacteria with diazotrophic abilities can add nitrogen to the system when under N-limited conditions (Hoffman et al., 2022). However, studies have shown the long-standing dogma that nitrogen fixers will dominate only under nitrogen limited conditions may not always hold true (Harding et al., 2014; Natwora & Sheik, 2021; Paerl et al., 2004; Paerl & Otten, 2016). Therefore, mechanisms initiating nitrogen fixation remain poorly understood and indicate that other abiotic and biotic drivers of cyanobacteria community assemblage may have stronger influences under certain conditions. It remains unclear when and why diazotrophs gain competitive advantages over other eukaryotic algae and non-N-fixing cyanobacteria by fixing atmospheric nitrogen (i.e., what metabolic requirements are fulfilled by N fixation?).

Cyanobacteria also have the unique ability to produce various toxic compounds and metabolites (a.k.a. cyanotoxins) in both freshwater and marine systems that are harmful to humans and other wildlife (Carmichael & Boyer, 2016). Commonly analyzed cyanotoxins include neurotoxins and hepatotoxins (liver toxins) such as saxitoxins, cylindrospermopsins, anatoxin-a, and the most observed, microcystins, with over 80 different known congeners (Table 9, Dietrich & Hoeger, 2005). Microcystin congeners are nitrogen and carbon (C) rich secondary metabolites, therefore, congener chemical composition is influenced by differing C:N stoichiometries. Microcystin-RR (MC-RR) is a N-rich compound compared to other congeners and have been shown to increase under high N and low C conditions (high N:C) (Van de Waal et al., 2009). Because of chemical differences, congeners also vary in toxicity, proving that congener-specific research is necessary to comprehensively understand MC toxicity levels. LD-50 studies have shown greater toxicity in variants of the MC-LR congener (Bouaïcha et al., 2019; Christensen & Khan, 2020), which is also most commonly targeted by management agencies. However, the variability of cyanotoxin production makes it difficult for managers to predict toxic bloom events and properly inform the public about potentially toxic and dangerous waters, leading to unwanted cyanotoxin exposure for humans, pets, and wild animals. Although large-scale models have previously been used to predict bloom occurrence and toxicity, these models cannot accurately be applied to individual lakes due to differing cyanobacteria assemblages and metabolic capabilities.

The ecology of the cyanobacteria community assemblage plays a vital role in whether cyanotoxins may be present or even produced. Toxin production varies at the cyanobacteria strain level, meaning there are both toxic and non-toxic strains of the same species that are morphologically indistinguishable. Researchers currently do not have a complete understanding of the competition between toxic and non-toxic strains (Lei et al., 2015; Sukenik et al., 2015; Van de Waal et al., 2011), further making it difficult to predict when toxic-producing strains are present. Additionally, there is also a temporal component to cyanotoxins, meaning that cyanobacteria strains capable of producing toxins (i.e., have toxin production genes) only produce toxins under certain environmental conditions. Differing cyanobacteria species may also produce differing cyanotoxins (Table 9). Some genera, such as *Aphanizomenon*, have been found to produce multiple types of cyanotoxins including cylindrospermopsin and anatoxin-a (Table 9). Considering the composition of cyanobacteria blooms is necessary to better predict bloom toxicity. Current research indicates that toxin production is driven by both chemical and biological mechanisms, including nitrogen form and concentration (Donald et al., 2011; Newell et al., 2019; Steffen et al., 2017; N. D. Wagner et al., 2021), as well as cyanophage infection (Steffen et al., 2017) and zooplankton presence (Jang et al., 2003, Table 10).

However, further research is necessary to expand current knowledge on toxin production.

Table 9. Cyanotoxin genera producers (Carmichael, 2001; Codd et al., 2005)

Cyanotoxin	Genera producers
Hepatotoxins	
Microcystin	*Microcystis, Anabaena, Nostoc, Aphanocapsa, Oscillatoria (Planktothrix), Anabaenopsis, Hapalosiphon*
Cylindrospermopsin	*Aphanizomenon, Cylindrospermum, Raphidiopsis, Umezakia*
Neurotoxin	
Anatoxin-a	*Anabaena, Aphanizomenon, Oscillatoria (Planktothrix)*

Table 10. Chemical and biological mechanisms of cyanotoxin production.

	Mechanism	Reference
Chemical	Nitrogen concentration	Donald et al., 2011; Glibert et al., 2016; Van de Waal et al., 2009
	Nitrogen form - Nitrates - Ammonium	Newell et al., 2019; Steffen et al., 2017 Beversdorf et al., 2015; N. D. Wagner et al., 2021 Donald et al., 2011
Biological	Cyanophage infection	Jiang et al., 2019; Steffen et al., 2017
	Zooplankton presence	Jang et al., 2003; Lampert, 1987

To address knowledge gaps of cyanobacteria phenology (development, maintenance, senescence) and triggers and timing of toxin production, we analyzed cHAB seasonality and drivers of cyanotoxin presence and concentrations across six temperate Minnesota lakes varying in latitude, land uses, and lake morphometry. We took an integrated, multidisciplinary approach by collecting limnological data, conducting nutrient analyses and phytoplankton quantification counts, and analyzing multiple cyanotoxins and microcystin congeners. Our primary objectives of this chapter were to (1) analyze phytoplankton and cyanobacteria bloom phenology characteristics across six sample lakes, (2) assess environmental drivers of cyanobacteria community composition, and (3) analyze seasonal variability and predict environmental and community drivers of cyanotoxin concentrations. I hypothesized that (1) cyanobacterial blooms will begin earlier in the season and last longer into the fall in southern lakes than northern lakes and that cyanobacteria communities will differ across a latitudinal gradient, such that different lakes will have different dominant cyanobacteria, (2) primary drivers of cyanobacteria community assemblages will be in-lake processes including nutrient availability and composition, and (3) toxin concentrations will be greatest in southern lakes than northern lakes and primary drivers of cyanotoxin concentrations with be nitrogen form and availability.

2 Methods

Study lakes and field sampling locations analyzed throughout this chapter are as described in Chapter 2; methods for field sampling and laboratory analyses of nutrients and phytoplankton quantification are also described in Chapter 2. Briefly, mixed surface water samples were taken using a two-meter integrated surface water column sampler at 0.5-m intervals to the thermocline depth (2 m maximum). Water samples were collected, stored on ice in the dark, and returned to the lab for further nutrient, pigment, toxin analyses, and phytoplankton quantification. Phytoplankton samples were prepared using the Utermöhl settling chamber technique and quantified at 400x on an inverted microscope. Cyanobacteria were identified to species level, when possible, and all other phytoplankton were identified to genus level (refer to Chapter 2 for more details). Toxin water samples remained frozen until analyses and went through a freeze-thaw cycle three times to lyse all cyanobacterial cells to release intracellular cyanotoxins. All samples were filtered before analyses.

Cyanotoxin Quantification

Water samples across all lakes and both sample seasons were analyzed for seven microcystin congeners (MC-LA, MC-LF, MC-LR, MC-LW, MC-LY, MC-RR, and MC-YR) via high performance liquid chromatography- quadrupole time of flight mass spectrometer (HPLC-qTOF-MS) following EPA Method 544). Additional toxin analyses were conducted on water samples from 2021 for cyanotoxins using Eurofins Abraxis kits (enzyme linked immunosorbent assays, ELISAs) for microcystins (No. 520011), anatoxin-a (No. 520060), and cylindrospermopsin (No. 522011). Northern lakes (White Iron and Tait) were not analyzed for anatoxin-a and cylindrospermopsin due to lower cyanobacteria biovolume and the lack of visible surface blooms during sampling. Samples were run in triplicate and average concentrations were reported.

Statistical Methods

One-way ANOVAs were used to assess differences in average phytoplankton and cyanobacteria biovolume across years within sample lakes (see Chapter 2 for more details), and community composition time series were used to assess phenology changes of phytoplankton and cyanobacteria assemblages. Non-metric multidimensional scaling (NMDS) was used to assess drivers of cyanobacteria species composition. Random forest models (RFM) were used to determine predictor importance of the cyanobacteria community composition, specifically *Dolichospermum* and *Microcystis* biovolumes in Peltier Lake, and cyanotoxin concentrations across all sample lakes. Drivers of species composition and cyanotoxins were categorized into predictor groups including watershed-level, lake morphometry, and in-lake growth conditions (Table 4). *Dolichospermum* and *Microcystis* top four predictor variables (greater than 20% importance) were analyzed for significance using Spearman’s correlation. A 0.1 was added to Peltier Lake genera biovolume to remove zero values prior to $\log_{10}$ transformation. All response variables were $\log_{10}$ transformed before conducting RFM analyses to remove skewness.

Table 4. Categorical predictor groups of cyanotoxin concentrations: watershed-level, morphometry, and in-lake growth conditions (also seen in Chapter 2).

Category	Predictor
Watershed-level	- Watershed area - Watershed: Lake area - Percent agriculture - Percent urban
Lake morphometry	- Lake area - Avg. depth - Max. depth
In-lake growth conditions	- Nutrients: TP, TN, SRP, NH4, NO3, DOC, N:P - Surface temp - Limnological variables: avg conductivity, pH, DO - Schmidt stability - Available light - Secchi disk depth

3 Results

3.1 Phenology and Community Composition

Peltier Lake

The Peltier Lake phytoplankton community was dominated by diatoms in early summer and transitioned to cyanobacteria dominance from June to October for both sample seasons (Figure 13A). The lake experienced nuisance algal blooms beginning in June and continuing into fall for both years. Overall, Peltier Lake phytoplankton biovolume reached a greater maximum in 2021 than 2020, however, there was no significant difference in average phytoplankton biovolume (t = -1.61, df = 8.49, p = 0.14) between 2020 ($\bar{x}$ = 16.30 mm^3L^{-1}) and 2021 ($\bar{x}$ = 24.45 mm^3L^{-1}). Both years follow a general pattern of having a decrease in biovolume occurring mid-July (Figure 12A).

The cyanobacteria community in Peltier Lake mainly consisted of *Dolichospermum* and *Microcystis* species, including *D. Mendota*, *D. circinale*, *D. crissum*, *M. aeruginosa*, and *M. wesenbergii* (Figure 12B). The community shifted from *Dolichospermum* dominant (a potential nitrogen fixer) to *Microcystis* dominant (non-nitrogen fixer) during early August for both sample years. The decrease in overall phytoplankton biovolume during 2020 and 2021 corresponds to the cyanobacteria community shift from *Dolichospermum* to *Microcystis.* There was ephemeral dominance by *Aphanocapsa elastica* and *Aphanizomenon flos-aquae* during the 2020 sample season. During the final sampling event for both sample years (9/30/20 and 10/1/21), the cyanobacteria community consisted of ~50% *Oscillatoria sp.* Cyanobacteria biovolume also reached a greater maximum in 2021 than 2020, however, there was more interannual variation in biovolume during 2020. There was no significant difference between average cyanobacteria biovolume (t = -1.98, df = 9.08, p = 0.079) between 2020 ($\bar{x}$ = 9.35 mm^3L^{-1}) and 2021 ($\bar{x}$ = 19.39 mm^3L^{-1}) in Peltier Lake (Figure 12B).

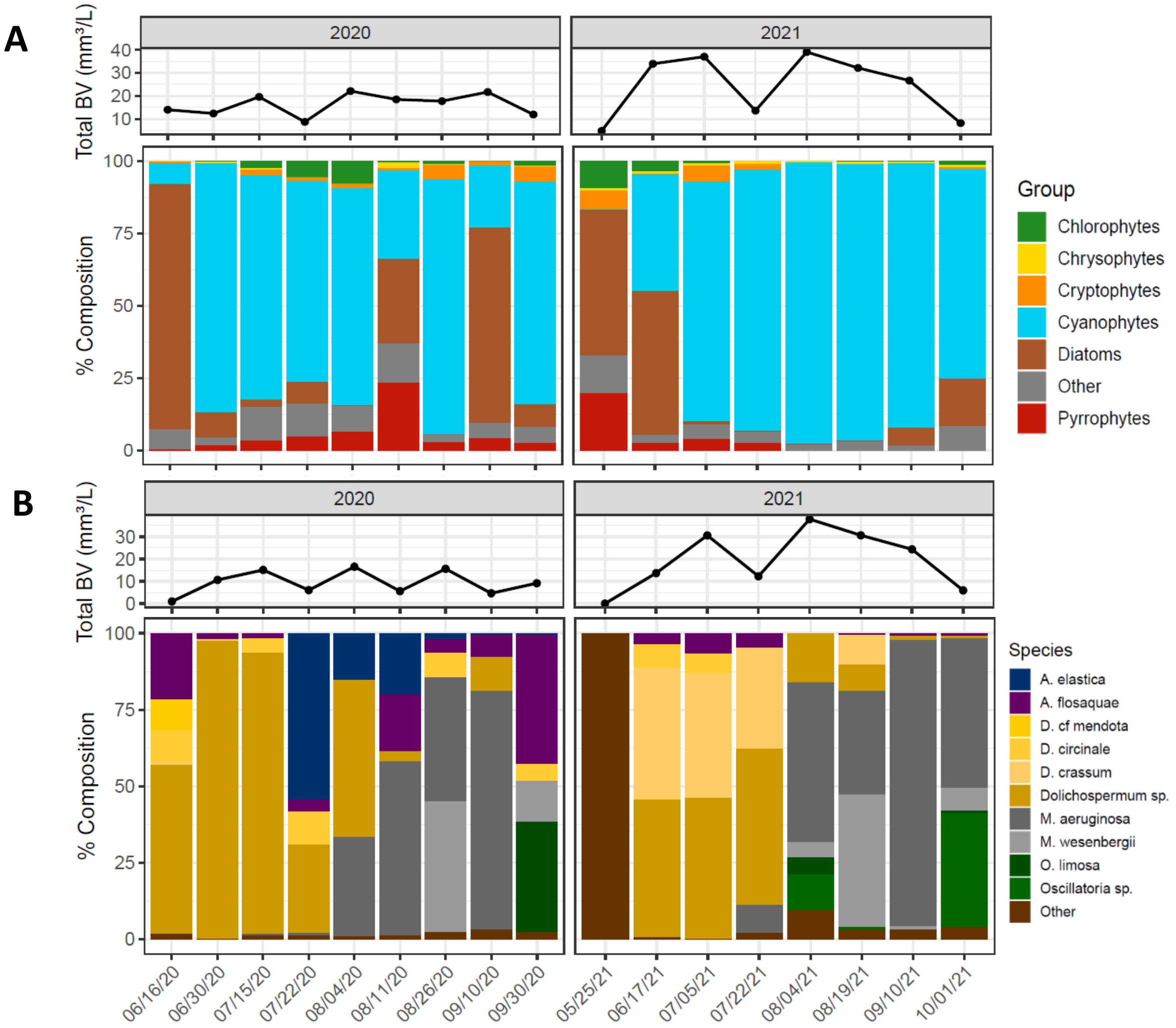


Figure 12. Peltier Lake 2020 and 2021 (A) phytoplankton groups and (B) cyanobacteria genera by biovolume (mm³/L) and community percent (%) composition (B. *Aphanocapsa elachista, aphanizomenon flos-aquae, Dolichospermum c.f. mendota, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum spp., Microcystis aeruginosa, Microcystis wesenbergii, Oscillatoria limosa, Oscillatoria sp.*, Other: *Aphanocapsa delicatissima, Aphanothece, Merismopedia sp., Pseudanabaena limnetica, Pseudanabaena mucicola, Planktothrix sp., Synechococcus, Woronichinia naegeliana, Woronichinia sp.).*

Carrie Lake

During 2020, the phytoplankton community within Carrie Lake was predominantly diatom dominated with an increase in cyanobacteria presence beginning mid-August (Figure 13A). Sample season 2021 did not follow the same trend, as the community had greater variation in phytoplankton group presence, including a lower proportion of diatoms and a greater proportion of dinoflagellates (pyrrophytes) and other phytoplankters (Other). Cyanobacteria ranged from 25-50% of the community from July to October both years. The overall phytoplankton biovolume in Carrie peaked at the end of June with a second increase in biovolume occurring mid-August 2021. The biovolume remained relatively constant for the remainder of the season. The phytoplankton biovolume in Carrie Lake was significantly lower (t = 5.21, df = 12.76, p < 0.001) in 2021 ($\bar{x}$ = 3.21 mm^3L^{-1}) than 2020 ($\bar{x}$ = 8.46 mm^3L^{-1}), with biovolume fluctuations between 1 and 5 mm^3L^{-1} during 2021 (Figure 13A).

The Carrie Lake cyanobacteria community consisted of *Pseudanabaena limnetica* during most of the summer and into fall (May – October) for both 2020 and 2021 (Figure 13B). *Microcystis* was present mid-August for both years, whereas *Dolichospermum spp*. and *O. limosa* were present in 2020 and not 2021. *O. limosa* made up >75% of the community at the end of September 2020. *Spirulina sp.* was present periodically during 2021, but increased beginning in early July into October 2021. There was no significant difference in average cyanobacteria biovolume (t = 1.57, df = 11.13, p = 0.14) between 2020 ($\bar{x}$ = 1.62 mm^3L^{-1}) and 2021 ($\bar{x}$ = 0.78 mm^3L^{-1}) in Carrie Lake (Figure 13B).

