Nutrient limitation of periphyton in agricultural streams: Implications for watershed management

by

Sydney Robyn Huculak

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ABSTRACT

Freshwater streams are ecologically important as sources of habitat, unique biodiversity, and valued ecosystem services. Yet, stream health can be threatened by intensified nutrient loading derived from adjacent anthropogenic land-uses such as agricultural and municipal developments. Since algal growth can be limited by nitrogen (N), phosphorus (P), or co-limited by both (N+P), increases in the supply of these nutrients can stimulate primary production, leading to eutrophication. Eutrophication is a primary stressor of freshwater streams as it can deplete dissolved oxygen, promote blooms of toxic algae, and cause the loss of critical biodiversity within affected ecosystems. Despite the well-known ecological implications of eutrophication, nutrient limitation is poorly understood in low-order streams found throughout the agricultural region of Alberta. Determining the limiting nutrients for algae associated with microbial biofilms, termed periphyton, is critical for the management of nutrient loading and the health of stream ecosystems. Past nutrient management efforts in Alberta have relied solely on correlations between within-stream nutrient concentrations and the standing stock of algae, primarily targeting P endpoints. However, empirical evidence that P input is always the key cause of eutrophication of streams in Alberta is lacking. Thus, experimentally identifying which nutrients are limiting will improve the efficacy of management practices designed to improve stream health within Alberta. Here, we performed *in-situ* nutrient diffusing substrate (NDS) bioassays, using a crossed factorial design (N x P), to experimentally identify the drivers of nutrient limitation (i.e., N, P, or N+P) and nutrient-driven shifts in algal community composition in freshwater streams across Alberta's agricultural region. NDSs were deployed in each of 30 streams, which were chosen to span three ecoregions, a gradient of land-use intensity, and ambient stream nutrient concentrations. Nitrogen, rather than phosphorus, was identified as the

limiting nutrient driving algal biomass within the streams studied. Yet, nutrient-driven shifts in algal community composition were detected as P differentially affected bacillariophytes and chlorophytes. N-limitation was driven primarily by the response of bacillariophytes, while co-limitation was driven primarily by chlorophyte response to P, not N. However, stimulation of algal growth via N additions was still universal across both algal groups. The magnitude of nutrient limitation was not found to vary across ecoregions, despite distinct differences in predominant vegetation and soil type. Unexpectedly, underlying abiotic stream characteristics did not significantly influence the magnitude of algal nutrient limitation. Overall, these results suggest that nutrient management efforts focused on limiting inputs of nitrogen will be the most effective at averting eutrophication of low-order streams in Alberta's agricultural region.

PREFACE

This thesis is an original work by Sydney R. Huculak. The research is part of a collaboration between Dr. S. E. Tank and Dr. R. D. Vinebrooke at the University of Alberta and Dr. G. S. Piorkowski and M. Kobryn at Alberta Agriculture and Forestry. I was responsible for the study design, data collection and analysis, and manuscript composition. All collaborators were involved with manuscript edits and suggestions for data analysis. Site selection was completed with the help of Dr. G. S. Piorkowski, M. Kobryn, and M. R. Baldwin.

This thesis is composed of three chapters. Chapter 1 is an introductory chapter that provides background information on the thesis topic and defines the research objectives. Chapter 2 is the data chapter intended for publication and is written in a manuscript format. Chapter 3 provides the general conclusions of the thesis and provides areas of improvement and future research directions. These chapters were written in the plural due to the collaborative nature of the research.

Chapter 2

Huculak, S. R., Tank, S. E., Piorkowski, G. S., Kobryn, M., and Vinebrooke, R. D. Assessment of nutrient limitation and algal community response to enrichment in agricultural streams across three ecoregions.

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Chapter 1: General Introduction

1.1 Background

1.1.1 Ecological Importance of Streams

Freshwater streams are highly valued as sources of habitat, unique biodiversity, and ecosystem services (Allan and Flecker 1993). Provisioning of water for agricultural and domestic consumption, power generation, and recreational (e.g., fishing) and aesthetic value are only some of the many ecosystem services provided by streams (Yeakley et al. 2016). Streams also regulate nutrient cycling and sediment transport, influencing the water quality and physical characteristics of downstream systems (Whiting et al. 1999; Dodds and Oakes 2008). Yet, due to their close linkages within terrestrial ecosystems, stream health can be heavily influenced by the surrounding landscape and anthropogenic land-use.

1.1.2 Agricultural Land-use and Stream Eutrophication

Streams can receive excessive nutrient inputs from various non-point sources, primarily due to land-use changes (Carpenter et al. 1998; Allan and Arbor 2004). In watersheds dominated by agricultural activity, cumulative land-use pressures have intensified inputs of nitrogen (N) and phosphorus (P) into adjacent streams, largely through fertilizer and manure applications and implications from livestock grazing (Carpenter et al. 1998). Nutrients can be exported to streams through runoff, soil leaching, and mobilization of stream bank sediments by livestock (Owens et al. 1996; Little et al. 2007; Casson et al. 2008). Reductions in riparian vegetation caused by intensive livestock grazing and direct access to streams (Scrimgeour and Kendall 2002) can also increase the potential for agricultural runoff to enter streams (Mapfumo et al. 2002; Olson et al. 2011). The intermittent release of municipal and industrial wastewater effluents can also contribute to nutrient inputs (Canadian Council of Ministers of the Environment 2009).

Excess nutrient inputs are primary stressors in freshwaters as they can stimulate primary production, leading to excessive algal growth and eutrophication (Dodds et al. 2002). These outbreaks (i.e., blooms) of algae can adversely affect aquatic biodiversity and ecosystem function. Proliferation of algae leads to excessive decomposition once senescence occurs, depleting oxygen concentrations, which can lead to deleterious outcomes such as fish kills (Carpenter et al. 1998). Nutrient enrichment is also linked to compositional shifts in algal

communities as it can reduce biodiversity and stimulate pollution tolerant, bloom-forming species (Dodds 1991; Chételat et al. 1999). Streams with diverse algal assemblages tend to be more resilient to the effects of enrichment through higher nutrient removal rates, resulting from niche partitioning (Cardinale 2011). Thus, critical losses in biodiversity may act as a positive feedback within streams, making them less resilient to future enrichment and additional anthropogenic stressors, thereby intensifying the ecological consequences of eutrophication.

Cyanobacteria are commonly found in enriched environments as their adaptations to extreme environmental conditions and ability to perform nitrogen (N_2) fixation enables the exploitation of nutrient-rich systems (Paerl and Otten 2013). Proliferation of cyanobacteria can have profound ecological impacts through the production of harmful algal blooms (HABs) that can result in the release of hepatotoxins and neurotoxins (Landsberg 2002). HABs can affect stream food-webs directly through the production of potentially lethal toxins, with the potential for biomagnification in higher trophic levels (Kozlowsky-Suzuki et al. 2012), and indirectly through reductions in food quality for grazers due to the filamentous nature of many HAB species (de Bernardi and Giussani 1990; Landsberg 2002). Combined, these effects can diminish top-down control of HAB species, resulting in a marked deterioration in the water quality of streams faced with nutrient loading.

Due to the ecological consequences of stream eutrophication, management efforts for preserving stream health are primarily focused on setting nutrient standards and minimizing nutrient loading events (Dodds and Welch 2000). However, the extent to which excess nutrient inputs contribute to stream eutrophication depends upon the nature and magnitude of algal nutrient limitation. Understanding nutrient limitation dynamics and nutrient-driven compositional shifts in algal communities within impacted streams is critical for preserving the biological integrity and ecosystem function of the stream and for the development of effective nutrient management strategies.

1.1.3 Nutrient Limitation

1.1.3.1 Nutrient Stoichiometry and Assessing Limitation

Algal growth, in part, depends upon nutrient availability, as algae require specific stoichiometric ratios of essential nutrients. The cellular ratio of C:N:P describes the cellular

requirements for optimal algal growth. Traditionally, algae were assumed to follow the classic "Redfield Ratio" (106C:16N:1P) (Redfield 1958) which was derived from cellular ratios of marine phytoplankton. More recently, optimal cellular ratios for freshwater periphyton have been confirmed (119C:17N:1P), varying only slightly from the traditional ratio (Hillebrand and Sommer 1999). Identifying these optimal ratios is critical as natural supply ratios of N and P may not always meet these algal cellular growth requirements, thereby limiting productivity.

One way to identify the nutrient limitation status of algae is through changes in their cellular nutrient ratios, as deviations from the optimal nutrient stoichiometry can indicate theoretical limitation. Experimental manipulation of periphytic communities has found that cellular N:P molar ratios <13 indicate N-limitation, 13-22 indicate co-limitation, and >22 indicate P-limitation (Hillebrand and Sommer 1999). Another method used to infer nutrient limitation is to investigate the ambient water column nutrient ratios of N and P (Bergström 2010; Keck and Lepori 2012). However, the success of solely using total N to total P (TN:TP) ratios to infer the nature of nutrient limitation in lotic ecosystems is underwhelming, as most of the bioavailable N is captured by dissolved inorganic nitrogen (DIN), not TN, measurements (Stelzer and Lamberti 2001; Lewis and Wurtsbaugh 2008; Keck and Lepori 2012). On the other hand, TP most accurately captures the true pool of bioavailable P because algae are able to use inorganic and organic forms (Wetzel 2001; Lewis and Wurtsbaugh 2008). More recently, DIN:TP ratios have gained traction as a more effective indicator of the type of limitation occurring in freshwater systems (e.g., <1 = N-limitation; 1 to 3.4 = co-limitation; >3.5 = P-limitation) (Bergström 2010; Keck and Lepori 2012). Regardless, cellular and stream water nutrient ratios have been found to be weakly predictive of nutrient limitation in experimental studies (Francoeur et al. 1999; Wold and Hershey 1999).

As a result of the shortcomings of cellular and stream nutrient ratios to infer nutrient limitation, the use of *in-situ* enrichment experiments has gained traction as an empirical tool for exploring the many different types of nutrient limitation and co-limitation (Elser et al. 2007; Harpole et al. 2011). Here, Nutrient Diffusing Substrates (NDSs) are commonly used to experimentally determine the causal relationship behind nutrient limitation in streams (Francoeur 2001; Tank and Dodds 2003; Bechtold et al. 2012; Reisinger et al. 2016). Construction of NDS bioassays has progressed over the years from clay pots (Fairchild 1985), to periphytometers (Matlock et al. 1998), to the now standard plastic cup method (Tank et al. 2017). This latter

method is a popular approach for assessing nutrient limitation across a variety of aquatic ecosystems due to its replicability and relatively low production cost.

1.1.3.2 Nutrient Limitation in Streams

Nutrient limitation within freshwater ecosystems has been studied for over 50 years. Much of the early research focused on nutrient limitation of phytoplankton within lake ecosystems that found P to be the primary limiting nutrient (Schindler 1971; Schindler 1977), creating an assumption that P was the primary limiting nutrient across all freshwater systems (Schindler 1974). In an effort to better understand the nutrient limitation dynamics specific to periphyton in lotic ecosystems, various nutrient enrichment experiments have been conducted in streams and rivers (Fairchild and Lowe 1984; Pringle and Bowers 1984; Bushong and Bachmann 1989; Stanley et al. 1990; Chessman et al. 1992; Francoeur et al. 1999). However, the observed nature of nutrient limitation did not strictly follow the P-limitation paradigm. Instead, nutrient limitation of stream periphyton varied from P-limitation (Peterson et al. 1983; Fairchild and Lowe 1984; Stanley et al. 1990; Matlock et al. 1998), to N-limitation (Chessman et al. 1992), to co-limitation by N and P (Pringle and Bowers 1984). Within specific study sites, nutrient limitation was also found to vary across algal species (Fairchild et al. 1985) and seasons (Francoeur et al. 1999), adding another layer of complexity when discerning nutrient limitation dynamics. More recently, NDS experiments and widespread meta-analyses show that N-limitation and co-limitation by N and P are the most common types of nutrient limitation detected across freshwater streams, once again challenging the existing paradigm of implicit P-limitation (Francoeur 2001; Tank and Dodds 2003; Elser et al. 2007).

Anthropogenic land-use within a watershed plays an important role in the type and magnitude of nutrient limitation within a stream. Streams experiencing high levels of agricultural and urban land-use within their catchments have been documented to experience a range of nutrient limitation (i.e., not nutrient limited to co-limited by N+P; Johnson et al. 2009), with the overall magnitude of limitation generally related to the intensity of adjacent land-use due to increases in nutrient concentrations (Reisinger et al. 2016). Thus, regional variations in nutrient limitation may develop across large spatial scales due to differing land-use intensities. In Alberta, only a few studies have investigated nutrient limitation dynamics in lotic ecosystems. The studies that have occurred were focused on forested headwater streams (Irvine and Jackson

2006), large northern rivers (Scrimgeour and Chambers 2000), or were conducted at a small spatial scale within a specific ecological region (the Cypress Hills Grassland Plateau; Scrimgeour and Kendall 2002). Thus, despite the substantial presence of agriculture throughout much of southern and central Alberta (see *Section 1.2*, below), a large-scale investigation of nutrient limitation dynamics in streams across Alberta's major agricultural regions has yet to be conducted.

1.1.3.3 Co-limitation Dynamics

The conceptual understanding of nutrient limitation was originally derived from Liebig's Law of the Minimum, which states that only a single nutrient can be limiting at any given time (Liebig 1842). Thus, this theory of single nutrient limitation would suggest that algae can be limited by either N or P (Figure 1.1). However, because this theory was developed based upon the limitation of a single crop species, the extension of this theory as it applies to diverse multi-species algal communities has been challenged (Danger et al. 2008). Given the complexities of periphyton communities, it is unlikely that only one nutrient will limit the entire algal community similarly (Harpole et al. 2011; McCormick et al. 2019). The lack of applicability of Liebig's Law as it applies to diverse ecological communities is further supported by the frequency of co-limitation that has been uncovered via experimental work (in algae: Francoeur 2001; Tank and Dodds 2003; Elser et al. 2007; Harpole et al. 2011).

At the whole-community level, limitation by N and P may be a result of "biochemically dependent co-limitation" (sensu Saito et al. 2008; Bracken et al. 2015), where limitation by one nutrient may inhibit the use of another. On the other hand, co-limitation may result from species-specific nutrient requirements (Borchardt 1996) that cause different species to be limited by different nutrients. Co-limitation may also result from an additive or synergistic response to simultaneous additions of N and P. Here, additions of the primary limiting nutrient would cause the second nutrient to be depleted and therefore become limiting itself (Francoeur 2001; Elser et al. 2007). Given the frequency and complexity of co-limitation dynamics within streams, using NDS bioassays to differentiate between the various types of co-limitation becomes even more important.

There are several types of co-limitation that can be defined to better understand the underlying mechanisms causing co-limitation to occur (Figure 1.1; Harpole et al. 2011).

Simultaneous co-limitation occurs when there is a biomass response only when N and P are added simultaneously, with the potential for a synergistic (i.e., more than additive) response. Independent co-limitation occurs when there is a biomass response to N and P when added separately. Here, additions of N and P together can be synergistic, additive, or sub-additive (Harpole et al. 2011). Serial co-limitation occurs when one nutrient added independently elicits a biomass response, but when both nutrients are added together, a synergistic response occurs. The nutrient eliciting the initial biomass response is referred to as the "driver" (e.g. if +N elicits the initial response, it would be identified as N-driven serial co-limitation) (Harpole et al. 2011).

It is important to note that algal biomass can also be limited by other environmental factors besides nutrients, including light (Hill et al. 1995; Von Schiller et al. 2007), temperature, scouring from high flow (Peterson 1996), and grazing activity (Hillebrand and Kahlert 2001). An absence of nutrient limitation may also occur in streams that are already highly nutrient-saturated, as the magnitude of limitation can decrease with increasing water column nutrient concentrations (Reisinger et al. 2016). Thus, other potentially limiting environmental factors have the potential to overrule nutrient limitation, such that streams may not always exhibit responses to nutrient enrichment as shown below (Figure 1.1) and can instead be classified as not nutrient limited.

1.2 Study region

Anthropogenic land-use pressures are prevalent throughout Alberta, as a large proportion of land has been converted for agricultural, municipal, and industrial uses. This study focused on regions experiencing varying degrees of agricultural land-use pressures within the Grassland, Parkland, and Boreal ecoregions of Alberta. Watersheds within these ecoregions contain a large proportion of cropland and pasture land-use (Alberta Biodiversity Monitoring Institute 2017). As is well documented across agricultural regions globally, agricultural land-use within this region can threaten the health of small streams through increased nutrient loading (Anderson et al. 1998), emphasizing the importance of developing nutrient standards and prioritizing beneficial management practices (BMPs) that can mitigate impacts to receiving streams (Dodds and Welch 2000).

Although nutrient standards for surface waters have been established by the federal government (National Agri-Environmental Standards Initiative; Chambers et al. 2012), nutrient management targets should ideally be developed on a localized scale as region-specific factors play an important role in the relevance and effectiveness of management plans (Canadian Council of Ministers of the Environment 2016). In 2014, surface water quality guidelines were updated by the provincial government, identifying the recommended concentrations of nutrients required to protect the current status of Albertan lakes and rivers (Government of Alberta 2014a). However, these guidelines were set based upon historical monitoring of large major rivers which may lack applicability to small streams. In addition, the type of nutrient limitation (i.e., N vs P limitation) within small streams in Alberta remains poorly understood. Thus, experimentally identifying region-specific limiting nutrients is critical for effective management of the health of small streams within Alberta by identifying the correct nutrients to target with management practices.

1.2.1 Establishing baseline nutrient concentrations

Baseline (or reference) nutrient concentrations are often unknown in anthropogenically impacted regions due to the lack of minimally impacted watersheds, particularly in regions without historical monitoring. A statistical method known as the "Y-intercept Model" was established by Dodds and Oakes (2004) to estimate reference nutrient conditions of streams in regions where unimpacted streams are rare. This approach uses multiple linear regressions between land-use variables (relative proportion of cropland, pasture, urban; independent variable) and water column nutrient concentrations (total N, total P; dependent variable) and identifies the intercept of the regressions as the reference nutrient concentration that is expected if land-use was equal to zero (Dodds and Oakes 2004). Determining baseline nutrient concentrations is a critical step in developing appropriate regional nutrient standards by identifying ecologically relevant endpoints.

Using a water quality dataset from streams within Alberta's agricultural watersheds, baseline nutrient conditions of streams facing long-term agricultural pressures were estimated. Data were collected as a part of *Nutrient Objectives for Small Streams in Agricultural Watersheds of Alberta* project being led by Alberta Agriculture and Forestry. Water quality data were collected from 62 streams within the Grassland (n=20) and Parkland (n=25) ecoregions

from 2016 to 2019 and from the Boreal ecoregion (n=17) in 2019. Statistical methods followed those described by Dodds and Oakes (2004). Preliminary ANCOVAs were performed using either the mean concentration of total N (TN) or total P (TP) as the response variable, ecoregion as the predictor variable, and relative proportion of cropland and pasture as the covariates to detect whether there was a significant ecoregion effect. Since no ecoregion effect was detected, ecoregion data was pooled for TN and TP (Table A1.1). Multiple linear regression (MLR) models were created for TN and TP using $\log_{10}$-transformed nutrient concentrations as the dependent variable and relative proportion of cropland and pasture in the watershed as the independent variable (Table A1.2, Figure A1.1). MLR model intercepts represent the estimated baseline nutrient concentration (i.e., interpreted as the nutrient concentration when cropland and pasture land-use is equal to zero). A 95% confidence interval (CI) was calculated around the intercept to estimate the upper and lower CI around the baseline nutrient concentration (Table 1.1).

Baseline nutrient concentrations of streams within Alberta's agricultural watersheds are estimated to be upwards of 270 and 330 µg L^{-1} for TN and TP, respectively (Table 1.1.). Thus, the baseline nutrient status of these streams are estimated to be considered oligotrophic based upon TN trophic status thresholds and eutrophic based upon TP trophic status thresholds (Dodds et al. 1998). Overall, these nutrient concentrations of streams within Alberta's agricultural regions are affected by land-use, warranting management of nutrient inputs. It is important to note that nutrient concentrations should be strongly correlated to land-use to appropriately extrapolate baseline nutrient concentrations. Here, cropland and pasture were highly correlated with TN (Table A1.2), but only cropland was significantly correlated with TP (Table A1.2). Given the inherent caveats of this type of analysis, baseline nutrient concentrations may be underestimated. Despite the limitations of this predictive modeling approach, it is important to estimate reference conditions of these streams when developing nutrient criteria and to understand what level of water quality can be achieved through the adoption of agricultural BMPs.

1.3 Research Goals and Objectives

The purpose of my thesis was to determine the nature of nutrient limitation in small streams within Alberta's agricultural watersheds. By identifying limiting nutrients, this thesis

advances understanding of the causal relationship between nutrient enrichment and algal response, including how limitation varies across gradients of water column nutrient concentrations and agricultural land-use. By conducting the study across three ecoregions, region-specific nutrient limitation can also be assessed. I also sought to explore the causal relationship between nutrient enrichment and algal community composition. Here, I investigated the response of algal assemblages to additions of different nutrients to identify the limitation status among the major algal groups. I also explored how algal response varied depending on the watershed and ecoregion classification, as well as the underlying environmental characteristics of the streams. Determining the nature of nutrient limitation and nutrient-driven compositional changes in algal communities is essential for the prevention of eutrophication and maintenance of stream health in Alberta's agricultural region.

This thesis contains one manuscript-style chapter (Chapter 2). The specific objectives of this chapter were to:

(1) experimentally determine the drivers of nutrient limitation (i.e., N, P, or N+P) within Alberta's agricultural regions;

(2) quantify algal response (total biomass and community composition) to nutrient enrichment across these same regions; and

(3) assess the extent to which physiochemical variables influence the magnitude of algal response to nutrient enrichment in Alberta.

In addition to these direct scientific objectives, I strove to generate information relevant to the determination of nutrient standards for managing the health of small streams in Alberta. Policy implications resulting from this research are briefly discussed in Chapter 3.

1.4 Significance of the Study

Eutrophication is a widespread threat to freshwater ecosystems. Anthropogenic activities have accelerated the rate and magnitude of cultural eutrophication across freshwater ecosystems through intensified nutrient loading (Carpenter et al. 1998), which can only be expected to increase with an expansion of agriculture to meet growing demands for food alongside increased urbanization. Freshwater ecosystems are threatened by eutrophication as it depletes dissolved

oxygen, promotes blooms of toxic algae, and causes the loss of critical biodiversity within affected ecosystems. These changes degrade overall ecosystem health, impacting food webs, fish, and humans through changes in water quality and aesthetic properties.

This study is the first to determine the nature of nutrient limitation in streams across the agricultural regions of Alberta, and the watershed and stream-specific characteristics (e.g., land-use, stream physiochemistry) that best predicts this limitation. Understanding the nature of nutrient limitation is essential for maintaining the structure and function of aquatic ecosystems and preventing eutrophication of streams experiencing agricultural land-use pressures. It is also critical for establishing effective management actions by identifying the need to control specific types of external nutrient inputs. Detecting regional patterns in nutrient limitation can also aid in nutrient management efforts through forming targeted management plans for specific agricultural watersheds. Identifying nutrient-driven compositional shifts in periphyton communities is important for understanding how nutrient enrichment will affect algal community structure under different limitation scenarios. Detecting alterations in community structure also provides important insight needed for prevention of the formation of HABs and losses in stream biodiversity.

1.5 Tables and Figures

Table 1.1. Estimated baseline nutrient concentrations and 95% confidence intervals for total N (TN) and total P (TP) for streams within Alberta's agricultural watersheds.

	Baseline (µg L^{-1})	Low 95% CI (µg L^{-1})	Upper 95% CI (µg L^{-1})
TN	0.50	0.001	270
TP	0.05	0.07	330

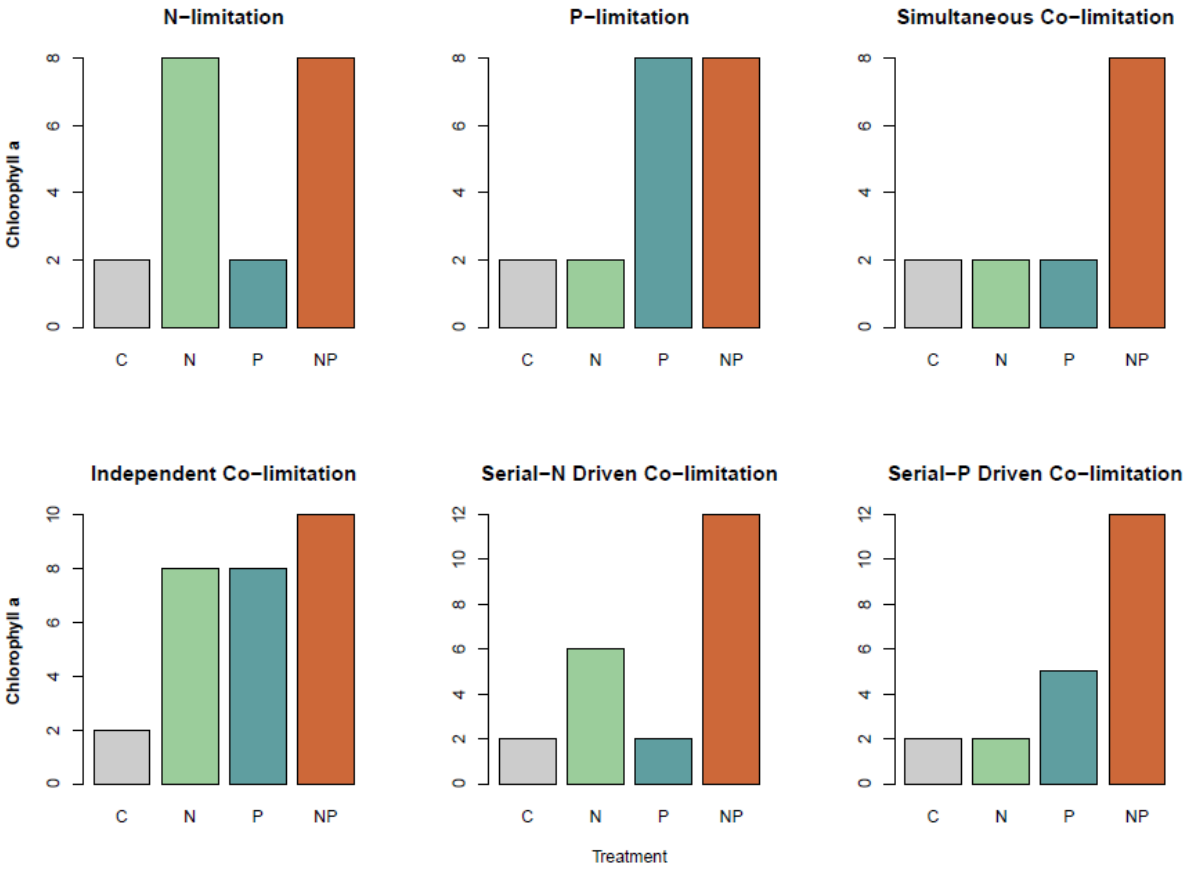

Figure 1.1. A conceptual illustration of the various types of nutrient limitation that can be observed, as defined by Tank and Dodds (2003) and Harpole et al. (2011).

