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Lara Stephanie Jansen
Portland State University

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Drivers and Dynamics of Phytoplankton Communities and Harmful Algal Blooms in Mountain Lakes

by

Lara Stephanie Jansen

A dissertation submitted in partial fulfillment of the
requirements for the degree of

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in
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Dissertation Committee:
Kelly Gleason, Chair
Angela Strecker
Daniel Sobota
Yangdong Pan

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Abstract

Harmful cyanobacterial blooms (cyanoHABs) are a complex and widespread disturbance in freshwater water bodies, impacting water quality for wildlife and human populations. While cyanobacteria often bloom in warm lakes impacted by human development like agriculture, blooms are increasingly reported in cooler waters with limited development in the surrounding watershed. As much of cyanoHAB research has focused on lakes in highly developed watersheds, the understanding of factors leading to cyanobacteria dominance and blooms in the absence of major development remains limited. Mountain lakes can serve as ideal systems to study bloom-forming cyanobacteria in watersheds with minimal development. In addition, mountain lakes span natural gradients of productivity and climate due to the varied elevation and topography. Mountain lakes are also highly valued for recreation and habitat for sensitive species due to perceived pristine conditions, but are vulnerable to multiple stressors such as climate warming and increased atmospheric deposition of nutrients, which limit cyanobacteria growth. Therefore, the potential for cyanoHABs in mountain lakes is likely to rise in the near future, making it crucial to understand the drivers of cyanobacteria dominance and blooms in these sensitive lakes.

The goal of this dissertation is to improve the understanding of drivers and dynamics of bloom-forming cyanobacteria in mountain lakes, including factors across lake to watershed scales, to temporal and spatial patterns in planktonic communities. I conducted field surveys to examine the physical and chemical conditions as well as the phytoplankton and other key biological communities in a set of Cascade Mountain lakes located in Oregon, USA. In Chapter 2, I used survey data and existing geospatial data to identify food web, lake, and watershed drivers, as well as interactions among drivers across scales on nitrogen (N)-fixing cyanobacteria, the common cyanoHAB taxa in the region. In Chapter 3, I examined the intra- and inter-annual variation in cyanoHABs and cyanotoxins relative to meteorological conditions and the bacterioplankton communities. Based on genetic sequencing and time integrated toxin monitoring, I determined possible temporal relationships and biological indicators of blooms and bloom toxicity. In Chapter 4, I used survey data as along with existing and interpolated trait data to assess how the top-down and bottom-up effects of grazing zooplankton and nutrients could have shaped phytoplankton communities functionally. I discuss the resulting implications for bloom-forming cyanobacteria dominance over other phytoplankton.

I identified watershed size and hypolimnetic dissolved oxygen as key drivers of N-fixing cyanobacteria biomass likely through the regulation of limiting nutrients such as phosphorus. Temperature and rainfall did not correspond strongly to the blooms and toxins of N-fixing cyanobacteria across lakes, suggesting other meteorological factors as well as lake productivity may be more important. Yet further study is needed with high-frequency sampling and better characterization of meteorological conditions. In terms of the food web, I found grazing cladocerans were a significant factor for N-fixing cyanobacteria, likely via consumption of other phytoplankton. These grazers may be in turn influenced by fish populations. I also found additional biological indicators for cyanobacteria blooms as well as bloom toxicity in heterotrophic bacteria. Finally, I found high grazer abundances favor fast growing and small unicellular phytoplankton at relatively low nutrients, while increasing phosphorus favors colonial cyanobacteria capable of toxin production, such as N-fixing taxa. At high levels of grazers and phosphorus, toxigenic cyanobacteria likely dominate phytoplankton communities in mountain lakes.

This dissertation sheds light on the key factors and indicators for N-fixing cyanobacteria dominance and cyanoHABs in mountain lakes. My results suggest that lake management agencies must consider how both landscape and within-lake factors may affect the risk for cyanoHABs, and how fish stocking increases risk via their impact on grazing zooplankton biomass and body size. As mountain lake monitoring is limited, my results suggest genetic identification of bacterial communities may provide a more time-integrated way to assess bloom development and toxicity. Determining how abiotic and biotic factors interact is needed to broaden management approaches since strict nutrient reduction programs have had mixed success in reducing cyanoHABs in lakes.

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Preface

Chapter 2 is currently under revision for the Limnology & Oceanography journal.

Chapter 3 and 4 will also be submitted for publication as separate manuscripts. Therefore, some repetition in introductory material is present. References are cited at the end of each chapter.

Chapter 1: Introduction to phytoplankton and blooms in mountain lakes

Freshwater phytoplankton are made up of unicellular and colonial photosynthetic organisms that support numerous ecosystem functions and services in water bodies (Reynolds, 2006; Naselli-Flores & Padisák, 2022). As the base of the food web, phytoplankton indirectly support fish populations through grazing zooplankton and invertebrates, and in turn, phytoplankton consumers readily moving carbon and other nutrients through the trophic levels (Naselli-Flores & Padisák, 2022). These photosynthetic organisms also affect water quality, such as through the production of toxic blooms and formation of hypoxia (Naselli-Flores & Barone, 2011; Lürling *et al.*, 2013). Therefore, the dynamics and drivers of freshwater phytoplankton remain an important area of ecological study, especially with the complex impacts of climate shifts and rising nutrient pollution (Paerl & Paul, 2012; Taranu *et al.*, 2015; Stoddard *et al.*, 2016; Wurtsbaugh, Paerl & Dodds, 2019; Oleksy *et al.*, 2020). The independent and interacting roles of abiotic and biotic factors in shaping phytoplankton communities and the resulting implications for harmful algal blooms in mountain lakes are central themes in this dissertation.

1.1 Freshwater phytoplankton

The multiple ecological roles of freshwater phytoplankton are driven partially by the large diversity of this group living in the open water (Reynolds, 2006). The major taxonomic groups of phytoplankton are chlorophytes, diatoms, chrysophytes, dinoflagellates, cryptophytes, euglenophytes, and cyanobacteria (Reynolds, 2006). Eukaryotic and prokaryotic phytoplankton are mostly photoautotrophs that have similar requirements for metabolism and growth and therefore compete for the same resources:

light, carbon, nitrogen, and phosphorus, as well as trace amounts of other elements such as iron and molybdenum (Hutchinson, 1961; Reynolds, 2006). The diversity of phytoplankton is a seeming paradox, as the open water of the pelagic environment is unstructured and relatively limited in all of these resources (Hutchinson, 1961). Yet the conditions in freshwater bodies change rapidly at a rate similar to phytoplankton reproduction, often on the order of days and weeks, which causes favored species to vary in time, allowing for coexistence (Hutchinson, 1961; Wetzel, 2001). In addition, phytoplankton species can have slight variations in resource requirements and vulnerabilities to zooplankton grazers, as well as mutualistic interactions, all of which facilitate coexistence (Brett, Müller-Navarra & Sang-Kyu, 2000; Reynolds, 2006). The major taxonomic groups can broadly be distinguished by these environmental filters and biotic interactions, such as diatoms often are favored in the nutrient-replete conditions and low grazer abundances, while dinoflagellates are favored in nutrient-poor conditions (typically late summer) and high grazer abundances (Reynolds, 1984, 2006; Sommer *et al.*, 1986). However, resource requirements and adaptations vary greatly within these major groups, demonstrating the wide diversity of phytoplankton (Reynolds *et al.*, 2002; Reynolds, 2006; Padisák, Crossetti & Naselli-Flores, 2009).

The dynamics of freshwater phytoplankton communities can be described more mechanistically through functional traits, which are traits that affect an organism's fitness, than taxonomy (Litchman & Klausmeier, 2008; Wentzky *et al.*, 2020; Loewen, Vinebrooke & Zurawell, 2021). The ecological niches of phytoplankton species, similar to terrestrial plants, can be defined by specific traits of morphology (e.g., cell size and shape), physiology (e.g., nutrient uptakes and affinities), behavior (e.g., motility), and life history (e.g., resting stages) (Litchman & Klausmeier, 2008). For example, cell size across taxa often reflects nutrient availability, with smaller cells favored in low nutrient conditions due to higher surface area to volume and smaller diffusion boundary layer, resulting in more efficient nutrient acquisition than larger cells (Litchman & Klausmeier, 2008; Hillebrand *et al.*, 2021). In addition, many traits are interrelated or co-occur together as trait syndromes, demonstrating ecological tradeoffs (Litchman & Klausmeier, 2008). Smaller taxa often also have higher nutrient uptake and growth rates than larger taxa, strengthening their competitive advantage with nutrients, but their small size makes them more susceptible to grazing (Thingstad *et al.*, 2005; Litchman & Klausmeier, 2008; Hillebrand *et al.*, 2021). Traits not only show phytoplankton responses to environmental conditions, but also the effects of phytoplankton on the environment (Litchman *et al.*, 2015; Breton *et al.*, 2017). Certain phytoplankton traits, such as diazotrophy, which is the ability to convert atmospheric nitrogen to ammonium, contribute significantly to nutrient cycles relative to external inputs (Quéré *et al.*, 2005; Cottingham *et al.*, 2015; Litchman *et al.*, 2015; Breton *et al.*, 2017). The roles of phytoplankton traits in ecosystem processes have been studied mostly in the ocean; thus, further study on freshwater phytoplankton is warranted (Weithoff & Beisner, 2019).

1.2 Harmful cyanobacterial blooms

Harmful blooms of cyanobacteria (cyanoHABs) are increasing in numerous freshwater bodies across the world, impacting water clarity and quality (Beaulieu, Pick & Gregory-Eaves, 2013; Paerl & Otten, 2013; Taranu *et al.*, 2015). The expansion of blooms is driven in part by a changing climate and expanding development, which favor multiple cyanobacterial traits (Reynolds, Oliver & Walsby, 1987a; Huisman *et al.*, 2004;

Paerl & Otten, 2013; Taranu *et al.*, 2015). The general conditions that promote dense masses of blooms are high nutrient levels, relatively warm temperatures, and stable stratified water columns, although there are exceptions (Beaulieu *et al.*, 2013; Paerl & Otten, 2013; Reinl *et al.*, 2021, 2023). Phosphorus (P) enrichment alone particularly favors diazotrophic cyanobacteria as they can overcome nitrogen (N) limitation, while N enrichment along with P often favors more non-diazotrophic species (Smith, 1983; Paerl & Otten, 2013). Water temperatures between 20 and 35°C are generally optimal for the growth of bloom-forming cyanobacteria species; but cyanobacteria can thrive at cooler temperatures depending on other environmental factors (Robarts & Zohary, 1987; Lürling *et al.*, 2013; Nalley, O'Donnell & Litchman, 2018; Reinl *et al.*, 2023). Thermal stratification due to density differences in the water column when mixing is limited can favor many bloom-forming cyanobacteria, as they can regulate their buoyancy with gas vesicles and carbon ballasts (Reynolds *et al.*, 1987a; Carey *et al.*, 2012). Cyanobacteria traits can also impact water quality, such as severe reduction of water clarity caused by buoyant colonies and filaments in a bloom that can cover much of the water surface (Carey *et al.*, 2012; Paerl & Otten, 2013). In addition, many cyanobacteria species have the capacity to produce secondary metabolites that are toxic to numerous organisms, and the type of toxins vary across cyanobacteria species (Carmichael, 2001). During blooms, toxin production can be high enough to be deadly for even large vertebrates, including humans (Carmichael, 2001). CyanoHABs are a major water quality issue that remain difficult to manage due to the species-specific preferences of cyanobacteria (Carey *et al.*, 2012; Paerl & Otten, 2013).

Human-driven nutrient loading of freshwater bodies has often been linked to a widespread rise in toxigenic (i.e., toxin-producing) cyanobacteria, yet cyanoHABs also occur in relatively nutrient-limited systems (Beaulieu *et al.*, 2013; Taranu *et al.*, 2015; Reinl *et al.*, 2021). There are several toxigenic cyanobacterial species that can bloom in oligotrophic and mesotrophic systems, such as from the filamentous orders of Nostocales and Oscillatoriales (Pomati *et al.*, 2012; Sorichetti, Creed & Trick, 2014; Carey *et al.*, 2014; Sterner *et al.*, 2020). These cyanobacteria are able to persist and even proliferate under nutrient limitation due to several traits such as diazotrophy and luxury P storage, which is the ability to sequester P in excess of metabolic demand (Carey *et al.*, 2012; Cottingham *et al.*, 2015; Reinl *et al.*, 2021). In addition, many cyanobacteria taxa are able to go dormant during stressful conditions, such as desiccation and low nutrients, by producing specialized resting cells, called akinetes, that settle into the sediments (Wildon & Mercer, 1963; Reinl *et al.*, 2021). Germination from akinetes can be triggered by increases in nutrients, temperature, and/or light (Reinl *et al.*, 2021). When nutrients become or are limiting in the epilimnion (i.e., the upper layer of stratified lakes), buoyancy regulation also allows cyanobacteria to descend to access nutrients in the hypolimnion (i.e., the lower lake layer) (Carey *et al.*, 2012). If light is relatively abundant, some cyanobacteria, such as *Planktothrix,* can maintain their buoyancy near the hypolimnion to access nutrients and are well adapted to the lower light environment due to their unique pigments such as phycobilin (Reynolds *et al.*, 1987a; Pomati *et al.*, 2012; Reinl *et al.*, 2021). These unique traits of certain cyanobacteria allow blooms to occur across the nutrient gradient, complicating management of harmful blooms through nutrient reduction (Pomati *et al.*, 2012; Reinl *et al.*, 2021).

While the abiotic factors of nutrients, temperature, and light drive blooms of cyanobacteria, zooplankton can also influence cyanoHABs through grazing and nutrient recycling (Vanni & Layne, 1997; Sarnelle, 2007; Ger *et al.*, 2016b). Bloom-forming cyanobacteria have multiple ways to resist grazing such as coloniality and toxin production, as well as being low in nutritional value relative to many eukaryotic phytoplankton (Gulati & Demott, 1997). Many zooplankton that are selective grazers, such as copepods, may actively avoid cyanobacteria if other food sources are available (DeMott & Moxter, 1991). In contrast, generalist grazers like *Daphnia* often readily consume cyanobacteria, including colonial and toxigenic taxa, especially if they have been exposed to high levels of cyanobacteria over multiple generations (DeMott, 1982; Ger *et al.*, 2016b). Toxin-tolerant cladocerans have been able to substantially reduce the biomass of toxigenic cyanobacteria, but this top-down control may depend upon the density and colony size of cyanobacteria, as well as the presence of fish (Vanni, 1987; Gliwicz, 1990; Sarnelle, 2007; Chislock *et al.*, 2013). In addition to the grazing of cyanobacteria, zooplankton contribute to the recycling of nutrients through excretion and decomposition, with nutrient stoichiometry varying with different zooplankton groups (Vanni & Layne, 1997). Yet the role of zooplankton in nutrient recycling has not been as well studied as the contribution of fish. The relative effects of zooplankton grazing and nutrient recycling on cyanobacteria blooms remains an area for further study.

Blooms of toxigenic cyanobacteria have long been studied in relation to other phytoplankton and eukaryotic grazers, yet cyanoHABs also result in significant shifts in the planktonic communities of bacteria, serving as another biological indicator (Louati *et al.*, 2015; Ger *et al.*, 2016b; Tromas *et al.*, 2017; Guedes *et al.*, 2018). With the advent of high-throughput sequencing, microbial ecologists are increasingly characterizing bacterioplankton communities in relation to harmful blooms from coastal oceans to lakes (Berg *et al.*, 2009; Louati *et al.*, 2015; Berry *et al.*, 2017; Tromas *et al.*, 2017). As toxigenic cyanobacteria make up the autotrophic portion of bacterioplankton communities, cyanoHABs inherently alter the composition of these communities, but not necessarily the diversity (Woodhouse *et al.*, 2016; Tromas *et al.*, 2017; Wang, Razzano & Mou, 2020). Multiple heterotrophic bacterial taxa that have the capacity to consume or degrade cyanobacterial compounds, including cyanotoxins, have been identified with increasing abundances during such blooms (Mou *et al.*, 2013; Lezcano *et al.*, 2017). In addition, decomposition of cyanobacteria biomass and related compounds by heterotrophic bacteria as part of the microbial loop results in the recycling of nutrients, creating a possible positive feedback loop with cyanoHABs (Paerl & Barnard, 2020). Therefore, the inclusion of bacterioplankton in predictive models of these blooms improves accuracy relative to models based only on environmental factors (Tromas *et al.*, 2017). Yet the responses and roles of bacterioplankton in cyanoHABs have not yet been examined in lakes across the trophic gradient and without substantial development in the watersheds (Eiler & Bertilsson, 2004; Berry *et al.*, 2017; Tromas *et al.*, 2017; Wang *et al.*, 2020).

1.3 Mountain lakes

The productivity of undeveloped mountain lakes is relatively low, yet can vary substantially due to different geological formations and age of formation, offering a unique setting to examine natural variation in ecosystems (Catalan *et al.*, 2006; Saros *et al.*, 2010; Nolin, 2012; Oleksy *et al.*, 2020). Mountain lakes are predominantly formed by glacial scour, especially morainal deposits damming river valleys, and volcanic activity creating depressions either from eruptions or collapses in lava flows (Wetzel, 2001). Therefore, mountain lakes can generally be either deep U-shaped glacial basins or more irregular and shallow volcanic basins, influencing the mixing regime (Wetzel, 2001). The light and thermal regimes of mountain lakes are also influenced by lake and watershed origin, such as through glacial flour inputs of remaining glaciers, as well as porous volcanic rock causing a groundwater-dominated hydrology (Catalan *et al.*, 2006; Nolin, 2012; Olson *et al.*, 2018). The formation of the lake and its watershed also affects the nutrient regime, with glacial systems often higher in N due to the nitrate-rich glacial melt than those without glaciers (Saros *et al.*, 2010). Yet many mountain lakes are also P-limited due to crystalline bedrock low in P relative to low elevation lakes with sedimentary bedrock, as well as mountain lakes with volcanic bedrock (Catalan *et al.*, 2006; Porder & Ramachandran, 2013). Therefore, the geology of the watersheds must be taken into consideration in studies of the mountain lake phytoplankton. The geological landscape and other physical characteristics also interact with climate change-induced warming as well as biotic factors, which in turn alters the effects of these factors on cyanobacteria. These altered effects are a form of cross-scale interactions, which is when driver and response variables act at different spatial or temporal scales, resulting in nonlinear dynamics (Soranno *et al.*, 2014). Therefore, landscape and local factors must be considered together to determine their influence on lake environments and their biota (Soranno *et al.*, 2014).

The elevation gradient of the mountains also affects lake nutrients, light, and temperature, which are highly influential for phytoplankton (Sommaruga, 2001; Catalan *et al.*, 2006). While water temperatures generally decline with elevation, light irradiance increases, especially ultraviolet (UV) radiation, and is often unaltered in the water due to low dissolved matter to adsorb radiation or attenuate light unless in the case of glacial inputs (Sommaruga, 2001). Elevation also negatively affects soil production and vegetation, which in turn reduces the amount of N in the lakes as productive soil is a major source of source of N (Stoddard, 1994; Baron *et al.*, 2000). Mountain lakes are more dependent on atmospheric deposition for N due to low soil productivity and greater orographic lift than lowland lakes (Stoddard, 1994; Baron *et al.*, 2000). Atmospheric deposition has been increasing in parts of the mountain ranges in western North America (Baron *et al.*, 2011). While factors influenced by elevation, such as snowpack and nutrients, have been found to alter phytoplankton biomass and composition in mountain lakes, phytoplankton composition does not seem to vary strongly with elevation (Larson *et al.*, 1998; Vinebrooke & Leavitt, 1999; Moraska Lafrancois, Nydick & Caruso, 2003; Oleksy *et al.*, 2020).

1.4 Objectives

For my dissertation, I examined the drivers and dynamics of phytoplankton, especially bloom-forming and toxigenic cyanobacteria, in the diverse environments of mountain lakes (Figure 1.1). I specifically studied phytoplankton communities of lakes in the Cascade Mountains within Oregon (USA), where the underlying bedrock is composed of permeable rock basalt or andesite and as a result, are primarily groundwater dominated and relatively rich in P (Peck, *et al.*, 1964; Nolin, 2012). The selection of lakes within this region of the Cascades minimized the variability in geology and hydrology. Additionally, certain lakes in this sub-region have documented harmful blooms of N-fixing cyanobacteria (Oregon Department of Environmental Quality, 2011). I first determined the potential watershed, lake, and food web characteristics that influence diazotrophic cyanobacteria from existing empirical watershed and lake data sources as well as surveys of lakes for phytoplankton, zooplankton, physical, and chemical parameters (Chapter 2). My main question for this chapter was: How do cross-scale interactions between variables from the watershed, lake, and food web influence diazotrophic cyanobacteria biomass in mountain lakes? To address this question, I used both an exploratory machine learning approach to test the significance of all variables and a focused modeling approach to test specific pathways of the different scales. In Chapter 3, I explored possible associations of bacterioplankton communities with climate characteristics, cyanoHABs and cyanotoxins at two temporal scales: intra-annually (i.e., within a year) and inter-annually (i.e., between years), using physicochemical measurements, time-integrated toxin monitoring, and 16S rRNA sequencing. I also examined how the same associations of bacterioplankton with climate, cyanoHABs, and cyanotoxins vary across spatial scales (i.e., within and between lakes). My main questions were: 1) How do cyanotoxin concentrations vary in relation to temperature and precipitation within and across lakes with varying cyanobacteria dominance? 2) How does bacterioplankton community structure change in relation to cyanoHABs and cyanotoxins and how do these relationships vary within and between lakes? I used amplicon sequencing to characterize the bacterioplankton communities across lakes and over time in relation to cyanotoxin concentrations and prevalence, based on specialized passive samplers, as well as climate variables. In Chapter 4, the final data chapter examined the variation of the entire phytoplankton communities in mountain lakes through a functional trait-based approach. I specifically addressed the following question: How do the traits of phytoplankton communities reflect gradients of nutrient availability and grazing pressure across mountain lakes? Functional traits provide more and different information on the environmental filters than taxonomy (Spitz, Ridoux & Brind'Amour, 2014; Weithoff, Rocha & Gaedke, 2015). Ultimately, analyses of phytoplankton communities, alongside environmental conditions in largely undeveloped mountain lakes that exist across steep gradients, may offer new insights into the variation in these key producers and specifically toxigenic cyanobacteria that increasingly threaten drinking and recreational water bodies.

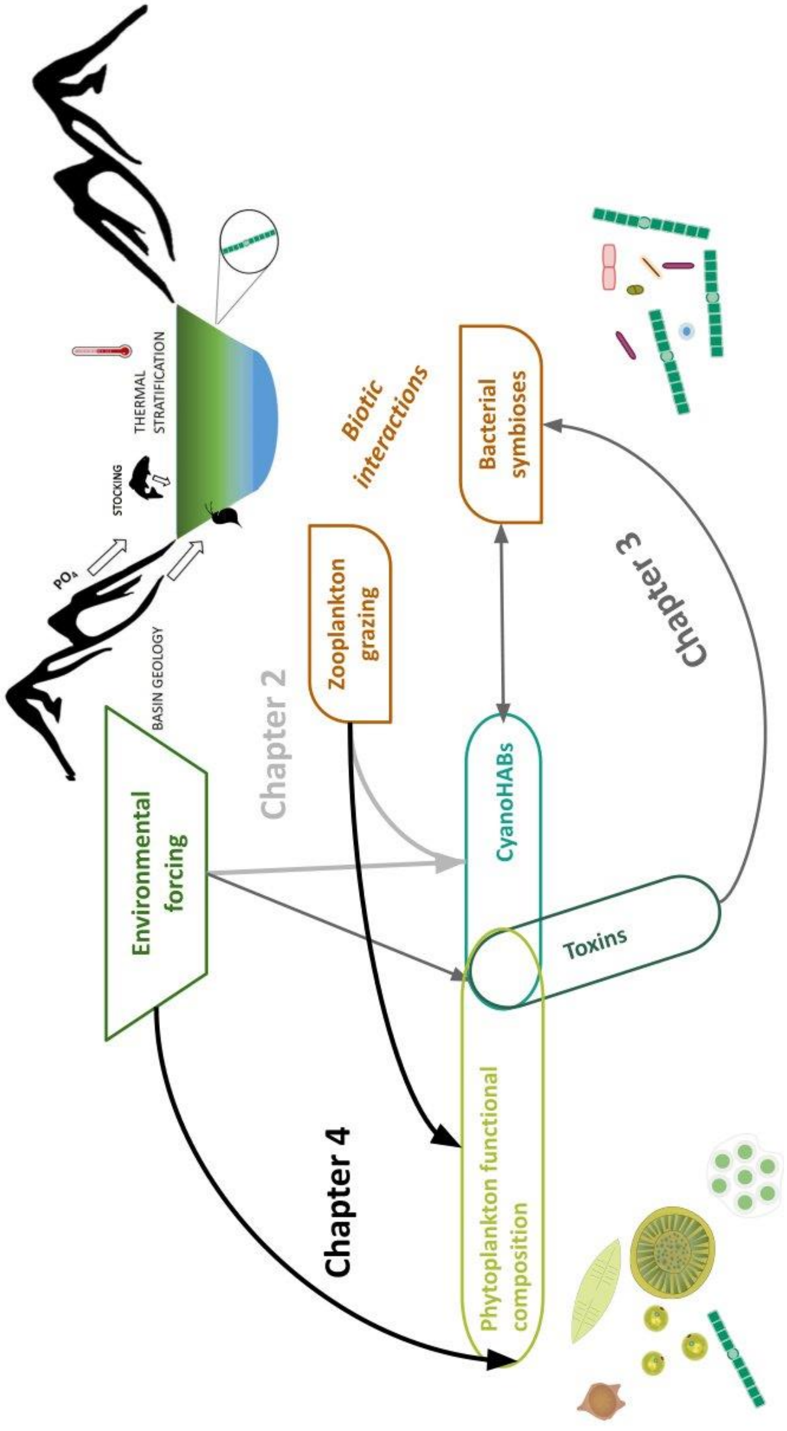

Figure 1.1 Schematic diagram of how each research chapter relate and interconnect. Chapter 2 examines the roles of environment factors at the watershed and lake level as well as the biotic factors such as grazers in diazotrophic cyanobacteria, which are the

common HAB taxa in the Cascade mountains. Chapter 3 examines how another set of biotic interactions through the bacterioplankton communities are affected by cyanoHABs and cyanotoxins. Chapter 4 examines how the whole phytoplankton community respond to environmental factors, using a trait-based approach.

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Chapter 2: Watershed, lake and, food web factors influence diazotrophic cyanobacteria in mountain lakes

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Lara S Jansen, Daniel Sobota, Yangdong Pan, Angela Strecker

Author contribution: L.S.J., A.L.S., D.S. and Y.P. designed the study. L.S.J. and D.S. conducted sampling and collected data. L.S.J. conducted taxonomic identification and nutrient analyses. L.S.J. analyzed data with assistance from A.L.S., D.S. and Y.P. L.S.J. wrote the manuscript with assistance from A.L.S. A.L.S., D.S., and Y.P. provided manuscript edits and approval of the final manuscript.

2.1 Introduction

Harmful blooms of cyanobacteria in freshwater bodies are influenced by bottom-up factors, such as nutrients, as well as top-down factors, such as zooplankton grazers

(Paerl & Otten, 2013). Increasing nutrient loads is a major factor driving increases in cyanobacteria abundance and harmful algal blooms in Northern Hemisphere lakes (Rigosi *et al.*, 2014; Taranu *et al.*, 2015). In addition, climate change-induced warming often magnifies the positive influence of nutrients, especially in eutrophic waterbodies, influencing nutrient availability as well as the dominance of cyanobacteria such as by extending stratification (Wagner & Adrian, 2009; Rigosi *et al.*, 2014). Food web shifts, including non-native fish introductions, may also contribute to harmful algal blooms, but the connection to cyanobacterial dominance and blooms is less certain (Paerl & Otten, 2013). The interplay of top-down and bottom-up controls on cyanobacterial blooms remains an area for further study, especially in regions isolated from anthropogenic development where blooms still occur (Paerl & Otten, 2013; Taranu *et al.*, 2015; Reinl *et al.*, 2021).

Mountain lakes are model systems to examine potential drivers of cyanobacteria, with natural gradients of temperature and nutrients (Catalan *et al.*, 2006). The topographic complexity and elevational gradient of mountains create distinct microclimates and vegetation zones, which in turn impacts lake thermal regimes, productivity, and food webs (Catalan *et al.*, 2006; Sadro, Nelson & Melack, 2012; Symons & Shurin, 2016). In addition, frequent and repeated stocking of non-native fishes in naturally fishless mountain lakes has been widespread throughout North America. This practice has demonstrably altered lake food webs with declines in large invertebrates and amphibians, as well as shifts in nutrient cycling (Knapp, Matthews & Sarnelle, 2001; Schindler, Knapp & Leavitt, 2001). The variation, remoteness, and sensitivity of mountain lakes has shed new light on climatic influences of ecosystem processes, including how climate change-induced warming enhances trophic cascades and increased primary production from summer storm events (Symons & Shurin, 2016; Oleksy *et al.*, 2020). Yet key determinants of bloom-forming cyanobacteria, such as nutrients and grazers, have not been well studied in mountain lakes, which could provide new insights on the complex issue of harmful algal blooms.

The interactions of external nutrient inputs, non-native fish stocking, and climate change-induced warming with landscape and lake characteristics may mediate effects on cyanobacteria. Therefore, examination of cross-scale interactions could provide new insights on drivers of cyanobacteria (Soranno *et al.*, 2014). Cross-scale interactions occur when driver and response variables operate at different spatial or temporal scales but still influence each other (Soranno *et al.*, 2014). For example, increasing amounts of agriculture within a region can shift wetlands from acting as a phosphorus source to a sink for adjacent waterbodies (Soranno *et al.*, 2014). These interactions may clarify why specific drivers have different effects depending on the region of interest. In oligotrophic mountain lakes where nutrient loading from the watershed is often minimal, the drivers of cyanobacteria are more likely a combination of several factors that influence nutrients, light, and temperature at different scales from the landscape to the lake (Reinl *et al.*, 2021).

Watershed characteristics along elevational gradients in mountain ranges can influence nutrient loads and thermal regimes of lakes (Catalan *et al.*, 2006; Sadro *et al.*, 2012). Atmospheric deposition of nitrogen (N) has increased substantially since the Industrial Revolution and now is the greatest single source of N for US mountain ranges

(Baron *et al.*, 2011). However, the extent of lake enrichment from atmospheric deposition of N depends not only on proximity to sources, but also upon the amount of vegetation within the watershed as terrestrial plants often are N limited (Kopáček *et al.*, 2000; Baron *et al.*, 2011). Yet this is dependent on the abundance of N-fixing species. Increasing vegetation density and soil complexity increases terrestrial uptake and retention of N in undeveloped watersheds, potentially reducing N loading downstream (Baron *et al.*, 2011). Therefore, lakes in the subalpine and montane regions, which have mostly forested watersheds in the Northern Hemisphere, may respond less readily to elevated atmospheric N deposition than barren alpine lakes (Baron *et al.*, 2011). Phytoplankton in lakes with forested watersheds are typically N-limited, making diazotrophic cyanobacteria, which are capable of N fixation, potentially more competitive (Sadro *et al.*, 2012; Hayes *et al.*, 2015; Williams *et al.*, 2016). In addition, watershed size, topography, hydrology, and geology influence nutrient loading to mountain lakes (Kamenik *et al.*, 2001; Sadro *et al.*, 2012). Topography, hydrology, vegetation, and ice cover can also modify lake responses to large-scale climate shifts, such as lake temperatures, which are somewhat buffered when groundwater is the major inflow to the lake (Schindler, 2006; Nolin, 2012). Therefore, responses of cyanobacteria need to be placed in the context of the watershed environment.

In addition to watershed factors, lake characteristics regulate nutrient and thermal dynamics that may ultimately affect cyanobacteria (Reynolds, 2006; Adrian *et al.*, 2009; Kraemer *et al.*, 2015). Mountain lakes typically formed from glacial scour or volcanic activity, producing deep U-shaped basins or more shallow depressions, (Wetzel, 2001). Shallow lakes may be more susceptible than deep lakes to elevated nutrient loading as nutrients quickly cycle through the ecosystem, favoring eutrophic and low light tolerant phytoplankton (Reynolds, 2006). In addition, climate change-induced warming will likely increase overall temperatures in most lakes, as well as alter the thermal stability of deep lakes, potentially prolonging stratification (Adrian *et al.*, 2009; Kraemer *et al.*, 2015; Preston *et al.*, 2016). Prolonged stratification favors cyanobacteria that can regulate their buoyancy and compete in nutrient-depleted conditions via N fixation, luxury phosphorus (P) uptake, and other adaptations (Reynolds, 2006; Litchman & Klausmeier, 2008). While temperatures are rising at faster rates in mountains than at lower elevations, the consequences of warming will vary with mountain lake morphology (Adrian *et al.*, 2009; Pepin *et al.*, 2015).

Although physical dynamics within lakes affect cyanobacteria, biotic processes can also have a substantial influence and should be considered in tandem. Lake food webs can also influence cyanobacteria and other phytoplankton, directly through top-down predation and indirectly through nutrient cycling, mediated by large-scale drivers like climate change (Knapp *et al.*, 2001; Schindler *et al.*, 2001; Symons & Shurin, 2016). Stocking in naturally fishless lakes can reduce the biomass of zooplankton by selectively feeding on larger taxa, releasing phytoplankton from grazing pressure in a trophic cascade (Knapp *et al.*, 2001). Moreover, the effects of fish on lower trophic levels increase with temperature, suggesting that climate change will amplify trophic cascades (Symons & Shurin, 2016). Stocking can result in higher P recycling even when compared to lakes with naturally reproducing trout populations due to higher growth rates from continual stocking of planktivorous fingerlings (Schindler *et al.*, 2001). Additional P may shift mountain lakes to be more N-limited, favoring diazotrophic cyanobacteria

(Schindler *et al.*, 2001). However, if lakes also receive elevated atmospheric deposition of N and P, the combined inputs may favor other eutrophic phytoplankton (e.g., chlorophytes) (Nydick *et al.*, 2011; Brahney *et al.*, 2015). Therefore biotic stressors, such as introduced predators, should be examined in the context of abiotic factors to determine the influence on phytoplankton biomass and composition (Symons & Shurin, 2016).

Despite minimally developed watersheds and limited nutrient loading from natural sources, a subset of lakes in the Cascade Mountains (USA) have experienced toxic cyanobacteria blooms in recent years (Oregon Department of Environmental Quality, 2011). Thus, this region serves as a model system to study the cross-scale drivers of diazotrophic cyanobacteria in mountain lakes. Our study addressed the following question: How do cross-scale interactions between variables from the watershed, lake, and food web influence diazotrophic cyanobacteria in mountain lakes? We tested three hypothesized pathways (Figure 2.1): 1) Trophic: The trophic control of zooplankton by fish stocking will influence diazotrophic cyanobacteria by reducing grazing pressure from large zooplankton (Knapp *et al.*, 2001); 2) Nutrients: Fish stocking biomass, as well as watershed land cover, size and topography will influence diazotrophic cyanobacteria by affecting nutrient concentrations. These influences will vary across lakes and that variation will be explained in part by lake thermal regime, as it may regulate internal nutrient loading (Kopáček *et al.*, 2000; Schindler *et al.*, 2001; Kamenik *et al.*, 2001); and 3) Thermal regime: Relative groundwater contribution of the watershed as well as watershed topography and land cover will influence diazotrophic cyanobacteria by affecting lake temperatures. We predict that watershed influence will vary across lakes, and that variation will be explained in part by lake thermal regime as it regulates the warming of the water column (Wetzel, 2001; Nolin, 2012; Kraemer *et al.*, 2015). Results from our study may inform management of waterbodies isolated from development yet still susceptible to diazotrophic cyanobacterial blooms.

2.2 Methods

2.2.1 Site description

The underlying geology of lakes in the Oregon portion of the Cascade Range is predominantly highly permeable basalt or andesite volcanic rocks (Oregon Department of Geology 2014), resulting in primarily groundwater fed lakes (Johnson, 1985; Nolin, 2012). In addition, basalts tend to contain and release significantly higher amounts of P with weathering compared other types of rocks; therefore, the Cascade lakes can be comparatively less P-limited (Porder & Ramachandran 2013). Oregon Cascade lakes range from ultra-oligotrophic to eutrophic, which is likely due to variation in geology and hydrology (Johnson, 1985). These lakes were primarily formed by glacial scour, such as morainal deposits damming river valleys, and volcanic activity creating depressions either from eruptions or collapses in lava flows (Johnson, 1985). Many of the Oregon Cascade lakes were naturally fishless. Stocking began in the early 1900s and today several hundred lakes are regularly stocked with brook (*Salvelinus fontinalis*), rainbow (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarkii*) trout (Oregon Department of Fish and Wildlife, 2011).

2.2.2 Data compilation

We compiled a data set of 132 Oregon Cascade lakes on fish stocking, water chemistry, and watershed characteristics from multiple sources to identify a potential set of study lakes (Johnson, 1985; USDA Forest Service, 1996; US Environmental

Protection Agency, 2009). We only considered lakes >10 hectares and with a maximum depth of ≥3 meters, to exclude extremely small lakes or ponds that can be seasonally variable in depth and emergent vegetation cover (US Environmental Protection Agency, 2009). We created a representative sample of lakes from this dataset using binary regression trees from the R package *rpart* v.4 (Therneau, Atkinson & Ripley, 2019). A binary regression tree repeatedly divides the response data into nodes to reduce variation within nodes based on predictor variables. Total P concentration was the response variable as P is often a crucial lake characteristic for diazotrophic cyanobacteria and varies significantly in the Cascades (Johnson, 1985; Williams *et al.*, 2016). In addition, total P was the most commonly available chemical analyte for all lakes from the compiled datasets. We chose elevation, 10-year annual fish stocking average rate, and surface area as the predictor variables. From the resulting binary regression tree, we selected a similar number of lakes from each node to make up the 29 lakes of the study, which were sampled in summer 2019 (Appendix A: Figure S1).

We compiled lake morphometry data from the Atlas of Oregon Lakes database(Johnson, 1985), including bathymetry to calculate Schmidt Stability Index (a measure of stratification strength), using the R package *rLakeAnalyzer* v. 11 (Winslow *et al.*, 2019). We obtained fish stocking records from 1978-2019 for the study lakes, including biomass, count, and species (Oregon Department of Fish and Wildlife, unpublished). The fish were mostly stocked as fingerlings that were < 250 mm long (fork length) and thus were primarily planktivores (Elser *et al.*, 1995).

For many watershed variables, we extracted data from the LakeCat dataset, which provides watershed characteristics of lakes within the conterminous US derived from existing spatial data such as land cover, geology, and long-term climate (Appendix A: Table S1, Hill *et al.*, 2018). Average slope and aspect of watersheds was derived from Digital Elevation Models with 10-m resolution (US Geological Survey, 2019). Maximum snow-water equivalent (SWE) for each watershed in the previous winter (2018-2019) was derived from the Snow Data Assimilation System daily estimated SWE, selecting the days based on the maximum SWE at the nearest SNOTEL site for each watershed (National Operational Hydrologic Remote Sensing Center, 2004; USDA Natural Resources Conservation Service, 2020). Percent change in forest cover in each watershed for the past 20 years was derived from the spatial Global Forest Change dataset for 2000-2019 (Hansen *et al.*, 2013). We calculated drainage ratio for each lake by dividing the watershed area by the lake surface area.

2.2.3 Lake & food web: field sampling

Each lake (n=29) was sampled twice in summer 2019 to capture within season variation, with the first sampling occurring between June 24-July 13 and the second between August 8-31. We measured physical and chemical variables at the deepest spot in the lake, including a complete depth profile of temperature and dissolved oxygen with a YSI ProODO meter (Yellow Springs, Ohio, USA). For the stratified lakes, the profile data were divided into the thermal layers (epilimnion, metalimnion, and hypolimnion) to calculate average epilimnion temperature, mixed layer depth (i.e., the bottom of the epilimnion), and average hypolimnetic dissolved oxygen. For unstratified lakes, average whole water column temperature was used in lieu of epilimnetic temperature and the bottom dissolved oxygen value was used in lieu of average hypolimnetic dissolved oxygen.

We sampled for phytoplankton with a Van Dorn sampler (Wildco, Yulee, Florida, USA) at one meter below the surface at the deepest point in the lake, identified using bathymetric maps and a depth sounder. Samples were collected in 250-mL brown Nalgene bottles and preserved for identification using Lugol's solution. An additional sample was taken at the deep spot and a known volume was filtered onto glass fiber filters (Whatman GF/C, 1.2-µm pore size) for chlorophyll-*a* analysis. We collected samples for nutrient analyses (total N and P) from the top 5 m of the water column, using a 5-m long, 2.54-cm diameter tygon tube and transferred them into 125-mL HDPE bottles, which were kept cool and preserved with H_2SO_4 until frozen in the laboratory. We conducted an integrated tow of the water column for crustacean zooplankton using a plankton net with 80-µm mesh and 25-cm diameter, starting two meters from the bottom and preserving the sample in 70% ethanol for identification.