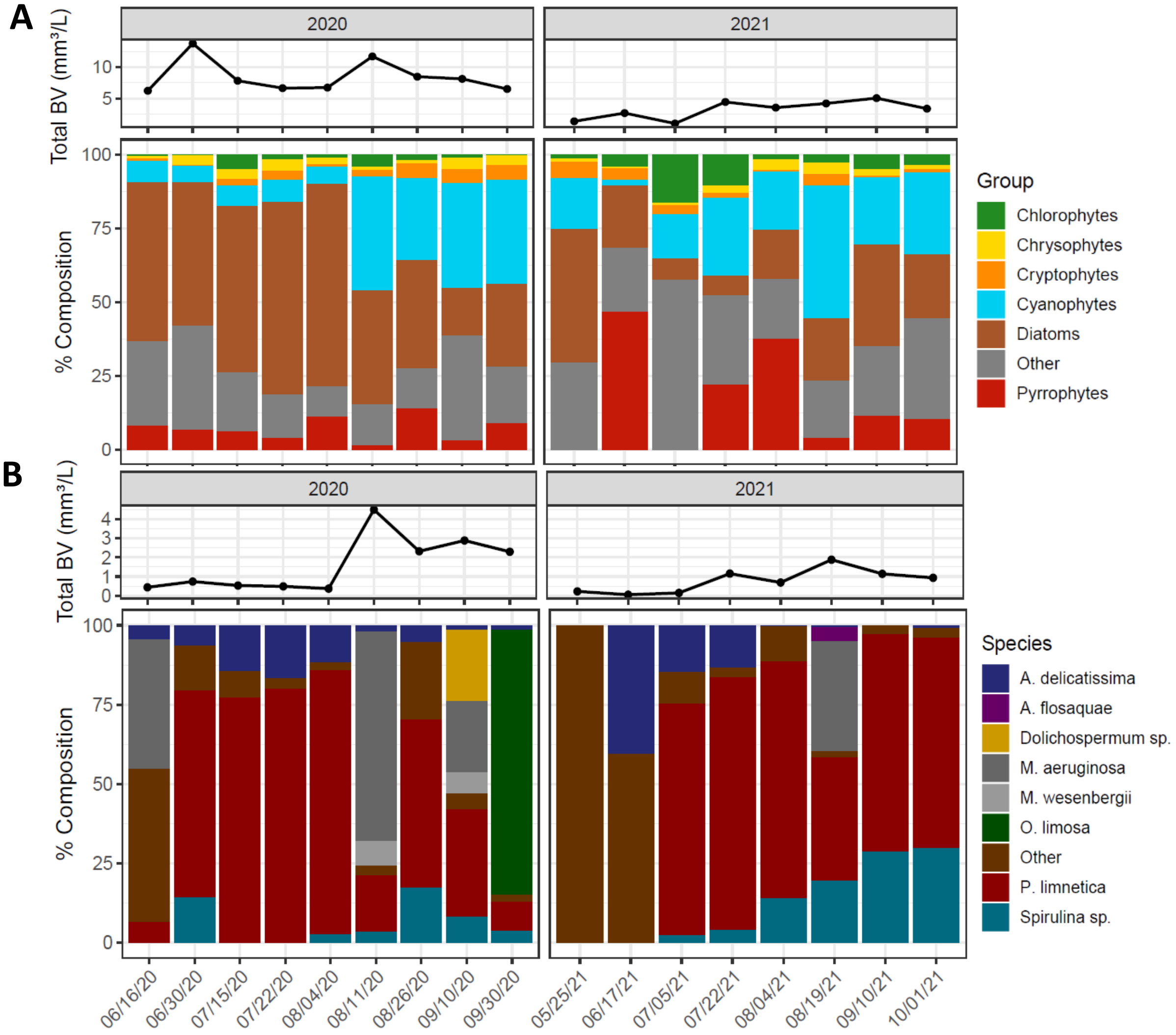


Figure 13. Carrie Lake 2020 and 2021 (A) phytoplankton group and (B) cyanobacteria genera by biovolume (mm³/L) and community percent (%) composition (B. *Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum spp., Microcystis aeruginosa, Microcystis wesenbergii, Oscillatoria limosa, Pseudanabaena limnetica. Spirulina sp.,* Other: *Aphanothece sp., Aphanocapsa elastica, Merismopedia sp., Merismopedia tenuissima, Synechococcus sp., Snowella lacustris, Woronichinia naegeliana*).

Hill Lake

The Hill Lake phytoplankton community assemblages were diverse over time for both sample seasons. As seen in Peltier and Carrie lakes, the phytoplankton community was diatom dominated early in the season (May and June) for both sample years (Figure 14A). Beginning in July 2020, phytoplankton groups mainly consisted of cyanobacteria, dinoflagellates, and others with percent composition fluctuating for all groups. Generally, the percent composition of cyanobacteria increased throughout the season. A less distinct pattern is observed in 2021, as the phytoplankton composition remained diverse throughout the summer. However, the final sampling event in mid-September 2021 consisted of > 50% Cyanophytes. There was no significant difference in average phytoplankton biovolume ($t = -0.77$, $df = 3.08$, $p = 0.50$) between 2020 ($\bar{x} = 3.23$ mm^3L^{-1}) and 2021 ($\bar{x} = 7.80$ mm^3L^{-1}) in Hill Lake (Figure 14A). However, late May 2021 had a total phytoplankton biovolume of 25.4 mm^3L^{-1} with 97% of the community being diatoms (consisting of *Fragilaria sp*.).

The cyanobacteria community was mainly comprised of *Dolichospermum spp*. and *A. flos-aquae,* both known nitrogen fixers, during 2020 (Figure 14B). Hill Lake was observed to have less cyanobacteria species evenness during 2021, however*, Dolichospermum sp., D. viguleri,* and others were primarily dominant during sampling events. The community shifted to *Oscillatoria sp*. at the end of September 2020 and *O. limosa* was also present at > 25% of the community mid-September 2021. Cyanobacteria biovolume increased throughout the summer during 2020 and 2021. There was no significant difference in average cyanobacteria biovolume ($t = 1.15$, $df = 9.20$, $p = 0.28$) between 2020 ($\bar{x} = 1.48$ mm^3L^{-1}) and 2021 ($\bar{x} = 0.58$ mm^3L^{-1}) in Hill Lake (Figure 14B).

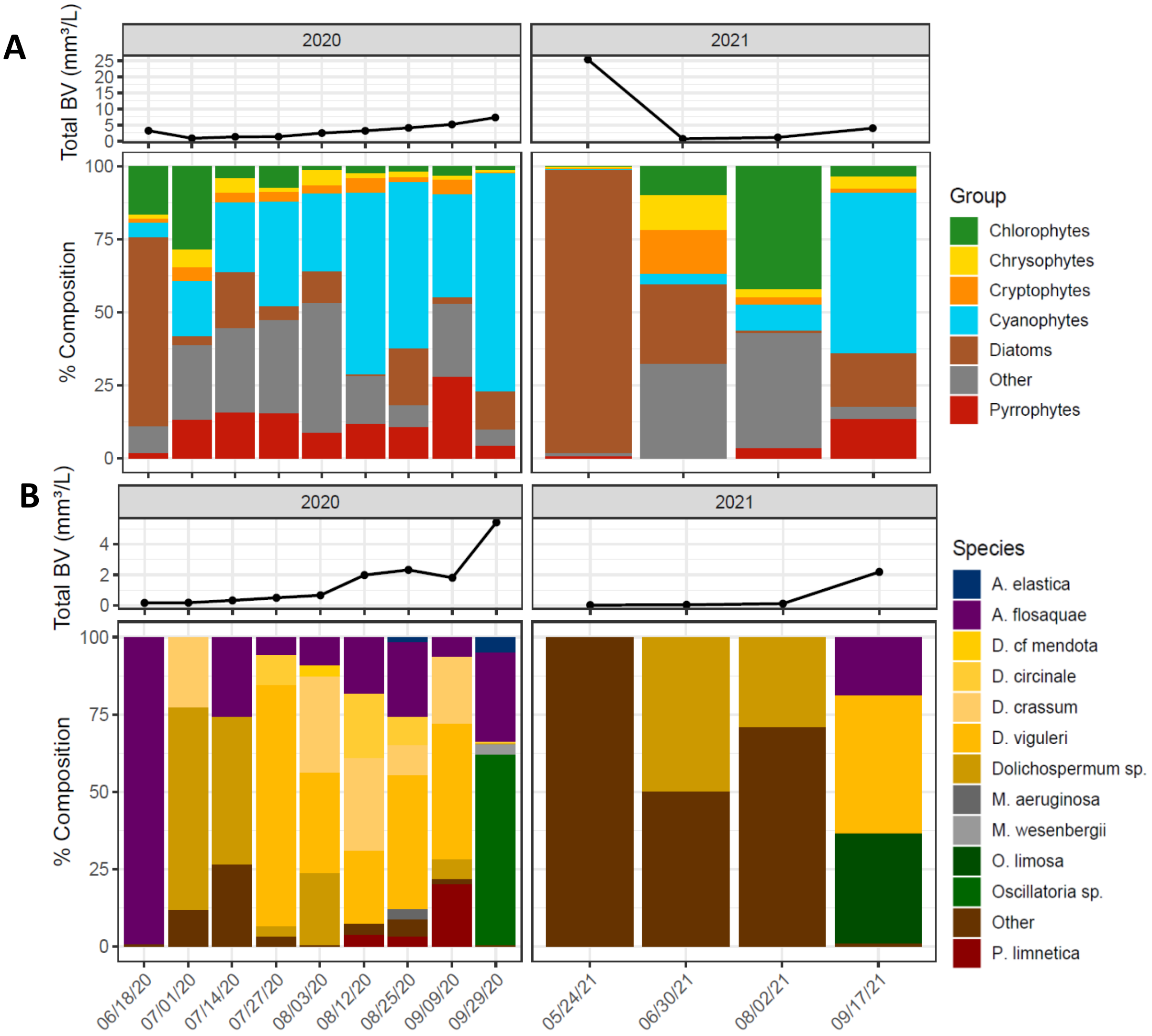


Figure 14. Hill Lake 2020 and 2021 (A) phytoplankton groups and (B) cyanobacteria genera by biovolume (mm³/L) and community percent (%) composition (B. *Aphanocapsa elastica, Aphanizomenon flos-aquae, Dolichospermum c.f. mendota, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum spp., Microcystis aeruginosa, Microcystis wesenbergi, Oscillatoria limosa, Oscillatoria sp., Pseudanabaena limnetica,* Other: *Aphanocapsa delicatissima, Aphanothece sp., Merismopedia sp., Spirulina sp., Synechococcus sp., Snowella lacustris, Snowella litoralis, Woronichinia naegeliana).*

Portage Lake

Portage Lake phytoplankton community assemblages remained diverse and dynamic throughout both sample years. Early 2020 consisted of diatoms and others, whereas early 2021 consisted of cryptophytes (Figure 15A). Cyanobacteria were present both years and were greatest during August. Phytoplankton biovolume in Portage Lake fluctuated throughout the 2020 sample season, with peak biovolume occurring early July. Biovolume continued to increase during the 2021 sample season, peaking mid-September. There was no significant difference in average phytoplankton biovolume (t = 0.74, df = 3.59, p = 0.50) between 2020 ($\bar{x}$ = 2.37 mm^{3}L^{-1}) and 2021 ($\bar{x}$ = 1.84 mm^{3}L^{-1}) in Portage Lake (Figure 15A).

Cyanobacteria predominantly present in Portage Lake included *A. delicatissima*, *A. flos-aquae*, *Dolichospermum spp.,* and others (Figure 16B). Both years had *P. limnetica* present within the community, however greater percent composition occurred during August 2020. *O. limosa* was only detected in early July 2020. Cyanobacteria biovolume in 2020 increased and peaked early August, and then began to decline. Cyanobacteria biovolume in 2021 continued to increase throughout the season, following the same trend as the phytoplankton biovolume. There was no significant difference in average cyanobacteria biovolume (t = 0.43, df = 6.91, p = 0.68) between 2020 ($\bar{x}$ = 0.35 mm^{3}L^{-1}) and 2021 ($\bar{x}$ = 0.26 mm^{3}L^{-1}) in Portage Lake (Figure 15B).

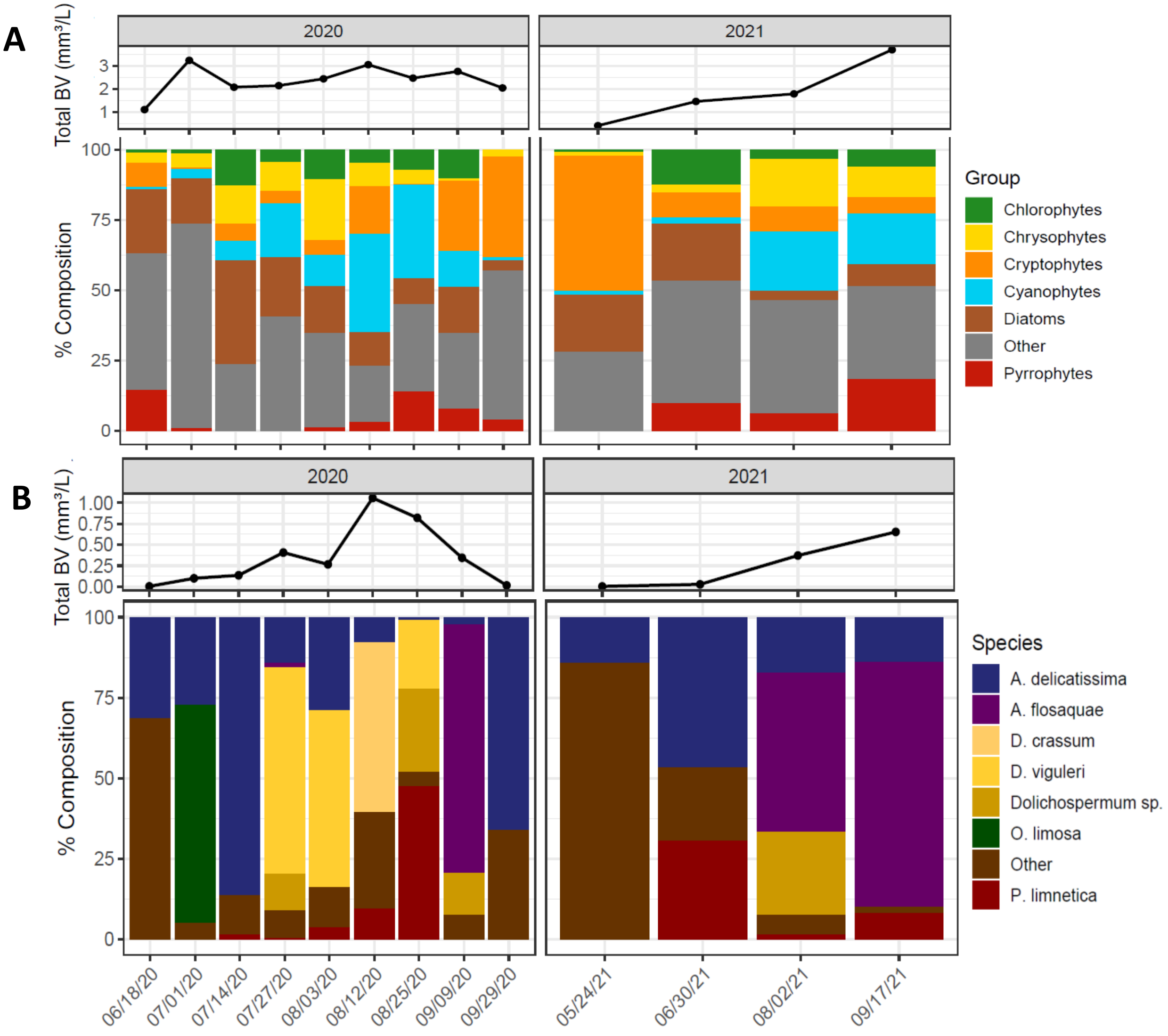


Figure 15. Portage Lake 2020 and 2021 (A) phytoplankton groups and (B) cyanobacteria genera by biovolume (mm³/L) and community percent (%) composition (B. *Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum spp., limosa, Pseudanabaena limnetica.* Other: *Aphanocapsa elastica, Aphanothece sp., Merismopedia sp., Pseudanabaena acicularis, Pseudanabaena mucicola, Synechococcus sp., Snowella lacustris, Snowella litoralis. Woronichina naegeliana).*

White Iron Lake

White Iron Lake phytoplankton communities showed diverse assemblages early in the season across both sample years (Figure 16A). Cyanobacteria percent composition generally increased over time during 2020, except for the 8/10/20 sampling event, in which diatoms were most abundant. The end of the season community structure consisted of cyanobacteria, diatoms, and others for both sample years. Phytoplankton biovolume in White Iron Lake fluctuated between 0.2 mm^{3}L^{-1} and 3 mm^{3}L^{-1} during the 2020 sample season, with the maximum biovolume occurring mid-September. Phytoplankton biovolume peaked during the end of September 2021 at 6 mm^{3}L^{-1}. However, there was no significant difference in average phytoplankton biovolume (t = -1.19, df = 3.44, p = 0.31) in White Iron Lake between 2020 ($\bar{x}$ = 1.49 mm^{3}L^{-1}) and 2021 ($\bar{x}$ = 2.92 mm^{3}L^{-1}; Figure 16A).