Chapter 2: Assessment of nutrient limitation and algal community response to enrichment in agricultural streams across three ecoregions

2.1 Introduction

Periphytic algal communities are critical components of streams, responsible for driving numerous ecosystem functions such as primary production and nutrient cycling. Due to their close linkages with terrestrial environments, streams can receive considerable excess nutrients from various non-point sources, primarily due to land-use change (Carpenter et al. 1998). In Alberta, agricultural land-use has intensified inputs of nitrogen (N) and phosphorus (P) into adjacent streams, through runoff and leaching of fertilizers and manure (Anderson et al. 1998). Since algae can be limited by N, P or co-limited by both (N+P), excess nutrient input can promote spikes in primary productivity and biomass accrual, often leading to eutrophication (Dodds et al. 2002; Dodds and Smith 2016). This can result in outbreaks of potentially harmful algae that can implicate aquatic biodiversity and ecosystem function (Landsberg 2002). Yet, the extent to which nutrient loading events contribute to eutrophication depends, in part, upon the type of nutrient limitation experienced within a stream. Thus, identifying algal nutrient limitation is critical for fully understanding the ecological implications of nutrient loading on the health of agricultural streams.

Nutrient limitation of periphytic algae occurs when nutrient supply rates do not meet cellular growth requirements, following a modified Redfield ratio specific to periphytic cells (119C:17N:1P; Hillebrand and Sommer 1999). Ambient water column nutrient ratios have been used as predictors of nutrient limitation (Bergström 2010; Keck and Lepori 2012). Here, the ratio of dissolved inorganic N (DIN) to total P (TP) is considered the strongest predictor of algal nutrient limitation (DIN:TP < 1.5 = N-limitation, 1.5-3.4 = co-limitation, and > 3.4 = P-limitation; Bergström 2010), as DIN and TP are most reflective of the bioavailable forms of N and P, respectively (Wetzel 2001; Bergström 2010; Keck and Lepori 2012). Thus, these ratios can be used to predict the overall fertilizing effect of enrichment on algal response. However, the predictive power of ratio-inferred limitation is generally poor compared to experimental studies (Francoeur et al. 1999). *In-situ* nutrient diffusing substrate (NDS) bioassays are a much stronger empirical tool for exploring the various types of nutrient limitation, especially for differentiation between co-limitation types (e.g., N-driven serial co-limitation, Independent co-limitation;

Harpole et al. 2011), and allow the causal relationship between enrichment and algal response to be experimentally identified (Tank and Dodds 2003; Reisinger et al. 2016). Nevertheless, since algal growth is largely dependent upon ambient nutrient availability, the overall fertilizing effect of N vs P on algal response will likely depend upon the ambient DIN:TP ratios. Investigating the concordance between predicted versus experimentally confirmed nutrient limitation can provide valuable insight on the predictive ability of nutrient ratios, which can be useful for future nutrient limitation investigations.

Despite the well-known ecological consequences of eutrophication and prevalence of land-use pressures across Alberta, a large-scale investigation of periphytic nutrient limitation in streams across Alberta's agricultural regions has yet to be conducted. Previous NDS-based studies in Alberta have focused on forested headwater streams (Irvine and Jackson 2006), large northern rivers (Scrimgeour and Chambers 2000), or were limited to a specific ecological region (the Cypress Hills Grassland Plateau; Scrimgeour and Kendall 2002). Since streams within agricultural regions experience a relatively higher degree of nutrient loading (Alberta Agriculture Food and Rural Development 1998), investigating the specific nature of nutrient limitation within these systems is required. Nutrient management efforts in Alberta have relied solely on correlations between nutrients and algal standing stock, primarily targeting P endpoints (Kalischuk et al. 2006; Soil Phosphorus Limits Committee and LandWise Inc 2006; Government of Alberta 2014b). Yet, algal nutrient limitation in these systems is poorly understood and empirical evidence that P input is the key limiting nutrient of Albertan stream is lacking. Historically, P has been assumed to be the primary limiting nutrient across all freshwater systems (Schindler 1977). However, this implicit assumption of widespread P-limitation has been increasingly challenged, largely through the use of NDS experiments (Elser et al. 2007). A growing number of studies have shown that streams are primarily N-limited or co-limited by N and P, with co-limitation generally resulting in the highest algal biomass accrual (Francoeur et al. 1999; Francoeur 2001; Tank and Dodds 2003; Elser et al. 2007; Marcarelli et al. 2009; Sanderson et al. 2009; Harpole et al. 2011; Reisinger et al. 2016). Thus, P can no longer be assumed to be the primary limiting nutrient of these streams. Experimentally identifying nutrient limitation dynamics in agricultural streams will allow for the detection of the correct nutrient inputs to target. By doing so, nutrient management efforts can be more ecologically effective in the prevention of eutrophication and maintenance of healthy agricultural streams.

Nutrient limitation can also vary spatially, as regional differences in underlying abiotic characteristics of streams can influence the magnitude of algal response to nutrient additions. Regional (e.g., ecoregion and land-use; Biggs and Gerbeaux 1993) and local factors (e.g., water column nutrient availability, light, and flow regime; Hill et al. 1995; Ghosh and Gaur 1998; Reisinger et al. 2016) can regulate algal growth in streams, which can subsequently intensify or diminish algal response to nutrient enrichment. Land-use and ambient nutrient availability are often inherently linked and have been identified as critical drivers of spatial variation in nutrient limitation (Johnson et al. 2009; Beck et al. 2017). Specifically, increasing ambient nitrate concentrations, resulting from more intensive land-use within a watershed, have been associated with diminished algal response to nutrient enrichment across the Midwest, USA (Reisinger et al. 2016). Thus, regions experiencing relatively higher nutrient or land-use pressures may not be as sensitive to enrichment as less impacted sites. Alternatively, algae may not exhibit a substantial response to nutrient enrichment if growth is limited more strongly by other factors, such as light (Von Schiller et al. 2007). Thus, the extent to which nutrient loading leads to algal proliferation can depend upon the underlying abiotic characteristics of streams. To generate a more comprehensive understanding of the ecological effects of nutrient loading on agricultural streams, the specific environmental drivers that influence the type and magnitude of algal nutrient limitation must be identified. Detecting potential regional patterns in nutrient limitation dynamics also has important implications for the formation of more targeted nutrient management efforts.

Nutrient enrichment can also promote compositional shifts in algal communities, as tolerant taxa are able to exploit enriched conditions (Dodds 1991; Hicks and Taylor 2019; Huttunen et al. 2020). Nutrient-driven taxonomic shifts can amplify the effects of enrichment on stream health, particularly if enrichment favours algal groups with relatively higher biomass production or growth rates (e.g., filamentous chlorophytes; Biggs 1996). Such a shift could accelerate the deleterious effects of eutrophication through higher decomposition and subsequent reductions in oxygen, magnifying the potential for fish kills (Carpenter et al. 1998). Of particular concern is the potential for nutrient-driven shifts towards potentially toxic bloom-forming species, such as cyanobacteria. Proliferation of cyanobacteria can result in the formation of harmful algal blooms (HABs) that have many deleterious effects on stream health such as toxin production (hepatotoxins and neurotoxins) and reduction in food quality for grazers due to the

filamentous nature of HABs (Landsberg 2002). Thus, if enrichment preferentially promotes cyanobacteria, the ecological consequences of nutrient loading may extend beyond just the direct effects of eutrophication. Experimentally revealing the taxonomic response to enrichment is thus essential when attempting to prevent losses in stream biodiversity and maintain stream health.

Algal groups may also be more valuable indicators of the presence of nutrient limitation than total community biomass alone. This is because nutrient-driven taxonomic shifts may not always be associated with changes in total algal biomass, as compensatory species dynamics may offset responses detected at the whole-community level (Stelzer and Lamberti 2001). Such functional compensation among algal groups would result in a lack of a nutrient effect detected at the community level, despite divergent responses among algal groups to enrichment (Frost et al. 1995). This may buffer streams against the direct ecological effects of excessive algal biomass. Yet, despite the known complexities of algal communities facing nutrient pressures, many NDS-based studies only quantify total community level responses (Stanley et al. 1990; Francoeur et al. 1999; Tank and Dodds 2003; Reisinger et al. 2016). Since periphytic algal communities are diverse and species can differ in their nutrient uptake, storage, and utilization abilities (Borchardt 1996), algal groups are likely limited to different extents by different nutrients (Fairchild et al. 1985; Harpole et al. 2011). Thus, investigating the specific response of algal groups to enrichment provides more detailed information regarding the effects of enrichment and is, therefore, a more valuable indicator of nutrient limitation than just quantifying total community response, as represented by biomass, alone. By quantifying both together, the implications of nutrient enrichment on periphytic communities in streams can be more fully understood.

The goal of this study was to determine the nature of nutrient limitation in small streams within Alberta's agricultural regions for the first time. Through this, we also investigated enrichment effects on algal community composition and identified important abiotic factors that drive the magnitude of observed nutrient limitation. To accomplish this, NDS bioassays were deployed within 30 small streams spanning a gradient of ambient nutrient concentrations and land-use intensities. The specific objectives were to: (1) experimentally determine the drivers of nutrient limitation (i.e., N, P, or N+P) of agricultural streams in Alberta; (2) quantify algal response (total biomass and community composition) to nutrient enrichment across these

streams; and (3) assess the extent to which physiochemical variables influence the magnitude of algal response to nutrient enrichment. We hypothesized that (1) the type of nutrient limitation will depend upon the relative *in-situ* ratio of ambient nutrients (DIN:TP), (2) algal groups will be more sensitive indicators of nutrient limitation than total community biomass, and (3) that streams along a gradient of increasing ambient nutrient concentration will have a diminished response to nutrient enrichment. We undertook this work across Alberta's three primary agricultural ecoregions, which allowed us to explore regional variability in nutrient limitation dynamics across gradients of agricultural land-use intensity. Advancing knowledge in these areas is essential for the prevention of eutrophication and maintenance of stream health and can influence decisions on how we manage streams and surrounding watersheds in Alberta.

2.2 Methods

2.2.1 Study Area

In-situ bioassays of periphytic nutrient limitation were conducted within the major agricultural ecoregions of Alberta, Canada. Thirty 3rd and 4th Strahler order streams were selected to span the Grassland, Parkland, and Boreal ecoregions (Figure 2.1). The Grassland ecoregion is characterized by a semi-arid climate with warm temperatures and low precipitation. Cross-regional mean daily temperatures peak at 17.8^{o}C in the summer and reach a low of -11.7^{o}C during the winter, with a mean annual precipitation of 374 mm (Natural Resource Committee 2006). This region supports intensive agricultural cultivation due to the presence of highly productive Chernozemic soils. Shrublands and grasslands characterize the Grassland ecoregion. The Parkland ecoregion is colder and wetter than the Grassland and represents a transition zone between the Grassland and Boreal ecoregions. Cross-regional mean daily temperatures peak at 16.4^{o}C in the summer and drop to a low of -14.4^{o}C during the winter, with a mean annual precipitation of 447 mm (Natural Resource Committee 2006). The Parkland ecoregion is also extensively cultivated for agricultural land-use, containing predominantly Chernozemic soils with aspen woodlands and fescue grasslands dominating the remaining native vegetation (Natural Resource Committee 2006). Although the Boreal ecoregion covers over 50% of Alberta (Natural Resource Committee 2006), streams selected for this study were concentrated in the southern reaches of this ecoregion where most agricultural land-use occurs. The Boreal ecoregion experiences the coldest and wettest climatic conditions. Cross-regional mean daily

temperatures reach a high of 15.7ºC during the summer and a low of -19ºC during the winter, with a mean annual precipitation of 469 mm (Natural Resource Committee 2006). Within the boreal, the Dry Mixedwood subregion, where most of the study streams are located, is made up of a mixture of cultivated land and aspen forest, with Gray Luvisolic and Brunisolic soils (Natural Resource Committee 2006)

Within each ecoregion, streams were chosen to reflect a gradient of agricultural land-use intensity (cropland and pasture) and runoff potential (Jedrych and Martin 2013), resulting in a subsequent gradient in nutrient concentrations. No reference streams were available for the study as all streams within the study region, which encompasses Alberta's privately-held land area where agriculture is permitted (Timberlake et al. 2008; the "white-zone"), have experienced at least some degree of anthropogenic impact. Land-use intensity was calculated as the proportion of crop and pasture land-use within the watershed of each stream based on the 2017 Alberta Human Footprint Inventory Dataset (Alberta Biodiversity Monitoring Institute 2017).

Following site selection, the watershed for each study site was also classified into watershed categories in an effort to identify variation in nutrient limitation dynamics on a more detailed regional scale. This is particularly beneficial for the development of beneficial management plans targeted to specific watersheds. Watersheds were classified as part of the larger research program based upon watershed size, drainage density, shape factor, watershed slope, annual precipitation, mean annual temperature, potential evapotranspiration, maximum snowpack depth, annual leaching and runoff, and other variables including aggregate soil properties. Watersheds were classified into three watershed categories using self-organizing maps (SOM). Category I sites are represented by low- to moderately-sloped watersheds that experience low temperatures, moderate runoff and leaching, and relatively higher precipitation and snowpack. Soils in category I watersheds are poorly drained Luvisolic soils. These watersheds are characterized by high proportions of tame pasture and forage and are located predominantly in the Boreal transition area. Category II sites are represented by low-sloped watersheds that experience moderate precipitation and temperatures, with an overall low runoff and leaching potential. Soils within category II watersheds consist of well-drained Chernozemic and Solonetzic order soils. These watersheds are found across the northern Grassland, Parkland, and Boreal regions. Category III sites are represented by moderate- to high-sloped watersheds

that experience high levels of precipitation and temperatures, with high levels of runoff potential and leaching. Soils in category III watersheds are well-drained, consisting of soils under the Chernozemic order. These watersheds are characterized by native grasslands and are located across southern Alberta.

2.2.2 Nutrient Diffusing Substrates

2.2.2.1 Construction and Calculation of Nutrient Release Rates

Nutrient Diffusing Substrates consisted of 30-mL polyethylene cups filled with a 2% agar solution amended with either: 0.5 M $NaNO_3$ (N treatment), 0.5 M KH_2PO_4 (P treatment), 0.5 M $NaNO_3$ and 0.5 M KH_2PO_4 (N+P treatment), or no amendment (control) (Tank et al. 2017). Nutrient salts were added to agar solutions after autoclaving to minimize H_2O_2 formation (Tanaka et al. 2014; Tank et al. 2017). After the agar solution cooled, each cup was topped with a sterile porous fritted glass disc (LECO Corporation, catalog no. 528-042). Holes (28-mm diameter) were pre-drilled into the center of each cup lid to secure the discs overtop the agar and enable nutrient diffusion and algal colonization.

Nutrient release rates from the NDSs were determined prior to their deployment in the streams. Three replicates of each nutrient amendment and control were constructed using the methods described above. An acid-washed glass beaker was filled with 500 mL of artificial stream water (90% MilliQ water and 10% Bold's Basal Medium; final concentration of 0.125 mg $NaNO_3$ and 0.087 mg KH_2PO_4). Stock solution was chosen instead of 100% distilled water to provide a more accurate representation of *in-situ* diffusion as a result of gradient diffusion effects. One replicate of each NDS treatment and control was suspended upside down in a beaker containing 500 mL of artificial stream water via a wire basket. Beakers were then covered with plastic wrap to avoid evaporation. Each beaker was placed on a stir plate with a stir bar to simulate flow. All beakers were stored in an environmental growth chamber (12:12 light: dark cycle at 14^{o}C) for the duration of the experiment.

Water samples were collected from each beaker on days 0, 3, 6, 9, 12, 15, 18, and 21. After each water sample was collected, beakers were completely emptied and replaced with 500 mL of fresh artificial stream water to simulate *in-situ* flow conditions and ensure that the concentration gradient, and thus diffusion, was maintained (Scrimgeour and Chambers 1997). Collected water samples were analyzed for both nitrate (NO_3^-) and phosphate (PO_4^{3-}) via Ion

Chromatography (Dionex DX-600) at the ISO/IEC 17025 certified Biogeochemical Analytical Service Laboratory (BASL) at the University of Alberta. Nutrient release from each NDS was calculated as the volume of water in each beaker multiplied by the nutrient concentration (either N or P), divided by the number of hours elapsed since the previous water sample was taken (mg N h^{-1} and mg P h^{-1}) (Rugenski et al. 2008). Release rates of N and P from NDS showed exponential decay over 21 days, supporting that NDS continually released nutrients during the duration of deployment (Figure A2.1, Figure A2.2).

2.2.2.2 Deployment

We deployed NDSs in all 30 streams to experimentally determine nutrient limitation. For each stream, five replicate NDS cups were constructed for each treatment (N, P, and N+P) and control (C). One replicate of each treatment was attached to a plastic L-bar rack using a randomized complete block design to account for natural variation in flow and light availability in the stream. Constructed NDS racks were then sealed in plastic wrap and stored in the dark at 4ºC until deployment.

Deployment of NDS assays was performed in July of 2019. Five racks of NDS were deployed in each stream and placed mid-stream within a run. Individual NDS racks were spaced 1m apart in an upstream-downstream arrangement, totaling a stream reach of approximately 8 m. Racks were secured in the stream using flotation devices attached to two pieces of rebar set into the stream bed, allowing for vertical movement along the rebar with changing water levels. The racks were deployed parallel to flow, in base flow conditions, and were positioned approximately 5-8 cm below the water surface to offset potential photoinhibition. After 21 days, NDS racks were retrieved from the stream and each disc was harvested from the underlying agar, placed in a petri dish, wrapped in aluminum foil, and placed in a zipper-seal bag. Discs were transported from the field on ice, and then frozen at -20ºC within five hours of collection until analyzed.

Storm events during the deployment period, concentrated exclusively in the Boreal and Parkland ecoregions, compromised the NDS bioassays at eight streams (DOG01, PIP01, WEI01, STW01, WED01, POP01, MDS02, NAM02; Table 2.1). By comparing the mean monthly average discharge during 2019 to historical normals from a monitoring station at STW01, we illustrate the severity of these storm events on mean discharge during the deployment period (Figure A2.3). Mean discharge increased 26-fold in July and 40-fold in August compared to

historical median discharge (Figure A2.3). As a result, no NDS data were collected from these streams and they were omitted from subsequent statistical analyses. Two streams (CNR02 and RSB03) lost a large number of NDS cups due to high flow, resulting in a low sample size for certain nutrient treatments.

2.2.2.3 Algal Pigment Analyses

Chlorophyll *a* (Chl *a*) was used as a surrogate metric for algal biomass on NDS discs. Chl *a* and accessory pigments (Table 2.2) were extracted from the discs within two months of retrieval. Frozen NDS discs were freeze-dried (Virtis Freezemobile FM25-XL) to negate the potential confounding influence of variation in the water content affecting pigment extraction efficiencies (Hansson 1988). Each disc was then placed in a glass vessel filled with 5 mL of an 80:20 methanol: acetone solution and left in the dark at 4ºC for 24 hours for pigment extraction. Extracts were then filtered (Whatman Grade GF/F; nominal pore size of 0.7µm) into vials and stored at -20ºC until analysis. Chl *a* was quantified using a spectrofluorophotometer (Shimadzu RF-1501) following the methods of Welschmeyer (1994). An algal Chl *a* standard (C6144, Sigma Aldrich) was used to create a series of dilutions to create the standard curve for calibration (0 to 0.941 mg L^{-1}). A liquid secondary standard (25mg of zinc-phthalocyanine in 500 mL of extraction solution, Sigma Aldrich) was also used to correct spectrofluorophotometer measurements against lamp degradation.

Concentrations of taxonomically diagnostic algal pigments (e.g., Chlorophyll *b* and various xanthophylls; Table 2.2) were quantified using high-performance liquid chromatography (HPLC) (Vinebrooke and Leavitt 1999). Aliquots (3 mL) of original NDS extracts were dried down under nitrogen gas and stored in a -80ºC freezer until each sample was reconstituted using a specific volume (either 500 or 1000 µL depending on spectrofluorometrically-determined Chl *a* concentration) of injection solution (70% acetone: 25% ion-pairing reagent: 5% methanol). The analyses were performed using an Agilent 1100 Series HPLC System with quaternary pump fitted with a Varian Microsorb 100 C-18 column (10-cm long, 5-µm particle size), an inline HP Series 1100 diode array detector (435-nm detection wavelength), and a fluorescence detector (435-nm excitation wavelength, 667-nm detection wavelength). Analytical separation involved uniform delivery (1.0 mL min^{-1}) of a mobile phase A (10% Ion Pairing Reagent in methanol) for 1.5 min, a linear succession to 100% solution B (27% acetone in methanol) over 7 min, and

constant hold for 12.5 min. The column was re-equilibrated by continued uniform delivery for 3 min, a linear return to 100% solution A over 3 min, and constant delivery for a final 4 min. All detected algal pigments were identified based on how well their respective chromatographic retention times and spectral profiles (400 – 700 nm wavelength band) matched with those of commercial standards (DHI Water and Environment, Agern Alle 5, DK-2970 Hørsholm, Denmark).

Algal community composition was inferred from concentrations of detected taxonomically specific pigments. The major algal groups identified were: Bacillariophytes, Chlorophytes, Cryptophytes, and Cyanophytes (Table 2.2). Relative abundance of each algal group was calculated as the sum of the concentration of individual pigments characteristic for each algal group for each replicate, averaged across replicates for all treatment combinations at each stream.

2.2.3 Environmental Characteristics

A suite of physiochemical variables was measured upon deployment and retrieval of NDS, providing a snapshot of the environmental characteristics of each stream. Temperature, pH, dissolved oxygen (DO; mg L^{-1} and % saturation) and specific conductance (SpC) were measured at each site using a handheld multiparameter sonde (smarTROLL, In-Situ, Fort Collins, CO, USA). Stream morphology and flow parameters (width, depth, velocity, and discharge) were measured using a handheld Acoustic Doppler Velocimeter (FlowTracker2, SonTek/Xylem Inc., San Diego CA). Incident photosynthetically active radiation (PAR) was measured for the duration of the NDS deployment using pendant light loggers (HOBO UA-002-64, Onset Computer Corporation, Bourne, MA). One light logger was deployed at each stream and was attached to the rebar of a randomly chosen NDS rack, above the water surface. Light measurements were recorded in lux units every 10 minutes for the entire 21-day deployment and converted to PAR (µmol photons m^{-2} s^{-1}) by calibrating each pendant logger with a LI-COR pyranometer and applying a constant of 1.96 to convert from irradiance (W m^{-2}) to PAR (Reis and Ribeiro 2020).

Samples for water chemistry were collected immediately below the water surface from mid-stream, mid-depth, and immediately downstream of NDS. Samples for total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC) were

filtered on-site into either pre-cleaned HDPE bottles (TDN, TDP) or glass vials (DOC) using a filter tower and pre-combusted (4 hours at 450ºC) Whatman GF/F filters (nominal pore size of 0.7µm). DOC samples were acidified on-site with Trace Metal Grade HCl (A508-P500; Fisher Scientific) to ensure preservation to pH < 2.0. Samples collected for nitrate/nitrite (NO_3^-+NO_2^-), ammonium (NH_4^+), and soluble reactive phosphorus (SRP) were filtered on-site into polypropylene centrifuge tubes using syringe filters (Whatman GF/F). Unfiltered samples were collected directly in pre-cleaned HDPE bottles and were used to analyze total nitrogen (TN) and total phosphorus (TP). All water samples were transported in the dark on ice until stored in a refrigerator at 4ºC (TN, TP, TDN, TDP, and DOC) or frozen at -20ºC (NH_4^+, NO_3^- + NO_2^-, and SRP) until analyzed at BASL. TN, TDN, NH_4^+, and NO_3^- + NO_2^- were measured following standard methods, using a Lachat QuikChem 8500 FIA automated ion analyzer with a detection limit of 6, 6, 3, and 2 µg L^{-1}, respectively. NO_3^-+NO_2^- and NH_4^+ concentrations were measured as ug L^{-1} of N. TP, TDP, and SRP were measured using a Lachat QuikChem 8500 FIA automated ion analyzer with a detection limit of 1, 2, and 1 µg L^{-1}, respectively. DOC was analyzed using a Shimadzu 5000A TOC Analyzer with a detection limit of 0.1 mg L^{-1}. When individual values were below the detection limit, half of the detection limit was used for calculation of the mean concentrations. The mass of TN and TP in µg L^{-1} were used to calculate the TN:TP molar ratio.

2.2.4 Statistical Analyses

2.2.4.1 Assessment of Nutrient Limitation

Nutrient limitation was assessed using a linear mixed model (LMM). At each site, the presence or absence of N and P in the NDS and their interaction were included as fixed effects and the NDS rack number of each replicate was included as the random effect to account for blocking. The models were fit using restricted maximum likelihood. Shapiro-Wilks' and Levene's tests were used to check the assumptions of normality and homogeneity of variances, respectively. If the normality and/or variance assumptions were violated, biomass data were log-transformed before statistical analyses were conducted. If transformations did not improve assumptions, a generalized linear mixed model (GLMM) was performed using the Gamma distribution with a log link function due to the positive continuous nature of the data (n=3). Tukey's honest significant difference (HSD) post hoc tests followed all LMMs and GLMMs to differentiate between mean biomass of the treatments. Tukey HSD contrasts were labeled based

on the combination of the absence/presence of N and P (e.g., absent: absent is a Control (C) while present: present is an NP treatment).

Mixed model results were used to classify the type of nutrient limitation observed at each site, following interpretations outlined by Tank and Dodds (2003) and definitions of co-limitation described by Harpole et al. (2011) (Figure 1.1). Identification of site-specific limiting nutrients followed a two-step approach. First, mixed model results were interpreted to indicate the potential limitation scenario (Table 2.3). Here, a significant positive biomass response to one of the amendments (i.e., N or P), without a significant interaction term demonstrated limitation by a single specific nutrient. Co-limitation by N and P was indicated by either: (1) a significant interaction term only; (2) main effects for N and P were significant but not the interaction; or (3) all three terms were significant (Table 2.3). Second, post hoc Tukey HSD contrasts were evaluated to either confirm the presence of single-nutrient limitation, or to differentiate between various co-limitation scenarios (Table 2.3).

N-limitation was detected when the main N effect for the LMM was significant and the post hoc contrasts of the nitrogen treatment to the control (C-N) and phosphorus treatment (N-P) were significant. P-limitation was detected when the main effect for P in the LMM was significant and the post-hoc contrasts of the phosphorus treatment to the control (C-P) and nitrogen treatment (N-P) were significant. General co-limitation was detected when one of the three possible results of the LMM main effect occurred, as described above. No strict combination of main LMM effect and post-hoc contrasts could be defined for one specific type of co-limitation because inhibitory effects likely diluted main LMM results. Identification of the specific type of co-limitation was detected using post-hoc contrasts (Table 2.3). Independent co-limitation was detected when all post hoc contrasts except N-P were significant. Simultaneous co-limitation was detected when the post hoc contrasts of C-NP, N-NP, and P-NP were significant. N-driven serial co-limitation was detected when the post hoc contrasts for C-N, C-NP, N-P, N-NP, and P-NP were significant. P-driven serial co-limitation was detected when the C-P, C-NP, N-P, N-NP, and P-NP post-hoc contrasts were significant. If all possible contrasts were significant, the site was classified as serially co-limited with the driving nutrient identified as the single nutrient treatment (either N or P) that elicited the higher biomass response. P-inhibition (biomass decrease relative to the control in the presence of P; Beck and Hall 2018)

was detected when the contrasts with P presence exhibited a negative effect on algal biomass. Sites were classified as Inconclusive if the main LMM and post-hoc results did not follow any of the above assessment criteria scenarios indicative of a specific type of limitation (e.g., RSB03; C-NP was significant, but no significant difference between C-N and N-NP).