2.2.4 Lake & food web: Lab analyses

We concentrated preserved phytoplankton samples before counting by gently mixing the sample for 5 minutes and then taking a 100-mL subsample for settling in a graduated cylinder. After 100 hours of settling, the top 98 mL were removed via a vacuum pump and reserved to dilute while the remaining 2 mL were used for counting. We counted and identified 300 natural units per concentrated sample in Palmer counting cells to the genus level or to the lowest taxonomic level possible, using taxonomic guides (Smith, 1950; Wehr, 2002; Matthews, 2016) with a Leica DM1000 microscope at 400X and ICC50 HD camera (Leica Microsystems Inc., Buffalo Grove, IL). Diazotrophic genera were determined based on current literature (Reynolds, 2006). We measured the dimensions of 20 individuals of each taxon in each sample to calculate biovolume using standardized equations based on the shapes of taxa (Appendix A: Table S4; Hillebrand *et al.*, 1999). For zooplankton, we counted and identified 250 individuals from each preserved sample to the order level for Copepoda and to the family level for Cladocera using taxonomic guides (Balcer, Korda & Dodson, 1984; Thorp & Covich, 2014) with a Leica M165C microscope at 100X and IC80HD camera. For further analyses, cladocerans were aggregated into two groups based on different feeding impacts, as *Daphnia*, large and efficient, and small cladoceran grazers, smaller and less efficient (e.g. *Bosmina, Holopedium, Ceriodaphnia*) (DeMott, 1982; Reynolds, 2006).

We extracted chlorophyll-*a* from filters using acetone for 20 hours in a dark refrigerator, and measured concentrations using a fluorometer following Arar & Collins (1997). We used a persulfate solution to digest total P samples heated to 100°C and then analyzed with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the molybdenum blue colorimetric method (detection limit: 0.002 mg/L; precision limit: +/- 0.004 mg/L) (APHA, 2018c). We also used a persulfate solution to digest total N samples heated to 100°C and then analyzed with a SmartChem 200 discrete analyzer (Guidonia, Italy) for colorimetric determination of nitrate and nitrite (detection limit: 0.01 mg/L; precision limit: +/- 0.01 mg/L) (APHA, 2018b).

2.2.5 Statistical analyses

The relative importance of lake, food web, and watershed characteristics to diazotrophic cyanobacteria biovolume were determined using a two-step process. First, machine learning methods were used to identify the most important variables for diazotrophic cyanobacteria. Second, these variables were used in a structural equation model to test the hypothesized direct and indirect effects of characteristics from the three different pathways (Figure 2.1).

First, we examined and ranked the potential influence of all variables, using two complementary machine learning methods, binary regression tree and random forest models. This approach was chosen because a single binary regression tree provides an interpretable graphic on the entire regression function but often is unstable. In comparison, a random forest model, which generates hundreds of trees based on bootstrap samples, provides robust estimates of variable importance but limited information on the overall regression function (Breiman, 1996; De'ath & Fabricius, 2000). The predictors were removed if they had zero or near zero variance based on percent of unique values (<10 %) and frequency ratio (i.e. frequency of the most common value divided by the frequency of the second most common value) (Kuhn, 2008; Kuhn & Johnson, 2013). Correlations between predictors were examined using the Spearman's rank coefficient due to non-normal distributions and one predictor of a pair was removed if $\rho > 0.80$ (Kuhn & Johnson, 2013). Epilimnetic dissolved oxygen and pH were dropped as predictors as variation may be mainly a consequence of algal production, especially in poorly buffered lakes such as in the Cascades (Wetzel, 2001; Reynolds, 2006). Hypolimnetic dissolved oxygen was kept as a predictor as it is a key factor for internal P loading through anoxic release of sediment-bound P though it can be indirectly influenced by algal production (Nürnberg, 1984). In addition, summer hypoxia in the hypolimnion occurred with a range of phytoplankton biomass, suggesting the role of other factors such as lake thermal regime (Appendix A: Figure S3).

After the pre-process steps, there were 38 predictor variables remaining from the lake, food web, and watershed categories (Appendix A: Table S1). Binary regression tree and random forest models are sensitive to non-constant variation in the response variable (De'ath & Fabricius, 2000); therefore, diazotrophic cyanobacteria biovolume was natural log (ln)-transformed. In addition, one lake lacked bathymetry data (thus mean depth was not calculated) and another lake had missing total N concentrations due to an analytical error. The missing data was imputed as the median value for the variable for the random forest models. The values of the response variable and predictor variables were averaged between the two sampling bouts to avoid temporal pseudoreplication. In addition, variation in diazotrophic cyanobacteria dominance was somewhat similar across bouts (Appendix A: Figure S4).

We built binary regression tree and random forest models using variance explained, model error and cross-validation to check and improve model fit. We adjusted the binary regression tree from the default settings to allow a minimum of eight observations per split due to the small sample size as well as a 10-fold cross validation and a complexity parameter of 0.01 to prevent overfitting (Breiman, 1996; De'ath & Fabricius, 2000; Therneau *et al.*, 2019). The final model was selected based on the minimum cross-validated relative error and root mean squared error (RMSE). Then we created random forest models with the R packages *caret* v. 6.0, *randomForest* v. 4.6 and *pdp* v. 0.7 (Breiman, 1996; Kuhn, 2008; Greenwell, 2017; Breiman & Cutler, 2018). For the random forest models, we used 10-fold cross-validation repeated three times on different random subsets to reduce the error in the estimated mean model performance. The number of predictors randomly selected at each split was set at the square root of the number of predictors. Variable importance was measured using the increase in mean squared error if the variable is randomly imputed. A reduced random forest model was developed to improve interpretation by dropping predictors iteratively based on their variable importance in the full model, starting with the least important, checking RMSE and pseudo-R^2. Once the model fit was no longer improved by the removal of a predictor (pseudo-R^2 decreased & RMSE increased) the process stopped. The fit of the reduced random forest model was compared against the fit of the full random forest model. The fit was fairly similar, so the reduced random forest model was used as the final model for further analyses.

Second, we used structural equation models (SEMs) to test the hypothesized single-scale and cross-scale effects of the food web, lake and watershed characteristics on diazotrophic cyanobacteria biovolume (Figure 2.1), using the R package *lavaan v.* 0.6 (Rosseel, 2012). SEMs were used as these models allow variables to be both independent and dependent so direct and indirect effects can be separated (Grace, 2006). The SEM was restricted in complexity due to small samples size; therefore, an indicator variable for each main component in the conceptual model (watershed, lake nutrients, thermal regime, food web) was selected based upon variable importance in the final random forest model and existing literature. We scaled all variables by calculating the Z-scores to correct for different measurement scales and normalize variance because SEMs are limited to estimating linear relationships. In addition, we fitted the SEM by fixing the independent variables' variances and covariances to one and zero, respectively, to limit the number of parameters estimated due to small sample size. We started with a full model as described in Figure 1. We examined nested models to test the relative importance of direct and indirect effects of predictors by constraining specific paths that are free in the full model (Grace, 2006). We used model Satorra-Bentler scaled χ^2, which is a robust test statistic for skewed data, as well as R^2 for endogenous variables to assess goodness of fit for each model (Rosseel, 2012). For model comparison, we used the Satorra-Bentler scaled χ^2 difference test. All path coefficients in the SEMs were standardized. All statistical analyses were conducted in R 4.1.1 (R Development Core Team, 2021).

2.3 Results

2.3.1 Data summary

Study lakes (n=29) were similar in watershed geology, mainly dominated by volcanic extrusive rock, and also mostly groundwater fed (Appendix A: Table S1). The lakes varied notably in surface area and average depth (Table 2.1). While the majority of lakes were oligotrophic, several were mesotrophic (Table 2.1; Appendix A: Table S1). Median biovolume of diazotrophic cyanobacteria was similar between the early and late summer, but the range in biovolume was greater in the late summer with a maximum of 1.93×10^6 $\mu m^3/mL$ (Table 2.1). The most common genera of diazotrophic cyanobacteria present were *Dolichospermum* and *Gloeotrichia*.

2.3.2 Identification of top predictors

The optimal binary regression tree included the following lake, watershed and food web predictors: hypolimnetic dissolved oxygen, small cladoceran grazers, epilimnetic temperature, *Daphnia*, and northness (Figure 2.2). The model had a pseudo-R^2 of 0.89 and a RMSE of 1.40 with five splits selected. The highest diazotrophic cyanobacteria biovolumes occurred in lakes with low hypolimnetic dissolved oxygen and relatively low densities of small cladoceran grazers (Figure 2.2). In contrast, the lowest diazotrophic cyanobacteria biovolumes occurred in lakes with some of the highest densities of small cladoceran grazers and the lowest summer epilimnetic temperatures (Figure 2.2). Lakes with lower diazotrophic cyanobacteria biovolumes also had greater representation of different phytoplankton groups (Figure 2.2).

We found the fit of the reduced and the full random forest model was similar, as was the variance explained, so the reduced random forest model was used as the final model for further analyses. The final random forest model contained 11 predictors and explained 76.1% of the variance in diazotrophic cyanobacteria biovolume with an RMSE=2.57 (Figure 2.3). The predictors were a mix of food web, lake, and watershed characteristics with the six most important predictors being hypolimnetic dissolved oxygen, drainage ratio, small cladoceran grazers, specific conductance, total P, and developed area (% of watershed) (Figure 2.3). Some predictors from the binary regression tree (i.e., epilimnetic temperature and degree of northern aspect) were not among the top predictors in the random forest models, which suggests they may have limited influence.

The marginal relationship, which is the relationship when all other predictors are kept fixed, that each predictor in the random forest model had with diazotrophic cyanobacteria biovolume varied in directionality and shape (Figure 2.4). The marginal relationship of hypolimnetic dissolved oxygen with diazotrophs was negative with biovolume declining most sharply as dissolved oxygen increased from 0.3 to 1.5 mg/L (Figure 2.4a). Drainage ratio, specific conductance and *Daphnia* abundance had similar positive relationships with diazotroph biovolume (Figure 2.4b,d,g). The marginal effect of small cladoceran grazer abundances was negative with biovolume declining as these grazers increased from 0 to 3,000 /L (Figure 2.4c). The relative amount of developed area had a more gradual positive relationship overall, but this relationship should be interpreted with caution as 85% of the lakes have less than 1% developed area in their watershed (Figure 2.4f). Total P had a positive marginal effect from 0.001 to 0.040 mg/L. Latitude had a gradual negative relationship with biovolume overall (Figure 2.4). Maximum depth and mixed layer depth had similar negative relationships with biovolume, although mixed layer depth had a more complex shape (Figure 2.4h, j). The relative flow from groundwater (% base flow) had a positive marginal effect, although this relationship should be interpreted with caution as most lakes are groundwater fed (Figure 2.4k).

2.3.3 Potential cross-scale interactions

We found the SEM, based on the original path diagram in Figure 2.1, to explain a substantial portion of diazotrophic cyanobacteria biovolume. A single variable was selected to represent each component in the full and nested SEMs based on the final random forest model and existing literature (Figure 2.5). The variables included: drainage ratio (watershed characteristics), maximum depth (lake thermal regime), total P (nutrients), and small grazing cladocerans (zooplankton). The full model produced a good fit with the data (Satorra-Bentler χ^2=5.69, df=5, p=0.34) and explained 37% of variance in the response variable. Among the nested models, the reduced model that dropped the thermal regime–nutrient path and the zooplankton–nutrient path had the best fit (χ^2=7.93, df=7, p=0.34) and explained the same amount of variance (R^2=0.35). The difference between the likelihood ratios for the reduced and full models was nonsignificant so the reduced model is the preferred final model (S-B scaled difference χ^2=2.28, df=2, p=0.32).

The final SEM showed direct effects through each pathway as well as a potential cross-scale interaction through the nutrient pathway (Figure 2.5). Drainage ratio affected maximum depth and total P. Small cladoceran grazers and maximum depth had direct negative effects on biovolume. Total P had a significant direct positive effect on biovolume (Figure 2.5). Drainage ratio explained 22% of the variation in the total P and 8% of the variation in maximum depth. The indirect effect of drainage ratio on diazotrophs via maximum depth (path coefficient = 0.09) was not significant (p=0.10). The indirect effect of drainage ratio on diazotrophs via total P was significant (path coefficient=0.133, p=0.007).

2.4 Discussion

Cross-scale interactions between watershed, lake, and food web characteristics influenced diazotrophic cyanobacteria biovolume in addition to independent effects of food web characteristics (Figure 2.5). The hypothesized nutrient pathway was partially supported as drainage ratio in the mostly forested watersheds was positively related to diazotrophic cyanobacteria likely through P loading. Lake internal nutrient cycling likely magnified watershed P loads via anoxic release of P from lake sediments (Figures 2.3, 2.4). The importance of cladocerans suggest planktivorous fish may play more of a role via the trophic pathway with top-down control of grazers more so than bottom-up control of nutrient cycling (Figures 2.3- 2.5). In addition, the extent of shallow habitat influenced the composition of zooplankton grazers and in turn diazotrophic cyanobacteria and their competitors. Therefore, trophic, nutrient and thermal pathways are all important for diazotrophic cyanobacteria in mountain lakes.

2.4.1 Trophic pathway

Daphnia had a positive relationship with diazotrophic cyanobacteria while small cladocerans had a negative relationship, which may be due to different grazing modes (Figures 2.4, 2.5). *Daphnia* in mesotrophic and eutrophic waters are more efficient grazers than other zooplankton due to filter feeding (DeMott, 1982; Reynolds, 2006). The non-selective feeding of *Daphnia* reduces phytoplankton biomass and diversity, leaving only a few indigestible species such as filamentous cyanobacteria to dominate, especially in the presence of fish (Vanni *et al.*, 1990). Diazotrophic cyanobacteria, which was comprised of only filamentous genera in our lakes, were often dominant when *Daphnia* made up a major portion of the zooplankton community (Figure 2.2). In contrast, small cladocerans are less efficient grazers, but more selective so they support a greater diversity of phytoplankton especially smaller species that are also well suited to resource-limited environments, competing with diazotrophic cyanobacteria (DeMott, 1982; Bergquist, Carpenter & Latino, 1985; Reynolds, 2006). When small cladocerans were abundant, phytoplankton communities were relatively diverse and diazotrophic cyanobacteria less common (Figure 2.2). Therefore, top-down control likely has a role in driving the dominance of diazotrophic cyanobacteria.

The influences of cladocerans on diazotrophic cyanobacteria also may have been due to varying tolerance to cyanotoxins and resources. *Daphnia* can be less sensitive to toxins produced by cyanobacteria than other zooplankton, especially over generations, allowing for local adaptation (DeMott, Qing-Xue Zhang & Carmichael, 1991; Sarnelle &

Wilson, 2005). In addition, zooplankton can be reflective of trophic status, with *Daphnia* more dominant in eutrophic lakes and small cladocerans more dominant in oligotrophic lakes (DeMott, 1982; Reynolds, 2006). While *Daphnia* abundances were positively correlated with total algal biomass (using chl-*a* as a proxy) (r=0.61, p<0.001) and total P (r=0.49, p=0.006), small cladocerans were only weakly correlated with these trophic status indicators (total P: r=-0.35, p=0.060; chl-*a*: r=-0.38, p=0.040). Therefore, zooplankton do not only reflect trophic status, but also likely top-down control on diazotrophic cyanobacteria.

We contend that differences in zooplankton composition likely reflect fish predation pressures in relation to lake habitat. However, *Daphnia* abundance was not correlated with fish stocking biomass, which is mostly made up of planktivorous trout fingerlings (r=-0.01, p=0.96) (Oregon Department of Fish and Wildlife, unpublished). A lack of observed relationship between stocked fish and zooplankton may be due in part to fish stocking records being weak proxies for actual population sizes as fish have variable survival in mountain lakes such as in the Cascades (Liss *et al.*, 1999). There also have been illegal introductions of bait fish in the region, but data for most lakes are lacking (Eilers *et al.*, 2011). Large zooplankton such as *Daphnia* are able to persist with fish when planktivory is fairly low and there are predation refugia, such as vegetated littoral areas (Burks, Jeppesen & Lodge, 2001; Mushet *et al.*, 2020). *Daphnia* abundance was somewhat negatively correlated with lake mean depth (r=-0.37, p=0.05), and the highest abundances were in the shallowest lakes. In addition, *Daphnia* can also locally adapt to fish predation such as by reducing body size over generations and changing behavior (De Meester, Boersma & Spaak, 1999). However, if refugia are minimal and planktivory is high then smaller zooplankton such as *Bosmina,* a smaller cladoceran, may become more abundant because they can largely avoid fish predation (Burks *et al.*, 2001). Therefore, the role of lake morphometry in shaping zooplankton communities, which consequently affected cyanobacterial abundance, likely reflected a cross-scale interaction between lake and food web characteristics.

2.4.2 Nutrient pathway

Diazotrophic cyanobacteria may only be partially influenced by P in the epilimnion. Total P had a significant but asymptotic positive relationship with diazotrophic cyanobacteria, suggesting a threshold effect of P (Figs. 2.4, 2.5). P may have a weaker role above a threshold of 0.03 mg/L relative to other factors, especially if sediment P is accessible. We recognize not all TP is bioavailable. Orthophosphate concentrations were on average 60% of TP concentrations in 12 of the same lakes resampled in 2020, suggesting most of TP was bioavailable (Jansen, unpublished data). Certain characteristics such as watershed size and lake morphometry are likely affecting external and internal P loading and ultimately, diazotrophic cyanobacterial biovolume.

The expected effect of fish on diazotrophic cyanobacteria by increasing P cycling as observed in similar studies was not supported (Vanni & Layne, 1997; Schindler *et al.*, 2001; Sharitt *et al.*, 2021). When planktivorous fish are relatively abundant, they are the dominant source of recycled P through excretion and decomposition (Vanni & Layne, 1997). When planktivorous fish are less abundant or absent, zooplankton are significant contributors to P recycling (Vanni & Layne, 1997). In addition, the amount of P excreted relative to N varies with zooplankton taxa: *Daphnia* excretions have low N:P relative to copepods, and therefore lakes dominated by *Daphnia* may be less favorable to diazotrophic cyanobacteria (Vanni & Layne, 1997). In contrast, *Daphnia* and diazotrophic cyanobacteria had a positive relationship in our study, suggesting other pathways, such as their role as algal grazers, may be more influential. We also found no significant relationship between stocked fish biomass and TP (r=-0.09, p=0.63). In addition, the effect of fish and zooplankton on P cycling has been highly variable across and within lakes (Vanni & Layne, 1997; Schindler *et al.*, 2001; Sharitt *et al.*, 2021).

Watershed size relative to lake area played an important role likely through nutrient loading in the context of other characteristics (Figure 2.3, 2.4). Most watersheds were within the subalpine and montane environments, and thus were heavily vegetated (median forest cover=81%; Appendix A: Table S1). In relatively undisturbed areas, such as mountains, terrestrial vegetation can be N limited, depending on the abundance of N-fixing species; therefore downstream runoff may also be low in N (Kopáček *et al.*, 2000; Baron *et al.*, 2011; Sadro *et al.*, 2012; Williams *et al.*, 2016). Watershed size magnifies this effect by increasing the amount of vegetation upstream (Sadro *et al.*, 2012). Watersheds in our study spanned gradients of precipitation and atmospheric deposition, increasing from south to north. Only the most northern watersheds have exceeded the critical N loads of atmospheric deposition estimated for the western Pacific Northwest mountains, which may explain the very low biomass of diazotrophic cyanobacteria in these lakes (Williams *et al.*, 2016, 2017). In addition, nearly all watersheds were dominated by relatively young volcanic rock, which is high in P, and groundwater fed (Catalan *et al.*, 2006; Nolin, 2012; Porder & Ramachandran, 2013). Increasing watershed size likely amplified the effect of geological contribution of P, while increasing forested watershed area in the subalpine and montane reduced the effect of atmospheric nitrogen deposition (Baron *et al.*, 2011). Some of the largest watersheds also had the greatest amount of developed area, but there were only five watersheds with more than 1% developed area (maximum=4.2%; Table 2.1). In addition, total developed area was only weakly correlated with TP (r=0.34, p=0.06), and not at all with TN (r=0.03, p=0.89). Further study is needed to disentangle the role of limited development relative to the other natural watershed drivers of diazotrophic cyanobacteria (Doubek, Carey & Cardinale, 2015).

Hypolimnetic dissolved oxygen (DO) has a crucial but complex relationship with diazotrophic cyanobacteria via internal nutrient cycling and decomposition. Hypolimnetic DO had a negative relationship with diazotrophic cyanobacteria (Figure 2.4a) with the higher abundances associated with anoxia (0 mg/L) and hypoxia (>0-2 mg/L), which were more common than expected (Appendix A: Figure S3). Anoxic conditions release sediment-bound P that can then be mixed physically as well as taken up by migrating cyanobacteria, such as certain diazotrophs, if the lake is not too deep (Nürnberg, 1984; Reynolds, 2006; Cottingham *et al.*, 2015). Relatively shallow stratified lakes with an anoxic hypolimnion had the highest biovolumes of diazotrophic cyanobacteria. However, depletion of hypolimnetic DO is partly influenced by decomposition of sinking organic matter such as algae in the hypolimnion (Reavie *et al.*, 2016; Paerl, 2017). Blooms of algae, including cyanobacteria, can indirectly result in hypolimnetic DO depletion as the bloom senesces (Paerl, 2017). However, hypolimnetic anoxia and hypoxia occurred across a range of total phytoplankton and diazotroph biovolume in our lakes (Appendix A: Figure S3). Therefore, diazotrophic cyanobacteria blooms likely contributed to hypolimnetic DO depletion but were not always the primary cause. Spring blooms of other algae such as diatoms can drive summer hypoxia in the hypolimnion (Reavie *et al.*, 2016). We could not fully disentangle the response and role of diazotrophic cyanobacteria with hypolimnetic DO depletion as we were limited to only two sampling points. We recommend further study with higher frequency sampling of lakes to discern the relationship and mechanisms involved (Reavie *et al.*, 2016).

Watershed size may mediate the effect of hypolimnetic DO depletion on internal nutrient loading as a potential cross-scale interaction. Increasing watershed area, especially in P rich areas, can produce greater pools of legacy P that can be mobilized from the sediments under anoxic conditions (Sharpley *et al.*, 2013; Porder & Ramachandran, 2013). When watershed inputs attenuate either seasonally or due to drought, anoxia can sustain P availability through internal cycling (Sharpley *et al.*, 2013). Therefore, lakes prone to hypolimnetic anoxia, as well as with relatively large watersheds, may be among the most susceptible characteristics to diazotrophic cyanobacteria dominance and blooms.

2.4.3 Thermal pathway

The biovolume of diazotrophic cyanobacteria may be less constrained by temperature than by the mixing regime of lakes. Temperature may explain temporal variation of diazotrophic cyanobacteria within lakes more so than variation across lakes (Taranu *et al.*, 2015; Monchamp, Spaak & Pomati, 2019). Certain species of diazotrophic cyanobacteria are well adapted to cool temperatures, so temperatures may be less influential than nutrients (Reynolds, 2006a; Carey et al., 2012; Reinl et al., 2021). In addition, variation in temperature within a region, such as the Cascades, may not be significant enough to strongly influence phytoplankton communities. However, lake stratification can often be more influential than water temperatures on phytoplankton and in particular cyanobacteria (Reynolds, 2006; Wagner & Adrian, 2009; Carey *et al.*, 2012).

Lake depth may have more of an indirect effect on diazotrophic cyanobacteria by regulating lake thermal regimes. Maximum depth was strongly positively correlated with stratification stability (i.e., Schmidt stability index: r=0.85, p<0.001), but had a negative relationship with diazotrophic cyanobacteria (Figures 2.4, 2.5). Increasing lake depth constrains access to hypolimnetic nutrients for migrating cyanobacteria, as well as the recruitment of resting stages, like akinetes from the sediments (Wetzel, 2001; Karlsson-Elfgren, Rengefors & Gustafsson, 2004; Carey *et al.*, 2012). Stable stratification, which depends on lake depth, often favors buoyant cyanobacteria, including diazotrophs like *Gloeotrichia* (Wagner & Adrian, 2009; Carey *et al.*, 2014). Yet diazotrophic cyanobacteria were not correlated with stratification strength (r=-0.09, p=0.64). The Schmidt stability index does not account for effective fetch of a lake so physical mixing may not have been captured (Schmidt, 1928). While turbulent conditions often favor other taxa such as diatoms over cyanobacteria, many cyanobacteria species can still thrive (Carey *et al.*, 2012; Reinl *et al.*, 2021). Further work is still needed to examine the effect of other important aspects of lake thermal regimes, such as stratification duration, on diazotrophic cyanobacteria.

Development in the watershed, especially in nearshore areas, can increase lake temperatures (Doubek, Carey, and Cardinale 2015). Although developed area was concentrated around the lakeshore in our study, there was no significant correlation with average lake temperatures (r=0.34, p=0.07) and development was quite limited within the watersheds. Watershed characteristics may be less of a factor for temperature than for nutrients in relation to diazotrophic cyanobacteria, but this depends upon regional variation in key aspects such as groundwater contributions.

2.5 Conclusions

Our study demonstrates that even without significant cultural eutrophication, diazotrophic cyanobacteria can thrive due to a combination of food web, lake and watershed factors. While most Cascades watersheds are naturally rich in P, P deposition is increasing in many regions to the point that formerly oligotrophic waterbodies, including mountain lakes, are becoming mesotrophic (Brahney *et al.*, 2015; Scholz & Brahney, 2022). This shift could allow diazotrophic cyanobacteria to become more prevalent in mountain ranges where P was previously limiting (Scholz & Brahney, 2022). Our study offers insights relevant to management of lakes removed from most direct anthropogenic impacts but where diazotrophic cyanobacteria and related harmful algal blooms (HABs) can occur.

Internal P loading via hypolimnetic anoxia is a well-known issue in HAB management in impaired eutrophic lakes (Reavie *et al.*, 2016; Paerl, 2017). Our study confirms its importance in undeveloped and less productive systems such as mountain lakes. Managers should monitor for hypolimnetic anoxia or estimate its likelihood based on lake characteristics to identify lakes that are vulnerable (Reynolds, 2006; Cottingham *et al.*, 2015). Oxygen depletion in the hypolimnion will likely only expand with climate change in temperate regions as the length of the stratified period will increase (Preston *et* *al.*, 2016). In addition, HABs contribute to hypolimnetic anoxia, creating a positive feedback loop that sustains the bloom (Paerl, 2017).

Top-down control also has a potential role with certain zooplankton grazers releasing filamentous cyanobacteria from resource competition with other phytoplankton that are more readily grazed. With fish, grazers like *Daphnia* can persist with refugia, but are size constrained so cannot readily consume large algae like long filaments or colonies (Vanni *et al.*, 1990; Burks *et al.*, 2001). Alterations of stocked fish populations, such as the removal or shifts in species composition, has been linked to HAB declines with the return of *Daphnia* (Eilers *et al.*, 2011). However, diazotrophic cyanobacteria may persist if fish remain. Fisheries management should consider how lake morphology, as well as the relative loading of N to P, may increase the likelihood of fish altering lower trophic levels in ways that favor cyanobacteria (i.e., reduced grazer size).

Diazotrophic cyanobacteria, which constitute most freshwater HAB species, may not be constrained by the conditions that are generally assumed to promote HABs, e.g., warm water and eutrophic conditions. While this study was conducted on mountain lakes, the consequences transcend mountain environments as similar conditions can occur elsewhere such as in the N-limited boreal forests of North America and Asia (Näsholm *et al.*, 1998). Therefore, scientists and managers must broaden their consideration of factors that can promote HABs when evaluating lake health and restoration. Lake management must account for bottom-up factors, such as lake morphometry and watershed size, as well as top-down factors, such as fish stocking, to effectively mitigate diazotrophic cyanobacterial blooms. Ongoing climate change in tandem with other stressors such as introduced aquatic species in lakes across the world makes such a holistic approach crucial for HAB management (Paerl & Otten, 2013).

2.6 Tables and Figures

Table 2.1 Summary statistics for diazotrophic cyanobacteria biovolume and the predictor variables that were candidates for the best binary regression tree and random forest models. Median, minimum and maximum were calculated for variable by each sampling bout (early & late summer) and summer average (averaging across dates).

	Early Summer	**Late Summer**	**Summer Average**
Parameter	**Median (min-max)**	**Median (min-max)**	**Median (min-max)**
Diazotrophic cyanobacteria biovolume (µm³/mL)	32 (0–217,000)	41 (0–1,930,000)	71 (0–967,000)
Food web			
***Daphnia* (#/L)**	771 (0–76,000)	1,781 (0–13,100)	2,140 (0–41,300)
Small cladoceran grazers (#/L)	588 (0–18,300)	842 (0–24,400)	886 (0–18,500)
Lake environment			
Specific conductance (µS/cm)	23.5 (2.0–492.0)	27.2 (2.3–590.0)	24.6 (2–541.0)
Total phosphorus (mg/L)	0.010 (0.001–0.370)	0.003 (0.001–0.080)	0.010 (0.001–0.220)
Total nitrogen (mg/L)	0.070 (0.005–0.161)	0.030 (0.005–0.136)	0.020 (0.000–0.360)
Mixed layer depth (m)	9.0 (1.0–19.2)	10.0 (1.0–19.2)	9.5 (1.0–19.2)
Maximum depth (m)	---	---	19.2 (4.9–86.0)
Hypolimnetic dissolved oxygen (mg/L)	7.0 (0.3–11.4)	6.2 (0.3–20.4)	6.8 (0.3–14.7)
Watershed			
Latitude (°)	---	---	43.8 (42.4–45.5)
Drainage ratio	---	---	12.3 (2.0–206.0)
Developed area (%)	---	---	0.01 (0.00–4.17)
Base-flow index (%)	---	---	79.7 (56.3–86.0)
Northness [cos(aspect)]			0.16 (-0.21–0.69)

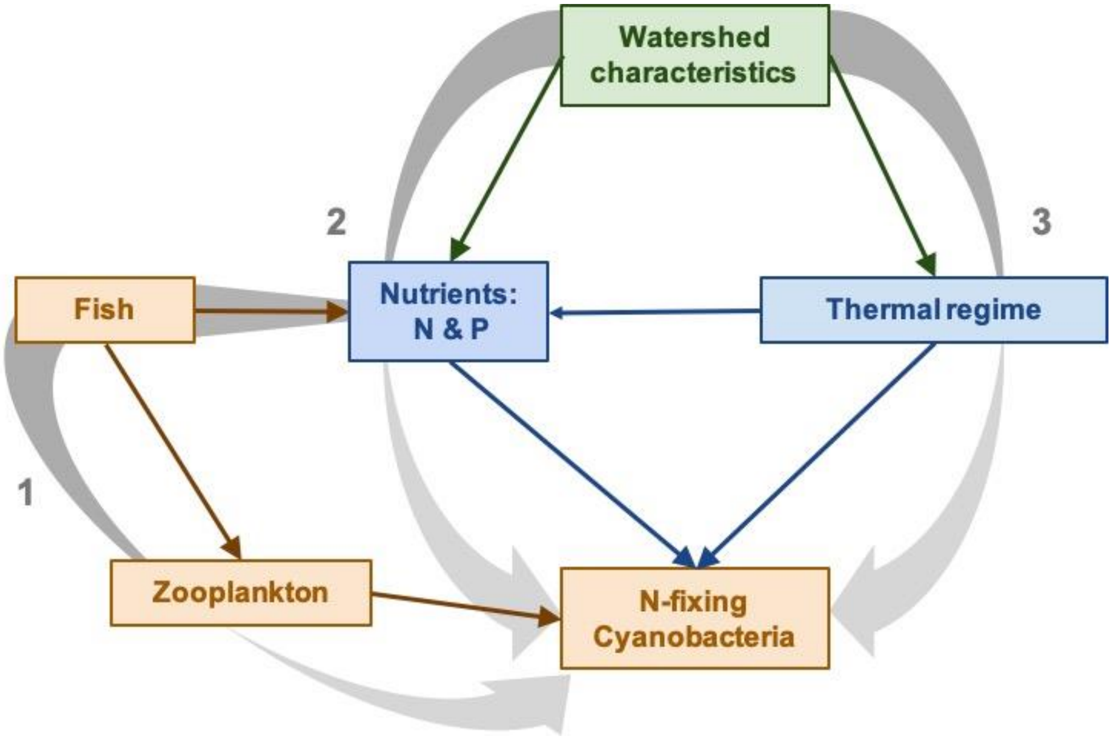

Figure 2.1 Conceptual diagram of hypothesized pathways with cross-scale interactions (grey arrows) and related direct effects (solid thin arrows) of food web (orange), lake (blue) and watershed (green) on nitrogen-fixing cyanobacteria. We hypothesize that there are three key pathways by which N-fixing cyanobacteria are influenced (numbered in grey): 1) Food web, 2) Nutrients, 3) Thermal.

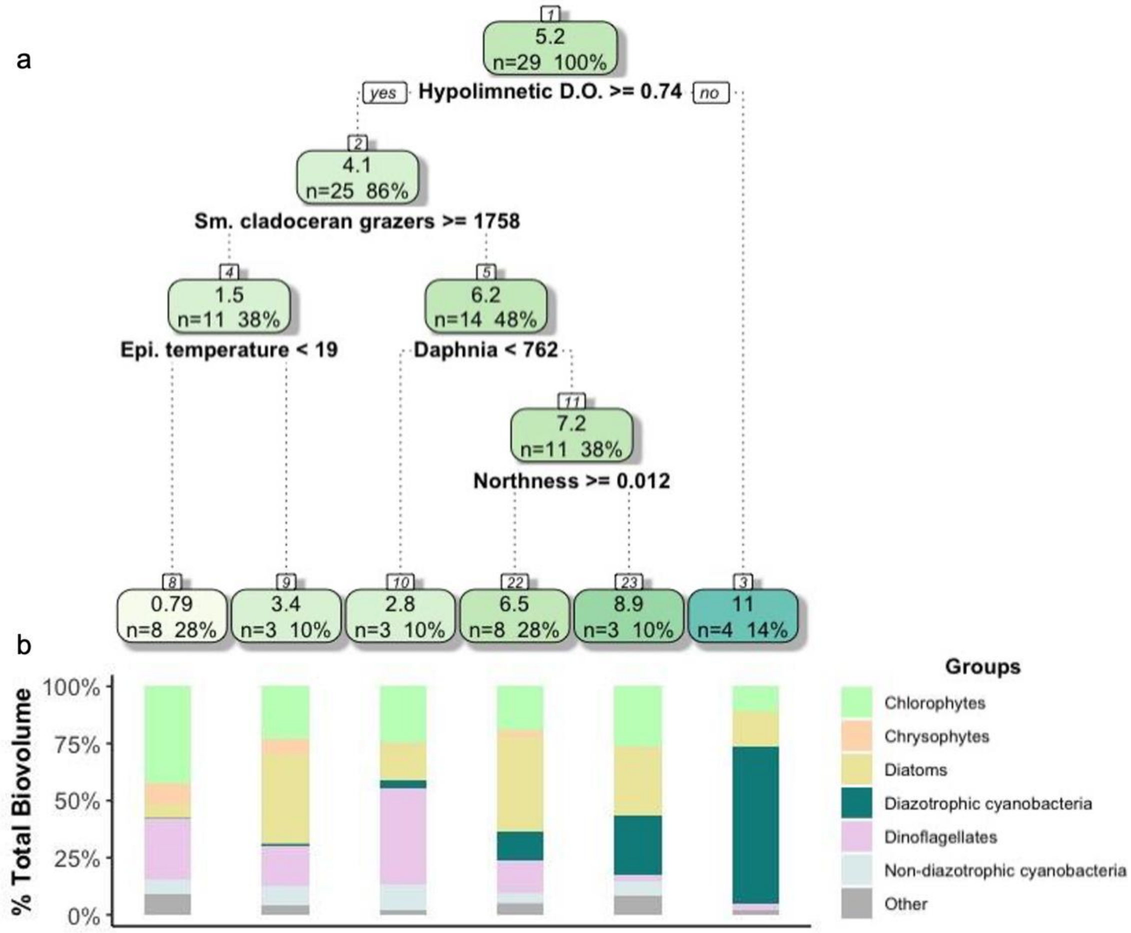

Figure 2.2 Binary regression tree for diazotrophic cyanobacteria with the five predictors driving the splits: hypolimnetic dissolved oxygen (D.O., mg/L), small (Sm.) cladoceran grazers (#/L), average epilimnetic (epi.) temperature (°C), Daphnia (#/L) and degree of northern aspect (cos[aspect]). Each node at the bottom of the tree represents the average diazotrophic cyanobacteria biovolume (μm3/mL, ln-transformed) and (n) number of lakes for that cluster. (b) Average phytoplankton composition (% total biovolume) of lakes in each terminal node by the six most common algal groups: chlorophytes, chrysophytes, diatoms, dinoflagellates, diazotrophic cyanobacteria, and non-diazotrophic cyanobacteria with a seventh 'other' group for rarer algal groups.

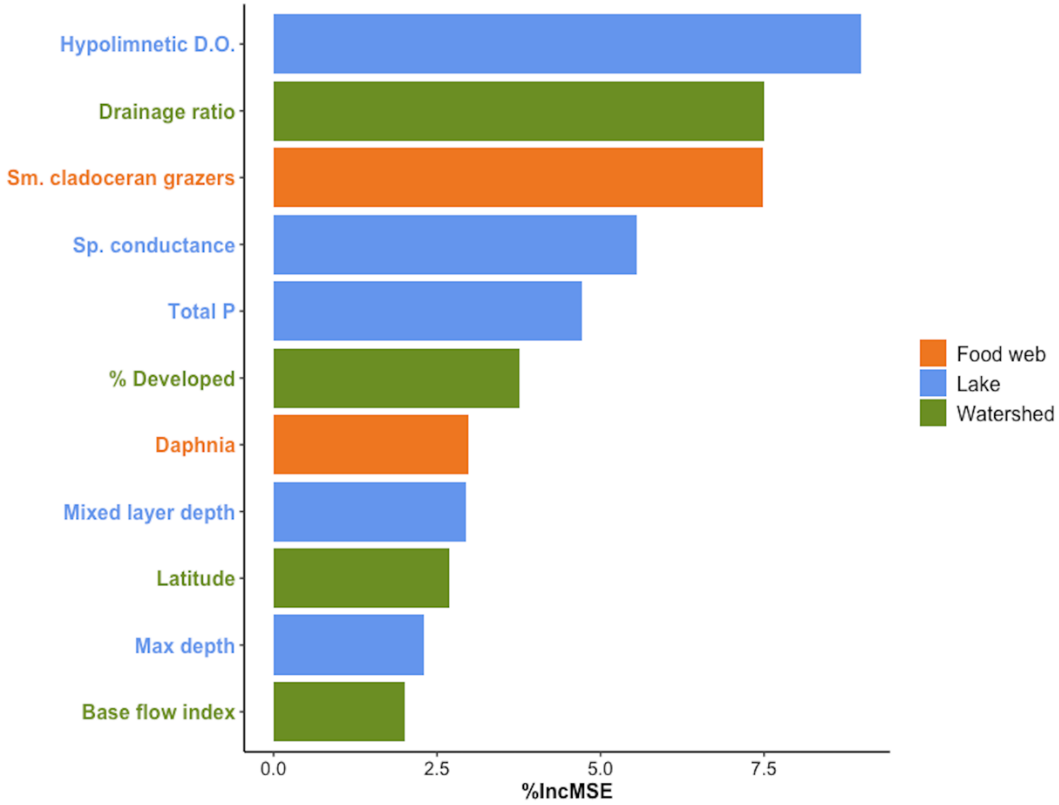

Figure 2.3 Variable importance plot of 11 most important predictors for nitrogen-fixing cyanobacteria biovolume (ln-transformed) based on the final reduced random forest model, categorized by food web, lake, and watershed. %IncMSE = decrease in mean model accuracy when each variable is dropped. Abbreviations: hypolimnetic dissolved oxygen (D.O.), small (sm.) cladoceran grazers, and specific (sp.) conductance.

