2022-06-06

Climate Change and the Global Nutrient Overload: The Microbial Response of Extreme Waterbodies to Environmental Change

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

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One of the defining characteristics of our current epoch—the Anthropocene—is modification of nutrient cycles. At regional to global scales, humans have fundamentally reshaped the availability of carbon, nitrogen, and phosphorus. These changes are particularly apparent in freshwater ecosystems, which receive surface and groundwater inputs of nutrients from agriculture, fossil fuel use, and wastewater. In this thesis, I investigated how the addition of nutrients affects microbial community and biogeochemistry in two extreme environments: the hypereutrophic shallow Utah Lake and nutrient-limited Arctic permafrost streams. In my first chapter, I used bioassay and dilution bioassay experiments to identify what factors control harmful algal blooms in Utah Lake. Specifically, I measured phytoplankton and cyanobacteria growth, cyanotoxin production, and aquatic N-fixation potential. I included physical factors, such as temperature, light, nutrient concentrations, and pH, as well as biological factors, such as top-down control by zooplankton grazers. Phytoplankton showed a threshold behavior at 0.005 mg/L for soluble reactive phosphorus and 0.14 mg/L for dissolved inorganic nitrogen. Surprisingly, nitrogen fixation rates were only high in active bloom samples and were augmented by the addition of both nitrogen and phosphorus. Also contrary to our hypothesis, zooplankton preferentially grazed cyanobacteria over total phytoplankton. In my second chapter, I investigated how permafrost degradation might influence dissolved organic matter (DOM) in Arctic stream networks. Specifically, I used nutrient and labile carbon additions to simulate the effects of permafrost thaw DOM degradation and microbial community in three distinct permafrost-covered catchments on the North Slope of Alaska. The alpine catchment had higher biodegradability but lower DOM concentration across seasons compared with the lake-influenced and tundra catchments. For all catchments, there were strong seasonal changes in microbial community and distinct responses to nutrient addition. The addition of nutrients stimulated DOM biodegradation in the late season—the period of the year when permafrost DOM release typically occurs. Microbial communities differed by catchment type, but overall diversity was similar. Together, these experiments highlight the diverse downstream consequences of human alteration of global carbon, nitrogen, and phosphorus cycles. Even in extreme systems, alteration of the microbial community regulating many of these cycles has potential to exacerbate ecosystem and climate change, so understanding our influence over biogeochemical cycles and microbial interactions is vital for informing future management practices and planetary boundaries.

Keywords: nitrogen, phosphorus, eutrophication, permafrost, zooplankton, cyanobacteria, dissolved organic carbon

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CHAPTER 1

Nutrient Thresholds to Algal Growth in a Shallow, Hypereutrophic Lake

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ABSTRACT

Humans have supercharged the availability of reactive nitrogen and phosphorus at global scales. This global nutrient overload has resulted in harmful overgrowth of algae and cyanobacteria in lakes, rivers, reservoirs, estuaries, and oceans worldwide. Referred to as harmful algal blooms (HABs), this overgrowth can result in ecological degradation of freshwater ecosystems, causing immense damage to public health and economy. Though efforts to reduce nutrient loading from point sources have been effective in many areas, climate change and the intensification of agriculture and urban development threaten to exacerbate HABs at regional to global scales. In this study, we investigated the drivers and internal dynamics of HABs in Utah Lake, one of the largest freshwater lakes in the western U.S. We used controlled microcosm experiments to assess how nitrogen and phosphorus concentration, microbial community, and zooplankton abundance affect cyanobacterial growth and toxin production. Phytoplankton showed a threshold behavior at 0.005 mg/L for soluble reactive phosphorus and 0.14 mg/L for dissolved inorganic nitrogen. In examining cyanobacterial influence in HABs, we did not observe correlations between cyanotoxin production and any environmental or community parameters, though aquatic N-fixation was highest in active bloom areas and was encouraged by both nitrogen and phosphorus addition. Surprisingly, we found that zooplankton grazed cyanobacteria preferentially over algae. These findings demonstrate that while the fundamentals of lake and reservoir restoration remain nutrient reduction and restoration of water flow, better understanding of food web response to temperature and nutrient pressure could improve ability to predict recovery timelines and successfully manage ecosystems as climate and land-use pressures increase.

INTRODUCTION

The prevalence and intensity of phytoplankton blooms of cyanobacteria and algae are becoming more widespread worldwide due to excess nutrient loading and climate change (Gobler 2020; Descy et al. 2016; Wells et al. 2015). Blooms can cause the formation of hypoxic dead zones leading to massive fish and invertebrate kills, destabilizing aquatic ecosystems. In addition to dead zones, the release of cyanotoxins by cyanobacteria can have negative effects across trophic levels and cause water to be unusable by humans (Le Moal et al. 2021; Dupas, Minaudo, and Abbott 2019; Brooks et al. 2016). Risk factors for bloom formation include increased concentrations of nitrogen (N) and phosphorus (P), water temperature, light availability, water residence time, drought, and trophic structure (Jankowiak et al. 2019, Fernández, Estrada, and Parodi 2015). Blooms are further complicated by a diverse assemblage of green algae and cyanobacterial species that may play either a minor or major role within the blooms depending on lake conditions (Gilbert and Durand 1990; Hartnell et al. 2020).

Cyanobacteria are perhaps the most important functional group in a world of greater nutrient availability and warmer waters. Cyanobacteria act as a keystone species, controlling overall primary productivity and the production of toxins, while also acting as an additional nutrient source by fixing atmospheric N. Common cyanotoxins are often harmful neurotoxins (Mahmood and Carmichael 1987; Kinnear 2010; Antoniou, de la Cruz, and Dionysiou 2005). Cyanotoxins endanger other primary producers, as well as higher trophic levels within aquatic systems, including humans consuming local fish and waterfowl (Weirich and Miller 2014; Valério, Chaves, and Tenreiro 2010)**.** The negative effects of cyanotoxins are compounded by cyanobacterial N fixation. Though cyanobacterial N-fixation is usually not the primary source of bioavailable N in eutrophic systems due to the high energy cost (16 ATP for each N_2 molecule) and faster denitrification rates (Paerl 2017), the additional input of bioavailable N from cyanobacteria may exacerbate cyanobacterial blooms further, which will in turn stimulate more

N-fixation creating a positive feedback loop (Paerl 2017). Due to these threats, reducing cyanobacterial populations is often the goal in reducing HABs.

One limitation on phytoplankton growth is predation (grazing) by zooplankton. In non-eutrophic conditions, zooplankton abundance is negatively correlated with algal biomass due to predation (Jackson 1980; Spencer and Ellis 1998). In eutrophic conditions where phytoplankton and zooplankton are much more abundant, predation of phytoplankton over cyanobacteria shifts blooms towards cyanobacterial dominance, and has often been attributed to cyanotoxin production (Yuan and Pollard 2018). This selective predation has been observed in multiple studies (Fernández, Estrada, and Parodi 2015; Jackson 1980; L. L. Yuan and Pollard 2018), though other factors may influence the selective predation of phytoplankton. Zooplankton size, cyanotoxin tolerance, and phytoplankton species could also shift dominance in blooms. Shift in the phytoplankton-dominated community towards cyanobacterial dominance selects against large-bodied daphnids, which may consume more algae than small-bodied zooplankton (Fulton and Paerl 1988). Shift towards cyanobacterial dominance has the possibility to eliminate the benefit of algal predation by zooplankton and further exacerbate issues associated with uncontrolled algal blooms by increasing the relative abundance of cyanobacteria.

In this context, we hypothesized the phytoplankton response to nutrient addition would be greatest in low ambient N and P conditions, with higher nutrient conditions favoring cyanobacterial dominance. We further hypothesized that aquatic N fixation would be lower in higher nutrient conditions, as this process is energetically costly (Vitousek and Howarth 1991; Marcarelli and Wurtsbaugh 2009). Finally, we hypothesized that grazing by zooplankton will favor cyanobacterial dominance because of grazer preference for algae. We tested these hypotheses with nutrient addition and removal bioassays the harmful algal season (April-September) of 2019 in Utah Lake—a large, shallow, and hypereutrophic lake in the semiarid southwestern U.S.

METHODS

We performed three incubation bioassay experiments over the summer of 2020. and performed early and late bloom season bioassay experiments in late spring and early fall with samples collected from three sites in Utah Lake (figure 1). We performed the dilution bioassay (described below) during mid-summer at the site which was most characteristic of the whole lake with moderate urbanization and nutrient concentrations. Each collection site represents different lake conditions due to nearby wastewater treatment plants, population density, and lake geomorphology.

Study site

Utah Lake is the third largest freshwater lake in the Western United States, with a surface area around 145 square miles and a watershed of 2950 square miles (Abbott et al. 2021). Draining to the Great Salt Lake through the Jordan River, it is an important source of water within a semi-arid desert ecosystem. It is considered hypereutrophic based on EPA standards, and is very shallow (9 feet average, 18 feet max depth), but was classified in the cleanest category for algal bloom intensity and extent in 2020 (Coffer et al. 2021). Utah Lake provides habitat for many species, including over 500 invertebrates, over 400 diatoms, 226 birds, 150 algae and cyanobacteria, 49 mammals, 18 fish, and 16 amphibians & reptiles (Abbott et al. 2021), and is a major stopping point in the Pacific Flyway for migrating birds. Utah Lake's watershed is rapidly urbanizing, with the current population of 600,000 expected to double by 2050 based on census projections.

Utah Lake has experienced blooms for more than 35 years (Tate 2019). Toxins produced by recent algal blooms have received media attention, resulting in poor local opinion of the lake. With projections of huge population growth in Utah Lake's watershed, algal bloom intensity and

prevalence has the potential to increase exponentially (Perlich et al 2017). Utah Lake has many characteristics that set it apart from other freshwater lakes and contribute to the complexity of algal growth, though anthropogenic sources such as agricultural lands and wastewater treatment plants comprise most of the nutrient inflow (Crandall et al. 2021; Bradshaw et al. 1973), with 77% of the P introduced to the lake through wastewater treatment plants ("Nutrient Loading Analysis: Utah Lake Water Quality Study" 2019). The other 23% of the P introduction Utah Lake occurs via urban and agricultural runoff, atmospheric deposition, and natural runoff. Due to the large surface area and shallow depths of Utah Lake, evaporation and mixing rates are high, resulting in high calcite formation rates, which removes some of the introduced P.

Bioassay Protocol

We performed bioassay experiments to establish growth responses to increased nutrient concentrations. We based bioassay protocol on methods in Xu et al 2015. We collected water samples from ~10 cm depth and filtered them through a Wisconsin net (153 micrometer mesh) to remove zooplankton and grazers (excluding late season zooplankton inclusion bioassay). We then divided samples into sterile 1-gallon Cubitainers (Hedwin Corporation, Newark, DE, 85% PAR transmittance) for incubation in floating corrals. We separated samples into four treatment groups: control, nitrogen (N), phosphorus (P), and a combination of N and P treatment (N+P). We performed the N, P, and N+P treatments by directly adding 1 mL of a specific stock solution to respective treatment Cubitainers: the P addition equaled an increase in 0.10 mg-P/L above background concentrations added as K_2HPO_4, the N addition equaled an increase in 0.72 mg-N/L added as NH_4NO_3 to achieve a 16:1 molar ratio of DIN:SRP, and the N+P treatment was the combination of the N and P additions. We also treated all samples with 1 mL of NaHCO3 to remove to alleviate CO_2 limitation to photosynthesis, at a rate to support production of 100 ug/L chlorophyll, based on preliminary inorganic C levels in the lake. Each treatment group

contained three replicates for pre and post incubation, totaling 72 samples per experiment (3 sample sites * 2 collections * 4 treatments * 3 replicates). During sample collection, we used a YSI EXO2 sonde (Yellow Springs Instrumentation, Yellow Springs, OH) to measure ambient conditions.

We incubated samples in floating corrals in an enclosed marina on the eastern shore of the lake to maintain similar temperature and light intensity to collection areas. We placed shade covers over the corrals to limit PAR by an additional 30% (total reduction ~45%) to avoid photoinhibition of photosystem II in phytoplankton (Aro, Virgin, and Andersson 1993; Häder et al. 2015). Wave action within Utah Lake State Park is limited by fetch but was sufficient for sample mixing. Depending on if we collected initial samples from an active bloom area or not, we incubated the samples for 48 hours (active bloom) or 72 hours (non-bloom) to allow for clear quantification of the growth and nutrient response. Following incubation, we collected subsamples from selected samples for acetylene reduction assay, then transported them all to the BYU Microbial Ecology Lab (Provo, Utah, USA) for filtering and analysis (details below).