The cyanobacteria community within White Iron Lake primarily consisted *of Dolichospermum spp.*, *A. flos-aquae*, and others across both sample years (Figure 16B). During 2020, the cyanobacteria community was dominated by *Woronichinia naegeliana* early in the season, however, *W. naegeliana* was not detected in the early 2021 season. *O. limosa* was observed only during 2020 and *Snowella lacustris* was observed only during 2021. There was also a greater diversity of *Dolichospermum* species present during 2020 than 2021. *A. flos-aquae* was the dominant genera present during the end of September/ early October during both years. The cyanobacteria biovolume remained relatively constant during 2020, always below 2 mm^{3}L^{-1}. Contrastingly, the cyanobacteria biovolume in 2021 continuously increased throughout the sample season, with the greatest biovolume at 5 mm^{3}L^{-1} at the end of September. There is no significant difference in average cyanobacteria biovolume (*t* = -0.62, *df* = 3.17, *p* = 0.58) between 2020 ($\bar{x}$ = 0.63 mm^{3}L^{-1}) and 2021 ($\bar{x}$ = 1.39 mm^{3}L^{-1}; Figure 16B).

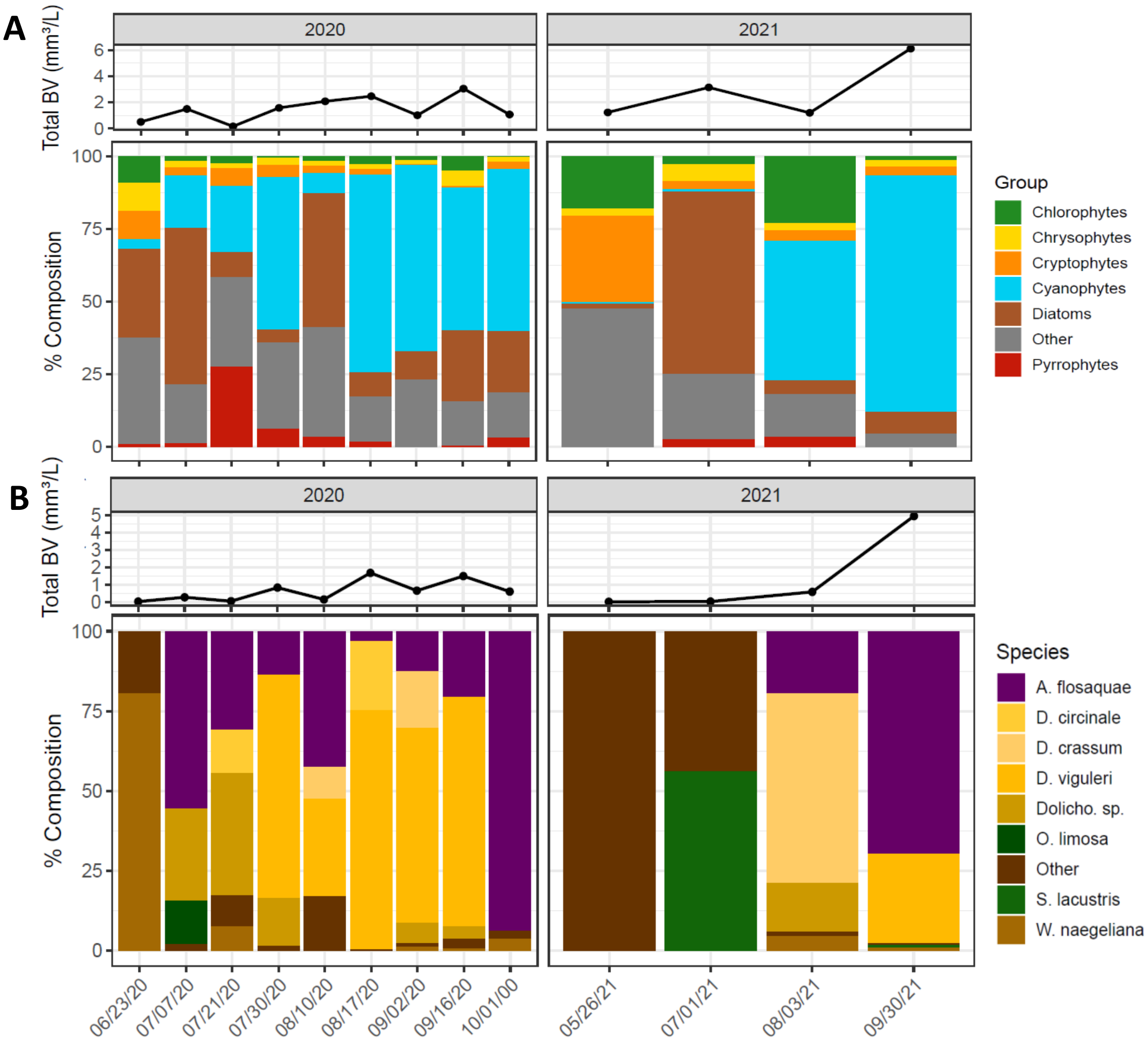


Figure 16. White Iron Lake 2020 and 2021 (A) phytoplankton groups and (B) cyanobacteria genera by biovolume (mm^3/L) and community percent (%) composition (B. *Aphanizomenon flos-aquae, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum spp., Oscillatoria limosa, Snowella lacustris, Woronichinia naegeliana,* Other: *Aphanocapsa delicatissima, Aphanocapsa elastica, Limnothrix planctonica, Merismopedia sp., Pseudanabaena limnetica, Synechococcus sp., Woronichinia sp.).*

Tait Lake

The phytoplankton community composition within Tait Lake was relatively diverse over time for both sample years. Communities primarily consisted of other with periodic diatom and cyanobacteria dominance during 2020 (Figure 17A). Cyanobacteria community percent composition was greatest during 2020 at the end of July, however, cyanobacteria did not dominate the community during any sampling events of 2021. Cryptophytes and others made up approximately 70% of the community structure at the beginning of the 2021 sample season (end of May), however, cryptophytes did not dominate the community during any 2020 sampling events. Phytoplankton biovolume increased at the end of July 2020 reaching the maximum biovolume of 1.6 mm^3L^{-1}. Biovolume declined throughout August 2020 and increased throughout September. During 2021, biovolumes did not fluctuate as observed in 2020 and remained constant formost of the summer. There was no significant difference between average cyanobacteria biovolumes ($t = -0.59$, $df = 9.16$, $p = 0.56$) in Tait Lake between 2020 ($\bar{x} = 1.04$ mm^3L^{-1}) and 2021 ($\bar{x} = 1.15$ mm^3L^{-1}; Figure 17A).

Unlike other study lakes, the cyanobacteria community in Tait Lake was dominated by *Synechococcus sp.* early season (May/ June) for both years (Figure 17B). The community during early and mid-July was primarily *A. delicatissima* and *Synechococcus sp*. During 2020, the cyanobacteria community began to diversify at the end of July with the presence of *W. naegeliana*, *S. lacustris*, *S. litoralis*, and *P. galeata*. However, these diverse community assemblages were not observed during 2021, as *A. delicatissima* remained the most prevalent species. By the end of September 2021, the community had shifted to *A. flos-aquae*, a nitrogen fixer, which was not observed during 2020. The cyanobacteria biovolume during 2020 remained constant except for a rapid increase and following decline during mid-August. Biovolume gradually increased throughout the season during 2021. The average cyanobacteria biovolume was not significantly different (*t* = 0.32, *df* = 9.91, *p* = 0.75) in Tait Lake between 2020 ($\bar{x}$ = 0.055 mm^{3}L^{-1}) and 2021 ($\bar{x}$ = 0.047 mm^{3}L^{-1}; Figure 17B).

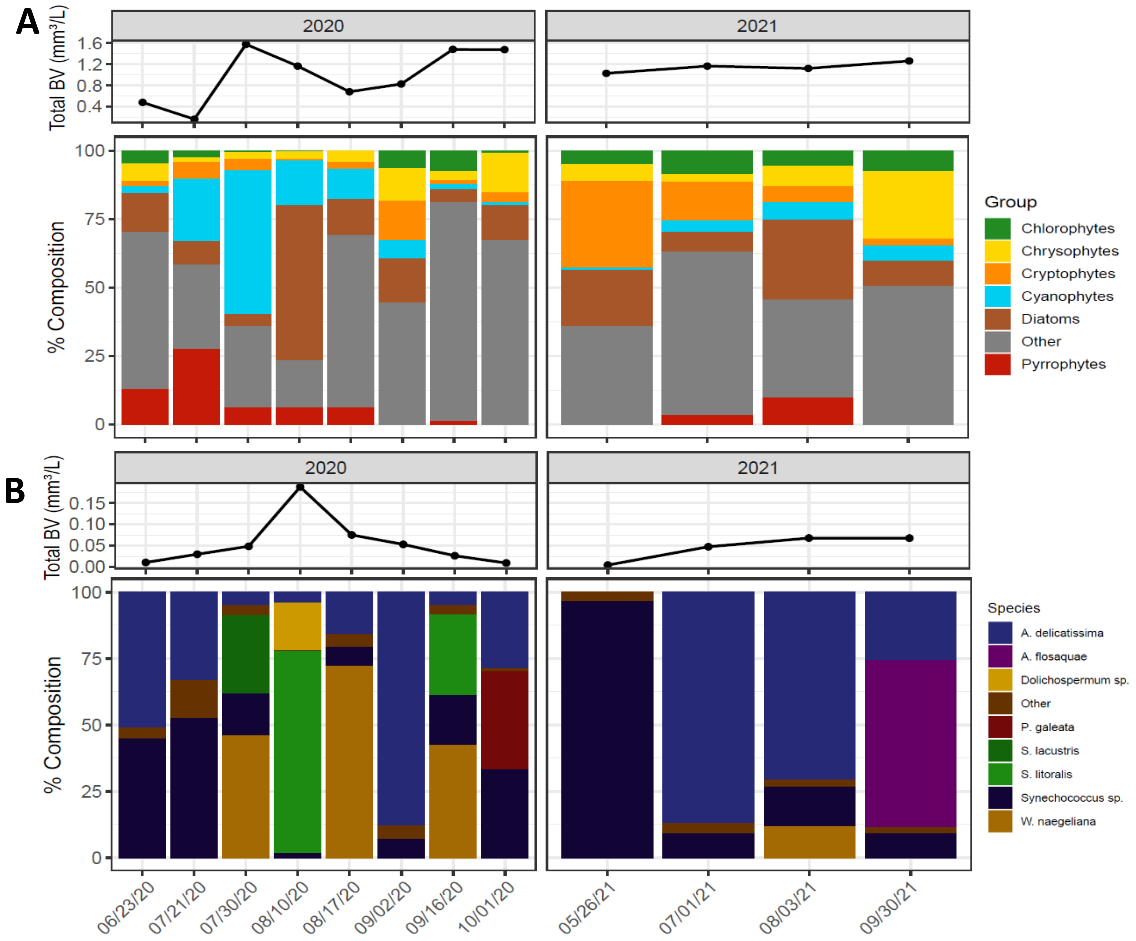


Figure 17. Tait Lake 2020 and 2021 (A) phytoplankton groups and (B) cyanobacteria genera by biovolume (mm³/L) and community percent (%) composition (B. *Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum spp., Pseudanabaena galeata, Snowella lacustris, Snowella litoralis, Synechococcus sp., Woronichina naegeliana,* Other: *Aphanothece sp., Merismopedia sp., Merismopedia tenuissima, Limnothrix planctonica, Pseudanabaena limnetica*).

3.2 Drivers of Cyanobacteria Community Composition

Cyanobacteria community assemblages were analyzed using non-metric multidimensional scaling to determine watershed-level, lake morphometry, and in-lake growth condition (Table 4) drivers of species composition (Figure 18). Line length indicates predictor strength. TP (p = 0.001), SRP (p = 0.001), NO_3^{-} (p = 0.001), DOC (p = 0.01), lake area (p = 0.004), watershed area (p = 0.011), watershed: lake area ratio (p = 0.001), percent agriculture (p = 0.001), and percent urban (p = 0.001) were found to be significantly associated with cyanobacteria species biovolume across all sample lakes; TN (p = 0.8), NH_4^+ (p = 0.3), average temperature (p = 0.4), average depth (p = 0.06), secchi disk depth (p = 0.1), and stability (p = 0.2) were not. Clustering of lakes indicates that the cyanobacteria communities are more similar within lakes than across lakes, especially Peltier (yellow) and Carrie (blue). The cyanobacteria community in Carrie Lake was most associated with NO_3^{-} and percent agriculture; Peltier Lake was most associated with percent urban, TP, DOC, SRP, and watershed: lake area ratio. As anticipate from Chapter 2, these lakes fall on opposite sides of the NMDS. TN and NH_4^+ showed weak association with all sample lakes. Hill Lake showed weak associations with stability and average depth.

Peltier Lake Cyanobacteria Community Drivers

Peltier Lake was further analyzed for specific drivers associated with the community shift observed both sample years (Figure 12). The top ten predictor variables for *Dolichospermum spp*. explained 54% variance, and predictors for *Microcystis spp.* explained 81% variance across genera biovolumes. *Dolichospermum* spp. biovolume had a significant, positive correlation with surface temperature (p < 0.001) and average pH (p = 0.015), and a significant, negative correlation with NH_4^+ (p = 0.03) and available light (*p* = 0.015; Figure 19) for both sample years. The cyanobacteria community shifted from *Dolichospermum* spp. on 8/4/2020 and between 7/22/2021 and 8/4/2021 to *Microcystis spp.* The main predictors of *Microcystis* spp. biovolume differed and showed a significant, positive correlation with TP (*p* < 0.001), SRP (*p* < 0.001), and DOC (*p* < 0.001), and a significant, negative correlation with N:P molar ratio (*p* < 0.001; Figure 19).

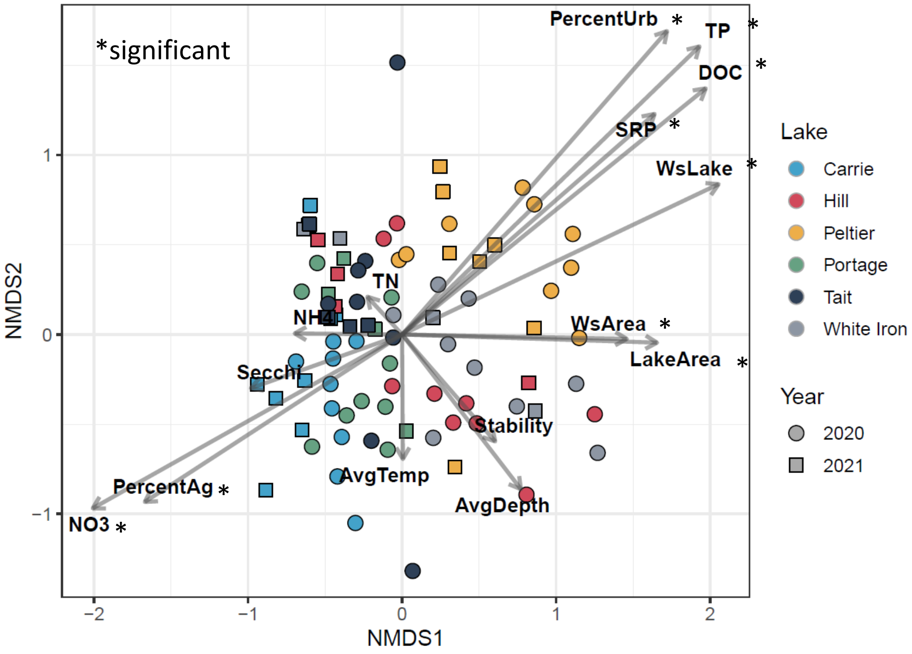


Figure 18. Cyanobacteria species biovolume for six sample lakes during 2020 and 2021 with watershed characteristics, lake morphometry, and in-lake processes as potential drivers. * Indicates significance. (In-lake processes: TP = total phosphorus (µgL^{-1}), SRP = soluble reactive phosphorus (µgL^{-1}), TN = total nitrogen (µgL^{-1}), NO3 = nitrite + nitrate (µgL^{-1}), NH4 = ammonium (µgL^{-1}), DOC = dissolved organic carbon (mgL^{-1}), stability = Schmidt's Stability Index,; Morphometric: avgdepth = average depth, avgtemp = average temperature, secchi_m = secchi disk depth (m); Watershed-scale: per_urb = % urban development, per_ag = % agricultural development, wsarea = watershed area, lakearea = lake area, wslake = watershed: lake area ratio).

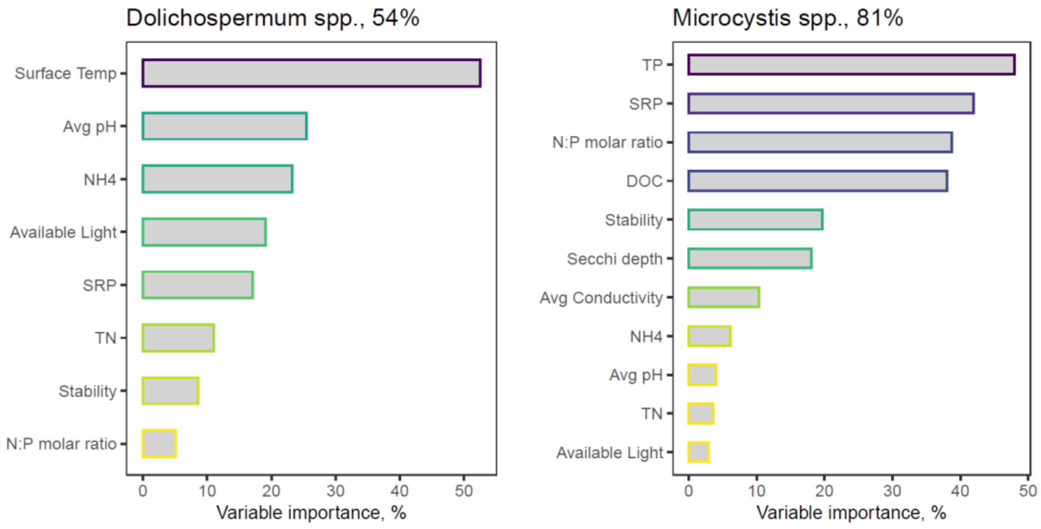


Figure 19. Random Forest model variable importance of $\log_{10}$ *Dolichospermum* spp. (54% explained variance) and *Microcystis* spp. (81% explained variance) biovolumes in Peltier Lake.