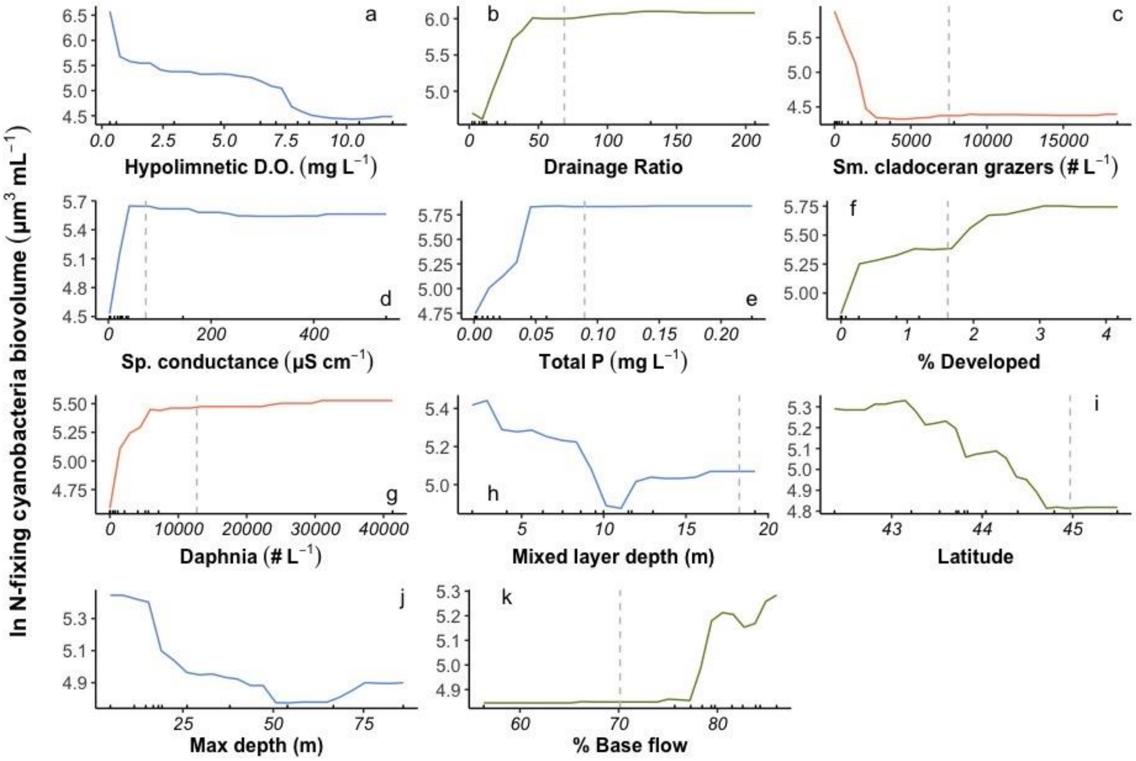

Figure 2.4 (a-k) Partial dependence plots of the 11 most important predictors based on the interquartile range above the third quartile or below the first quartile (Tukey 1977). The dashed line represents the upper outlier; for all predictors except Base flow and Max depth, the curve to the right of the line should be interpreted with caution. The curve to the left of the dashed outlier line (lower outlier) for Base flow should be interpreted with caution. Abbreviations: hypolimnetic dissolved oxygen (D.O.), small (sm.) cladoceran grazers, and specific (sp.) conductance.

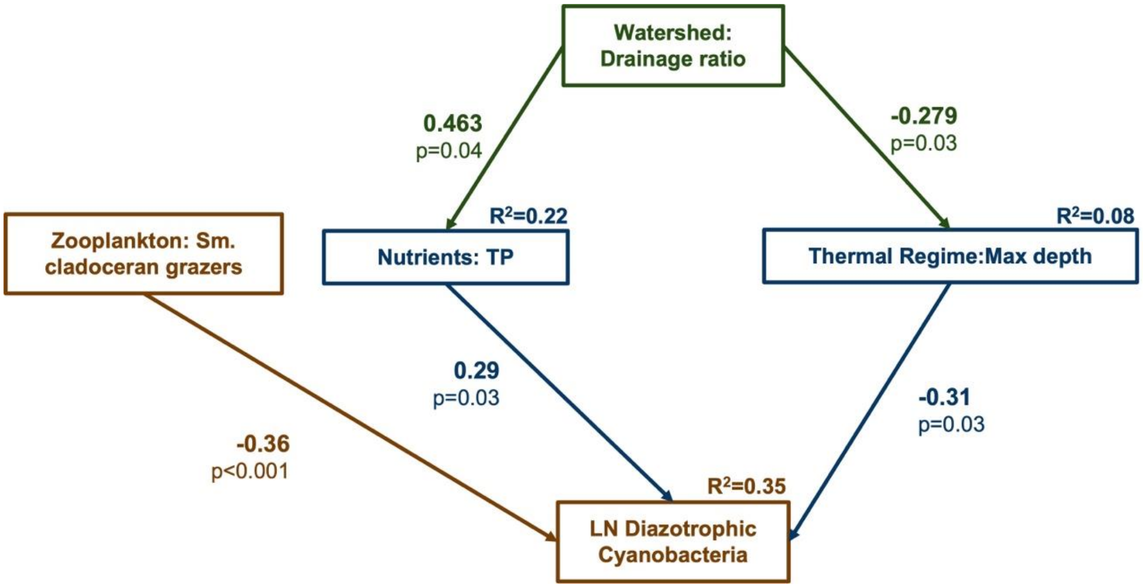

Figure 2.5 Path diagram of the final SEM of the effects of drainage ratio, small cladoceran grazers, total phosphorus, and maximum depth on naturally log transformed (ln) diazotrophic cyanobacteria biovolume. The values near each path are the standardized coefficients with the associated p-values below ($p<0.05$ in bold and thick lines) and the variance explained (R^2) being reported for total phosphorus (TP), maximum depth, and diazotrophic cyanobacteria.

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Chapter 3: Differing dynamics and toxicity of cyanobacterial blooms are reflected in bacterioplankton communities in mountain lakes

External collaborators: Nicolas Tromas, Université de Montréal & Jesse Shapiro, McGill University

3.1 Introduction

Harmful cyanobacterial blooms (cyanoHABs) are occurring across lakes spanning the trophic gradient from oligotrophic to hypereutrophic (Carey, Weathers & Cottingham, 2008; Pomati *et al.*, 2012; Huisman *et al.*, 2018; Reinl *et al.*, 2021). Cyanobacteria in oligotrophic and mesotrophic lakes can develop into blooms, sometimes even toxic, at the water surface or in the metalimnion when light is not limiting (Pomati *et al.*, 2012; Reinl *et al.*, 2021). As much of the focus of cyanoHABs studies has been on eutrophic waterbodies, new insights on HAB factors as well as bloom dynamics can be gained from studies in less productive systems, especially as management strategies like the reduction of external nutrient loading may have limited impact (Reinl *et al.*, 2021). In addition, future climate shifts will continue to increase water temperatures and alter precipitation patterns, potentially promoting cyanoHABs as well as cyanotoxin formation in temperate lakes regardless of trophic state (Chapra *et al.*, 2017; Reinl *et al.*, 2021).

Temperature and precipitation play important roles in the development and termination of cyanoHABs by influencing growth rates and toxin production (Figure 3.1). Temperatures above 20°C are often optimal for growth of toxigenic cyanobacteria, but energetically costly toxin production may drop as a result (Yin, Carmichael & Evans, 1997; Davis *et al.*, 2009; Peng *et al.*, 2018). Cyanobacteria can store more nutrients at cooler temperatures (<20°C), which is advantageous for toxin production, especially in nutrient-limited conditions (Layden *et al.*, 2022). Extended periods of dry warm weather can result in lake stratification, which facilitates the surface accumulation of buoyant cyanobacteria (Wagner & Adrian, 2009; Wood *et al.*, 2017). Although prolonged elevated temperatures favor toxigenic cyanobacteria, intermittent rainfall supplies limiting nutrients directly through deposition and indirectly through watershed runoff, driving proliferations of cyanobacteria, including toxic blooms (Yin *et al.*, 1997; Wood *et al.*, 2017; Celikkol *et al.*, 2021).While heavy rainstorms can break up blooms via mixing and rapid changes in surface temperatures (Reichwaldt & Ghadouani, 2012), less intense rainfall results in limited mixing and can provide inputs of nitrogen and phosphorus (Reichwaldt & Ghadouani, 2012; Celikkol *et al.*, 2021). Nutrient additions, especially in oligotrophic lakes, can drive cyanobacterial population growth, especially of toxigenic strains (Davis *et al.*, 2010; Reichwaldt & Ghadouani, 2012; Taranu *et al.*, 2019). However, toxin production may also increase in sub-optimal conditions, such as oxidative stress of cyanobacteria cells (Briand *et al.*, 2008; Zilliges *et al.*, 2011). As abiotic factors may have different effects on cyanobacterial biomass compared to toxins, biotic indicators should also be considered for predicting cyanoHABs and their toxicity.

The heterotrophic bacterioplankton closely interact with phytoplankton communities through their similar and different roles in nutrient cycles like carbon and phosphorus (P), and therefore are responsive to blooms like cyanoHABs (Cole, Findlay & Pace, 1988; Niu *et al.*, 2011; Tromas *et al.*, 2017). Phytoplankton provide organic carbon to heterotrophic bacteria, which in turn provide inorganic nutrients through mineralization (Currie, 1990; Brett *et al.*, 1999). Although the extent of mutualistic interactions depends upon the availability of nutrients for heterotrophic bacteria also require nutrients like P so competition can occur when nutrients are relatively low (Currie, 1990). In the case of cyanoHABs, the relatively large biomass of cyanobacteria can drive shifts in heterotrophic bacteria (Niu *et al.*, 2011; Berry *et al.*, 2017). Yet bloom related changes in heterotrophic bacterioplankton diversity vary across lakes, with decreases in richness and evenness observed in some lakes, while no changes were observed in other lakes (Woodhouse *et al.*, 2016; Tromas *et al.*, 2017; Wang *et al.*, 2020). These variable responses in bacterioplankton diversity may be in part due to different local environmental conditions influencing bloom dynamics, as well as bacterioplankton communities (Yannarell & Triplett, 2004; Shade, Jones & McMahon, 2008; Isles & Pomati, 2021). While heterotrophic bacterioplankton have predictable responses to cyanoHABs (Tromas *et al.*, 2017), this community should be examined beyond broad measures such as diversity to effectively characterize cyanoHABs.

CyanoHABs can alter bacterioplankton composition, with increases in specific cyanobacteria strains, as well as shifts in specific heterotrophic taxa (Louati *et al.*, 2015; Woodhouse *et al.*, 2016; Berry *et al.*, 2017; Guedes *et al.*, 2018). These community shifts during cyanobacterial blooms can be predictable, as certain heterotrophic taxa utilize cyanobacterial exudates such as polysaccharides and detritus (Louati *et al.*, 2015; Woodhouse *et al.*, 2016; Berry *et al.*, 2017; Guedes *et al.*, 2018). Some of these bloom-associated heterotrophs produce compounds that are complementary to cyanobacterial metabolism (Louati *et al.*, 2015; Pérez-Carrascal *et al.*, 2021). In addition, specific heterotrophic bacterioplankton can be more abundant in the presence of cyanotoxins, as they can break down these complex organic molecules (Mou *et al.*, 2013; Lezcano *et al.*, 2017). The response of bacterial degraders of toxins can be more consistent than the relationship between cyanobacteria biomass and toxin concentrations (Sinang, Reichwaldt & Ghadouani, 2013; Tromas *et al.*, 2017). The capacity for bacterioplankton communities to be an indicators of cyanobacteria toxins has only been studied in a limited number of lakes, all eutrophic and most impacted by development (Eiler & Bertilsson, 2004; Berry *et al.*, 2017; Tromas *et al.*, 2017; Wang *et al.*, 2020). The response of bacterioplankton communities to cyanoHABs in lakes differing in key characteristics, such as trophic state, remains understudied. As bacterioplankton taxa associated with cyanoHABs can be similar across regions, bacterioplankton communities have the potential to be a common indicator of cyanoHABs regardless of location (Louati *et al.*, 2015; Woodhouse *et al.*, 2016; Berry *et al.*, 2017; Guedes *et al.*, 2018) .

Distinct shifts in specific heterotrophic bacteria taxa due to cyanoHABs have often been examined in a single lake within a year (Woodhouse *et al.*, 2016; Berry *et al.*, 2017; Wang *et al.*, 2020), but species variation across years and lakes provides key insights into the predictability of community dynamics (Eiler & Bertilsson, 2004; Tromas *et al.*, 2017). While inter-annual variation of lake bacterioplankton may be somewhat consistent, bacterioplankton communities may be more variable in lakes where blooms are sporadic relative to lakes with persistent blooms (Tromas *et al.*, 2017; Isles & Pomati, 2021). A few studies have examined bacterioplankton communities in relation to cyanoHABs across multiple water bodies, but with only one sampling event (Eiler & Bertilsson, 2004; Berg *et al.*, 2009). Variation of bacterioplankton communities within lakes (e.g., between nearshore and offshore) has also been observed before, during, and after cyanoHAB events (Yannarell & Triplett, 2004; Shade *et al.*, 2008; Berry *et al.*, 2017). Additional studies are needed that examine the spatial and temporal variation in bacterioplankton communities in lakes with and without cyanoHABs.

The lakes of the Cascade Mountains (USA) offer an optimal setting to examine cyanotoxin variation, as well as associated shifts in bacterioplankton communities, due to documented toxic cyanoHABs across the region. Using physicochemical measurements, time-integrated toxin monitoring, and 16S rRNA sequencing of bacterioplankton, I explored possible associations of bacterioplankton communities with select meteorological characteristics, cyanoHABs and cyanotoxins at two temporal scales: intra- and inter-annually. I also examined how the same associations of bacterioplankton with weather, cyanoHABs, and cyanotoxins vary across spatial scales (i.e., within and between lakes). Specifically, I addressed the following questions: 1) How do cyanotoxin concentrations vary in relation to temperature and precipitation within and across lakes with varying cyanobacteria dominance? 2) How does the bacterioplankton community structure change in relation to cyanoHABs and cyanotoxins and how do these relationships vary within and between lakes? I hypothesized that: 1) Air temperature will have positive lagged relationships with toxin concentrations because water temperatures change more slowly due to the high specific heat of water, as well as the surface area and depth of the lakes (Wetzel, 2001). Rainfall will also have a positive lagged relationship, due to the delay in subsequent runoff in the lake catchments, and the relationship will depend upon rainfall intensity and lake stratification (Yin *et al.*, 1997; Davis *et al.*, 2009; Gallina, Anneville & Beniston, 2011; Celikkol *et al.*, 2021). 2a). Bacterioplankton communities will shift during cyanoHABs relative to non-HAB periods as cyanoHABs favor certain taxa such as organic compound degraders, which can potentially consume cyanotoxins (Mou *et al.*, 2013; Lezcano *et al.*, 2017). These shifts will vary in part with the toxicity of the HABs. 2b) Bacterioplankton composition will vary within and across lakes due to local characteristics, such as depth and nutrient availability, as well as geographic location (Yannarell & Triplett, 2004; Shade *et al.*, 2008; Schulhof *et al.*, 2020). Additionally, community composition across lakes will be more similar during HABs than in non-HAB periods due to the similar selective pressure of HABs (Wang *et al.*, 2020). As cyanoHABs are highly variable across lakes and regions, determining relationships with meteorological factors, as well as biological indicators of cyanoHABs across natural gradients with minimal human impact, will provide new insights because this ecological disturbance is not limited to developed eutrophic systems (Reinl *et al.*, 2021).

3.2 Methods

3.2.1 Study region

Several lakes in the southern Cascade Mountains (Oregon, USA) experience regular cyanobacterial blooms, with some having microcystin, a cyanotoxin, reported above Environmental Protection Agency (EPA) approved test detection limits (Fischer *et al.*, 2001). Some blooms have been prolonged, occurring for several weeks or more, while others are episodic lasting for as little as a few days (Oregon Department of Environmental Quality, 2011). The dominant genera in these blooms are *Dolichospermum* and *Gloeotrichia,* which are capable of producing toxins, including microcystins (Oregon Department of Environmental Quality, 2011; Dreher, Davis & Mueller, 2021). The dominance of diazotrophic cyanobacteria capable of fixing atmospheric nitrogen in certain Cascade lakes, likely reflects nitrogen (N) limitation, due partially to the underlying volcanic geology supplying relatively high levels of phosphorus (see Chapter 2). The Cascade Mountains receive on average 70% of precipitation between November and March; summers are relatively dry with only occasional rainfall (Nolin, 2012).

3.2.2 Field sampling & data compilation

Lakes were sampled in 2019 and 2020 to capture the spatial, inter-annual, and intra-annual variation of microcystins and bacterioplankton in relation to key meteorological and environmental variables. To examine the three components of variation, different subsets of lakes were sampled (Figure 3.2). To capture spatial variation, 29 lakes were initially selected from a binary regression tree using historical data to choose a representative sample from a larger set of lakes in the region (n=132; see Chapter 2). These lakes were sampled between August 8-31, 2019. I measured physical and chemical parameters at the deepest point in the lake (determined with bathymetric maps and a depth sounder), including a complete depth profile of temperature and dissolved oxygen with a YSI ProODO meter (Yellow Springs, Ohio, USA). I sampled for bacterioplankton and microcystin with a Wildco Van Dorn sampler (Yulee, Florida, USA) at 1 m below the surface at the deepest point in the lake and in the littoral zone. If the lake was stratified, I also sampled 1 m above the thermocline. A 250-mL sample was filtered onto 0.2-µm Sterlitech polyethersulfone membrane filters (Auburn, WA, USA) and kept frozen on dry ice in the field and at -80°C back in the laboratory for subsequent bacterioplankton DNA analyses. An additional sample with the Van Dorn sampler was taken at the deep spot and 500 mL of each sample was kept in brown Nalgene bottles, while another 50 mL was kept cool in a Falcon tube for microcystin analysis. A known volume from the brown Nalgene bottle was filtered onto Whatman glass fiber filters (GF/C, 1.2-µm pore size) for chlorophyll-a analysis. I collected samples for nutrient analyses (total N and P) from the top 5 m of the water column, using a 5-m long, 2.54-cm diameter tygon tube and transferred water into 125-mL HDPE bottles kept cool and preserved with H_2SO_4 to a pH <2 until frozen in the laboratory.

In order to capture inter-annual variation, 12 of the 29 lakes from the spatial set were resampled once between July 20-24, 2020 (Figure 3.2). The 12 lakes were chosen to be a representative subset of the original 29 lakes based on surface area, depth, elevation, and trophic status. Samples were taken with a Wildco Van Dorn sampler at 1 m below the surface in the littoral zone at three separate locations in each lake, either a dock or wading, due to COVID-19 restrictions that did not permit field work of >one person. Samples were taken for DNA sequencing, as well as microcystin, chlorophyll-a, and nutrient (total N and P) analyses as described previously. An additional 40-mL sample was placed into Falcon tubes after filtration through 0.45-µm Sterlitech polyethersulfone membrane syringe filters for orthophosphate (OP) and nitrite+nitrate (NO_2+NO_3). Both sets of nutrient samples were kept frozen until laboratory analysis. An additional 40-mL Falcon tube sample was acid preserved with 0.25 mL of hydrochloric acid (1 M) and filtered through 0.45-µm polyethersulfone membrane syringe filters (Sterlitech) kept cool for ammonia (NH_3) laboratory analysis.

For intra-annual variation (2020) of microcystins and bacterioplankton, three lakes were sampled biweekly (Figure 3.2). Solid Phase Absorption Toxin Tracking (SPATT) passive samplers with DIAION HP20 (Fisher Scientific) resin were constructed and activated for tracking microcystin prevalence with time-integrated samples following Kudela (2011). SPATTs were secured to a weighted rope with a plastic zip-tie at ~0.5-m depth from docks in three lakes (two with known cyanoHABs, Diamond and Odell; one without known cyanoHABs, Crescent). An ONSET HOBO pendant temperature data logger (Bourne, MA, USA) was also attached to the rope at each lake and recorded water temperature hourly from the start of the first SPATT deployment to the end of the final SPATT deployment. Two SPATT samplers were deployed biweekly in all three lakes from July 1 to October 17 from two docks at least 1 km apart in each lake. During each SPATT deployment and final removal, a grab sample was collected in a 40-mL Falcon tube at 0.5-m water depth at the docks for microcystin analysis for comparison with the SPATT sampler data. Another grab sample was collected and filtered as described previously for DNA sequencing of the bacterioplankton community at each SPATT deployment and final removal.

Daily air temperature and precipitation data for the lakes at 4-km resolution were obtained from the PRISM Climate Group (2022). Due to the major wildfires in the region that occurred in 2020, smoke was also included as a covariate as it can affect primary productivity (Scordo *et al.*, 2021). Daily aerosol optical depth data, a measure of smoke, was obtained at 1-km resolution for the lakes from MODIS Terra & Aqua (NASA, 2022). Daily air temperatures, aerosol optical depths, and water temperature (from HOBO loggers) were averaged, while precipitation was summed over 3, 5, 7, and 14-d periods prior to each sampling day, as these time periods have been found to be influential for cyanoHAB formation and termination (Persaud *et al.*, 2015; Tromas *et al.*, 2017; Celikkol *et al.*, 2021). These values were calculated at the three scales: 1) lake; 2) catchment, which was defined as the upstream area that drains directly and only into the lake; and 3) watershed, which was defined as the upstream area that was hydrologically connected to the lake, which can include other lakes (Hill *et al.*, 2018). As temperature and aerosol optical depth influence cyanobacteria and other bacterioplankton most strongly at the lake level, only lake-level measures were considered (Persaud *et al.*, 2015; Tromas *et al.*, 2017). As precipitation influences cyanobacteria and other bacterioplankton through both direct deposition and runoff, only catchment and watershed level measures were considered (Reichwaldt & Ghadouani, 2012; Celikkol *et al.*, 2021).

Correlations between predictors were examined using the Spearman's rank coefficient due to non-normal distributions. As expected, the different mean temperatures at the various time lags and spatial scales were highly correlated with each other. The same was true for the different cumulative precipitations and the different mean aerosol optical depths. The measure for temperature, precipitation, and aerosol optical depth that was most highly correlated across the time periods and spatial scales with each response variable were selected for statistical analyses. The 5-d mean air temperature and water temperature (intra-annual only), 5-d mean aerosol optical depth, and 14-d cumulative precipitation were thus selected for further consideration in statistical analyses.

3.2.3 Algal biomass & nutrient analyses

I extracted chlorophyll-a from filters using acetone for 20 hours in a dark refrigerator, and measured concentrations using a fluorometer (Arar & Collins, 1997). In 2019, I used a persulfate solution to digest total P samples heated to 100°C and then analyzed with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the molybdenum blue colorimetric method (detection limit: 0.002 mg L^{-1}; precision limit: +/- 0.004 mg L^{-1}) (Patton & Kryskalla, 2003; APHA, 2018c). In 2019, I also used a persulfate solution to digest total N samples heated to 100°C and then analyzed with a SmartChem 200 discrete analyzer (Guidonia, Italy) for colorimetric determination of NO_2-NO_3 (detection limit: 0.01 mg L^{-1}, precision limit: +/- 0.01 mg L^{-1}) (APHA, 2018b). In 2020, nutrient samples were sent to Western Washington University's Institute of Watershed Studies for colorimetric analyses with a Xylem OI FS3700 Continuous Flow Analyzer (College Station, Texas, USA). A sodium hydroxide and persulfate solution were used to digest total N and P samples, respectively. Total N and NO_2/NO_3 were analyzed using the automated cadmium reduction method (total N detection limit: 0.025 mg L^{-1}, precision limit: +/-0.023 mg L^{-1}; NO_2-NO_3 detection limit: 0.020 mg L^{-1}, precision limit: +/-0.015 mg L^{-1}) (APHA, 2018b). NH_3 was analyzed using the automated phenate method (detection limit: 0.010 mg L^{-1}, precision limit: ± 0.010 mg L^{-1}) (APHA, 2018a). Total P and orthophosphate were analyzed using the molybdenum blue colorimetric method (total P detection limit: 0.005 mg L^{-1}, precision limit: ± 0.003 mg L^{-1}; orthophosphate detection limit: 0.003 mg L^{-1}, precision limit: ± 0.001 mg L^{-1}) (APHA, 2018c).

3.2.4 SPATT & grab sample microcystin analysis

I triple rinsed each SPATT sampler in deionized water and then conducted a methanol extraction following the protocol developed by Kudela (2011). I quantified microcystin concentrations from SPATT extracts and whole water grab samples using Microcystin LR Enzyme-Linked Immunosorbent Assay (ELISA) kits (Eurofin-Abraxis, Warminster, PA, USA) and a plate reader (Tecan Infinite Pro 200, Männedorf, Switzerland) (detection limit: 0.15 ng ml^{-1}). For SPATT extracts, I created microcystin standards in 2% methanol solutions for the ELISA tests. I used Eurofin-Abraxis software to calculate the standard curve parameters and then calculated sample concentrations. The SPATT extract concentrations were normalized by dividing by grams of HP20 resin in each SPATT and the deployment time.

3.2.5 DNA extraction & sequencing

I extracted DNA from frozen filters using the QIAGEN Dneasy PowerSoil kits (Hilden, Germany) following manufacturer's protocol with the addition of an initial bead beating step (2 minutes at 30Hz) for increased mechanical lysis (Turk-Kubo *et al.*, 2012). The concentrations of a subset of DNA extracts were quantified using a ThermoFisher Scientific Qubit fluorometer (Waltham, MA, USA). DNA sequencing was performed at Université de Montréal. DNA libraries were prepared for paired-end sequencing (2x250 bp) on two Illumina MiSeq runs (San Diego, CA, USA). A two-step polymerase chain reaction (PCR) was performed, targeting the V4 region of the 16s rRNA gene with primers U515_F and E786_R as described in (Preheim *et al.*, 2013). Libraries were pooled, denatured, and then sequenced using the MiSeq reagent Kit V2. Each MiSeq run had negative controls and a mock community DNA sample containing identified 16s rRNA clone libraries from American Type Culture Collection MSA-1002 mock community (Manassas, VA, USA).

3.2.6 Sequence data processing

Barcoded FASTQ files were demultiplexed from the sequencing data using *idemp* v.3.4 (Wu, 2016). Adapters and primers were removed using *cutadapt* v.4.0 (Martin, 2011). I used the *dada2* v.1.22 package in R to filter and trim reads with the default filtering parameters with a maximum expected error score of two (Callahan *et al.*, 2016). Sequence reads shorter than 200 bp for forward reads and 150 bp for reverse reads were discarded. I then used *dada2* to infer amplicon sequence variants (ASVs), remove chimeras and then obtain an ASV count matrix in each sample for each MiSeq run separately. I used the default parameters of the "learning error rates" function with the multi-thread option enabled. The average raw reads per sample was 187,133 and the average nonchimeric reads per sample was 125,802 thus 32.8% of the reads were excluded due to filtering and chimera removal. PCR negative controls and extraction blanks produced 186 nonchimeric reads on average. These were excluded from the downstream analyses as I only included samples with a minimum of 1,000 reads. Of the 20 expected sequences from the mock community, *dada2* found 18 exact sequence matches. I joined the ASV abundance matrices of each MiSeq run and then used TaxAss (Rohwer *et al.*, 2018) to assign taxonomy with the FreshTrain database (Newton *et al.*, 2011), which was curated for freshwater bacterioplankton, and the general Greengenes database (DeSantis *et al.*, 2006). The ASV matrices along with taxonomic assignments and related environmental data were joined as an object in the *phyloseq* v.1.38 package (McMurdie & Holmes, 2013). I removed sequence data assigned as chloroplast or mitochondria. I also only included ASVs that had ≥ 10 total reads across samples to remove extremely rare ASVs.

3.2.7 Statistical analyses

To address the variation of cyanotoxins relative to weather patterns (Question 1), I examined the SPATT and cyanotoxin grab samples in relation to temperature, precipitation, and aerosol optical depth. As SPATTs are considered a semi-quantitative method, I binned the SPATT data into three numerical levels: 0) 0-0.15 ng/g/day, which is less than or equal to the lower detection limit (LDL) of the ELISA microcystin-LR test; 1) 0.16-1.60 ng/g/day, which is greater than the LDL, but less than the EPA drinking water limit concentration (1.60 ng/ml); 2) 1.61-8.00 ng/g/day, which is greater than the drinking water limit, but less than the EPA recreational water limit (US EPA, 2013; Kudela, 2017). For the SPATT data, there were no concentrations above 1.61 ng/g/day so there were only two categories (0, 1). I then used binomial generalized linear mixed effect models (GLMMs) due to the high number of zeros (i.e., no detections), with lake and month as random effects, to test the influences of fixed effects precipitation, aerosol optical depth, air and water temperature on SPATT levels with the *lme4* v.1.1-28 package (Bates *et al.*, 2015). I checked the assumptions of GLMMs using the *DHARMa* v.0.4.6 package (Hartig, 2022). The assumption of uniform distribution in the model residuals was checked through QQ plots and tested via the Kolmogorov-Smirnov non-parametric test of equality. The assumption of evenly dispersed residuals was checked with scatterplots and tested via a simulation-based dispersion test. The presence of zero-inflated data was tested via a ratio test of observed versus expected zeros for a binomial model. I found the data did not have significantly more zeros than expected for a binomial model. I used a similarly structured Poisson GLMM for the grab microcystin concentrations in 2020. Both GLMMs met model assumptions.

To assess the spatial and temporal variation of the bacterioplankton communities (Question 2), I examined the communities in relation to cyanoHABs and toxigenic cyanobacteria. I defined the presence of cyanoHABs on three conditions: 1) SPATT levels >1 (see definitions above); 2) grab samples with microcystin concentrations >1 ng ml^{-1}; and 3) toxigenic cyanobacteria >20% of the community abundance (Tromas *et al.*, 2017). As cyanoHABs were not present in any lakes during the sampling period in 2019, I instead examined the abundance of toxigenic cyanobacteria relative to all cyanobacterial taxa in the lake spatial set (n=29). In the spatial set only, groupings of toxigenic cyanobacteria were based on quartiles of the data: none (0-0.08%), low (>0.08-0.44%), medium (>0.44-4.75%), and high (>4.75-100%).

I examined bacterioplankton community variation in terms of alpha and beta diversity. I calculated the estimated Shannon-Weiner and Gini-Simpson Diversity Indices for each sample using the *DivNet* package v.4.0, which accounts for sampling bias such as missing rare taxa (Willis & Martin, 2022). I calculated weighted UniFrac distances for each sample using the *phyloseq* package (Lozupone & Knight, 2005). Weighted UniFrac distances are based on phylogenetic distances and relative abundance of taxa, which makes it less sensitive to rare taxa or ASVs (Lozupone & Knight, 2005). The weighted UniFrac distances were square-root transformed for principal coordinates analysis (PCoA). Differences in community structure between groups were tested through permutational multivariate analysis of variance (PERMANOVA) with the *adonis2* function in the *vegan* v.2.4-6 package (Oksanen *et al.*, 2009). For the three scales of analysis, different response variables were analyzed with PERMANOVA. The response variable for spatial and inter-annual variation was toxigenic cyanobacteria groups (see above), whereas intra-annual variation was analyzed with cyanoHAB presence or absence. As PERMANOVA tests can be sensitive to differences in dispersion, this assumption was tested via an analysis of multivariate homogeneity (PERMDISP) with the permuted *betadisper* function from the *vegan* package.

I used redundancy analyses (RDA) to examine how the bacterioplankton communities vary across lakes (spatial) and over a season (2020 only) in relation to climate (air and water temperature, precipitation, aerosol optical depth), cyanotoxin (grab microcystin concentrations and SPATT levels), and environmental (total P, total N, lake surface area, and maximum depth) variables. The community data was Hellinger-transformed to down-weight low abundance ASVs (Legendre & Legendre, 2012). The predictor variables were log-transformed and then centered and scaled due to varying measurement units. Multicollinearity between explanatory variables was assessed by checking correlation coefficients and variance inflation factors. If variables had either $r > 0.80$ or a variance inflation factor > 10, then one of highly correlated variables was dropped. Model selection was performed using the function *step*, which carries out backwards selection of explanatory models from the full model based on AIC (Akaike information criterion) at each step to determine the most parsimonious model. Analyses were performed using the *vegan* package.

For identification of ASVs and genera indicators of cyanoHABs (Question 2), I conducted differential abundance analysis using *ALDEx2* v.1.26 (Fernandes *et al.*, 2014). I conducted a differential abundance analysis of the bacterioplankton communities with Welch’s T-test and Monte Carlo simulations to estimate the amount of variation caused by technical errors (Fernandes *et al.*, 2014). Following Tromas et al. (2017), the ASVs and genera with a $p < 0.05$ based on the Wilcoxon Rank Sum test, after Benjamini-Hochberg correction, were considered biomarkers of toxic blooms. All statistical analyses were conducted in R v. 4.1.1 (R Development Core Team, 2022).

3.3 Results

3.3.1 Physicochemical and meteorological conditions for spatial, inter-annual, and intra-annual contrasts

The full set of 29 lakes sampled in 2019 (spatial variation) were similar in geology and hydrology but varied substantially in surface area and maximum depth (Table 3.1 and see Chapter 2). The lakes ranged in elevation from 958 – 1,980 m, spanning the montane to alpine zones (Table 3.1). During the late summer of 2019, total nitrogen concentrations varied between 0.005 – 0.890 mg L^{-1} and total phosphorus varied between 0.001 – 0.080 mg L^{-1} (Figure 3.3 a,d). At the catchment scale, cumulative precipitation over the 14 days prior to the sampling dates ranged from 0.0 – 16.9 mm at the catchment scale with a median of 6.2 mm (Figure 3.3j, Table 3.2). Air temperatures averaged over the five days prior to sampling ranged from 11.1 – 18.1°C with a median of 15.0°C (Table 3.2).

Based upon the 12 lakes resampled in 2020 (inter-annual variation), total nitrogen and phosphorus concentrations were higher in 2020 than 2019 (Table 3.1). Cumulative precipitation was similar between the two years, although there was a larger precipitation event in 2019 (Table 3.1). Air temperatures were somewhat higher in 2020 compared to 2019 (Table 3.1).

The intra-annual comparison lakes differed in temperature with Crescent having the lowest median surface water temperatures and Diamond had the highest median water temperature, but the greatest range (Figure 3.3i). The three lakes had similar median cumulative precipitation, but Diamond had higher rainfall events than Odell and Crescent, which are adjacent watersheds (Figure 3.3l, 3.4b). The 5-d mean aerosol optical depth was similar in Crescent (median: 92.9) and Odell (median: 96.2), peaked in early July, while Diamond was on average lower (median: 55.0) and peaked in late August. These peaks in aerosol optical depth tracked with nearby fires in the region. For overall temporal variation in the summer 2020, average 5-day water temperatures varied from 12.7 – 22.3°C, cumulative 14-day precipitation varied from 0.0 – 44.5 mm, and average 5-day aerosol optical depth varied from 18.8 – 183. Total phosphorus and nitrogen were lower in Crescent than Diamond and Odell, while Diamond had consistently higher concentrations than Odell (Figure 3.3c,f).

3.3.2 Question 1: Relationships between microcystin and select meteorological factors

While microcystin concentrations did not vary substantially across the spatial set of lakes in 2019 (0.01 –0.25 ng mL^{-1}) (Figure 3.3p), toxigenic cyanobacteria (in proportion to the total bacterioplankton community) exhibited substantial variation in both 2019 (0 – 29.7%) and 2020 (0 – 56.7%) (Figure 3.3). The prevalence of toxigenic cyanobacteria relative to the rest of bacterioplankton community was higher in 2020 compared to 2019 likely due to HAB events (Figures 3.3n, 3.4c). For the inter-annual comparison, microcystin concentrations were similar between years (2019 median: 0.06 ng mL^{-1}; 2020 median: 0.10 ng mL^{-1}), but the maximum concentrations were higher in 2020 compared to 2019 (2019 maximum: 0.15 ng mL^{-1}; 2020 maximum: 8.60 ng mL^{-1}; Figure 3.3q).

In the intra-annual subset, microcystin concentrations were highest in Odell in 2020 (maximum: 8.60 ng mL^{-1}; Figure 3.4). The maximum concentrations in Odell occurred in late July, coinciding with toxigenic cyanobacteria exceeding 50% of the bacterioplankton community, tracking with the harmful bloom advisory issued by Oregon

Health Authority. Microcystin concentrations in Crescent stayed below the detection limit of 0.15 ng mL^{-1}. The maximum concentrations in Diamond occurred in early October (1.01 ng mL^{-1}) when toxigenic cyanobacteria also peaked at 31% of the bacterioplankton community (Figure 3.4).

The binomial GLMM testing the relationships of SPATT levels with water temperature, precipitation, and aerosol optical depth was not significant ($\chi^2=3.99$, df=3, p=0.26; Table 3.3). The Poisson GLMM testing the relationships of microcystin grab concentrations with the same variables was also not significant ($\chi^2=1.11$, df=3, p=0.77; Table 3.3).

3.3.3 Question 2: Spatial variation in bacterioplankton communities

The bacterioplankton communities varied somewhat with the percent of toxigenic cyanobacteria across the lakes, but not within the lakes (Figure 3.5). Community composition shifted when toxigenic cyanobacteria were increasingly prevalent, including the non-cyanobacterial portion of the communities, based on weighted UniFrac (Figure 3.4a, b). The differences in community composition could not be assessed with PERMANOVA statistics due to the groups of toxigenic cyanobacteria being differently dispersed (Appendix B: Table S1). There was no variation in alpha diversity indices in relation to the percent of toxigenic cyanobacteria (Appendix B: Figure S2). However, the results qualitatively suggest that communities with increasing toxigenic cyanobacteria differ beyond the Cyanobacteria phylum, with variation in the heterotrophs also present (Figure 4b).

The potential of environmental variables to explain community variation in relation to the toxigenic cyanobacteria was explored through redundancy analysis (Figure 3.6a). The final reduced RDA model explained 25.6% of the spatial variation in bacterioplankton with four variables: latitude, longitude, 7-d mean air temperature, and total phosphorus (TP) (Figure 3.6; ANOVA: $F_{4,24}$ =2.07, p=0.001). Air temperature and latitude were mostly associated with the first axis but in opposing directions (Figure 3.6a). TP was mostly associated with the second axis in the same direction. Most of the lakes with the highest amounts of toxigenic cyanobacteria were associated with higher TP and air temperatures (Figure 3.6a). The two ASVs that explained the most variation were heterotrophs from two classes (Betaproteobacteria-*betIII-A*, and Cytophagia-*bacIII-A*), acting in the opposite direction of toxigenic cyanobacteria (Figure 3.6b).

3.3.4 Question 2: Inter-annual variation in bacterioplankton communities

Community composition was relatively similar between 2019 and 2020 based upon weighted UniFrac (PERMANOVA: F_1=1.03, p=0.40). Alpha diversity did not significantly change between the years when compared with paired t-tests (Shannon: t_{11}=0.59, p=0.56; Gini-Simpson: t_{11}=0.94, p=0.36). These results suggest the bacterioplankton communities were relatively stable between the two study years.

3.3.5 Question 2: Intra-annual variation in bacterioplankton communities

Bacterioplankton communities of the three lakes were different from one another, with two of the lakes experiencing cyanoHABs (Figure 3.7, 3.8). Generally, cyanoHABs were a significant negative factor in the variation in diversity, but the identity of the lake also explained substantial variation (i.e., as a random effect; Table 3.4). In Odell Lake, both diversity measures dropped during the cyanoHAB event, whereas in Diamond Lake, diversity did not change during the cyanoHAB event (Figure 3.7). During the cyanoHABs, 41 cyanobacterial ASVs made up 31% of the bacterioplankton in terms of relative abundance in Diamond, while 16 cyanobacterial ASVs made up over 55% of the bacterioplankton in Odell (Figure 3.7). The 41 cyanobacterial ASVs in the Diamond cyanoHAB included multiple toxigenic genera: *Dolichospermum*, *Woronichinia* and *Microcystis.* The 16 cyanobacterial ASVs in the Odell cyanoHAB included only one toxigenic genus: *Dolichospermum*. Within just the non-cyanobacterial portion of the communities, both diversity measures did not change during the cyanoHAB in Odell and Diamond Lake (Figure 3.7). Outside of the cyanoHAB periods, the lake communities were mainly made up of taxa from the phyla Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia (Figure 3.7). Actinobacteria dropped in relative abundance during both cyanoHABs, while Verrucomicrobia only dropped during the cyanoHAB in Odell (Figure 3.7).

Total and non-cyanobacterial bacterioplankton composition shifted under cyanoHABs based on weighted UniFrac, although these shifts were dependent on month and lake (Table 3.5, Figure 3.8). Composition varied over the months in each of the lakes. The bacterioplankton communities of Odell during the cyanoHAB were more strongly separated from the other communities when comparing both the total community and the non-cyanobacterial portion (Figure 3.8). However, the communities of Diamond during the cyanoHAB were not as strongly separated from the other communities for the total community, and there was no differentiation between cyanoHAB and non-cyanoHAB periods for the non-cyanobacterial portion of the bacterioplankton community (Figure 3.8).