Dilution Bioassay

We performed a dilution bioassay experiment to identify thresholds when N and P become limiting to algal growth. We followed the methods from Xu et al. (2015). We used samples from the East site, which experiences median algal bloom intensity and human impact between our three sites. Dilution of bioassay samples reduces the amount of ambient nutrients and enables nutrient additions to uncover growth thresholds (Xu et al. 2015; Carrick et al. 1993; Anderson et al. 2019).

In the dilution bioassay experiment, we followed the same protocol as above, but diluted samples with a major ion solution which contained ionic concentrations similar to Utah Lake at

the same collection site excluding N and P (concentrations shown in Table 1), resulting in samples that contained 50% (1.5 L) lake samples and 50% (1.5 L) MIS. As above, we injected nutrients based on treatment group, but separated additions into "high" and "low" additions, with 1mL and 0.5mL of stock solution added, respectively. We included undiluted samples as a control to assess if the major ion solution influenced bloom response. We collected samples after 2 and 5 days of incubuation.

Acetylene Reduction Assay

We used an acetylene reduction assay to measure N fixation potential in bioassay samples. After sample incubation, we set aside a portion of each samplefor acetylene reduction assay. We followed protocols from Marcarelli and Wurtsbaugh 2009, with some adaptations for purely water samples. This experiment indirectly measures N fixation rates by measuring activity of nitrogenase. N fixation, which is usually performed by converting dinitrogen gas to ammonium, is performed by microbes using nitrogenase (Berman-Frank et al. 2007). However, this enzyme "fixes" acetylene into ethylene when dinitrogen is unavailable (Breitbarth et al. 2004). By depriving our samples of dinitrogen and adding a known amount of acetylene, we measure nitrogenase activity and therefore N fixation potential.

We measured N fixation potential by incubating samples for 4 hours with pure acetylene and measuring resulting ethylene concentrations before and after incubation. We produced Acetylene-site by reacting calcium carbonate pellets with water in an air-tight environment and funneling the resulting acetylene gas into a sterile balloon. We placed samples into an air-tight mason jar along with the acetylene balloon and checked for air bubbles. To start the incubation, we popped the balloons, exposing the sample to acetylene gas, and placed them into the floating corrals described above to maintain similar temperature and mixing among samples. We collected gas samples before and after incubation and analyzed the samples via gas

chromatography using a PoraBOND Q (Agilent, Santa Clara, CA) column. We calculated N-fixation rates as change in ethylene concentrations over incubation time.

DNA Collection and Pigment Analysis

Following incubation, we transported samples to the lab and immediately filtered 250-500 mL through 0.2 μm pore size GF/F filters for DNA collection and 500mL through .45 μm pore size filters for pigment analysis. Until extraction, we stored DNA filters at -80° Celsius and pigment filters at -20° Celsius. We evaluated total phytoplankton and cyanobacteria as shifts in chlorophyll-a and phycocyanin concentrations, respectively. We analyzed chlorophyll-a via ethanol extraction and evaluation on a microplate spectrophotometer (Spectramax Plus, Molecular Devices, LLC, San Jose, CA) at a wavelength of 665 and 750 nm. Shifts in chlorophyll-a represented general trends in total phytoplankton. We measured phycocyanin, a major phycobiliprotein pigment produced by cyanobacteria, via a phosphate buffer extraction and spectrophotometry (Kasinak et al 2014). We extracted DNA from 0.2 μm filters using D6011 Zymo Research Quick-DNA kits (Zymo Research, Irvine, CA), strictly following provided protocols, with an extra step during initial sample collection after bead beating, where we removed filters to make sample collection easier. Despite this extra step, gel electrophoresis indicated extractions were viable. We matched sequences to bacterial taxa via QIIME2 (Bolyen et al. 2018), and exported matched reads for further filtering and analysis using the "Phyloseq" package (McMurdie and Holmes 2013) in R Studio.

Cyanotoxin Quantification

We measured three cyanotoxins–microcystin, cylindrospermopsin, and anatoxin-a using ADDA, anatoxin-a, and cylindrospermopsin enzyme-linked immunosorbent assays or ELISA.

We chose specific toxins based on the dominant cyanotoxins found in Utah Lake (Collins 2019): anatoxin-a by *Aphanizomenon flos-aquae* (Mahmood and Carmichael 1987), cylindrospermopsin primarily by *Cylindrospermopsis raciborskii* (Kinnear 2010), and microcystin by *Microcystis aeruginosa* (Backer et al. 2010). We collected Water for the cyanotoxin analyses from samples before filtration in ashed amber glass vials with a PTFE-lined lids. Anatoxin-a samples received a preservative immediately upon collection to prevent sample degradation. We then analyzed the toxins using the appropriate enzyme-linked immunosorbent assay kit (Eurofins Abraxis, Warminster, PA). Detection limits were as follows: 0.10 ppb microcystin, 0.10 ppb anatoxin-a, 0.04 ppb cylindrospermopsin. Because of the high cost of analysis, we measured the three cyanotoxins in only two of the three replicates for each location, season, and treatment combination.

Statistical Tests and Figures

We used two-way analysis of variance (ANOVA) to test statistical significance of change in photosynthetic pigment, cyanotoxin concentration, and N-fixation potential, each using site and treatment as explanatory variables. A significance value of ≤ .05 applied to all statistical tests. We created all figures and performed statistical tests in R version 4.1.1 (R Core Team (2022)). A full list of R packages used is provided in table 3.

RESULTS

Nutrient Thresholds for Algal Blooms

Total phytoplankton increased in all treatment groups after full incubation time, with a clear division between groups with and without P additions around 3.5 mg/L chlorophyll-a (figure 2). We treated all groups that experienced higher amounts of growth with P, indicative of

P limitation in original samples. Total cyanobacteria showed fewer differences, though treatment groups that experienced the most growth were the high P addition and the high N+P addition. Our intermediate timestep contained very low concentrations of phycocyanin, indicating a cyanobacterial population crash sample processing error.

Treatment groups that experienced less growth over 120 hours all contained lower amounts of soluble reactive phosphorus (SRP). The three treatment groups that never crossed the 3.5 mg/L division (Low N, High N, dilution control) contained the smallest amounts of SRP (.005 mg/L). All groups that crossed this threshold had higher concentrations of SRP. However, treatment groups on each site of the 3.5 mg/L threshold contained variable amounts of dissolved inorganic nitrogen (DIN), with no correlation between growth and DIN concentration. The high correlation between SRP and growth and the lack of correlation between DIN and growth is indicative of single P limitation.

SRP concentrations decreased in all treatments after the full incubation time, with the largest decreases occurring in groups with added P, showing the uptake of newly available P to previously limited conditions (figure 2C). In most medium nutrient treatments, SRP declined quickly in the first 48 hours, while SRP declined gradually in high nutrient addition treatments. No such effect was apparent in DIN concentrations, which did not decline during the incubations.

Cyanobacterial influence

Cyanobacteria are capable of intensifying algal blooms by producing harmful toxins and fixing atmospheric N, so understanding the community composition of algal blooms is key to understanding the threat that algal blooms pose. Bacterial community composition of samples from two sites is shown in figure 3, one of which (Provo Bay) was collected during bloom

conditions. The top six phyla shown compose 97.9% of reads. Dominant phyla differ mostly between sites, with East samples containing much more *Proteobacteria* and *Bacteroidetes* than Provo Bay samples, which contained relatively more *Cyanobacteria*, *Verrucomicrobia*, and *Planctomycetes*.

Nutrient additions altered relative abundance of phyla. In non-bloom conditions, most of the variation in relative abundance was determined by whether the samples were from a nutrient-treated group or a control sample. Control group samples contained a much higher percentage *Proteobacteria* than nutrient groups (Control: 52.45%, Nutrient addition: 29.50%) and a much lower percentage *Bacteroides* (Control: 8.32%, Nutrient: 18.52%) and *cyanobacteria* (Control: 17.69%, Nutrient: 39.41%). This was consistent with the PCoA plots (figure 5), demonstrating that variation post-incubation was divided between control group and all nutrient groups. When only considering nutrient-treated samples, there were several differences between the N and P groups. N-treated samples contained a higher percentage of *cyanobacteria* than P-treated samples (N: 43.11%, P: 29.60%) and a lower percentage of *Bacteroidetes* (N: 16.59%, P: 22.65%).

Nutrient addition largely determined the community composition in Provo Bay. The largest difference in composition is between the N and P treatment groups, with the N+P group exhibiting characteristics of both. The N group contained a lower percentage of *Verrucomicrobia* than the P group (N: 20.05%, P: 30.06%) and a higher *cyanobacteria* percentage (N: 43.87%, P: 33.79%). Despite the compositional differences between collection sites, there are some common patterns. In both locations, samples that we treated with N resulted in higher percentage of *cyanobacteria*. This could indicate a N limitation on blue-green algae growth across different lake conditions, despite the N-fixing potential of *cyanobacteria*.

The cyanobacterial community (figure 4) varied among sites and treatment groups. All samples were dominated by three main cyanobacterial genera: *Synechococcus,*

Dolichospermum, and Planktothrix. Small amounts of *Cylindrospermopsis, Microcystis, Snowella*, and unidentified families were measured in certain samples. Relative abundance of *Synechococcus* was much higher in East samples, especially in control group samples, while *Dolichospermum* relative abundance was much higher in Provo Bay samples.

Some of the patterns described above are reinforced by PCoA plots of microbial communities. When all samples are included, the variance between control and nutrient samples is further reinforced, with T_0 samples being distant from the tightly clustered T_1 samples (figure 5A). Note that Provo Bay T_0 DNA extractions did not yield enough data for sequencing. Individual examination of the nutrient-treated samples from each site (figure 5 B/C) shows no clear patterns in the East samples, though N-treated samples from Provo Bay are not as tightly clustered with P and N+P samples.

Nitrogen Fixation Potential

Based on cyanobacterial community, a potentially large input of N occurs through aquatic nitrogen fixation. In our active bloom samples, the higher relative abundance of cyanobacteria, especially *Dolichospermum*, could indicate increased N-fixing potential. Active bloom samples exhibited the highest rates of N-fixation across all treatments (figure 6). Two-way ANOVA results indicate sample site as statistically significant ($P = 0.001$) in determining N-fixation rates. Based on the pairwise comparison, East and West samples are not significantly different from each other (adjusted P value: 0.904). Between Provo Bay samples, N-fixation rates were highest in N+P samples. Control and N samples indicated similar rates, whose average was lower than P and N+P groups. P samples had much more variability but exhibited a higher mean N-fixation rate than control and N groups.

Toxin production

The other concern in active blooms is the production of cyanotoxins by cyanobacteria. Of the three toxins measured, anatoxin-a had the highest concentration at all sites (mean: 0.42 µg/L, SE=0.01), microcystin slightly lower (mean: 0.32 µg / L, SE=0.01), and cylindrospermopsin was only present in trace amounts (mean: 0.01 µg / L, SE=0.003). This did not differ among sites or treatments enough to be considered statistically significant (P-value (site): 0.53, P-value (treatment): 0.92). Cylindrospermopsin samples from the West site in N and N+P treatments did not contain enough cylindrospermopsin to be measured.

Despite measurements of multiple toxins across all treatments and collection sites, there was little relationship found between toxin concentrations and biotic or abiotic parameters. Results of two-way ANOVAs of toxin concentrations by collection site and nutrient addition indicated no significant relationships existed (all P-values > 0.05). Toxin concentrations were consistent across all collection sites despite differing algal abundance and nutrient concentrations.

Zooplankton Influence

Predation by zooplankton may influence the phytoplankton to cyanobacteria ratio, altering bloom function and composition. During the incubation, algae were preferentially grazed over cyanobacteria only in active bloom conditions (table 2). In the main body of the lake, the inclusion of zooplankton relative to the exclusion of zooplankton caused chlorophyll-a and phycocyanin concentrations to decrease by at least 3.8 and 54-fold respectively. In the East and West water, zooplankton grazed the cyanobacteria, measured as phycocyanin concentrations, to almost non-detection levels. Alternatively, in Provo Bay water, the inclusion

of zooplankton led to an increase in chlorophyll-a concentrations across all treatments and the control. For cyanobacteria in the bay, the inclusion of zooplankton induced at least a 1.8-fold decrease phycocyanin concentrations.