3.3 Seasonal Variability and Predictors of Cyanotoxin Concentrations

Cyanotoxin Seasonal Trends

For the 2021 sample season, all lakes were analyzed for seasonal trends of total microcystins (TMC), and the southern and central lakes (Peltier, Carrie, Portage, Hill) were analyzed for anatoxin-a (ANA) and cylindrospermopsin (CYL) via ELISAs (Figure 20). White Iron Lake showed increasing TMC during the duration of the 2021 season, however, only in the final sampling event in October were TMC above the detection limit. Tait Lake TMC were below detection limit and showed little variation over time. The Central lakes showed greater toxin concentrations and variability over time than the Northern lakes. Portage Lake showed increasing concentrations of TMC, low and stable concentrations of CYL, and a peak in ANA concentrations at the beginning of July followed by a decrease and stabilization. In comparison, Hill Lake showed a decreasing trend in ANA over time and low concentrations with little variability in CYL and TMC, with a slight increase in TMC from September to October. The Southern lakes showed the greatest variability in cyanotoxin concentrations over time. Carrie Lake had the greatest concentrations of ANA that displayed a cyclic trend, peaking mid-June, late August and early October. TMC concentrations remained stable most of the season with a gradual increase beginning mid-August and continuing into the fall. CYL concentrations remained low and stable during the duration of the sample season. Peltier Lake had the greatest TMC concentrations compared to all other lakes. TMC concentrations began increasing early July, peaked mid-August, and declined during September. ANA exhibited a similar trend to TMC, however, peaked at 4 µgL^{-1} early August and returned to non-detectable concentrations. CYL concentrations remained stable and below the detection limit (Figure 20).

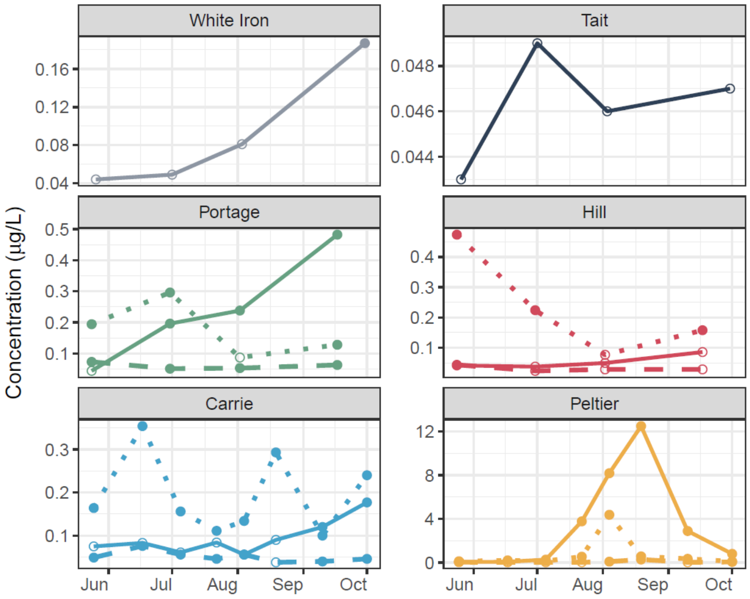


Figure 20. ELISA toxin analyses for total microcystins (solid, TMC), anatoxin-a (dotted, ANA), and cylindrospermopsin (dashed, CYL). Open circle indicates below detection limit, closed circle indicates above detection limit (detection limits: microcystin 0.10 µgL^{-1}, anatoxin-a 0.10 µgL^{-1}, and cylindrospermopsin 0.04 µgL^{-1}). Only total microcystins were measured for Northern Lakes, White Iron and Tait. Y-scales differ to focus on seasonal variability.

Table 11. Mean, coefficient of variance (%), and ranges of the sum of microcystin congeners (MC) from 2020 and 2021, and total microcystins (TMC), anatoxin-a (ANA), and cylindrospermopsin (CYL) from 2021. NA = not analyzed.

Lake	∑ MC congeners ng mL^{-1} (2020)	∑ MC congeners ng mL^{-1} (2021)	∑ MC congeners (2020 – 2021)	TMC ng mL^{-1} (2021)	ANA ng mL^{-1} (2021)	CYL ng mL^{-1} (2021)
Carrie	0.55	0.11	0.34	0.09	0.19	0.05
	63.9	54.1	98.0	39.2	44.3	22.2
	0.22-1.20	0.05-0.25	0.05-1.20	0.06-0.18	0.10-0.35	0.04-0.08
Hill	0.17	0.08	0.14	0.05	0.23	0.03
	53.4	81.0	66.3	35.1	63.5	23.8
	0.06-0.33	0.01-0.17	0.01-0.33	0.04-0.09	0.08-0.47	0.02-0.04
Peltier	5.63	2.64	4.22	3.56	0.80	0.08
	94.8	86.8	105.3	119.0	169.0	107.7
	0.27-16.10	0.09-6.30	0.09-16.10	0.06-12.47	0.11-4.37	0.03-0.29
Portage	0.17	0.21	0.18	0.24	0.18	0.06
	63.1	47.1	64.6	65.5	44.8	14.6
	0.01-0.42	0.05-0.31	0.01-0.42	0.04-0.48	0.09-0.30	0.05-0.07
Tait	0.06	0.02	0.04	0.05	NA	NA
	47.8	36.7	60.1	4.68		
	0.02-0.11	0.02-0.04	0.02-0.11	0.04-0.05		
White Iron	0.09	0.04	0.07	0.09	NA	NA
	48.9	72.8	60.3	63.9		
	0.01-0.14	0.01-0.09	0.01-0.14	0.04-0.19		

In addition to ELISAs, we analyzed both sample years for seven common microcystin (MC) congeners. Peltier Lake had all seven MC congeners present with MC-LA and MC-LR most prominent during 2020 and MC-LR during 2021 (Figure 21). Although cyanobacteria reached greater biovolumes during 2021, MC concentrations were greater during 2020 (Table 11). Across both sample years, lowest toxin concentrations correlated to a *Dolichospermum* dominated community. The community shift from *Dolichospermum* to a *Microcystis* correlated to an increase in MC concentrations. During 2020, MC reached the greatest concentration on 9/10/20, corresponding to a *M. aeruginosa* dominated community. The greatest MC concentrations in 2021 were observed on 8/19/21 and correlates to a community consisting of >76% *Microcystis*, both *M. aeruginosa* and *M. wesenbergii* were present at similar biovolumes. TMC concentrations showed similar trends to the MC congener concentrations, however, TMC concentrations detected were 2-fold greater than the sum of MC congener concentrations, suggesting that other congeners were present in samples (Figures 20, 21). *Microcystis* and *Dolichospermum* biovolumes were further analyzed against the sum of MC congeners (Figure 22). There was a positive, significant relationship between *Microcystis* biovolume and the sum MC congeners ($p < 0.001$); no significant relationship existed among *Dolichospermum* and MC concentrations.

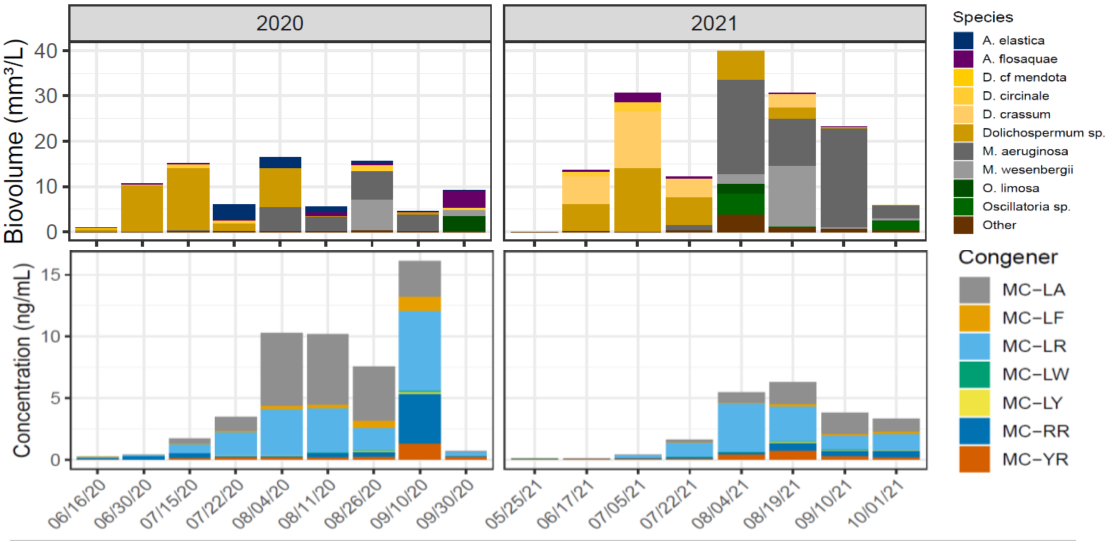


Figure 21. Peltier Lake 2020 and 2021 cyanobacteria community assemblages and microcystin congener concentrations (*Aphanocapsa elachista, Aphanizomenon flos-aquae, Dolichospermum c.f. mendota, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum spp., Microcystis aeruginosa, Microcystis wesenbergii, Oscillatoria limosa, Oscillatoria sp.*, Other: *Aphanocapsa delicatissima, Aphanothece, Merismopedia sp., Pseudanabaena limnetica, Pseudanabaena mucicola, Planktothrix sp., Synechococcus, Woronichinia naegeliana, Woronichinia sp.*).

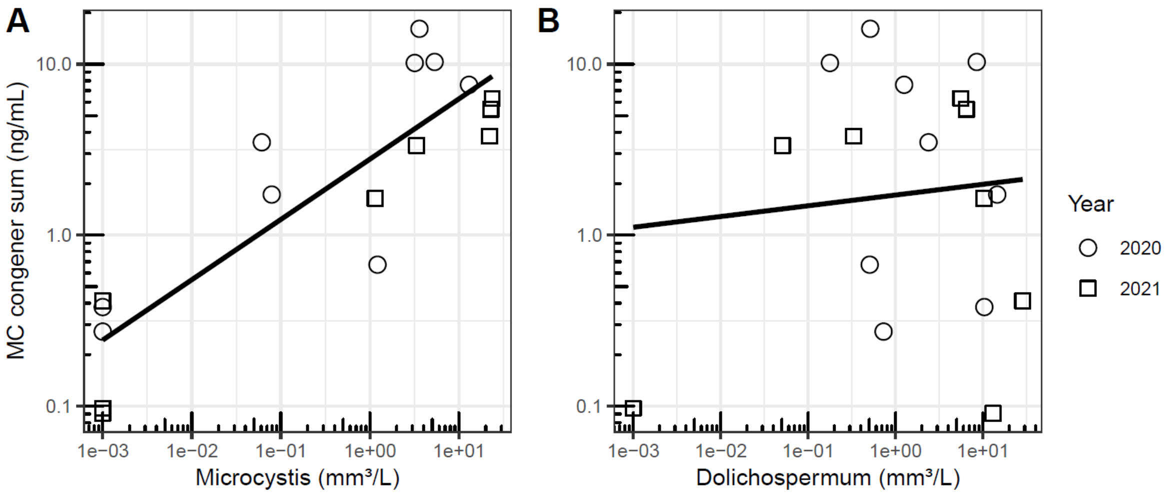


Figure 22. (A) *Microcystis* ($\text{mm}^3\ \text{L}^{-1}$) and (B) *Dolichospermum* ($\text{mm}^3\ \text{L}^{-1}$) biovolumes compared to the sum of microcystin congeners (ng mL^{-1}) from Peltier Lake.

Similar to Peltier Lake, all seven MC congeners were detected within Carrie Lake, however, at much lower concentrations. Toxin concentrations show comparably different trends across time between the two sample years. MC-LR was the most prominent congener within Carrie Lake but was found at much greater concentrations during 2020 than 2021 (Table 11), although never surpassed 1.25 ng mL⁻¹ (Figure 23). During 2020, Carrie Lake experienced a gradual increase in MC concentrations peaking on 8/26/20 at 1.20 ng mL⁻¹ and declined into the fall. This increase in MC followed the occurrence of a sharp increase in *M. aeruginosa* on 8/11/20. The cyanobacteria biovolume remained between 2 and 3 mm³ L⁻¹ for August and September being primarily dominated by *Dolichospermum* sp., *M. aeruginosa*, and *O. limosa*, all of which are MC producing genera. During 2021, MC concentrations did not surpass 0.25 ng mL⁻¹ and remained stable through the season. There was a minor increase in *M. aeruginosa* mid-August, however, no corresponding increases in MC were detected. Similar trends and concentrations were observed across TMC and MC congener concentrations (Figure 20, 23).

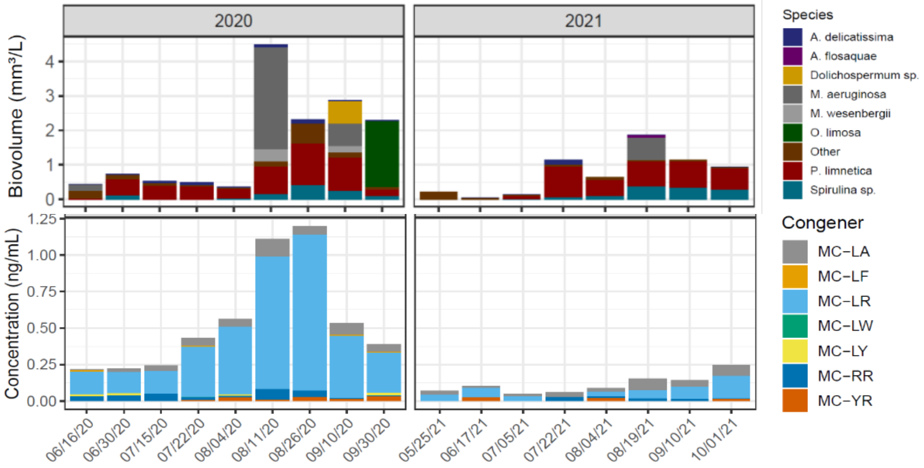


Figure 23. Carrie Lake 2020 and 2021 cyanobacteria assemblages and MC congener concentrations (*Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum sp., Microcystis aeruginosa, Microcystis wesenbergii, Oscillatoria limosa, Pseudanabaena limnetica. Spirulina sp., Other: Aphanothece sp., Aphanocapsa elastica, Merismopedia sp., Merismopedia tenuissima, Synechococcus sp., Snowella lacustris, Woronichinia naegeliana*).

MC congener results in Portage Lake were comparable across sample seasons in terms of concentration and congener composition (Figure 24). Although MC-YR, MC-RR, and MC-LY were quantified at minimal concentrations during 2020 and not observed at the same quantity during 2021, the dominant congeners across both years were MC-LA and MC-LR. MC congener concentrations remained relatively constant throughout most of 2020 (~2 ng mL¯¹), however an increase was observed on 9/9/20 (4 ng mL¯¹) followed by a substantial decrease at the end of September. MC concentrations increased at the end of June 2021 and gradually increased the remainder of the season; TMC data from ELISA runs confirm this trend (Figure 20). Dominant genera present, such as *Dolichospermum* during 2020 and *A. flos-aquae* during 2021, are likely the producers of MC in Portage Lake (Österholm et al., 2020).