The final reduced RDA model explained 44.2% of the intra-annual variation with five variables: week, 5-d mean water temperature, 5-d mean aerosol optical depth, microcystin concentrations, and SPATT level (Figure 3.9; ANOVA: $F_{6,34}$=4.49, p<0.001). Aerosol optical depth and week were mostly associated with the first RDA axis but in opposite directions, which is likely due to the late summer fires (Figure 3.9). Water temperatures and microcystin were mostly associated with the second RDA axis in the same direction (Figure 3.9). There were 13 ASVs that explained most of the variation, including an ASV of *Dolichospermum* as well as heterotrophic ASVs from the phyla Actinobacteria (2-*C111*,*acl-A*), Armatimonadetes (1-*CL0-1*), Bacteroidetes (4-*bacl-A,* unnamed Cryomorphaceae, *bacll-A,* unnamed Sphingobacteriales), Firmicutes (1-*Streptococcus sobrinus*), Proteobacteria (2-*betl-A*, *betVll-B*), and Verrucomicrobia (1-*Xip-Bl*) (Figure 3.7). The *Dolichospermum*, Cryomorphaceae, Sphingobacteriales, C111, and CL0-1 ASVs appear to be most strongly related to microcystins in terms of SPATT levels 1-2 and grab sample concentrations (Figure 3.9).

3.3.6 Question 2: Intra-annual indicators of cyanoHABs

The potential of biological indicators of cyanoHABs was explored through differential abundance analyses at the ASV and genus levels using ALDEx2. The results for the ASV and genus level were relatively similar (Table 3.6, Appendix B: Table S2). Several genera were identified as indicators of cyanoHABs as well as non-cyanoHAB periods (Table 3.6, Figure 3.10). *Dolichospermum*, as well as an undefined genus from the order Cytophagales, increased the most in relative abundance during the cyanoHABs than in the non-HAB periods of Odell and Diamond as well as in the entire sampling period for Crescent (Table 3.6, Figure 3.9). The cyanobacteria communities of both

HABs were dominated by *Dolichospermum* belonging to different ASVs, although Diamond had other toxigenic cyanobacteria genera present such as *Woronichinia* and *Microcystis* (Appendix B: Table S2). In addition, the genus *Rubrivivax* (order Burkholderiales) was a strong cyanoHAB indicator for Odell in particular (Figure 3.10). Genera that were most strongly indicative of a non-cyanoHAB period were an undefined Cerasicoccaceae genus (Phylum Verrucomicrobia), *aclV-A* (Actinobacteria) and *betlll-A* (Proteobacteria) (Table 3.6).

3.4 Discussion

In my study, intra-annual and inter-annual variation in cyanotoxins and cyanoHABs were examined in relation to bacterioplankton communities, as well as meteorological and physicochemical factors across and within mountain lakes. Rainfall and temperature did not significantly relate with cyanotoxins across lakes even though these factors varied from by nearly 50 mm and 10ºC, respectively. The lack of effects suggests other factors such as nutrient availability may be more influential on toxin production as well as bloom dynamics. Bacterioplankton community composition changed during cyanoHABs, as cyanobacteria and potential cyanotoxin-degrading taxa increased in relative abundance within lakes. While this shift in cyanobacteria and related heterotrophs was relatively consistent across lakes with cyanoHABs, bacterioplankton composition was not more similar between lakes during cyanoHABs than in a non-cyanoHABs period. Bacterioplankton composition was also relatively stable between years, suggesting regularity in the intra-annual dynamics. Across the Southern Cascade mountains, bacterioplankton communities as well as cyanoHABs vary across lakes, reflecting in part variation in nutrient availability. I explore these key results below.

3.4.1 Question 1: Relationships between microcystin and meteorological factors

Contrary to my predictions, microcystin levels during the cyanoHABs were not consistently related to meteorological variables, which is likely due to the different bloom dynamics (Isles & Pomati, 2021). The highly toxic bloom in mesotrophic Odell Lake was a sporadic bloom, defined as variable spikes in biomass and, unlike the more persistent blooms, which are near-peak biomass over a prolonged period, such as the observations in Diamond Lake and other eutrophic lakes (Wood *et al.*, 2017; Celikkol *et al.*, 2021). Sporadic blooms in less productive systems are often driven more by external, physical processes, such as prolonged warm weather, than internal processes (Wagner & Adrian, 2009; Isles & Pomati, 2021). For instance, the highly toxic cyanoHAB in Odell occurred soon after the cessation of precipitation and the rise in temperature, but before the peak (Figure 3.4). This warm and dry period, with indirect inputs of nutrients transported from the watershed by recent precipitation, may set the stage for a cyanoHAB. In addition, sporadic blooms are usually a combination of increased cyanobacterial growth near the lake surface as well as vertical migration of cyanobacteria from deeper depths (Leach *et al.*, 2018; Isles & Pomati, 2021). A deep chlorophyll-a and phycocyanin maximum was observed in Odell Lake prior to the cyanoHAB period; this deep layer of cyanobacteria likely contributed to the cyanoHAB via vertical migration (Oregon Department of Environmental Quality, 2019). Thus, sporadic blooms, including their toxins, may be better predicted by monitoring pre-bloom cyanobacteria growth and movement in relation to meteorological conditions such as temperature and precipitation (Isles & Pomati, 2021).

Persistent blooms in eutrophic systems are driven less by physical processes than by internal feedback loops, such as nutrient recycling, including by cyanobacteria (Cottingham *et al.*, 2015; Isles & Pomati, 2021). The less toxic bloom in eutrophic Diamond Lake occurred late in the summer even as water temperatures began to drop. In addition, phosphorus typically declines in the epilimnion by the late summer in stratified lakes (Nürnberg, 1984). Yet in this eutrophic lake, total phosphorus has been regularly higher or at similar levels in late summer relative to earlier in the ice-free season (Table S3), coinciding with the rise of toxigenic cyanobacteria biomass in the epilimnion (Eilers *et al.*, 2007; Miller & Sytsma, 2020). *Gloeotrichia*, a cyanobacterium that can move substantial amounts of phosphorus from the sediments into the water column, is one of the most common cyanobacteria taxa in Diamond Lake and therefore likely contributes substantially to epilimnetic phosphorus in the lake (Eilers *et al.*, 2007; Cottingham *et al.*, 2015). In addition, Diamond Lake experiences hypolimnetic anoxia in the summer (see Chapter 2), allowing for the release of sediment-bound phosphorus, which can be taken up by germinating *Gloeotrichia* cells (Carey *et al.*, 2008). The further decline in temperatures in late September likely influenced the termination of the bloom in Diamond Lake (Isles & Pomati, 2021). Therefore, the role of temperature and rainfall in cyanoHABs and cyanotoxins may be weaker in eutrophic lakes relative to less productive lakes as internal nutrient cycling plays a more substantial role in eutrophic systems. Yet further study is needed to determine the effect of meteorological factors like temperature, rainfall as well as wind, which are highly variable in time.

3.4.2 Question 2: Shifts in cyanobacteria relative to HAB toxicity and local factors

The cyanobacterial fraction of the bacterioplankton community during HABs varied in relation to the toxicity of the blooms as well as the local lake conditions; this variation limited the compositional overlap across lakes that was expected with HABs (Figure 3.9). *Dolichospermum*, toxigenic cyanobacteria somewhat common across the Southern Cascades (see Chapter 2), was more dominant during the highly toxic bloom in Odell Lake than in the less toxic bloom in Diamond Lake (Figure 3.7, 3.10). CyanoHABs in Diamond and other eutrophic lakes often contain multiple toxigenic taxa so the cyanobacterial diversity of HABs may be greater in these lakes than in less productive lakes like Odell (Scherer *et al.*, 2017; Tromas *et al.*, 2017; Wang *et al.*, 2020). The most common ASVs of *Dolichospermum* were also different between the two HABs, with toxin-producing strains likely more abundant in the highly toxic bloom in Odell than in the less toxic bloom in Diamond. In addition, differences in microcystin production between lakes may be, in part, a stress response to greater nutrient limitation (Eilers *et al.*, 2007; Briand *et al.*, 2008; Oregon Department of Environmental Quality, 2019). Odell has lower average nitrogen and phosphorus than Diamond (Appendix B: Table S3), which supports the hypothesis that cyanotoxin production may be triggered by limited nutrients. Thus, cyanobacterial sequence data can be indicative of HAB toxicity and key lake characteristics such as productivity in mountain lakes.

3.4.3 Question 2: Shifts in heterotrophs relative to HAB toxicity & local factors

Heterotrophic taxa from multiple phyla were bioindicators of cyanoHABs as well as microcystins in my study. Bioindicators were relatively consistent between lakes but with some variation in relation to toxicity, as expected (Figure 3.10, Table 3.5). As the same cyanobacteria genus, *Dolichospermum* dominated both HABs, the heterotrophic bacterioplankton associated with the HABs may have been more similar than if the HABs were dominated by different cyanobacteria taxa (Louati *et al.*, 2015). The cyanobacteria associated with HABs may provide different resources to the broader bacterioplankton community, such as diazotrophic taxa providing bioavailable nitrogen (Louati *et al.*, 2015). In my study, the heterotrophic taxa indicative of the HABs were also similar to the taxa associated with HABs in other lake studies (e.g., Cytophagales), suggesting some commonality in cyanoHAB communities across lakes and regions (Berg *et al.*, 2009; Tromas *et al.*, 2017; Lezcano *et al.*, 2017; Guedes *et al.*, 2018). Following my prediction, some of the HAB-associated heterotrophic taxa are considered potential cyanoHAB and cyanotoxin degraders. One heterotrophic indicator of HABs was from the order Cytophagales, which has the capacity to degrade large complex organic compounds (van Hannen *et al.*, 1999; Lezcano *et al.*, 2017). This Cytophagales member made up 3.6× more of the bacterioplankton community in the less toxic bloom than in the highly toxic bloom, suggesting it may consume other cyanobacterial exudates beside microcystin during the HAB (van Hannen *et al.*, 1999). By contrast, another HAB indicator from the order Burkholderiales made up 5× more of the bacterioplankton community in toxic bloom than in the less toxic bloom (Figure 3.10). Several taxa from Burkholderiales are known to be responsive to cyanoHABs, as well as microcystin, indicating their potential as microcystin degraders (Mou *et al.*, 2013; Woodhouse *et al.*, 2016; Tromas *et al.*, 2017). Therefore, bacterioplankton appear to be responsive to cyanoHABs and cyanotoxins, as the prevalence of specific heterotrophic taxa reflected the toxicity of the cyanoHABs in my study.

Other heterotrophs declined with cyanoHABs and toxigenic cyanobacteria in my study, potentially reflecting differences in nutrient availability across lakes. Some taxa from the order Actinobacteria, such as from the genus *acl-A,* declined during the cyanoHABs: *acl-A* was somewhat negatively correlated (r=-0.30, p=0.12) with toxigenic cyanobacteria across lakes in 2019 (Table 3.5, Appendix B: Figure S2). This negative association has occurred in other lakes experiencing cyanoHABs (Louati *et al.*, 2015; Berry *et al.*, 2017). Taxa from Actinobacteria are also often negatively associated with nutrients (Haukka *et al.*, 2006), although the relative abundance of Actinobacteria was not correlated with nutrients (TN: r=-0.12, p=0.54; TP: r=-0.25, p=0.19). On the other hand, Verrucomicrobia taxa are positively associated with high nutrients, in particular nitrogen (Haukka *et al.*, 2006). While taxa from the phyla Verrucomicrobia have responded positively to *Microcystis* and *Dolichospermum* HABs in other lakes (Louati *et al.*, 2015; Tromas *et al.*, 2017), these taxa did not increase during cyanoHABs in my study lakes, with a few taxa even decreasing (Table 3.5). The difference in cyanoHAB response in Verrucomicrobia may reflect variation between lakes, with previous studies centered on eutrophic lowland lakes, which often have higher nutrient levels, especially nitrogen, than mountain lakes (Louati *et al.*, 2015; Williams *et al.*, 2017; Tromas *et al.*, 2017). In accordance with my prediction, nutrient levels were key in driving the overall variation in bacterioplankton across my study lakes (Figure 3.5). Therefore, the response of heterotrophic bacterioplankton to cyanoHABs may dependent partially on lake nutrient availability.

3.4.4 Questions 1 & 2: Limited variation across years and within lakes

While cyanotoxins and bacterioplankton varied intra-annually in some lakes, limited variation was observed inter-annually, as well as in different regions within lakes. Bacterioplankton communities and microcystin levels in my study lakes were relatively similar between years (Figure 3.3q). In addition, microcystin and bacterioplankton communities did not significantly vary between the epilimnion, thermocline, and littoral regions, suggesting relatively even distributions in the Cascade lakes (Figure 3.5). Therefore, intra-annual dynamics of bacterioplankton likely occur regularly as observed in other studies (Shade *et al.*, 2008; Tromas *et al.*, 2017). In addition, variation in bacterioplankton composition within lakes have been linked with isolated regions, such as restricted bays, which were not present in the lakes of this study (Yannarell & Triplett, 2004). Bacterioplankton communities in the Cascade lakes have the potential to be consistent cyanoHAB indicators regardless of sampling location in a lake and across years.

3.4.5 Questions 1 & 2: Study limitations

My study on intra- and inter-annual variation of cyanoHABs and bacterioplankton in mountain lakes did have some limitations. While two years of sampling captures some variation across years, additional years of sampling would provide more robust evidence on the degree of repeatability in seasonal bacterioplankton dynamics as well as HAB events (Tromas *et al.*, 2017). In terms of intra-annual variation, my study's biweekly sampling is not sufficient to completely resolve the dynamics of cyanoHABs and cyanotoxins, as well as weather, which can vary greatly even in a single day (Li *et al.*, 2022). Additionally, the sample size was relatively small (3 lakes by 7 sample points), resulting in low statistical power. In terms of weather, I did not examine certain variables for which I had little information, such as cloud coverage and wind, which can have substantial effects on cyanoHABs (Reichwaldt & Ghadouani, 2012).The SPATT samplers used in this study, which captured microcystins throughout the study period, suggested peak microcystin levels occurred around the time toxigenic cyanobacteria dominance in the bacterioplankton communities was observed (Kudela, 2011). However, high frequency sampling (i.e., daily) of the bacterioplankton along with cyanotoxins would offer greater insight into the pre-bloom and post-bloom periods as well as the role of meteorological conditions. For bloom toxicity, 16s rRNA sequencing cannot determine which cyanobacteria strains were toxin-producing. Complete genomic sequencing would identify the toxin-producers, and if the entire bacterioplankton community was also sequenced then possible functions related to toxin degradation could also be identified (Mou *et al.*, 2013). Therefore, additional studies are needed to understand the unique dynamics of the cyanoHABs in the Cascade mountains and other mountain ranges.

3.5 Conclusions

The intra-annual dynamics of toxigenic cyanobacteria and cyanotoxins across lakes are variable within the Southern Cascades, reflected in heterotrophic bacterioplankton communities with increases in potential cyanotoxin degraders as well as decreases in oligotrophic taxa. The effects of precipitation and thermal regimes on cyanotoxins will depend upon lake and watershed characteristics that influence productivity (Taranu *et al.*, 2019). Yet further research is needed on the spatial variation in cyanotoxins within a region, such as a mountain range, with lakes sampled at multiple points within a year to capture seasonal shifts. CyanoHAB monitoring is often limited to a few grab samples for toxins and cell density, especially in remote regions such as mountain lakes (Moser *et al.*, 2019; Chaffin *et al.*, 2021). Therefore, the analysis of bacterioplankton communities could provide more time-integrated data of a cyanoHAB, such as bloom toxicity, than a grab sample, as bacterial composition can reflect changing conditions over multiple days (Tromas *et al.*, 2017; Lezcano *et al.*, 2017). As toxin monitoring of cyanoHABs also remains expensive to account for the numerous toxins and variants, bacteria identification can complement traditional toxin sampling. Bacterioplankton are increasingly being recognized as cyanoHAB indicators, but as cyanoHABs can occur across a range of productivity and thermal regimes, research must adjust its focus from warmer, eutrophic lakes to encompass more oligotrophic and colder lakes (Berg *et al.*, 2009; Tromas *et al.*, 2017; Reinl *et al.*, 2021, 2023). Heterotrophic bacteria reflect cyanoHAB properties as well as other local factors (Yannarell & Triplett, 2004; Woodhouse *et al.*, 2016; Berry *et al.*, 2017), and therefore these microbes can elucidate how blooms vary across thermal and productivity gradients.

3.6 Tables and Figures

Table 3.1 Summary information for non-meteorological environmental predictor variables of the spatial set (n=29), the inter-annual subset (n=12), and the intra-annual subset (n=3). Summary statistics include median, minimum, and maximum for each variable with exception of the intra-annual subset.

Parameter	**Spatial** *median (min, max)*	**Inter-annual** *median (min, max)*	**Intra-annual** *Crescent, Diamond, Odell*
Elevation (m)	1460 (958, 1980)	1430 (1050,1720)	4839, 5183, 4787
Surface area (ha)	102 (17, 1550)	87 (17, 1550)	1547, 1301, 1382
Z_{max} (m)	19.2 (4.9, 86.0)	21.1 (5.5, 86.0)	81.0, 15.8, 86.0
Z_{mean} (m)	9.8 (1.9, 49.7)	13.5 (2.4, 40.2)	37.8, 7.3, 40.2
Latitude (º)	43.9 (42.4, 45.5)	43.8 (43.2, 44.4)	43.5, 43.8, 43.6
Longitude (º)	-121.9 (-122.2, -121.2)	-121.9 (-122.2, -121.7)	-122.0, -121.9, -122.0

Table 3.2 Summary statistics of the meteorological variables used in the generalized linear mixed effect models for toxins by lake, which was the random effect. The median is presented along with the minimum and maximum in parentheses. The variable names are shortened for brevity: 5-d mean air temperature (air temp), 14-d cumulative precipitation (precip), and 5-d mean aerosol optical depth (AOD).

Parameter	**Crescent**	**Odell**	**Diamond**
5-d air temp.(°C)	14.9 (5.8, 19.9)	14.3 (5.8, 19.7)	15.4 (6.0, 19.3)
14-d precip. (mm)	4.6 (0.0, 30.8)	4.1 (0.0, 39.5)	7.7 (0.0, 44.5)
5-d AOD	92.9 (49.9, 175.1)	55.0 (25.3, 154.6)	96.2 (18.8, 183.5)

Table 3.3 Summary of generalized linear mixed effect models (GLMM) for SPATT levels (binomial distribution) and microcystin grab samples (Poisson distribution). The coefficients (± standard error) for the fixed effect of 5-d mean air temperature (air temp), 14-d cumulative precipitation (precip), and 5-d mean aerosol optical depth (AOD) are reported along with the model's degrees of freedom (df), T-value, and p-value. Neither model was significant ($\alpha = 0.05$). R^2m = marginal R^2, R^2c = conditional R^2 .

Factor	**SPATT Level**					
Fixed	**Coefficients**	**Df**	**T-value**	**P-value**	**R^2m**	**R^2c**
5-d air temp.	0.690(±0.480)	9	1.43	0.10	0.27	0.61
14-d precip.	0.160(±0.120)		1.27	0.15		
5-d AOD	0.020(±0.030)		0.50	0.20		
Random	**Variance**	**SD**				
Lake	4.110	2.03				

Factor	**Grab sample**					
Fixed	**Coefficients**	**Df**	**T-value**	**P-value**	**R^2m**	**R^2c**
5-d air temp.	-0.080(±0.250)	9	-0.31	0.76	0.09	0.21
14-d precip.	-0.002(±0.010)		0.14	0.89		
5-d AOD	-0.040(±0.070)		-0.61	0.54		
Random	**Variance**	**SD**				
Lake	0.450	0.67				

Table 3.4 Summary of linear mixed effect models (LMM) for Shannon Diversity Index and Gini-Simpson Index. The coefficients (± standard error) for the fixed effect of harmful algal blooms (HAB) are reported along with the model's degrees of freedom (df), T-value, and p-value. R^2m = marginal R^2, R^2c = conditional R^2 . Significant p-values ($\alpha = 0.05$) are denoted by an asterisk.

Factor	**Shannon Diversity Index**					
Fixed	**Coefficients**	**Df**	**T-value**	**P-value**	**R^2m**	**R^2c**
HAB	-0.74(±0.15)	31	-5.12	<0.0001*	0.39	0.54

Random	**Variance**	**SD**
Month	0.01	0.11
Lake	0.78*	0.88
Residual	0.07	0.27

Factor	**Gini-Simpson Index**					
Fixed	**Coefficients**	**Df**	**T-value**	**P-value**	**R^2m**	**R^2c**
HAB	-0.18(±0.02)	35	-8.96	<0.0001*	0.65	0.71

Random	**Variance**	**SD**
Month	0.00	0.01
Lake	0.01	0.09
Residual	0.00	0.00

Table 3.5 Results of the analysis of multivariate homogeneity (PERMDISP) and permutational MANOVA (PERMANOVA) to test beta diversity differences between HAB and non-HAB samples, month, and lake for the total and non-cyanobacterial communities of intra-annual 2020 lakes. The beta diversity distances were calculated based on weighted-UniFrac. PERMDISP tested if the dispersion between the groups were significantly different. PERMANOVA tested if the centroids of the groups are significantly different. p<0.01 are denoted by an asterisk.

Metric	**Analysis**	**Factor**	**df**	**Pseudo-F**	**R^2**	**p-value**
Intra-annual Weighted-UniFrac	**PERMDISP**	HAB	1	0.55	-	0.500
	PERMANOVA	HAB	1	13.8	0.20	0.001*
		Month	1	6.12	0.09	0.001*
		Lake	2	3.52	0.10	0.001*
		HAB*Month	1	2.80	0.04	0.001*
		HAB*Lake	1	2.67	0.08	0.001*
Intra-annual NC Weighted UniFrac	**PERMDISP**	HAB	1	0.003	-	0.950
	PERMANOVA	HAB	1	6.90	0.11	0.001*
		Month	1	7.47	0.12	0.001*
		Lake	2	3.86	0.12	0.001*
		HAB*Month	1	2.75	0.04	0.003*
		Month*Lake	1	3.23	0.10	0.001*

Table 3.6 Differential abundance analysis result from ALDEx2 with 1,000 Monte Carlo samples for HAB and non-HAB communities. Difference indicates the median log difference between HAB and non-HAB samples. BH p-values = Wilcoxon's t-test p-values with the Benjamini-Hochberg (BH) correction for multiple tests. Select taxonomic levels (Phylum, Order, Genus) are reported for brevity.

Group	**Phylum**	**Order**	**Genus**	**Difference**	**BH p-value**
HAB	Proteobacteria	Burkholderiales	*Rubrivivax*	6.20	0.004
	Cyanobacteria	Nostocales	*Dolichospermum*	5.99	0.003
	Proteobacteria	Rhodobacterales	unnamed	4.78	0.026
	Bacteroidetes	Cytophagales	unnamed	3.77	0.003
	Proteobacteria	Rhodospirillales	unnamed	2.57	0.006
no HAB	Proteobacteria	Methylophilales	*betIV-A*	-1.49	0.042
	Actinobacteria	Actinomycetales	*acI-A*	-1.76	0.024
	Verrucomicrobia	Cerasicoccales	unnamed	-2.57	0.008
	Verrucomicrobia	Opitutales	*Opitutus*	-2.66	0.038
	Actinobacteria	Acidimicrobiales	*acIV-D*	-3.09	0.023
	Proteobacteria	Burkholderiales	*betIII-A*	-3.58	0.014
	Actinobacteria	Acidimicrobiales	*acIV-A*	-4.46	0.012
	Verrucomicrobia	Verrucomicrobiales	*Luteolibacter*	-5.41	0.018

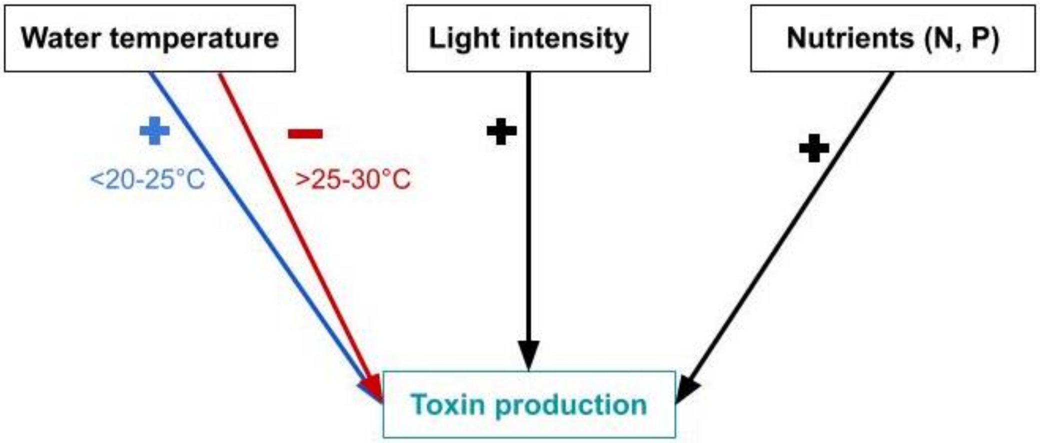

Figure 3.1 Conceptual diagram of the major drivers for cyanobacterial toxin production based on current literature (Davis et al. 2009, 2019; Peng et al. 2018, Utkilen et al. 1992).

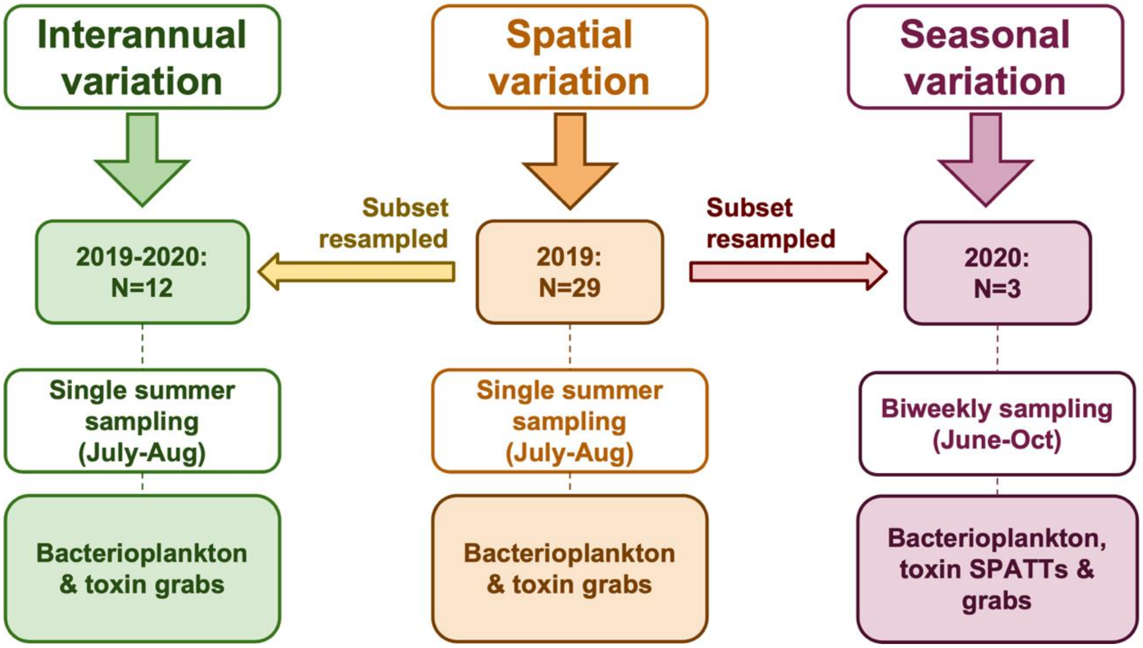

Figure 3.2 Sampling design for the three study components (spatial, inter-annual, and intra-annual) with number of lakes for each (N), period of sampling, and main biological samples taken. SPATT = Solid Phase Adsorptive Toxin Tracking samplers.

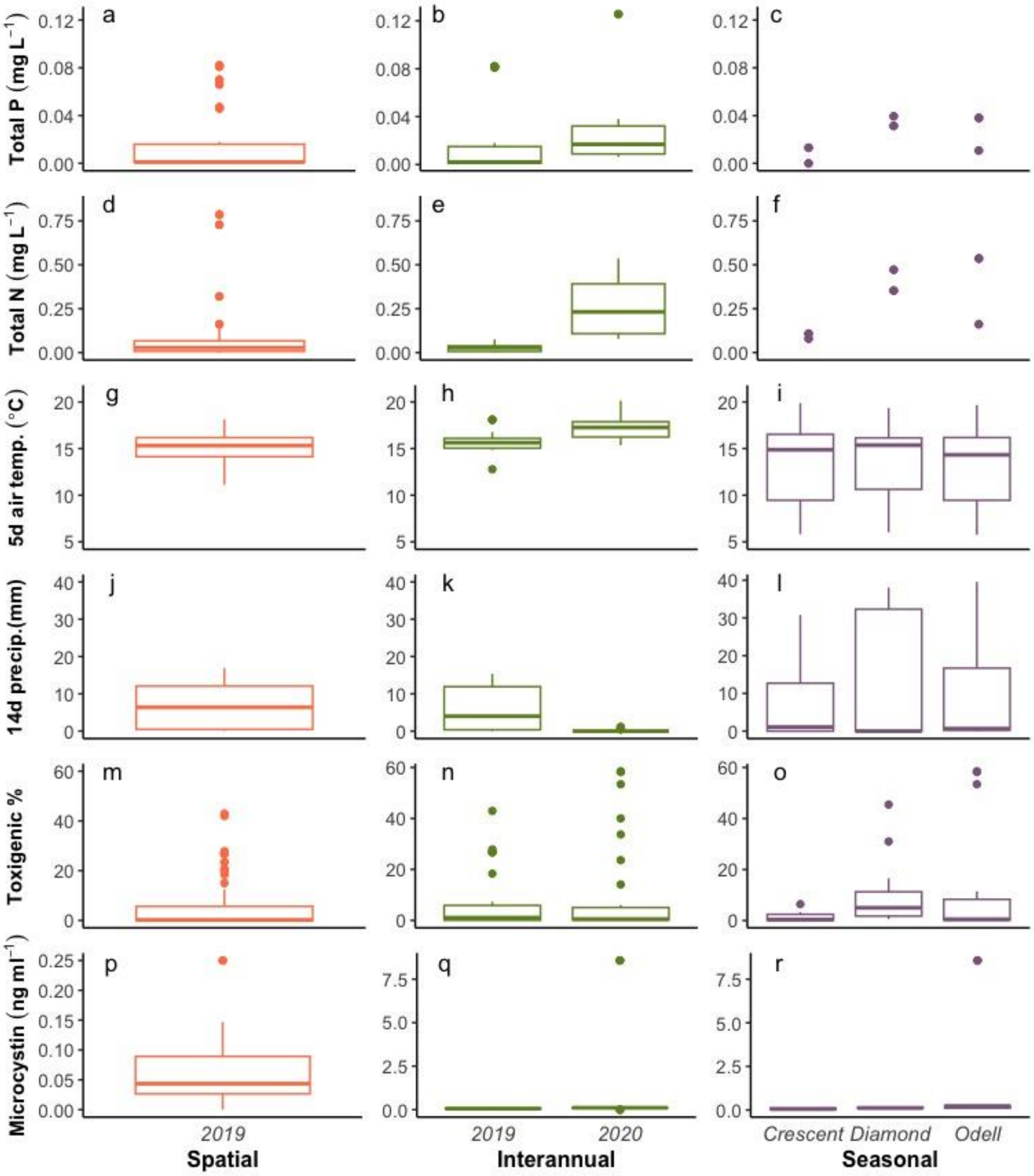

Figure 3.3 Summary graphs for dynamic variables for the spatial set (left), inter-annual subset (middle), and intra-annual (right) subsets. Variables included (a,b,c) total phosphorus (P) and (d,e,f) total nitrogen (N) (mg L^{-1}); (g,h,i) 5-d mean air temperatures (°C); (j,k,l) 14-d cumulative precipitation (mm), (m,n,o) % of toxigenic cyanobacteria, and (p,q,r) microcystin concentrations (ng ml^{-1}). The boxes of each boxplot are defined by the interquartile range, the bold line in the box is the median, and the points are outliers. The (c,f) graphs of intra-annual total phosphorus and nitrogen only had 2 samples per lake, which is not enough for a boxplot. The y-axes of the (p,q,r) microcystin graphs vary across the subsets to allow for better visualization of the substantial differences between subsets.

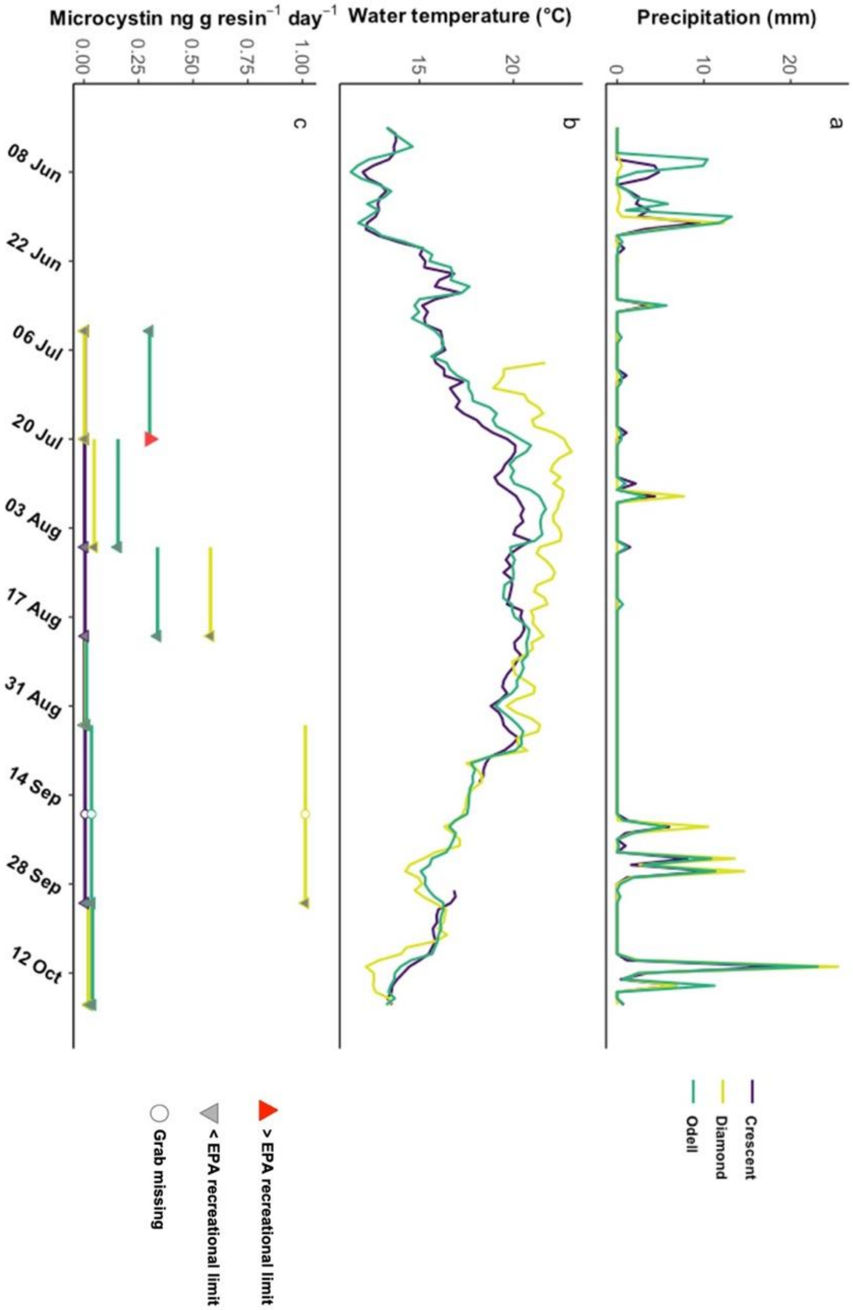

Figure 3.4 a) Daily total precipitation (mm) at the catchment scale by lake; b) daily mean water temperature (°C) by lake; and c) microcystin concentrations by lake (ng per g of resin per day) from the SPATTs for the intra-annual comparison in 2020. In c), the grab samples concentrations are denoted as either below (gray triangle) or above (red triangle) the EPA recreational limit of 8 ng ml^{-1} or missing (open circle).

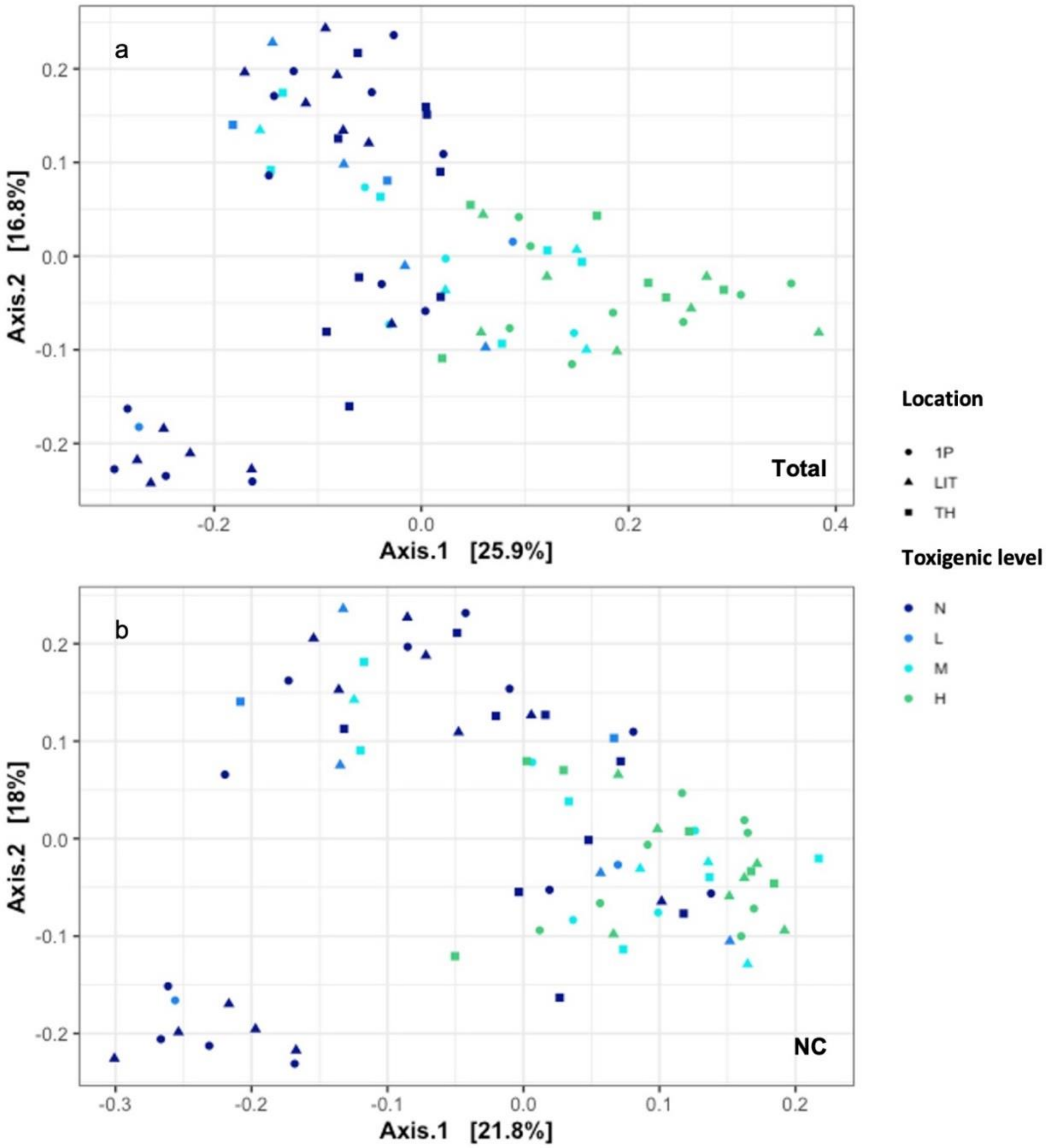

Figure 3.5 Differences in total (a) and non-cyanobacterial (b) bacterioplankton community composition between sampling location (1P: pelagic 1m, circle; LIT: littoral, triangle; TH:1m above thermocline, square) and levels of toxigenic cyanobacteria (see text for classifications) using Principal Coordinates Analysis. Each point represents a sample and distances calculated using weighted UniFrac. Toxigenic level is represented by color (None: dark blue, Low: light blue, Medium: cyan, High: green).