DISCUSSION

Despite the large and growing population in Utah Lake's watershed, it is extremely resilient to harmful algal blooms (HABs), mostly due to features common to shallow lakes. At the current level of nutrient runoff into the lake, HABs would be continuous, but are discouraged due to the following factors: 1) Utah Lake has a large surface area (2950 square miles) which results in high evaporation rates and rapid calcite formation. The process of calcite formation causes nutrients and dissolved organic carbon to precipitate from the water column and be buried in sediments, effectively shielding them from algal use (Küchler-Krischun and Kleiner 1990). 2) With calcite formation, large fetch, and shallow depths (average 9 feet), Utah Lake is very mixed and barely ever stratifies. This keeps the surface of the lake very cloudy, reducing light penetration, and therefore, primary productivity, reducing algal growth. This also eliminates dead zones that would usually form beneath HABs and lead to massive fish kills. 3) This constant mixing also keeps sediments oxygenated, which limits the amount of nutrient release from sediments. These factors reduce the amount and severity of HABs within Utah Lake, which would otherwise exhibit constant, severe blooms (Abbott et al. 2021).

Despite Utah Lake's resilience factors to HABs, there are some factors that threaten to destabilize the ecosystem and increase HABs: 1) Utah Lake's watershed, especially on the East side, has one of the United States' highest rates of population growth by county (Utah County), with a population doubling occurring by 2060 (from census population in 2000, David Eccles School of Business). Increased population has potential to increase the nutrient runoff into the lake. 2) Climate change increases average temperatures worldwide, and is increasing drought

conditions in Utah Valley (Gillies, Wang, and Booth 2012). This reduces the water level of Utah Lake and increases residence time of nutrients, both of which exacerbate HABs. 3) Capitalizing on population growth projections and poor public opinion of Utah Lake, proposed dredging and island formation threatens to destabilize Utah Lake's ecosystem and eliminate the previously mentioned resilience factors that reduce HABs. Despite these risk factors, recent studies have found that algal bloom prevalence has been decreasing in recent centuries due to remediation efforts (Tate n.d.; Abu-Hmeidan, Williams, and Miller 2018; Coffer et al. 2021). Restoration of wetlands, reduction of nutrient outflow from wastewater treatment plants, and removal of invasive species have reduced the amount and severity of HABs. Further efforts in these areas, supplemented by peer-reviewed research into shallow lake ecosystems, will further reduce HABs.

Nutrient Thresholds for Limitation:

Our dilution bioassay experiment identified thresholds for phytoplankton at soluble reactive phosphorus (SRP) concentrations ≤ 0.005 mg/L and dissolved inorganic nitrogen (DIN) concentrations ≤ 0.14 mg/L (figure 1). Our threshold values were within range of similar studies that identified nutrient thresholds to limit algal growth, such as Anderson et al 2019, who found a significant growth limitation at 0.56 mg/L DIN and a marginally significant growth limitation at 0.005 mg/L SRP. This is supported by Xu et al 2015, who found significant growth limitations at 0.63 mg/L DIN and a marginally significant growth limitation at 0.049 mg/L SRP.

Based on seasonal data from around Utah Lake, SRP concentrations exceed our threshold of 0.005 mg/L primarily during spring runoff and late summer (figure 8). Data from 2017 (the most recent year where SRP was monitored), indicates SRP concentrations exceeding our threshold of 0.005 mg / L during spring runoff and late summer, which is highly correlated with algal bloom presence. DIN concentrations primarily exceed our threshold of 0.14 mg / L

from spring to early summer, and slightly during mid-summer. When examining seasonal trends in SRP and DIN concentrations (figure 8), SRP concentrations are much better correlated with HAB presence, which peaks in mid to late summer. This may represent a cause or an effect of HABs, where either the presence of SRP is a product or a result of HABs. To address this, SRP (and DIN) could be measured at wastewater treatment plant outflow and at fixed points throughout the lake to determine the source of this correlation.

Cyanobacterial Composition and Activity

Cyanotoxin production by cyanobacteria remains one of the key threats posed by HABs, though relationships between cyanobacterial species, abiotic factors, and toxin production rates are difficult to define and are probably the result of a combination of many conditions (Neilan et al. 2013; Hartnell et al. 2020). We were not able to correlate toxin concentration with any environmental or community parameters, though we did find consistent concentrations across lake zones. When examining the relationship between cyanobacterial genus relative abundance and toxin concentration, there was one relationship (P value: 0.02) between Synechococcus and microcystin. Synechococcus is capable of producing microcystin (Gulledge et al. 2002), though overall reactive abundance of specific cyanobacterial families was not a good metric to determine toxin concentrations. Based on our three most abundant genera, various non-measured toxins could be produced, such as cyanopeptolin, homoanatoxin-a, saxitoxin, or BMAA (β-N-methylamino-l-alanine) by planktothrix alone (Rastogi, Madamwar, and Incharoensakdi 2015).

Unlike toxin production, N-fixation rates differed depending on collection site and nutrient treatment. Samples from Provo Bay (active bloom) exhibited significantly higher N-fixation rates, especially in the N+P treatment group. Sites that were not in active bloom exhibited much lower rates of N-fixation, though rates were slightly increased in treatments

including both N and P. This could seem counter-intuitive since N-fixation is used by cyanobacteria to address N limitation but is still encouraged by N additions. However, N-fixation is a very costly process (16 ATP per N_2 molecule), so utilization of dissolved N is much easier for cyanobacteria. The addition of nitrogen in our samples likely primed the cyanobacteria prior to acetylene reduction assay and enabled higher rates of N-fixation. This reflects in important dynamic within blooms, where the amount of N released into blooms does have consequences for further N additions via cyanobacterial N-fixation. Runoff that has high concentrations of N and P will result in increased N-fixation potential in cyanobacterial blooms.

Utah Lake Zooplankton Reduce Harmfulness of Blooms

The zooplankton inclusion experiment occurred during late season bioassay experiments, and unexpectedly indicated selective grazing of cyanobacteria over phytoplankton (figure 7). Several previous studies have shown the opposite effect, where zooplankton selectively feed on other forms of phytoplankton rather than cyanobacteria (Fernández, Estrada, and Parodi 2015; Jackson 1980; L. L. Yuan and Pollard 2018), citing a few possible explanations including lack of highly unsaturated fatty-acids in cyanobacteria (Bednarska and Dawidowicz 2007), cyanobacterial colony-forming (Persson et al. 2007), and cyanotoxin production (DeMott, Zhang, and Carmichael 1991). However, Utah Lake's special qualities provide an explanation for our unexpected result. Utah Lake has unusually low zooplankton species richness, with zooplankton that are often smaller than those found in nearby water bodies (Richards 2019), which may result from the unnatural top-down effect of introduced planktivorous fish feeding on zooplankton communities (Sondergard et al 2008). In addition, the genus that dominated the cyanobacterial component of our samples, *Synechococcus*, is significantly smaller than other cyanobacteria (Partensky, Blanchot, and Vaulot 1999). The smaller size of Utah Lake zooplankton could be better adapted to consuming small, unicellular

Synechococcus than larger filamentous green algae and other phytoplankton. This would explain the preferential grazing of cyanobacteria over total phytoplankton and could indicate the positive effect of Utah Lake zooplankton on bloom harmfulness.

LITERATURE CITED

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FIGURES

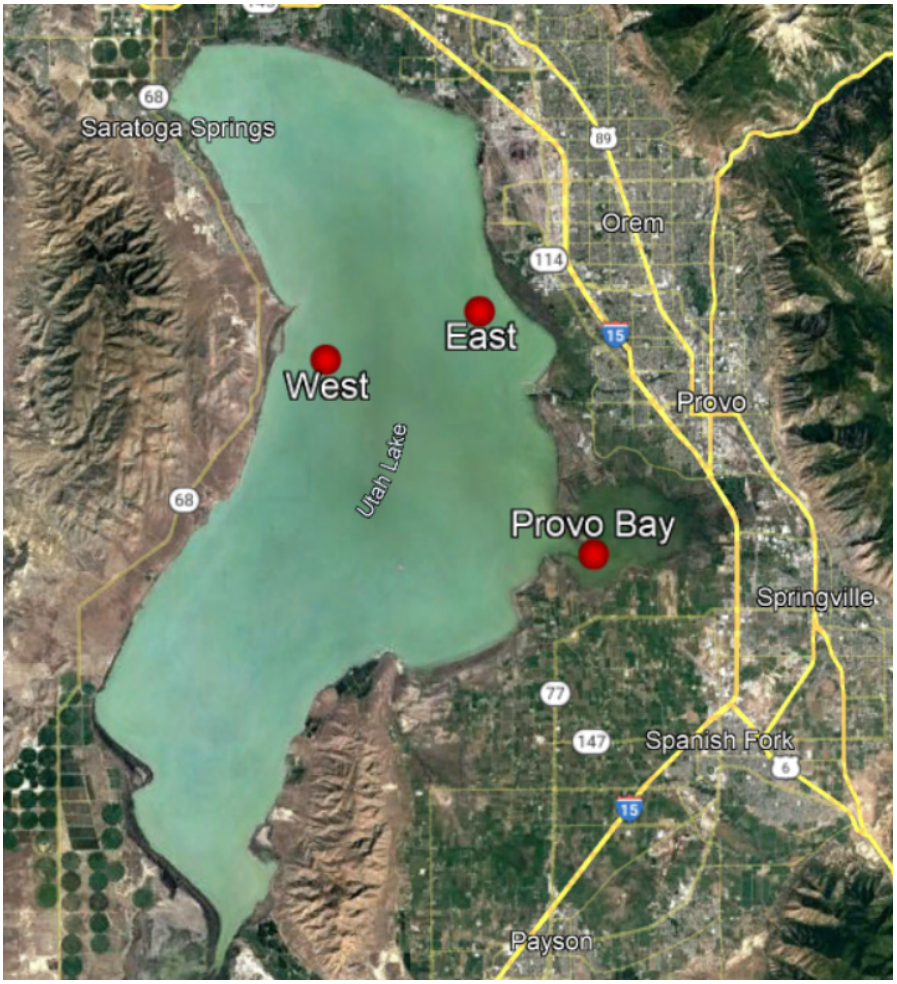

Figure 1. Map of Utah Lake with Sampling Points. Map collected by Google Earth Pro. Coordinates for sampling points are: East: 40°14'16"N, 111°45'56"W, West: 40°15'33"N, 111°50'22"W, and Provo Bay: 40°10'42"N, 111°42'41"W

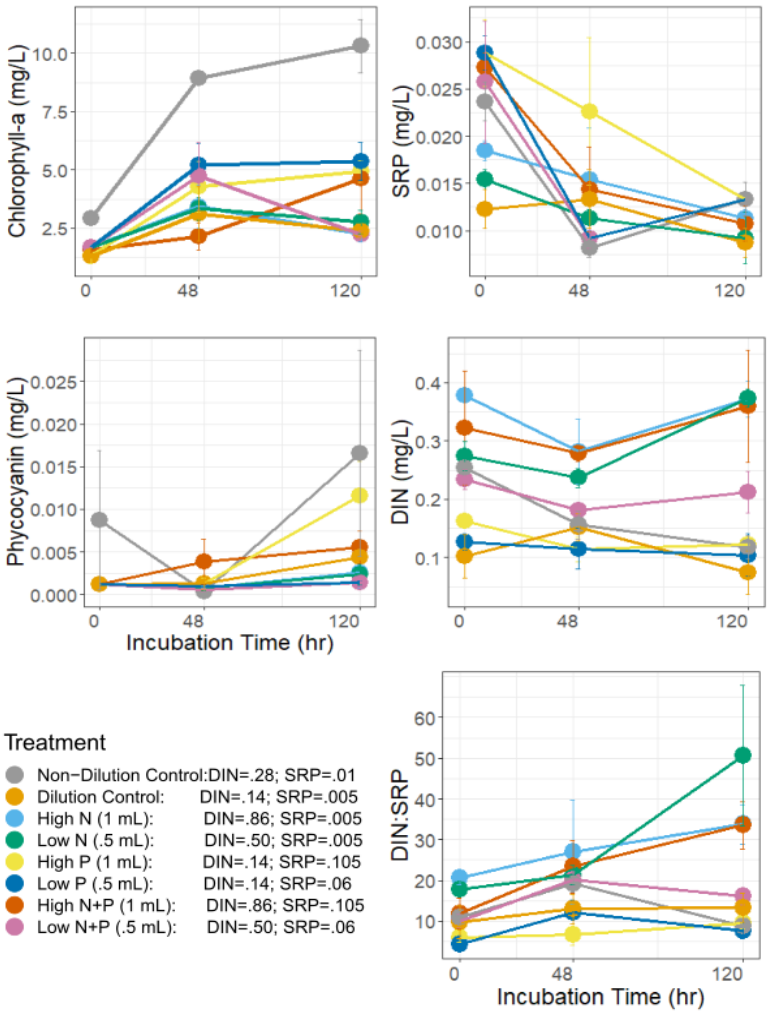

Figure 2. Nutrient limitation of Cyanobacteria and phytoplankton. Measurements were taken at 0, 48, and 120 hours. A) Chlorophyll-a concentrations as proxy for phytoplankton abundance. B) Phycocyanin concentration as proxy for cyanobacteria abundance C) Soluble Reactive Phosphorus (SRP) concentrations, measured due to bioavailability D) Dissolved Inorganic Nitrogen (DIN) concentrations, measured due to bioavailability E) SRP:DIN ratio.