The TN:TP molar ratios and NDS bioassay results were compared at each site to determine how well nutrient ratios predicted observed nutrient limitation. Nutrient limitation thresholds were adapted from TN:TP molar ratios outlined by Bergström (<19 = N-limitation, 19-41 = co-limitation, > 41 = P-limitation; 2010). Since DIN concentrations were frequently below detect, TN had to be used as the metric for ambient N concentrations, despite it being a poorer predictor of bioavailable N (Stelzer and Lamberti 2001; Bergström 2010; Keck and Lepori 2012), disallowing the application of our original hypothesis. To enable the comparison with prediction thresholds, observations of simultaneous, independent, and serial co-limitation were all classified under "co-limited".

2.2.4.2 Response Ratios

Response ratios (RRs) were calculated to quantify the magnitude of nutrient limitation by normalizing algal response to nutrient treatments relative to the control. At each site, the RR was calculated as the logarithmic ratio of the mean Chl *a* concentration on treatment $_X$ discs (e.g., averaged concentration on N discs) divided by the mean Chl *a* concentration on control discs. A RR greater than zero indicates a positive effect of enrichment on algal biomass, while a RR less than zero indicates an inhibitory effect. Two-tailed t-tests were performed on each RR (RR_N, RR_P, and RR_{NP}), pooled across all sites, to detect if RRs were significantly less than or greater than zero. If normality assumptions were not met, a one-sample Wilcoxon Signed Rank test was performed. One-way ANOVAs were then used to assess whether RRs varied significantly across ecoregions and watershed categories. A Kruskal-Wallis (KW) test was used to assess differences among all RRs (RR_N, RR_P, and RR_{NP}) across the study region as a whole. Tukey HSD and pairwise Wilcoxon rank sum post hoc tests followed ANOVAs and KW tests, respectively.

Multiple linear regressions were used to infer the relationship between RRs and physicochemical (water temperature, pH, SpC, DO, discharge, velocity, TP, TDP, SRP, TN, TDN, DOC, PAR) and land-use parameters (relative proportion of cropland and pasture) across sites. Global models were run separately for each nutrient treatment (RR_N, RR_P, and RR_{NP}). A

Durbin-Watson test was performed on each of the full models to test autocorrelation of parameters before model selection. Variance inflation factors (VIF) were calculated for all variables in the global model. Relative proportion of crop and pasture on a watershed were added together and included as a "land-use" parameter after high VIF of 245 (crop) and 304 (pasture) indicated collinearity. TDP and SRP were excluded from the final global model due to collinearity with TP (TDP: Pearson's correlation coefficient; $r = 0.99$, $p < 0.0001$; SRP: $r = 0.97$, $p < 0.0001$). TDN and DOC were also excluded due to collinearity with TN (TDN: $r = 0.98$, $p < 0.0001$); DOC: $r = 0.85$, $p < 0.0001$). Models were compared and selected using an Information-Theoretic approach based on Akaike Information Criteria adjusted for small sample sizes (AIC_C) (Burnham and Anderson 2002). All possible model combinations were considered and those with the highest support (within Δ 2 AIC_C of the top model) were selected. Akaike weights were then calculated as the likelihood that model *i* is the best model based on the suite of top models. Multi-model averaging was conducted on the top models to estimate the relative importance of each model parameter. This was calculated as the sum of the Akaike weights of a specific model parameter across all models in which that parameter occurred.

2.2.4.3 Algal Groups

Nutrient limitation of each detected algal group was assessed using the statistical approach outlined for the biomass-based analyses (Table 2.3). Response ratios were also calculated for each algal group at each site. RRs were calculated as the logarithmic ratio of the mean pigment concentration on treatment $_X$ discs divided by the mean pigment concentration on control discs. Here, pigment concentrations refer to the concentration of an algal group (e.g., Bacillariophytes) and were calculated as the sum of the concentrations of the individual diagnostic pigments for each replicate and averaged across replicates at each stream. RRs were analyzed following the same statistical approach as the chlorophyll-based data above (*Section 2.2.4.2).* However, t-tests were only performed when the algal group was detected in a measurable concentration across more than 10 sites (only RR_{BACILL} and RR_{CHLORO}; see *Section 2.3.4*, below) and either a one-way ANOVA or KW test was conducted for ecoregion and watershed category analyses, depending on the normality of the data. MLR were used to infer the influence of physiochemical factors on algal group RRs using the same methodology as for the

chlorophyll-based data (*Section 2.2.4.2*), using a separate global model for each nutrient treatment within each algal group (e.g., $RR_{BACILL\text{-}N}$ and $RR_{CHLORO\text{-}NP}$).

For all algal group analyses, any algal group that had replicates with concentrations of zero had the mean concentration for that algal group added as a constant to all values to remove zeros. This allowed RRs to be calculated and transformations and Gamma distributions to be used for the assessment of nutrient limitation.

A non-metric multidimensional scaling (NMDS) ordination was performed to assess taxonomic turnover of algal communities among the four nutrient treatment combinations. The NMDS was conducted using non-pooled pigment data (i.e., individual pigments) instead of algal groups to better enable separation of samples in ordination space. All pigment data were Hellinger-transformed to normalize data prior to analysis (Legendre and Gallagher 2001). An ANOSIM test was conducted to determine statistical differences in algal pigments among all treatments using ranked dissimilarities (Bray-Curtis). The higher the R value for the ANOSIM, the more dissimilar the species among each treatment are. A redundancy analysis (RDA) was also conducted on the individual control NDS pigment data to identify the suite of environmental predictors that best explained taxonomic variation among algal communities. A reduced subset of environmental variables (TN, TP, and PAR) were included in the RDA to minimize VIFs in the environmental data. Forward selection was used to identify significant and independent environmental variables that would best explain variance in the pigment data across the different treatment combinations. Permutation testing was performed for all RDA axes to describe the overall significance of the final model, RDA axes, and model variables.

2.2.4.4 Statistical Software

All statistical analyses were performed in R 4.0.0 (R Core Team 2020). The basic pre-programmed '*stats-package*' in R was used for ANOVAs, Kruskal Wallis tests, Wilcox tests, and t-tests. The package *car* (John et al. 2020) was used for testing model assumptions and calculating VIF, *emmeans* (Lenth et al. 2020) was used for the post-hoc tests, *lme4* (Bates et al. 2020) was used for LMM and GLMM, *MuMIn* (Berton 2020) was used for the MLR model selection and averaging, *lmtest* (Millo and Mitchell 2020) was used for the Durbin Watson tests, and *vegan* (Oksanen et al. 2019) was used for the NMDS, ANOSIM, and RDA analyses and

ordinations plots. All graphics (box plots and bar graphs) were created using the package *ggplot2* (Wickham et al. 2020) and *ggpubr* (Kassambara 2020).

2.3 Results

2.3.1 Stream Characteristics

Study streams spanned a wide range of environmental characteristics and anthropogenic land-use intensities. Anthropogenic land-use as the percentage of cropland ranged from 4% to 92% coverage (median 67%), while pasture coverage ranged from 2% to 89% (median 24%) (Table 2.1). Study sites from the Grassland and Parkland ecoregions contained a higher percentage of cropland compared to the Boreal. Mean cropland coverage within Boreal, Grassland, and Parkland study sites was 36%, 72%, and 71%, respectively. Pasture land-use was highest for Boreal study sites at 39% but was generally similar across all three ecoregions (Grassland 31%, Parkland 31%) (Table 2.1).

Mean physiochemical data are presented in Tables 2.4 and 2.5. Across all sites, water temperature ranged from 15.1 to 22.9 ºC (median 19.3 ºC), DO ranged from 0.82 to 11.81 mg L^{-1} (median 7.57 mg L^{-1}), and stream water was neutral to alkaline with pH ranging from 7.31 to 9.16 (median 8.15). Mean stream velocity ranged from 0.003 to 0.447 m s^{-1} (median 0.150 m s^{-1}) and mean discharge ranged from 0.006 to 4.274 m^3 s^{-1} (median 0.309 m^3 s^{-1}). TN ranged from 273.5 to 3830 µg L^{-1} (median 1535 µg L^{-1}) and TP ranged from 8.7 to 991.5 µg L^{-1} (median 142.5 µg L^{-1}). Concentrations of NO_3^- + NO_2^- -N and NH_4^+ -N were below detection on both sampling days in eight and seven of the 22 streams, respectively. NO_3^- + NO_2^--N ranged from below the detection limit of 2 µg L^{-1} to 309.5 µg L^{-1} (median 2.5 µg L^{-1}) and NH_4^+ -N ranged from below the detection limit of 3 µg L^{-1} to 138.5 µg L^{-1} (median 6 µg L^{-1}). Dissolved organic carbon (DOC) ranged from 3.15 to 45.65 mg L^{-1} (median 17.45 mg L^{-1}). Mean PAR ranged from 116.72 to 1169.35 mol m^{-2} day^{-1} (median 844.58 mol m^{-2} day^{-1}). This large range may have resulted, at least in part, from macrophyte coverage of PAR sensors at some sites during incubation of NDS. Coverage of NDS by macrophytes was documented at 12 streams (55%). The percent coverage of NDS was estimated visually and varied across impacted streams, ranging from less than 5% to 100% coverage.

2.3.2 Nutrient Limitation and Response Ratios of Algal Biomass

Chl *a* concentrations on control NDS discs ranged from 0.31 to 23.51 µg cm^{-2} across all streams (median 7.43 µg cm^{-2}). Chl *a* ranged from 0.46 to 44.23 µg cm^{-2} (median 9.64 µg cm^{-2}) for N treatments, 0.16 to 20.10 µg cm^{-2} (median 4.72 µg cm^{-2}) for P treatments, and 0.18 to 48.39 µg cm^{-2} (median 14.54 µg cm^{-2}) for N+P treatments. Nutrient limitation was observed in 14 of the 22 streams (63%). Streams were primarily N-limited, or co-limited by N and P (Figure 2.2). Of the 14 sites that were nutrient limited, single N-limitation was detected in six streams (Figure A2.3), Simultaneous co-limitation was detected in seven streams (Figure A2.4), and N-driven serial co-limitation was detected once (Figure A2.4) (Figure 2.2, Table A2.1). Seven streams were classified as Not Nutrient Limited (NNL) (Figure 2.2, Figure A2.5, Table A2.1) and one stream was classified as Inconclusive due to uninterpretable results (Table A2.1, Figure A2.5). Single P-limitation was not detected in any of the study streams. P-inhibition (e.g., negative response compared to the control) of algal biomass was observed in four streams (Table A2.1).

Predicted nutrient limitation inferred from TN:TP ratios agreed with observed nutrient limitation 71% of the time, once streams classified as not nutrient limited were excluded (10 out of 14 streams; Figure 2.3). Five streams predicted to be N-limited (i.e., TN:TP < 19) agreed with the bioassay results (83% agreement). For co-limitation, five of the sites predicted to be co-limited by N and P agreed with bioassay results (83%). However, TN:TP ratios were not successful at predicting P-limitation (0%).

The effect size of nutrient enrichment was most pronounced for N (RR_N) and N+P (RR_{NP}) treatments, which both stimulated algal biomass similarly across all streams (Table A2.2, Figure 2.4). P treatments (RR_P) resulted in an overall inhibitory effect on algal biomass that was significantly lower than the RR for both the N and NP treatments (Figure 2.4, Table A2.2). RRs did not vary across the three ecoregions for any of the nutrient treatments (Figure 2.5, Table A2.3), and were also similar across watershed categories for both N and P treatments (Figure 2.5, Table A2.3). For N+P treatments, RRs were higher in watershed category 3 (WC3) than in watershed category 2 (WC2) (Figure 2.5, Table A2.3).

MLR models examining environmental drivers of RRs, ranked using AIC_C model selection and averaging, resulted in seven top models for RR_N, five top models for RR_P, and four

top models for RR_{NP} (Table 2.6). RR_N was positively related to PAR, proportion of land-use, and TP, and negatively related to DO, velocity, and discharge (Table 2.6, Table A2.4). However, only DO was identified as significant after model-averaging (Table A2.4). RR_P was positively related to TP and proportion of land-use, and negatively related to SpC, PAR, and DO (Table 2.6, Table A2.4). After model-averaging, SpC and TP were identified as the significant predictors (Table A2.4). RR_{NP} was positively related to PAR and negatively related to both velocity and SpC (Table 2.6, Table A2.4). However, only velocity was identified as a significant predictor after model-averaging (Table A2.4). Overall, the explanatory power of the models were weak (e.g., $R^2 < 30$; Table 2.6).

2.3.4 Nutrient Limitation and Response Ratios of Algal Groups

Four major algal groups were detected on the NDS bioassays. The relative abundance of each major algal group on control NDS is summarized in Table A2.5. Bacillariophytes were the most dominant group occurring on NDS in all 22 streams. Bacillariophyte concentrations ranged from 0.02 to 7.44 μg cm^{-2} across all nutrient treatments (median 1.23 μg cm^{-2}). Chlorophytes were detected in 21 streams at concentrations ranging from 0.02 to 11.38 μg cm^{-2} across all nutrient treatments (median 0.54 μg cm^{-2}). Cyanophytes were detected in 10 streams at concentrations ranging from 0.003 to 0.44 μg cm^{-2} across all nutrient treatments (median 0.05 μg cm^{-2}). Cryptophytes were the least abundant algal group, only appearing in four streams at concentrations ranging from 0.003 to 0.08 μg cm^{-2} (median 0.006 μg cm^{-2}). N-amended NDS were dominated by bacillariophyte-based pigments (median 2.01 μg cm^{-2}) relative to chlorophytes (median 0.38 μg cm^{-2}). Conversely, chlorophytes dominated N+P treatment (median 3.46 μg cm^{-2}) relative to bacillariophytes (median 1.06 μg cm^{-2}).

Nutrient limitation of chlorophytes was detected in 15 of the 22 streams (68%) (Figure 2.2). Chlorophytes within six streams were classified as Not Nutrient Limited and one stream was classified as Inconclusive (Table A2.6, Figure A2.6). Chlorophytes were primarily co-limited by both N and P (Figure 2.2, Table A2.6). Simultaneous co-limitation of chlorophytes was detected in six streams (Figure A2.7), Independent co-limitation was detected in one stream (Figure A2.8), N-driven serial co-limitation was detected in two streams (Figure A2.9), P-driven serial co-limitation was detected in five streams (Figure A2.10), and single P-limitation was detected in one stream (Figure A2.11). Bacillariophytes were only nutrient-limited in six of the

22 streams and single N-limitation was the only type of limitation observed (Figure 2.2, Table A2.7, Figure A2.12). Bacillariophytes within the remaining 16 streams were classified as Not Nutrient Limited (Figure 2.2, Table A2.7, Figure A2.13, Figure A2.14). P-inhibition was common in bacillariophytes, occurring in 12 of the streams (Table A2.7). Nutrient limitation was not assessed for cyanophytes and cryptophytes, due to their low occurrence across streams and replicates.

For chlorophytes, the effect size of nutrient enrichment was greater than zero for all nutrient treatments (Figure 2.6, Table A2.8). However, it was more pronounced for P ($RR_{CHLORO-P}$) than for N and N+P, which stimulated chlorophyte biomass similarly across all streams (Figure 2.5, Table A2.8). The magnitude of the response of chlorophytes to nutrient treatments did not vary across ecoregions or watershed categories (Figure 2.7, Table A2.9).

Bacillariophytes were only stimulated by N treatments (Figure 2.6, Table A2.8). Both P and N+P treatments suppressed bacillariophytes to a similar extent, indicating an overall inhibitory effect of P on this group (Figure 2.6, Table A2.8). The magnitude of bacillariophyte response to nutrient treatments did not vary across ecoregions for either N or N+P treatments (Figure 2.7, Table A2.9). However, the extent to which P was inhibiting varied significantly among ecoregions. P treatments suppressed bacillariophytes in both Grassland and Parkland ecoregions but were stimulating within the Boreal ecoregion (Figure 2.7, Table A2.9). No variation in bacillariophyte RRs was detected across watershed categories for N treatments (i.e., non-significant post-hoc contrasts), despite a marginally significant main effect ($p = 0.04$; Figure 2.6, Table A2.9). For P treatments, bacillariophyte RRs were stimulated by P in WC1 but inhibited by P in WC2 and WC3 (Figure 2.6, Table A2.9). Bacillariophyte RRs for the N+P treatment were higher in WC1 than in WC2, however, this difference was only marginally significant and an overall inhibitory effect across all watershed categories was maintained (Figure 2.7, Table A2.9).

MLR assessing environmental drivers of chlorophyte RRs resulted in three top models for $RR_{CHLORO-N}$, six top models for $RR_{CHLORO-P}$, and eight top models for $RR_{CHLORO-NP}$ (Table 2.7). $RR_{CHLORO-N}$ was positively associated with SpC, velocity, and PAR (Table 2.7, Table A2.10). However, only SpC was identified as significant after model-averaging (Table A2.10). $RR_{CHLORO-P}$ was positively associated with PAR, TN, SpC, and land-use, and negatively

associated with velocity (Table 2.7, Table A2.10). $RR_{CHLORO-NP}$ was positively related to TN, TP, pH, SpC, and velocity, and negatively associated with DO (Table 2.7, Table A2.10). However, the null model for $RR_{CHLORO-NP}$ had the highest overall model weight (Table 2.7). None of the selected variables for either P or N+P models were identified as significant after model-averaging (Table A2.10). Overall, the explanatory power of the models were weak for P and N+P treatments (e.g., $R^2 < 14$) and modest for N treatments (e.g., $R^2 < 33$; Table 2.7).

MLR assessing environmental drivers of bacillariophyte RRs resulted in seven top models for $RR_{BACILL-N}$, four top models for $RR_{BACILL-P}$, and six top models for $RR_{BACILL-NP}$ (Table 2.8). $RR_{BACILL-N}$ was positively associated with PAR, TN, and TP, and negatively related to DO, velocity, pH, and SpC (Table 2.8, Table A2.10). After model-averaging, DO, PAR, and velocity were identified as significant predictors (Table A2.10). $RR_{BACILL-P}$ was negatively related to DO, TN, TP, SpC, and discharge (Table 2.8, Table A2.10). However, only DO was identified as significant after model-averaging (Table A2.10). $RR_{BACILL-NP}$ was positively associated with TP and negatively associated with DO, velocity, pH, and SpC (Table 2.8 and Table A2.10). However, none of the variables were identified as significant after model-averaging (Table A2.10). Overall, the explanatory power of the models were modest for N treatments (e.g., $R^2 < 63$) and weak for P and N+P treatments (e.g., $R^2 < 30$; Table 2.8).

2.3.5 Drivers of Algal Community Composition

NMDS ordination coupled with ANOSIM analysis showed that pigments indicative of specific algal groups separated out in ordination space based upon nutrient treatments ($R = 33.07$, $p=0.0001$, Figure 2.8). Community composition within N+P treatments was significantly different than in other treatments, consisting of more chlorophyte-associated pigments (Chl *b,* lutein, and violaxanthin) relative to the controls and N-amended communities, which contained higher concentrations of bacillariophytes (diadinoxanthin and fucoxanthin) (Figure 2.8). Neoxanthin, also associated with chlorophytes, exhibited a positive relationship along the first NMDS axis (NMDS1), occurring in both P and N+P treatments. However, because neoxanthin was detected less frequently than the other chlorophyte pigments, it is separated out in ordination space. Nutrient treatment combinations were not differentiated on the basis of cyanobacteria (zeaxanthin, canthaxanthin) (Figure 2.8). The negative association of zeaxanthin and, to a lesser extent, canthaxanthin along NMDS1 was attributed to the low detection within samples, which

were mainly detected in only control and N treatments. Interestingly, the NMDS suggested that P rather than N drove the overall shifts in community composition in N+P treatments. In general, nutrient amendments were associated with a shift from bacillariophytes to chlorophytes.

RDA showed controls on community composition did not differ significantly across streams as the full model (R^2= 0.11, p = 0.66) and both RDA1 (p = 0.808) and RDA2 (p = 0.805) axes were not significant (Figure 2.9). This was further supported by forward-selection showing that the null model best explained variation in detected algal pigments. It is important to note that the overall lack of significance was potentially a result of the limited data that were available for the analysis. Overall, these results suggest that control communities were not significantly affected by baseline environmental conditions among sites. RDA also revealed that compositional differences detected on treatment NDS discs were driven by the enrichment treatments themselves, not by underlying environmental differences.

2.4 Discussion

Nitrogen, rather than phosphorus, was identified as the primary driver of nutrient limitation of periphytic algae in the studied agricultural streams. Total algal biomass was primarily N-limited or simultaneously co-limited by both N and P. However, major algal groups responded differently to nutrient enrichments. While bacillariophytes were only responsive to N amendments, chlorophytes showed greater variation in their responses, being predominantly stimulated by additions of P. In general, algal responses to nutrient amendments did not differ among ecoregions or watershed categories. Differences in stream velocity, DO, and SpC among the study sites were weak, yet significant, predictors of the responses of algae to added N or P. Overall, these findings highlight the potential to avert eutrophication of small streams within Alberta's agricultural regions by primarily focusing efforts on limiting inputs of nitrogen.

2.4.1 Nutrient Limitation

2.4.1.1 Dominance of N-limitation of total community biomass in Albertan streams

Nutrient limitation of algal growth was detected in 63% of streams, agreeing in general with the proportions of detected limitation documented in previous studies (Francoeur 2001; Marcarelli et al. 2009). Similar to our study, it has been well-established that algae within streams are most commonly limited by either N or co-limited by N and P (Francoeur 2001; Tank

and Dodds 2003; Elser et al. 2007; Marcarelli et al. 2009; Harpole et al. 2011; Bechtold et al. 2012; Reisinger et al. 2016). The magnitude of algal response to additions of N and N+P was similar, indicating the key role of N in defining nutrient limitation of periphyton in many streams across North America (Tank and Dodds 2003).

The dominance of N-driven nutrient limitation in agricultural streams in Alberta is likely driven by the low ambient DIN concentrations, coupled by the potential for relatively higher non-point source pollution of P. Although DIN loading can be substantial within agricultural watersheds (Carpenter et al. 1998), loss of bioavailable N via bacterial denitrification can also substantially deplete $NO_3^- + NO_2^-$, via transformation to N_2 gas (Hill 1979). Moreover, soils in Alberta are naturally low in P (Paterson et al. 2006), so application of P-rich fertilizers and manure are required for crop cultivation (McKenzie and Middleton 2013). Application of high-P fertilizer and manure can also lead to an accumulation of P in soils, increasing the potential for P runoff and leaching into adjacent streams (Carpenter et al. 1998). Additionally, when manure is applied in quantities necessary to meet crop N requirements, P is inadvertently added in excess (manure ratio 2:1 N:P) (Alberta Agriculture and Forestry 2015). Taken together, agricultural streams in Alberta are likely subject to relatively higher levels of P which would inadvertently lower the N:P ratio of stream water, contributing towards the prevalence of N-limitation.

Interestingly, P was never found to be the sole limiting nutrient of total algal biomass, despite the focus on phosphorus control in watershed management strategies throughout Alberta (Government of Alberta 2019). In fact, the magnitude of algal response to additions of P suggests an overall inhibitory effect of P on algal biomass. Suppression of algal biomass via nutrient enrichment bioassays has been previously documented in similar studies (Francoeur 2001; Tank and Dodds 2003; Reisinger et al. 2016) and a recent meta-analysis found that P-inhibition occurred in 12.9% of analyzed NDS experiments (Beck and Hall 2018). There are several proposed mechanisms thought to contribute to biomass inhibition which can be separated into two categories: (1) experimental artefacts of NDS construction contributing to direct P-toxicity, and (2) naturally occurring interactions among biotic communities which may diminish algal response to P additions. A commonly referred to artefact of NDS construction is the production of hydrogen peroxide (H_2O_2) gas which can occur when P salts are autoclaved with agar, and thus inhibit algal growth on agar mediums (Tanaka et al. 2014). However, this artefact

was controlled for in our study by only adding P salts after the agar heating process. The chemical form of P salt (i.e., monobasic vs dibasic) used in P amendments is another potential experimental artefact (Beck et al. 2017; Beck and Hall 2018). Monobasic forms of P (i.e., KH_2PO_4) have been suggested to influence algal biomass response, through reduction in stream water pH (Beck and Hall 2018). Although a monobasic P salt was used in our study, the streams were generally well-buffered (i.e., pH levels suggest the presence of bicarbonate buffering), so it is unlikely that this was a dominant mechanism driving the observed suppression. Moreover, the effect was not ubiquitous across all sites, indicating that other factors may have been important. Biotic interactions such as heterotrophic competition (Bernhardt and Likens 2004) and selective grazing (Hood et al. 2014) have also been proposed as potential mechanisms for P-inhibition. Yet, the occurrence of preferential grazing of algae on P-rich substrates has yet to be thoroughly explored and a recent meta-analysis found that neither heterotrophic competition nor preferential grazing pressure explained suppression by P (Beck and Hall 2018). Nevertheless, since these specific biotic interactions were not tested in this study, they cannot be ruled out as potential mechanisms behind observed P-inhibition.

Algal biomass did not exhibit nutrient limitation within 31% of streams. An absence of nutrient limitation has been previously documented in streams within agricultural watersheds (Johnson et al. 2009), and a meta-analysis found that 42.6% of NDS experiments analyzed reported a lack of nutrient limitation (Francoeur 2001). There are numerous possible explanations for the absence of detected nutrient limitation. First, algae can be limited by other factors besides nutrients. Light availability can play a critical role in algal growth (Hill et al. 1995) and has the potential to be a stronger limiting factor than nutrient enrichment (Von Schiller et al. 2007). Although most of the study streams had an open canopy such that riparian shading was not a concern, high turbidity is often observed in streams experiencing anthropogenic land-use pressures which can lead to increased light attenuation (Davies-Colley and Smith 2001). Most importantly, direct macrophyte coverage of NDS was documented in 12 streams, of which seven were classified as not nutrient limited, indicating that – in at least some cases – macrophyte coverage may have induced light limitation, thereby diluting the response to nutrient enrichment (Von Schiller et al. 2007). Additionally, unanticipated storm events during the NDS deployment period may have contributed to the scouring of algal biomass off the NDS. Although there was no observation of grazing activity on the NDS, grazers can also limit algal

biomass accrual and potentially contribute to a lack of observed nutrient limitation (Hillebrand and Kahlert 2001). Finally, detection of nutrient limitation can depend upon the statistical power of the experiment (Francoeur 2001). A number of streams experienced loss of replicates, resulting from the above-described high flow events that either detached individual replicate NDS or compromised entire sets of NDS bars. The nutrient limitation analyses performed for streams that experienced substantial loss of replicates would have had a lower statistical power, which likely contributed to the lack of detected limitation.