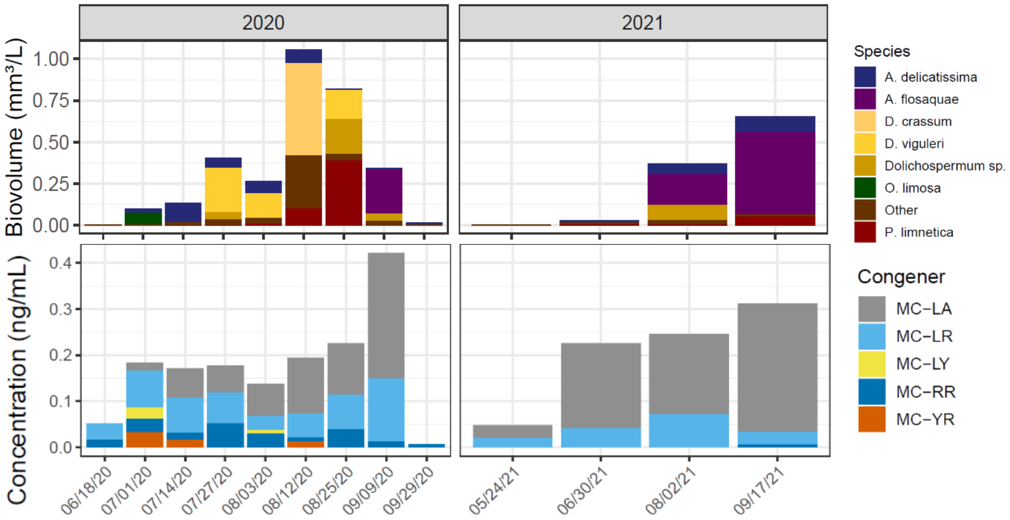


Figure 24. Portage Lake 2020 and 2021 cyanobacteria assemblages and MC congener concentrations (*Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum sp., limosa, Pseudanabaena limnetica.* Other: *Aphanocapsa elastica, Aphanothece sp., Merismopedia sp., Pseudanabaena acicularis, Pseudanabaena mucicola, Synechococcus sp., Snowella lacustris, Snowella litoralis. Woronichina naegeliana).*

A diversity of MC congeners was observed in Hill Lake both sample years (Figure 25). Like Portage Lake, MC-LA and MC-LR were most prominent. MC congeners reached greatest concentrations on 9/26/20 at 0.33 ng mL^{-1} consisting of 35% MC-LA and on 8/2/21 at 0.17 ng mL^{-1} consisting of 90% MC-LA. During 2020, MC concentrations remained stable until a gradual increase beginning 8/25/20 and remaining the rest of the season. Overall, lower MC concentrations were observed during 2021 and our results from TMC ELISA runs further support this observation (Figure 20). Although MC concentrations persist at low levels, MC was likely produced by *Dolichospermum spp.,* as that genus was continuously present during the duration of both sample seasons. Likewise, the decreasing trend of ANA (Figure 20) corresponds to a decreasing biovolume of *A. flos-aquae* (Figure 14), a known ANA producer (Codd et al., 2005).

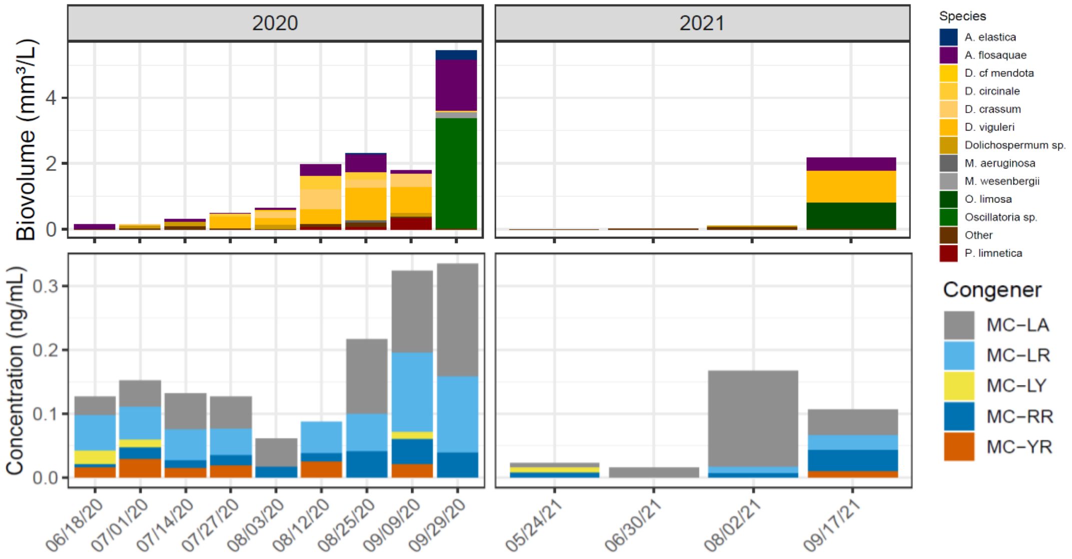


Figure 25. Hill Lake 2020 and 2021 cyanobacteria assemblages and MC congener concentrations (*Aphanocapsa elastica, Aphanizomenon flos-aquae, Dolichospermum c.f. mendota, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum sp., Microcystis aeruginosa, Microcystis wesenbergi, Oscillatoria limosa, Oscillatoria sp., Pseudanabaena limnetica,* Other: *Aphanocapsa delicatissima, Aphanothece sp., Merismopedia sp., Spirulina sp., Synechococcus sp., Snowella lacustris, Snowella litoralis, Woronichinia naegeliana).*

White Iron Lake, a northern, mesotrophic system, had detectable concentrations of MC congeners and TMC. Greater variation among MC congeners was observed during 2020 compared to 2021; MC-LA, MC-LR, MC-LY, MC-RR, and MC-YR were detected throughout 2020, whereas only MC-LA and MC-LR were present in 2021 (Figure 26). Trends between MC congeners and TMC differed across time throughout 2021; TMC showed increasing concentrations throughout the summer reaching a maximum of 0.18 ng mL^{-1} (Figure 20), whereas MC congener concentrations peaked on 8/3/21 at 0.09 ng mL^{-1} and declined by late September. White Iron Lake cyanobacteria community was primarily composed of *Dolichospermum spp. and A. flos-aquae*, which are both known N-fixers and potential toxin producers (Österholm et al., 2020).

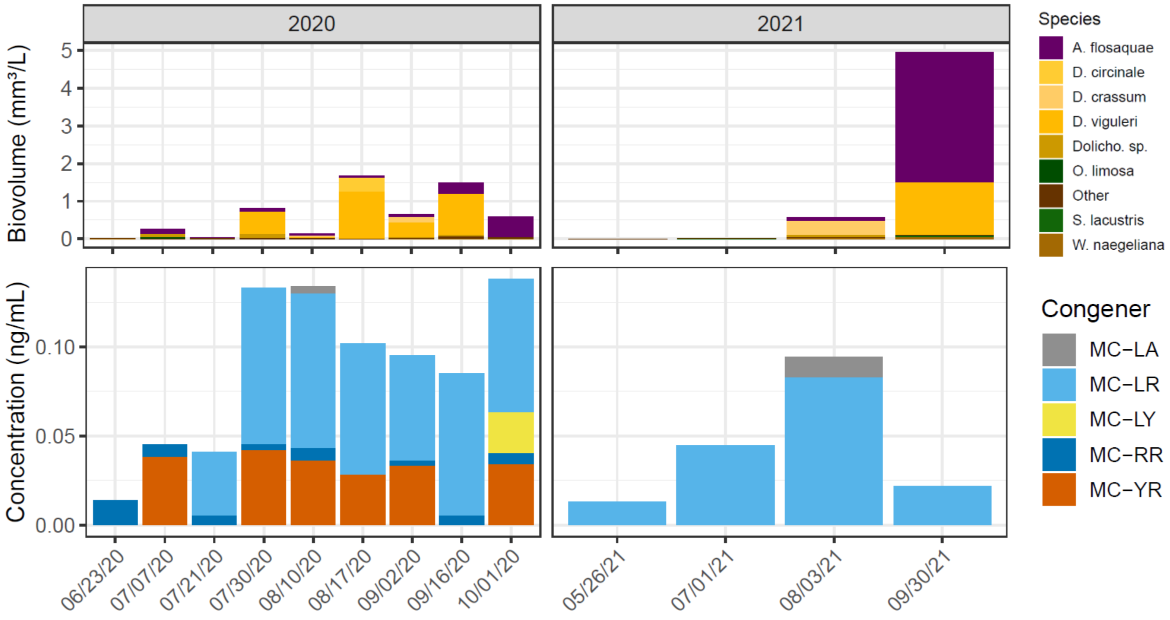


Figure 26. White Iron Lake 2020 and 2021 cyanobacteria assemblages and MC congener concentrations (*Aphanizomenon flos-aquae, Dolichospermum circinale, Dolichospermum crassum, Dolichospermum viguleri, Dolichospermum sp., Oscillatoria limosa, Snowella lacustris, Woronichinia naegeliana,* Other: *Aphanocapsa delicatissima, Aphanocapsa elastica, Limnothrix planctonica, Merismopedia sp., Pseudanabaena limnetica, Synechococcus sp., Woronichinia sp.).*

Tait Lake is also a northern, temperate system that showed toxin production potential, similarly seen in White Iron Lake. Unlike other sample lakes, Tait Lake did not have a prominent presence of MC-LA within the MC congener composition. Rather, MC-LR, MC-LY, and MC-YR were the most dominant congeners observed within Tait Lake (Figure 27). As previously seen in other sample lakes, MC congener and TMC data showed differing trends across time (Figure 20). Although quantified concentrations were similar, TMCs peaked on 7/1/21, whereas MC congener concentrations peaked the following sampling event on 8/3/21. Compared to other sample lakes, Tait Lake had contrastingly different cyanobacteria community assemblages, with *Snowella litoralis* and *Aphanocapsa delicatissima* being prominent within the cyanobacteria community, indicating that the presence of a toxin producer in even minor biovolume quantities can still result in detectable toxin concentrations.

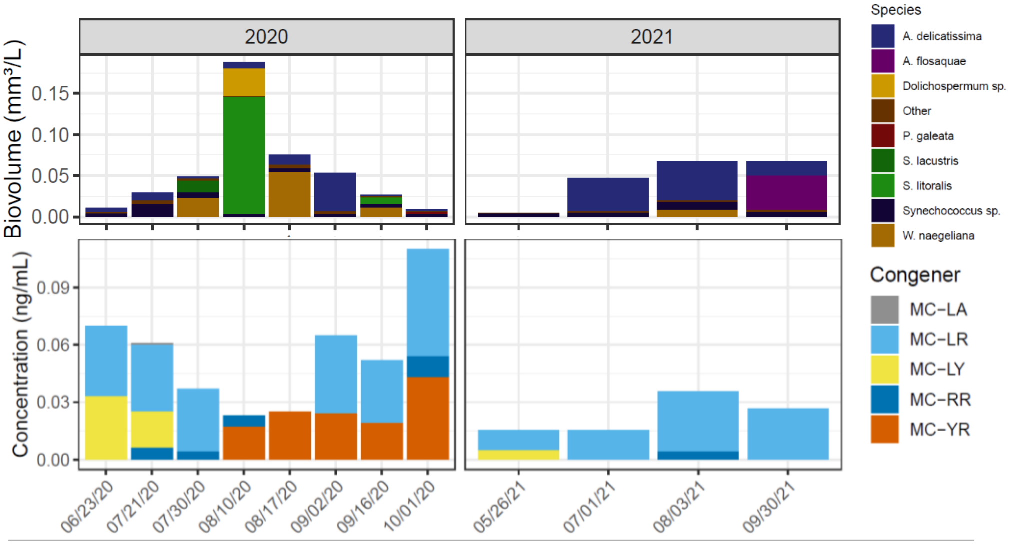


Figure 27. Tait Lake 2020 and 2021 cyanobacteria assemblages and MC congener concentrations (*Aphanocapsa delicatissima, Aphanizomenon flos-aquae, Dolichospermum sp., Pseudanabaena galeata, Snowella lacustris, Snowella litoralis, Synechococcus sp., Woronichina naegeliana,* Other: *Aphanothece sp., Merismopedia sp., Merismopedia tenuissima, Limnothrix planctonica, Pseudanabaena limnetica*).

Predictors of Cyanotoxin Concentrations

RFM analyses were used to assess predictor importance of the $\log_{10}$ sum of MC congener concentrations for six sample lakes. Similar to the analyses for cyanobacteria biovolume, Peltier Lake was removed and RFMs were run again. This distinction was considered because of the significantly greater concentrations of cyanobacteria biovolume and cyanotoxins present in Peltier Lake, a likely outlier influencing results of RFMs (Figure 28). However, excluding Peltier Lake in cyanotoxin predictor analyses did not drastically change predictor order or importance. When including and excluding Peltier Lake in RFM analyses, phycocyanin was the most important predictor, showing a significant ($p < 0.001$), positive relationship with cyanotoxin concentrations. TN also showed a significant ($p < 0.001$), positive relationship and was ranked within the top three predictors when including and excluding Peltier Lake. TP was, however, an important and significant ($p < 0.001$, positive) predictor of cyanotoxin concentrations only when including Peltier Lake. Contrastingly, DOC showed a significant ($p < 0.001$), negative relationship with cyanotoxin concentrations when excluding Peltier Lake. Although nitrates were important predictors of cyanotoxins, there was no significant relationship (including Peltier, $p = 0.8$; excluding Peltier, $p = 0.4$). These results indicate that in-lake growth conditions, such as nutrient form and availability, are the most important predictors of cyanotoxins in these lakes.

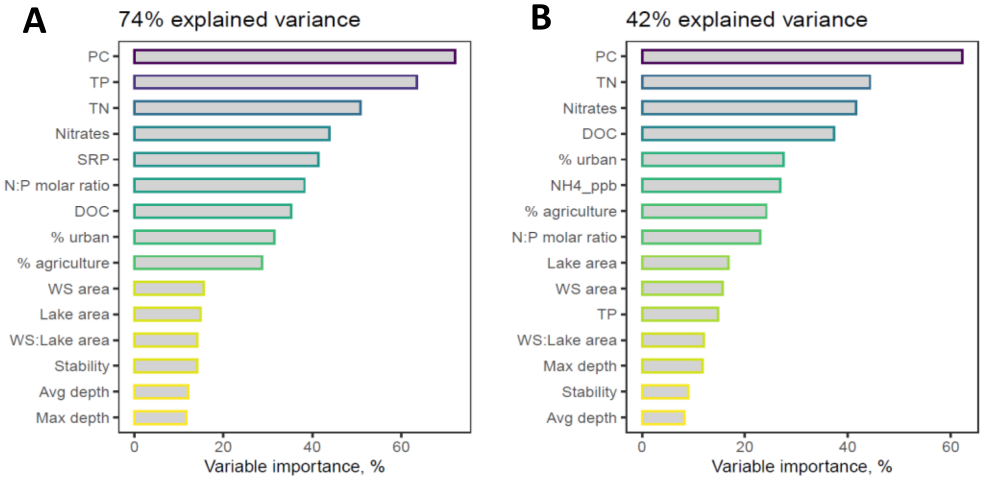


Figure 28. RFM variable importance for $\log_{10}$ MC concentrations for (A) six sample lakes (including Peltier) and (B) five sample lakes (excluding Peltier).

All lakes were analyzed for in-lake growth conditions to determine lake-specific predictors of MC concentrations. Hill Lake did not indicate any significant predictors of MC concentrations (Figure 29). All remaining sample lakes, except Peltier, exhibited a form of nitrogen within the top three predictors of MC concentrations. White Iron, Tait, and Carrie lakes were highly influenced by nitrates, and Portage Lake by ammonium. Peltier Lake, however, had the greatest explained variance at 60%, following by White Iron (36%), Carrie (34%), Tait (27%), and Portage (26%). Due to the high importance of nitrogen forms, TN was further analyzed across all lakes (Figure 30) and showed a positive, significant relationship with MC congener concentrations ($p < 0.001$).

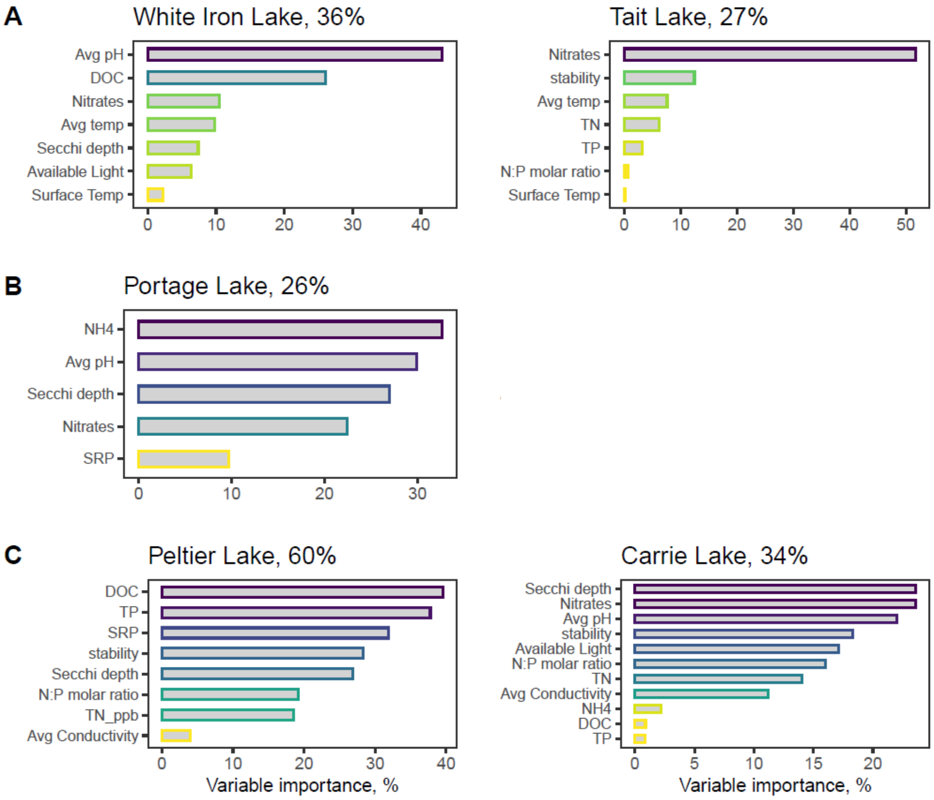


Figure 29. Random Forest model of in-lake predictor importance of $\log_{10}$ MC concentration. A) Northern lakes: White Iron and Tait, B) Central lakes: Portage, C) Southern lakes: Peltier and Carrie. Hill Lake did not indicate any important predictors of toxin concentration.