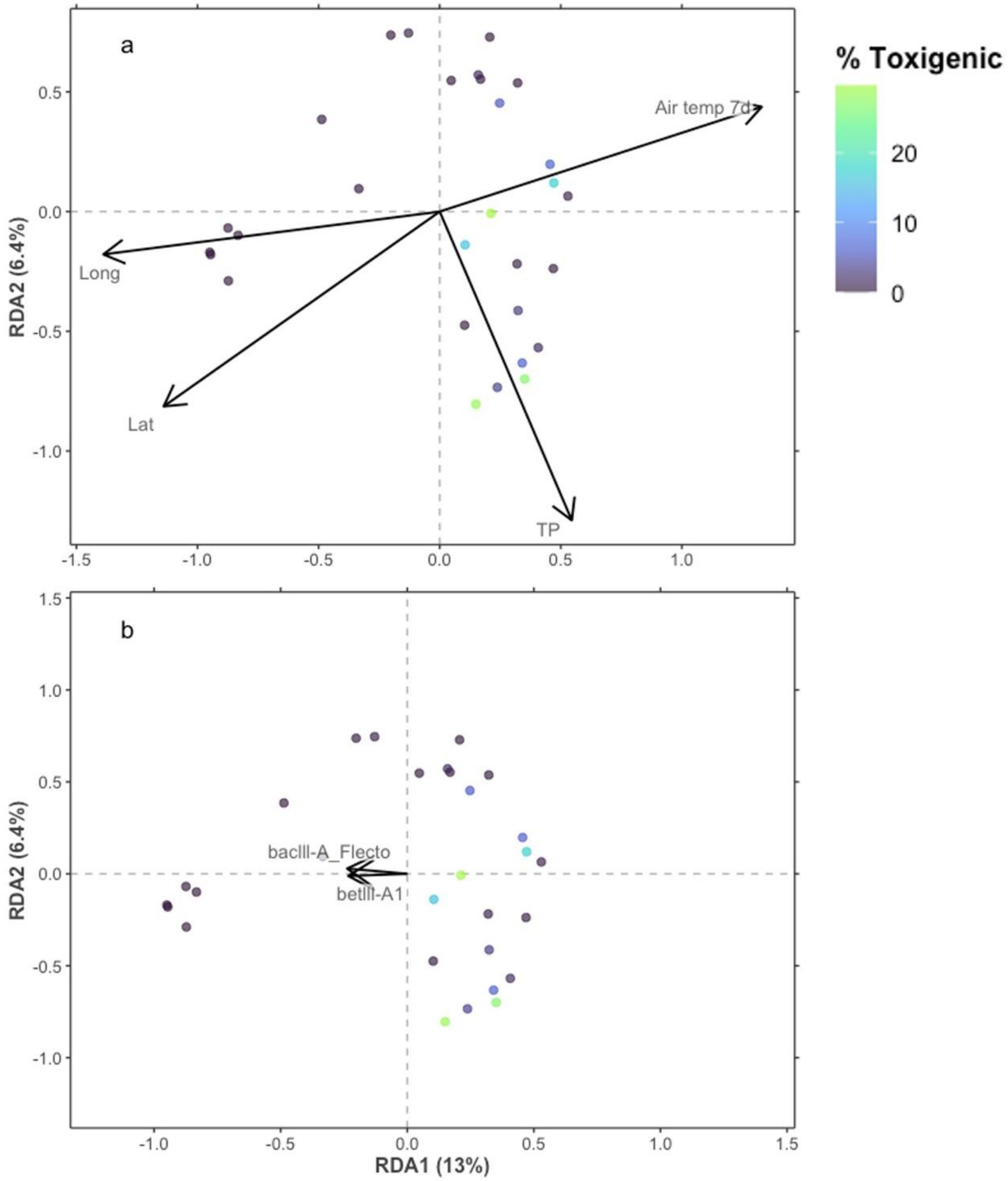

Figure 3.6 Redundancy analysis biplot of (a) the significant environmental predictors, and (b) ASVs explaining at least 85% of the variation in the spatial set of lakes (2019). The environmental predictors were latitude (Lat), longitude (Long) , 7-d air temperature (Air temp 7d), and total phosphorus (TP). The ASVs are betlll-A1 (Betaproteobacteria), and baclll flecto (Cytophagia).

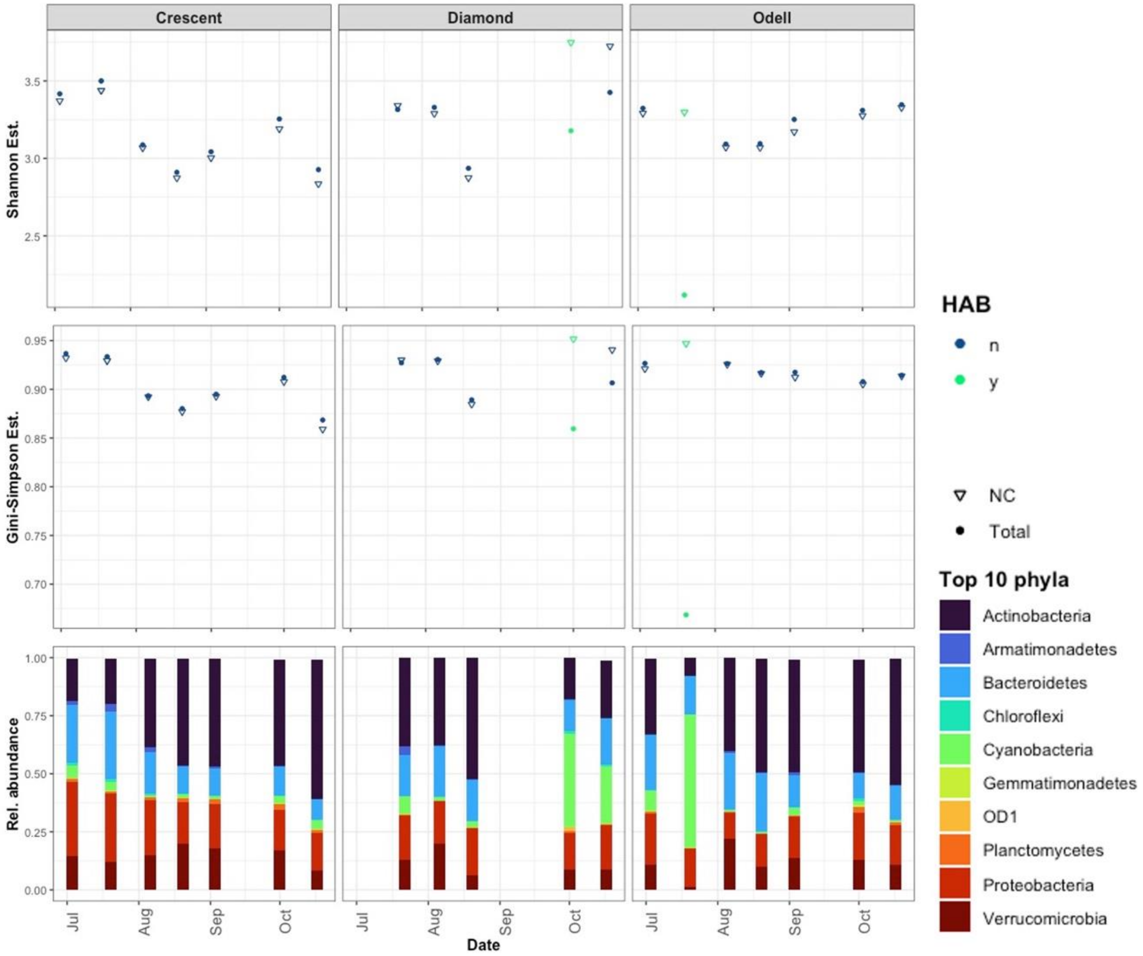

Figure 3.7 Changes in bacterioplankton communities during the summer of 2020: (top) Shannon Diversity Index, (middle) Gini-Simpson Index, and (bottom) relative abundance of the top 10 phyla. The diversity indices of the entire community of each sample are estimated with DivNet, which accounts for sampling bias. The samples in the diversity indices are colored by the presence (bright green) or absence (dark blue) of harmful cyanobacterial blooms (cyanoHABs) as well as the portion of the communities in terms of shapes by total (circle) and non-cyanobacterial (NC, triangle).

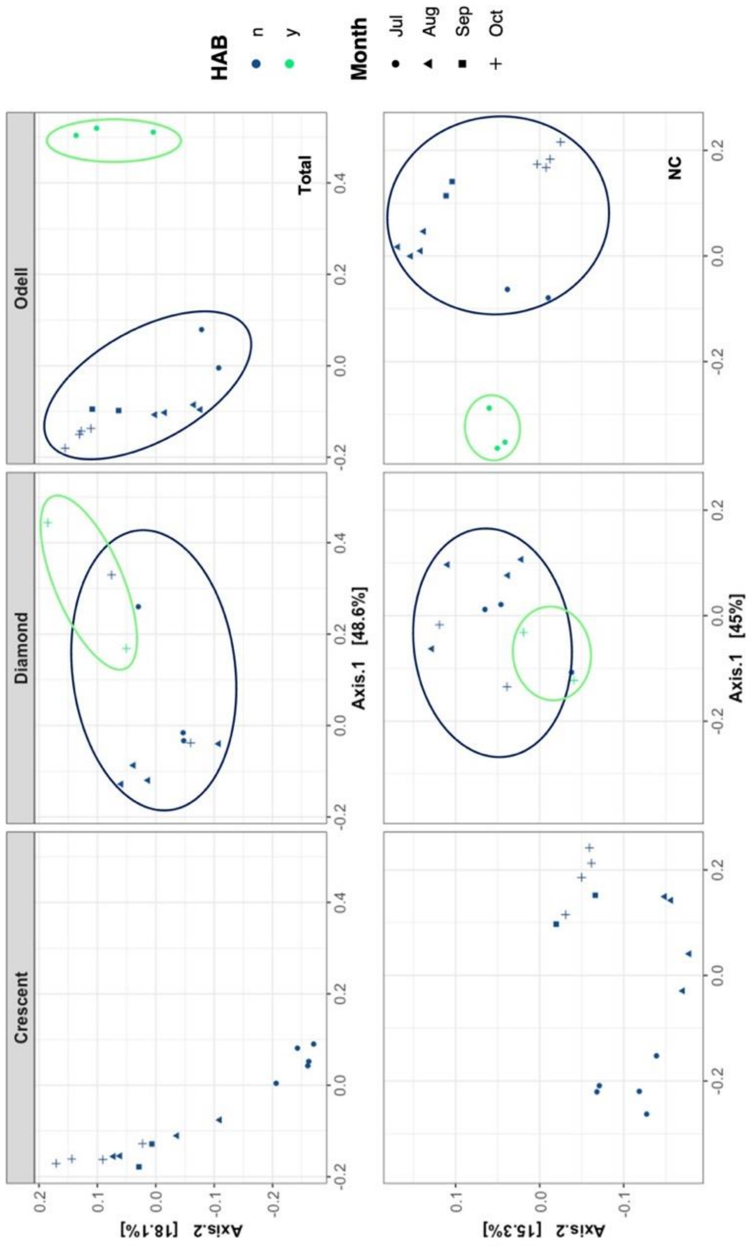

Figure 3.8 Differences in total (top) and non-cyanobacterial (bottom) bacterioplankton community composition between months and HABs and non-HABs separated by lake. Each point in the principal coordinate analyses (PCoAs) represent a sample and distances calculated using weighted UniFrac. The points are colored based on the presence (green) or absence (blue) of cyanoHABs. The month is symbolized by shape (July: circle, August: triangle, September: square, October: cross). The ellipses group the HAB (green) and non-HAB (blue) communities in Diamond and Odell.

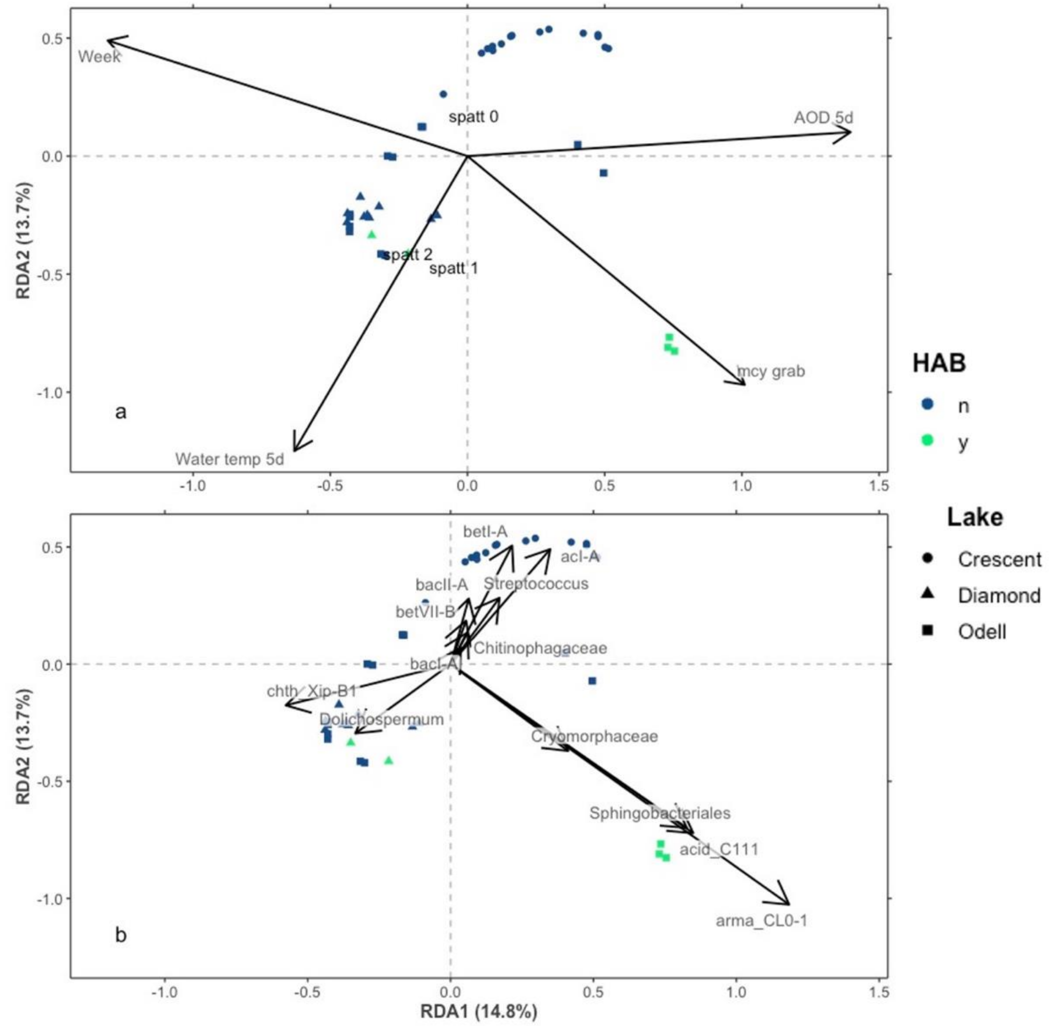

Figure 3.9 Redundancy analysis biplot of (a) the significant environmental predictors and (b) ASVs explaining at least 85% of the variation for the intra-annual subset (2020). The predictors were week, 5-d mean water temperature (water temp 5d), microcystin concentrations (mcy_grab), 5-d mean aerosol optical depth (AOD 5d), and SPATT level (spatt). The points are colored based on the presence (green) or absence (blue) of cyanoHABs. The lake is symbolized by shape (Crescent: circle, Diamond: triangle, Odell: square). The ASVs are an undefined Dolichospermum species, an undefined Cryomorphaceae species, an undefined Sphingobacteriales, C111-Acidimicrobiales, CL0-1-Armatimonadaceae, Xip-61-Chitinophagaceae, an undefined Streptococcus species, acl-A-Actinomycetales, betl-A and betVll-B -Burkholderiales, bacll-A and bacl-A -Bacteroidetes.

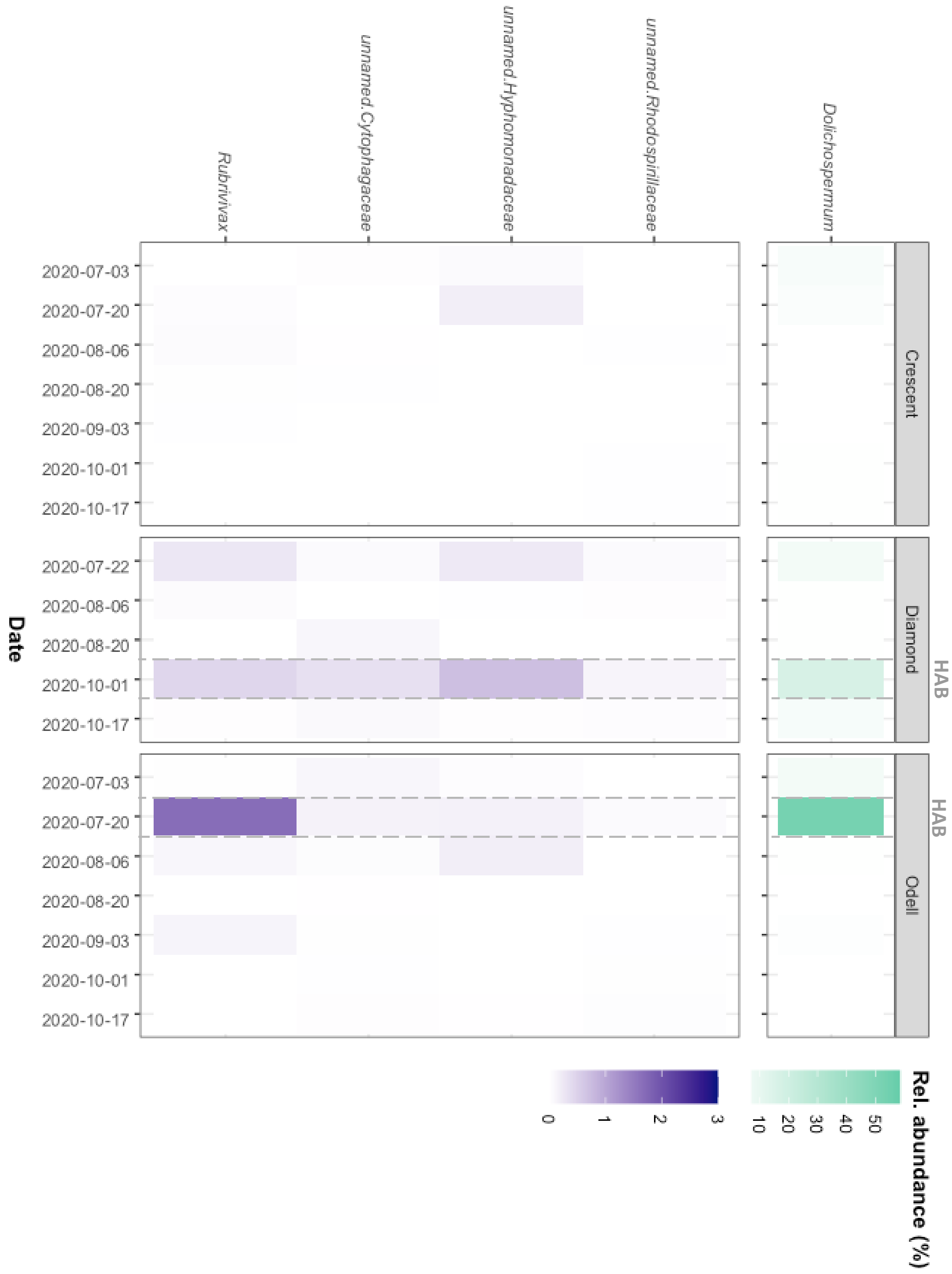

Figure 3.10 Temporal shifts of HAB indicator genera by lake based on ALDEx2 differential abundance analysis. The colors indicate the relative abundance of the genus over the entire bacterioplankton community of each lake and date. There are two separate color scales to enhance the views of the low and high relative abundances. For genera that were unnamed, the next lowest taxonomic level was provided. The cyanoHAB events are denoted by gray dash lines.

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Chapter 4: Functional traits in phytoplankton communities reflect gradients of nutrients and grazing pressure across mountain lakes

External collaborator: Robin Matthews, Western Washington University

4.1 Introduction

Biological communities are sensitive indicators of environmental shifts, such as climate change, and are often studied in terms of taxonomic composition. Yet functional traits of organisms can provide more direct and transferable links to ecosystem processes and environmental disturbances (Lavorel & Garnier, 2002; Moretti & Legg, 2009; Bello *et al.*, 2010). For example, individual traits clarify the roles of biological communities in key ecosystem processes such as carbon and nitrogen (N) cycling (Lavorel & Garnier, 2002; Suding *et al.*, 2008; Litchman *et al.*, 2015). The utility of individual functional traits is well recognized for freshwater communities, yet traits remain underutilized to examine ecological patterns in freshwater relative to marine and terrestrial communities (Litchman & Klausmeier, 2008; Bello *et al.*, 2010; Weithoff & Beisner, 2019b; Wentzky *et al.*, 2020). Studies of functional traits can provide generalizable information on abiotic and biotic drivers of phytoplankton, as well as the potential consequences for ecosystem processes (Litchman & Klausmeier, 2008).

Phytoplankton have developed a variety of physiological, morphological and behavioral traits to scavenge and compete for nutrients such as phosphorus (P), which are often variable and limiting in lakes (Hutchinson, 1961; Litchman & Klausmeier, 2008). Cell size and growth rate often respond positively to nutrient availability: greater cell size results in a lower surface area:volume ratio; thus nutrient uptake is less efficient, but biomass-specific production increases (Droop, 1973; Grover, 1991; Peter & Sommer, 2015). Physiologically, some phytoplankton have developed higher uptake affinities, which is the ratio of the maximum uptake rate to the half-saturation constant for uptake for nutrients (Healey & Hendzel, 1980; Litchman *et al.*, 2007). The ability to fix atmospheric nitrogen (N) (i.e., diazotrophy) by some cyanobacteria species is one key physiological trait to overcome N limitation (Smith, 1983; Litchman *et al.*, 2015). One physiological and behavioral trait for nutrient scavenging is mixotrophy, allowing taxa like dinoflagellates to not only photosynthesize but also consume smaller prey and organic compounds through phagotrophy or osmotrophy (Granéli, Carlsson & Legrand, 1999). Mixotrophic taxa are also often motile, increasing their encounter rate with particles and prey (Reynolds, 1984, 2006). In addition, motility can reduce the loss to sinking, which is important as motile mixotrophs are often relatively slow growing (Reynolds 2006, Wentzky 2020). Multiple nutrient scavenging traits often co-occur as trait syndromes, working in tandem to survive in nutrient-limited waters (e.g., motile mixotrophs with high P affinity; Sommer *et al.*, 1986; Reynolds, 2006). Yet nutrients are rarely the only driver of lake phytoplankton, so trait variation must be examined in the context of multiple abiotic and biotic factors (Reynolds, 2006; Pomati *et al.*, 2020).

Many phytoplankton have adopted or modified physiological, behavioral, and morphological traits to resist the top-down control of grazers, ranging from ciliates to small crustaceans (Lürling, 2021). Phytoplankton resist ingestion by grazers by altering their cell size and shape, such as developing spines, as well as forming colonies or filaments (Bergquist *et al.*, 1985; Sterner, 1989; Hillebrand *et al.*, 2022). Some phytoplankton even have the ability to produce compounds that are toxic to grazers

(DeMott & Moxter, 1991; Ger *et al.*, 2016a). Highly grazer-resistant taxa often have a combination of these traits such as large cells with long spines (Sterner, 1989; Jakobsen, Everett & Strom, 2006; Hillebrand *et al.*, 2022). Alternatively, some taxa have high enough growth rates to compensate for the losses from grazing (Lürling, 2021). In addition, some traits like cell shape that provide grazing resistance also play a role in reducing sinking rates, which is another major form of biomass loss (Reynolds 2006). Grazing and nutrient availability may similarly influence traits such as cell size, yet some grazing defenses like toxin production are metabolically expensive so the competitive ability of phytoplankton is reduced (Lürling, 2021). In oligotrophic lakes, the nutrient acquisition trait of mixotrophy may not be directly affected by grazers: some mixotrophs such as chrysophytes and dinoflagellates can have relatively low nutritional quality for grazing zooplankton, as well as thick plates or shells (Reynolds, 2006; Vad *et al.*, 2020). Therefore, selective feeders like copepods may more readily consume other phytoplankton that have higher nutritional value and more vulnerability to grazing (Pomati *et al.*, 2020; Vad *et al.*, 2020). While the interaction of these bottom-up and top-down pressures on phytoplankton traits has been examined temporally within lakes, their joint influence across lakes remains an area for further study (Weithoff & Gaedke, 2017; Pomati *et al.*, 2020; Wentzky *et al.*, 2020; Schulhof *et al.*, 2022).

Lakes in remote regions largely removed from direct anthropogenic activities have communities that vary due to multiple factors from large-scale climate to local biotic interactions (Schindler *et al.*, 1993; Parker *et al.*, 2001; Moorhouse *et al.*, 2018). Studies of freshwater invertebrate communities in remote regions have revealed important environment-trait associations, such as links between feeding strategies and climate (Ilg & Castella, 2006; Schriever *et al.*, 2015; Boersma *et al.*, 2016). In regions with significant portions of developed land, external nutrient inputs from anthropogenic sources often override other drivers of phytoplankton composition, including climate (Maloufi *et al.*, 2016; Moorhouse *et al.*, 2018). Trait-based responses and roles of phytoplankton in more remote regions are important in predicting ecosystem-level shifts in lake productivity, especially with the far-reaching impacts of climate change and atmospheric deposition (Weithoff & Gaedke, 2017; Moser *et al.*, 2019; Cook *et al.*, 2023)

Mountain lakes are mostly undeveloped but can range naturally in productivity, representing an important case study with which to examine environment-trait relationships of phytoplankton across lakes (Larson *et al.*, 1998; Kopáček *et al.*, 2000; Kamenik *et al.*, 2001; Sadro *et al.*, 2012). Phytoplankton in mountain lakes are primarily shaped by light, nutrients, and grazers (Larson *et al.*, 1998; Nydick *et al.*, 2011; Kuefner *et al.*, 2021; Cook *et al.*, 2023). While chrysophytes often dominate in oligotrophic mountain lakes, cyanobacteria and chlorophytes become more prevalent with increasing nutrient availability (Larson *et al.*, 1998; Nydick *et al.*, 2011). Across the elevation gradient from montane to alpine, most algal groups stay fairly consistent in relative abundances with the exception of cryptophytes, which decline with increasing elevation due to sensitivity to temperature and organic matter (Vinebrooke & Leavitt, 1999; Kuefner *et al.*, 2021). Yet phytoplankton communities in mountain lakes have not been examined in terms of functional trait variation. A trait-based approach may reveal novel patterns in the spatial distribution of phytoplankton in remote mountain lakes.

The lakes of the Cascade Mountains (USA) are model systems to examine phytoplankton functional traits as communities can range from sparse populations of small flagellates and chlorophytes to blooms of filamentous cyanobacteria (Chapter 2), but remain understudied at a community level. Therefore, I examined the spatial variation in phytoplankton functional traits in relation to bottom-up and top-down pressures: nutrients and zooplankton grazers (Bergquist *et al.*, 1985; Litchman & Klausmeier, 2008). As certain traits vary together due to their shared roles in nutrient acquisition or grazing resistance, I also identified potential trait syndromes, which are defined as a suite of co-occurring or co-varying traits (Mouillot *et al.*, 2013). I asked the following question: How do the traits of phytoplankton communities reflect gradients of nutrient availability and grazing pressure across mountain lakes? I posed the following hypotheses (Table 4.1): 1) The nutrient gradient from low to high will be reflected by a transition from nutrient scavengers to nutrient competitors and will be represented in select traits (e.g., maximum growth rate) and depend, in part, on the nutrient(s) (Healey & Hendzel, 1980; Smith, 1983; Grover, 1991; Granéli *et al.*, 1999). 2) A grazing pressure gradient from low to high will be represented by a transition from grazer-vulnerable taxa to grazer-resistant taxa, reflected in select traits such as cell size (Bergquist *et al.*, 1985; Sterner, 1989; Jakobsen *et al.*, 2006). 3) The interaction of grazing pressure and nutrient gradients will be reflected by dominant trait syndromes shifting from nutrient scavengers to competitors that also vary in resistance to grazing (Litchman *et al.*, 2007; Pomati *et al.*, 2020; Lürling, 2021).

4.2 Methods

4.2.1 Study area

In the southern portion of the Cascade Mountain Range (Oregon, USA) (Appendix A: Figure S1), lakes range in trophic status from ultra-oligotrophic to eutrophic, which is likely due to watershed-scale variation in geology and hydrology (Johnson, 1985). The region's volcanic geology can provide more phosphorus (P) to the lakes through weathering relative to other rock types (see Chapter 2). Crustacean zooplankton communities of the Oregon Cascade lakes vary not only in abundance, but also composition, with some communities dominated by calanoid copepods, while others are a mixture of cladocerans and cyclopoid copepods (US Environmental Protection Agency, 2009, 2016). The variation of the zooplankton communities is likely due in part to differing predation pressures, as most of lakes have been stocked with non-native fish since the early 1900s (see Appendix A: Table S3) (Oregon Department of Fish and Wildlife, 2011).

4.2.2 Field sampling

I selected 29 lakes from a binary regression tree model on historical data to obtain a representative sample that reflects regional variation in elevation, size, and productivity (see Chapter 2). Study lakes were sampled between June 24-July 13 and August 8-31, 2019. I measured physical and chemical parameters at the deepest point, including a complete depth profile of temperature and dissolved oxygen with a YSI ProODO meter (Yellow Springs, Ohio, USA). I sampled for phytoplankton with a Wildco Van Dorn sampler (Yulee, Florida, USA) at 1 m below the surface at the deepest point in the lake, identified using bathymetric maps and a depth sounder. If the lake was stratified, I also sampled for phytoplankton at 1 m above the thermocline at the deepest point. Samples were collected in 250-mL brown Nalgenes and preserved for identification using Lugol's iodine. I collected samples for nutrient analyses (total N and P) from the top 5 m of the water column, using a 5-m long, 2.54-cm diameter tygon tube and transferred them into 125-mL HDPE bottles, which were kept cool and preserved with H_2SO_4 until frozen in the laboratory. I conducted an integrated tow of the water column for zooplankton using a plankton net with 80-µm mesh and 25-cm diameter, starting two meters from the bottom and preserving the sample in ethanol to a final concentration of 70% for identification.

4.2.3 Lab analyses

I concentrated preserved phytoplankton samples before counting by gently mixing the sample for 5 minutes and then taking a 100-mL subsample for settling in a graduated cylinder. After 100 hours of settling, the top 98 mL were removed via a vacuum pump and reserved to dilute while the remaining 2 mL were used for counting. I counted and identified 300 natural units per concentrated sample in Palmer counting cells to the genus level or to the lowest taxonomic level possible, using taxonomic guides (Smith, 1950; Wehr, 2002; Matthews, 2016) with a Leica DM1000 microscope at 400X and ICC50 HD camera (Leica Microsystems Inc., Buffalo Grove, IL). I measured the cell and colony dimensions of 20 individuals of each taxa in each sample to calculate the average maximum linear dimension based on single cell measurements and biovolume based on the whole individual measurements using standardized equations based on the shapes of taxa (Hillebrand *et al.*, 1999). A total of 82 taxa were identified with an average of 17 found in each lake. Only taxa that occurred in at least 10% (i.e., 3 of 29) of the lakes and at least 0.1% relative abundance were included for statistical analyses; therefore only 61 taxa were included. For zooplankton, I counted and identified at least 250 individuals from each preserved sample to the order level for Copepoda and to the family level for Cladocera using taxonomic guides (Balcer *et al.*, 1984; Thorp & Covich, 2014) with a Leica M165C microscope at 100X and IC80HD camera (Leica Microsystems Inc., Buffalo Grove, IL).

I used a persulfate solution to digest total P samples heated to 100°C and then analyzed with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the molybdenum blue colorimetric method (detection limit: 0.002 mg/L; precision limit: ± 0.004 mg/L) (Patton & Kryskalla, 2003; APHA, 2018c). I also used a persulfate solution to digest total N samples heated to 100°C and then analyzed with a SmartChem 200 discrete analyzer (Guidonia, Italy) for colorimetric determination of nitrate and nitrite (detection limit: 0.01 mg/L; precision limit: ± 0.01 mg/L) (APHA, 2018b).

4.2.4 Trait compilation & assignment

I selected functional traits that would likely be responsive to nutrients and grazers based upon previous literature (Table 4.2; Litchman & Klausmeier, 2008; Cottingham *et al.*, 2015; Klais *et al.*, 2017; Wentzky *et al.*, 2020). Maximum linear dimension (MLD) represented the cell size trait and was based on previously mentioned biovolume measurements. The other morphological trait (colony/filament), the behavioral traits (motility, mixotrophy, buoyancy), and certain physiological traits (diazotrophy, silica demand, mixotrophy) were assigned values based on available trait compilations, as well as additional literature review (Reynolds, 1998; Wehr, 2002; Weithoff, 2003; Bruggeman, 2011; Cottingham *et al.*, 2015; Klais *et al.*, 2017; Kruk *et al.*, 2017; Laplace-

Treyture *et al.*, 2021). The taxon-specific values for these traits were possible to compile as many are represented simply as a binary 0 (absence) or 1 (presence) (Table 4.2).

Some other physiological and behavioral traits were not available for all taxa as they are quantitative traits that must be measured in cultures in laboratories. Therefore, I used a method of trait inference based on phylogenetic relationships and morphology-based power-law relationships for the following traits: maximum growth rate, P affinity, light affinity, the optimum irradiance before the onset of inhibition, and edibility for *Daphnia* (Bruggeman, Heringa & Brandt, 2009; Bruggeman, 2011; Wentzky *et al.*, 2020). This trait inference method is a statistically consistent method to estimate trait values with cross-validation checks for bias and accuracy (Bruggeman *et al.*, 2009). I developed qualitative phytoplankton phylogenetic trees based on current taxonomic classifications from National Center for Biotechnology Information (NCBI), using phyloT tree generator software (Letunic, 2015). I did not assume a common ancestor for the phytoplankton so a separate rooted tree was generated for each phylum (Letunic, 2015). As these trees were based on NCBI taxonomic structure, they did not have branch lengths or clade support values (Letunic, 2015). Trees were not generated for cryptophytes, euglenophytes, tribophytes, and charophytes as the present taxa either had trait values in the literature (cryptophytes and charophytes) or had too few taxa and trait values for inference (tribophytes and euglenophytes). The light traits (affinity, optimum irradiance) were not found nor could be inferred for 26% of the taxa, which included most taxa that were either a dinoflagellate, euglenophyte, and tribophyte. As these taxa make up some of the most common mixotrophs and non-cyanobacterial filamentous taxa in my study lakes, light traits were not considered for statistical analyses. Ultimately, 13.1% of the taxa had values inferred. The functional traits for each taxa are available in Appendix C: Table S1, S2.

4.2.5 Statistical analysis

First, I analyzed the early and late summer community samples to examine seasonal variation with sampling bout (early vs. late) as an environmental variable. I tested for significant differences in trait composition between the early and late summer based on a community-weighted average for each trait for each lake and bout (site × trait). As the trait values were not normally distributed, I used the Wilcoxon signed rank test, which is a non-parametric version of a paired t-test.

Co-variation between traits as well as between environment variables were examined through correlation matrices, using Spearman's rank correlation coefficient (ρ). Collinearities between the variables within the trait matrix, as well as the environment matrix, were examined through the variance inflation factors (VIF). Based on VIF, environmental variables were not collinear (VIF <10). Buoyancy and motility were not included for trait-based analyses due to the high correlations with diazotrophy and mixotrophy, respectively (Spearman's ρ >0.85). Based on VIF, once buoyancy and motility were removed, the remaining traits did not have high collinearity (VIF <10).

The taxon × trait and site × environment matrices were also analyzed separately to understand the spatial distribution of environmental conditions as well as the functional trait space of the taxa. For the taxon × trait matrix, I examined the variation by first converting the data into a dissimilarity matrix based on Gower's distance, which can handle mixed data types (i.e., categorical, numerical), and then conducted a principal coordinates analysis (PCoA) on the dissimilarity matrix, using the R packages *cluster* v.

2.1.4 and *vegan* v. 2.4-6 (Oksanen *et al.*, 2009; Maechler *et al.*, 2022). The site × environment matrix included the following variables for each lake: sampling bout, total P and N, specific conductance, epilimnetic mean temperature, mean depth, maximum depth, elevation, surface area, and total abundance of zooplankton grazers. Grazing zooplankton were defined as those taxa known to consume only phytoplankton and heterotrophic bacteria instead of other zooplankton (Appendix C: Table S3; Thorp & Covich, 2014). Epilimnetic pH was not included as it is highly variable throughout the day, especially in poorly buffered lakes such as in the Cascades, and time of day was not controlled for in sampling (Wetzel, 2001; Reynolds, 2006). I examined the environmental variation via a principal component analysis (PCA) after natural log-transforming any skewed variables and then centering and scaling the data to correct for differences in parameter units, using the R package *stats* v. 4.3.0 (R Development Core Team, 2021)

As traits and environmental variables did not notably vary between the two sampling bouts, I averaged the species abundance and environmental data across both dates for further analyses (see Results). I directly tested the relationships of traits and the environment that define the phytoplankton communities using a form of direct gradient analysis to integrate traits with species and environment matrices. R linked to Q-mode (RLQ) analysis was used to test the covariance between the environment conditions and trait values mediated by taxon abundance, using the R package *ade4* v. 1.4-3 (Dolédec *et al.*, 1996; Dray & Dufour, 2007). RLQ analysis is a three-table ordination method using correspondence analysis (CA) to quantify site and taxa weights for the PCA of the centered and scaled R table (site × environment) and the Hill-Smith ordination of the Q table (taxon × trait), using the relative biovolume of each taxon in each lake and time point (L table; site × taxon). RLQ maximizes the covariance between the linear combinations of the R and Q tables through co-inertia analysis (Dolédec *et al.*, 1996). I assessed the global significance of the trait-environment relationships using a multivariate permutation test (n=4,999 permutations) based upon the total inertia of the RLQ analysis (Dray & Dufour, 2007; Dray *et al.*, 2014). The global significance test evaluates two null models: 1) the environmental conditions do not influence the distribution of species with fixed traits; and 2) traits do not influence the composition of species assemblages in sites with a given set of environmental conditions.

I used fourth corner analysis to test for significant individual environment-trait relationships (Legendre, Galzin & Harmelin-Vivien, 1997; Dray *et al.*, 2014). The fourth corner analysis quantifies and tests for the multiple associations, one trait and one environmental variable at a time (Legendre *et al.*, 1997). The permutations of the environment and traits were used to calculate the distributions of fourth corner statistics based on their null hypotheses and determine the significance of the observed values (n=4,999 permutations). I adjusted the p-values for multiple comparisons with the Benjamini-Hochberg, or false discovery rate, correction (Benjamini & Hochberg, 1995; Dray *et al.*, 2014).

To determine potential trait syndromes, I conducted hierarchical clustering based on distances between species along the first two axes of the RLQ model, using the R package *stats*. I used the agglomerative method of Ward's minimum variance. I selected the optimal clusters based on the Calinski-Harabasz stopping criterion (Kleyer *et al.*, 2012). All statistical analyses were conducted in R 4.1.1 (R Development Core Team, 2021).

4.3 Results

4.3.1 Environmental variation in time and space

The study lakes varied substantially in size, water chemistry, and zooplankton abundance, but with limited shifts between the early and late summer. Maximum depth of the study lakes ranged from 4.9 m to 86.0 m with a median of 19.2 m, while lake surface area ranged from 17 to 1,550 ha with a median of 102 ha. The elevation of the lakes ranged from 958 to 1,980 m with a median of 1,460 m. Most lakes were relatively low in TP (<0.02 mg L^{-1}) and TN (<0.20 mg L^{-1}), with only two lakes rich in either TP (≥ 0.10 mg L^{-1}) or TN (≥ 0.40 mg L^{-1}). While TP was lower in the late summer, TN did not vary notably across the summer (Figure 4.1a, b). Epilimnetic temperature was higher on average in the late summer than early summer (Figure 4.1c). Hypolimnetic dissolved oxygen (DO) was fairly similar across the summer, but more lakes in the late summer had an anoxic hypolimnion (Figure 4.1d). Zooplankton abundance ranged from 455 to 86,241 individuals m^{-3} and did not vary greatly across the summer (Figure 4.1f).

The major environmental gradients were reflected in the two main PCA axes (Figure 4.2, Appendix C: Figure S3). By comparing the eigenvalues of the PCA with a broken stick model that tests which components are significant enough for interpretation, only the first two components were significant (Appendix C: Figure S4). The first PCA axis explained 26.7% of the variance in the environmental variables, while the second PCA axis explained 23.4%. Zooplankton abundance and total P were in the opposing direction of maximum depth, surface area and elevation along the first PCA axis (Figure 4.2). Hypolimnetic DO was in the opposing direction of total N and specific conductance along the second PCA axis (Figure 4.2). Additional correlation analyses supported this trend: hypolimnetic DO was negatively correlated with total N (ρ =-0.538, p=0.003) and specific conductance (ρ =-0.598, p=0.001). The variables N and P were orthogonal to each other, suggesting these nutrients are partially influenced by different factors.