2.4.1.2 TN:TP ratios as predictors of observed nutrient limitation

TN:TP ratios were largely successful at predicting nutrient limitation within the streams where nutrient limitation was detected (71% agreement). The remaining discrepancy between predicted and observed nutrient limitation for the remaining 29% of streams is likely a result of the weak predictive ability of TN:TP ratios. It is well-described that DIN:TP ratios are the strongest predictor of nutrient limitation in freshwater systems (Bergström 2010; Ptacnik et al. 2010). Although DIN was the preferred N metric for this study, the frequency of concentrations of NO_3^- + NO_2^- and NH_4^+ that occurred below detection limits disallowed the calculation of ambient DIN for the nutrient ratios. TN is not the best metric to reflect the bioavailable N that algae can use for growth, as it can vary substantially in the relative composition of DIN to dissolved organic nitrogen (DON) (Wetzel 2001; Bergström 2010). Given the lack of detected DIN in the streams, TN concentrations likely consisted of mainly organic, non-bioavailable forms of N. Regardless, these findings do support that nutrient limitation does depend, in part, upon the relative ratio of ambient nutrients, even though DIN could not be used. Nevertheless, experimental investigations using NDS bioassays are still critical for accurately identifying limiting nutrients of streams and quantifying the causal relationship between enrichment and algal response.

2.4.1.3 Divergent response of chlorophytes and bacillariophytes to enrichment

Chlorophytes and bacillariophytes were differentially affected by nutrient enrichment, indicating that enrichment can promote shifts in algal community composition via contrasting taxon-specific nutrient requirements. Divergent nutrient limitation among algal species has been reported previously (Fairchild et al. 1985). However, a majority of previous studies using NDS assays have not quantified the response of specific algal groups (e.g., Francoeur et al. 1999; Tank

and Dodds 2003; Johnson et al. 2009; Reisinger et al. 2016), limiting our ability to compare taxon-specific results from this study to previous assessments. In our study, chlorophytes were most frequently classified as experiencing simultaneous co-limitation or P-driven serial co-limitation, with additions of P resulting in the greatest biomass response. Stimulation of chlorophytes under P-enriched conditions is common, as an increase in chlorophytes is frequently associated with higher TP levels (Chételat et al. 1999; Pearce et al. 2020). In contrast, bacillariophytes were only ever limited by N. Bacillariophytes have been found to be N-limited in a variety of stream ecosystems (Fairchild et al. 1985; Stephens et al. 2012), although limitation can vary among species within this taxonomic group (Keithan et al. 1988).

In contrast to chlorophytes, bacillariophytes were suppressed by P amendments, even in the presence of N (i.e., N+P additions). The ubiquitous nature of P-inhibition of bacillariophytes suggests that this group drove the overall inhibitory effect of P observed on total algal biomass. Shifts in bacillariophytes assemblages from low- to high-P tolerant species have been observed along increasing gradients of TP (Chételat et al. 1999; Hicks and Taylor 2019; Huttunen et al. 2020), suggesting that whether P additions stimulate or suppress the response of bacillariophytes could depend upon the taxa-specific tolerance in the pre-existing species pool (Pringle and Bowers 1984). However, it is unlikely that the suppression of bacillariophytes was driven solely by an abundance of low-P tolerant species, as mean ambient TP concentrations in study streams were generally high and often exceeded the 0.12 mg L^{-1} benchmark set by Hicks and Taylor (2019) for expected shifts towards high-P tolerant assemblages. Instead, bacillariophytes may have been more susceptible to direct P-inhibition than chlorophytes, as bacillariophytes experienced P-inhibition in 12 of the study streams compared to one observation for chlorophytes.

Competitive exclusion of bacillariophytes by chlorophytes may have also contributed to the observed P inhibition (Borchardt 1996; McCormick 1996; Stevenson 1997). Since the divergent response to P additions promoted a shift in community composition from bacillariophytes to chlorophytes, it is likely that chlorophytes out-competed bacillariophytes for nutrients, even in the combined presence of N, inhibiting bacillariophytes ability to use nutrient additions whenever P was present. Moreover, bacillariophytes may favour uptake and storage of silica over P, which can make them poor competitors for P (Sommer 1988, as cited in Borchardt

1996). Heterotrophic competition can also occur in freshwater systems (Halvorson et al. 2020). However, competition between periphytic algae and heterotrophs for nutrients is not commonly reported in streams (Rier and Stevenson 2002; Carr et al. 2005). Although investigating such competitive interactions was not the focus of this study, these results broadly suggest that chlorophytes may have been the dominant competitor for P. Overall, these results highlight that the specific responses of algal groups are more valuable indicators than total community biomass alone as their responses may be blurred by the lack of response by more dominant algal groups.

Cyanobacteria were not abundant relative to the other major algal groups detected in this study, suggesting that this algal group played a minor role in the ecology of the periphyton communities. Such a low prevalence of cyanobacteria is surprising given the low ambient DIN concentrations that would typically favor N_2-fixing species (Scott and Marcarelli 2012). However, a lack of response by cyanobacteria to enrichment has been documented in previous stream studies, which have found that cyanobacteria were either not stimulated by enrichments (Nelson et al. 2013) or accounted for only a small percentage (<7%) of the total algal biomass across gradients of both TN and TP (Chételat et al. 1999). Moreover, a recent investigation into the composition of algal communities across nutrient gradients in a number of our study streams also documented a relatively low proportion of cyanobacteria, compared to bacillariophytes and chlorophytes (van Klaveren 2020). Cyanobacteria may also become more dominant later in the summer (i.e., mid- to late-August) as water temperatures peak (Paerl and Otten 2013), so the duration of this study (July – early August) may have been too short to capture a response. Nevertheless, these results highlight that cyanobacteria are not the dominant algal group responding to enrichment during mid-summer. This is an important finding as it suggests a low potential for mid-summer periphytic cyanobacterial blooms to occur in these streams.

2.4.2 Regional Variation in Nutrient Limitation

There were no regional differences in whole-community biomass or chlorophyte-specific response to enrichment, despite the study occurring across three ecoregions with strong differences in predominant vegetation and soil types, and watersheds with statistically discernible differences in characteristics such as slope, runoff, leaching potential, and precipitation. These findings contrast with previous research which has detected differences in both the type and magnitude of nutrient limitation across regions in the Midwest, USA

(Reisinger et al. 2016). However, previously-described regional differences were driven primarily by variation in background NO_3^- concentrations and land-use intensity (Reisinger et al. 2016). Thus, locally-controlled abiotic stream characteristics appear to be more influential to the magnitude of response to enrichment than regional classification.

In contrast, the extent to which bacillariophytes were suppressed versus stimulated by additions of P varied across both ecoregions and watershed categories. Specifically, P additions stimulated growth in the Boreal ecoregion and WC1 streams but suppressed growth in all other regions. Bacillariophytes often lack a universal response to enrichment across large spatial scales as intermediate factors such as land-use and drainage basin characteristics can influence diatom assemblages (Snyder et al. 2002). It may be that bacillariophyte within the Boreal and WC1 streams consisted of more nutrient-tolerant taxa allowing it to bypass P-inhibition experienced in the other regions. Nevertheless, ecoregion and watershed categories appear to be poor predictors of algal nutrient limitation overall, emphasizing the importance of investigating how gradients of abiotic variables may influence algal response to enrichment.

2.4.3 Environmental Drivers of Algal Response

Unexpectedly, water column nutrient concentrations (i.e., TN and TP) were not important drivers of algal response to enrichments. Although TP was identified as a significant predictor of algal biomass response to P, this relationship appears to be an artefact of the MLR approach as no direct correlation between RR_P and TP was identified. In general, these findings contrast previous research that observed reduction in response ratios with increased ambient nutrient concentrations (Reisinger et al. 2016). It is possible that the true concentration of bioavailable nutrients in the water column was not captured by total nutrient measurements (Wetzel 2001). Given the frequency of DIN concentrations recorded below the detection limit, TN was likely comprised primarily of dissolved organic N or particulate N, and therefore, did not capture the effect of low bioavailable N on algal response. Regardless, these findings do not support our initial hypothesis that the magnitude of nutrient limitation will decrease under higher ambient nutrient concentrations. In general, these results suggest that total nutrient concentrations are poor predictors of algal response to enrichments across these agricultural streams.

Increasing stream velocity decreased the response of total algal biomass to additions of N+P, as well as the response of bacillariophytes to additions of N. Increases in stream velocity

can encourage algal growth when flows are low enough to cause substantial boundary layer effects (Stevenson 1996). However, higher velocities, particularly if resulting from a storm event, can decrease biomass accrual through reduction of immigration to colonize new surfaces and through shear stress and mobilized suspended sediments that can scour attached algae (Francoeur and Biggs 2006). As stated above, scouring of stream periphyton by spates was a possible explanation of the absence or muted response of certain algae to the nutrient amendments.

PAR was also detected as an important positive predictor of the response of bacillariophytes to N additions. The role of PAR was expected as it is well known that increased light availability stimulates algal biomass (Hill et al. 1995; Hill 1996; Johnson et al. 2009). Additionally, total algal biomass and bacillariophyte response to N and P was negatively associated with increasing DO. Since DO can increase with primary production (Dodds and Whiles 2010), this response may be reflecting the overall productivity of the stream which may not be thoroughly captured by ambient TN and TP concentrations in the models. Specific conductivity was a strong driver that exerted divergent influence on algal response. Total algal response to P was negatively associated, while chlorophyte response to N was positively associated with increased SpC. High levels of conductivity can reflect an increased availability of inorganic nutrients (Dodds and Whiles 2010) and have also been associated with higher proportions of land-use within a watershed (Mapfumo et al. 2002). Therefore, response of algae to nutrient enrichments may be muted under conditions of high conductivity if it is reflective of higher ambient nutrient concentrations (Johnson et al. 2009; Reisinger et al. 2016). Alternatively, high conductivity can also reflect higher concentrations of minor nutrients that are required for many biological processes (e.g., iron is important for nitrate assimilation) (Dodds and Whiles 2010). In this case, increased conductivity can stimulate the response of algae as they may be better able to use nutrient additions. Thus, depending on what is driving the increase, high conductivity may have divergent effects on algal response to enrichments. Overall, these results highlight the complex interactions between abiotic stream conditions and algal growth and how these interactions can influence the magnitude of algal nutrient limitation observed.

In general, the high number of top models and their relatively low R^2 values suggest that the magnitude of response to enrichment is not strongly explained by the underlying

physiochemical characteristics of streams included in the models. This stands out particularly for chlorophytes where model-selection resulted in the inclusion of the null model for both P and N+P treatments. It is possible that the models were weakly predictive of explanatory effects because of the overruling effect of N-limiting conditions (i.e., low ambient DIN concentrations) on algal response across all sites. Although some environmental variables (e.g., SpC) were identified as important drivers of algal response, the extent of their effect on the response of algae to enrichments is likely to be small and gradients of underlying abiotic conditions do not appear to exert a strong influence on algal nutrient limitation across study streams. Inclusion of landscape variables (e.g., land cover type, riparian vegetation) could have also increased predictive power of models and should be included in future studies.

2.4.4 Environmental Drivers of Baseline Algal Community Composition

Algal community composition on control treatment discs did not vary across study streams, suggesting that baseline environmental conditions were not driving the observed compositional differences across treatments. It is unlikely that the streams chosen did not span a large enough gradient of nutrients for it to substantially influence algal community structure as observed nutrient gradients extended from oligo-meso to meso-eutrophic nutrient thresholds (Dodds 2006; Dodds 2007). The range of stream TN (274 – 3830 $\mu g\ L^{-1}$) and TP (9 – 992 $\mu g\ L^{-1}$) concentrations were also similar to that of Chételat et al. (1999) who did observe differences in community composition across nutrient gradients. Instead, it is possible that the low sample size of controls (n=22) used in the pigment-based analysis may have contributed to a low statistical power and therefore reduced the ability to detect drivers of control algal community structure. Nevertheless, these results support that the observed shift in algal community composition were attributable to nutrient treatments, rather than underlying compositional differences in algal assemblages.

2.5 Conclusion

Eutrophication is a primary stressor of freshwater streams, especially in regions subject to agricultural land-use. Identifying the nature of nutrient limitation of streams within agricultural watersheds is critical for understanding how, and to what extent, nutrient loading may promote stream eutrophication. These results indicate that algae within streams across Alberta's agricultural regions are frequently N-limited or co-limited by N and P. Importantly, the results

highlight the divergent response of chlorophytes and bacillariophytes to nutrient enrichments. N-limitation was driven primarily by bacillariophytes, while co-limitation was driven primarily by chlorophytes. Interestingly, chlorophyte response was attributable more strongly to additions of P, not N. Although both N and N+P enrichment led to similar total community biomass, the underlying taxonomic shift may affect community function as species can vary in their photosynthetic capacities (Guasch et al. 1995; Rosemond and Brawley 1996). Additionally, if P additions stimulate filamentous bloom-forming chlorophytes (e.g., *Cladophora*), streams may experience more substantial effects beyond just higher productivity, potentially impacting both food webs (via reduced consumption efficiency) and ecosystem services (via inability for consumptive use of water, reduced recreational value, and diminished maintenance of downstream water quality; Dodds 1991; Dodds and Gudder 1992). These results highlight that both N and P are important regulators of periphytic algae and emphasize the potential for nutrient-driven compositional shifts in algal communities under P enrichment. The lack of strong spatial variation in nutrient limitation dynamics of these small streams suggests that broad nutrient management efforts can be applied across Alberta's agricultural regions. Overall, a dominance of N-driven nutrient limitation was detected across all streams and algal groups, suggesting that management efforts in Alberta should put more emphasis towards managing inputs of N to prevent eutrophication of agricultural streams.

2.6 Tables

Table 2.1. Study streams, site codes, sample size (n), site latitude and longitude (in decimal degrees), ecoregion, watershed category, and anthropogenic land-use (area cropland and pasture, as relative proportion of the total watershed area).

Stream	Site Code	n	Latitude	Longitude	Eco-region	Watershed Category	Crop-land	Pasture
Bigstone Creek	BGS01	16	53.0293	-113.4141	Parkland	II	0.77	0.17
Buffalo Creek	BUF01	19	53.0073	-110.8693	Parkland	II	0.78	0.19
Bullshead Creek	BUL02	20	49.9609	-110.6059	Grassland	III	0.41	0.51
Big Valley Creek	BVY01	20	51.9407	-112.8386	Parkland	II	0.47	0.47
Clearwater Creek	CLR01	20	54.4164	-114.6817	Boreal	I	0.43	0.52
Connor Creek	CNR02	11	54.0125	-114.8204	Boreal	I	0.25	0.71
Dogpound Creek	DOG01	0	51.7942	-114.3613	Parkland	III	0.83	0.11
Eagle Creek	EGL01	16	51.9450	-114.4265	Parkland	I	0.52	0.42
Foothill Creek	FTH02	18	49.4069	-113.7021	Grassland	III	0.40	0.54
Grizzlybear Creek	GRZ01	20	53.1077	-110.6438	Parkland	II	0.71	0.25
Goose Creek	GSE01	18	54.3368	-114.9515	Boreal	I	0.30	0.56
Horse Creek	HRS01	13	54.3339	-114.6893	Boreal	I	0.53	0.41
Kneehills Creek	KNE03	20	51.4803	-113.1100	Grassland	II	0.92	0.03
Modeste Creek	MDS01	0	53.1341	-114.5838	Boreal	I	0.04	0.89
Mosquito Creek	MSQ02	20	50.2521	-113.5537	Grassland	III	0.72	0.22
Matzhiwin Creek	MTZ01	20	50.8419	-111.9321	Grassland	II	0.82	0.11
Namepi Creek	NAM02	0	54.0777	-112.9756	Boreal	I	0.70	0.25
Onetree Creek	ONE01	15	50.7344	-111.6903	Grassland	II	0.64	0.22
Seven Persons Creek	PER01	17	49.9020	-110.8457	Grassland	III	0.80	0.14
Pipestone Creek	PIP01	0	53.0270	-113.2698	Parkland	II	0.70	0.23
Poplar Creek	POP01	0	53.0875	-114.4824	Boreal	I	0.05	0.88
Pothole Creek	POT01	19	49.5238	-112.7987	Grassland	III	0.76	0.17
Romeo Creek	ROM01	19	54.0723	-114.902	Boreal	I	0.16	0.82
Rosebud Creek	RSB03	14	51.3179	-113.3343	Grassland	II	0.90	0.05
Shanks Creek	SHN01	19	49.0577	-112.7375	Grassland	III	0.79	0.17
Sturgeon River	STU03	19	53.8333	-113.2828	Parkland	II	0.59	0.17
Strawberry Creek	STW01	0	53.2250	-114.3428	Boreal	I	0.53	0.41
Threehills Creek	THR01	19	51.9975	-113.5684	Parkland	II	0.85	0.11
Weed Creek	WED03	0	53.2211	-114.0682	Boreal	I	0.63	0.30
Weiller Creek	WEI01	0	52.9856	-113.2205	Parkland	II	0.88	0.02

Table 2.2. Taxonomically diagnostic carotenoids and chlorophylls and the associated major freshwater algal groups

Pigment	Algal Group
Chl *a*	All algae
Chl *b*	Chlorophytes
Alloxanthin	Cryptophytes
Diadinoxanthin	Bacillariophytes
Diatoxanthin	Bacillariophytes
Canthaxanthin	Cyanophytes
Fucoxanthin	Bacillariophytes
Lutein	Chlorophytes
Neoxanthin	Chlorophytes
Violaxanthin	Chlorophytes
Zeaxanthin	Cyanophytes

Table 2.3. Interpretation of nutrient limitation using main LMM effects and post hoc Tukey HSD contrasts ($p < 0.1$). Black triangles indicate significant terms. Grey triangles indicate special cases where the term may or may not be significant (see notes). The greater and less than symbols (> and <) indicate which treatment term resulted in a higher mean biomass response (e.g., if N-C = > ▲, the mean for N treatments was significantly higher than for controls).

	Interpretation					
Main effects	*N-limited*	*P-limited*	*Co-limited*[*]			
N	▲		▲		▲	
P		▲	▲		▲	
Interaction (N*P)				▲	▲	
Contrasts	*N-limited*	*P-limited*	*Independent co-limitation*	*Simultaneous co-limitation*	*N-driven serial co-limitation*	*P-driven serial co-limitation*
N-C	>▲		>▲		>▲	
P-C		>▲	>▲	<△[3]		>▲
NP-C	>△[1]	>△[1]	>▲	>▲	>▲	>▲
N-P	>▲	<▲		>△[3]	>▲	<▲
NP-N			>△[2]	>▲	>▲	>▲
NP-P			>△[2]	>▲	>▲	>▲

*Co-limitation was detected when either (1) significant interaction term only, (2) main effects for N and P were significant but not the interaction, or (3) all three terms were significant.
[1]NP-C may not be significant for single nutrient-limited (either N or P) sites if P-inhibition occurs.
[2]NP-N and NP-P will only be significant if the co-limited response is additive or synergistic.
[3]P-C and/or N-P may be significant if P inhibition occurs. Only when N-C or P-C was significant was the site classified as Serial co-limitation.

Table 2.4. Mean (± SE) physiochemical and water chemistry characteristics of study streams. Measured variables include water temperature, dissolved oxygen (DO), specific conductance (SpC), pH, total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), nitrate + nitrite (NO_3^- + NO_2^--N), ammonium (NH_4^+-N), dissolved organic carbon (DOC), and photosynthetically active radiation (PAR). Values for PAR represent a mean of daily values, summed from 10-min recordings over the 3-week deployment period; all other measurements represent the mean of pre- and post-deployment measurements. Note: < symbol indicates that both values were below the noted detection limit and asterisks (*) indicate that one of the two values (pre- or post-deployment measurement) was below detection and that half the detection limit was used to calculate the mean concentration.

Study Stream	Water Temperature (°C)	DO (mg L^{-1})	SpC (µS cm^{-1})	pH	TN (µg L^{-1})	TP (µg L^{-1})	TDN (µg L^{-1})	TDP (µg L^{-1})	SRP (µg L^{-1})	NO_3^- + NO_2^- (µg L^{-1})	NH_4^+ (µg L^{-1})	DOC (mg L^{-1})	PAR (mol m^{-2} day^{-1})
BGS01	17.2 (0.6)	8.80 (0.52)	651 (8)	8.06 (0.06)	2040 (220)	259 (9)	2140 (290)	184 (77)	159 (70)	26* (25)	28* (27)	24 (4)	416.72
BUF01	18.1 0.5)	6.62 (1.28)	1303 (42)	8.58 (0.10)	1615 (115)	230 (49)	1595 (35)	205 (49)	186 (43)	9* (8)	41 (15)	23 (2)	873.06
BUL02	21.4 (0.6)	7.69 (0.74)	334 (19)	8.86 (0.24)	1315 (45)	146 (30)	1020 (20)	94 (16)	81 (43)	44 (8)	139 (28)	7 (0.1)	116.72
BVY01	20.2 (2.6)	5.84 (0.04)	1664 (67)	8.09 (0.03)	3830 (300)	897 (80)	3765 (455)	820 (86)	749 (7)	<2 (0)	11 (1)	46 (1)	890.85
CLR01	18.1 (0.3)	4.41 (0.17)	261 (6)	7.38 (0.01)	1215 (25)	103 (7)	1300 (0)	84 (15)	58 (22)	<2 (0)	14 (7)	30 (0.4)	267.13
CNR02	19.1 (0.6)	0.82 (2.20)	462 (45)	7.31 (0.05)	2540 (510)	992 (379)	2505 (505)	835 (326)	588 (234)	<2 (0)	75 (50)	44 (11)	312.37
EGL01	15.1 (1.0)	7.87 (0.53)	669 (26)	8.06 (0.09)	925 (49)	37 (6)	829 (65)	24 (7)	14 (6)	5 (1)	6* (4)	15 (1)	565.56
FTH02	16.9 (1.9)	6.90 (1.79)	789 (160)	7.86 (0.27)	444 (49)	9* (8)	463 (64)	3* (3)	4 (1)	3* (2)	<3 (0)	7 (1)	980.70
GRZ01	17.5 (0.4)	6.52 (1.34)	2740 (201)	8.69 (0.06)	3360 (70)	775 (193)	3335 (35)	596 (97)	567 (29)	<2 (0)	43* (41)	42 (1)	782.18
GSE01	19.1 (0.3)	7.29 (0.03)	309 (4)	7.83 (0.04)	1555 (135)	139 (34)	1235 (95)	71 (10)	46 (19)	69 (52)	34* (33)	28 (2)	766.04
HRS01	18.5 (0.1)	7.90 (0.30)	422 (6)	7.89 (0.04)	1515 (65)	44 (10)	1445 (25)	33 (7)	20 (10)	18* (17)	6* (5)	35 (0.2)	497.21
KNE03	20.5 (1.5)	9.94 (0.10)	1345 (47)	8.48 (0.01)	1580 (30)	129 (11)	1355 (105)	35 (7)	12 (5)	<2 (0)	<3 (0)	15 (0.1)	737.89
MSQ02	19.7 (0.3)	6.20 (0.34)	348 (33)	8.17 (0.10)	274 (53)	18 (5)	299 (44)	9 (2)	4 (2)	2* (1)	<3 (0)	5 (1)	1080.41
MTZ01	19.6 (0.2)	7.96 (0.42)	561 (48)	8.29 (0.05)	584 (19)	81 (12)	655 (14)	61 (7)	52 (14)	<2 (0)	3* (1)	7 (0.2)	1037.20
ONE01	20.8 (0.02)	8.95 (0.06)	598 (46)	8.38 (0.15)	729 (16)	206 (59)	833 (41)	184 (57)	191 (71)	6 (4)	3* (1)	8 (1)	1041.50

PER01	22.4 (0.4)	7.75 (1.26)	328 (18)	8.39 (0.24)	592 (12)	68 (10)	606 (22)	53 (6)	33 (8)	13 (9)	21 (15)	5 (0.3)	1034.78
POT01	22.9 (0.5)	8.96 (0.65)	458 (72)	8.52 (0.13)	357 (134)	65 (34)	387 (54)	16 (3)	13 (4)	5 (0)	<3 (0)	3 (0.4)	1078.91
ROM01	18.3 (0.7)	1.25 (0.88)	550 (4)	7.51 (0.06)	1785 (205)	185 (48)	1785 (40)	139 (26)	95 (12)	2* (1)	4* (3)	36 (2)	827.41
RSB03	20.7 (1.6)	11.81 (1.01)	1086 (81)	8.72 (0.09)	1455 (115)	225 (27)	1445 (145)	190 (3)	108 (8)	<2 (0)	<3 (0)	16 (0)	1029.85
SHN01	20.7 (0.3)	10.93 (1.88)	707 (57)	9.16 (0.00)	1825 (1025)	132 (112)	1350 (250)	33 (21)	12 (5)	2* (1)	<3 (0)	15 (4)	1169.35
STU03	18.9 (1.1)	7.45 (0.17)	697 (91)	7.75 (0.02)	1710 (110)	228 (68)	1955 (315)	133 (36)	92 (35)	310 (49)	83 (58)	19 (3)	855.43
THR01	20.1 (2.9)	7.03 (1.05)	1154 (5)	8.14 (0.07)	1800 (100)	318 (44)	1705 (5)	295 (47)	228 (1)	<2 (0)	<3 (0)	25 (2)	833.74

Table 2.5. Mean (±SE) stream morphology and flow data for study streams. Asterisks (*) indicate that only one value was recorded for the specific parameter.

Site	Depth (m)	Velocity (m sec^{-1})	Discharge (m^3 s^{-1})
BGS01	0.42 (0.09)	0.120 (0.10)	0.276 (0.24)
BUF01	0.40 (*)	0.052 (*)	1.370 (*)
BUL02	0.36 (0.04)	0.166 (0.07)	0.302 (0.15)
BVY01	0.67 (0.05)	0.048 (0.02)	0.317 (0.19)
CLR01	1.00 (*)	0.213 (*)	0.635 (*)
CNR02	0.71 (0.01)	0.198 (0.02)	0.756 (0.20)
EGL01	0.45 (0.04)	0.048 (0.03)	0.100 (0.05)
FTH02	0.33 (0.01)	0.018 (0.02)	0.023 (0.02)
GRZ01	0.33 (0.01)	0.014 (0.01)	0.014 (0.01)
GSE01	0.97 (*)	0.334 (*)	2.130 (*)
HRS01	0.84 (*)	0.194 (*)	0.502 (*)
KNE03	0.28 (0.04)	0.086 (0.03)	0.255 (0.12)
MSQ02	0.63 (0.02)	0.225 (0.00)	1.246 (0.10)
MTZ01	0.58 (0.01)	0.199 (0.09)	0.714 (0.32)
ONE01	0.71 (0.08)	0.240 (0.13)	1.049 (0.62)
PER01	0.36 (0.15)	0.177 (0.03)	0.207 (0.12)
POT01	0.41 (0.01)	0.134 (0.00)	0.240 (0.03)
ROM01	0.84 (0.01)	0.035 (0.01)	0.157 (0.05)
RSB03	0.31 (0.11)	0.447 (0.09)	0.914 (0.52)
SHN01	0.51 (0.01)	0.003 (0.00)	0.006 (0.00)
STU03	0.84 (0.15)	0.242 (0.03)	4.274 (1.39)
THR01	0.62 (0.05)	0.006 (0.00)	0.009 (0.00)

Table 2.6. Top models (Δ AIC < 2) assessing controls on RR of Chl *a* for N, P, and NP treatments. Signs indicate the direction of the effect of the variable. Variables included in final full global model included: water temperature, pH, specific conductance (SpC), dissolved oxygen (DO), discharge, velocity, total phosphorus (TP), total nitrogen (TN), photosynthetically active radiation (PAR) and relative proportion of land-use (the sum of the relative proportion of both crop and pasture) in watershed. Bold text indicates that the variable was significant ($p < 0.05$) after top-models were averaged (Table A1.4).