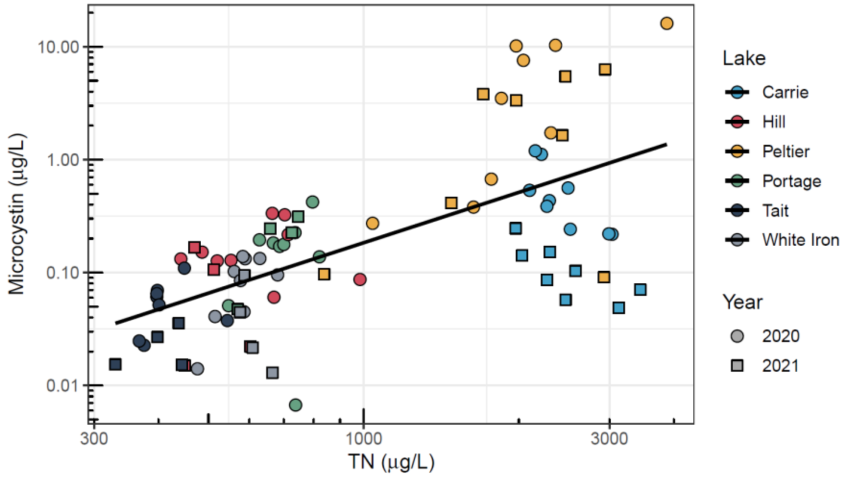


Figure 30. Sum of MC congeners (ng mL^{-1}) and TN (µg L^{-1}) across six sample lakes from 2020 and 2021.

4 Discussion

Our results indicate cyanobacteria bloom phenology, community assemblages, and toxin concentrations differed across sample lakes due to differing watershed characteristics, lake morphometry, and in-lake processes. Our hypothesis that cyanobacterial blooms would start earlier in the season in southern lakes than northern lakes was supported by Peltier Lake (Figure 12), however, Carrie Lake (Figure 13) experienced a gradual increase in cyanobacteria dominance, similar to the northern lake, White Iron (Figure 16). Hence, such a trend may be weak within the narrow geographical gradient of Minnesota. Our second hypothesis that cyanobacteria community assemblages would be primarily driven by in-lake processes was supported, however, watershed characteristics (% urban, & agriculture) were also significant drivers (Figure 18). Our final hypothesis that toxin concentrations would be greatest in southern lakes had mixed support (supported by Peltier Lake, not Carrie Lake), and that primary drivers of cyanotoxin concentrations would be nitrogen forms was supported although several caveats arose.

It is well documented that phytoplankton community assemblages change based on trophic status and therefore are related to nutrient availability (Schindler et al., 1978). Peltier Lake, classified as hypereutrophic due to high phosphorus concentrations, was the only study lake in which cyanobacteria dominated for the entirety of the season (Figure 12). Watson et al. (1997) observed, across a large-scale study, that oligotrophic systems were primarily dominated by diatoms, chrysophytes, and cryptophytes; mesotrophic systems showed no clear dominant phytoplankton; and eutrophic systems had few groups present if not already dominated by cyanobacteria. Carrie Lake, however, also classified as eutrophic due to significantly high concentrations of nitrogen, did not have a prominent cyanobacteria community until mid-August 2020 and early July

2021, rather the phytoplankton community was dominated by diatoms during 2020 and a mixed assemblage during 2021 (Figure 14); therefore, the phytoplankton assemblages did not follow the expected nutrient regimes. The central and southern lakes, Portage (eutrophic) and Tait (mesotrophic), were not cyanobacteria dominated for a significant duration of the season (Figures 15 & 17). However, White Iron (mesotrophic) and Hill (eutrophic), both displayed gradual increases in cyanobacteria dominance within the phytoplankton community during 2020 and 2021 (Figures 14 & 16). The question arises as to *why* phytoplankters distribute across this trophic spectrum. Reynolds (1998) suggests that interspecific competition and habitat adaptations to better survive under specific conditions are driving this trophic distribution. Cyanobacteria have adapted to extreme conditions and have evolved to become better competitors, specifically of NH_4^+ uptake. Glibert et al. (2016) analyzed N forms on phytoplankton assemblages and N metabolism and showed that biomass and MC concentrations increased with increasing N. However, this pattern of increased biomass and MC concentrations was not observed in our high N lake (Carrie), indicating that some other predictor variable may be inhibiting cyanobacteria biomass and toxin concentrations. RFM for both cyanobacteria biovolume (Figure 10) and MC concentrations (Figure 28) indicate nitrates and TN are important predictors of biovolume and concentrations. However, it is difficult to predict phenomenon based on single variables as individual phytoplankters are responding to numerous stressors.

Drivers of cyanobacteria assemblages have been studied across various trophic statuses, lake types, and differing morphological systems, however, similar drivers of community assemblages have been observed. Generally, cyanobacteria genera and successional changes have been associated with temperature, light availability, residence time, water column stability, and ability to tolerate high UV (Paerl & Otten, 2016; Roelke et al., 2004; Soares et al., 2009), similarly observed as drivers of cyanobacteria biovolume. The community shift, in Peltier Lake, from *Dolichospermum* (diazotroph, nitrogen-fixer) to *Microcystis* (non-diazotroph, non-nitrogen-fixer) was an interesting dynamic we chose to focus on. Similar community shifts have been observed in lakes such as Lake Chaohu, China and cultured in laboratory experiments. Zhang et al. (2020) studied the annual shift between *Microcystis* and *Dolichospermum* in Lake Chaohu and found that *Microcystis* biovolume was strongly associated with higher temperatures and TP concentrations, in which *Dolichospermum* was outcompeted during those environmental conditions. Specifically, researchers found that *Dolichospermum* was always dominant at temperatures below 17°C, indicating a physiological difference between the two genera. Similar trends were observed in Peltier Lake, in which *Microcystis* become dominant later in the season while TP concentrations were greater. The top predictor from RFMs of *Dolichospermum* biovolume in Peltier Lake was surface temperature, however, we did not observe the community shift being specifically correlated with 17°C because Peltier Lake temperatures were typically greater beginning in May. Additionally, top predictors from RFMs of *Microcystis* biovolume in Peltier Lake were TP and SRP, both significantly associated with *Microcystis* biovolume. Associations have also been made between *Aphanizomenon*, another common diazotrophic cyanobacteria, and *Microcystis*, in which weak correlations have been observed between increasing temperatures and decreasing nitrogen fixers and increasing non-nitrogen-fixers (Paerl & Otten, 2016).

Our results are further supported by a laboratory experiment studying allelopathic interactions between *Microcystis* and *Anabaena* (now *Dolichospermum*) in which

Anabaena dominated under low nitrogen conditions, similarly seen in Peltier Lake (Figures 6 & 12). However, this study additionally showed *Microcystis* dominant during low TP concentrations, which was not observed in Peltier Lake (Chia et al., 2018). Moisander et al. (2009) conducted delta 15N tracers to further study N forms and transformations across cyanobacteria genera in the Klamath River (CA, OR), in which the community shifts from *Aphanizomenon* to *Microcystis*. Researchers found that the N form *Microcystis* used, to potentially fuel toxin production, did not come from the N fixed by *Aphanizomenon*, rather the N came from N available within the water column or via sediment regeneration. Our research, along with these studies show the complexity of cyanobacteria assemblages both spatially and temporally, and indicate further research is necessary to better understand lake-specific community dynamics and drivers of assemblage changes.

Cyanotoxins, such as microcystins, have previously been associated with eutrophic to hypereutrophic lakes (Tanvir et al., 2021), yet our results indicate that cyanotoxin production, including toxin type and quantity, varied across time and within lakes. Peltier Lake, a southernly located, eutrophic system, had the greatest microcystin concentrations, however, there were detectable concentrations within all sample lakes, including the northernly located White Iron Lake. Due to our multifaceted approach of analyzing MC by both ELISAs and HPLC, we were able to compare detection quantities across methods. White Iron Lake, for example, had greater MC concentrations detected via ELISA compared to the sum of MC congeners using HPLC. This could indicate that other MC congeners may be present that were not specifically targeted during HPLC analyses. This could provide implications in the future if total MC is not considered and targeted during analyses. Our results showed a positive relationship between TN and the sum of MC congeners across all lakes (Figure 30) which is supported by others (Downing et al., 2005; Glibert et al., 2016; Lee et al., 2000; Van de Waal et al., 2009; Vézie et al., 2002). Although recent research has emphasized the importance of both N and P, our southernly located, P-limited system did not follow expectations. Carrie Lake had significantly higher TN concentrations than all lakes, however, did not show exceptionally high MC concentrations. MC concentrations also showed variability between years, Carrie Lake (Figure 23), further emphasizing the unpredictability of toxin production across time within systems. Therefore, further genomic research is needed to explore genetic capabilities of cyanobacteria to determine toxin production abilities.

5 Conclusions

Our findings emphasize the importance of moving beyond microcystin as the sole indicator of toxicity. Anatoxin-a concentrations were detected at higher concentrations than microcystins in several sample lakes. Hence, it is important to consider, and analyze for, additional toxins to determine total toxicity. However, we have also demonstrated the influence cyanobacteria community composition consequently has on toxicity, such that toxin production is dependent on both a species' secondary metabolite capabilities as well as environmental conditions, including nutrient availability. Understanding species-specific functional traits, such as cell buoyancy regulation or ecosystem functions like nitrogen fixation, will provide necessary knowledge for lake-specific management plans. As discussed in Chapter 2, we have further shown that lake-specific models for management plans are necessary and will be most effective at future mitigation efforts.

REFERENCES

Arbuckle, K. E., & Downing, J. A. (2001). The influence of watershed land use on lake N: P in a predominantly agricultural landscape. *Limnology and Oceanography*, *46*(4), 970–975. https://doi.org/10.4319/lo.2001.46.4.0970

Beaulieu, M., Pick, F., & Gregory-Eaves, I. (2013). Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. *Limnology and Oceanography*, *58*(5), 1736–1746. https://doi.org/10.4319/lo.2013.58.5.1736

Bertos-Fortis, M., Farnelid, H. M., Lindh, M. V., Casini, M., Andersson, A., Pinhassi, J., & Legrand, C. (2016). Unscrambling Cyanobacteria Community Dynamics Related to Environmental Factors. *Frontiers in Microbiology*, *7*. https://www.frontiersin.org/articles/10.3389/fmicb.2016.00625

Beversdorf, L. J., Chaston, S. D., Miller, T. R., & McMahon, K. D. (2015). Microcystin mcyA and mcyE Gene Abundances Are Not Appropriate Indicators of Microcystin Concentrations in Lakes. *PLOS ONE*, *10*(5), e0125353. https://doi.org/10.1371/journal.pone.0125353

Bibby, T. S., Mary, I., Nield, J., Partensky, F., & Barber, J. (2003). Low-light-adapted Prochlorococcus species possess specific antennae for each photosystem. *Nature*, *424*(6952), Article 6952. https://doi.org/10.1038/nature01933

Bouaïcha, N., Miles, C. O., Beach, D. G., Labidi, Z., Djabri, A., Benayache, N. Y., & Nguyen-Quang, T. (2019). Structural Diversity, Characterization and Toxicology of Microcystins. *Toxins*, *11*(12), Article 12. https://doi.org/10.3390/toxins11120714

Brooks, B. W., Lazorchak, J. M., Howard, M. D. A., Johnson, M.-V. V., Morton, S. L., Perkins, D. A. K., Reavie, E. D., Scott, G. I., Smith, S. A., & Steevens, J. A. (2016). Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry*, *35*(1), 6–13. https://doi.org/10.1002/etc.3220

Carmichael, W. W. (1994). The Toxins of Cyanobacteria. *Scientific American*, *270*(1), 78–86.

Carmichael, W. W. (2001). Health Effects of Toxin-Producing Cyanobacteria: “The CyanoHABs.” *Human and Ecological Risk Assessment: An International Journal*, *7*(5), 1393–1407. https://doi.org/10.1080/20018091095087

Carmichael, W. W., & Boyer, G. L. (2016). Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae*, *54*, 194–212. https://doi.org/10.1016/j.hal.2016.02.002

Cha, Y., Park, S. S., Kim, K., Byeon, M., & Stow, C. A. (2014). Probabilistic prediction of cyanobacteria abundance in a Korean reservoir using a Bayesian Poisson model. *Water Resources Research*, *50*(3), 2518–2532. https://doi.org/10.1002/2013WR014372

Chia, M. A., Jankowiak, J. G., Kramer, B. J., Goleski, J. A., Huang, I.-S., Zimba, P. V., do Carmo Bittencourt-Oliveira, M., & Gobler, C. J. (2018). Succession and toxicity of Microcystis and Anabaena (Dolichospermum) blooms are controlled by nutrient-dependent allelopathic interactions. *Harmful Algae*, *74*, 67–77. https://doi.org/10.1016/j.hal.2018.03.002

Christensen, V. G., & Khan, E. (2020). Freshwater neurotoxins and concerns for human, animal, and ecosystem health: A review of anatoxin-a and saxitoxin. *Science of*

The Total Environment, *736*, 139515. https://doi.org/10.1016/j.scitotenv.2020.139515

Codd, G. A., Morrison, L. F., & Metcalf, J. S. (2005). Cyanobacterial toxins: Risk management for health protection. *Toxicology and Applied Pharmacology*, *203*(3), 264–272. https://doi.org/10.1016/j.taap.2004.02.016

Dao, T.-S., Nimptsch, J., & Wiegand, C. (2016). Dynamics of cyanobacteria and cyanobacterial toxins and their correlation with environmental parameters in Tri An Reservoir, Vietnam. *Journal of Water and Health*, *14*(4), 699–712. https://doi.org/10.2166/wh.2016.257

David, G. M., Moreira, D., Reboul, G., Annenkova, N. V., Galindo, L. J., Bertolino, P., López-Archilla, A. I., Jardillier, L., & López-García, P. (2021). Environmental drivers of plankton protist communities along latitudinal and vertical gradients in the oldest and deepest freshwater lake. *Environmental Microbiology*, *23*(3), 1436–1451. https://doi.org/10.1111/1462-2920.15346

Dick, G. J., Duhaime, M. B., Evans, J. T., Errera, R. M., Godwin, C. M., Kharbush, J. J., Nitschky, H. S., Powers, M. A., Vanderploeg, H. A., Schmidt, K. C., Smith, D. J., Yancey, C. E., Zwiers, C. C., & Denef, V. J. (2021). The genetic and ecophysiological diversity of Microcystis. *Environmental Microbiology*, *23*(12), 7278–7313. https://doi.org/10.1111/1462-2920.15615

Dietrich, D., & Hoeger, S. (2005). Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): A reasonable or misguided approach? *Toxicology and Applied Pharmacology*, *203*(3), 273–289. https://doi.org/10.1016/j.taap.2004.09.005

Dodson, S. (2004). *Introduction to Limnology*. McGraw-Hill Education.

Dolman, A. M., Rücker, J., Pick, F. R., Fastner, J., Rohrlack, T., Mischke, U., & Wiedner, C. (2012). Cyanobacteria and Cyanotoxins: The Influence of Nitrogen versus Phosphorus. *PLOS ONE*, *7*(6), e38757. https://doi.org/10.1371/journal.pone.0038757

Donald, D. B., Bogard, M. J., Finlay, K., & Leavitt, P. R. (2011). Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnology and Oceanography*, *56*(6), 2161–2175. https://doi.org/10.4319/lo.2011.56.6.2161

Downing, J. A., & McCauley, E. (1992). The nitrogen: Phosphorus relationship in lakes. *Limnology and Oceanography*, *37*(5), 936–945. https://doi.org/10.4319/lo.1992.37.5.0936

Downing, T. G., Meyer, C., Gehringer, M. M., & van de Venter, M. (2005). Microcystin content of Microcystis aeruginosa is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology*, *20*(3), 257–262. https://doi.org/10.1002/tox.20106

Duan, H., Ma, R., Xu, X., Kong, F., Zhang, S., Kong, W., Hao, J., & Shang, L. (2009). Two-Decade Reconstruction of Algal Blooms in China’s Lake Taihu. *Environmental Science & Technology*, *43*(10), 3522–3528. https://doi.org/10.1021/es8031852

Elliott, J. A. (2010). The seasonal sensitivity of Cyanobacteria and other phytoplankton to changes in flushing rate and water temperature. *Global Change Biology*, *16*(2), 864–876. https://doi.org/10.1111/j.1365-2486.2009.01998.x

Elliott, J. A., Jones, I. D., & Thackeray, S. J. (2006). Testing the Sensitivity of Phytoplankton Communities to Changes in Water Temperature and Nutrient

Load, in a Temperate Lake. *Hydrobiologia*, *559*(1), 401–411. https://doi.org/10.1007/s10750-005-1233-y

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Ewing, H. A., Weathers, K. C., Cottingham, K. L., Leavitt, P. R., Greer, M. L., Carey, C. C., Steele, B. G., Fiorillo, A. U., & Sowles, J. P. (2020). “New” cyanobacterial blooms are not new: Two centuries of lake production are related to ice cover and land use. *Ecosphere*, *11*(6), e03170. https://doi.org/10.1002/ecs2.3170

Filstrup, C. T., & Downing, J. A. (2017). Relationship of chlorophyll to phosphorus and nitrogen in nutrient-rich lakes. *Inland Waters*, *7*(4), 385–400. https://doi.org/10.1080/20442041.2017.1375176

Filstrup, C. T., Hillebrand, H., Heathcote, A. J., Harpole, W. S., & Downing, J. A. (2014). Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. *Ecology Letters*, *17*(4), 464–474. https://doi.org/10.1111/ele.12246