4.3.2 Trait variation in time and algal groups

The average trait values and frequencies by lake only varied slightly between early and late summer, with no significant differences based on Wilcoxon signed rank tests (Figure 4.3, Appendix C: Table S4). The proportion of taxa that required silica moderately but non-significant declined from early to late summer. Average phosphate affinity had a moderate but non-significant increase from early to late summer.

The variation in phytoplankton of my study lakes was examined by separating taxa according to their functional traits. As shown in the PCoA and the correlation matrix, multiple traits co-varied (Figure 4.4, 4.5). The frequency of buoyancy and diazotrophy in the trait by species matrix were perfectly correlated (Spearman's ρ=1.00, p<0.001), as the only buoyancy-regulating taxa were diazotrophs. The frequency of motility and mixotrophy were nearly perfectly correlated (ρ=0.99, p<0.001), with only one motile taxa, *Chlamydomonas,* that was not mixotrophic (Figure 4.5). Toxin production was highly correlated with buoyancy and diazotrophy, with the most common toxin producers being diazotrophic cyanobacteria (ρ=0.68, p<0.001). Maximum growth rate was positively correlated with silica demand (ρ=0.55, p<0.001) and edibility (ρ=0.56, p<0.001).

The functional trait space of the phytoplankton was defined by a range of strategies from quick growing silica users to motile mixotrophs (Figure 4.4). The 61 taxa were from the following groups: charophytes (desmids), chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, euglenophytes, and tribophytes (xanthophytes). Chlorophytes were the most taxa rich group (n=27), followed by diatoms (n=20). The first and second axes of the PCoA explained 54.0% and 35.6%, respectively, of the dissimilarity variation in the taxon × trait matrix. The traits buoyancy and diazotrophy were highly related as they only occurred in certain cyanobacteria genera, which occupied the lower left corner of PcoA (Figure 4.4). In the lower right corner, phosphate affinity along with mixotrophy and motility were positively correlated. Maximum growth rate and silica demand was located opposite of the motile mixotrophs in the upper right corner, suggesting the mixotrophs are relatively slow growing. Edibility to *Daphnia* was located directly opposite in the upper left corner of the buoyant diazotrophs, which suggests these cyanobacteria have relatively low edibility. Surprisingly, maximum linear dimension (MLD) was in a similar location to edibility. Most algal groups were well separated in the trait space. Dinoflagellates, cryptophytes, and euglenophytes were constrained in the area around motility and mixotrophy, while diatoms were confined in a slightly broader area near maximum growth rate and silica demand. Chlorophytes occupied the broadest area in the trait space, reflecting a range of taxa from colonies to motile single cells.

4.3.3 RLQ and fourth corner analyses

The relationships between phytoplankton traits and environmental variables were primarily summarized by the first two RLQ axes with 65.2% of the cross-covariance of the traits and environment explained by the first axis and 25.0% explained by the second axis (Table 4.3, Figure 4.6). The permutation test of global significance for the RLQ analysis indicated a significant relationship between traits and environmental variables (p=0.03). The strong negative association of hypolimnetic DO with the traits of diazotrophy and (potential) toxin production primarily represented the first axis. Total P was also positively associated with diazotrophy and toxin production along the first axis. The secondary axis was represented by multiple associations that may indicate alternative strategies in phytoplankton. The positive associations of zooplankton abundance with the traits of edibility and maximum growth rate represented the upper end of the second axis. The opposite end of the second axis was mainly represented by the positive associations of maximum depth and epilimnetic temperature with the traits of mixotrophy and maximum linear dimension. Total P also had a negative association with mixotrophy, but not total N. Total N also had a positive association with MLD. The traits of phosphate affinity and silica demand as well as the environmental variables of specific conductance and elevation had no strong associations.

Based on the fourth corner analysis, there was a single significant bivariate relationship between traits and environmental variables after correcting for multiple comparisons (Table 4.5). Hypolimnetic DO and diazotrophy were significantly negatively associated (p=0.04), reflecting the first RLQ axis (Figure 4.6). While other associations noted in the RLQ, such as edibility and zooplankton abundance, were significant before correcting for multiple comparisons, they were not significant following the correction (Table 4.5, Figure 4.6).

4.3.4 Trait syndromes by hierarchical clustering

Phytoplankton taxa clustered into five groups or trait syndromes associated with certain environmental variables based on hierarchical clustering (Figure 4.7, Appendix C: Figure S1). There was one cluster (E) made up of a single genus *Gloeotrichia,* which is a toxigenic and colonial diazotroph that was only found in a few nutrient-rich lakes with anoxic hypolimnia. The limited distribution of *Gloeotrichia* likely resulted in it being clustered separately. The other cluster (B) besides *Gloeotrichia* on the right side of the first axis was a cluster defined mostly by coloniality, the potential for toxin production and yet also high edibility for *Daphnia* (Figure 4.7b). This toxigenic colonial cluster was associated with total P and shallow maximum depth (Figure 4.7a). Another cluster (C) associated with abundant zooplankton but on the left side of the first RLQ axis, was defined by high edibility, fast maximum growth rate, and small cell size (low MLD) as well as somewhat associated with low total N (Figure 4.7b). On the other hand, cluster A was defined by relatively moderate maximum growth rate and medium MLD as well as mostly unicellular taxa (Figure 4.7b). Finally, the fifth cluster (D) was most associated with greater maximum depth as well as low zooplankton abundance and total P (Figure 4.7a). This cluster was primarily defined by mixotrophy and low edibility as well as silica demand (Figure 4.7b). The cluster of large mixotrophs and diatoms (Appendix C: Figure S2) was positioned directly opposite of the toxigenic colonial cluster, reflecting the productivity gradient in terms of nutrients and grazers, as well as the range in lake depths.

4.4 Discussion

4.4.1 Summary of key findings

Phytoplankton in Southern Cascades lakes ranged from slow growing, large scavengers to fast growing, small competitors to colonies capable of producing toxins. Scavenging mixotrophs were notably and negatively associated with P, but less so with N (Figure 4.6). In contrast to expected relationships, fast-growing nutrient competitors, which are relatively edible taxa, were primarily positively associated with grazing pressure and not nutrients, suggesting rapid growth can somewhat overcome grazing losses (Figure 4.6, 4.7). Interactions in nutrient acquisition and grazing resistance strategies were reflected in trait syndromes with small single cells under high grazing and low P availability versus toxigenic colonies under high grazing and P availability (Figure 4.6, 4.7). The spatial variation in phytoplankton traits demonstrated the independent and interacting pressures of nutrients and grazers in mountain lakes.

4.4.2 Regulation by nutrients

The transition in phytoplankton communities from mixotrophs to colonies capable of toxin production primarily reflects the P gradient of these mountain lakes (Figure 4.7), ranging from oligotrophic to eutrophic. In lakes low in P, mixotrophic taxa that were also motile, such as dinoflagellates and chrysophytes, were abundant and likely acted as effective nutrient scavengers (Reynolds, 1984; Granéli *et al.*, 1999). While phosphate affinity was not strongly associated with the P gradient, some of these mixotrophic taxa have high P uptake affinity (Bruggeman, 2011). At the upper end of P, colonial cyanobacteria were more dominant, including diazotrophic and toxigenic taxa. Coloniality reduces phytoplankton nutrient uptake because it increases the organism's dimensions, which in turn increases the thickness of the diffusion boundary layer (Ploug, Stolte & Jørgensen, 1999). Therefore, the relative abundance of colonies depends in part upon the availability of nutrients like P. In addition, the prevalence of potential toxin production and N fixation, which are metabolically expensive processes, requires and therefore reflects nutrients such as P at levels above or optimal for phytoplankton growth (Smith, 1983; Sivonen, 1990; Sañudo-Wilhelmy *et al.*, 2001; Wagner *et al.*, 2019). While unicellular mixotrophs, colonial diatoms and chlorophytes, and toxigenic cyanobacteria did overlap somewhat in their distribution in the Southern Cascades lakes, their dominance likely depends upon the P concentration in each lake.

N availability in the mountain lakes was also represented somewhat by increasing coloniality but more so increasing cell size (Figure 4.6, 4.7). Colonial diatoms and cyanobacteria were among the most abundant phytoplankton in relatively N-rich lakes. Colonial diatoms like *Fragilaria* are known to be sensitive to N additions, especially in low productivity mountain lakes (Saros *et al.*, 2005; Williams *et al.*, 2016). Many of these colonial diatoms also were relatively long compared to other taxa, driving the positive relationship between N and cell size (Figure 4.7). As the cells of phytoplankton become more elongated, their surface area:volume ratio declines, reducing the efficiency of nutrient uptake, which becomes less crucial as nutrient levels rise (Naselli-Flores, Padisák & Albay, 2007). In addition, large mixotrophs (e.g., chrysophytes), while effective P-scavengers, can be limited by N availability and therefore favored in lakes with higher N:P (Jansson *et al.*, 1996; Deininger, Faithfull & Bergström, 2017). Mixotrophs can be limited by N more so than P because the bacteria they consume often have a lower N:P (Jansson *et al.*, 1996). In addition, mixotrophic phytoplankton have a higher P incorporation efficiency than for N (Jansson *et al.*, 1996). In lakes low in N, small and oblate taxa, mostly chlorophytes, were more dominant. Unexpectedly, the most abundant cyanobacteria (i.e., *Gloeotrichia*) in the N-rich lakes were diazotrophic, suggesting these taxa may contribute notable inputs of N to these lakes (Cottingham *et al.*, 2015). Yet further research involving controlled experiments is needed to elucidate the mechanisms underlying this relationship. Additions of N have been shown to result in increases in some cyanobacteria, such as *Microcystis*, in mountain lakes (Nydick *et al.*, 2011; Williams *et al.*, 2016). While P may be more influential for phytoplankton nutrient acquisition strategies, N may be more influential in phytoplankton size and form in mountain lakes.

Hypolimnetic dissolved oxygen and lake depth may have partially influenced phytoplankton functions through their indirect roles in internal nutrient cycling (Jäger, Diehl & Schmidt, 2008; Carey *et al.*, 2012; Cottingham *et al.*, 2015). The occurrence of anoxia in the hypolimnion allows for the release of sediment-bound P and ferrous iron, which can be physically mixed upwards or taken up by buoyancy-regulating diazotrophs, as well as motile mixotrophs (Nürnberg, 1984; Reynolds, 2006; Molot *et al.*, 2014; Cottingham *et al.*, 2015). Through leakage and mortality, these migrating taxa, especially blooms of diazotrophic cyanobacteria, can increase the availability of P back to the photic zone (Cottingham *et al.*, 2015). In turn, the decomposition of phytoplankton contributes to the depletion of hypolimnetic dissolved oxygen, creating a positive feedback loop (Cottingham *et al.*, 2015). Further research with higher frequency sampling is needed to determine the relative contribution and response of migrating phytoplankton to hypolimnetic anoxia. The potential for phytoplankton to access hypolimnetic nutrients depends partially upon lake depth, which influences the volume accessible to migrating phytoplankton (Nürnberg, 1984; Cottingham *et al.*, 2015). For example, cyanobacteria migration may be limited beyond 15 m, so the hypolimnion was likely inaccessible in the deeper study lakes (mean depth>16 m). In addition, depth regulates the mixing regime of the lake and therefore the movement of sediment-bound nutrients (Nürnberg, 1984; Kraemer *et al.*, 2015). The role of nutrients in the functional structure of phytoplankton communities should be considered relative to lake morphometry and vulnerability to hypolimnetic anoxia.

4.4.3 Regulation by zooplankton grazing

The abundance of grazing zooplankton varied by over two orders of magnitude across the lakes (Figure 4.2). This gradient of grazers may have reflected in shifts from slow growing, large phytoplankton taxa where grazers were less abundant to fast growing, small taxa with increasing edibility where grazers were abundant (Figure 4.6, 4.7). This finding diverges in part from hypothesized relationships (Sterner, 1989; Jakobsen *et al.*, 2006). The unexpected dominance of vulnerable small chlorophytes and cryptophytes observed with high grazer abundances has been noted in other lakes during summer (Agrawal, 1998; Wentzky *et al.*, 2020). While these taxa are highly edible to *Daphnia*, which were fairly common in several lakes (see Chapter 2), they may not be readily consumed by other zooplankton like copepods (Agrawal, 1998; Sommer *et al.*, 2001). Copepods often prefer larger phytoplankton than *Daphnia*: size-selective predation by copepods may allow small phytoplankton to thrive even when *Daphnia* are relatively common (Agrawal, 1998; Wentzky *et al.*, 2020). Another way these small taxa are able to cope under high grazing pressure is through rapid growth rates, allowing them to compensate for grazing loss (Reynolds, 1984; Wentzky *et al.*, 2020). On the other hand, larger taxa, such as dinoflagellates and desmids, grow slowly and are potentially vulnerable to large copepods (Sommer *et al.*, 2001; Lürling, 2021). An alternative explanation is the abundance of vulnerable phytoplankton reflects a positive bottom-up effect on grazing zooplankton as sampling was restricted to a single season. Yet as these relationships were based on abundances averaged across early and late summer, the composition of phytoplankton at least partially reflected the top-down effect of grazing zooplankton.

4.4.4 The interacting roles of nutrients and grazing pressure

The spatial variation across lakes in phytoplankton trait syndromes were primarily driven by nutrient availability and secondarily by zooplankton grazer abundance. For example, in lakes with low P yet abundant grazers, relatively small unicellular taxa with relatively high growth rates were prevalent (Figure 4.6, 4.7). In contrast, lakes with relatively high levels of P, as well as abundant zooplankton grazers, were dominated by toxigenic colonies (Figure 4.6, 4.7). The shift in grazing resistance strategies likely was driven by nutrients, as toxin production requires additional nutrients and coloniality lowers rates of nutrient uptake (Ploug *et al.*, 1999; Davis *et al.*, 2010; Wagner *et al.*, 2019). While small taxa are vulnerable to large generalist grazers like *Daphnia*, they are also less readily grazed by selective feeders like many copepods, which were fairly common in many of the study lakes (see Chapter 2) (Bergquist *et al.*, 1985; Sommer *et al.*, 2001; Lürling, 2021). Additionally, many of these smaller taxa can compensate for grazing losses with higher growth rates than larger taxa (Reynolds, 2006; Lürling, 2021). While fast growth rates are typically associated with increasing nutrient levels, relatively fast growing taxa can also be selected by grazing through apparent competition as well as highly fluctuating conditions, providing pulses of resources (Droop, 1973; Grover, 1991; Holt, Grover & Tilman, 1994; Ehrlich, Kath & Gaedke, 2020). When P and grazer abundance was low, mixotrophic dinoflagellate and chrysophytes were common (Figure 4.7, Appendix C: Figure S2), which may be due in part to their low nutritional quality relative to other phytoplankton for zooplankton (Vad *et al.*, 2020). Therefore, the trait syndromes of summer phytoplankton communities in mountain lakes can reflect the interacting pressures of nutrients and grazers.

4.4.5 Limitations

While this study provides evidence of nutrient and grazers shaping phytoplankton community traits in mountain lake, there were limitations. Sampling was restricted to summer, precluding characterization of the full range of seasonal patterns in the phytoplankton communities. Sampling across the seasons can provide more robust evidence of the abiotic and biotic drivers of phytoplankton assemblages (Wentzky *et al.*, 2020; Loewen *et al.*, 2021). In addition, the study lakes spanned a limited elevation gradient (~1000m) so further study is needed across the montane to alpine ecozones to determine the role of elevation in shaping the functional structure of phytoplankton. Previous studies in the Northern Cascades and Canadian Rockies did not find substantial shifts in phytoplankton with elevation, but functional traits were not examined (Larson *et al.*, 1998; Vinebrooke & Leavitt, 1999). This study also was limited to genus-level identification or for a few instances at the family or phylum level: phytoplankton traits can vary substantially at the species-level and even within a single species; therefore, additional studies at a finer taxonomic resolution would provide further insights

(Weithoff & Beisner, 2019b). In addition, local environmental conditions can influence the expression of traits so the static trait values derived or inferred from other sources (Appendix C: Table S1) may not be representative of traits in the Southern Cascades lakes (Weithoff & Beisner, 2019b). Future research should directly measure trait expression of each species from the samples when possible. Regardless of these factors, prior studies relying on traits compiled from existing literature have provided consistent evidence for the roles of environmental factors in structuring phytoplankton communities functionally, which have implications for ecosystem processes (Wentzky *et al.*, 2020; Loewen *et al.*, 2021).

4.5 Conclusions

This study offered new insights on how phytoplankton communities in mountain lakes are structured functionally in relation to top-down and bottom-up forces. The distinct groupings or syndromes of traits based on grazing resistance and nutrient acquisition strategies were often polyphyletic, suggesting studies based on taxonomy alone may overlook key relationships. Temporal shifts in mountain lake phytoplankton from small fast-growing chlorophytes and cryptophytes towards toxigenic colonial cyanobacteria, including diazotrophs, may be indicative of increasing P availability. Numerous mountain lakes may be experiencing increased P inputs from atmospheric deposition and greater soil activity with climate warming (Stoddard *et al.*, 2016; Scholz & Brahney, 2022). Rising P loads can potentially increase and expand toxigenic cyanobacteria dominance in these remote lakes (Scholz & Brahney, 2022). Shifts in dominant phytoplankton groups may further drive nutrient availability such as nitrogen fixation by diazotrophs (Cottingham *et al.*, 2015; Litchman *et al.*, 2015). Trait-based studies of phytoplankton can provide evidence of ecosystem shifts that are generalizable beyond mountain lakes (Weithoff & Beisner, 2019b). While some phytoplankton species are rather unique to mountain lakes, species with similar traits are found in low elevation lakes (Reynolds, 2006). Future work should disentangle the response and effect of freshwater phytoplankton in terms of functional traits in these remote lakes where anthropogenic alterations remain limited. While external inputs of nutrients are likely increasing in even mountain lakes (Baron *et al.*, 2011; Scholz & Brahney, 2022), managers must also account for phytoplankton that can increase the internal cycling of nutrients, accelerating and maintaining lake eutrophication (Cottingham *et al.*, 2015).

4.6 Tables and Figures

Table 4.1 Hypothesized individual relationships of key environmental variables and phytoplankton functional traits for Hypotheses 1-3.

Hypothesis	**Environmental variable(s)**	**Trait**	**Expected relationship**	**Reference**
1) Nutrient gradient reflected in a transition from nutrient scavengers to nutrient competitors.	Total N and P	Maximum growth rate	Positive asymptotic	(Droop, 1973; Grover, 1991)
		PO_4 affinity	Negative with total P	(Healey & Hendzel, 1980)
		Diazotrophy	Negative with total N; positive with total P	(Smith, 1983; Litchman *et al.*, 2015)
		Mixotrophy	Negative	(Granéli *et al.*, 1999)
		Cell size & colony/filament	Positive	(Ploug *et al.*, 1999; Peter & Sommer, 2015)
		Toxin production	Positive	(Sivonen, 1990; Wagner *et al.*, 2019)
2) Grazing pressure from low to high is represented by a transition from grazer vulnerable to grazer resistant.	Total grazer density	Maximum growth rate	Positive	(Lürling, 2021)
		Cell size & colony/filament	Positive	(Bergquist *et al.*, 1985; Sterner, 1989)
		Toxin production	Positive	(DeMott & Moxter, 1991; Ger *et al.*, 2016a)
		Edibility	Negative	(Litchman & Klausmeier, 2008)
		Syndrome		**Examples**
3) The interaction of grazing and nutrients will be represented by shifts from nutrient scavengers to competitors that vary in their edibility.	Low total N and P /low grazers	Edible scavengers		Small, slow growing mixotrophs
	Low total N and P / high grazers	Inedible scavengers		Large armored mixotrophs
	High total N and P/ low grazers	Edible competitors	Small fast growers	Large colonies with relatively fast growth
	High total N and P/ high grazers	Inedible competitors		

Table 4.2 Phytoplankton functional traits used in this study by trait type based on Litchman and Klausmeier (2008). See text for further definitions.

Trait type	**Trait**	**Units/Categories**	**Definition or Description**
Morphological	Maximum linear dimension	µm	Longest single dimension of the cell
	Colony/filament	Presence or absence	Potential to form colonies or filaments
Physiological and behavioral	PO_4 affinity	μmol^{-1} day^{-1}	Ratio of maximum growth rate to half saturation coefficient
	Light affinity	μmol $quanta^{-1}$ m^2 s day^{-1}	Initial slope of the growth-irradiance curve
	Maximum growth rate	day^{-1}	Fastest growth rate in relation to nutrient uptake
	Silica demand	Presence or absence	Need for silica for cell wall material
	Diazotrophy	Presence or absence	Potential to fix atmospheric N
	Mixotrophy	Presence or absence	Ability to perform photosynthesis and phagotrophy
	Motility	Presence or absence	Possession of a flagella
	Buoyancy	Presence or absence	Possession of gas vacuoles
	Edibility for *Daphnia*	Numerical scale	The rate of prey consumption relative to the rate at which the preferred prey is consumed
	Toxin production	Presence or absence	Potential to produce toxins or irritants

Table 4.3 Summary of the RLQ analysis to assess the alteration of the structure of each matrix (R-environment, L-taxon, Q-trait) to maximize the covariance between the environment and traits. The eigenvalues were presented along with the total variance (%) in parentheses. The cumulative inertia or variance of the environmental scores and trait scores in the RLQ analysis for each axis are presented. The correlation between the taxon and site scores for each axis are presented. The percent preserved from the original ordination is in parentheses.

Axis	Eigenvalue	Covariance	Cumulative inertia of R	Correlation L	Cumulative inertia of Q
1	0.9 (65.2%)	0.97	1.9 (81.8%)	0.5 (55.1%)	2.0 (69.9%)
2	0.4 (25.0%)	0.06	3.7 (83.0%)	0.3 (43.0%)	4.1 (76.4%)

Table 4.4 The unadjusted p-values for the fourth corner analysis of the environment (enviro)-trait relationships based on the R, L and Q matrices. The Benjamini-Hochberg correction was used to adjusted the p-values for multiple comparisons. The environment-trait relationships that were significant without adjustment are colored with positive relationships in blue and negative in red. The environment-trait relationship that was significant with adjustment had a colored background and in bold. The significance level was set at α =0.05. The environment variables were lake surface area (SA), maximum depth (max depth), total nitrogen (TN), total phosphorus (TP), specific conductance (Cond.), zooplankton grazers (Zoop.), Epilimnetic temperature (Temp.), elevation, and hypolimnetic dissolved oxygen (Hypo. DO). The traits were maximum growth rate (max growth rate), maximum linear dimension (MLD), edibility, phosphate affinity (P affinity), coloniality/filamentous (Colony/fila.), silica demand, diazotrophy, mixotrophy and potential for toxin production (Toxin production).

Enviro.→ Trait ↓	**SA**	**Max depth**	**TN**	**TP**	**Cond.**	**Zoop.**	**Temp.**	**Elevation**	**Hypo DO**
Max growth rate	0.91	0.89	0.10	0.75	0.97	0.17	0.13	0.67	0.31
MLD	0.59	0.72	**0.04**	0.51	0.18	0.35	0.32	0.67	0.29
Edibility	0.21	0.16	0.09	0.26	0.65	**0.03**	**0.02**	0.88	0.90
P affinity	0.43	0.50	0.27	0.82	0.52	0.40	0.40	0.34	0.76
Colony/Fila.	0.55	0.80	0.47	0.35	0.08	0.95	0.16	0.84	0.26
Silica	0.42	0.35	0.99	0.79	0.17	0.90	0.87	0.83	0.74
Diazotrophy	0.51	0.08	**0.03**	**0.03**	0.06	0.46	0.90	0.83	**<0.001**
Mixotrophy	0.55	0.26	0.63	0.11	0.64	0.25	0.69	0.81	0.30
Toxin prod.	0.38	0.12	0.09	**0.04**	0.43	0.60	0.62	0.62	**0.02**

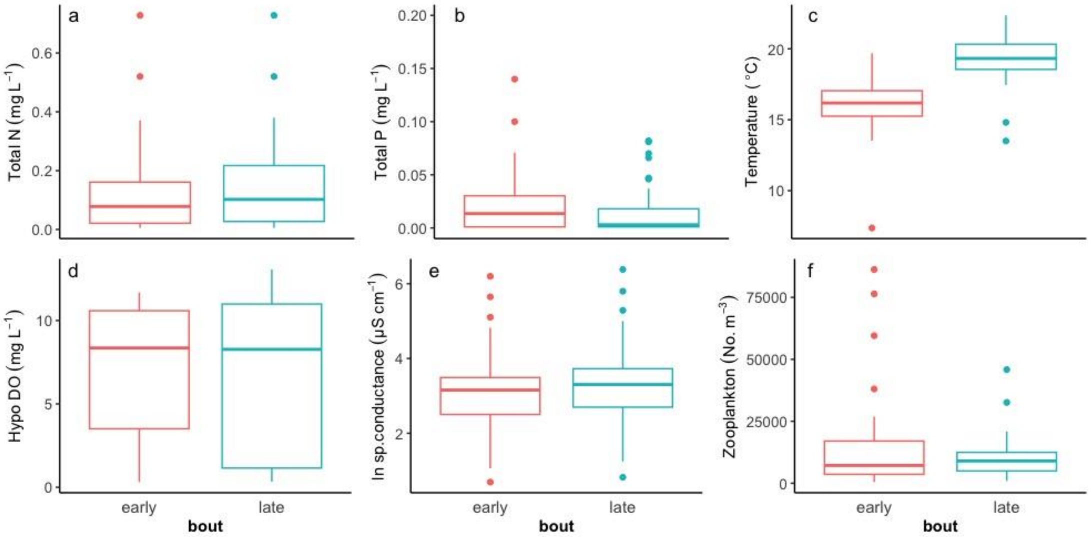

Figure 4.1 Boxplots of the environmental variables for the early (red) and late blue) summer sampling bouts. Variables included: (a) total nitrogen (N) and (b) phosphorus (P) (mg L-1); (c) mean epilimnetic temperature (°C); (d) hypolimnetic dissolved oxygen (hypo DO; mg L-1); (e) ln-transformed specific conductance (ln sp.conductance; μS cm-1); (f) total zooplankton abundance (no. m-3).

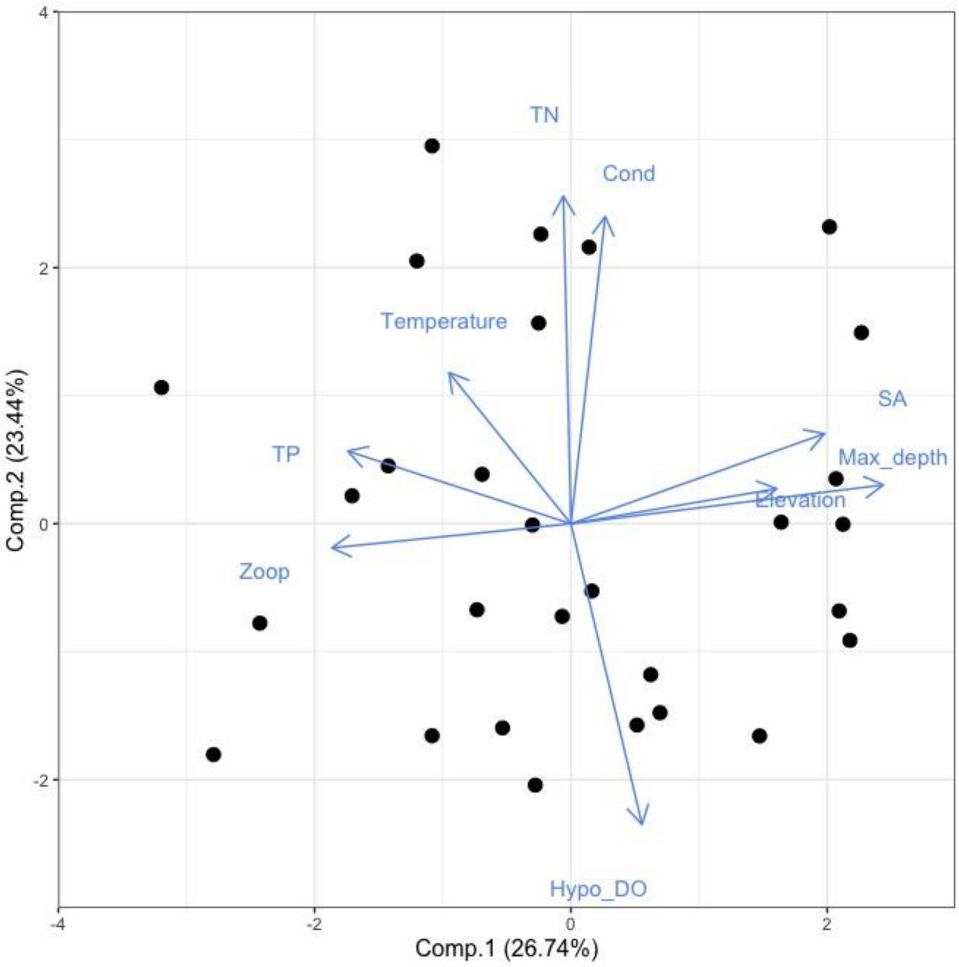

Figure 4.2 Biplot of the first two axes of the principal component analysis (PCA) of the environmental variables of each lake averaged across the two sampling bouts. The environmental variable loadings are plotted as vectors in blue: zooplankton abundance (Zoop), total phosphorus (TP), temperature, total nitrogen (TN), specific conductance (Cond), surface area (SA), maximum depth (Max depth), elevation, and hypolimnetic dissolved oxygen (Hypo DO). The PCA lake site scores are plotted as black points.

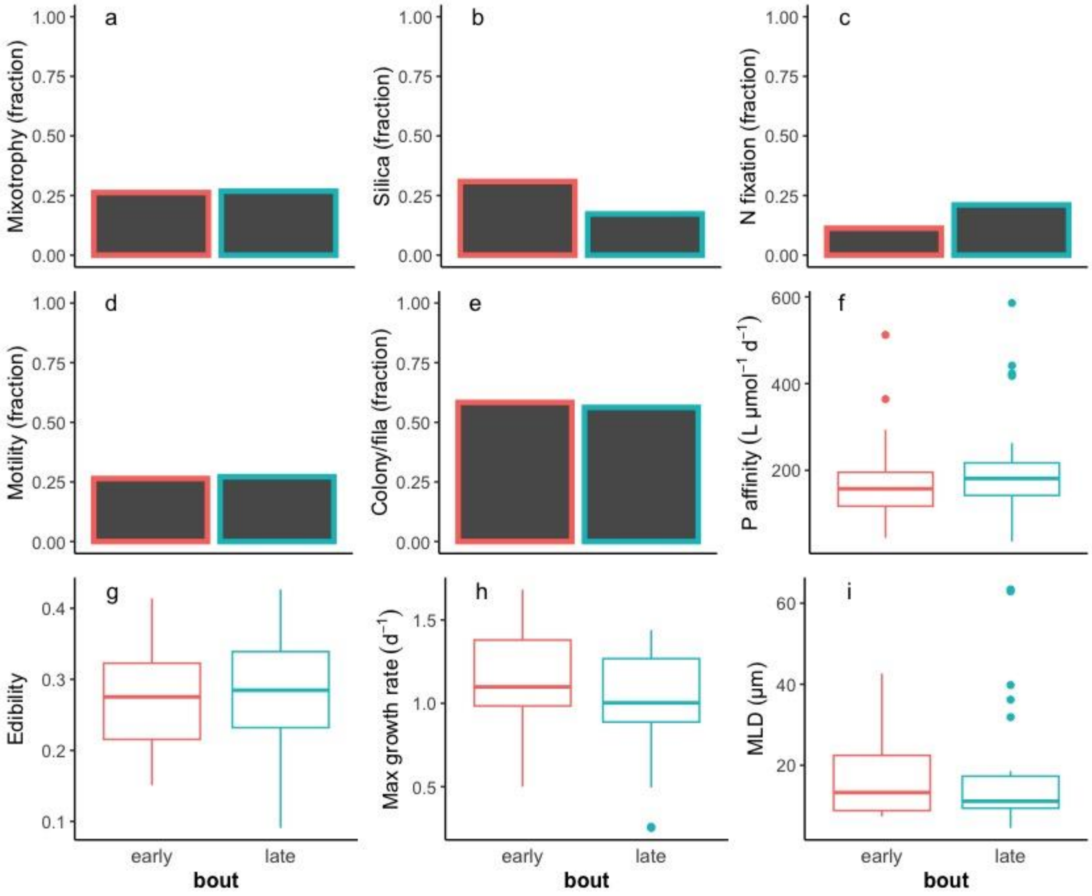

Figure 4.3 Bar graphs of the categorical traits and boxplots of the quantitative traits for early (red) and late (blue) summer sampling bouts. The categorical traits are in proportion of the total community (a-e). The numerical traits are (f) scaled phosphate affinity (L µmol^{-1} d^{-1}), (g) edibility to *Daphnia* (unitless), (h) maximum growth rate (d^{-1}), (i) maximum linear dimension (MLD in µm).

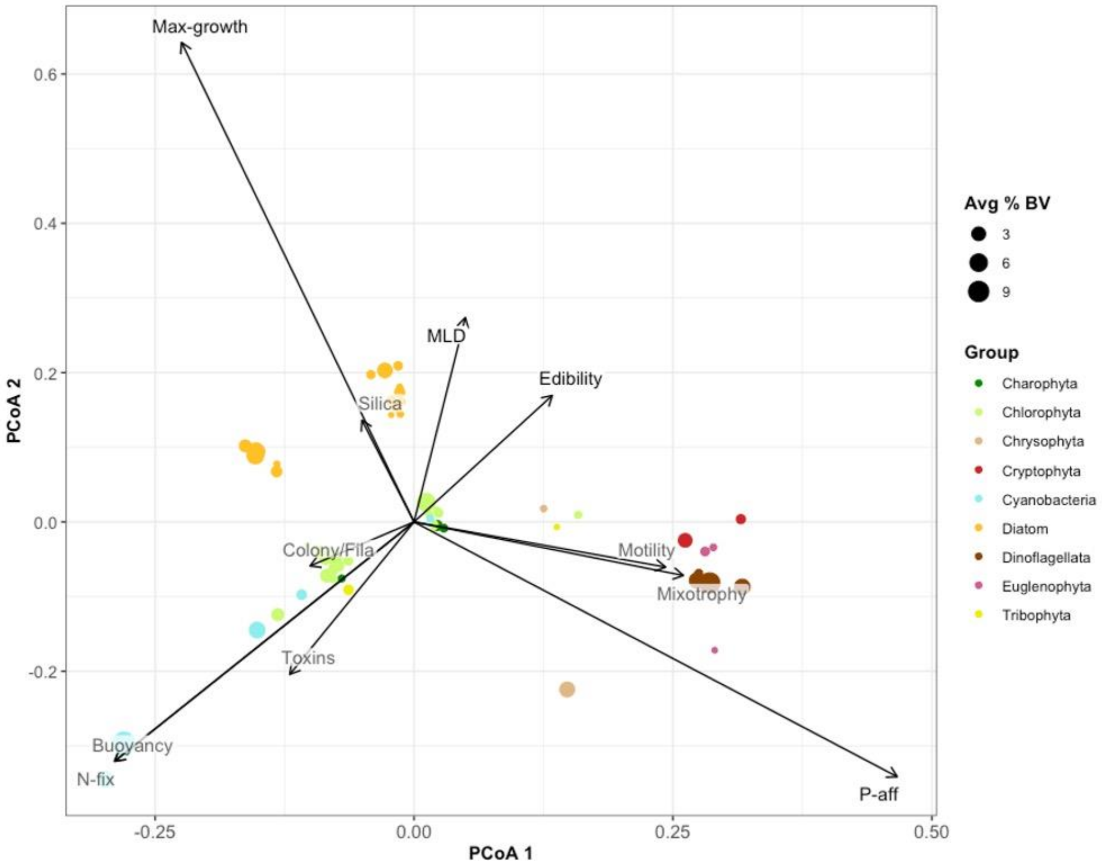

Figure 4.4 The biplot of the principal coordinate analysis (PCoA) of the trait × taxon matrix based on the Gower distance. The loadings of the traits are plotted as vectors. The taxon scores are points colored by algal group and sized by the average relative biovolume across lakes. The traits are: maximum growth rate (max-growth), Silica (Silica demand), maximum linear dimension (MLD), edibility for *Daphnia* (edibility), motility, mixotrophy, phosphate affinity (P-aff), toxin production (Toxins), coloniality/filamentous (Colony/fila), buoyancy, and diazotrophy (N-fix).

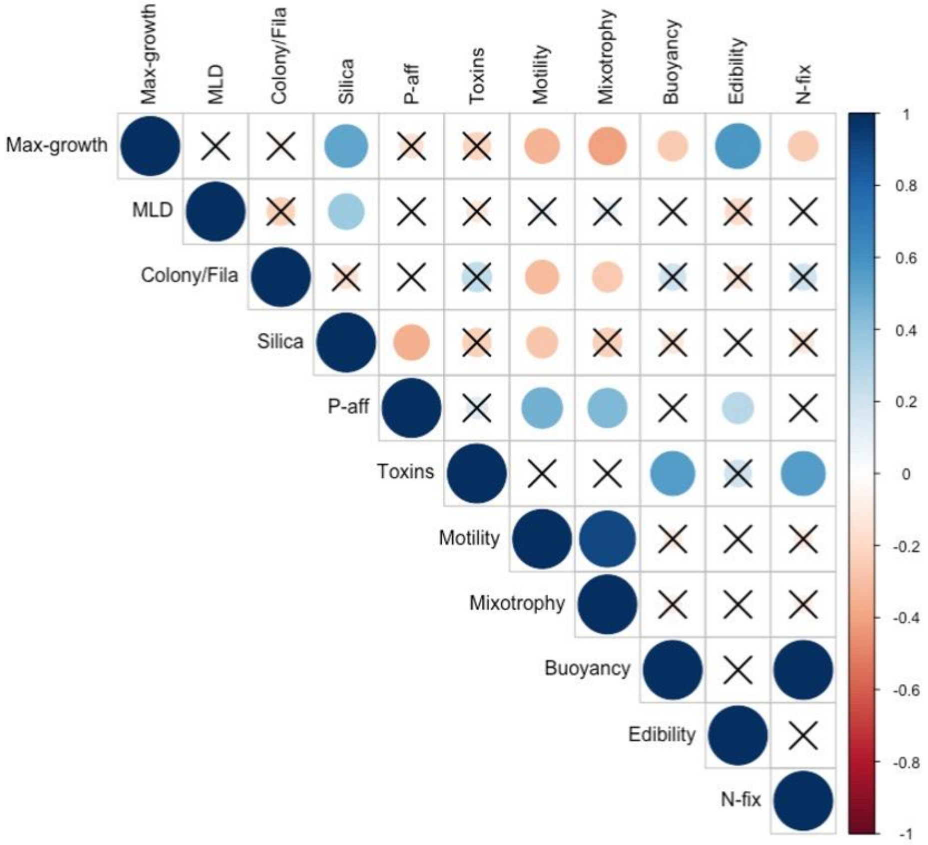

Figure 4.5 Spearman correlation matrix between the functional traits of the phytoplankton. The areas of the circles show the absolute value of the corresponding correlation coefficient. The color of the circles signifies the strength and direction of the correlation (dark blue: strong positive correlation; dark red: strong negative correlation). The crossed-out points signify insignificant correlations with the significance $\alpha = 0.05$. The trait names are defined in Figure 4.4.