Response Variable	Model	AIC_C	Δ AIC_C	Weight	Adjusted R^2
RR_N	**-DO**	-9.1	0.00	0.23	0.22
	-DO, +PAR	-8.4	0.65	0.17	0.26
	-DO, +Land-use	-8.3	0.78	0.16	0.25
	-DO, -Velocity	-8.2	0.91	0.15	0.25
	-DO, +Land-use, +PAR	-7.5	1.59	0.10	0.30
	-DO, -Discharge	-7.5	1.59	0.10	0.23
	-DO, +TP	-7.2	1.91	0.09	0.21
RR_P	**+TP**, **-SpC**	-8.3	0.00	0.31	0.21
	-PAR	-7.4	0.89	0.20	0.10
	+TP, **-SpC**, +Land-use	-7.2	1.06	0.18	0.25
	-DO	-7.1	1.24	0.17	0.09
	Null Model	-6.6	1.73	0.13	-
RR_{NP}	**-Velocity**	6.4	0.00	0.34	0.16
	-Velocity, -SpC	7.1	0.67	0.24	0.21
	-Velocity , -SpC, +PAR	7.2	0.79	0.23	0.28
	-Velocity , +PAR	7.5	1.07	0.20	0.19

Table 2.7. Top models (Δ AIC < 2) assessing controls on RR of chlorophytes for N, P, and NP treatments. Signs indicate the direction of the effect of the variable. Variables included in final full global model included: water temperature, pH, specific conductance (SpC), dissolved oxygen (DO), discharge, velocity, total phosphorus (TP), total nitrogen (TN), photosynthetically active radiation (PAR) and relative proportion of land-use (the sum of the relative proportion of both crop and pasture) in watershed. Bold text indicates that the variable was significant ($p < 0.05$) after top-models were averaged (Table A1.10).

Response Variable	Model	AIC_C	Δ AIC_C	Weight	Adjusted R^2
$RR_{CHLORO-N}$	**+SpC**	-37.3	0.00	0.44	0.29
	+SpC, +Velocity	-36.7	0.51	0.34	0.33
	+SpC, +PAR	-35.8	1.42	0.22	0.31
$RR_{CHLORO-P}$	+PAR	7.0	0.00	0.29	0.12
	+PAR, +TN	8.3	1.30	0.15	0.14
	Null Model	8.4	1.34	0.15	-
	+PAR, -Velocity	8.4	1.35	0.15	0.14
	+PAR, +SpC	8.6	1.57	0.13	0.13
	+PAR, +Land-use	8.9	1.83	0.12	0.12
$RR_{CHOLRO-NP}$	Null Model	-32.8	0.00	0.20	-
	+TN	-32.5	0.34	0.17	0.05
	-DO, +pH	-32.4	0.41	0.17	0.13
	+pH	-31.6	1.22	0.11	0.02
	+TN, +pH	-31.3	1.46	0.10	0.09
	+TP	-31.1	1.72	0.09	-0.01
	+SpC	-31.0	1.76	0.08	-0.01
	+pH, -DO, +Velocity	-30.9	1.91	0.08	-0.01

Table 2.8. Top models (Δ AIC < 2) assessing controls on RR of bacillariophytes for N, P, and NP treatments. Signs indicate the direction of the effect of the variable. Variables included in full global model included: water temperature, pH, specific conductance (SpC), dissolved oxygen (DO), discharge, velocity, total phosphorus (TP), total dissolved phosphorus (TDP), total nitrogen (TN), total dissolved nitrogen (TDN), photosynthetically active radiation (PAR), and proportion of cropland and pasture in watershed. Bold text indicates that the variable was significant ($p < 0.05$) after all top-models were averaged (Table A1.10).

Response Variable	Model	AIC_C	Δ AIC_C	Weight	Adjusted R^2
$RR_{BACILL-N}$	**+PAR**, **-DO**, **-Velocity**	-40.6	0.00	0.22	0.55
	+PAR, **-DO**, **-Velocity**, +TN	-40.3	0.30	0.19	0.60
	+PAR, **-DO**, **-Velocity**, +TP	-39.9	0.73	0.16	0.59
	+PAR, **-Velocity**, +TP, -pH	-39.4	1.26	0.12	0.58
	+PAR, **-DO**, **-Velocity**, +TN, -SpC	-39.1	1.49	0.11	0.63
	+PAR, **-DO**, +TN	-39.1	1.54	0.10	0.52
	-DO, **-Velocity**	-39.0	1.64	0.10	0.47
$RR_{BACILL-P}$	**-DO**	-18.2	0.00	0.40	0.26
	-DO, -TP	-17.3	0.85	0.26	0.29
	-DO, -SpC	-16.5	1.70	0.17	0.27
	-DO, -Discharge	-16.4	1.82	0.16	0.26
$RR_{BACILL-NP}$	-DO	-29.3	0.00	0.32	0.15
	-DO, -Velocity	-27.9	1.41	0.16	0.17
	-Velocity, -pH	-27.6	1.66	0.14	0.16
	-Velocity, -SpC	-27.5	1.78	0.13	0.16
	-Velocity, -SpC, +TP	-27.4	1.90	0.12	0.24
	-DO, -Velocity, -SpC	-27.3	1.99	0.12	0.23

2.7 Figures

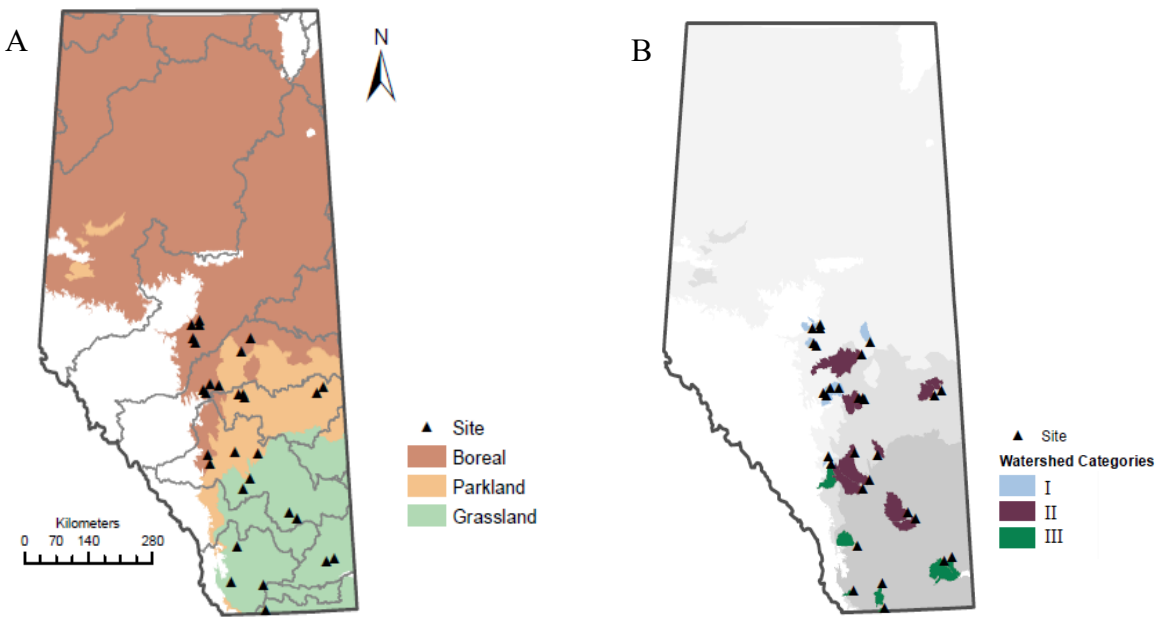

Figure 2.1. Map of the 30 study streams across (A) three major ecoregions and (B) watershed categories in Alberta, Canada

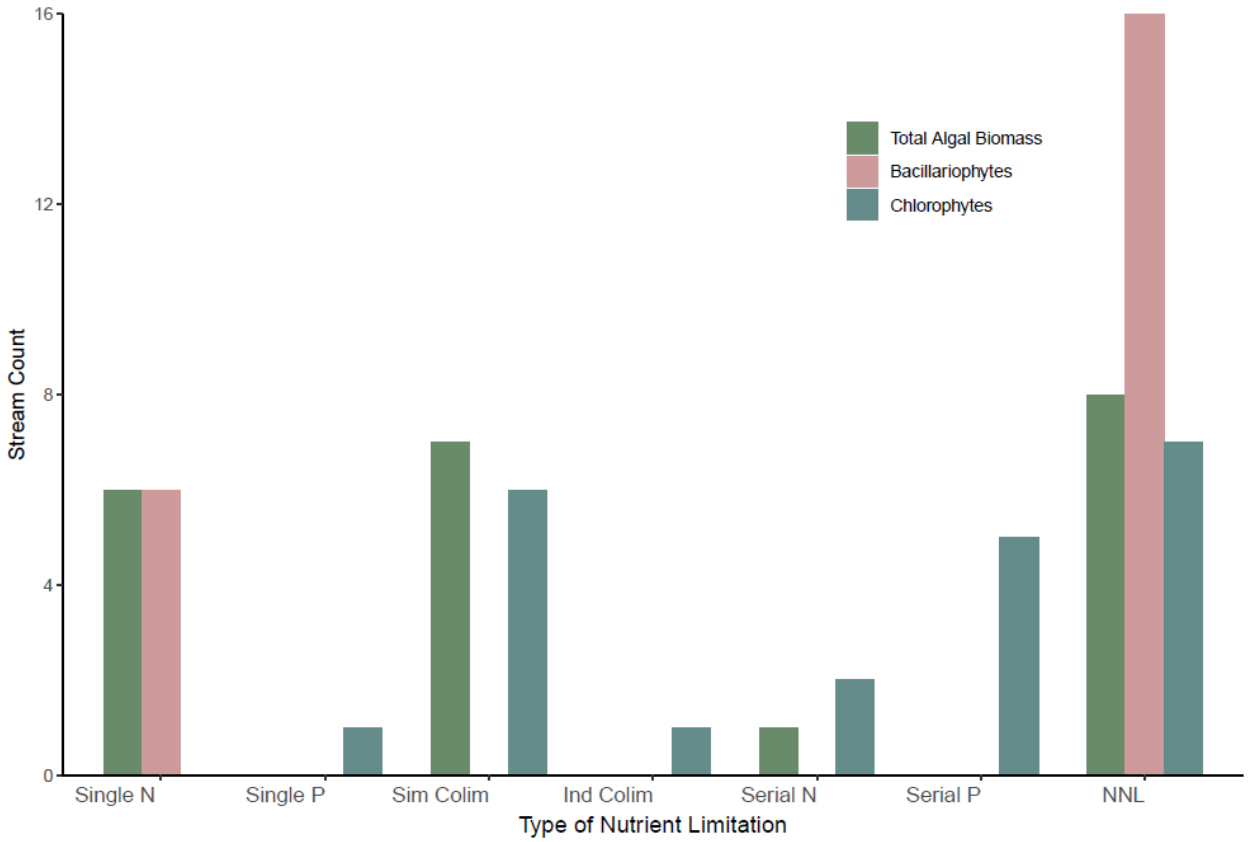

Figure 2.2. Bar plot summarizing the frequency of the types of nutrient limitation (Single N, Single P, Simultaneous co-limitation (Sim Colim), Independent co-limitation (Ind Colim), N-driven serial co-limitation (Serial N), P-driven serial co-limitation (Serial P), and Not Nutrient Limited (NNL) detected for both total algal biomass (i.e., Chlorophyll *a*) and each detected algal group (Bacillariophytes and Chlorophytes) across all streams (n = 22).

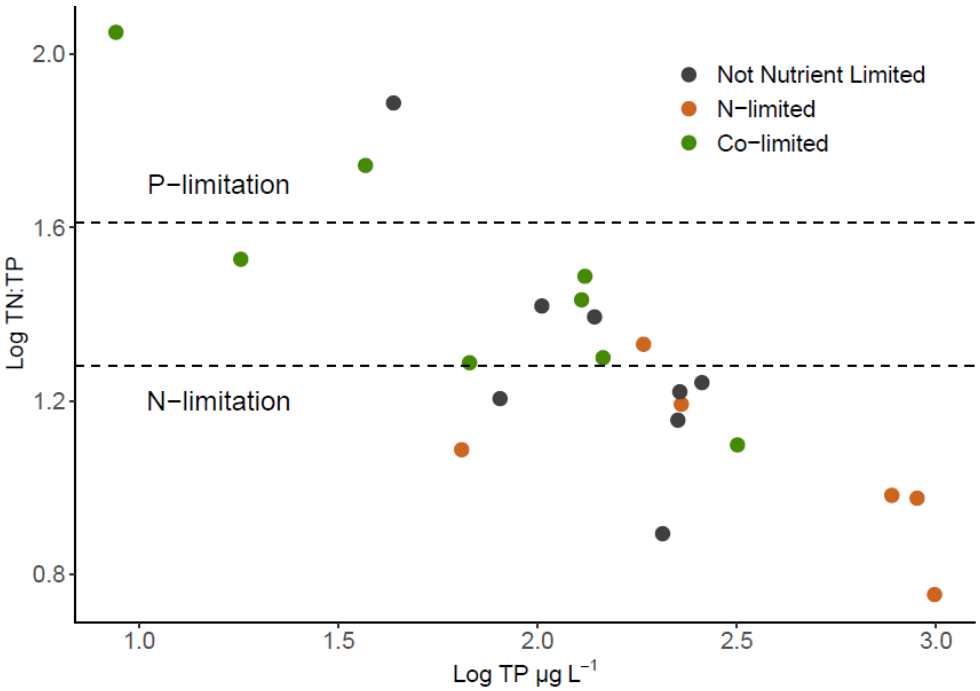

Figure 2.3. Predicted versus observed nutrient limitation of streams (n=22) across TP (µg L^{-1}) gradient of study streams. Predicted limitation was inferred using TN:TP molar ratios described by Bergström (2010) as follows: <19 = N-limitation, 19-41 = co-limitation, > 41 = P-limitation. Dashed lines represent theoretical N- and P-limitation thresholds. Streams are plotted as individual points, with colour representing the observed nutrient limitation via NDS bioassays. Note the log scale used for both axes.

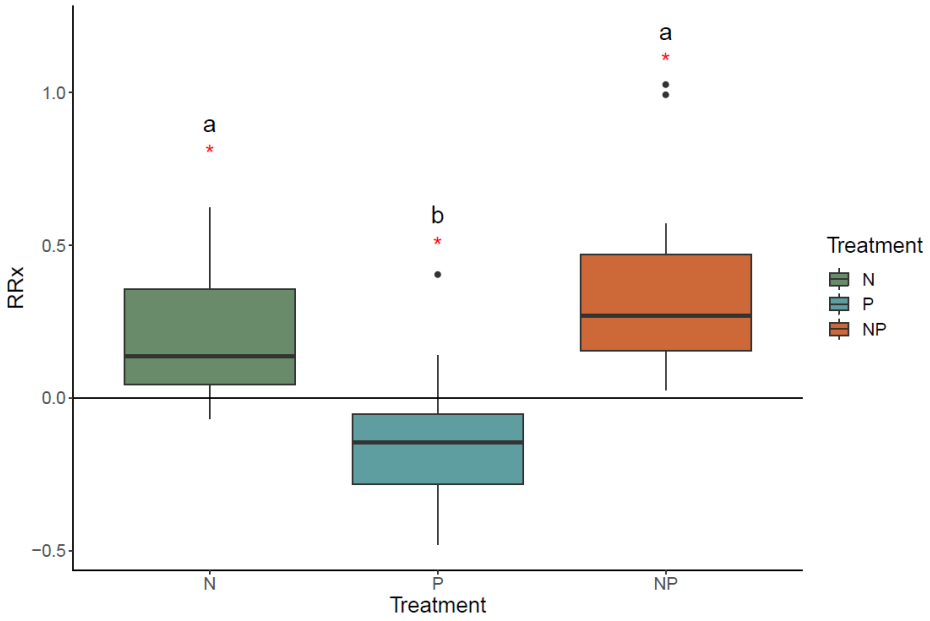

Figure 2.4. Boxplot comparing RR_N, RR_P, and RR_{NP} across all study sites. Asterisks (*) indicate RR is significantly different ($p < 0.05$) from zero. Significant differences among treatments ($p < 0.05$) are indicated by letters. The boxes represent the interquartile range, including the median value (centre line). Outliers are plotted as points.

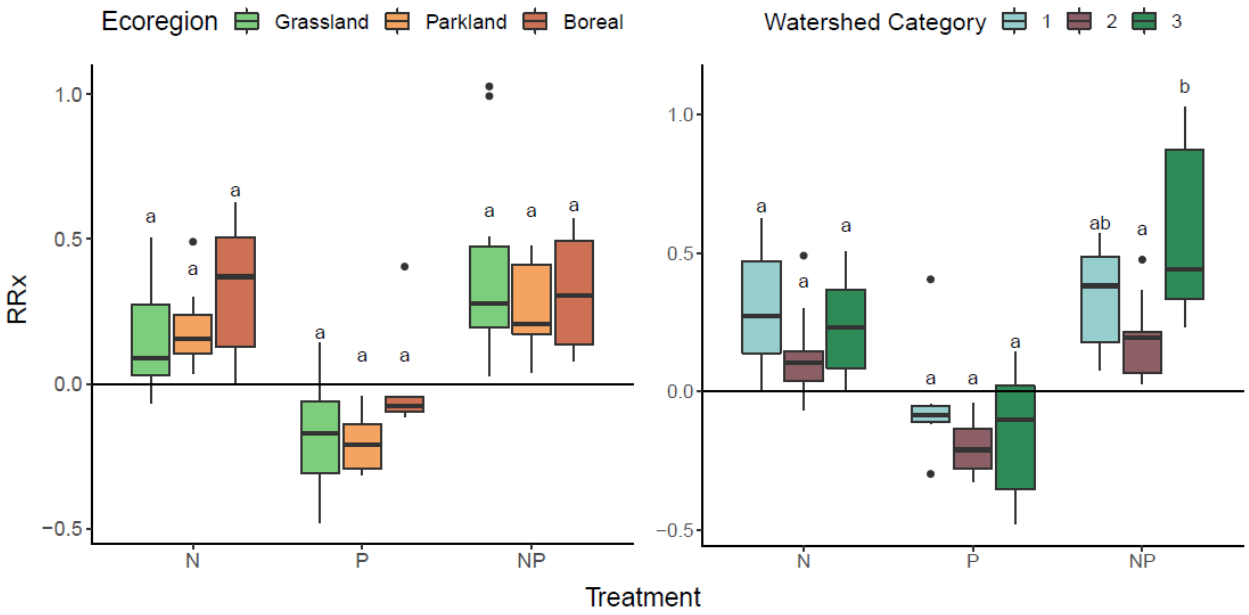

Figure 2.5. Boxplots of RR for each nutrient amendment (N, P, and NP) across both ecoregions: Boreal (n=5), Grassland (n=10), Parkland (n=7) and watershed categories: 1 (n=6), 2 (n=10), 3 (n=6). Within ecoregion or watershed categories, significant differences among treatment types (p <0.05) is indicated by letters. The boxes represent the interquartile range, including the median value (centre line). Outliers are plotted as points.

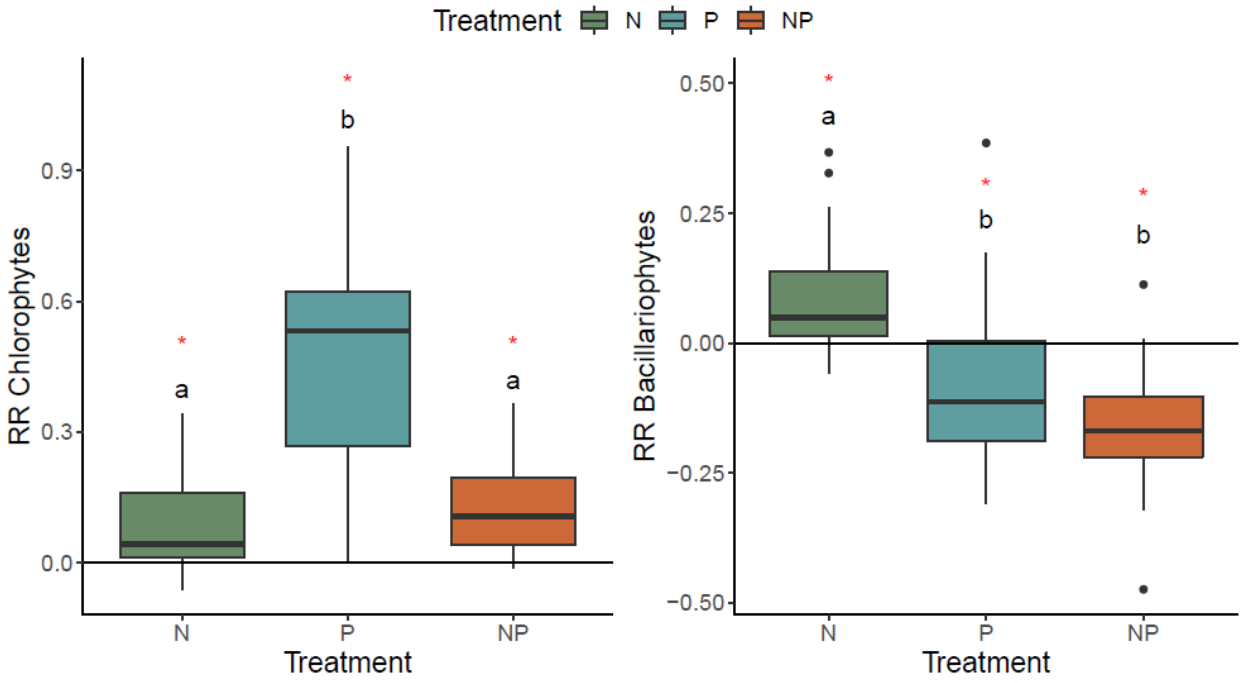

Figure 2.6. Boxplot comparing RR for chlorophytes (RR_{CHLORO}) and bacillariophytes (RR_{BACILL}) across all treatments (N, P, and NP). Asterisks (*) indicate RR is significantly different ($p < 0.05$) from zero. Within each panel, significant differences ($p < 0.05$) among treatments are indicated by letters. The boxes represent the interquartile range, including the median value (centre line). Outliers are plotted as points.

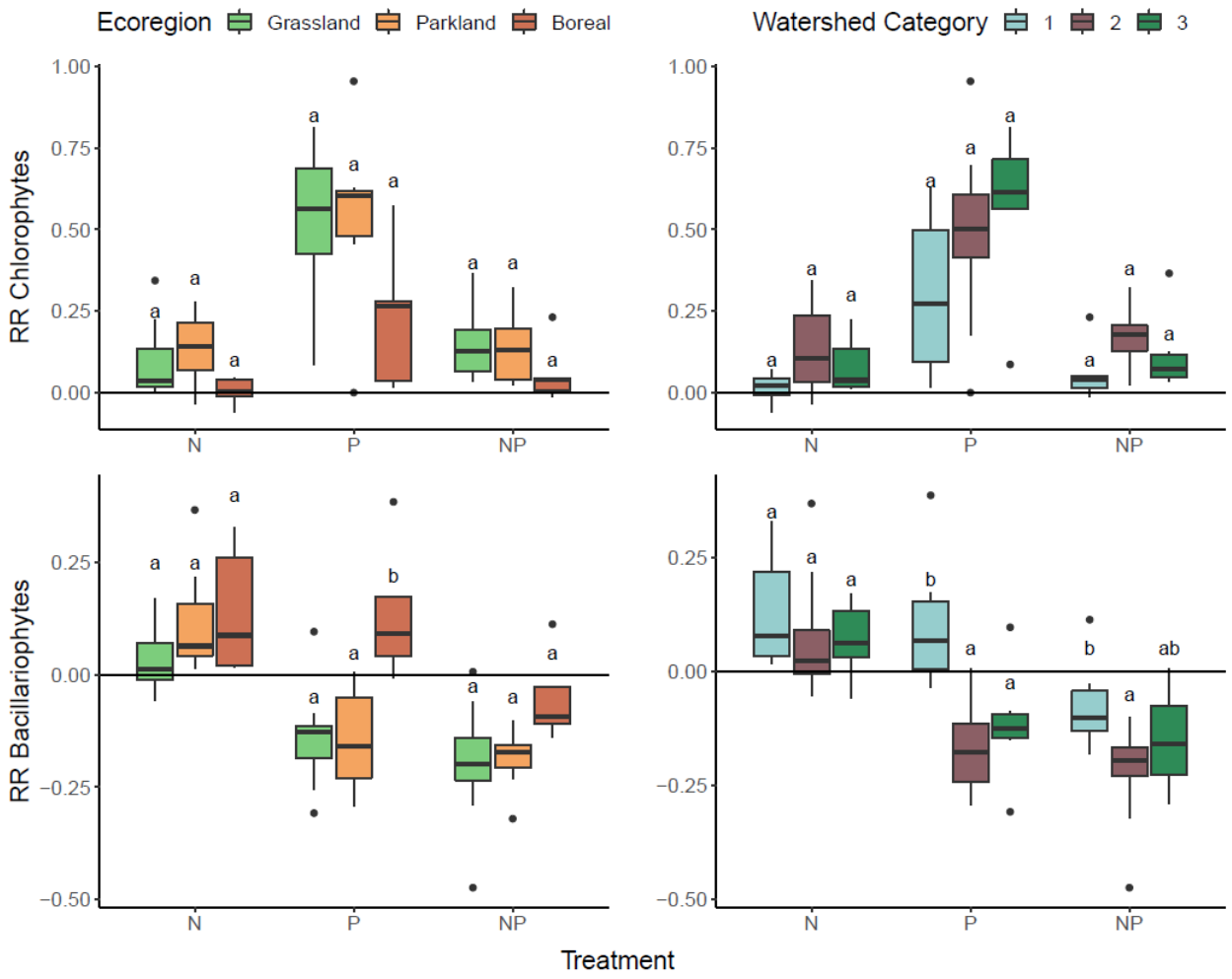

Figure 2.7. Boxplot comparing RR for chlorophytes (RR_{CHLORO}) and bacillariophytes (RR_{BACILL}) across ecoregions (Grassland, Parkland, and Boreal) and watershed categories (1, 2, and 3) for each nutrient treatment (N, P, and NP). Within ecoregion or watershed categories, significant differences among treatment types (p <0.05) is indicated by letters. The boxes represent the interquartile range, including the median value (centre line). Outliers are plotted as points.