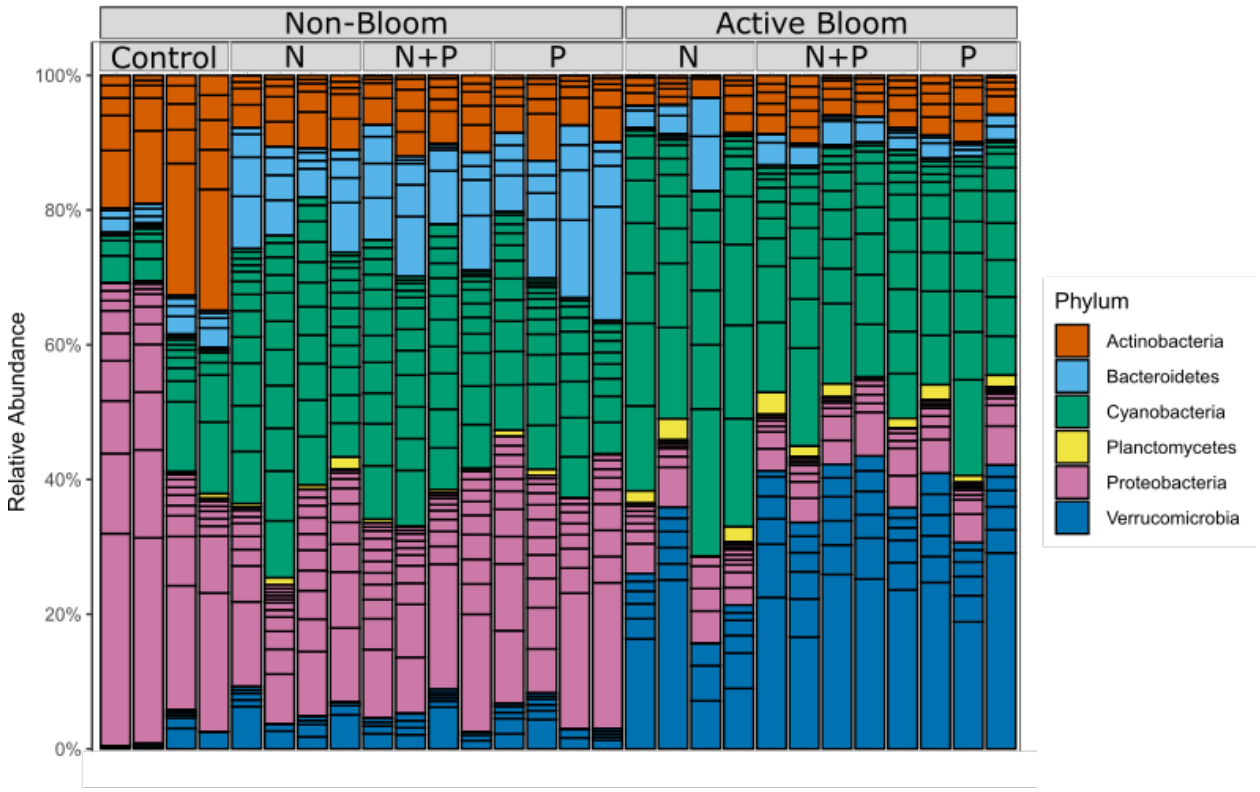

Figure 3. Bacterial relative abundance among non-bloom and active bloom conditions. Samples shown are from post-incubation samples during late season bioassay and are separated by treatment group. Data from active bloom control group were too low quality to include.

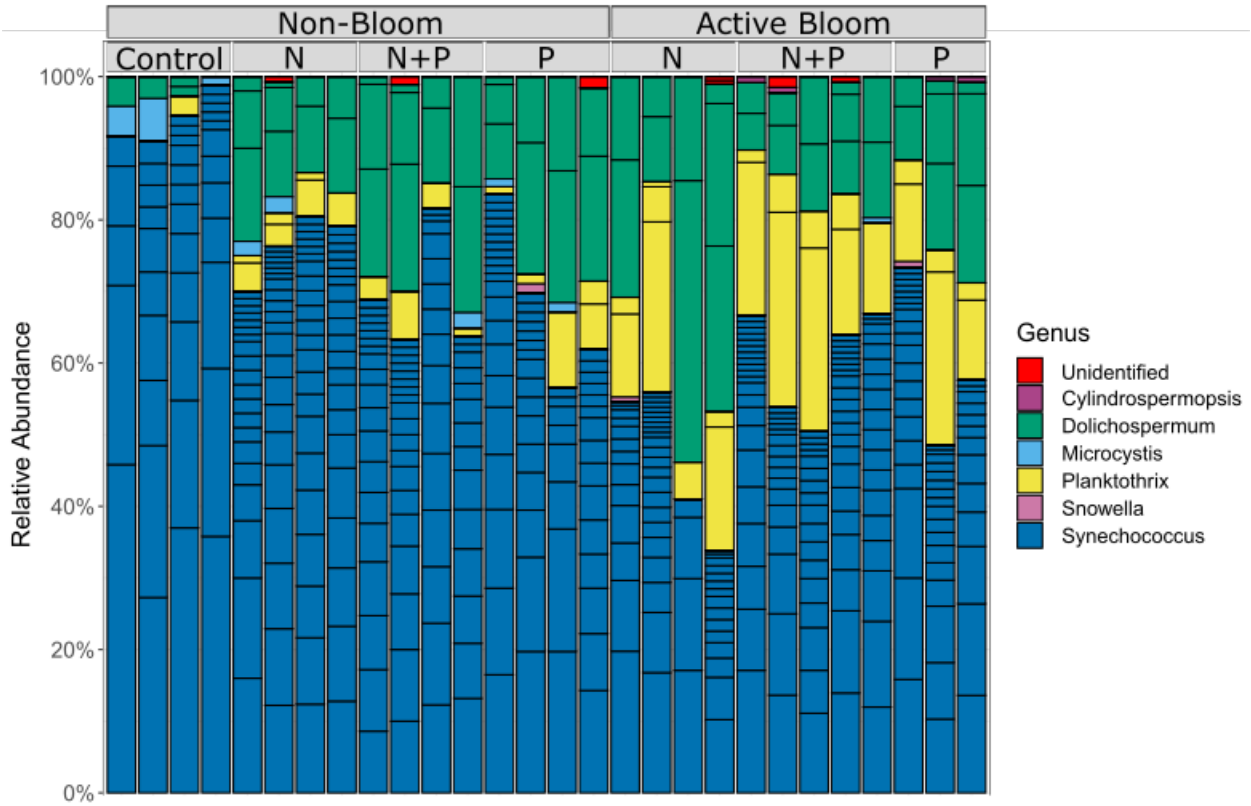

Figure 4. Cyanobacterial relative abundance among non-bloom and active bloom conditions. Samples shown are from post-incubation samples during late season bioassay and are separated by treatment group. Data from active bloom control group were too low quality to include.

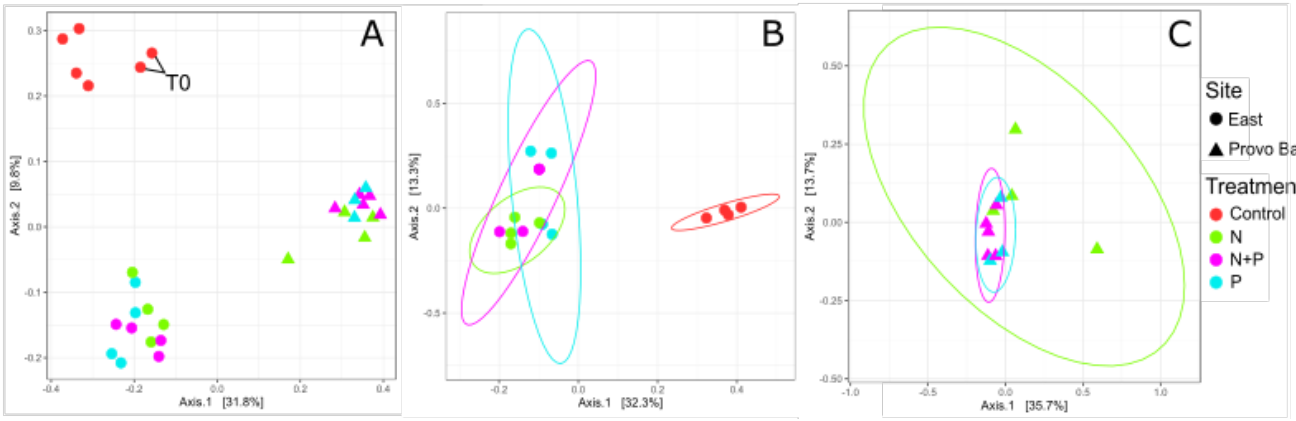

Figure 5. Principal Coordinate Analysis plots based on bacterial community of late season samples. A) All samples collected, including all sites, time points, and treatments, B) All East post-incubation samples, C) All Provo Bay post-incubation samples.

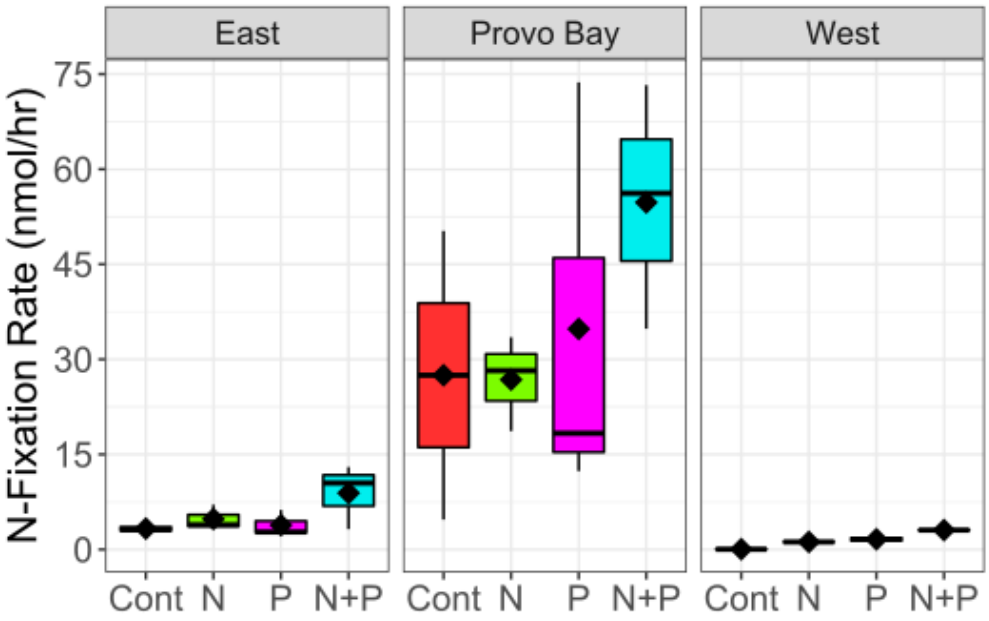

Figure 6. Nitrogen fixation rates were highest in active bloom samples and nitrogen + phosphorus additions. All acetylene incubations were performed on samples post-bioassay incubation.