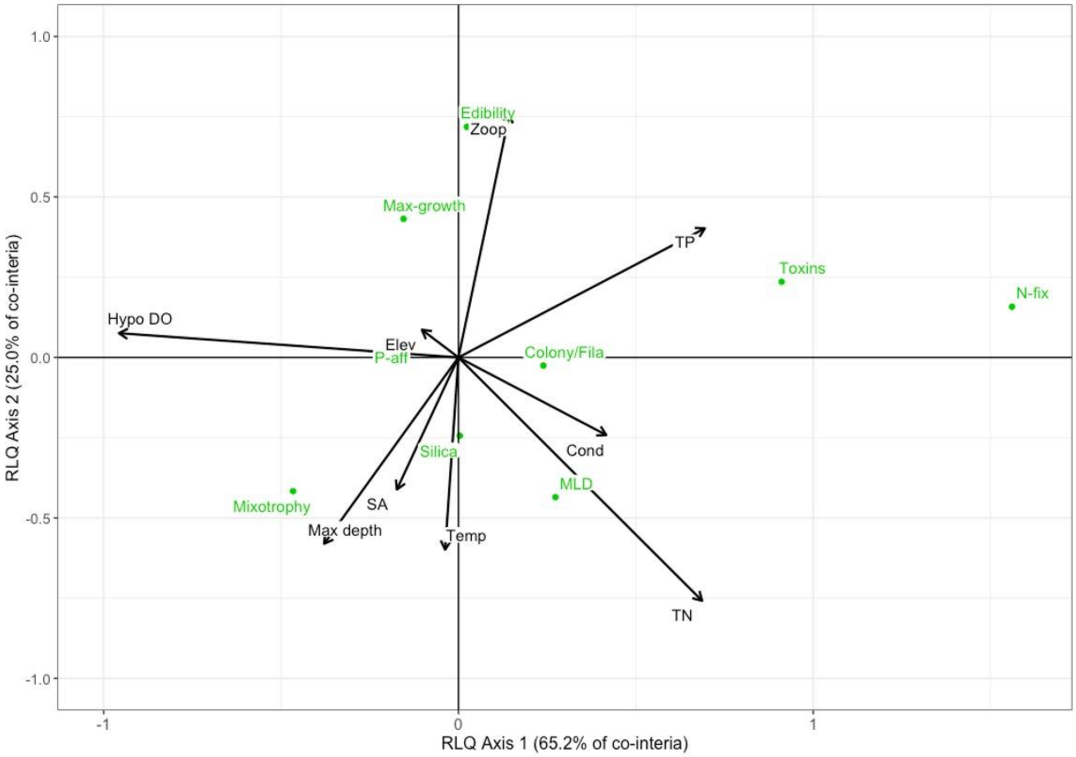

Figure 4.6 Biplot of the RLQ analysis, showing the cross-covariance between the traits (61 taxa × 9 traits) and environmental variables (29 lakes × 9 variables), constrained by the taxon relative biovolume table (61 taxa × 29 lakes). The trait loadings plotted as points in green and the environmental variable loadings as vectors in black. The environment variables and traits are defined previously in Figure 2 and 4, respectively.

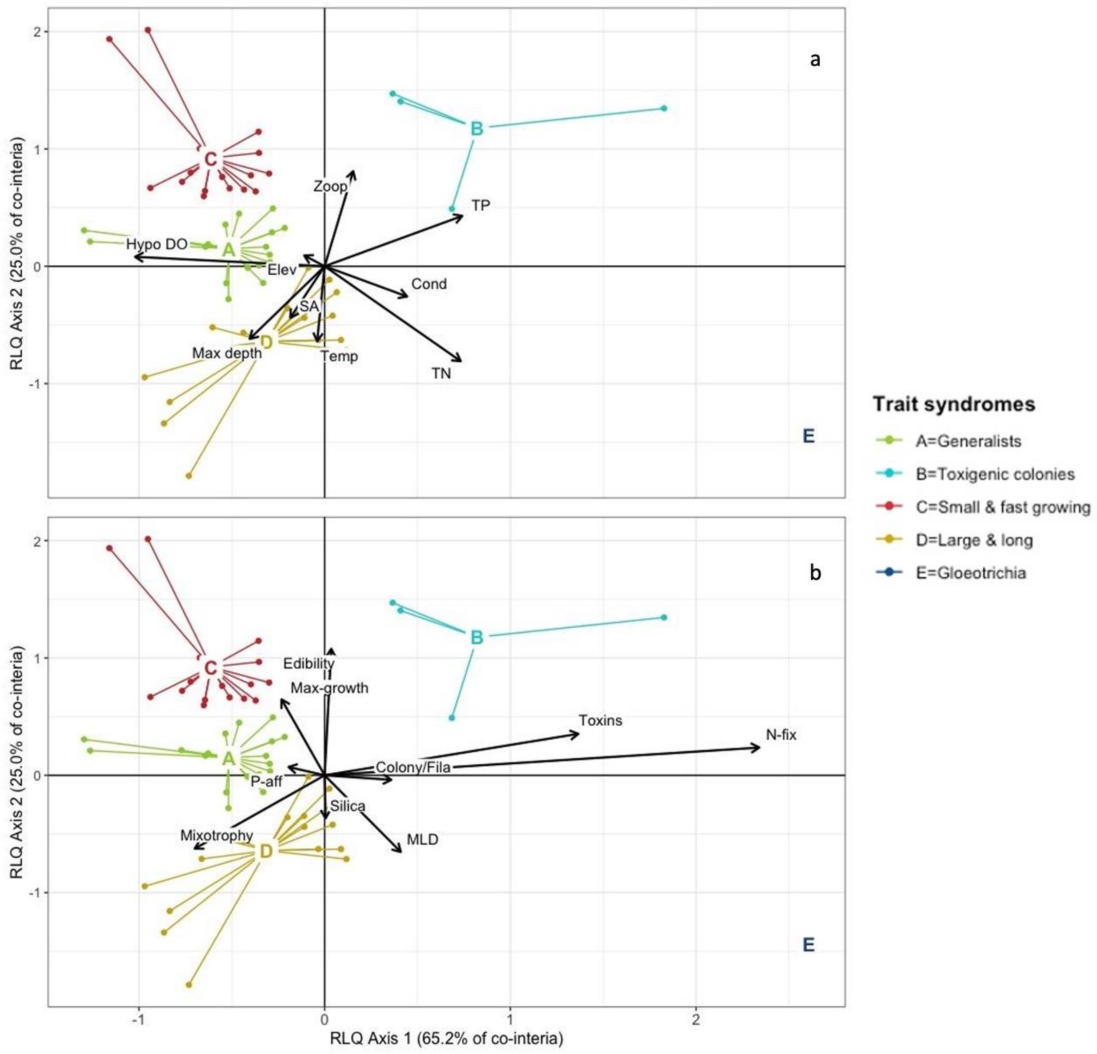

Figure 4.7 Biplots of the RLQ analysis, showing the taxa scores relative to the environmental variables (a) and traits (b). The trait loadings and the environmental variable loadings are plotted as vectors in black. The taxa scores are plotted as points. The points are clustered and colored by the five trait syndrome groups. The groups are A=generalists (green), B=toxigenic colonies (cyan), C=small & fast growing (red), D=large & long (yellow), and E=*Gloeotrichia* (dark blue). The environment variables are defined previously in Figure 2 and 4, respectively.

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Chapter 5: Conclusion

Freshwater phytoplankton support lake ecosystems by making up a substantial portion of the base of the food web when allochthonous organic matter inputs are limited (Naselli-Flores & Padisák, 2022). Yet phytoplankton can also negatively affect higher trophic levels through forming harmful blooms (Naselli-Flores & Padisák, 2022). Cyanobacteria typically form harmful blooms (cyanoHABs) in freshwater bodies with high nutrient loading and warm temperatures (Reynolds, Oliver & Walsby, 1987b; Huisman *et al.*, 2004; Paerl & Otten, 2013; Taranu *et al.*, 2015). Yet recent studies have revealed cyanoHABs occurring across nutrient levels and thermal regimes (Reinl *et al.*, 2021, 2023). Remote mountain lakes, while generally low in nutrients and temperatures, still exhibit a range of productivity due to steep elevational gradients and topographic complexity within a mountain range (Catalan *et al.*, 2006). Accordingly, mountain lakes serve as ideal systems to examine the natural drivers and dynamics of phytoplankton communities and in particular cyanoHABs (Oleksy *et al.*, 2020). However, phytoplankton and cyanoHABs in mountain lakes remain understudied (Larson *et al.*, 1998; Vinebrooke & Leavitt, 1999; Cook *et al.*, 2023).

The overall objective of my dissertation was to determine indicators and drivers of phytoplankton communities and cyanoHABs in mountain lakes. My specific goals were to: (1) to determine potential cross-scale interactions between food web, lake and watershed factors that drive diazotrophic cyanobacteria, which are capable of fixing atmospheric nitrogen (N) to ammonium and are common cyanoHAB taxa; (2) elucidate relationships between cyanotoxins and meteorological parameters as well as identify cyanoHAB indicators within bacterioplankton communities; and (3) determine how spatial variation in phytoplankton functional composition is defined by top-down and bottom-up forces.

Through a field survey (Chapter 2), I found that lakes spanned a range in diazotrophic cyanobacteria biomass as well as potential drivers of the spatial variation. However, cyanoHABs are fairly short-lived disturbances, often lasting only a few days or weeks (Isles & Pomati, 2021). Therefore, I also examined temporal shifts in bacterioplankton communities in relation to cyanoHABs and bloom toxicity (Chapter 3). The ability for cyanoHABs to form hinges upon the traits of not only the cyanobacteria species of the HABs, but also the other phytoplankton species competing with cyanoHAB species for nutrients and light (Litchman, 2022). Therefore, I identified distinct trait syndromes that reflect grazer abundance and nutrient levels. These studies collectively shed light on drivers of cyanoHABs in mostly undisturbed watersheds, contributing to the knowledge of mountain lake ecology and phytoplankton dynamics. These studies also have implications for management of water quality and fisheries in these lakes highly valued for recreation and habitat (Chiapella, Nielsen-Pincus & Strecker, 2018; Ebner *et al.*, 2022).

In Chapter 2, I identified drivers of diazotrophic cyanobacteria in mountain lakes at the food web, lake, and watershed scales, as well as possible cross-scale interactions. Similar to other studies (Molot *et al.*, 2021; MacKeigan *et al.*, 2023), I found phosphorus (P) positively influenced diazotroph biomass. I also found watershed size had a positive and indirect influence on diazotrophs by likely increasing inputs of P. Internal nutrient cycling was also found to be likely crucial as hypolimnetic dissolved oxygen had a strong negative relationship with diazotrophs. When dissolved oxygen drops to anoxic levels (near or close to zero) at the lake bottom, sediment-bound nutrients such as P and iron, which are essential for diazotrophs, are released into the water column (Molot *et al.*, 2014). My findings suggest natural external and internal inputs of P in some mountain lakes may be sufficient to support high biomass, even blooms, of diazotrophs. Increasing relative area of the watershed to the lake, in combination with low hypolimnetic dissolved oxygen levels, can increase the release of legacy phosphorus bound to sediments as a potential cross-scale interaction. The combination of relatively high external and internal nutrient loading facilitates the succession of the lakes towards a eutrophic state. With climate warming, weathering rates as well as atmospheric deposition of nutrients like P may increase, thus increasing watershed inputs of nutrients to lakes (Hartmann *et al.*, 2014; Scholz & Brahney, 2022). As the climate warms, periods of stratification can lengthen and so can hypolimnetic anoxia, increasing sediment P release in the ice-free season (Wagner & Adrian, 2009; Kraemer *et al.*, 2015; Preston *et al.*, 2016). Therefore, management should consider watershed size and likelihood of hypolimnetic anoxia to assess the vulnerability of lakes to cyanoHABs. In addition to these abiotic factors, I found cladocerans influenced diazotrophs by likely grazing on other phytoplankton that compete with these filamentous cyanobacteria. As fish stocking is prevalent in mountain lakes, fishery management should assess how current fish populations and stocking practices may impact zooplankton in terms of composition and abundance (Knapp *et al.*, 2001; Pister, 2001). The role of abiotic and biotic factors in cyanobacterial biomass has been recently recognized (MacKeigan *et al.*, 2023). By studying diazotrophs in lakes with minimal development, my study provides new information about natural drivers of cyanoHABs.

In Chapter 3, I examined the intra-annual and inter-annual variation in cyanoHABs and cyanotoxins in mountain lakes in relation to the bacterioplankton communities and meteorological conditions. I characterized the bacterioplankton composition by 16sRNA sequencing and meteorological parameters by using interpolated PRISM daily climate data. While intra-annual patterns in precipitation and temperature did not influence cyanotoxins, bacterioplankton significantly changed in composition during cyanoHABs relative to non-cyanoHAB periods. The influence of climate likely hinges upon lake and watershed characteristics that affect HAB dynamics such as length and regularity (Isles & Pomati, 2021). My study not only demonstrated cyanoHABs in mountain lakes are reflected in cyanobacteria dominance, but also a rise in certain heterotrophic bacteria that have been previously identified as potential degraders of cyanotoxins and other cyanobacterial metabolites (Berg *et al.*, 2009; Tromas *et al.*, 2017; Lezcano *et al.*, 2017; Guedes *et al.*, 2018). I also found specific heterotrophs that were more abundant in a highly toxic bloom than a low toxic bloom, which suggests they are cyanotoxin degraders or at least indicators of bloom toxicity. While more research is needed with higher frequency sampling, this study demonstrates there are heterotrophic taxa that can be consistent indicators of cyanoHABs and cyanotoxins across lakes. Identification of bacteria via sequencing can be complementary to cyanotoxin monitoring, which can be costly and often underestimates toxin levels due to many toxin variants (Pacheco, Guedes & Azevedo, 2016). In addition, bacterioplankton communities were not notably different within lakes at different depths, indicating a single sampling point may be sufficient for monitoring cyanobacteria blooms. As monitoring of cyanoHABs in relatively remote lakes is often limited, sequencing of bacterial communities can provide more time-integrated information about cyanoHABs than a grab sample analyzed for only toxins. Future studies should directly compare the bacterioplankton communities in cyanoHABs across regions such as along an elevational gradient to determine how much spatial overlap exists in HAB taxa as well as indicator heterotrophic taxa. These studies should also account for key environmental factors in relation to the blooms as these will likely vary between lakes and affect bloom dynamics.

Identifying the indicators and drivers of cyanoHABs without considering the rest of the phytoplankton community precludes a complete understanding of the dominance and expansion of bloom-forming cyanobacteria (Litchman, 2022). In Chapter 4, I leveraged a trait-based approach to determine how phytoplankton communities in mountain lakes are shaped by the bottom-up and top-down pressures of nutrients and grazers, respectively. I found the gradient of P was reflected in a switch in dominance from unicellular mixotrophs at the low end to toxigenic colonies at the high end. Along the N gradient, colonies became more prevalent with increasing N, but cell size also increased with larger colonial diatoms. In terms of grazing pressure, I found slow growing, large taxa dominant at the low end of grazer abundance and faster growing, small taxa dominant at the high end of grazer abundance. Yet at relatively high P and grazing zooplankton abundance, the dominant strategy in phytoplankton was instead toxigenic colonies, including diazotrophic cyanobacteria. Chapter 4 expands upon the findings of Chapter 2 by illustrating how nutrients and grazers not only influence the prevalence of diazotrophy, but also other key phytoplankton traits such as size. The spatial variation across lakes in the defense and nutrient acquisition strategies suggests regional shifts in nutrients such as increased atmospheric deposition could change the dominant strategy (Scholz & Brahney, 2022). For example, greater P availability under high grazing pressure could shift the dominance away from fast growing chlorophytes to toxigenic cyanobacteria. As trait-based approaches are generalizable, these relationships have implications beyond mountain lakes.

The combined findings of my chapters on phytoplankton in mountain lakes shed light on the roles of abiotic and biotic factors in cyanoHABs. In Chapters 2 and 4, I identified possible drivers of diazotrophic cyanobacteria. In particular, I found P alone, rather than N relative to P, was the primary nutrient driver of biomass and dominance of these cyanobacteria, similar to other lakes (Molot *et al.*, 2021; MacKeigan *et al.*, 2023). P availability in lakes in relatively undisturbed watersheds are affected by external and internal loading, which can be magnified by watershed size and hypolimnetic anoxia (Chapter 2). Temperature appears to have a limited direct effect on cyanoHABs in mountain lakes, which may be due to cold-water adaptation of the diazotrophic cyanobacteria species (Chapter 2, 3; Reinl 2023). I also determined possible biotic indicators of diazotrophic cyanobacteria and their blooms in terms of microbial degraders and cladoceran grazers (Chapter 2, 3). Certain oligotrophic phytoplankton and bacterioplankton taxa (Chapter 3, 4) were found inversely related to bloom-forming cyanobacteria in mountain lakes, and therefore a decline in these taxa may indicate increasing vulnerability to cyanoHABs. While abiotic factors of cyanoHABs have long been recognized (Smith, 1983; Paerl & Otten, 2013; Taranu *et al.*, 2015), biotic interactions likely also play a role in bloom formation as well as termination, especially when nutrient loading is limited. Future research of cyanoHABs should encompass environmental factors as well as the planktonic communities, especially in lakes with relatively limited nutrients.

Further work should focus upon determining the ecological niche of these taxa further in terms of nutrient thresholds in relation to traits key for HABs (Litchman, 2022). Critical loads of atmospheric N deposition have been estimated to shift phytoplankton communities within western U.S mountain lakes from N-limitation to P-limitation, with N deposition increasing in multiple regions (Baron *et al.*, 2011; Williams *et al.*, 2017). Yet P loading in mountain ranges may also be increasing via atmospheric deposition, with warming soils leaching P, as well as increasing rates of weathering (Stoddard *et al.*, 2016; Scholz & Brahney, 2022), therefore critical loads of P should also be estimated, especially in the context of HAB species like diazotrophs. The Southern Cascade Mountain lakes exhibit a range of P availability from very low to relatively high as well as a range of diazotrophic cyanobacteria abundance, which are positively associated with each other (Chapter 2). P enrichment bioassays in these Cascade lakes alongside lakes in other mountain ranges would help establish critical P thresholds for diazotrophic and other bloom-forming cyanobacteria (Williams *et al.*, 2017). These bioassays should examine not only changes in biomass, but also changes in key traits such as coloniality, diazotrophy, and P storage, as well as toxicity (Litchman, 2022). These bioassays should include N and P fertilization, as well as N-only fertilization, to compare the response of biomass and key traits across different nutrient scenarios (Williams *et al.*, 2016). Many western US mountain lakes are N-limited so diazotrophic cyanobacteria may become more dominant in many lakes if P becomes more available while N remains relatively low (Williams *et al.*, 2017). In addition, estimates of critical P loads must also account for lake and watershed factors such as morphometry, watershed size, and forest cover (Chapter 2) that may naturally elevate P loading as well as reduce N loading. The critical P loads based on HAB traits as well as key lake and watershed drivers would provide resource managers information to identify lakes that are or may become vulnerable to cyanoHABs with ongoing climate warming and shifts in atmospheric deposition.

While trophic interactions likely contribute to diazotrophic cyanobacterial dominance and blooms, the exact effects of zooplankton and other biological communities remains less certain (Sweeney, Rollwagen-Bollens & Hampton, 2022; MacKeigan *et al.*, 2023). Grazing zooplankton like *Daphnia* may be able to effectively reduce bloom-forming cyanobacteria biomass, but dense blooms of large colonies may become too difficult for these filter feeders to consume (Ghadouani, Pinel-Alloul & Prepas, 2003; Chislock *et al.*, 2013). The positive association of large cladocerans and diazotrophic filamentous cyanobacteria observed in Cascade lakes, as well as other lakes, may indicate these generalist grazers more readily consume other phytoplankton that compete with diazotrophs (MacKeigan *et al.*, 2023). With widespread fish stocking in mountain lakes, the impact of fish on zooplankton grazers must be further examined in the context of phytoplankton (Knapp *et al.*, 2001; Schindler *et al.*, 2001). Fish predation generally reduces zooplankton body size, which in turn reduces the upper size limit of ingestible particles so they cannot easily graze large phytoplankton, such as long filaments (Vanni *et al.*, 1990; Sarnelle, 2007). Controlled experiments testing the top-down effect of fish on filamentous cyanobacteria via zooplankton grazers in mountain lakes are needed. In addition, smaller non-crustacean zooplankton like ciliates, which are prey for crustacean zooplankton, may readily consume filamentous cyanobacteria (Bec, Martin-Creuzburg & von Elert, 2006; Sweeney *et al.*, 2022). The smaller non-crustacean zooplankton are more edible and have higher nutritional quality than the cyanobacteria, providing an alternative pathway for large crustacean zooplankton to thrive during cyanoHABs (Bec *et al.*, 2006; Paerl & Barnard, 2020). Therefore, the non-crustacean and crustacean zooplankton communities should be characterized in food web manipulation experiments on cyanoHABs. As some diazotrophic cyanobacteria can produce multiple toxins that may be detrimental to zooplankton and higher trophic levels (DeMott *et al.*, 1991; Carmichael, 2001; Ger *et al.*, 2016a), toxicity should also be assessed. While nutrients and thermal regimes often play a predominant role in cyanoHABs, the food web likely facilitates as well as constrains the growth of bloom-forming cyanobacteria. Relatively undisturbed mountain lakes serve as model systems to examine food web effects across gradients of productivity and climate (Catalan *et al.*, 2006; Symons & Shurin, 2016).

Mountain lakes are important habitat for sensitive species as well as highly valued recreational sites (Chiapella *et al.*, 2018), but the capacity for HAB research and monitoring is limited and requires tiered and cost-effective approaches (Srivastava *et al.*, 2013). Surveys are essential to identify lakes with HABs or vulnerable to HABs as well as for further research, but field monitoring in mountainous areas is intensive and challenging (Shahgedanova *et al.*, 2021). For a first step, web-based applications using cyanobacteria abundance estimates from satellite sensors (Schaeffer *et al.*, 2018) alongside watershed geospatial data, in conjunction with field validation at a subset of sites can be an efficient method for identifying lakes of concern. Once cyanoHAB vulnerable lakes are identified, these lakes or at least a stratified sample of them should be sampled. For early warning assessment, samples should be taken for nutrient analysis as well as cyanobacteria community identification, including toxin-producing genes through quantitative real-time PCR (polymerase chain reaction), which has a relatively quick turn-around time (Srivastava *et al.*, 2013). As mountain lake sampling may likely be restricted to one or two time points within a year, eukaryotic phytoplankton and heterotrophic bacteria communities should eventually be identified, using genetic sequencing, along with cyanobacteria as bioindicators of trophic status and potentially HABs. Bacterioplankton and algae have been found to be consistent integrators of environmental change (Pan *et al.*, 1996; Eiler, Heinrich & Bertilsson, 2012). Lakes that are prone to cyanoHABs should be monitored on an annual basis to track temporal shifts, Sampling efforts could be supported in part by citizen science programs, which have been leveraged for HAB monitoring in other regions (Castilla *et al.*, 2015) with relatively simple sampling protocols from docks and shorelines. As climate change is occurring at an accelerated rate in mountain ranges, the potential for cyanoHABs in these remote lakes may rise with changes in thermal regimes, precipitation as well as watershed processes (Wagner & Adrian, 2009; Pepin *et al.*, 2015; Preston *et al.*, 2016).

The management of lakes in the mountains should consider additional actions when lake vulnerability to cyanoHABs is high. As fish populations can influence the grazing community of the cyanoHAB, managers should assess how current fish species and densities may impact the food web (Knapp *et al.*, 2001; Eilers *et al.*, 2007). If certain fish species appear to likely impact key grazers of cyanoHABs, then managers should consider actions to reduce the species' population in the lake (Eilers *et al.*, 2007). At the watershed scale, management agencies should develop nutrient budgets, especially for P, for cyanoHAB-vulnerable lakes to determine the inputs due to development and fish stocking relative to natural sources, using isotope tracers. While Cascade watersheds can often be naturally rich in P due to volcanic geology, other mountain ranges have relatively low P in their bedrock such as the granitic Sierra Nevadas (Porder & Ramachandran, 2013). Therefore, lakes in other mountain ranges may be more sensitive than Cascade lakes to even limited anthropogenic sources of nutrients within the watershed. If the estimated P loads from development are substantial relative to natural sources, managers should work with locals to mitigate any uncontrolled sources such as septic system leaks. While the management of mountain lakes is often limited relative to lowland lakes, the assessment of fish and development related impacts on cyanoHABs can provide specific plans of action.

Mountain lakes, while distinct from lowland lakes in multiple ways, still offer insights on cyanobacteria blooms that transcend the high elevation environments (Catalan *et al.*, 2006). High elevation lakes experience higher UV radiation and ice cover as well as lower temperatures and dissolved organic matter than most lowland lakes (Sommaruga, 2001; Juetten *et al.*, 2022). Mountain watersheds generally have less vegetation, especially wetlands and deciduous forests, but also are much less developed than lowland lakes (e.g., agriculture, urbanization) (Juetten *et al.*, 2022). Food webs in mountain lakes are also more simplistic with fewer species than low elevation lakes (Sánchez-Hernández, Cobo & Amundsen, 2015). Therefore, findings from mountain lakes cannot be directly translated to all lowland lakes, but comparisons with similar environments could identify coherent and differing trends between the high and low elevations. Oligotrophic and mesotrophic lowland lakes with limited developed area in the northern temperate zone may be the most similar to mountain lakes, especially those in the vegetated zones of the subalpine and montane (Rose *et al.*, 2009). Blooms of cyanobacteria, including diazotrophs, have been observed in such lowland lakes even at relatively low temperatures (<15ºC) (Reinl *et al.*, 2021, 2023). Therefore, comparisons between these low and high elevation lakes could assess the overlap in drivers of cyanoHABs. As nutrient reduction efforts by management have at times limited success in preventing cyanoHABs (Pomati *et al.*, 2012), key factors for blooms in less productive water bodies can suggest new avenues for management strategies.

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Appendix A: Supporting tables and figures for Chapter 2

Table S1. Additional summary information for the predictor and response variables for watershed. Summary statistics included median, minimum and maximum for each variable.

Variable names	Units	Median (Min, Max)
Alkaline intrusive volcanic rock	%	24 (0,100)
Silicic rock	%	0 (0, 61.4)
Extrusive volcanic rock	%	87.0 (0,100)
Coarse glacial till	%	0.73 (0, 61.9)
Glacial lake fines	%	0 (0,66.5)
Drainage ratio		12.3 (2.12, 207)
Ice cover	%	0 (0, 11.7)
Developed area	%	0.01 (0, 4.17)
Barren ground	%	2.53 (0, 66.3)
Coniferous forest cover	%	80.6 (12.7, 97.5)
Deciduous forest cover	%	0 (0,0.49)
Shrub cover	%	1.76 (0, 21.8)
Grass cover	%	0.21 (0, 55.8)
Woody wetland cover	%	0.01 (0, 0.40)
Herbaceous/emergent wetland cover	%	0.01 (0, 1.18)
Base-flow index	%	79.8 (56.3, 86)
Annual precipitation, 30-year average: 1981-2010	mm $year^{-1}$	1,760 (592, 3,075)
Maximum air temperature, 30-year average: 1981-2010	° C	21.4 (7.18, 23.9)
Mean air temperature, 30-year average: 1981-2010	° C	4.95 (2.00, 6.98)
2018 average of precipitation-weighted deposition for NH_4^+	kg ha^{-1} $year^{-1}$	1.28 (0.32,6.03)
2018 average of precipitation-weighted deposition for NO_3^-	kg ha^{-1} $year^{-1}$	0.45 (0.12, 2.91)
Median 2019 maximum snow water equivalent	m	0.88 (0.46, 1.42)
Mean forest cover loss for 2015-2019	%	0.07 (0, 27.9)
Mean forest cover loss for 2000-2019	%	0.70 (0.02,74.3)
Mean slope	%	13.1 (7.05,33.7)
Mean eastness [sin(aspect)]	°	-0.08 (-0.44,0.34)
Mean northness [cos(aspect)]	°	0.16 (-0.21,0.69)
Mean elevation	m	1,460 (958, 1,980)

Table S2. Additional summary information for the predictor and response variables for lake scale. Summary statistics included median, minimum and maximum for each variable and by each sampling bout (early & late summer) for variables that varied between bouts.

Variable Names	Units	Early Summer Median (Min, Max)	Late Summer Median (Min, Max)	Overall
N-fixing cyanobacteria biovolume	µm^3 mL^{-1}	31.8 (0, 217,000)	40.5 (0, 1,930,000)	71.0 (0.00, 967,000)
Maximum depth	m	---	---	19.2 (4.90, 86.0)
Mean depth	m	---	---	9.75 (1.90, 49.7)
Volume	hm^3	---	---	8.80 (0.40, 699)
Surface area	ha	---	---	102 (17.0, 1,550)
Mixed layer depth	M	9.00 (1.00-19.2)	10.0 (1.00-19.2)	9.50 (1.00-19.2)
Photic depth	M	---	---	20.7 (2.31, 39.8)
Mean epilimnetic temperature	° C	16.2 (7.37, 19.7)	19.3 (13.5, 22.4)	17.6 (10.7, 20.5)
pH		8.06 (6.61, 9.48)	8.27 (6.75, 9.48)	8.10 (6.78, 9.48)
Hypolimnetic dissolved oxygen	mg L^{-1}	8.27 (0.33,13.1)	8.35 (0.31,11.7)	8.27 (0.31, 13.1)
Total Phosphorus	mg L^{-1}	0.01 (0.001, 0.37)	0.003 (0.001, 0.08)	0.01(0.001, 0.22)
Total Nitrogen	mg L^{-1}	0.067 (0.001, 0.89)	0.03 (0.001, 0.16)	0.02 (0.00, 0.36)
Mean epilimnetic specific conductance	µS cm^{-1}	23.5 (2.00, 492)	27.2 (2.28, 590)	24.6 (2.14, 541)
Mean epilimnetic dissolved oxygen	mg L^{-1}	9.20 (8.30, 12.7)	8.98 (8.23, 11.4)	8.98 (8.47, 11.3)
Chlorophyll-a	mg L^{-1}	0.42 (0.05, 15.3)	0.26 (0.04, 4.90)	0.52 (0.06, 12.7)
Schmidt Stability Index	Joules m^{-2}	259 (1.38, 2,850)		297 (1.34, 4,140)

Table S3. Additional summary information for the predictor and response variables for food web scale. Summary statistics included median, minimum and maximum for each variable and by each sampling bout (early & late summer) for variables that varied between bouts.

Variable Names	**Units**	**Early Summer**	**Late Summer**	**Overall**
		Median (Min, Max)	**Median (Min, Max)**	
10 year mean fish stocking biomass	kg ha^{-1}	---	---	0.52 (0.00, 135)
Calanoida abundance	# L^{-1}	726 (0.00, 13,500)	619 (0.00, 8,200)	834 (0.00, 10,300)
Cyclopoida abundance	# L^{-1}	2, 800 (0.00, 42,600)	1,810 (0.00, 8,300)	3,070 (0.00, 25,300)
Copepod nauplii	# L^{-1}	194 (0.00, 11,600)	188 (0.00, 2,050)	330 (6.60, 6,000)
Daphnia	# L^{-1}	771 (0.00, 76,000)	1,781 (0.00, 13,100)	2,140 (0.00, 41,300)
Small cladoceran grazers	# L^{-1}	588 (0.00, 18,300)	842 (0.00, 24,400)	886 (0.00, 18,500)

Table S4. Biovolume equations based on geometric shapes adapted from Hillebrand et al. (1999). The equations are given using standard abbreviations for the linear dimensions to be measured. Abbreviations: A=surface area; V=volume; r=radius; d=diameter; h=height; a=apical axis (length); b=transapical axis (width); c=pervalvar axis (height/head pole for Gomphonemoid); z=height of cone; l=length of one side; m=height of a triangle, β=angle between the two transapical sides; f=length from transapically widest part to head pole; e=length from transapically widest part to head pole.

Shape	**Equation**
Sphere	$V = {}^{\pi}/_{6} \times d^3$
Prolate spheroid	$V = {}^{\pi}/_{6} \times d^2 \times h$
Rectangular box	$V = a \times b \times c$
Cylinder	$V = {}^{\pi}/_{4} \times d^2 \times h$
Elliptic prism	$V = {}^{\pi}/_{6} \times a \times b \times h$
Cymbelloid	$V = {}^{4\pi}/_{6} \times b^2 \times a \times {}^{\beta}/_{360}$
Gomphonemoid	$V = b \times c \times \left(\left[\pi - {}^{e}/_{4}\right] + \left[f - {}^{e}/_{3}\right] \right)$
Cone	$V = {}^{\pi}/_{12} \times d^2 \times z$

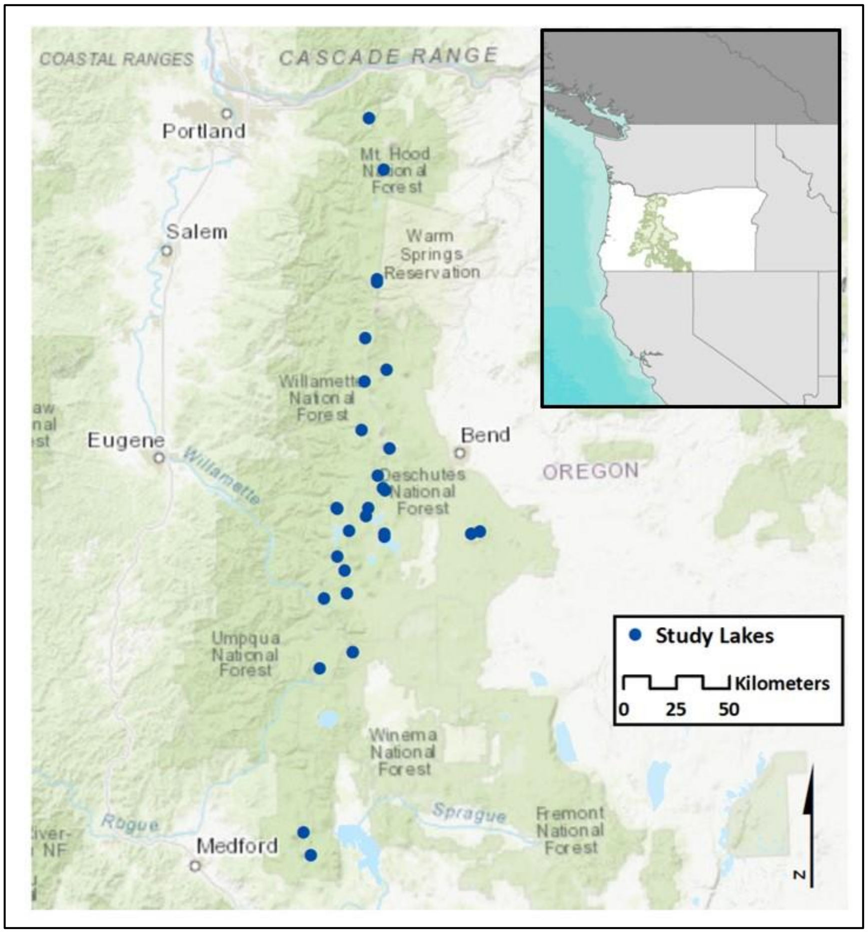

Figure S1. Map of the central Cascade Range, showing the location of the study lakes. The boundaries of the National Forests are denoted in dark green. Base map credit to ESRI.

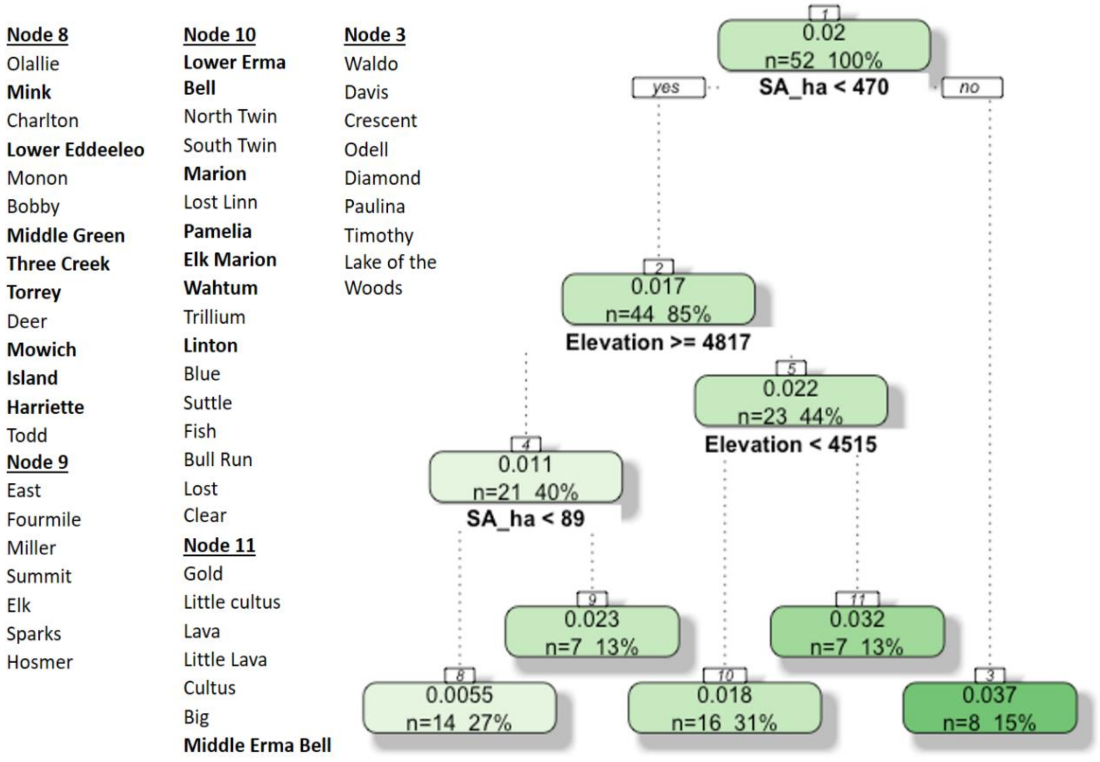

Figure S2. Binary regression tree of the compiled lake set with total phosphorus in milligrams per liter (mg/L) as the response variable and elevation in meters (m) and lake size in hectares (SA_ha) as the main predictors of the splits. 10-year fish stocking averages were also included in the model, but were not found to the main variable for any spilt. Each intermediate or final node has the average phosphorus concentration of the group of lakes, the number of lakes (n) and the percent of the total lakes. The lakes in each final node (3,8,9,10,11) are listed on the left. Lakes in wilderness areas are in bold.

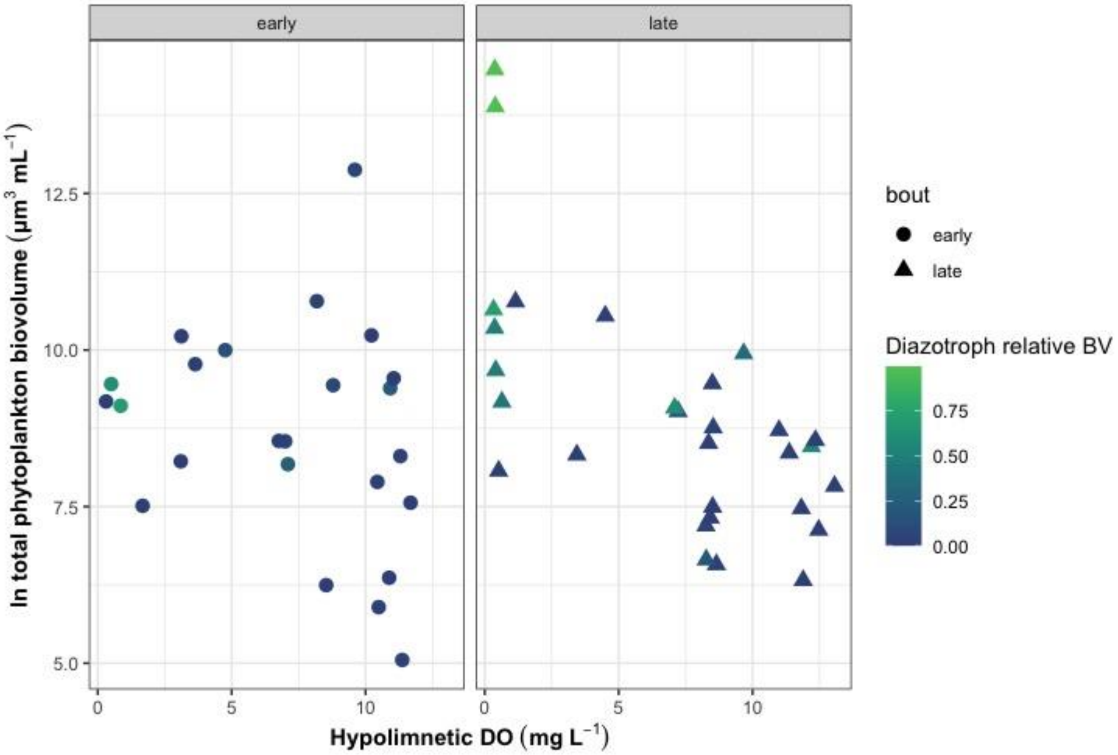

Figure S3. Scatterplots of hypolimnetic dissolved oxygen (DO) and natural log-transformed (ln) total phytoplankton biovolume (right) by each sampling bout: early (left; circles) and late (right; triangle). The relative biovolume (BV) of diazotrophic cyanobacteria is symbolized by a color gradient from dark blue (0.0: absence) to bright green (1.0: complete dominance).