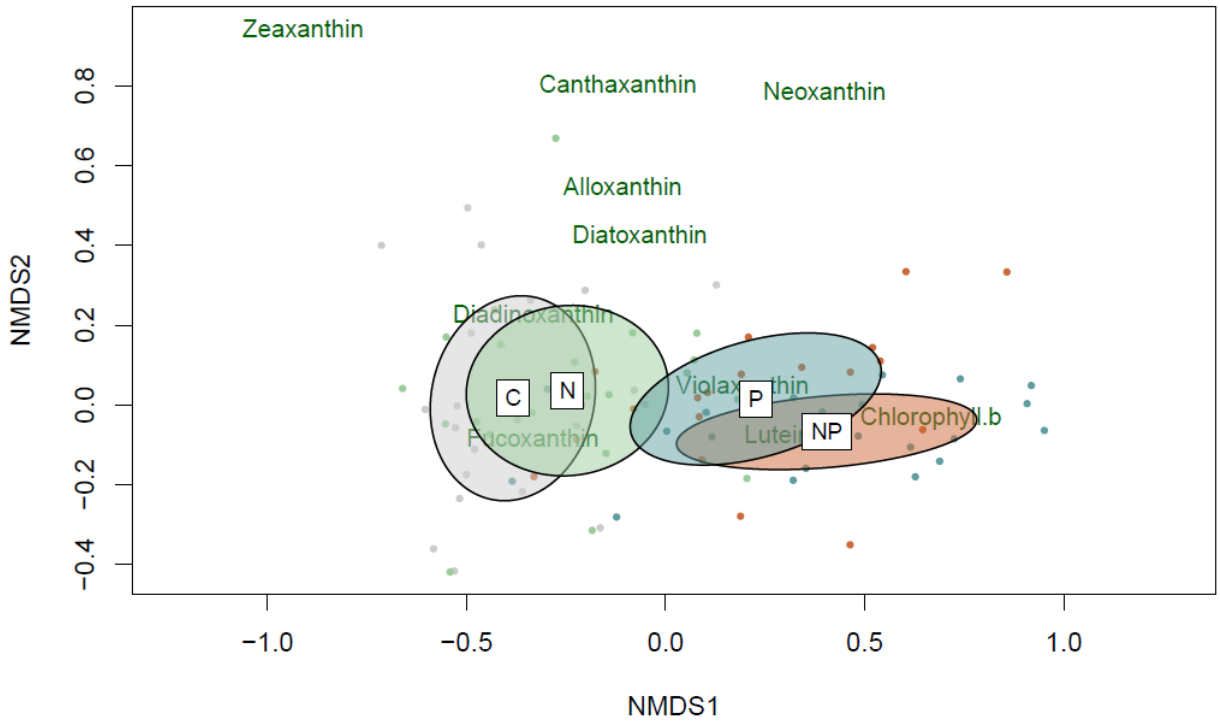

Figure 2.8. Plot of non-metric multidimensional scaling (NMDS) ordination to visualize pigment-inferred algal community composition among nutrient treatments across all sites. Ellipses were determined using the standard deviations of point (i.e., site) scores.

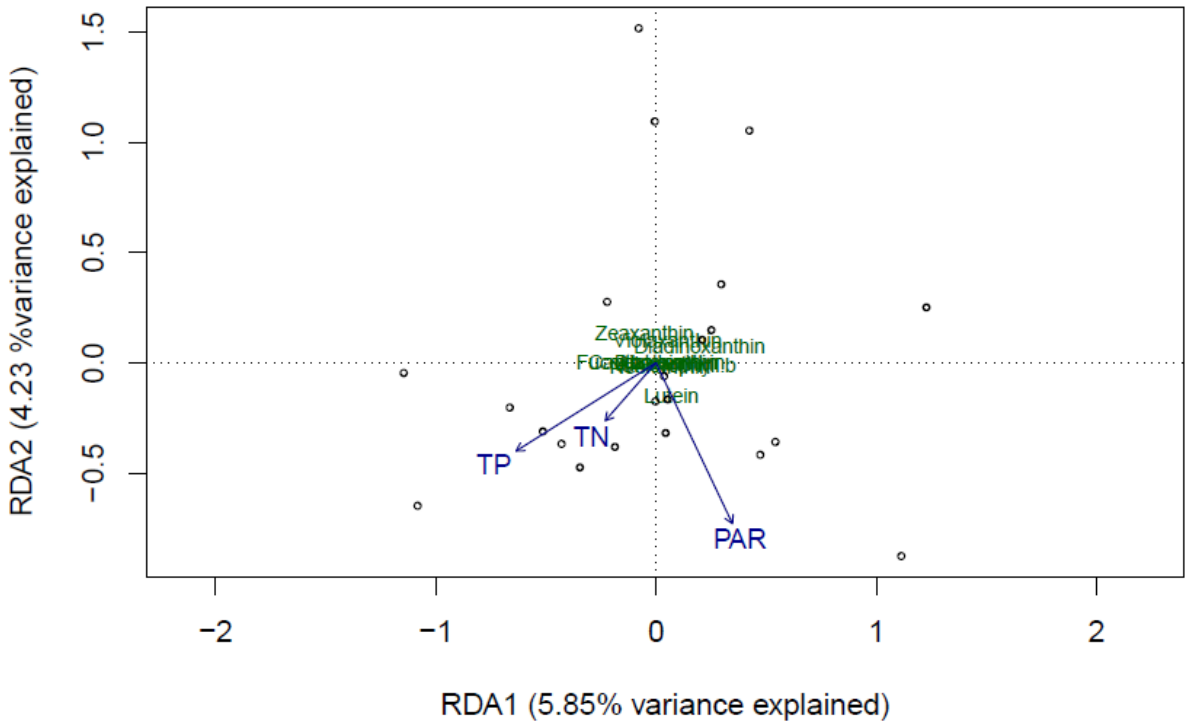

Figure 2.9. Redundancy analysis (RDA) of algal pigment data showing the first two RDA axes and the variance explained by both RDA1 (5.85%) and RDA2 (4.23%) using symmetrical scaling. Neither RDA1 ($p = 0.808$) nor RDA2 ($p= 0.805$) axes were significant. Vectors show environmental variables included in analyses. Points are sites.

Chapter 3: General Conclusion

3.1 Summary of Findings

In this thesis, I conducted the first large-scale investigation of nutrient limitation dynamics in small streams across the agricultural regions of Alberta. Using NDS bioassays, I found that N was the primary driver of algal growth. These results agree with a growing number of studies emphasizing the prevalence of N-limitation within streams across North America (Tank and Dodds 2003; Elser et al. 2007; Reisinger et al. 2016). Through quantification of taxonomically-diagnostic algal pigments on NDS discs, I found that chlorophytes and bacillariophytes experienced contrasting types of nutrient limitation. Here, N-limitation was driven primarily by bacillariophytes, while co-limitation was driven primarily by chlorophytes, emphasizing the potential for nutrient-driven shifts in algal community structure within these streams. The dominance of chlorophytes and suppression of bacillariophytes whenever P was present suggests competitive interactions for nutrients may have driven this shift. These divergent responses and underlying interactions support that identifying the specific response of algal groups to enrichment is critical for a comprehensive understanding of limitation dynamics, supporting our initial hypothesis that algal group response is a more valuable indicator of nutrient limitation than solely quantifying the response of total community biomass.

The results outlined in Chapter 2 also demonstrate that ambient nutrient ratios were relatively effective predictors of the causal relationship between nutrient enrichment and algal response when nutrient limitation was detected. The lack of significant response of cyanobacteria to enrichment suggests there is a low potential for mid-summer cyanobacterial blooms within these streams. Moreover, nutrient limitation did not vary across ecoregions or watershed categories, despite differences in characteristics such as dominant vegetation, soil types, precipitation, and runoff and leaching potential. Lastly, the abiotic characteristics of streams were largely irrelevant in regulating the magnitude of nutrient limitation, likely due to the overriding effect of low DIN. Taken together, these results suggest that broad nutrient management efforts can be applied across all impacted regions, despite underlying physiochemical differences across streams.

Overall, the finding that N is the primary limiting nutrient of agricultural streams has major implications for the development of nutrient management efforts in the province, as efforts

to date have focused primarily on controlling inputs of P (e.g., Alberta Phosphorus Watershed Management Tool; Bow River Phosphorus Management Tool; Government of Alberta 2014b; Government of Alberta 2019). These results largely suggest that the management of non-point source pollution entering streams within agricultural regions should focus on targeting inputs of N to limit excessive algal biomass accrual. Yet, I also found that P addition played an important structuring role, driving the observed shift in algal community composition. Taken together, a dual-nutrient management approach at controlling inputs of both N and P will likely be the most ecologically effective at averting potentially problematic shifts in algal assemblages and eutrophication of streams within agricultural regions of Alberta. Mitigating eutrophication of these small streams will also benefit downstream receiving ecosystems through maintenance of the beneficial ecosystem services provided by streams.

3.2 Considerations and Future Research

The use of NDS bioassays provides strong empirical evidence of algal response to nutrient enrichment. However, since these bioassays occur over a short time frame, they provide only a snapshot of nutrient limitation dynamics within streams. Our study was performed solely during the summer of 2019, so temporal variation in nutrient limitation across seasons within these streams is still unknown. Since seasonal variation in nutrient limitation dynamics has been previously documented in other systems (Francoeur et al. 1999; Sanderson et al. 2009), future research should focus on investigating how limitation may vary across the spring, summer, and fall to understand nutrient limitation dynamics in these regions more fully. Doing so will aid in the formation of more robust nutrient management efforts.

Algal growth can be limited by light, even in the presence of nutrient enrichment (Hill et al. 1995). Macrophyte coverage of NDS was documented at over half of the streams which could have induced light limitation of attached algae. Therefore, the absence of nutrient limitation documented across many streams may have been a result of the macrophyte-induced light limitation, diminishing the overall response of algae to nutrient additions. Future NDS studies should consider constructing cages surrounding the NDS racks to prevent coverage by macrophytes and other objects (e.g., tree branches) that can catch onto racks as they are swept downstream, particularly in streams with high discharge. This will help in minimizing avoidable light limiting scenarios.

The abnormally high levels of precipitation that occurred during the summer of 2019 in the Boreal and Parkland ecoregions compromised entire NDS bioassay experiments at several sites. This not only reduced the sample size of our entire study, but it may have also contributed to the scouring of algal biomass at other less-impacted sites, potentially diluting the effects of enrichment. Thus, the detected response of algae to nutrient enrichments in our study may be conservative. To gain a more complete understanding of nutrient limitation within these streams, conducting annual experiments across the entire ice-free season will reduce the influence of random climatic events on observed nutrient limitation and will help document long-term temporal patterns in nutrient limitation.

High-performance liquid chromatography (HPLC) is a standardized and well-suited methodology to quantify concentration of major algal groups within streams (Lauridsen et al. 2011). Yet, it only allows for broad-scale quantification of changes in algal assemblages and species-specific responses to nutrient addition are not detectable. Albeit more costly and time-consuming, taxonomic identification of algal species via microscopy would likely provide a more sensitive metric of the effects of nutrient amendments on periphytic algal community composition. In turn, multivariate analyses of relationships between environmental factors and algal traits that reflect their ecological roles in streams could potentially provide valuable insights into how nutrient loading impacts their ecosystem functioning.

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APPENDICES

Appendix 1. Supporting Information for Chapter 1

Table A1.1. Summary of ANCOVA results on log_{10}-transformed total N (TN) and total P (TP) including ecoregions (Grassland, Parkland, and Boreal) as the predictor and relative proportion of cropland and pasture as the covariates.

		Sum of squares	df	F	p
TN	Intercept	0.03	1	0.53	0.47
	Ecoregion	0.11	2	1.06	0.35
	Cropland	0.05	1	1.01	0.31
	Pasture	0.04	1	0.71	0.40
	Ecoregion*Cropland	0.10	2	0.94	0.39
	Ecoregion*Pasture	0.07	2	0.65	0.52
	Cropland*Pasture	0.03	1	0.46	0.49
	Ecoregion*Cropland*Pasture	0.07	2	0.63	0.53
	Error	2.78			
TP	Intercept	0.30	1	2.64	0.11
	Ecoregion	0.17	2	0.76	0.47
	Cropland	0.24	1	2.06	0.15
	Pasture	0.22	1	1.91	0.17
	Ecoregion*Cropland	0.17	2	0.73	0.48
	Ecoregion*Pasture	0.21	2	0.89	0.41
	Cropland*Pasture	0.003	1	0.03	0.87
	Ecoregion*Cropland*Pasture	0.007	2	0.03	0.97
	Error	5.97			

Table A1.2. Summary of multiple linear regressions examining the relationship between log_{10}-transformed total N (TN) and total P (TP) concentrations (ug L^{-1}) and relative proportion of cropland and pasture pooled across three ecoregions in Alberta. Bolded numbers indicate significance ($p < 0.05$).

		B	Standard Error	t_{61}	p
TN ($R^2 = 0.08$)	Intercept	-3.27	1.35	-2.42	**0.019**
	Cropland	3.65	1.43	2.56	**0.013**
	Pasture	3.61	1.46	2.48	**0.016**
TP ($R^2 = 0.17$)	Intercept	-4.30	1.91	-2.25	**0.028**
	Cropland	3.94	2.02	1.95	**0.055**
	Pasture	3.40	2.05	1.65	0.104

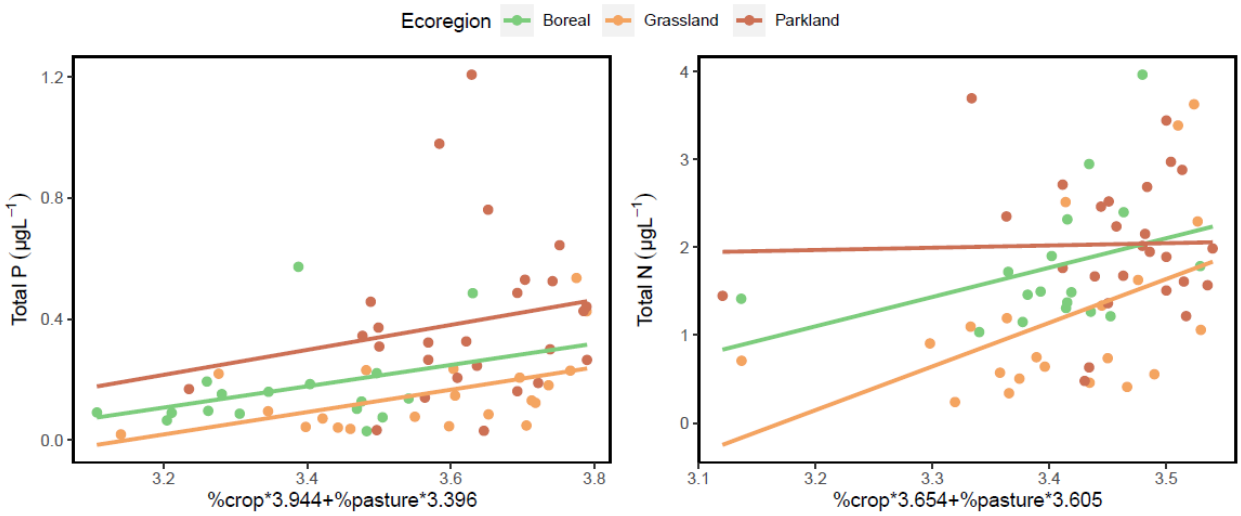

Figure A1.1. Regression relationship between land-use (% cropland and % pasture) and Total P and Total N across three ecoregions (Boreal, Grassland, and Parkland) within Alberta's agricultural region. X-axis was calculated from regression analysis conducted across all three ecoregions. Lines represent individual regression within ecoregion.

Appendix 2. Supporting Information for Chapter 2

Table A2.1. Summary of mixed effect model and Tukey HSD post hoc comparison *p*-values, and the type of nutrient limitation detected at each study stream. Significance (p <0.1) is indicated by bolded numbers. Negative sign (-) indicates nutrient response was inhibitory.

Study Stream	N effect	P effect	Interaction (N*P)	N:C	P:C	NP:C	N:P	NP:N	NP:P	Limitation Type
BGS01	0.750	0.587	0.681	0.987	0.939	0.982	0.807	1.000	0.788	Not nutrient limited
BUF01	**0.008**	0.777	0.492	**0.033**	0.910	0.276	**0.028**	0.556	0.213	N-limitation
BUL02	0.882	0.927	**0.062**	0.999	1.000	**0.044**	0.995	**0.057**	**0.029**	Simultaneous co-limitation
BVY01	**<0.0001**	**0.011**	0.116	**<0.0001**	**(-) 0.048**	**<0.0001**	**<0.0001**	0.934	**<0.0001**	N-limitation
CLR01	0.748	0.921	0.940	0.987	1.000	0.999	0.972	0.997	0.996	Not nutrient limited
CNR02	**0.014**	0.437	0.260	**0.048**	0.832	0.113	**0.071**	0.7746	0.201	N-limitation
EGL01	**0.082**	**0.005**	**0.001**	0.271	**(-) 0.020**	**0.004**	**0.001**	**0.065**	**0.0001**	Simultaneous co-limitation
FTH02	**0.055**	0.535	**0.0003**	0.195	0.916	**<0.0001**	**0.061**	**0.0002**	**<0.0001**	Simultaneous co-limitation
GRZ01	**0.013**	**0.023**	0.121	**0.057**	**(-) 0.093**	**0.085**	**0.001**	0.995	**0.001**	N-limitation
GSE01	**0.083**	0.566	0.854	0.277	0.932	0.355	0.132	0.988	0.188	Not nutrient limited
HRS01	**0.094**	0.544	0.707	0.289	0.914	0.375	0.127	1.000	0.177	Not nutrient limited
KNE03	0.543	0.151	**0.010**	0.936	0.449	**0.026**	0.204	**0.070**	**0.002**	Simultaneous co-limitation
MSQ02	0.499	0.369	**0.044**	0.858	0.806	**0.031**	0.329	0.207	**0.002**	Simultaneous co-limitation
MTZ01	0.724	**0.097**	0.232	0.983	0.320	0.985	0.189	1.000	0.195	Not nutrient limited
ONE01	0.783	0.404	0.228	0.991	0.811	0.918	0.893	0.786	0.506	Not nutrient limited
PER01	0.664	**0.066**	**0.006**	0.986	0.226	**0.043**	**0.081**	**0.059**	**0.001**	Simultaneous co-limitation
POT01	**0.0001**	0.159	0.241	**0.001**	0.465	**0.001**	**0.009**	0.997	**0.012**	N-limitation
ROM01	**0.0002**	0.971	0.907	**0.001**	1.000	**0.002**	**0.001**	0.996	**0.001**	N-limitation
RSB03	0.817	**<0.0001**	**<0.0001**	0.996	**(-) <0.0001**	**0.024**	**0.0004**	0.197	**<0.0001**	Inconclusive
SHN01	**0.002**	0.768	**0.0001**	**0.008**	0.990	**<0.0001**	**0.021**	**<0.0001**	**<0.0001**	N-driven serial co-limitation
STU03	0.391	0.142	**0.078**	0.809	0.427	0.229	0.131	0.664	**0.022**	Not nutrient limited
THR01	0.425	0.265	**0.009**	0.840	0.655	**0.009**	0.205	**0.027**	**0.001**	Simultaneous co-limitation

Table A2.2. Summary statistics for Figure 2.4 including one sample, two-tailed t-tests, Wilcoxon Rank Sum test, and Kruskal Wallis Test for RR. Significance (p <0.05) is indicated by bolded numbers.

Test	Response variable	Test Statistic	p-value	
T-test	RR_N	t(21) = 4.87	**< 0.0001**	
	RR_P	t(21) = -3.49	**0.002**	
Wilcoxon Rank Sum Test	RR_{NP}	v(21) = 253	**<0.0001**	
Kruskal Wallis Test	RRx	$X^2(2) = 34.60$	Pairwise Wilcox Tests	
			N:P	**< 0.0001**
			N:NP	0.085
			P:NP	**< 0.0001**

Table A2.3. Summary statistics for Figure 2.5 including one-way ANOVAs detecting differences in RR among ecoregions (B = Boreal, G = Grassland, P = Parkland) and watershed categories. Significance (p <0.05) is indicated by bolded numbers.

One-way ANOVAs		Main effect p-value	Tukey HSD contrast p-values	
Ecoregion	N	$p = 0.369$	B:G	0.347
			B:P	0.533
			G:P	0.951
	P	$p = 0.105$	B:G	0.148
			B:P	0.118
			G:P	0.952
	NP	$p = 0.674$	B:G	0.877
			B:P	0.957
			G:P	0.659
Watershed Cluster	N	$p = 0.357$	1:2	0.338
			1:3	0.836
			2:3	0.703
	P	$p = 0.267$	1:2	0.240
			1:3	0.549
			2:3	0.876
	NP	**$p = 0.014$**	1:2	0.424
			1:3	0.200
			2:3	**0.010**

Table A2.4. Final model variables, direction of coefficients, and *p*-values ($p < 0.05$) after model-averaging of top models summarized in Table 2.6.

Response Variable	Model-averaged coefficients	Weighted Importance	*p*-value
RR_N	-DO	1	**0.023**
	+PAR	0.27	0.166
	+Land-use	0.26	0.178
	-Velocity	0.15	0.195
	-Discharge	0.10	0.291
	+TP	0.09	0.352
RR_P	-SpC	0.49	**0.022**
	+TP	0.49	**0.017**
	-PAR	0.20	0.079
	+Land-use	0.18	0.184
	-DO	0.17	0.096
RR_{NP}	-Velocity	1.01	**0.022**
	-SpC	0.47	0.131
	+PAR	0.43	0.159

Table A2. 5. Relative abundance (as concentration of each algal group in µg cm^{-2}) on Control NDS across all study streams.

Site	Bacillariophytes	Chlorophytes	Cryptophytes	Cyanophytes
BGS01	2.85	0.94	0	0
BUF01	2.34	0.15	0	0
BUL02	1.61	0.08	0	0.24
BVY01	2.35	0.08	0	0
CLR01	0.55	0.04	0	0
CNR02	0.30	0.03	0	0
EGL01	1.56	0.14	0.01	0
FTH02	0.64	0.10	0	0
GRZ01	1.23	0.07	0	0.05
HRS01	1.07	0.06	0	0.01
KNE03	2.47	0	0	0.16
GSE01	3.25	0.55	0	0
MSQ02	1.70	0.07	0	0.09
MTZ01	2.93	0.13	0	0.03
ONE01	1.13	0.10	0	0
PER01	1.63	0.32	0	0
POT01	0.86	0.05	0	0
ROM01	0.95	0.06	0	0
RSB03	3.40	0.40	0	0.15
SHN01	0.30	0.25	0.01	0.003
STU03	2.26	0.09	0	0
THR01	1.56	0.58	0	0.01

Table A2.6. Summary of chlorophyte-based mixed effects models and Tukey HSD post hoc comparison *p*-values, and the type of nutrient limitation detected at each study stream. Significance (p <0.1) is indicated by bold numbers. Negative sign (-) indicates nutrient response was inhibitory.

Site	N effect	P effect	Interaction (N*P)	N:C	P:C	NP:C	N:P	NP:N	NP:P	Limitation Type
BGS01	0.737	0.801	0.953	0.984	0.993	1.000	0.926	0.984	0.993	Not nutrient limited
BUF01	**0.0004**	**<0.0001**	0.828	**0.003**	**<0.0001**	**<0.0001**	**0.004**	**<0.0001**	**0.003**	P-driven serial co-limitation
BUL02	0.716	**<0.0001**	0.114	0.983	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**0.050**	P-driven serial co-limitation
BVY01	**0.012**	**0.002**	**<0.0001**	**0.059**	**0.0108**	**<0.0001**	0.934	**<0.0001**	**<0.0001**	Simultaneous co-limitation
CLR01	0.618	0.446	0.936	0.955	0.857	0.511	0.992	0.801	0.921	Not nutrient limited
CNR02	0.122	0.155	0.195	0.349	0.421	0.487	0.996	0.981	0.998	Not nutrient limited
EGL01	0.227	0.423	**<0.0001**	0.623	0.854	**<0.0001**	0.977	**<0.0001**	**<0.0001**	Simultaneous co-limitation
FTH02	**<0.0001**	**0.018**	**0.019**	**<0.0001**	**0.085**	**<0.0001**	**0.007**	**<0.0001**	**<0.0001**	N-driven serial co-limitation
GRZ01	**0.008**	**0.087**	**0.088**	**0.035**	0.291	**<0.0001**	0.581	**0.004**	**0.0004**	N-driven serial co-limitation
GSE01	0.783	0.953	0.139	0.991	0.999	0.233	0.996	0.184	0.252	Not nutrient limited
HRS01	0.613	0.165	**0.005**	0.948	0.453	**0.002**	0.219	**0.001**	**0.007**	Simultaneous co-limitation
KNE03	0.147	**<0.0001**	**<0.0001**	0.469	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	**<0.0001**	P-driven serial co-limitation
MSQ02	**0.096**	0.212	**0.004**	0.342	0.596	**<0.0001**	0.975	**(-)<0.0001**	**(-)<0.0001**	Inconclusive
MTZ01	**0.080**	**0.002**	0.522	0.276	**0.011**	**0.002**	0.266	**0.057**	0.767	P-driven serial co-limitation
ONE01	0.992	0.200	0.618	1.000	0.529	0.236	0.482	0.199	0.908	Not nutrient limited
PER01	0.746	0.163	0.000	0.988	0.502	**<0.0001**	0.658	**<0.0001**	**<0.0001**	Simultaneous co-limitation
POT01	0.389	0.146	0.662	0.807	0.434	**0.034**	0.885	0.150	0.493	Not nutrient limited
ROM01	**0.003**	**<0.0001**	0.591	**0.013**	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**	**0.002**	P-driven serial co-limitation
RSB03	**0.001**	**0.004**	0.861	**0.006**	**0.017**	**0.000**	0.412	**0.058**	**0.003**	Independent co-limitation
SHN01	0.125	**0.025**	**0.0003**	0.387	0.101	**<0.0001**	0.743	**<0.0001**	**<0.0001**	Simultaneous co-limitation
STU03	0.276	0.297	0.072	0.671	0.699	**0.001**	1.000	**0.009**	**0.013**	Simultaneous co-limitation
THR01	**0.035**	**0.0002**	**0.0002**	0.133	**0.001**	0.857	**(-) 0.026**	0.239	**(-) 0.001**	P-limitation

Table A2.7. Summary of bacillariophyte-based mixed effects model and Tukey HSD post hoc comparison *p*-values and the type of nutrient limitation detected at each study stream. Significance ($p < 0.1$) is indicated by bold numbers. Negative sign (-) indicates nutrient response was inhibitory.