Giordanino, M. V. F., Strauch, S. M., Villafañe, V. E., & Helbling, E. W. (2011). Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. *Journal of Photochemistry and Photobiology B: Biology*, *103*(1), 68–77. https://doi.org/10.1016/j.jphotobiol.2011.01.013

Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C. L., Leavitt, P. R., Parker, A. E., Burkholder, J. M., & Kana, T. M. (2016). Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnology and Oceanography*, *61*(1), 165–197. https://doi.org/10.1002/lno.10203

Gobler, C. J., Burkholder, J. M., Davis, T. W., Harke, M. J., Johengen, T., Stow, C. A., & Van de Waal, D. B. (2016). The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. *Harmful Algae*, *54*, 87–97. https://doi.org/10.1016/j.hal.2016.01.010

Harding, L. W., Batiuk, R. A., Fisher, T. R., Gallegos, C. L., Malone, T. C., Miller, W. D., Mulholland, M. R., Paerl, H. W., Perry, E. S., & Tango, P. (2014). Scientific Bases for Numerical Chlorophyll Criteria in Chesapeake Bay. *Estuaries and Coasts*, *37*(1), 134–148. https://doi.org/10.1007/s12237-013-9656-6

Harris, T. D., Smith, V. H., Graham, J. L., Van de Waal, D. B., Tedesco, L. P., & Clercin, N. (2016). Combined effects of nitrogen to phosphorus and nitrate to ammonia ratios on cyanobacterial metabolite concentrations in eutrophic Midwestern USA reservoirs. *Inland Waters*, *6*(2), 199–210. https://doi.org/10.5268/IW-6.2.938

Heathcote, A. J., Filstrup, C. T., Kendall, D., & Downing, J. A. (2016). Biomass pyramids in lake plankton: Influence of Cyanobacteria size and abundance. *Inland Waters*, *6*(2), 250–257. https://doi.org/10.5268/IW-6.2.941

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., & Zohary, T. (1999). Biovolume Calculation for Pelagic and Benthic Microalgae. *Journal of Phycology*, *35*(2), 403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x

Ho, M.-Y., Soulier, N. T., Canniffe, D. P., Shen, G., & Bryant, D. A. (2017). Light regulation of pigment and photosystem biosynthesis in cyanobacteria. *Current Opinion in Plant Biology*, *37*, 24–33. https://doi.org/10.1016/j.pbi.2017.03.006

Hoffman, D. K., McCarthy, M. J., Boedecker, A. R., Myers, J. A., & Newell, S. E. (n.d.). The role of internal nitrogen loading in supporting non-N-fixing harmful cyanobacterial blooms in the water column of a large eutrophic lake. *Limnology and Oceanography*, *n/a*(n/a). https://doi.org/10.1002/lno.12185

Holland, D. P., Pantorno, A., Orr, P. T., Stojkovic, S., & Beardall, J. (2012). The impacts of a high CO2 environment on a bicarbonate user: The cyanobacterium Cylindrospermopsis raciborskii. *Water Research*, *46*(5), 1430–1437. https://doi.org/10.1016/j.watres.2011.11.015

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbiology*, *16*(8), Article 8. https://doi.org/10.1038/s41579-018-0040-1

Huisman, J., & Hulot, F. D. (2005). Population Dynamics of Harmful Cyanobacteria. In J. Huisman, H. C. P. Matthijs, & P. M. Visser (Eds.), *Harmful Cyanobacteria* (pp. 143–176). Springer Netherlands. https://doi.org/10.1007/1-4020-3022-3_7

Ingrid Chorus, I. R. F., Henry J. Salas, Jamie Bartram. (2000). Health Risks Caused by Freshwater Cyanobacteria in Recreational Waters. *Journal of Toxicology and Environmental Health, Part B*, *3*(4), 323–347. https://doi.org/10.1080/109374000436364

Jang, M.-H., Ha, K., Joo, G.-J., & Takamura, N. (2003). Toxin production of cyanobacteria is increased by exposure to zooplankton. *Freshwater Biology*, *48*(9), 1540–1550. https://doi.org/10.1046/j.1365-2427.2003.01107.x

Jefferies, R. L. (2000). Allochthonous inputs: Integrating population changes and food-web dynamics. *Trends in Ecology & Evolution*, *15*(1), 19–22. https://doi.org/10.1016/S0169-5347(99)01758-9

Jiang, X., Ha, C., Lee, S., Kwon, J., Cho, H., Gorham, T., & Lee, J. (2019). Characterization of Cyanophages in Lake Erie: Interaction Mechanisms and Structural Damage of Toxic Cyanobacteria. *Toxins*, *11*(8), Article 8. https://doi.org/10.3390/toxins11080444

Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, *14*(3), 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Kasinak, J.-M. E., Holt, B. M., Chislock, M. F., & Wilson, A. E. (2015). Benchtop fluorometry of phycocyanin as a rapid approach for estimating cyanobacterial biovolume. *Journal of Plankton Research*, *37*(1), 248–257. https://doi.org/10.1093/plankt/fbu096

Katsiapi, M., Mazaris, A. D., Charalampous, E., & Moustaka-Gouni, M. (2012). Watershed land use types as drivers of freshwater phytoplankton structure. In N. Salmaso, L. Naselli-Flores, L. Cerasino, G. Flaim, M. Tolotti, & J. Padisák (Eds.), *Phytoplankton responses to human impacts at different scales* (pp. 121–131). Springer Netherlands. https://doi.org/10.1007/978-94-007-5790-5_10

Kebede, E., & Belay, A. (1994). Species composition and phytoplankton biomass in a tropical African lake (Lake Awassa, Ethiopia). *Hydrobiologia*, *288*(1), 13–32. https://doi.org/10.1007/BF00006802

Kosten, S., Huszar, V. L. M., Bécares, E., Costa, L. S., van Donk, E., Hansson, L.-A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., De Meester, L., Moss, B., Lürling, M., Nõges, T., Romo, S., & Scheffer, M. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biology*, *18*(1), 118–126. https://doi.org/10.1111/j.1365-2486.2011.02488.x

Lampert, W. (1987). Laboratory studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 483–490. https://doi.org/10.1080/00288330.1987.9516244

Lee, S. j., Jang, M.-H., Kim, H.-S., Yoon, B.-D., & Oh, H.-M. (2000). Variation of microcystin content of Microcystis aeruginosa relative to medium N:P ratio and growth stage. *Journal of Applied Microbiology*, *89*(2), 323–329. https://doi.org/10.1046/j.1365-2672.2000.01112.x

Lei, L., Li, C., Peng, L., & Han, B.-P. (2015). Competition between toxic and non-toxic Microcystis aeruginosa and its ecological implication. *Ecotoxicology*, *24*(7), 1411–1418. https://doi.org/10.1007/s10646-015-1456-2

Lepori, F., & Keck, F. (2012). Effects of Atmospheric Nitrogen Deposition on Remote Freshwater Ecosystems. *AMBIO*, *41*(3), 235–246. https://doi.org/10.1007/s13280-012-0250-0

Loeks, B. M., & Cotner, J. B. (2020). Upper Midwest lakes are supersaturated with N2. *Proceedings of the National Academy of Sciences*, *117*(29), 17063–17067. https://doi.org/10.1073/pnas.1921689117

Lu, X., Tian, C., Pei, H., Hu, W., & Xie, J. (2013). Environmental factors influencing cyanobacteria community structure in Dongping Lake, China. *Journal of Environmental Sciences*, *25*(11), 2196–2206. https://doi.org/10.1016/S1001-0742(12)60297-6

Luo, W., Chen, H., Lei, A., Lu, J., & Hu, Z. (2014). Estimating Cyanobacteria Community Dynamics and its Relationship with Environmental Factors. *International Journal of Environmental Research and Public Health*, *11*(1), Article 1. https://doi.org/10.3390/ijerph110101141

Marcarelli, A. M., Fulweiler, R. W., & Scott, J. T. (2022). Nitrogen fixation: A poorly understood process along the freshwater-marine continuum. *Limnology and Oceanography Letters*, *7*(1), 1–10. https://doi.org/10.1002/lol2.10220

McCormick, P. V., & Cairns, J. (1994). Algae as indicators of environmental change. *Journal of Applied Phycology*, *6*(5), 509–526. https://doi.org/10.1007/BF02182405

Mischke, U. (2003). Cyanobacteria associations in shallow polytrophic lakes: Influence of environmental factors. *Acta Oecologica*, *24*, S11–S23. https://doi.org/10.1016/S1146-609X(03)00003-1

Moisander, P. H., Ochiai, M., & Lincoff, A. (2009). Nutrient limitation of Microcystis aeruginosa in northern California Klamath River reservoirs. *Harmful Algae*, *8*(6), 889–897. https://doi.org/10.1016/j.hal.2009.04.005

Morales-Williams, A. M., Wanamaker Jr., A. D., & Downing, J. A. (2017). Cyanobacterial carbon concentrating mechanisms facilitate sustained CO_2 depletion in eutrophic lakes. *Biogeosciences*, *14*(11), 2865–2875. https://doi.org/10.5194/bg-14-2865-2017

Natwora, K. E., & Sheik, C. S. (2021). Assessment of nitrogen fixation rates in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *47*(5), 1288–1295. https://doi.org/10.1016/j.jglr.2021.07.005

Newell, S. E., Davis, T. W., Johengen, T. H., Gossiaux, D., Burtner, A., Palladino, D., & McCarthy, M. J. (2019). Reduced forms of nitrogen are a driver of non-nitrogen-fixing harmful cyanobacterial blooms and toxicity in Lake Erie. *Harmful Algae*, *81*, 86–93. https://doi.org/10.1016/j.hal.2018.11.003

Nürnberg, G. K., & LaZerte, B. D. (2016). More than 20years of estimated internal phosphorus loading in polymictic, eutrophic Lake Winnipeg, Manitoba. *Journal of Great Lakes Research*, *42*(1), 18–27. https://doi.org/10.1016/j.jglr.2015.11.003

Olokotum, M., Mitroi, V., Troussellier, M., Semyalo, R., Bernard, C., Montuelle, B., Okello, W., Quiblier, C., & Humbert, J.-F. (2020). A review of the socioecological causes and consequences of cyanobacterial blooms in Lake Victoria. *Harmful Algae*, *96*, 101829. https://doi.org/10.1016/j.hal.2020.101829

Onderka, M. (2007). Correlations between several environmental factors affecting the bloom events of cyanobacteria in Liptovska Mara reservoir (Slovakia)—A simple regression model. *Ecological Modelling*, *209*(2), 412–416. https://doi.org/10.1016/j.ecolmodel.2007.07.028

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Österholm, J., Popin, R. V., Fewer, D. P., & Sivonen, K. (2020). Phylogenomic Analysis of Secondary Metabolism in the Toxic Cyanobacterial Genera Anabaena, Dolichospermum and Aphanizomenon. *Toxins*, *12*(4), Article 4. https://doi.org/10.3390/toxins12040248

Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of The Total Environment*, *409*(10), 1739–1745. https://doi.org/10.1016/j.scitotenv.2011.02.001

Paerl, H. W., & Huisman, J. (2008). Blooms Like It Hot. *Science*, *320*(5872), 57–58. https://doi.org/10.1126/science.1155398

Paerl, H. W., & Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, *1*(1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x

Paerl, H. W., & Otten, T. G. (2013). Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, *65*(4), 995–1010. https://doi.org/10.1007/s00248-012-0159-y

Paerl, H. W., & Otten, T. G. (2016). Duelling 'CyanoHABs': Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. *Environmental Microbiology*, *18*(2), 316–324. https://doi.org/10.1111/1462-2920.13035

Paerl, H. W., Valdes, L. M., Joyner, A. R., Piehler, M. F., & Lebo, M. E. (2004). Solving Problems Resulting from Solutions: Evolution of a Dual Nutrient Management Strategy for the Eutrophying Neuse River Estuary, North Carolina. *Environmental Science & Technology*, *38*(11), 3068–3073. https://doi.org/10.1021/es0352350

Persaud, A. D., Paterson, A. M., Dillon, P. J., Winter, J. G., Palmer, M., & Somers, K. M. (2015). Forecasting cyanobacteria dominance in Canadian temperate lakes. *Journal of Environmental Management*, *151*, 343–352. https://doi.org/10.1016/j.jenvman.2015.01.009

Qin, B., Yang, G., Ma, J., Wu, T., Li, W., Liu, L., Deng, J., & Zhou, J. (2018). Spatiotemporal Changes of Cyanobacterial Bloom in Large Shallow Eutrophic

Lake Taihu, China. *Frontiers in Microbiology*, *9*. https://www.frontiersin.org/articles/10.3389/fmicb.2018.00451

Rabalais, N. N., Díaz, R. J., Levin, L. A., Turner, R. E., Gilbert, D., & Zhang, J. (2010). Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences*, *7*(2), 585–619. https://doi.org/10.5194/bg-7-585-2010

Reinl, K. L., Brookes, J. D., Carey, C. C., Harris, T. D., Ibelings, B. W., Morales-Williams, A. M., De Senerpont Domis, L. N., Atkins, K. S., Isles, P. D. F., Mesman, J., North, R. L., Rudstam, L. G., Alegre Stelzer, J. A., Venkiteswaran, J. J., Yokota, K., & Zhan, Q. (2021). Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology*. https://doi.org/10.1111/fwb.13791

Reynolds, C. S. (1998). What factors influence the species composition of phytoplankton in lakes of different trophic status? *Hydrobiologia*, *369*(0), 11–26. https://doi.org/10.1023/A:1017062213207

Reynolds, C. S. (2006). *The Ecology of Phytoplankton*. Cambridge University Press.

Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L., & Melo, S. (2002). Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research*, *24*(5), 417–428. https://doi.org/10.1093/plankt/24.5.417

Reynolds, C. S., Oliver, R. L., & Walsby, A. E. (1987). Cyanobacterial dominance: The role of buoyancy regulation in dynamic lake environments. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 379–390. https://doi.org/10.1080/00288330.1987.9516234

Richardson, J., Miller, C., Maberly, S. C., Taylor, P., Globevnik, L., Hunter, P., Jeppesen, E., Mischke, U., Moe, S. J., Pasztaleniec, A., Søndergaard, M., & Carvalho, L. (2018). Effects of multiple stressors on cyanobacteria abundance vary with lake type. *Global Change Biology*, *24*(11), 5044–5055. https://doi.org/10.1111/gcb.14396

Roelke, D., Buyukates, Y., Williams, M., & Jean, J. (2004). Interannual variability in the seasonal plankton succession of a shallow, warm-water lake. *Hydrobiologia*, *513*(1), 205–218. https://doi.org/10.1023/B:hydr.0000018185.41861.07

Rose, V. J., Forney, W. M., Norton, R. A., & Harrison, J. A. (2019). Catchment characteristics, water quality, and cyanobacterial blooms in Washington and Oregon Lakes. *Lake and Reservoir Management*, *35*(1), 51–63. https://doi.org/10.1080/10402381.2018.1518940

Schindler, D. W., Fee, E. J., & Ruszczynski, T. (1978). Phosphorus Input and Its Consequences for Phytoplankton Standing Crop and Production in the Experimental Lakes Area and in Similar Lakes. *Journal of the Fisheries Research Board of Canada*, *35*(2), 190–196. https://doi.org/10.1139/f78-031

Shapiro, J. (1997). The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. *Freshwater Biology*, *37*(2), 307–323. https://doi.org/10.1046/j.1365-2427.1997.00164.x

Soares, M. C. S., Rocha, M. I. de A., Marinho, M. M., Azevedo, S. M. F. O., Branco, C. W. C., & Huszar, V. L. M. (2009). Changes in species composition during annual cyanobacterial dominance in a tropical reservoir: Physical factors, nutrients and grazing effects. *Aquatic Microbial Ecology*, *57*(2), 137–149. https://doi.org/10.3354/ame01336

Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., Jeppesen, E., Lürling, M., Molinero, J. C., Mooij, W. M., van Donk, E., & Winder, M. (2012). Beyond the Plankton Ecology Group (PEG) Model: Mechanisms

Driving Plankton Succession. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 429–448. https://doi.org/10.1146/annurev-ecolsys-110411-160251

Soo, R. M., Hemp, J., Parks, D. H., Fischer, W. W., & Hugenholtz, P. (2017). On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. *Science*, *355*(6332), 1436–1440. https://doi.org/DOI: 10.1126/science.aal3794

Steffen, M. M., Davis, T. W., McKay, R. M. L., Bullerjahn, G. S., Krausfeldt, L. E., Stough, J. M. A., Neitzey, M. L., Gilbert, N. E., Boyer, G. L., Johengen, T. H., Gossiaux, D. C., Burtner, A. M., Palladino, D., Rowe, M. D., Dick, G. J., Meyer, K. A., Levy, S., Boone, B. E., Stumpf, R. P., … Wilhelm, S. W. (2017). Ecophysiological Examination of the Lake Erie Microcystis Bloom in 2014: Linkages between Biology and the Water Supply Shutdown of Toledo, OH. *Environmental Science & Technology*, *51*(12), 6745–6755. https://doi.org/10.1021/acs.est.7b00856

Sterner, R. W., Reinl, K. L., Lafrancois, B. M., Brovold, S., & Miller, T. R. (2020). A first assessment of cyanobacterial blooms in oligotrophic Lake Superior. *Limnology and Oceanography*, *65*(12), 2984–2998. https://doi.org/10.1002/lno.11569

Stumpf, R. P., Johnson, L. T., Wynne, T. T., & Baker, D. B. (2016). Forecasting annual cyanobacterial bloom biomass to inform management decisions in Lake Erie. *Journal of Great Lakes Research*, *42*(6), 1174–1183. https://doi.org/10.1016/j.jglr.2016.08.006

Sukenik, A., Quesada, A., & Salmaso, N. (2015). Global expansion of toxic and non-toxic cyanobacteria: Effect on ecosystem functioning. *Biodiversity and Conservation*, *24*(4), 889–908. https://doi.org/10.1007/s10531-015-0905-9

Tanvir, R. U., Hu, Z., Zhang, Y., & Lu, J. (2021). Cyanobacterial community succession and associated cyanotoxin production in hypereutrophic and eutrophic freshwaters. *Environmental Pollution*, *290*, 118056. https://doi.org/10.1016/j.envpol.2021.118056

Taranu, Z. E., Gregory-Eaves, I., Leavitt, P. R., Bunting, L., Buchaca, T., Catalan, J., Domaizon, I., Guilizzoni, P., Lami, A., McGowan, S., Moorhouse, H., Morabito, G., Pick, F. R., Stevenson, M. A., Thompson, P. L., & Vinebrooke, R. D. (2015). Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. *Ecology Letters*, *18*(4), 375–384. https://doi.org/10.1111/ele.12420

Tilman, D., Kilham, S. S., & Kilham, P. (1982). Phytoplankton Community Ecology: The Role of Limiting Nutrients. *Annual Review of Ecology and Systematics*, *13*, 349–372.