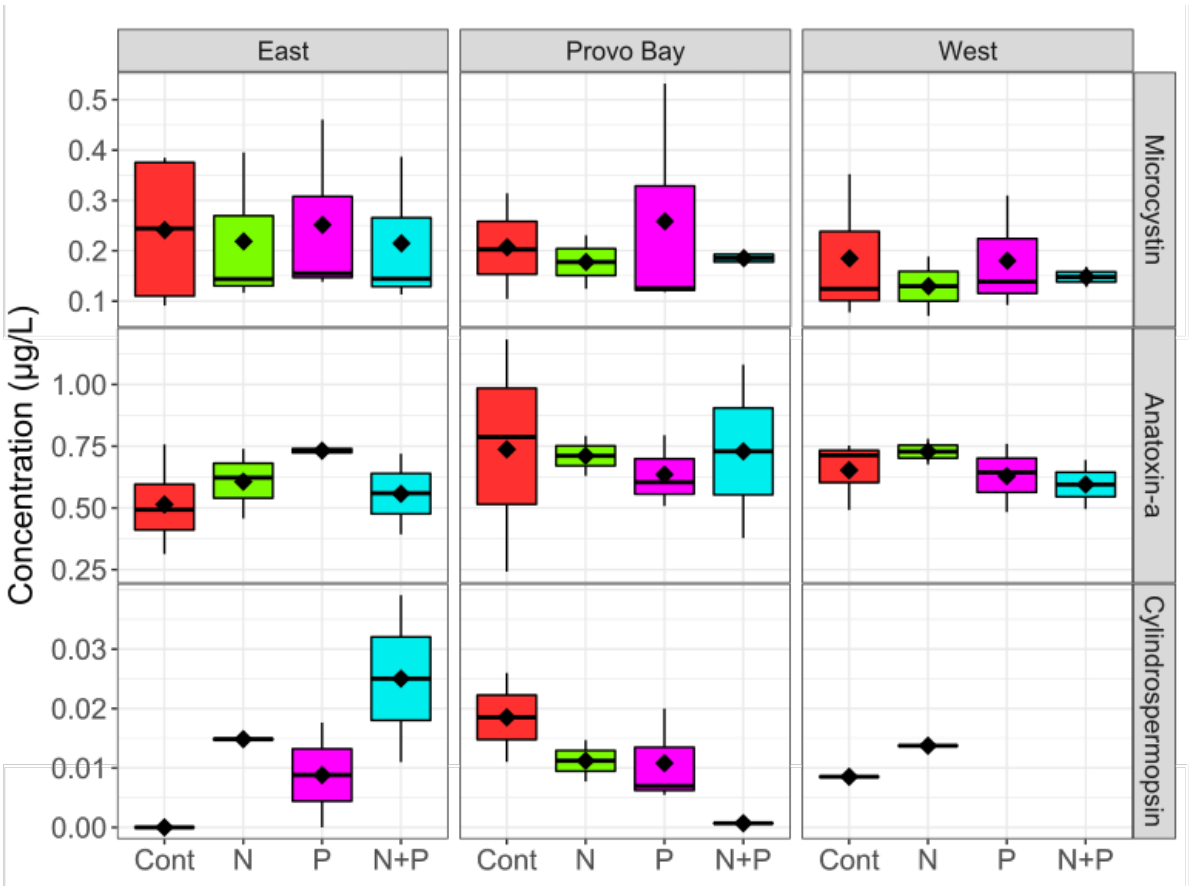

Figure 7. Cyanotoxin concentrations did not differ between sample collection site or treatment. All measurements were taken from post-incubation samples in late season bioassay.

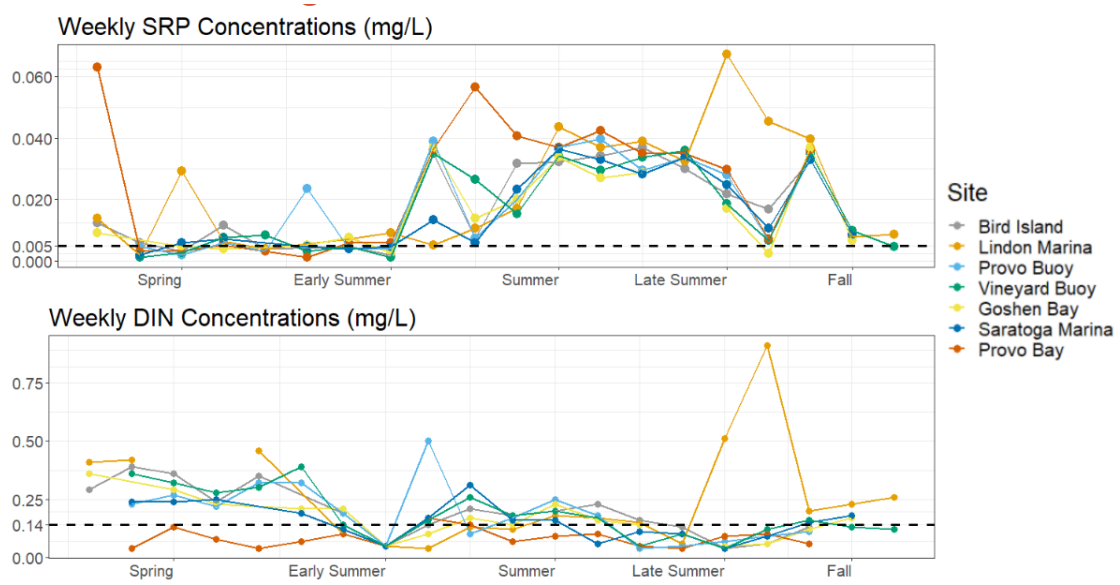

Figure 8. Weekly soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) concentrations from sites around Utah Lake during 2017. Previously defined nutrient thresholds are indicated with dotted lines. Data collected by Scott Collins of the Central Utah Water Conservancy District.

TABLES

Table 1. Concentrations of major ions in Major Ion Solution and chemical forms added, based on ionic concentrations from previous Utah Lake samples. MIS was used to in dilution bioassay experiment to dilute lake samples.

Ion	Chemical Form	Final Concentrations in MIS (mg/L)
Si^{4+}	$Na_2SiO_3*9H_2O$	.037
Ca^{2+}	$CaCl_2*2H_2O$	44.0
Mg^{2+}	$MgSO_4*7H_2O$	77.0
Na^{+}	Na_2SO_4	50.0
K^{+}	K_2SO_4	10.6
SO_4^{2-}	$MgSO_4*7H_2O$	304
Cl^{-}	$CaCl_2*2H_2O$	165

Table 2. Concentrations (μg/L) of chlorophyll-a and phycocyanin with zooplanktonic grazers included and excluded in the nutrient treatments. Values are means for grazers excluded (n=3) and included (n=2) from a EXO2 multi-parameter sonde.

Location	Treatment	Chlorophyll-a		Phycocyanin	
		plus grazers	minus grazers	plus grazers	minus grazers
EAST	Control	2.28 ±0.870	8.72 ±0.344	0.01 ±0.005	0.540 ±0.56
	N	2.48 ±1.07	48.2 ±4.81	0	2.62 ±0.254
	P	4.84 ±3.44	40.2 ±8.84	0.01 ±0.035	2.08 ±0.344
	N+P	3.90 ±2.49	55.8 ±5.64	0.01 ±0.045	2.64 ±0.333
WEST	Control	2.56 ±1.17	21.5 ±0.558	0.01 ±0.01	0.960 ±0.051
	N	2.41 ±1.01	18.4 ±0.649	0.01 ±0.005	0.870 ±0.006
	P	4.49 ±3.09	22.1 ±2.51	0.01 ±0.055	0.953 ±0.087
	N+P	3.97 ±2.57	23.6 ±4.78	0.01 ±0.050	0.990 ±0.107
PROVO BAY	Control	78.2 ±10.4	41.5 ±5.57	3.27 ±0.340	5.21 ±2.00
	N	101 ±12.9	55.7 ±2.61	4.26 ±0.645	7.71 ±0.254
	P	76.5 ±12.1	44.8 ±2.13	3.22 ±0.390	7.26 ±0.155
	N+P	89.3 ±0.660	57.7 ±2.61	3.76 ±0.145	7.09 ±0.274

Table 3. Full list of R packages used in sample analysis.

R Package	Full Citation
phyloseq v. 1.36.0	McMurdie and Holmes (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217
ggplot2 v. 3.3.6	Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York.
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CHAPTER 2

Nutrient Effects on DOM Degradation and Microbial Community in Arctic Streams

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ABSTRACT

Climate change is warming arctic ecosystems much faster than the worldwide average, potentially reactivating a huge amount of bioavailable carbon in permafrost soils. Changes in seasonal water flow encourage the movement of these newly liberated nutrients toward streams and rivers where nutrients and dissolved organic carbon (DOC) are rapidly mineralized and partially released as greenhouse gasses, exacerbating climate change and further permafrost thaw. I used nutrient and labile carbon additions to simulate the effects of permafrost thaw DOM degradation and microbial community in three distinct permafrost-covered catchments on the North Slope of Alaska. The alpine catchment had higher biodegradability but lower DOM concentration across seasons compared with the lake-influenced and tundra catchments. For all catchments, there were strong seasonal changes in microbial community and distinct responses to nutrient addition. The addition of nutrients stimulated DOM biodegradation in the late season—the period of the year when permafrost DOM release typically occurs. Microbial communities differed by catchment type, but overall diversity was similar. These results indicate potential changes to permafrost DOC biodegradability and community response to increased permafrost nutrient flux.

INTRODUCTION

Arctic permafrost thaw is accelerating due to increasing Arctic temperatures, fundamentally changing aquatic system dynamics (Biskaborn et al. 2019; Nitze et al. 2018). A concern associated with permafrost thaw is the increased activity of soil and aquatic microbes, resulting in the release of soil carbon as CO2 or other greenhouse gasses that exacerbate global warming (Cory et al. 2013; Olefeldt et al. 2016; Schuur et al. 2015). Permafrost thaw exposes an enormous storage of nutrients which have been frozen for potentially tens of thousands of years but remain highly labile (Drake et al. 2015; Ewing et al. 2015; Abbott et al. 2014; Vonk, Tank, Mann, et al. 2015). Changes in seasonal water flow encourage the movement of these newly liberated nutrients toward streams and rivers where nutrients and DOM (dissolved organic matter) are rapidly mineralized in headwaters (Kendrick et al. 2018; Vonk, Tank, Bowden, et al. 2015; Wickland et al. 2018) by opportunistic microbes. Mineralization rates of these DOM and nutrients vary due to permafrost type, nutrient source, light interaction, and topography (Stubbins et al. 2017; Wickland et al. 2018; Cory et al. 2014).

In the permafrost zone, several compelling longitudinal patterns in dissolved organic matter have been observed, including decreases in DOM radiocarbon age, biodegradability, and photodegradation (Cory et al. 2014; Mann et al. 2015; Drake et al. 2015; Vonk, Tank, Mann, et al. 2015). These patterns are typically attributed to in-stream processing, which would suggest that DOM and nutrients released from degrading permafrost are quickly consumed or retained in headwaters. However, residence time in permafrost river networks is typically on the scale of days to weeks, limiting the time biotic and abiotic reactions can modify DOM, even compared with laboratory rates of DOM processing at elevated temperatures (Cory et al. 2013; Abbott et al. 2014; Vonk, Tank, Mann, et al. 2015).

As warmer temperatures increase permafrost thaw depth and hydrologic connectivity, microbial activity, community, and dispersal become less constrained by prolonged frozen conditions (Bottos et al. 2018; Liu et al. 2021). Changes in permafrost physicochemical

conditions are reflected in altered community composition and functional gene abundance, with communities adjusting to carbon lability (M. M. Yuan et al. 2018; Liu et al. 2021). These changes to community structure and functional gene abundance optimize organic carbon mineralization rates, resulting in accelerated greenhouse gas release (Kwon et al. 2019). Due to increased hydrologic connectivity from increased permafrost thaw, the changes in soil microbial community and function extend to permafrost streams, where soil organic carbon is rapidly mineralized (O'Donnell et al. 2016; Paul J. Mann et al. 2015; Bottos et al. 2018). Thus, understanding how stream microbial composition is altered by carbon lability and permafrost thaw is vital to understanding how permafrost streams contribute to greenhouse gas release and permafrost climate feedback.