Site	N effect	P effect	Interaction (N*P)	N:C	P:C	NP:C	N:P	NP:N	NP:P	Limitation Type
BGS01	0.501	**0.032**	0.966	0.8920	0.1190	0.3311	**0.041**	0.1290	0.8670	Not nutrient limited
BUF01	**0.011**	**0.038**	0.178	**0.047**	0.1440	0.4112	**0.001**	**(-) 0.003**	0.8265	N-limitation
BUL02	0.265	**0.002**	0.119	0.6560	**(-) 0.009**	**(-) 0.07**	**(-) 0.07**	0.4431	0.6360	Not nutrient limited
BVY01	**<0.0001**	**0.018**	**0.0005**	**0.0001**	**(-) 0.073**	0.1320	**(-) <0.0001**	**(-) <0.0001**	0.9800	N-limitation
CLR01	0.394	0.644	0.649	0.8910	0.9630	0.8960	0.9750	0.9970	0.9950	Not nutrient limited
CNR02	**0.040**	0.895	0.231	0.1320	0.9980	0.7120	0.1000	0.3270	0.7100	Not nutrient limited
EGL01	0.236	**0.024**	0.572	0.6010	**(-) 0.091**	0.9232	**(-) 0.013**	0.2960	0.2230	Not nutrient limited
FTH02	**0.010**	**0.002**	0.032	**0.051**	**(-) 0.010**	**(-) 0.003**	**(-) <0.0001**	**(-) <0.0001**	0.9831	N-limitation
GRZ01	0.699	**0.003**	0.496	0.9780	**(-) 0.012**	**(-) 0.004**	**(-) 0.006**	**(-) 0.002**	0.9306	Not nutrient limited
GSE01	**0.035**	0.156	0.478	0.1310	0.4530	0.9980	**(-) 0.014**	0.1032	0.5408	Not nutrient limited
HRS01	0.367	0.187	0.146	0.7710	0.4980	0.3604	0.1584	0.8214	**0.0617**	Not nutrient limited
KNE03	0.784	**0.001**	0.201	0.9919	**(-) 0.005**	**(-) 0.086**	**(-) 0.008**	0.1370	0.3970	Not nutrient limited
MSQ02	0.844	**0.099**	0.940	0.9970	0.3250	0.3690	0.2450	0.2810	0.9990	Not nutrient limited
MTZ01	0.974	**0.002**	0.767	1.0000	**(-) 0.007**	**(-) 0.003**	**(-) 0.006**	**(-) 0.003**	0.9780	Not nutrient limited
ONE01	0.893	**0.023**	0.596	0.9989	**(-) 0.085**	0.1945	**0.0767**	0.1852	0.9294	Not nutrient limited
PER01	0.306	**<0.0001**	0.223	0.7354	**(-) <0.0001**	**(-) <0.0001**	**(-) <0.0001**	**(-) <0.0001**	0.9081	Not nutrient limited
POT01	**0.0001**	0.812	**0.086**	**0.0003**	0.9946	**0.0120**	**0.0006**	0.1161	**0.0261**	N-limitation
ROM01	**<0.0001**	0.107	**0.011**	**<0.0001**	0.3421	0.3623	**<0.0001**	**(-) 0.0003**	**0.0153**	N-limitation
RSB03	0.592	**0.0001**	0.117	0.9503	**(-) 0.0007**	0.2305	**(-) 0.068**	0.7266	0.3092	Not nutrient limited
SHN01	**0.002**	**0.002**	**0.001**	**0.008**	**(-) 0.008**	**(-) 0.0002**	**<0.0001**	**(-) <0.0001**	0.2046	N-limitation
STU03	0.317	**0.008**	0.274	0.7256	**(-) 0.036**	**(-) 0.009**	**0.007**	**(-) 0.001**	0.9316	Not nutrient limited
THR01	**0.030**	0.549	**0.004**	0.1177	0.9230	0.2670	0.2430	**(-) 0.002**	**(-) 0.0765**	Not nutrient limited

Table A2.8. Summary statistics for Figure 2.6 including one sample, two-tailed t-tests, Wilcoxon Rank Sum tests, and Kruskal Wallis Tests for RR. Significance (p <0.05) is indicated by bold numbers.

Algal Group	Test	Response Variable	Test Statistic	p-value
Chlorophytes	t-test	RR_N	t_{21}= 3.62	**0.001**
	t-test	RR_P	t_{21}= 7.98	**<0.0001**
	t-test	RR_{NP}	t_{21}= 5.46	**<0.0001**
	Kruskal Wallis Test	RR_X	X^2 = 22.96	Pairwise Wilcox Tests N:P **<0.0001** N:NP 0.178 P:NP **0.0001**
Bacillariophytes	Wilcoxon Rank Sum	RR_N	v_{21} = 225	**0.0006**
	t-test	RR_P	t_{21}= -2.19	**0.03**
	t-test	RR_{NP}	t_{21}= -6.32	**<0.0001**
	Kruskal Wallis test	RR_X	X^2 = 27.84	Pairwise Wilcox Tests N:P **0.0003** N:NP **<0.0001** P:NP 0.119

Table A2.9. Summary statistics for Figure 2.7 including one-way ANOVAs detecting differences in RR among ecoregions for chlorophytes and bacillariophytes (B = Boreal, G = Grassland, P = Parkland) and watershed categories. Significance (p <0.05) is indicated by bold numbers. Asterisk (*) indicates that a Kruskal Wallis Test was performed due to non-normality in data.

Algal Group	Test	Treatment	Main effect p-value	Post-hoc contrasts p-values	
Chlorophytes	Ecoregion	N	$p = 0.110$	B:G	0.286
				B:P	0.093
				G:P	0.644
		P	$p = 0.122$	B:G	0.121
				B:P	0.125
				G:P	0.989
		NP*	$p = 0.090$	B:G	0.810
				B:P	0.300
				G:P	0.400
	Watershed Cluster	N	$p = 0.202$	1:2	0.103
				1:3	0.503
				2:3	0.649
		P	$p = 0.341$	1:2	0.352
				1:3	0.196
				2:3	0.824
		NP	$p = 0.168$	1:2	0.141
				1:3	0.585
				2:3	0.663
Bacillariophytes	Ecoregion	N*	$p = 0.123$	B:G	0.190
				B:P	1.000
				G:P	0.190
		P	**$p = 0.0001$**	B:G	**0.0015**
				B:P	**0.0024**
				G:P	0.995
		NP	$p = 0.054$	B:G	0.054
				B:P	0.104
				G:P	0.977
	Watershed Cluster	N*	**$p = 0.04$**	1:2	0.660
				1:3	0.710
				2:3	0.710
		P	**$p = 0.001$**	1:2	**0.001**
				1:3	**0.012**
				2:3	0.723
		NP	$p = 0.052$	1:2	**0.035**
				1:3	0.436
				2:3	0.406

Table A2. 10. Final model variables, direction of coefficients, and *p*-values ($p < 0.05$) after model-averaging of top models summarized in Tables 2.7 and 2.8.

Algal Group	Response Variable	Model-averaged coefficients	Weighted Importance	*p*-value
Chlorophytes	RR_N	+SpC	1	**0.003**
		+Velocity	0.34	0.586
		+PAR	0.22	0.261
	RR_P	+PAR	0.85	0.059
		+TN	0.15	0.244
		-Velocity	0.15	0.251
		+SpC	0.13	0.244
		+Land-use	0.12	0.335
	RR_{NP}	+TN	0.27	0.148
		-DO	0.25	0.065
		+pH	0.27	0.168
		+TP	0.09	0.368
		+SpC	0.08	0.380
		+Velocity	0.08	0.234
Bacillariophytes	RR_N	-DO	0.88	**0.001**
		+PAR	0.90	**0.026**
		-Velocity	0.90	**0.024**
		+TN	0.29	0.103
		+TP	0.28	0.078
		-pH	0.12	**0.003**
		-SpC	0.11	0.143
	RR_P	-DO	0.99	**0.006**
		-TP	0.26	0.188
		-SpC	0.17	0.310
		-Discharge	0.16	0.334
	RR_{NP}	-DO	0.60	0.061
		-Velocity	0.68	0.108
		-pH	0.14	0.077
		-SpC	0.25	0.095
		+TP	0.12	0.112

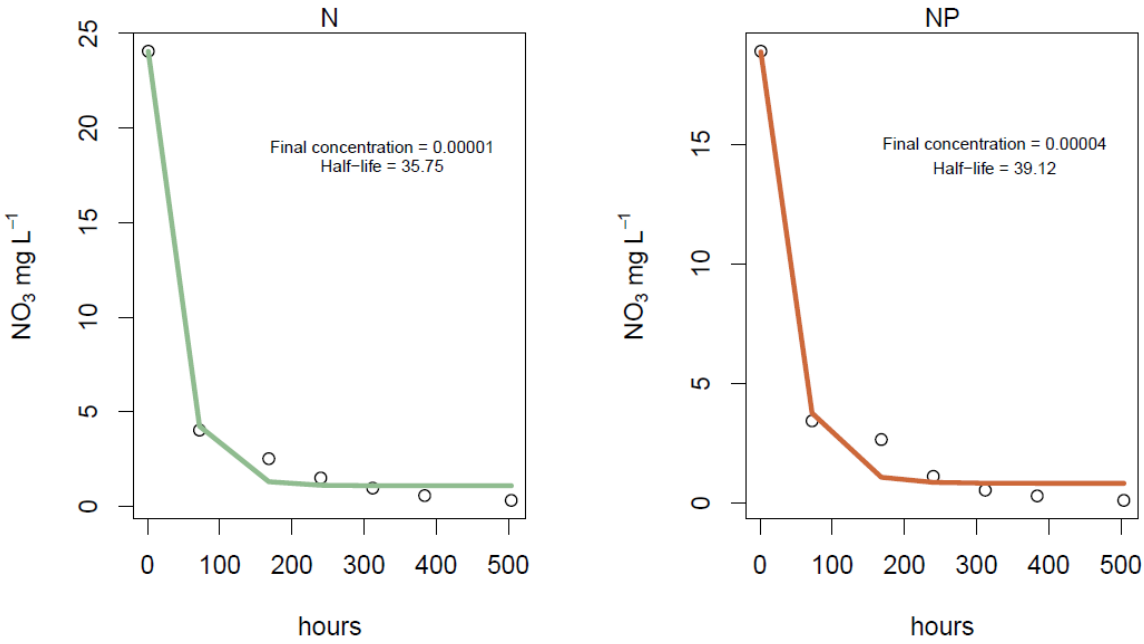

Figure A2.1. Exponential decay of nitrogen (NO_3^-) using release rate of both N and NP amended nutrient diffusing substrates over 21 days (500 hours). Final concentration of NO_3^- in units of mg L^{-1} and half-life is in units of number of hours.

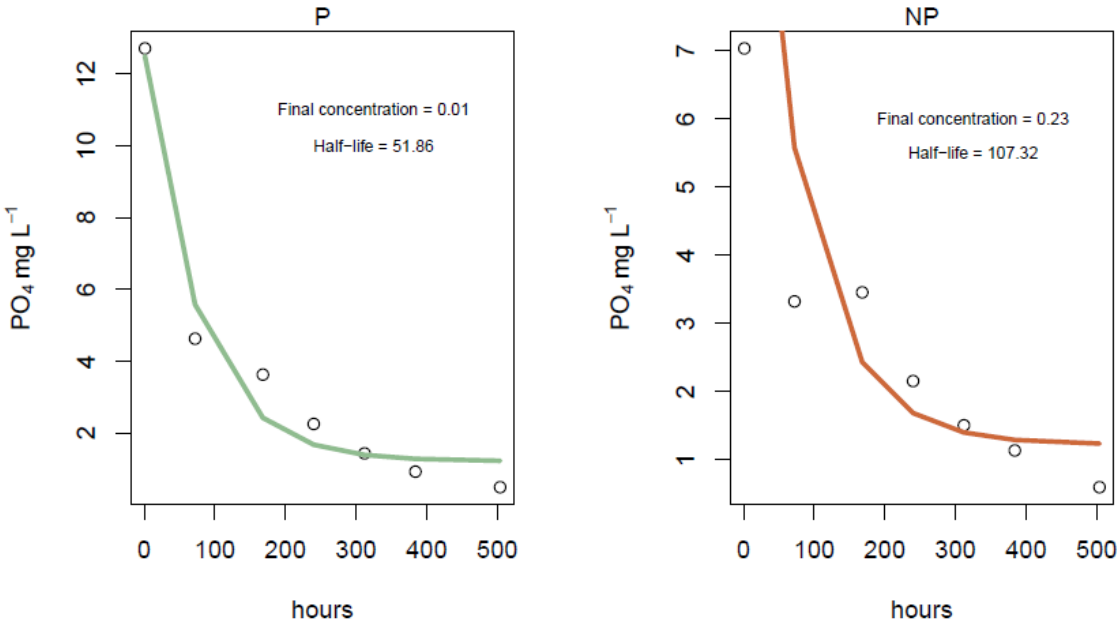

Figure A2.2. Exponential decay of phosphorus (PO^{3-}) using release rates of both P and NP amended nutrient diffusing substrates over 21 days (500 hours). Final concentration of PO^{3-} in units of mg L^{-1} and half-life is in units of number of hours.

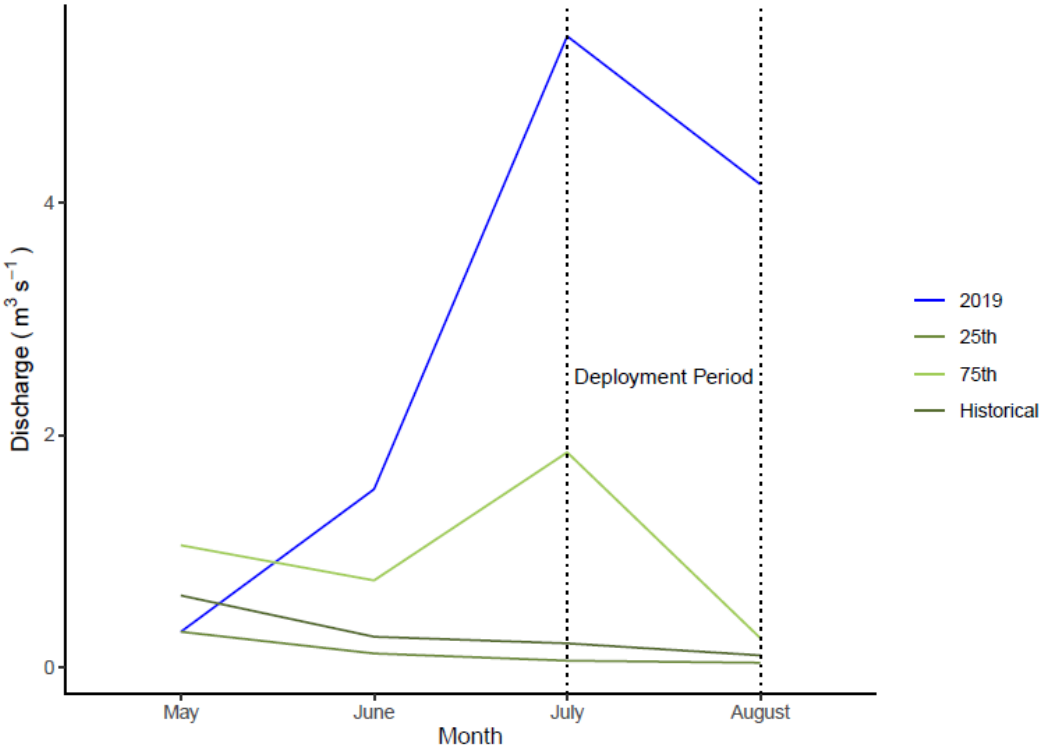

Figure A2.3. Mean monthly discharge (m^3 s^{-1}) from May to August in 2019 and historical median monthly discharge (including the 25th and 75th percentiles) from 1967 to 2018 at Strawberry Creek (STW01; Station ID: 05DF004).

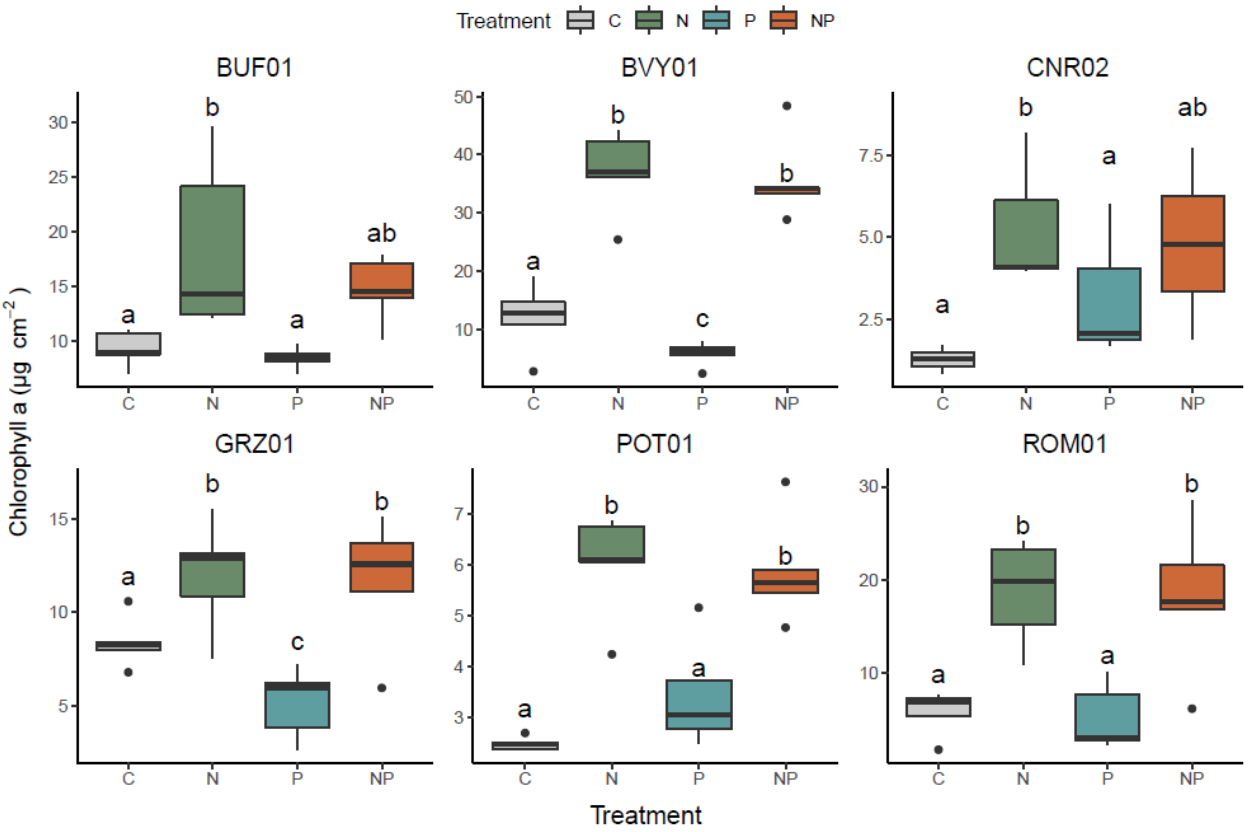

Figure A2.4. Boxplots of study streams classified as "N-limited", showing chlorophyll *a* (µg cm^{-2}) concentration across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

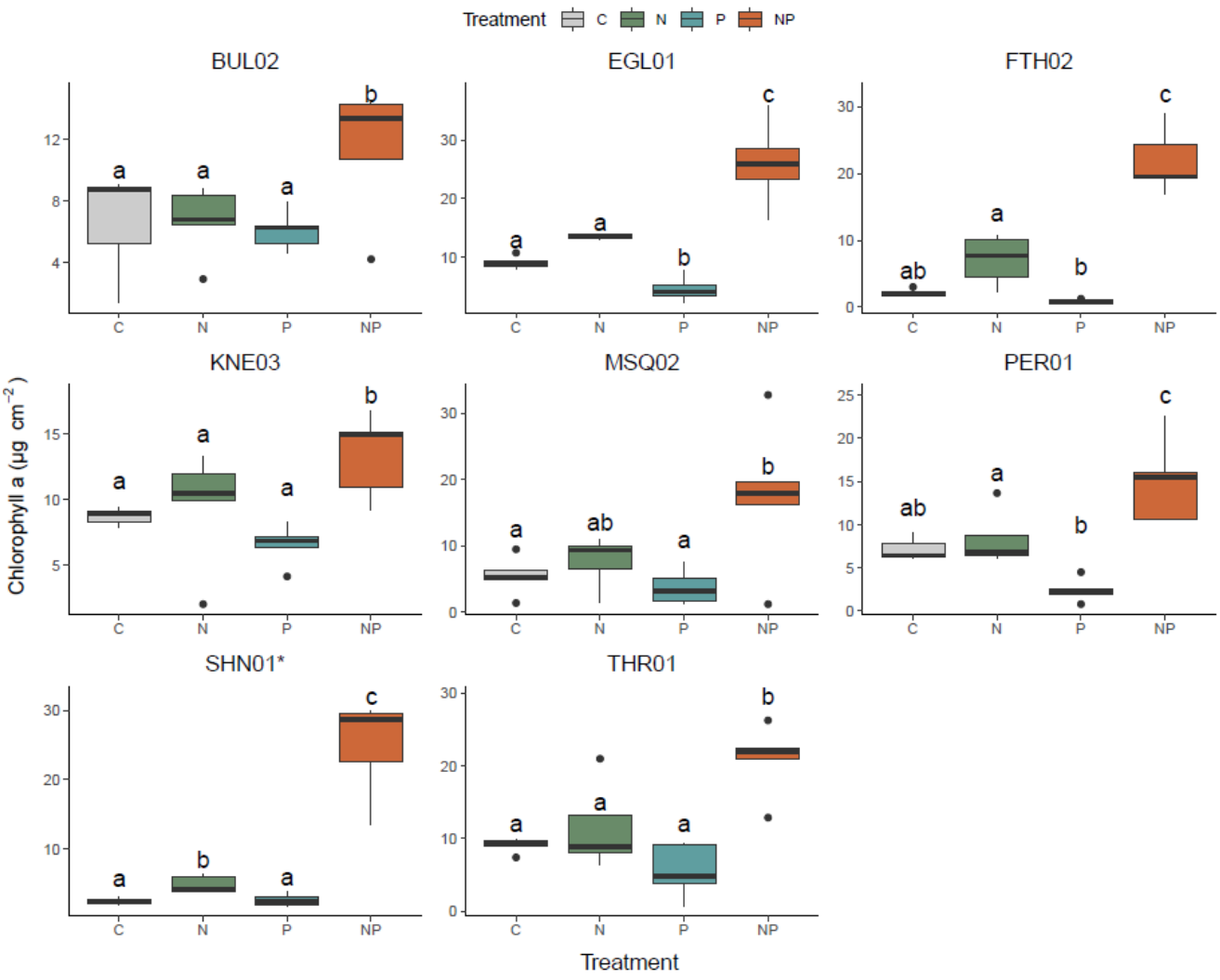

Figure A2.5. Boxplots of study streams classified as "Simultaneous Co-limited", showing chlorophyll *a* (µg cm^{-2}) concentration across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p <$ 0.1) are displayed as letters. *Note: SHN01 was classified as N-driven Serial Colimitation

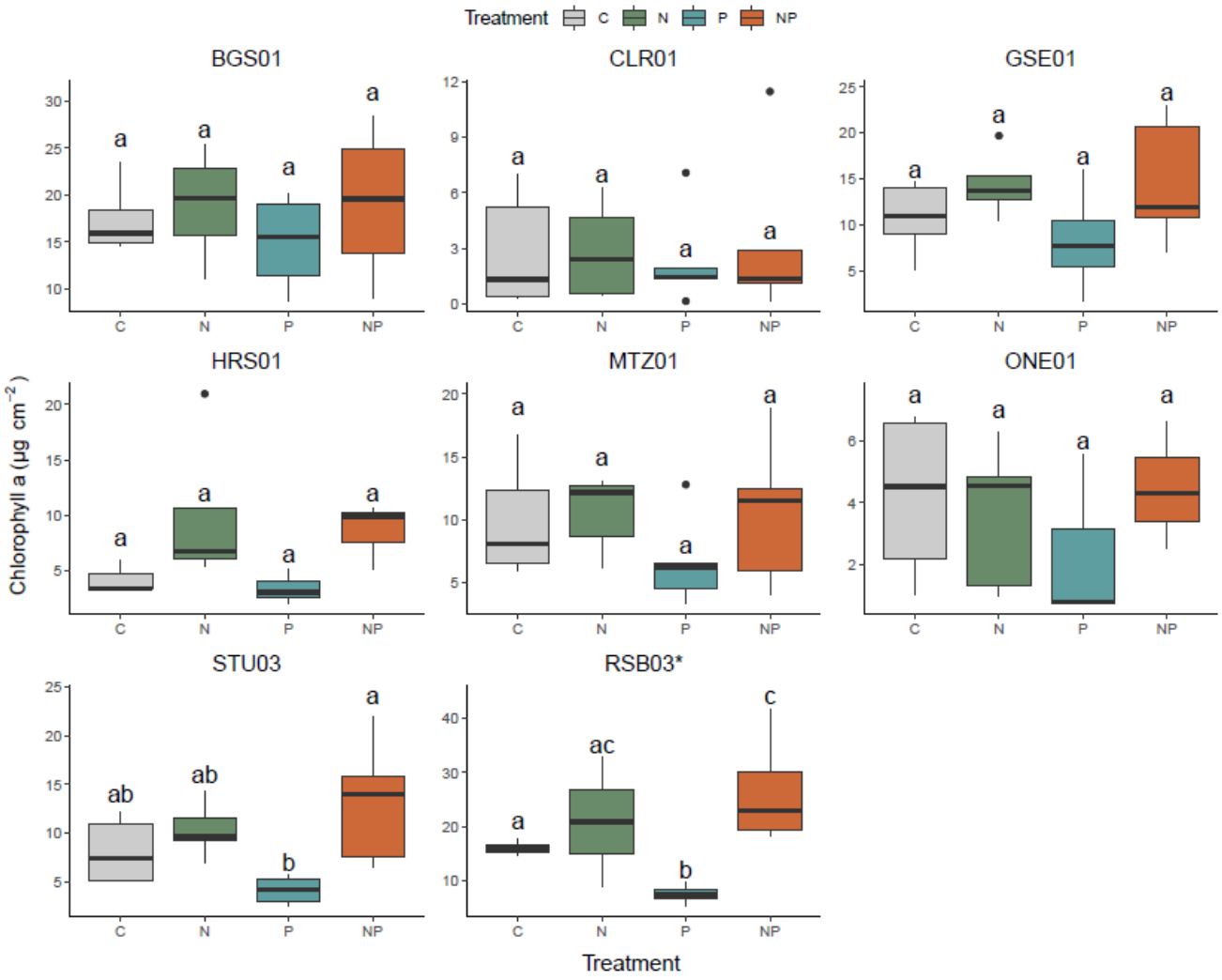

Figure A2.6. Boxplots of study streams classified as "Not Nutrient Limited", showing chlorophyll *a* (µg cm^{-2}) concentration across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters. *Note: RSB03 classified as Inconclusive.