Tu, J. (2009). Combined impact of climate and land use changes on streamflow and water quality in eastern Massachusetts, USA. *Journal of Hydrology*, *379*(3), 268–283. https://doi.org/10.1016/j.jhydrol.2009.10.009

Utermöhl, H. (1958). Methods of collecting plankton for various purposes are discussed. *SIL Communications, 1953-1996*, *9*(1), 1–38. https://doi.org/10.1080/05384680.1958.11904091

Van de Waal, D. B., Verspagen, J. M., Finke, J. F., Vournazou, V., Immers, A. K., Kardinaal, W. E. A., Tonk, L., Becker, S., Van Donk, E., Visser, P. M., & Huisman, J. (2011). Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO2. *The ISME Journal*, *5*(9), Article 9. https://doi.org/10.1038/ismej.2011.28

Van de Waal, D. B., Verspagen, J. M. H., Lürling, M., Van Donk, E., Visser, P. M., & Huisman, J. (2009). The ecological stoichiometry of toxins produced by harmful cyanobacteria: An experimental test of the carbon-nutrient balance hypothesis. *Ecology Letters*, *12*(12), 1326–1335. https://doi.org/10.1111/j.1461-0248.2009.01383.x

Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., & Sivonen, K. (2002). Effect of Nitrogen and Phosphorus on Growth of Toxic and Nontoxic Microcystis Strains and on Intracellular Microcystin Concentrations. *Microbial Ecology*, *43*(4), 443–454.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, D. G. (1997). Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications*, *7*(3), 737–750. https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wagner, C., & Adrian, R. (2009). Cyanobacteria dominance: Quantifying the effects of climate change. *Limnology and Oceanography*, *54*(6part2), 2460–2468. https://doi.org/10.4319/lo.2009.54.6_part_2.2460

Wagner, N. D., Quach, E., Buscho, S., Ricciardelli, A., Kannan, A., Naung, S. W., Phillip, G., Sheppard, B., Ferguson, L., Allen, A., Sharon, C., Duke, J. R., Taylor, R. B., Austin, B. J., Stovall, J. K., Haggard, B. E., Chambliss, C. K., Brooks, B. W., & Scott, J. T. (2021). Nitrogen form, concentration, and micronutrient availability affect microcystin production in cyanobacterial blooms. *Harmful Algae*, *103*, 102002. https://doi.org/10.1016/j.hal.2021.102002

Walsby, A. E. (1994). Gas vesicles. *Microbiological Reviews*, *58*(1), 94–144. https://doi.org/10.1128/mr.58.1.94-144.1994

Watson, S. B., McCauley, E., & Downing, J. A. (1997). Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnology and Oceanography*, *42*(3), 487–495. https://doi.org/10.4319/lo.1997.42.3.0487

Weyhenmeyer, G. A., Peter, H., & Willén, E. (2013). Shifts in phytoplankton species richness and biomass along a latitudinal gradient – consequences for relationships between biodiversity and ecosystem functioning. *Freshwater Biology*, *58*(3), 612–623. https://doi.org/10.1111/j.1365-2427.2012.02779.x

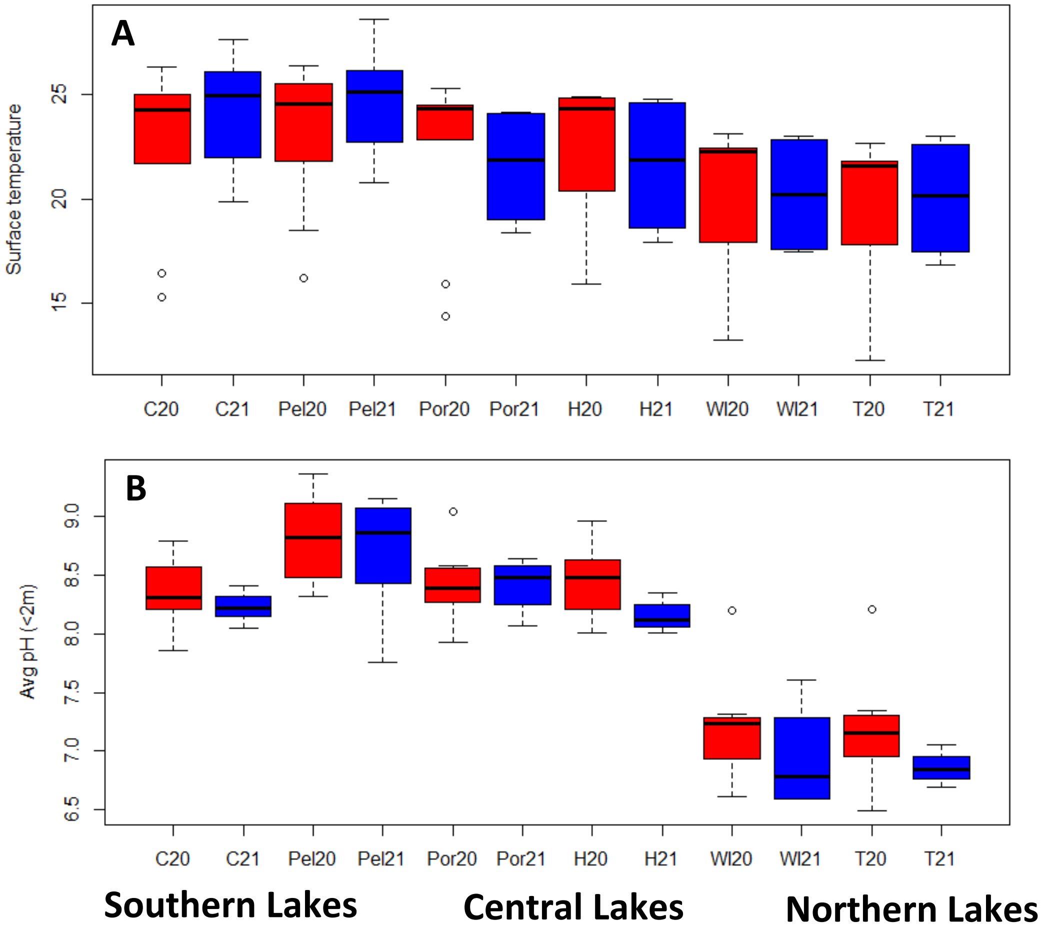
Wilhelm, S. W., Bullerjahn, G. S., Eldridge, M. L., Rinta-Kanto, J. M., Poorvin, L., & Bourbonniere, R. A. (2006). Seasonal Hypoxia and the Genetic Diversity of Prokaryote Populations in the Central Basin Hypolimnion of Lake Erie: Evidence for Abundant Cyanobacteria and Photosynthesis. *Journal of Great Lakes Research*, *32*(4), 657–671. https://doi.org/10.3394/0380-1330(2006)32[657:SHATGD]2.0.CO;2

Wilkinson, G. M., Walter, J. A., Buelo, C. D., & Pace, M. L. (2022). No evidence of widespread algal bloom intensification in hundreds of lakes. *Frontiers in Ecology and the Environment*, *20*(1), 16–21. https://doi.org/10.1002/fee.2421

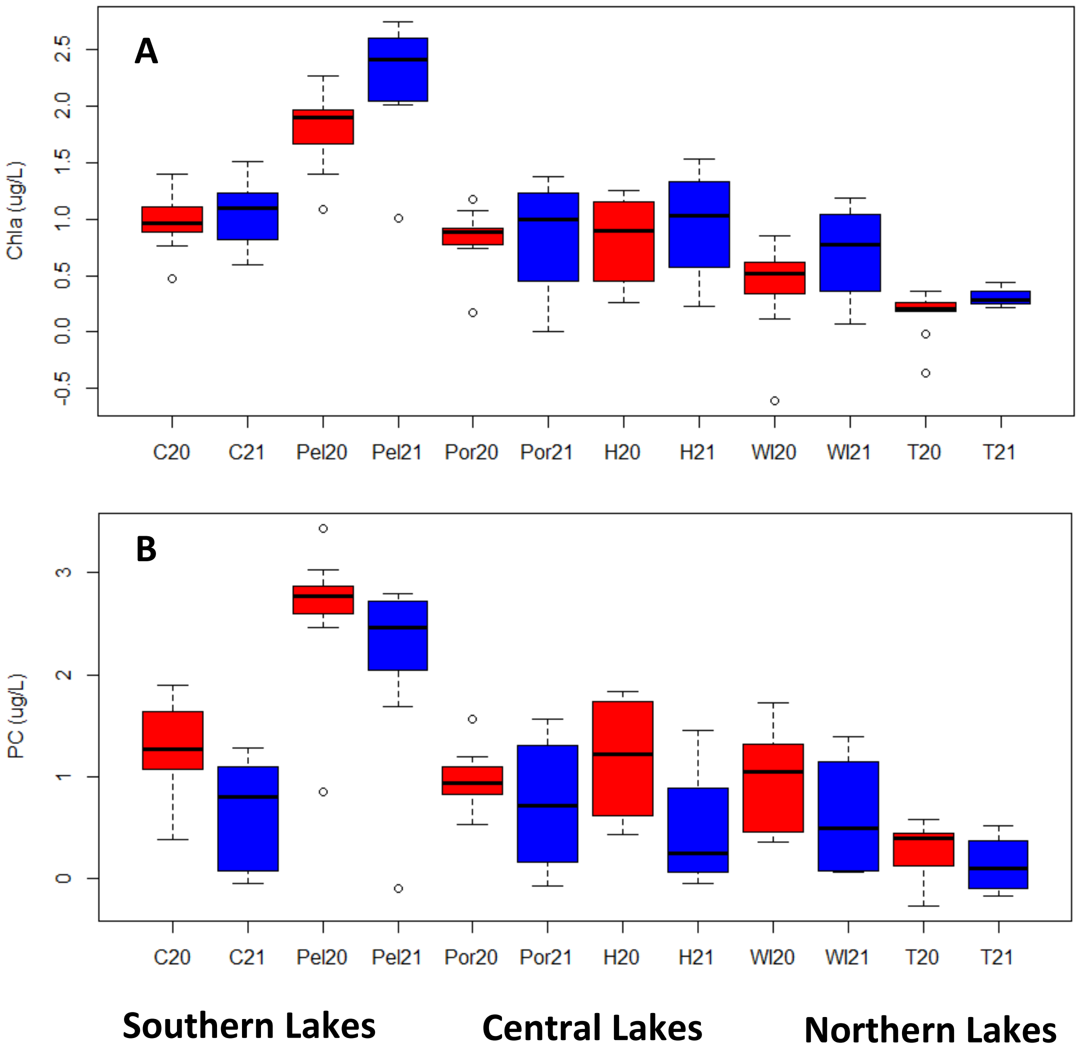
Wu, Y., Li, L., Zheng, L., Dai, G., Ma, H., Shan, K., Wu, H., Zhou, Q., & Song, L. (2016). Patterns of succession between bloom-forming cyanobacteria Aphanizomenon flos-aquae and Microcystis and related environmental factors in large, shallow Dianchi Lake, China. *Hydrobiologia*, *765*(1), 1–13. https://doi.org/10.1007/s10750-015-2392-0

Zhang, M., Yang, Z., Yu, Y., & Shi, X. (2020). Interannual and Seasonal Shift between Microcystis and Dolichospermum: A 7-Year Investigation in Lake Chaohu, China. *Water*, *12*(7), Article 7. https://doi.org/10.3390/w12071978

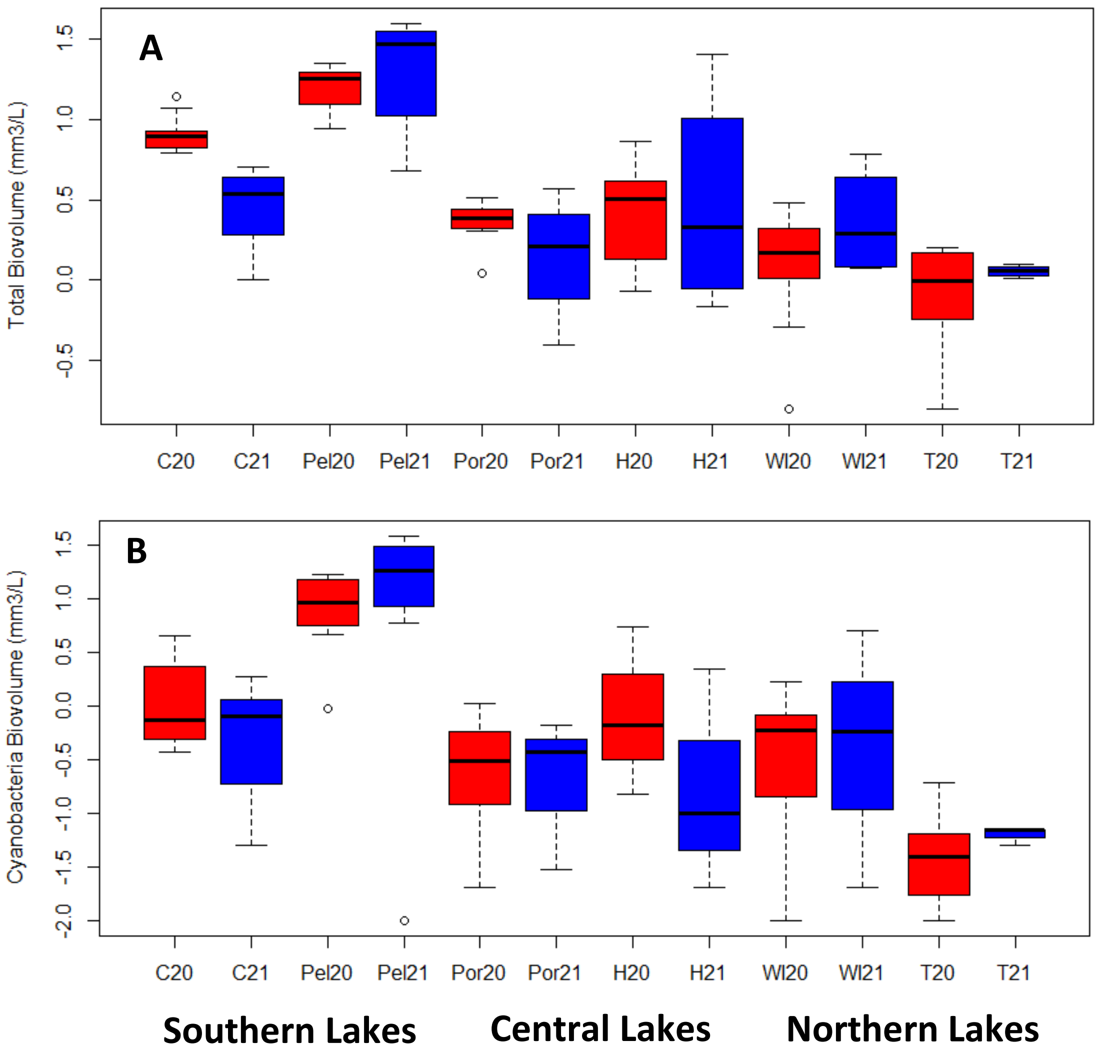
APPENDIX



Appendix 1. Interannual variability of (A) surface temperature (°C) and (B) average water column pH across six sample lakes for 2020 (red) and 2021 (blue). Carrie (C), Peltier (Pel), Portage (Por), Hill (H), White Iron (WI), Tait (T).



Appendix 2. Interannual variability of $\log_{10}$ transformed (A) chlorophyll-a (chla) and (B) phycocyanin (PC) concentrations across six sample lakes during 2020 (red) and 2021 (blue). Carrie (C), Peltier (Pel), Portage (Por), Hill (H), White Iron (WI), Tait (T).



Appendix 3. Interannual variability of $\log_{10}$ transformed (A) phytoplankton and (B) cyanobacteria biovolumes across six samples lakes for 2020 (red) and 2021 (blue). Carrie (C), Peltier (Pel), Portage (Por), Hill (H), White Iron (WI), Tait (T).