Using stream chemistry data, eDNA, and incubation analysis from multiple points within three distinct permafrost-covered catchments, we test the effects of nutrient addition and priming on DOM degradation and microbial community. We expand on past studies that have focused on single catchments or disturbance affected areas (Abbott et al. 2014; Vonk, Tank, Mann, et al. 2015) by sampling multiple points along three characteristically different permafrost-dominated catchments during early and late season flow. We hypothesized that early season samples across all catchments would exhibit higher DOM degradation rates over incubation time due to shorter hydrological residence times and recent leaf litter DOM sources during spring freshet (P. J. Mann et al. 2012). We also hypothesized that microbial communities would be more homogenous in permafrost zones with more hydrologic connectivity, where dispersal is less limited (Bottos et al. 2018).

METHODS

Study site

To test our hypotheses, we sampled 140 catchments near the Toolik Field Station on the North Slope of Alaska in the early and late season of 2016 and 2017. These catchments were selected across three permafrost zones, each representative of different permafrost "zones". This included the Kuparuk, Oksrukuyik, and Trevor Creek catchments, which represent tundra permafrost, lake-rich permafrost, and alpine permafrost zones respectively. These catchments have been the focus of previous studies conducted by the Arctic Long Term Ecological Research Network (LTER). These three catchments characterize distinct and important areas of the permafrost zone. We stratified sampling by catchment size, including small catchments with diverse landscape positions and catchment characteristics (e.g., surficial geology, vegetation, disturbance history, and topography). Sample catchment sizes varied from 1-1000 km^2 and covered a range of landscape positions.

Sample collection, treatment, and incubation

We collected samples via two synoptic samplings of each catchment during the summer of 2017. We performed the sampling parallel to biannual sampling of Arctic LTER study sites. After collection, we immediately transported the samples back to the Toolik Field Station and filtered them through .7 μm GF/F filters and subset by treatment and incubation time. Incubation methods closely followed standardized BDOM methods outlined in Vonk et al 2015. We divided the treatments into three incubation times of 0 (initial sample), 7, and 28 days (referred to as T0, T7, and T28). We incubated the samples in 1-liter amber bottles to avoid photodegradation of DOM. We created five nutrient treatments to test how variable nutrient concentrations would affect BDOM and microbial community. Our control group did not receive any additional nutrients. Nitrogen and phosphorus treatments received 1 g N and 1 g P, respectively. To test

priming effects, we created two treatment groups by adding 1g acetate, which are referred to as "A+N" and "A+P". This resulted in samples from 28 collection sites, collected in two seasons, subset into three incubation times and five treatment groups (28 sites * 2 seasons * 3 incubation times * 5 treatment groups = ~870 samples). We completed sample filtering and nutrient addition, and incubation initiated within 24 hours of initial sample collection.

Sample analysis

We analyzed initial (T_0) samples on a S::CAN spectrophotometer to obtain initial turbidity, nitrate, TOC, and DOC measurements before freezing and transporting them. We transported these frozen samples and incubating samples were to BYU, while maintaining a consistent temperature within samples, and re immediately frozen once incubation ended. Table 3 provides an overview of the analyzers used to measure specific parameters.

We also collected We also collected ggenetic data from initial (T_0) and full-length (T_{28}) incubation samples. We filtered samples through 0.7 GF/F filters to collect eDNA from stream samples. We immediately froze these samples at -80 degrees C, where they remained until extraction. We extracted the samples via Qiagen Powerwater kits (Qiagen, Hilden, Germany) strictly following the provided protocol. We checked the DNA concentrations via Nanodrop One Microvolume UV-Vis Spectrophotometer (Thermo-Scientific, Waltham, MA) for sample viability. We amplified and sequenced viable samples and sequences to bacterial taxa via QIIME2 (Bolyen et al. 2018), and exported them for further filtering and analysis using the "Phyloseq" package (McMurdie and Holmes 2013) in R Studio.

Statistical tests

We used one-way analysis of variance (ANOVA) to test statistical significance of DOC degradation rates and Shannon diversity, using catchment, nutrient treatment, and season as

explanatory variables and site ID as a blocking factor. We used a significance value of ≤ 0.05 in all statistical tests. We used random forest testing to identify potential explanatory variables for DOC biodegradability. We performed pairwise permutational multivariate analysis of variance (PERMANOVA) to find significant differences in microbial community between catchments, treatment groups, and sampling dates. We created all figures and performed all statistical tests in R version 4.1.1 (R Core Team (2022)). A full list of R packages used is provided in table 3.

RESULTS

BDOC Incubation

Ambient DOC concentrations (figure 9) were very similar between permafrost and lake-dominated permafrost areas (permafrost: mean=6.27, stdev=1.88; lake: mean=6.56, stdev=1.81), while alpine tundra showed much more variability, with a lower average concentration and higher standard deviation (mean=3.07, stdev=3.18). Despite the lower average DOC concentration in alpine tundra, the high standard deviation is a result of three of the four highest DOC concentrations originating from alpine samples.

Percentage DOC degraded after 7- and 28-days incubations showed similar patterns to ambient DOC concentrations but highlights the high percentage of DOC loss in alpine catchments (figure 10). There was a relatively similar amount of degradation between tundra and lake-dominated tundra sites among all treatments. Alpine samples, despite having lower initial DOC concentrations, had a strong response to nutrient addition.

Random forest testing results indicated the best predictors of DOC biodegradability (full incubation) were light absorbance at 254 nm, initial DOC concentration, and total dissolved phosphorus, with Spearman correlation values of -0.5, -0.51, and -0.31 respectively (figure 14). Watershed characteristics regressions (figure 15) indicated higher correlation between DOC percent loss and area (p-value: 0.03), mean slope (p-value: 0.01), and mean normalized difference vegetation index (NDVI, p-value: 0.03).

Microbial community

Shannon diversity decreased through incubation time in all treatments and seasons except for the late season alpine samples (figure 11). Diversity values post-incubation in tundra and lake were generally similar within each season, with early season values ~4.5 and late season values ~3.5. Alpine sample diversity values were most responsive to nutrient treatment, though responses were different between seasons. In early season alpine samples, priming treatments (A+N and A+P) indicated higher diversity than other post-incubation samples.

Principal coordinates analysis (PCoA) of microbial composition indicated close similarity of Tundra and Lake communities, with Alpine community more dissimilar (figure 12). Though tundra and lake communities are tightly grouped, the tundra community show slightly more similarity to alpine communities. This is reflected in pairwise PERMANOVA results, where comparisons between catchments only show a significant relationship between Tundra and Lake catchments (P-value: 0.03). Comparisons between the Alpine catchment and other were not significant (Alpine-Tundra P-value:0.13, Alpine-Lake P-value: 0.39). In all catchments, T_0 samples were most distant from T_1 treatment groups, showing a strong incubation effect on microbial community. This is most apparent in Tundra catchment samples, where pairwise comparisons indicated significant differences between T_0 samples and all treatment groups (all P-values >0.05), as opposed to Lake where only three (N, P, A+P treatments) comparisons were significant, and Alpine samples where only one comparison (A+P) was significantly different. Within all catchments, none of the T_1 treatment groups had significantly different microbial composition.

Further examination of bacterial community indicates the strong effect of nutrient addition and season on community composition (figure 13). Overall, the *Proteobacteria* phylum dominates across season, catchment, and treatment. Other phyla present in all catchments

include *Actinobacteria, Bacteroidetes,* and *Verrucomicrobia*. In all early season ambient samples, *Actinobacteria* relative abundance decreased through incubation time, except in A+N treatments. Seasonal response to incubation was very different especially in tundra samples, where early season *Proteobacteria* relative abundance was drastically decreased.

Some bacterial phyla are only present in specific catchments and seasons. Unlike other catchments, alpine bacterial communities include *Firmicutes* and higher relative abundance of *Proteobacteria*. *Firmicutes* are primarily present in late season baseline samples, though relative abundance is not amplified by any treatment group. Lake samples contained the highest relative abundance among all samples of *Verrucomicrobia* in early season, post-incubation samples and the highest relative abundance of *Armatimonadetes* in late season post-incubation samples. In both seasons, *Actinobacteria* was present in ambient samples, though relative abundance decreased over incubation. Tundra samples contained a high relative abundance of unidentified taxa, and high concentrations of *Planctomycetes* in early season post-incubation samples. We matched 16S reads to the National Center for Biotechnology Information databases, so unidentified taxa could represent unsequenced bacterial phyla. These unidentified taxa were most relatively abundant in Tundra site 17.4, whose watershed area was much smaller than other Tundra sites with microbial data. Only tundra samples contained *Planctomycetes*, which are widespread and often associated with biofilms (Lage and Bondoso 2014).

DISCUSSION

In permafrost streams, rates of DOC (dissolved organic carbon) mineralization have been linked to temperature, microbial community, chemical composition, and radiocarbon age (B. W. Abbott et al. 2014; Frei et al. 2020; Marín-Spiotta et al. 2014; Zarnetske et al. 2018), though most studies have focused on single catchments or disturbance events. In this study, we examined how nutrient addition and priming altered DOM mineralization and microbial

community across three distinct permafrost-types, while controlling for temperature and light effects. By performing incubation experiments in early and late season samples, we examined how DOM source and other seasonal effects alter DOM lability and microbial community. We found that there is a large amount of variation in microbial community across seasons, potentially due to changes in temperature, carbon lability, and thaw depth.

DOC degradation

We found very similar initial DOC concentrations and nutrient responses in the Lake and Tundra catchments, while Alpine DOC concentrations were much lower but had increased percent degraded over incubation time (figures 9 & 10). Alpine samples represented much smaller catchments than Tundra or Lake samples, which means baseline DOC would not have traveled as far as in other catchments. This indicates that though less DOC is released from alpine permafrost, it is more labile than lake-induced or tundra permafrost. This is consistent with previous research indicating high biodegradability of newly liberated permafrost DOC (Vonk et al. 2015; O'Donnell et al. 2016). This is especially evident in early season samples, where percent DOC loss is much higher in all alpine treatments. However, in late season alpine samples, DOC degradation rate was very low in the control group and was triggered by addition of either nitrogen or phosphorus. This could indicate a nutrient limitation on late season microbial activity, where nitrogen and/or phosphorus addition encourages growth of certain taxa. However, based on PERMANOVA results, late season arctic microbial community (figure 12) is not significantly different between N and P treatments (P-value: 0.99) or AN and AP treatments (P-value: 0.99), so addition of different nutrients did not show selection for different taxa.

Based on random forest testing (Ho 1995) results, the best predictors of DOC biodegradability (full incubation) were light absorbance at 254 nm, initial DOC concentration,

and total dissolved phosphorus, with Spearman correlation values of -0.5, -0.51, and -0.31 respectively. Absorbance at 254 nm is often used to define the aromaticity of organic material and was expected to be highly correlated with DOC mineralization rates. However, despite being selected by random forest testing as the best explanatory variables for DOC biodegradability, their high correlation values indicate a poor relationship, and visualization does not indicate a close relationship between these parameters (figure 14). These poor correlation values indicate the complexity of explaining DOC mineralization rates, and the necessity of using additional information, such as microbial community, when uncovering complex processes.

Watershed characteristics (area, mean slope, mean NDVI) had a significant relationship (all relationships P-value < 0.05) with DOC biodegradability. Due to the high biodegradability of permafrost organic carbon and strong nutrient limitations on stream metabolism, DOC has a long residence time in streams, and is rapidly mineralized in headwaters. Thus sites with smaller areas and steeper slopes indicate DOC that was released into streams more recently, which is more biodegradable.

Bacterial Community and Response

Similar to DOC degradation rates, baseline microbial communities of Tundra and Lake sites are more similar to each other than Alpine samples. Principal coordinate analysis plots (figure 12) show Tundra and Lake areas overlap much more with each other than with Alpine samples. Shannon diversity values of Tundra and Lake samples also show a similar response to nutrient incubation (figure 11), though late season Lake samples had a smaller decrease in diversity over incubation time than Tundra samples. Priming did not have any apparent effect on Shannon diversity for either catchment or season.