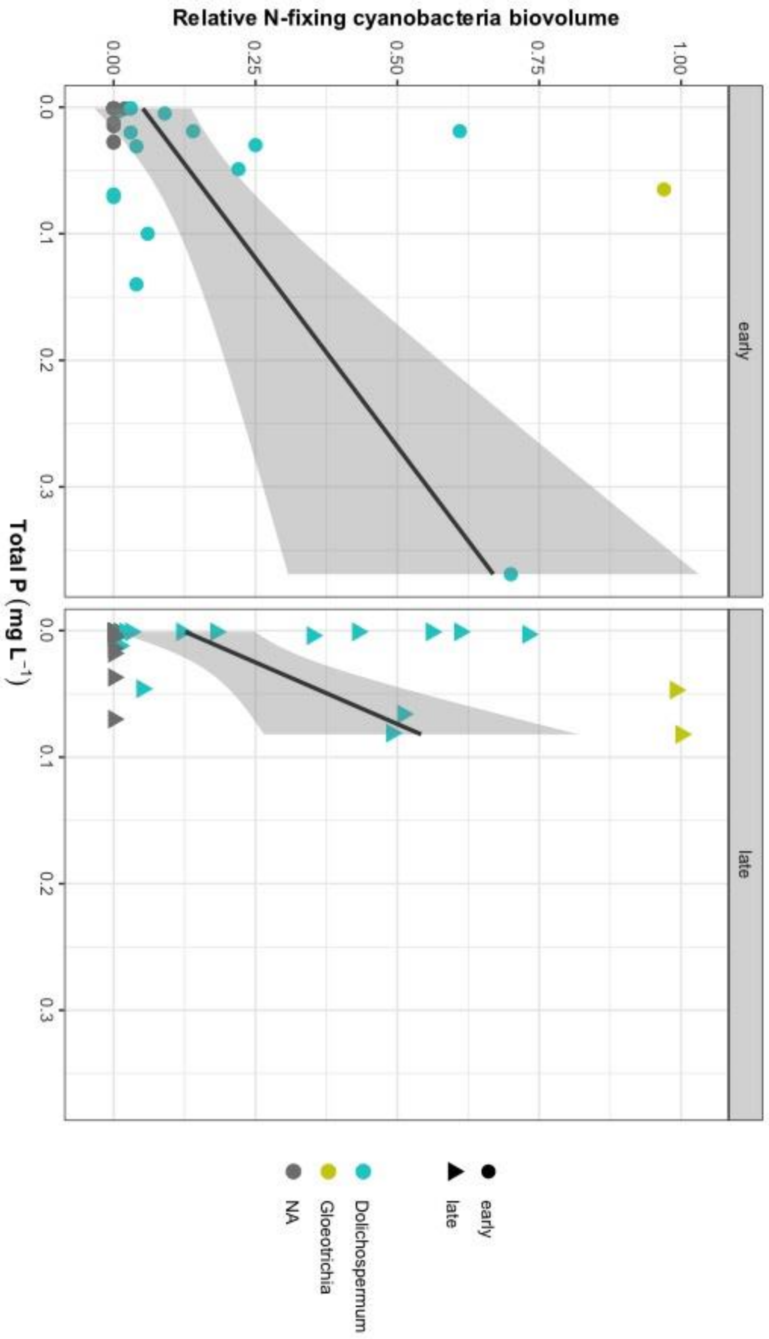

Figure S4. Scatterplots of total phosphorus with diazotrophic cyanobacteria biovolume proportion of total biovolume (right) by each bout: early (left; circles) and late (right; triangles). Fitted lines of simple linear regression models were estimated with the 95% confidence intervals in light grey. The dominant genus of diazotrophic cyanobacteria is symbolized by color: *Dolichospermum* (teal), *Gloeotrichia* (yellow-green), or NA-no diazotrophs present (grey).

Appendix B: Supporting tables and figures for Chapter 3

Table S1. The results of the PERMDISP and PERMANOVA tests for the total bacterioplankton and non-cyanobacterial (NC) communities. The following parameters were reported: degree of freedom (df), sum of squares (Sum of Sqs.), mean squares (Mean Sqs), Pseudo F-ratio for the model (Pseudo-F), R-squared (R^2), and p-value.

Total Weighted-UniFrac metric	**Analysis**	**Factor**	**df**	**Sums of Sqs**	**Mean Sqs**	**Pseudo-F**	**R^2**	**p-value**
	PERMDISP	Toxigenic cyano level	3	0.001	0.004	5.10	-	0.001*
	PERMANOVA	Toxigenic cyano level	3	1.140	-	3.31	0.12	0.001*
		Location	2	0.150	-	0.67	0.02	0.986
		Toxigenic cyano level *Location	6	0.520	-	0.76	0.06	0.992
NC Weighted-UniFrac metric	**PERMDISP**	Toxigenic cyano level	3	0.018	0.006	9.08	-	0.001*
	PERMANOVA	Toxigenic cyano level	3	0.690	-	2.11	0.08	0.001*
		Location	2	0.150	-	0.68	0.02	0.990
		Toxigenic cyano level*Location	6	0.500	-	0.76	0.06	0.990

Table S2. Differential abundance analysis result from ALDEx2 with 1,000 Monte Carlo samples for HAB and non-HAB communities at the ASV level. Difference indicates the median log difference between HAB and non-HAB samples. BH p-values correspond to Wilcoxon's t-test p-values corrected for multiple tests based on the Benjamini-Hochberg (BH) correction.

Group	**Phylum**	**Order**	**Genus**	**Species**	**Difference**	**BH corrected p-value**
HAB	Proteobacteria	Burkholderiales	*Rubrivivax*	unnamed	6.94	<0.001
	Cyanobacteria	Nostocales	*Dolichospermum*	unnamed	11.90	<0.001
	Cyanobacteria	Nostocales	*Dolichospermum*	unnamed	11.40	0.002
	Bacteroidetes	Sphingobacteriales	*unnamed*	unnamed	8.45	0.013
	Proteobacteria	Rhodobacterales	*unnamed*	unnamed	4.78	0.026
	Bacteroidetes	Cytophagales	*Emticicia*	unnamed	5.28	0.040
	Proteobacteria	Rhodospirillales	*unnamed*	unnamed	6.25	0.009
	Proteobacteria	BD7-3	*unnamed*	unnamed	4.47	0.050
No-HAB	Actinobacteria	Acidimicrobiales	*acIV-B*	*Iluma-B1*	-3.57	0.020
	Actinobacteria	Acidimicrobiales	*acIV-A*	*Iluma-A2*	-5.27	0.020

Table S3. Additional data from 2020 for total nitrogen (N) and phosphorus (P) concentrations for the three lakes in the seasonal subset in early summer (early July) and late summer (late August-early September**)**. The two sources were Oregon Department of Environmental Quality (2019) for Odell and Crescent Lakes and US Forest Service (Miller & Sytsma, 2020) for Diamond Lake.

	Early summer			**Late summer**		
Lake	**Sample Date**	**Total N (mg L^{-1})**	**Total P (mg L^{-1})**	**Sample Date**	**Total N (mg L^{-1})**	**Total P (mg L^{-1})**
Crescent	7/15/20	0.097	0.001	8/26/20	0.090	0.001
Diamond	7/8/20	0.360	0.086	9/4/20	0.400	0.071
Odell	7/15/20	0.240	0.020	8/26/20	0.163	0.010

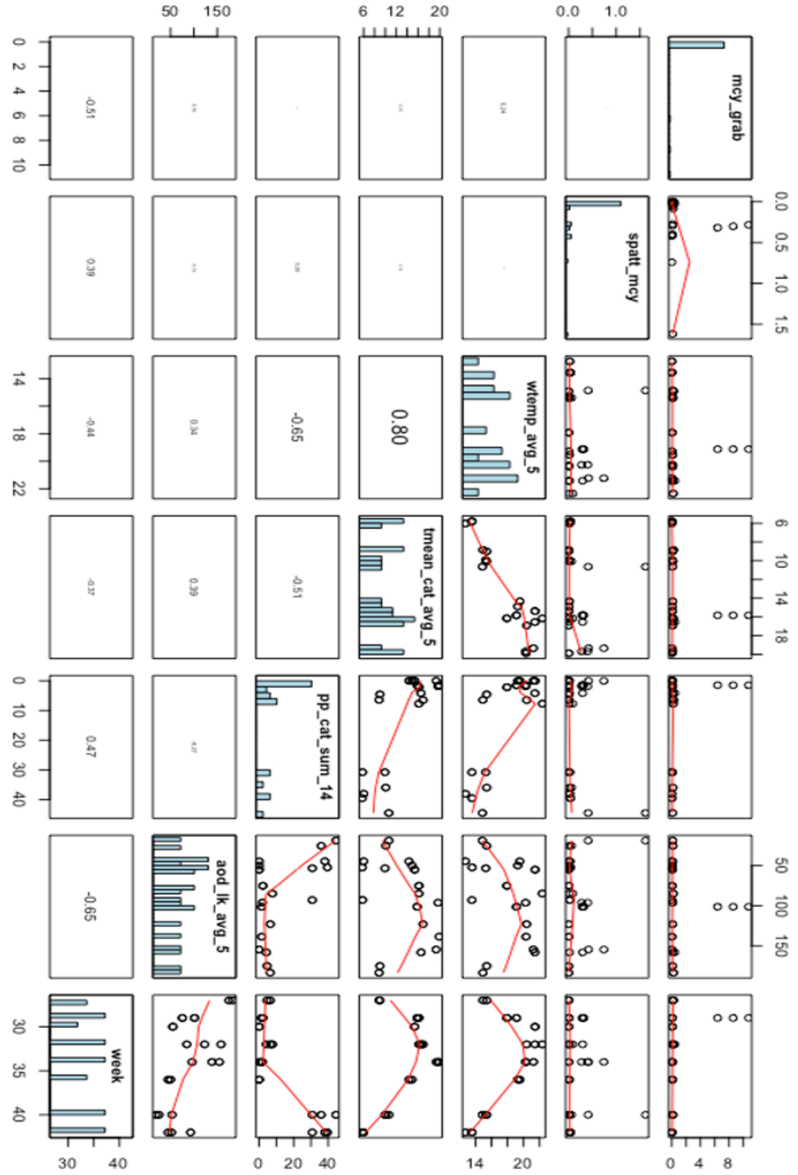

Figure S1. Correlation matrix of microcystin-grab and SPATT concentrations, week of sampling, and climate variables: five day mean water temperature (wtemp_avg_5), five day mean air temperature at the catchment scale, 14 day cumulative precipitation at the catchment scale (pp_cat_sum_14), five day mean aerosol optical depth at the lake scale (aod_lk_avg_5). The left side of the matrix are Spearman rank correlation coefficients for each combination of variables, which were used to due to non-normal data distribution. Along the top left to bottom right diagonal are the variable histograms. The right side of the matrix are scatterplots for each combination of variables with a line of best fit in red based on locally weighted scatterplot smoothing.

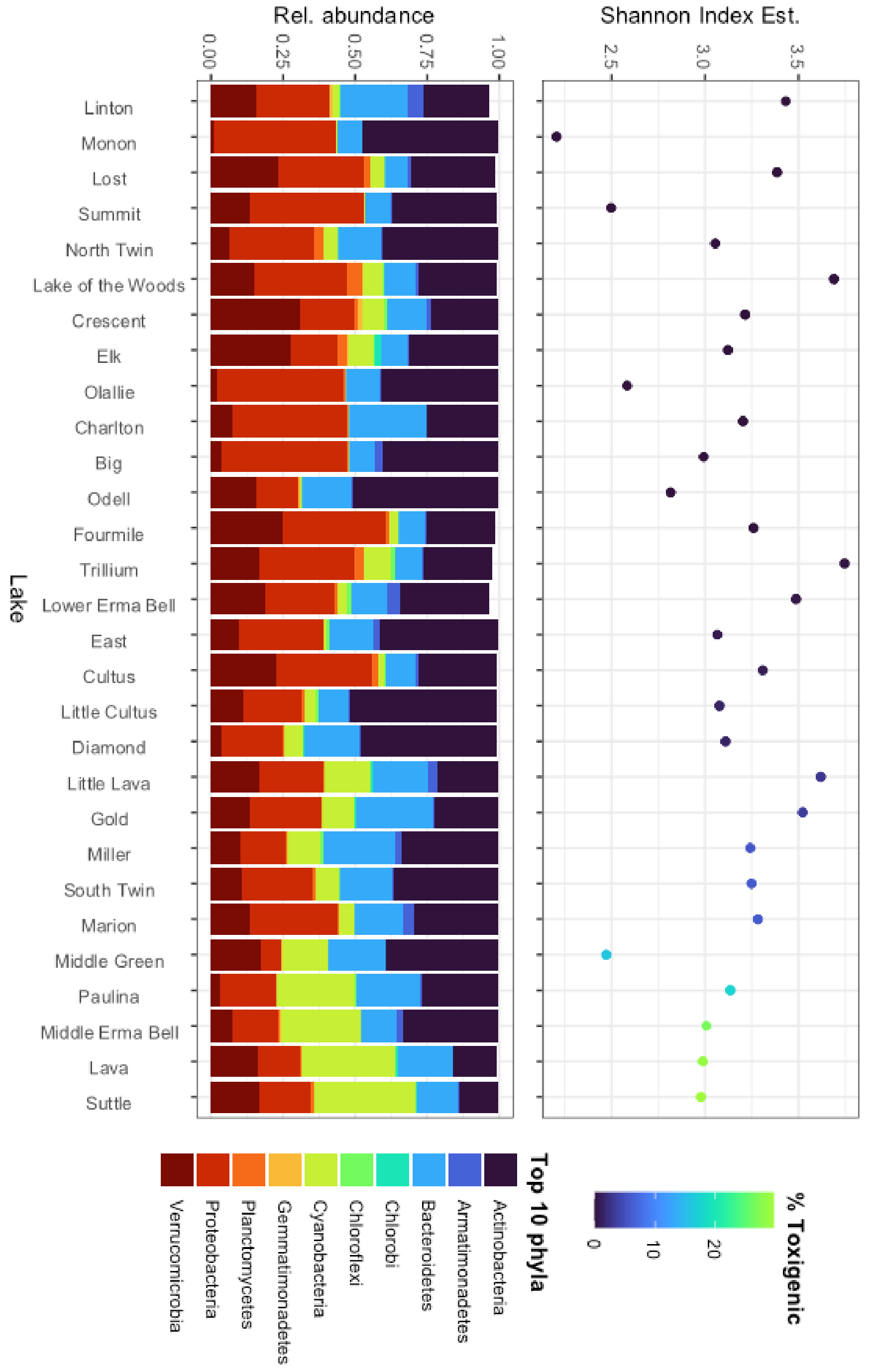

Figure S2. Top-Shannon Diversity estimates for each of the 29 lakes in the summer of 2019. The lake points are colored by the relative abundance of toxigenic cyanobacteria. Bottom-the relative abundance of the top ten phyla for each of the 29 lakes. The lakes are ordered by the relative abundance of toxigenic cyanobacteria in both graphs.

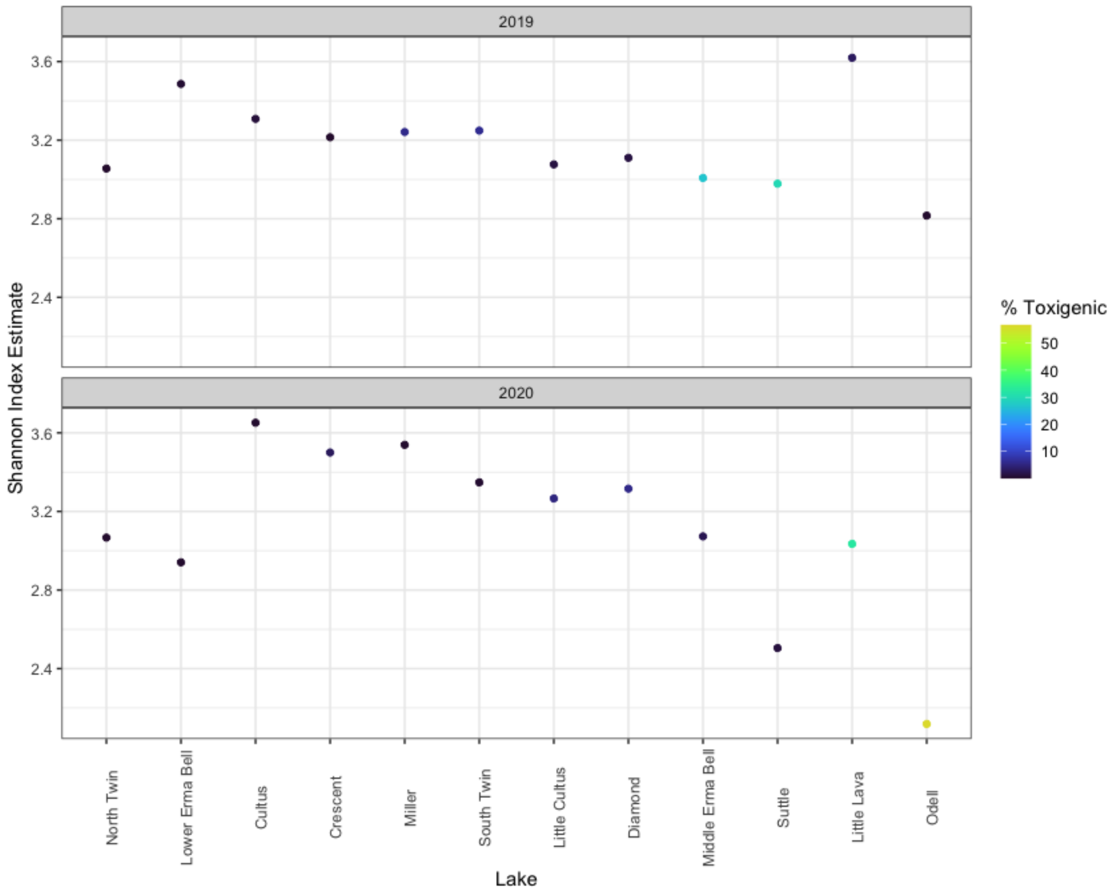

Figure S3. Shannon Diversity Index estimated for each lake and by year (2019-top, 2020-bottom). The points are colored by the relative abundance of toxigenic cyanobacteria.

Appendix C: Supporting tables and figures for Chapter 4

Table S1. Taxon × trait matrix of the phytoplankton for the study lakes. Some trait names were shortened for brevity: mean BV-biovolume, MLD-maximum linear dimension, maximum (max) growth rate, edib-edibility for *Daphnia*, col/fila-colonial/filamentous and PO_4 -phosphate affinity. The trait values besides mean BV and MLD were compiled from Reynolds, 1998; Wehr, 2002; Weithoff, 2003; Bruggeman, 2011; Cottingham *et al.*, 2015; Klais *et al.*, 2017; Kruk *et al.*, 2017; Laplace-Treyture *et al.*, 2021.

Taxon	**Mean BV**	**MLD**	**Max growth rate**	**Edib.**	**Col /fila**	**PO_4 affinity**	**Light affinity**
Achnanthidium	253.9	13.0	2.10	0.46	n	150	0.048
Aphanocapsa-Aphanothece	39.1	2.0	1.20	0.44	y	130	0.079
Asterionella	172.3	34.3	1.50	0.23	y	100	0.025
Aulacoseira-Melosira	357.8	8.4	1.50	0.23	y	18	0.072
Botryoccocus	1038.0	15.9	1.30	0.31	y	190	0.018
Ceratium	9081.4	38.3	0.25	0.11	n	910	0.004
Ceratoneis	93.3	9.3	1.40	0.27	n	130	0.037
Chlamydomonas	22.0	3.5	1.80	0.62	n	330	0.015
Chroococcus	181.8	4.7	0.58	0.1	y	4	0.081
Chrysosphaerella	563.2	9.3	1.00	0.26	y	120	NA
Cocconeis	592.4	10.6	1.70	0.38	n	120	0.050
Cosmarium	422.0	9.0	0.95	0.16	n	72	0.006
Crucigenia	101.5	4.1	1.40	0.32	y	130	0.026
Cryptomonas	218.4	14.6	1.20	0.55	n	140	0.011
Cyclotella	239.7	4.9	1.60	0.23	n	4	0.040
Cymbella	506.1	12.4	1.40	0.3	n	77	0.015
Desmidium	179.0	12.1	0.97	0.17	y	120	0.045
Diatoma	222.0	9.2	1.40	0.24	y	210	0.019
Dictyosphaerium	257.3	2.8	1.20	0.28	y	290	0.018
Dinobryon	57.2	5.4	0.74	0.15	y	110	0.063
Dolichospermum	408.2	4.2	0.96	0.46	y	170	0.076
Elakathroix	46.6	8.5	1.40	0.33	y	760	NA
Epithemia	315.6	11.7	1.40	0.3	n	77	0.047
Euglena	189.3	11.3	1.10	0.37	n	2700	0.203
Eunotia	34.9	20.7	1.40	0.3	n	77	0.044
Fragilaria	641.5	39.1	1.40	0.2	y	98	0.009
Franceia	134.3	8.3	1.50	0.37	y	170	0.013
Glenodinium	2695.4	16.4	0.47	0.16	n	220	NA

Taxon	**Mean BV**	**MLD**	**Max growth rate**	**Edib.**	**Col /fila**	**PO_4 affinity**	**Light affinity**
Gloeocystis	158.4	4.2	1.20	0.31	y	150	0.024
Gloeotrichia	288637.8	63.5	0.25	0.09	y	87	0.077
Golenkinia	121.9	6.2	1.00	0.24	n	100	0.039
Gomphonema	1088.0	24.9	1.40	0.3	y	77	0.130
Koliella	58.1	13.6	1.30	0.31	n	120	0.018
Merimsopedia	101.2	2.6	1.20	0.44	y	130	0.078
Mougeotia	502.4	36.1	1.50	0.23	y	87	NA
Navicula	796.5	30.7	1.40	0.3	n	77	0.047
Nitzschia	178.0	18.8	1.80	0.46	n	150	0.014
Odontidium	286.4	4.9	1.40	0.23	n	130	NA
Oocystis	117.6	6.6	1.50	0.4	y	190	0.003
Ophiocytium	292.4	28.5	1.10	0.31	n	140	NA
Parvodinium	438.4	12.8	0.34	0.12	n	250	NA
Pediastrum	238.6	14.5	1.30	0.31	y	310	0.005
Planktosphaeria	174.4	6.8	1.20	0.31	y	150	0.024
Psuedosphaerocystis	773.2	4.7	1.00	0.22	y	140	NA
Rhodomonas	32.9	12.9	1.70	0.9	n	550	0.012
Scenedesmus	47.6	5.0	2.10	0.37	y	45	0.030
Schroderia	65.4	18.3	1.20	0.31	n	150	0.024
Sphaerocystis	201.8	4.5	1.10	0.19	y	85	0.059
Staurastrum	801.3	16.8	0.84	0.15	n	150	0.003
Stephanodiscus	6934.6	22.4	1.80	0.36	n	13	0.042
Tabellaria	206.8	15.3	1.20	0.19	y	130	0.025
Tetraedron	1109.5	6.6	0.83	0.19	n	59	0.024
Trachelomonas	1067.8	8.6	1.10	0.37	n	1600	NA
Tribonema	418.6	18.5	0.64	0.18	y	300	NA
Ulnaria	1044.6	150.1	1.74	0.335	n	420	0.009
Unknown Chlorophyte	73.5	4.2	1.30	0.31	n	120	0.038
Unknown cyanobacteria	180.8	4.7	1.10	0.32	n	68	NA
Unknown diatom	1651.8	21.0	1.40	0.3	n	77	0.042
Unknown dinoflagellate	11461.5	8.6	0.47	0.16	n	220	NA
Unknown Euglenophyte	148.3	12.9	1.10	0.375	n	2670	NA

Table S2. Taxon × trait matrix of the phytoplankton for the study lakes. Some trait names were shortened for brevity: I_{opt}-optimal irradiance before the onset of inhibition, Si use-silica use, diazo-diazotrophy, mixo-mixotrophy, buoy-buoyancy regulation, and toxin-potential toxin production. The trait values were compiled from Reynolds, 1998; Wehr, 2002; Weithoff, 2003; Bruggeman, 2011; Cottingham *et al.*, 2015; Klais *et al.*, 2017; Kruk *et al.*, 2017; Laplace-Treyture *et al.*, 2021.

Taxon	**I_{opt}**	**Si Use**	**Diazo**	**Mixo**	**Motile**	**Buoy**	**Toxin**
Achnanthidium	127.6	y	n	n	n	n	n
Aphanocapsa-Aphanothece	85.5	n	n	n	n	n	y
Asterionella	118.9	y	n	n	n	n	n
Aulacoseira-Melosira	99.5	y	n	n	n	n	n
Botryococcus	144.2	n	n	n	n	n	Y
Ceratium	60.0	n	n	y	y	n	N
Ceratoneis	165.2	y	n	n	n	n	N
Chlamydomonas	144.9	n	n	n	y	n	N
Chroococcus	79.3	n	n	n	n	n	N
Chrysosphaerella	NA	y	n	y	y	n	N
Cocconeis	128.0	y	n	n	n	n	N
Cosmarium	204.9	n	n	n	n	n	N
Crucigenia	191.9	n	n	n	n	n	N
Cryptomonas	163.2	n	n	y	y	n	N
Cyclotella	70.1	y	n	n	n	n	N
Cymbella	90.9	y	n	n	n	n	N
Desmidium	227.9	n	n	n	n	n	N
Diatoma	111.8	y	n	n	n	n	N
Dictyosphaerium	204.5	n	n	n	n	n	N
Dinobryon	NA	n	n	y	y	n	N
Dolichospermum	93.8	n	y	n	n	y	Y
Elakathroix	NA	n	n	n	n	n	N
Epithemia	127.6	y	n	n	n	n	N
Euglena	NA	n	n	y	y	n	Y
Eunotia	118.4	y	n	n	n	n	N
Fragilaria	105.4	y	n	n	n	n	N
Franceia	133.0	n	n	n	n	n	N
Glenodinium	NA	n	n	y	y	n	N
Gloeocystis	160.5	n	n	n	n	n	N
Gloeotrichia	89.7	n	y	n	n	y	Y
Golenkinia	136.1	n	n	n	n	n	N
Gomphonema	127.8	y	n	n	n	n	N
Koliella	144.2	n	n	n	n	n	N

Taxon	**I_{opt}**	**Si Use**	**Diazo**	**Mixo**	**Motile**	**Buoy**	**Toxin**
Merimsopedia	87.6	n	n	n	n	n	Y
Mougeotia	NA	n	n	n	n	n	N
Navicula	127.6	y	n	n	n	n	N
Nitzschia	182.3	y	n	n	n	n	N
Odontidium	NA	y	n	n	n	n	N
Oocystis	108.7	n	n	n	n	n	N
Ophiocytium	NA	n	n	n	y	n	N
Parvodinium	NA	n	n	y	y	N	N
Pediastrum	110.6	n	n	n	n	N	N
Planktosphaeria	160.5	n	n	n	n	N	N
Psuedosphaerocystis	NA	n	n	n	n	N	N
Rhodomonas	195.7	n	n	y	y	N	N
Scenedesmus	223.4	n	n	n	n	N	N
Schroderia	160.5	n	n	n	n	N	N
Sphaerocystis	NA	n	n	n	n	N	N
Staurastrum	250.8	n	n	n	n	N	N
Stephanodiscus	60.4	y	n	n	n	N	N
Tabellaria	110.6	y	n	n	n	N	N
Tetraedron	160.5	n	n	n	n	N	N
Trachelomonas	NA	n	n	y	y	N	N
Tribonema	NA	n	n	n	n	N	N
Ulnaria	110.6	y	n	n	n	N	N
Unknown chlorophyte	130.5	n	n	n	n	N	N
Unknown cyanobacteria	NA	n	n	n	n	N	N
Unknown diatom	109.3	y	n	n	n	N	N
Unknown dinoflagellate	NA	n	n	y	y	N	N
Unknown Euglenophyte	NA	n	n	y	y	N	N

Table S3. The zooplankton taxa found in the study lakes. Cladocerans were identified to the genus level. Copepods were identified to the order level. Each taxon's feeding ecology was defined as either a grazer of phytoplankton or not (y/n) based on Thorp & Covich (2014). Those that did not graze phytoplankton were predators of smaller zooplankton.

Class	**Order/Suborder**	**Family**	**Genus/Taxon**	**Grazer (y/n)**

Branchiopoda	Cladocera	Daphniidae	*Daphnia*	y
Branchiopoda	Cladocera	Daphniidae	*Ceriodaphnia*	y
Branchiopoda	Cladocera	Bosminidae	*Bosmina*	y
Branchiopoda	Cladocera	Leptodoridae	*Leptodora*	n
Branchiopoda	Cladocera	Holopedidae	*Holopedium*	y
Copepoda	Calanoida	--	Calanoida	y
Copepoda	Cyclopoida	--	Cyclopoida	y
Copepoda	--	--	Nauplii	y

Table S4. The mean values for the early and late summer for each trait based on the community weighted average based on relative biovolume (site × trait). The difference in the two bouts were tested with Wilcoxon signed rank test (two-tailed) with the test statistic (*W*), degrees of freedom (df) and p-value with the significance level set at α = 0.05.

Traits	**Early mean**	**Late mean**	***W* value**	***Df***	**p-value**
Phosphate affinity	173	206.2	132	28	0.07
Mixotrophy frequency	0.20	0.24	175	28	0.37
Diazotrophy frequency	0.11	0.21	111	28	0.17
Maximum growth rate	1.26	1.49	285	28	0.15
Maximum linear dimension	17.6	17.3	252	28	0.47
Silica demand frequency	0.31	0.17	307	28	0.05
Coloniality frequency	0.58	0.56	231	28	0.78
Motility frequency	0.26	0.27	205	28	0.80
Toxin production frequency	0.13	0.24	117	28	0.09
Edibility	0.27	0.28	218	28	1.00

Table S5. Summary of the first two axes of the ordinations for the RLQ analysis. The eigenvalues are presented and the total variance (%) projected in parentheses.

Axis	**R/PCA (environment)**	**L/CA (genus composition)**	**Q/Hill-Smith (traits)**
1	2.4 (26.7)	0.8 (14.5%)	3.9 (35.9%)
2	2.1 (23.4%)	0.5 (9.7%)	3.07 (27.9%)

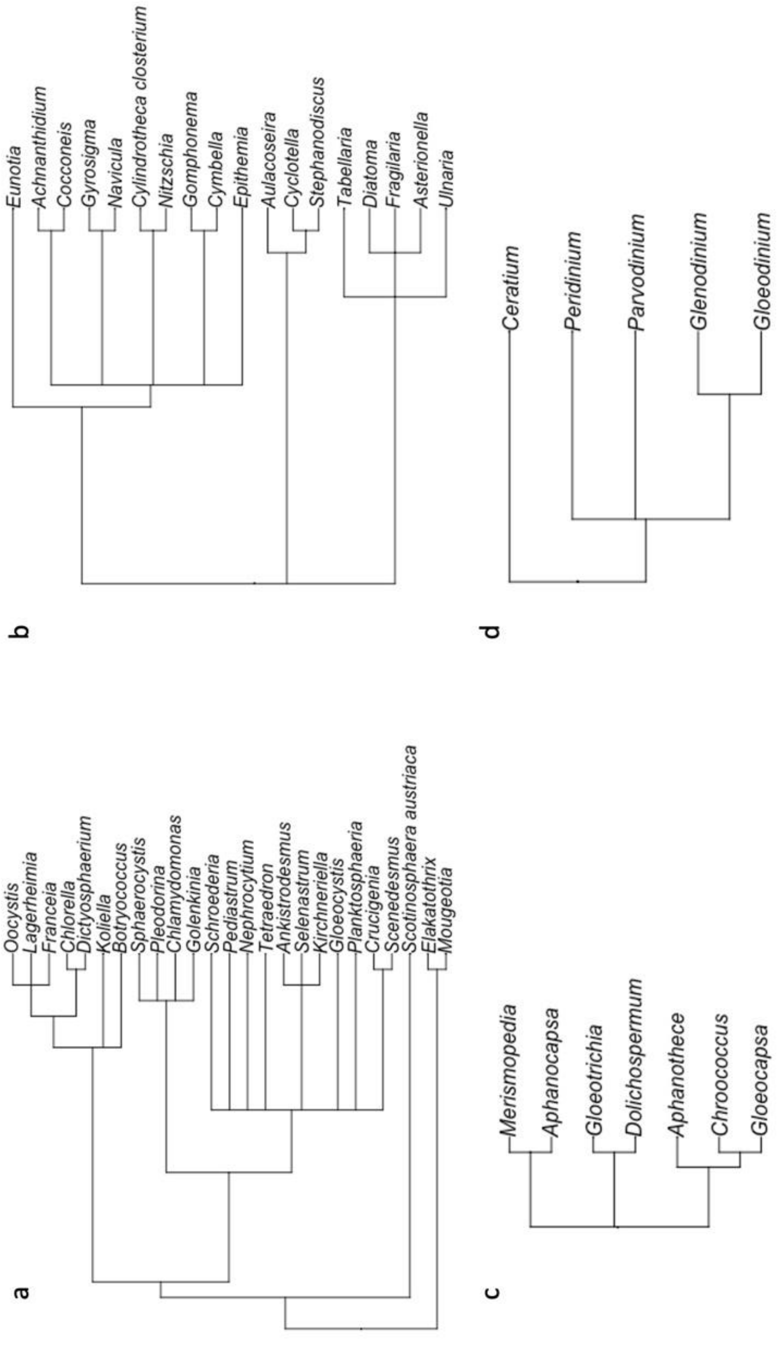

Figure S1. The four phylogenetic trees based on the National Center for Biotechnology Information taxonomic structure for the four main divisions of phytoplankton: (a) chlorophytes, (b) diatoms, (c) cyanobacteria, and (d) dinoflagellates. These trees were used for trait inference for missing trait values for specific genera.

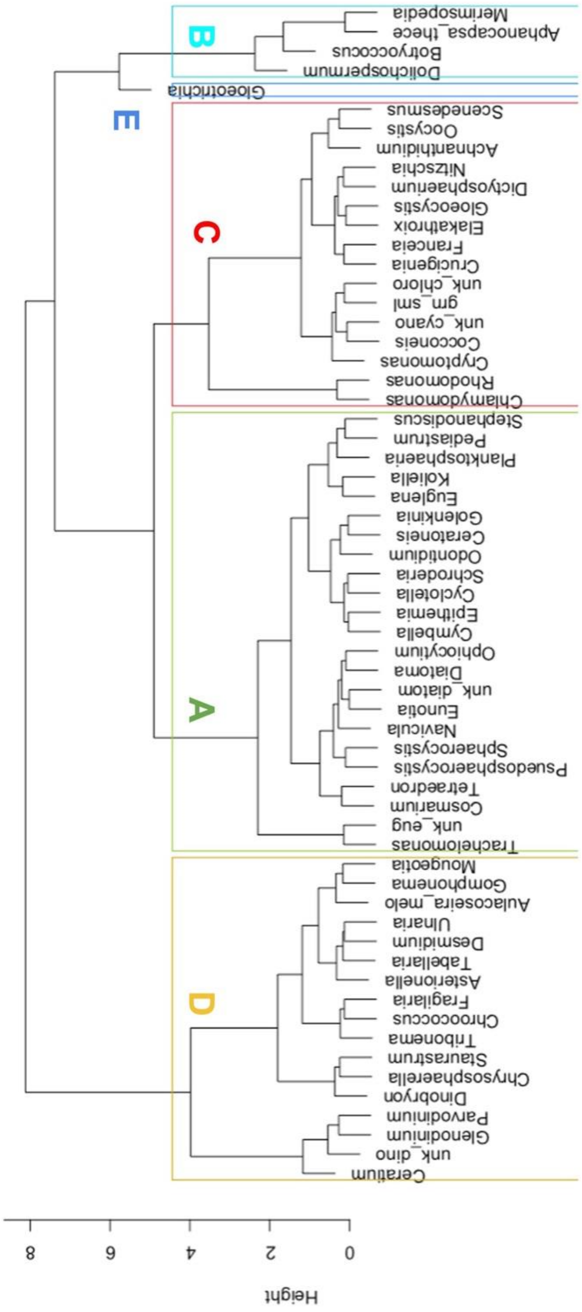

Figure S2. The dendrogram of the hierarchical clustering based on Ward’s minimum variance with the five cluster groups outline by different colors: A (green), B (cyan), C (red), D (yellow), and E (blue).

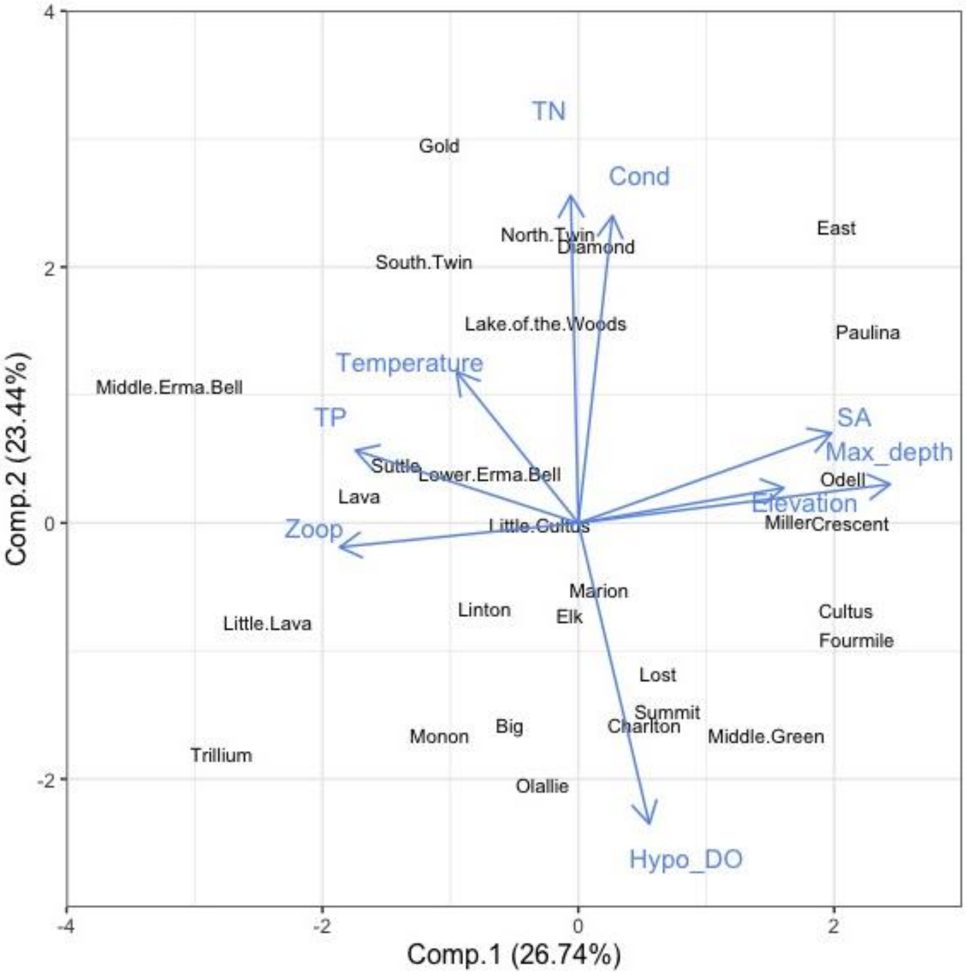

Figure S3. Biplot of the first two axes of the principal component analysis (PCA) of the environmental variables of each lake averaged across the two sampling bouts. The environmental variable loadings are plotted as vectors in blue: zooplankton abundance (Zoop), total phosphorus (TP), temperature, total nitrogen (TN), specific conductance (Cond), surface area (SA), maximum depth (Max depth), elevation, and hypolimnetic dissolved oxygen (Hypo DO). The PCA lake site scores are plotted as labels in black.

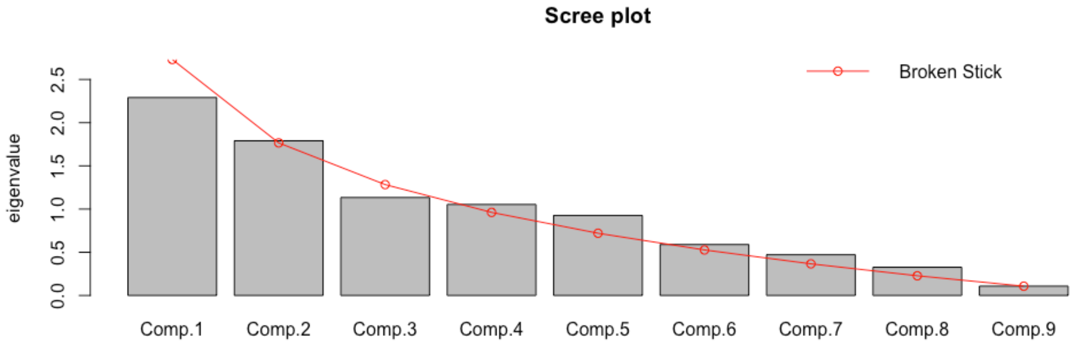

Figure S4. Scree plot of the eigenvalues (Comp.# values) of the PCA for the environmental variables averaged across the two sampling bouts by lake. A broken stick model was overlaid. The model randomly breaks a line into the same number of components in the PCA and then laid in order of decreasing length. If the eigenvalues are greater than the broken stick model length, then they should be considered for interpretation.