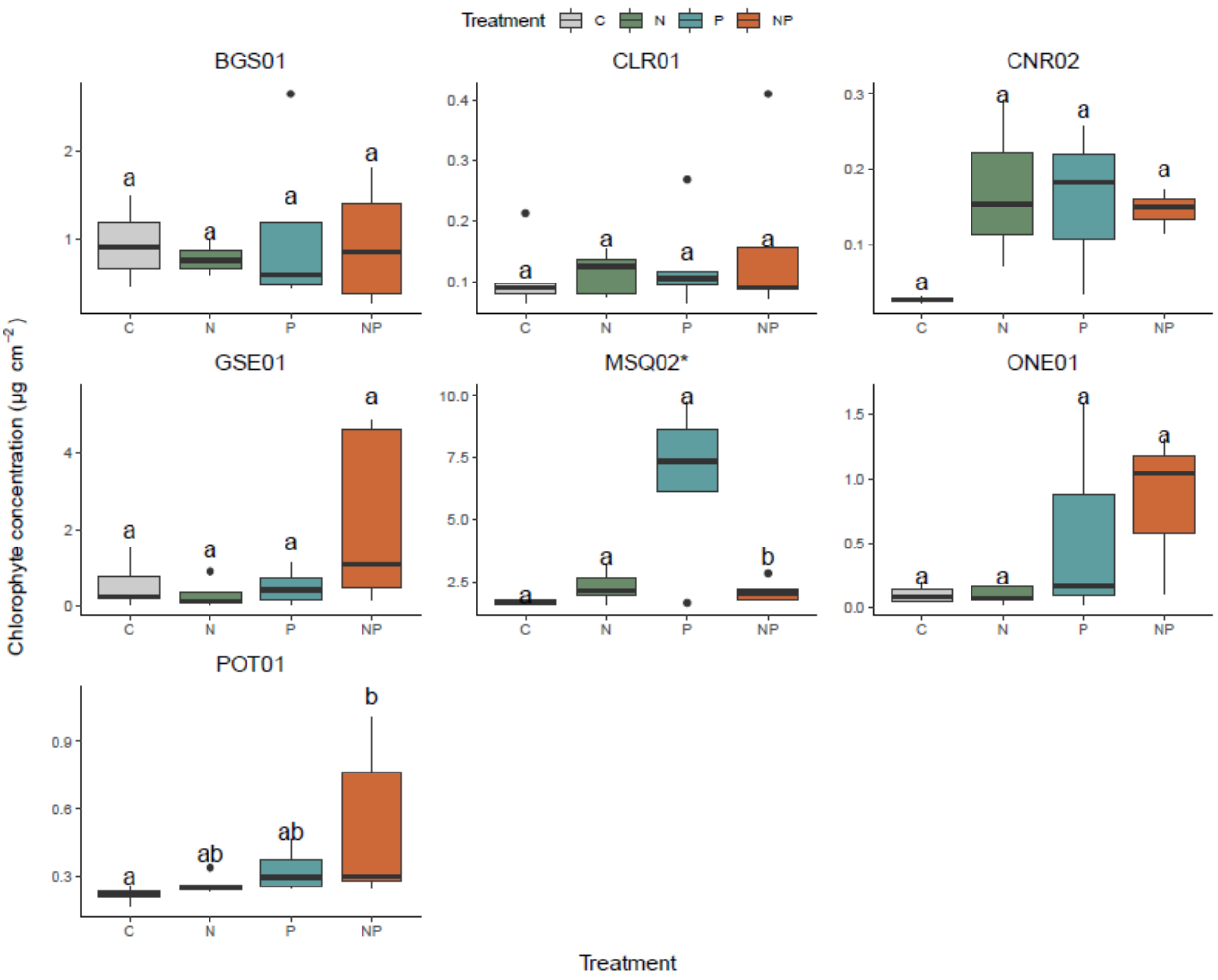

Figure A2.7. Boxplots of study streams with chlorophytes classified as "Not Nutrient Limited", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

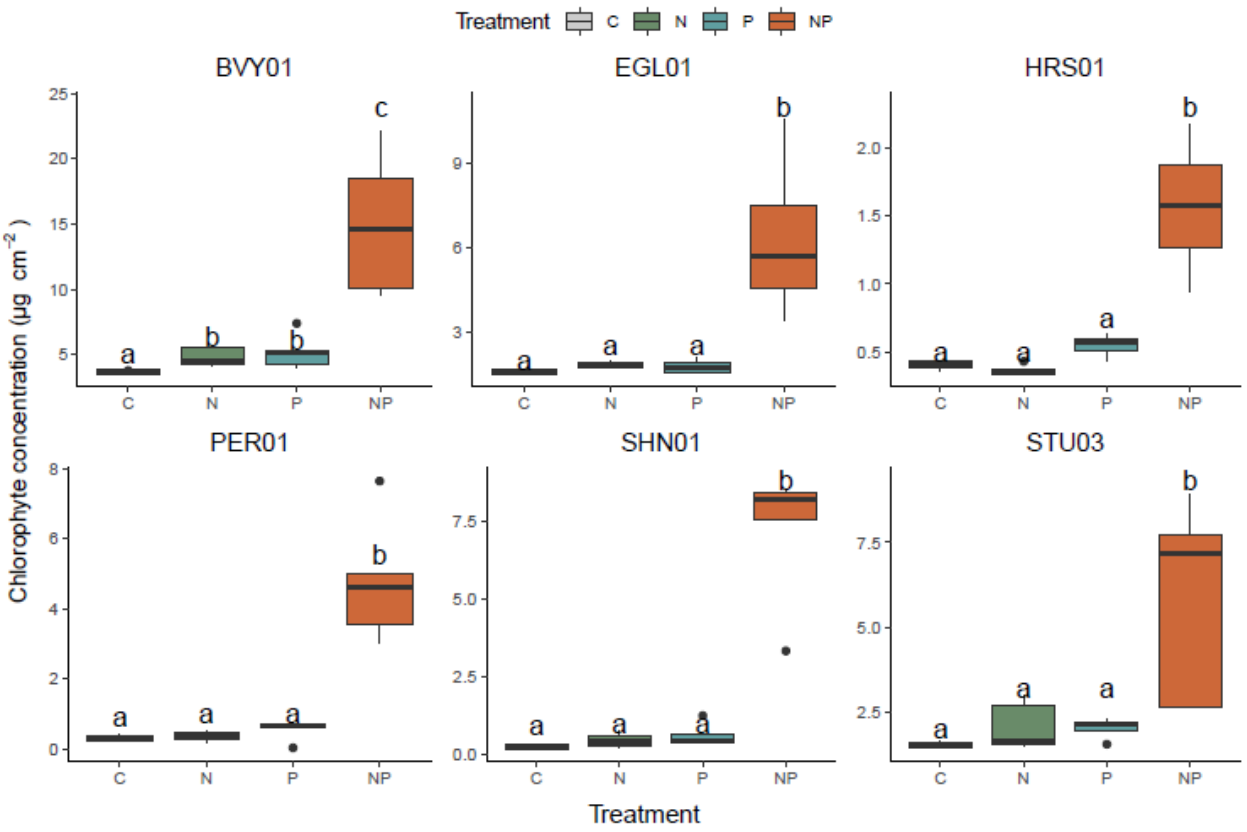

Figure A2.8. Boxplots of study streams with chlorophytes classified as "Simultaneous co-limitation", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

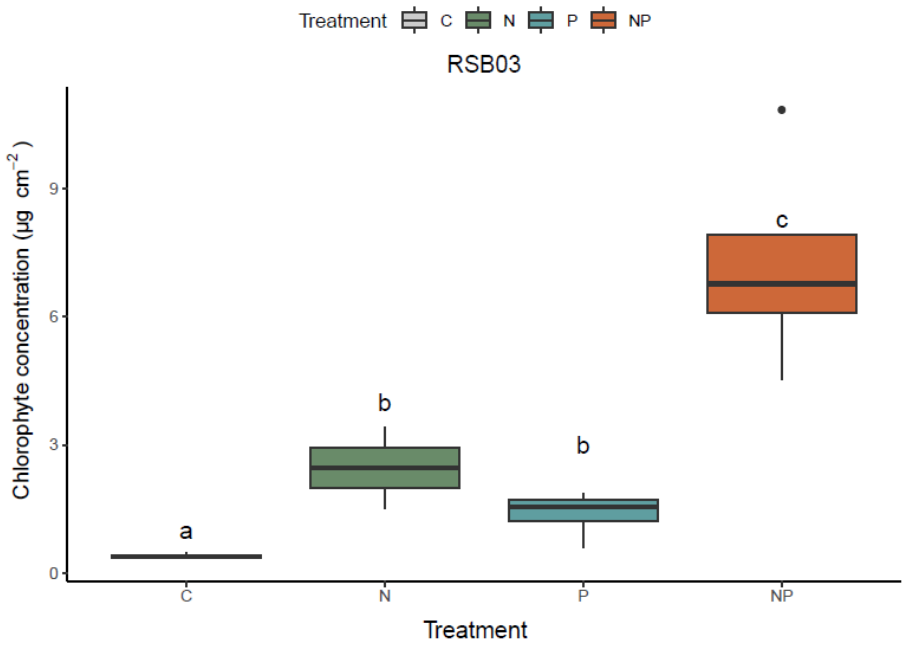

Figure A2.9. Boxplots of study streams with chlorophytes classified as "Independent co-limitation", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

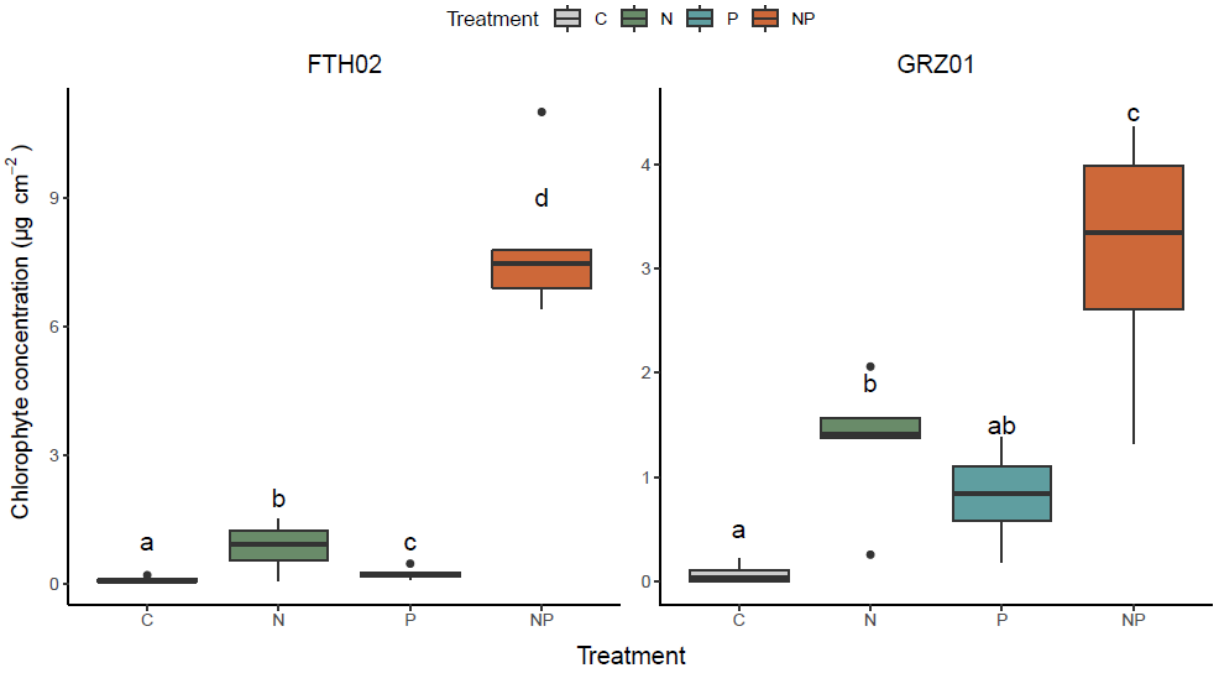

Figure A2.10. Boxplots of study streams with chlorophytes classified as "N-driven serial co-limitation", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

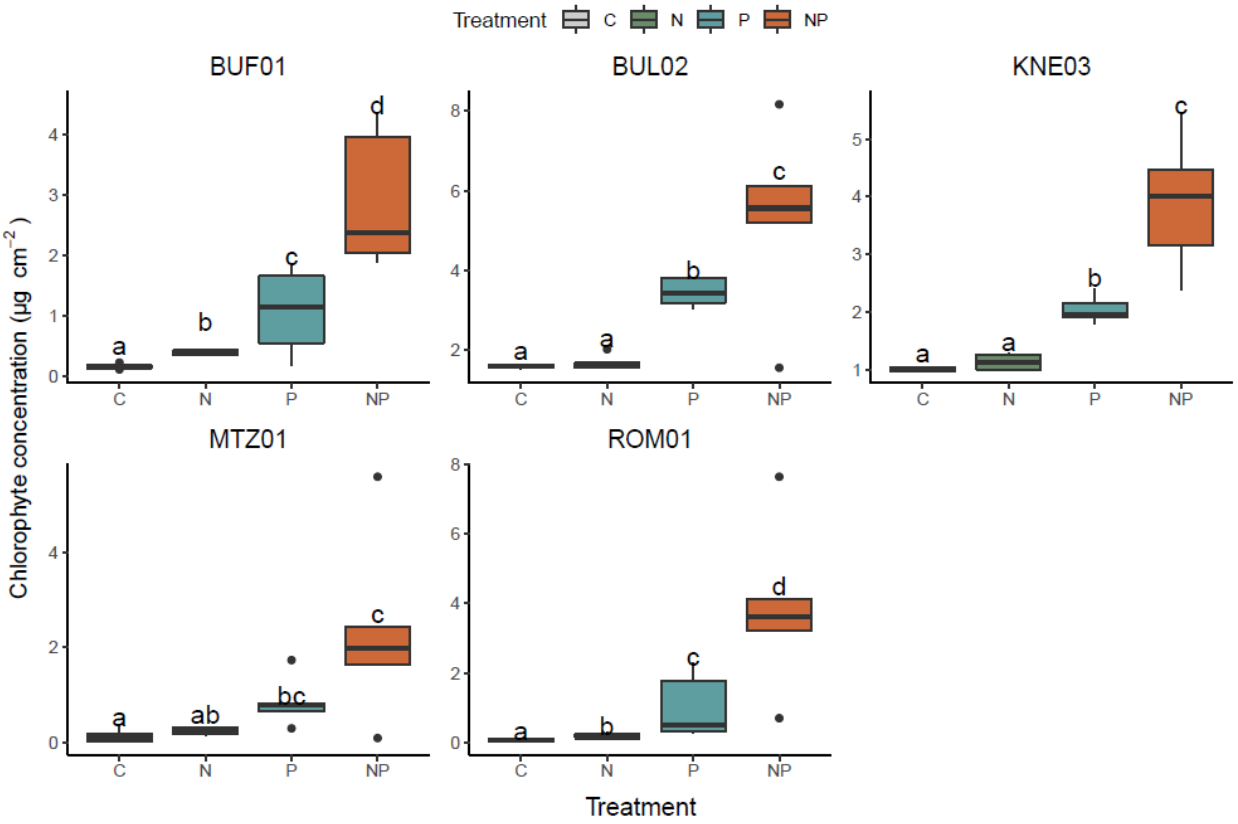

Figure A2.11. Boxplots of study streams with chlorophytes classified as "P-driven serial co-limitation", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

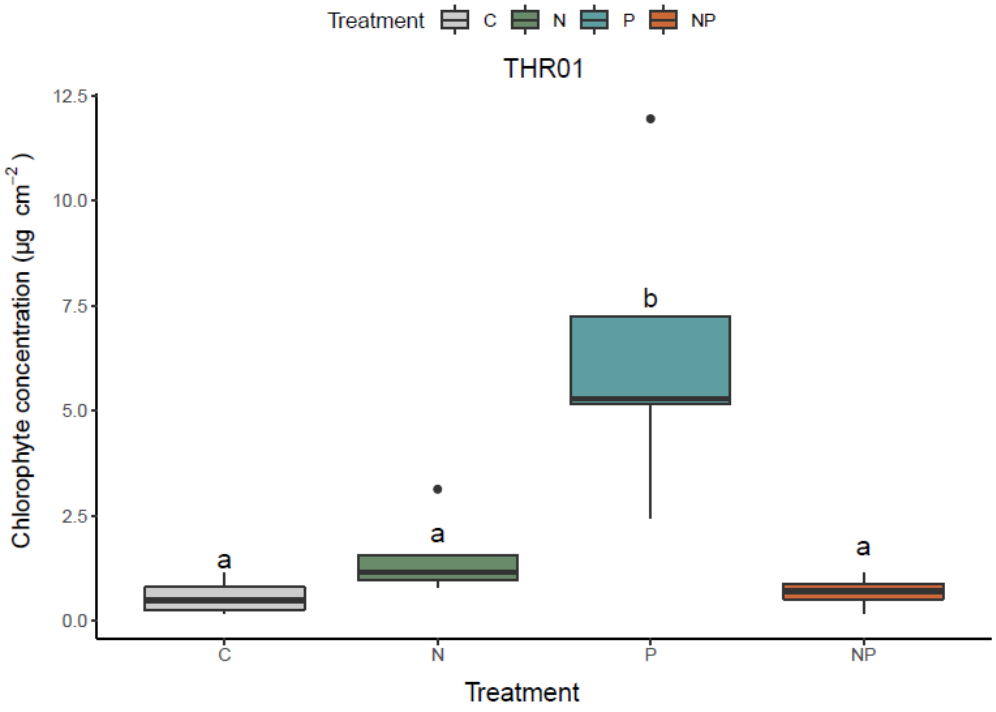

Figure A2.12. Boxplots of study streams with chlorophytes classified as "P-limitation", showing chlorophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

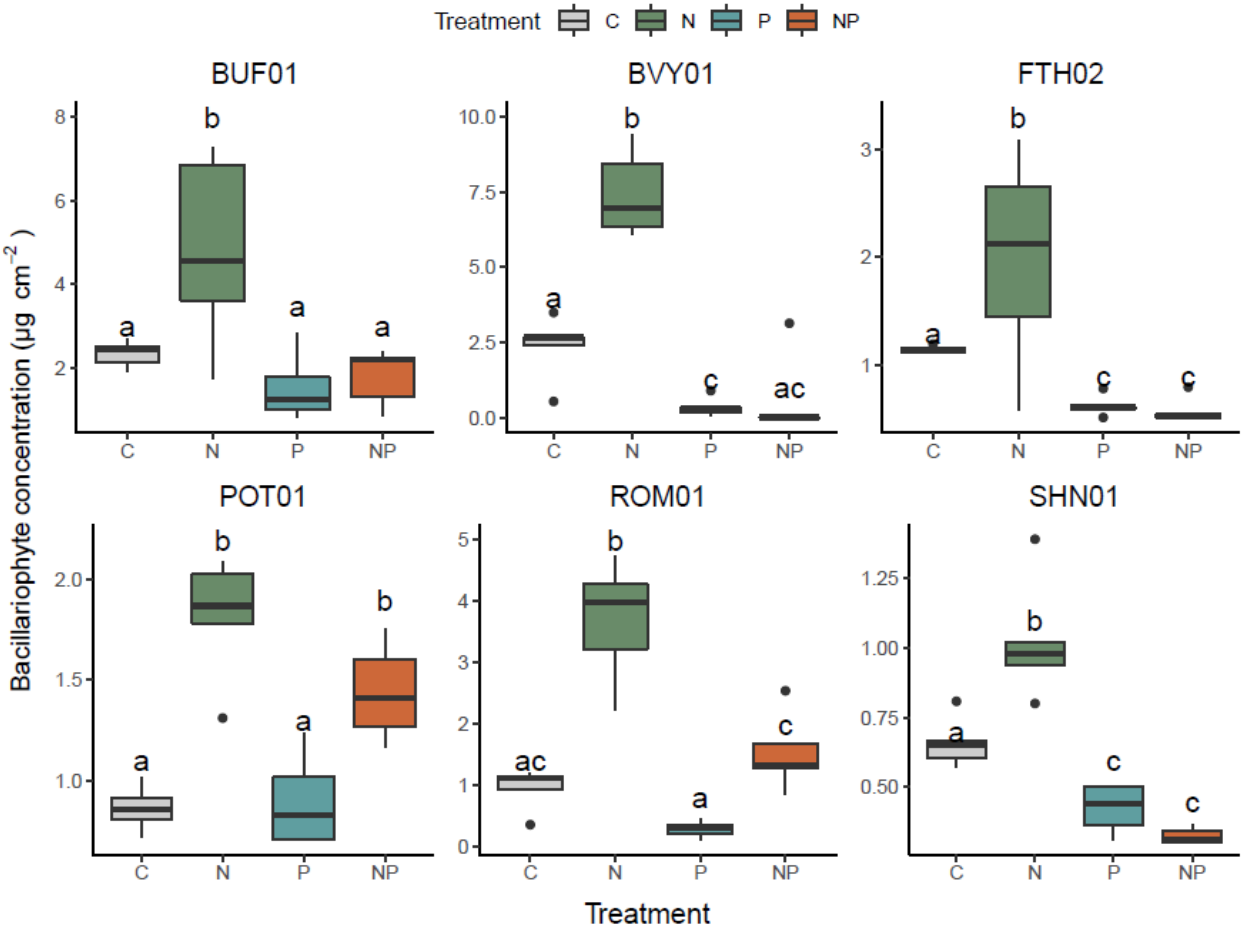

Figure A2.13. Boxplots of study streams with bacillariophytes classified as "N-limitation", showing bacillariophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

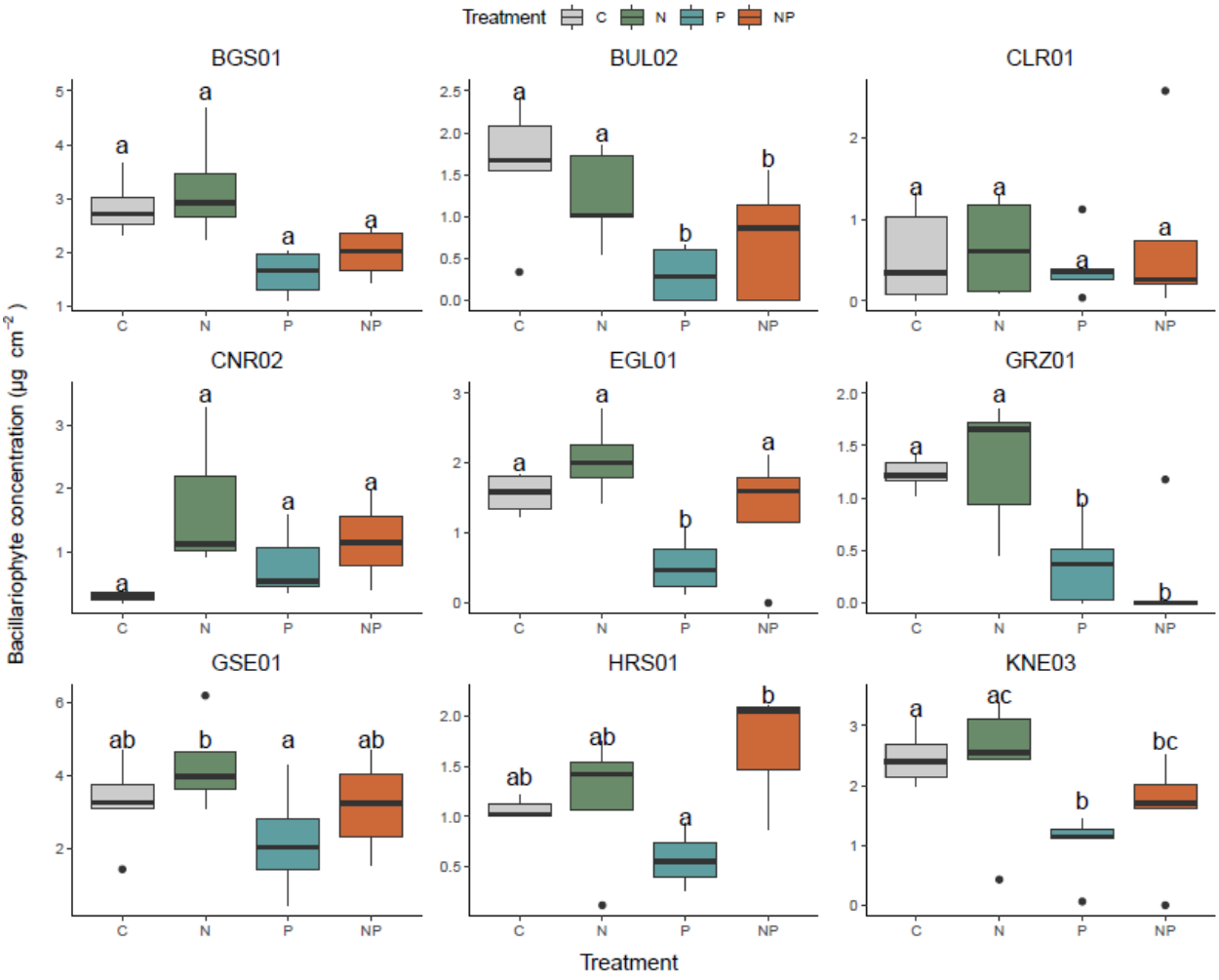

Figure A2.14. Boxplots of study streams with bacillariophytes classified as "Not Nutrient Limited", showing bacillariophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.

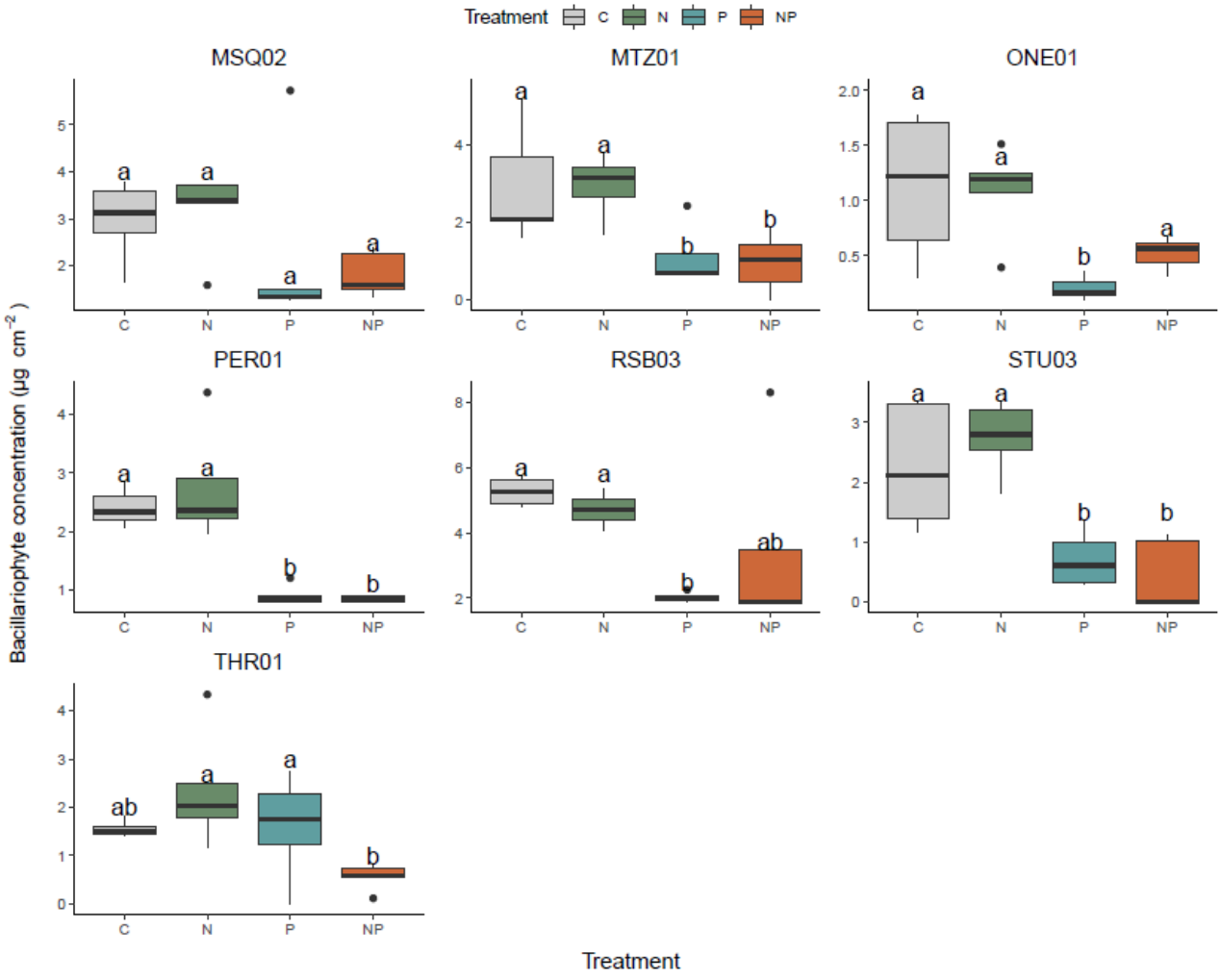

Figure A2.15. Boxplots of study streams with bacillariophytes classified as "Not Nutrient Limited", showing bacillariophyte concentration (µg cm^{-2}) across all nutrient treatments: nitrogen (N), phosphorus (P), nitrogen + phosphorus (NP), and control (C). Significant differences among treatments ($p < 0.1$) are displayed as letters.