Relative abundance plots (figure 13) indicate the strong seasonal change in microbial community. Though *Proteobacteria* dominated all communities, each catchment had distinct taxa that either were only present in one season or were selected for based on nutrient treatment. For example, in Alpine samples, early season samples contained good amounts of *Actinobacteria* and *Bacteroidetes* and shifted towards *Firmicutes* in late season. The response of the microbial community was also different between seasons. In Tundra samples, early season incubation overall increased the relative abundance of *Armatimonadetes, Planctomycetes,* and *Verrucomicrobia*, while late season incubation increased the relative abundance of *Proteobacteria*.

Certain methods used in our nutrient incubation experiment could have influenced the relative abundance of certain phyla. We are changing the conditions in our samples to examine a response, though some of the controlled conditions may exert unwanted pressure on certain taxa. For example, *Firmicutes*, which was most common in our late season Alpine samples and often is photosynthetic (Bryant and Frigaard 2006), could have been selected against due to the light limitation of the amber bottles we used. Other taxa that rely on light or flowing water for energy or nutrient acquisition could have been suppressed by the 28-day incubation. This shows the limitation of incubation methods in determining bacterial response to increased nutrient concentrations.

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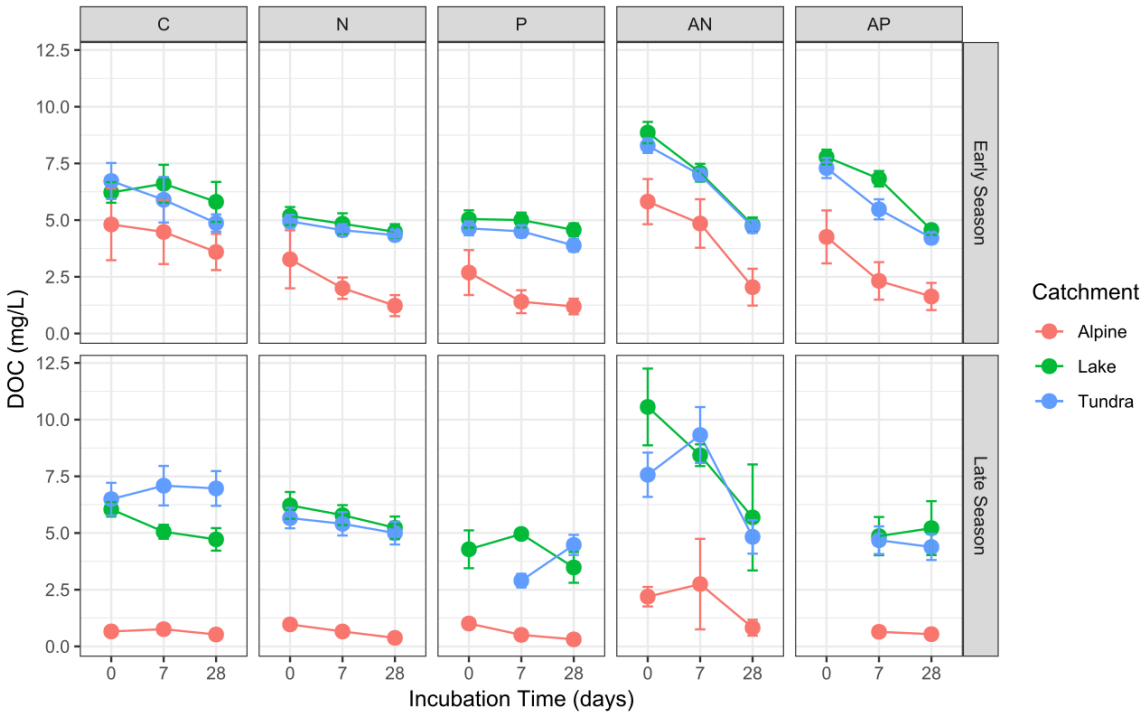

Figure 9. Mean dissolved organic carbon (DOC) concentrations through incubation time, separated by season, nutrient treatment, and catchment.

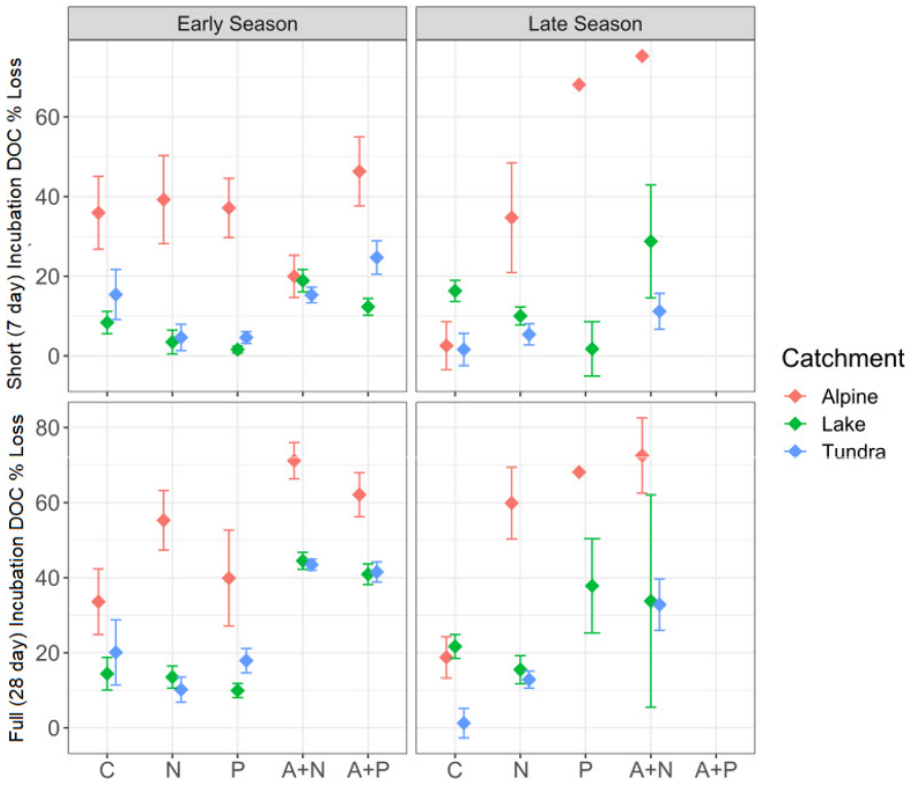

Figure 10. Mean percent dissolved organic carbon (DOC) loss through short and full incubation time. Short incubation: 7 days, full incubation: 28 days.

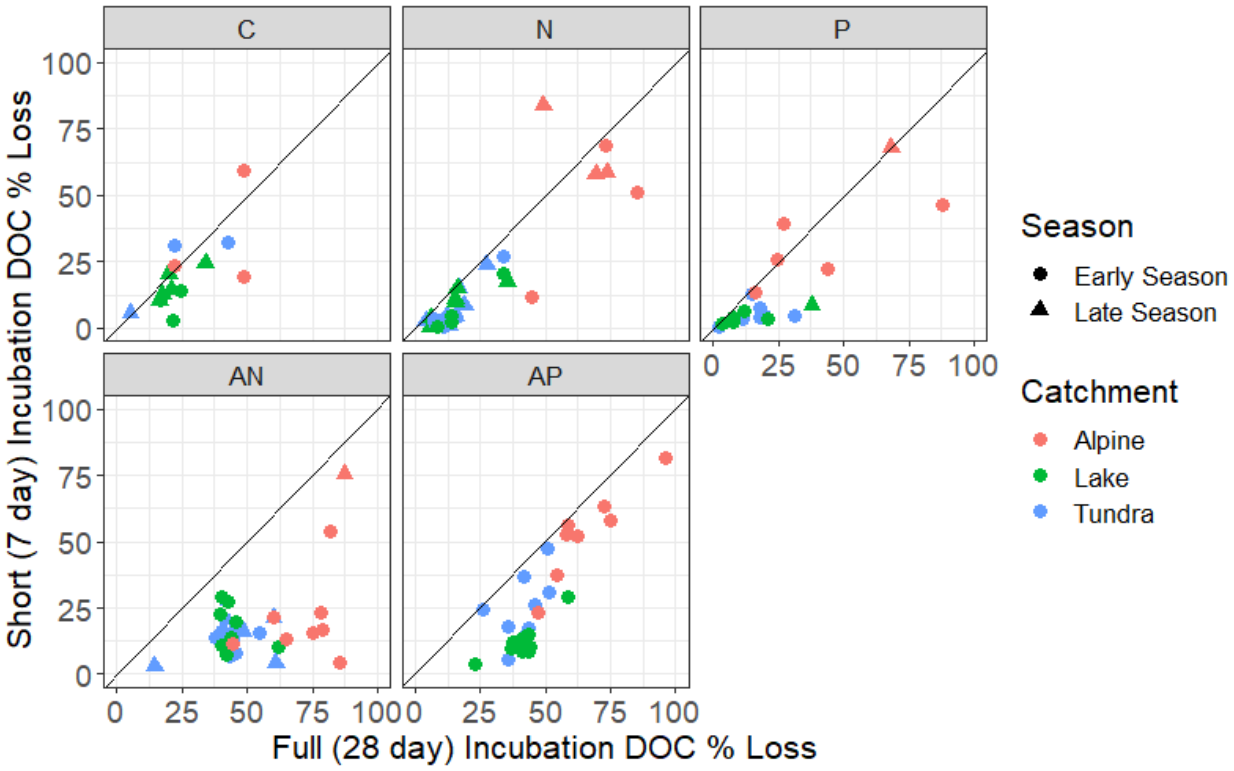

Figure 118. Scatter plots indicating short (7 day) and full (28 day) incubation dissolved organic carbon (DOC) loss. Black line indicative of 1:1 ratio or constant DOC loss rate throughout incubation. Points to the right of the black line indicate faster DOC loss in first 7 days of incubation.

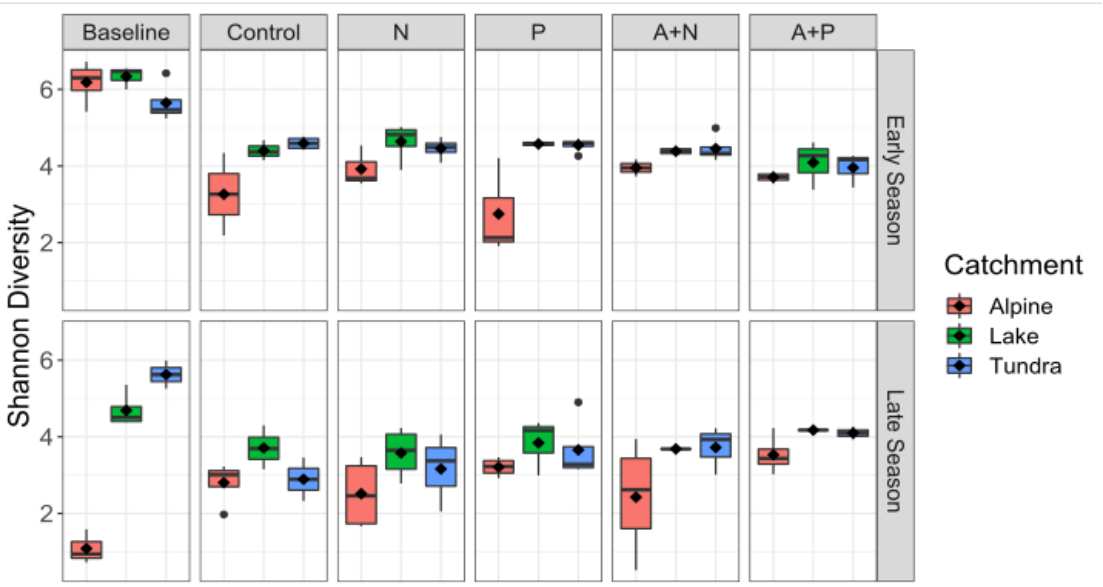

Figure 129. Shannon Diversity response to nutrient incubation. Samples were rarefied to 8000 reads prior to diversity calculations.

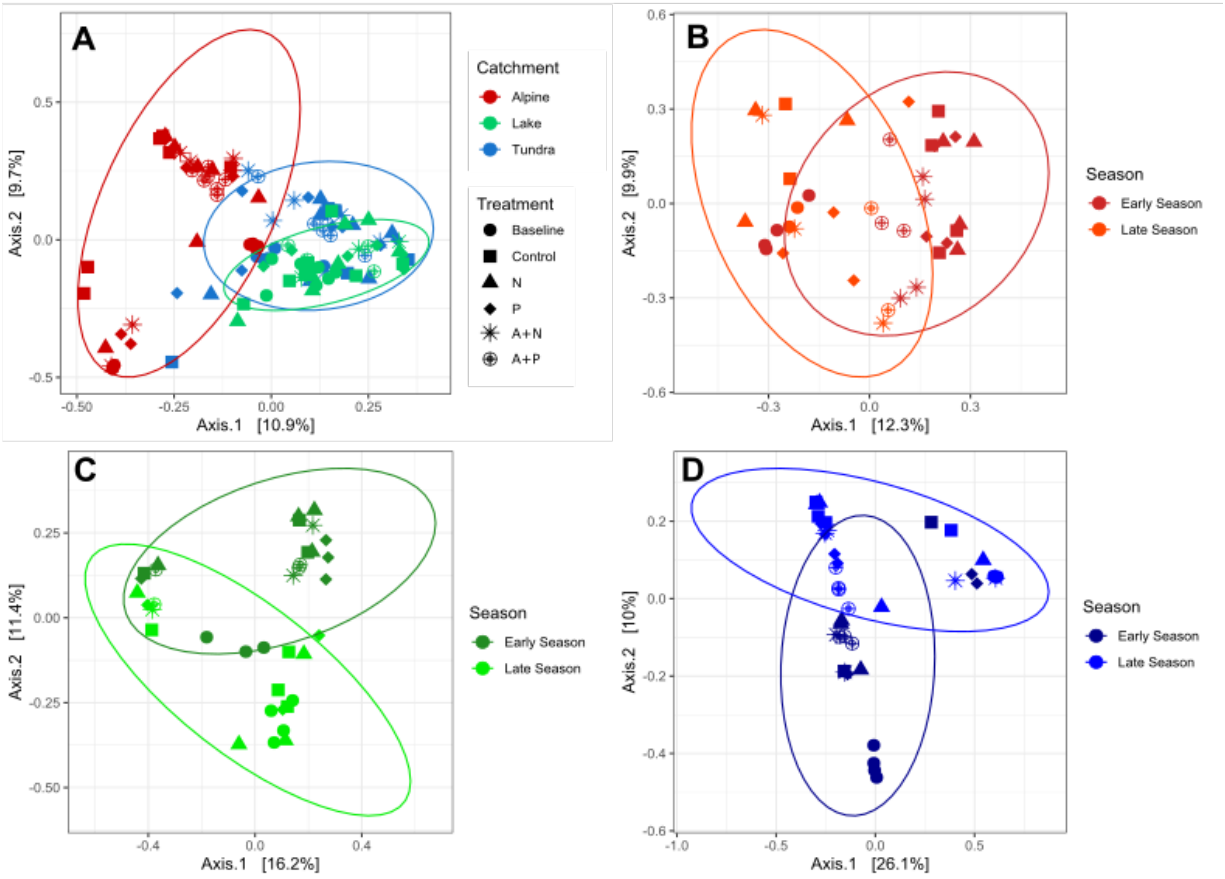

Figure 1310. Principal Coordinates Analysis (PCoA) plots of microbial communities. A) Comparison of all catchments, treatments, and seasons. B) Alpine samples across treatments and seasons. C) Lake samples across treatments and seasons. D) Tundra samples across treatments and seasons.

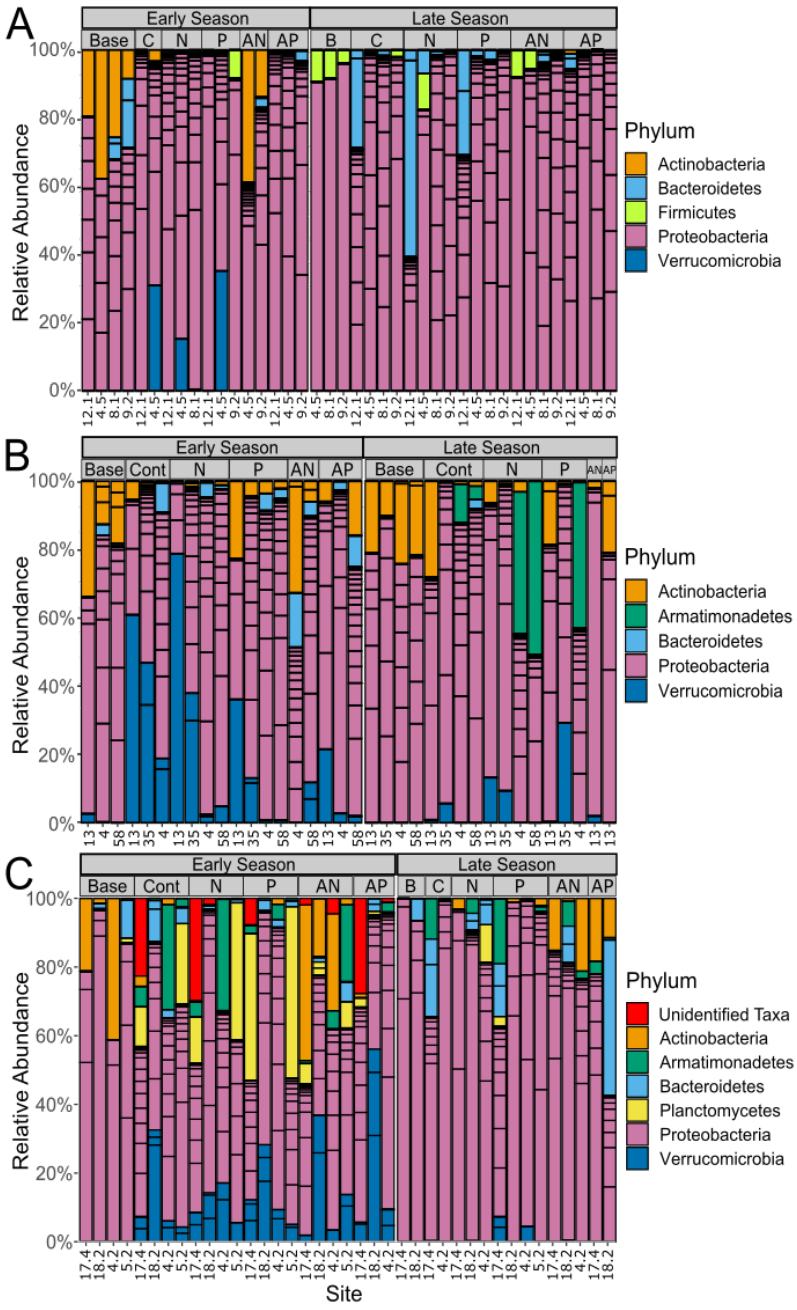

Figure 1411. Relative Abundance of bacterial community by catchment, season, treatment, and site. Plots are faceted by catchment: A) Alpine, B) Lake, and C) Tundra. Included taxa were limited to the 25 most abundant OTU's across individual catchments. Pre-incubation samples are designated by "Base" (or B), with other treatments designating post-incubation samples.

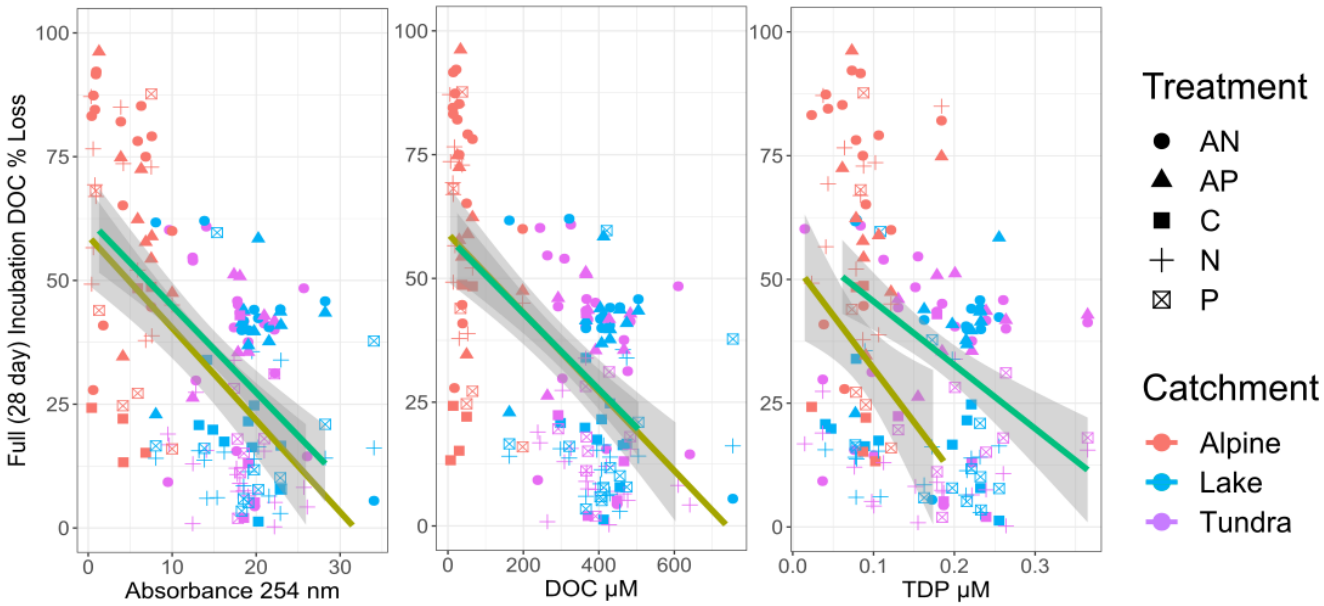

Figure 1512. Correlation plots of parameters deemed most explanatory by random forest testing, with corresponding Spearman correlation values. Left to right, panels are absorbance at 254 nm, dissolved organic carbon (DOC), and total dissolved phosphorus (TDP). Regression lines are June (yellow) and August (green) mean values, with shaded areas representing standard error.

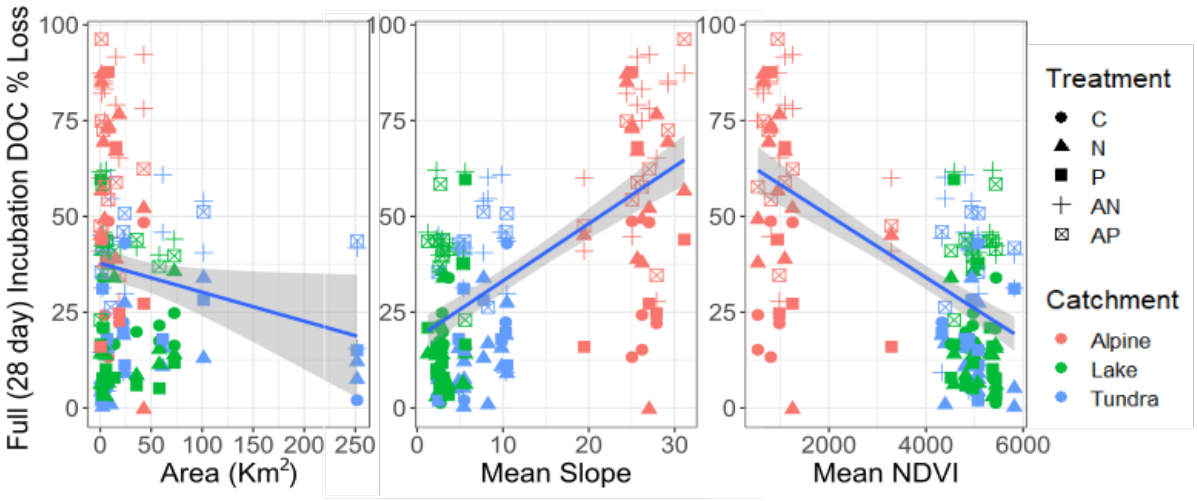

Figure 1613. Regression plot of DOC % loss through full incubation time by various watershed characteristics. Regression lines are in blue, with the shaded region representing the standard error of the relationship.

TABLES

Table 4. Water chemistry parameters collected, and analyzers used.

Analyzer Used:	Data Provided:
Ion Chromatograph	Anions / Cations: Al, As, B, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, K, Mg, Mo, Ni, Li, P, Pb, S, Se, Si, Sr, Ti, V, Zn
Elementar TOC Cube	Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), Total Nitrogen Bound (TNb)
S::CAN Spectrophotometer	Turbidity, NO_3, TOC, DOC, Absorbance 254 nm