Assessing Algal Community Structure and Nutrient Uptake Kinetics Across a Nutrient Gradient in Agricultural Streams

By

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ABSTRACT

Streams provide important ecosystem services, such as the transformation of organic matter and water purification, while transporting water from headwaters to larger receiving waterbodies downstream. Excess nutrients introduced through anthropogenic land use put stress on aquatic ecosystems and disrupt the ecosystem services we rely on. Current ecosystem health can be determined through structural and functional stream assessments to identify criteria required to maintain ecosystem services. Periphytic algal communities occupy a key position in stream ecosystems through coupling the abiotic environment with aquatic food webs, and are therefore a strong candidate for structural assessment. Nutrient cycling is a critical ecosystem service and a dynamic functional indicator of stream health as uptake saturation limits the capacity of the system to take up nutrients through biotic and abiotic processes. Ecosystem responses to anthropogenic land use and nutrient loading may differ between ecoregions such as the Grassland and Parkland ecoregions found in Alberta, Canada, due to the inherent differences in physicochemical variables affecting the biotic components. Therefore, region-specific nutrient criteria may be required to reflect these differences. Here, we explore both structural and functional metrics of stream function at 55 streams in watersheds that are agriculturally dominated, but with varying degrees of land use pressures such that a gradient in nutrient concentration is established. Periphyton samplers were deployed in each stream for one month in late-spring and again in mid-summer, and harvested algae were identified to genus. Ordination and threshold analyses were conducted to assess the impact of nutrient loading on the biotic components of the stream ecosystems and to identify bioindicator taxa. Nutrient injection experiments were performed in a subset of these streams to assess nutrient uptake kinetics and saturation dynamics, and to determine the limiting nutrient. Nitrogen was determined to be the limiting nutrient in this region by both approaches, but no threshold could be identified through either algal community shifts or uptake saturation. Algal communities appear to be resilient to the nutrient gradient sampled in this study, and continue to contribute to nutrient uptake even at the highest concentrations of nutrients. The results of this research could inform watershed management programs in Alberta's agricultural region by suggesting nutrient criteria that maintain aquatic ecosystem health.

PREFACE

This thesis is an original work by Nikki E. van Klaveren. The research conducted in this thesis forms part of a research collaboration between Dr. G. S. Piorkowski and M. Kobryn at Alberta Agriculture and Forestry, Dr. S. E. Tank and Dr. R. D. Vinebrooke at the University of Alberta, and students at the University of Alberta. I was responsible for data collection and analysis as well as the manuscript composition. G. S. Piorkowski, M. Kobryn, and M.-R. Baldwin assisted with the data collection along with additional field technicians. S. E. Tank and R. D. Vinebrooke were the supervising authors and were involved with storyline formation and manuscript composition. Manuscript edits and suggestions for data analysis were provided by S. E. Tank, R. D. Vinebrooke, G. S. Piorkowski, and M. Kobryn.

The first chapter is an introductory chapter that provides background on the thesis and outlines the research objectives. Chapters 2 and 3 are data chapters written in manuscript format intended for publication in the peer-reviewed literature. The final chapter provides concluding statements drawn from the complete thesis, future research potential, and suggests improvements to the research. This thesis is written in the plural as this work incorporates input from multiple collaborators.

Chapter 2

van Klaveren, N. E., Tank, S. E., Vinebrooke, R. D., Piorkowski, G. S., and Kobryn, M. Periphytic Algal Communities and Physicochemical Characteristics of Small Streams in the Agricultural Region of Alberta

Chapter 3

van Klaveren, N. E., Tank, S. E., Vinebrooke, R. D., Piorkowski, G. S., and Kobryn, M. Nutrient Uptake Dynamics Across a Gradient of Nutrient Concentration and Anthropogenic Land Use in Albertan Streams

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Ch1. 1: General Introduction

1.1. Background

1.1.1. Ecosystem Services and Eutrophication

Streams provide important ecosystem services such as the transformation of organic matter, nutrient cycling, provision of water for livestock and irrigation, and fish habitat, while transporting water from headwaters to larger receiving waterbodies downstream. Utilization of ecosystem services and introduction of anthropogenic stressors without regulation can disrupt the very ecosystem services we rely on (Grizzetti et al. 2019). Conservation of ecological condition is preferable over restoration, as restored ecosystems do not necessarily provide equivalent ecosystem services (Dodds et al. 2008). Streams run through anthropogenically altered areas including municipalities, crop lands, and pastures, which can affect in-stream nutrient status through point and non-point source inputs. Excess nutrients have long been identified as a primary stressor in aquatic ecosystems, where they lead to eutrophication and alteration of food webs (Irvine and Murphy 2009).

The cycling of the key nutrients nitrogen (N) and phosphorus (P) are intimately linked to algal communities, as nutrient supply drives and limits algal growth, and nutrients are released back into the water column with algal senescence (Elser et al. 2007). Increasing nutrient enrichment is associated with changes in algal community structure, as communities tend to shift towards lower diversity when exposed to high nutrient concentrations, which causes the communities to become less resilient to further disturbances (Irvine and Murphy 2009; Cardinale 2011). Eutrophication is often associated with blue-green algal blooms because cyanobacteria thrive and come to dominate the algal community, particularly when P concentrations are high relative to N (Scheffer 1997). Cyanobacteria are difficult to consume due to their filamentous nature, which lowers the availability of food at the bottom of the food chain and leads to bottom-up effects that disrupt food web function (Schindler 1971). Cyanobacteria blooms are also undesirable to humans due to cyanotoxin production (Paerl and Otten 2013). Alterations in biotic communities then cause changes in important functional processes such as stream metabolism, measured through the balance between oxygen production and consumption, as well as nutrient uptake and processing (Welti et al. 2017). Nutrient cycling in streams includes the exchange of chemicals between the streambed, subsurface water, biota, and water column, and is a measure of how nutrients are taken up and transported downstream (Bernot and Dodds 2005). Nutrient cycling is strongly affected by nutrient concentrations, and enrichment can saturate the attenuation of nutrient inputs. In the circumstance of uptake saturation, nutrients added to the stream through runoff, effluent, or groundwater input are taken up less quickly and are more readily transported to downstream receiving rivers and lakes.

1.1.2. Nutrient Limitation and Criteria

Nutrient criteria, or guidelines, are one way to communicate the concentration of nutrients at which streams or rivers are expected to become nutrient impaired, and should be based on region-specific data (Dodds and Welch 2000; Elser et al. 2007). Region-specific and broad nutrient criteria have been identified based on desirable levels of algal biomass or toxic algae using data from systems outside of Alberta (Dodds and Welch 2000). Federal guidelines in Canada are set by the Canadian Council of Ministers of the Environment, and based on international trophic status trigger points. However, they recommend setting region-specific guidelines to provide criteria relevant to the ecosystem type and locality (CCME 2004). Nutrient criteria are closely related to the concept of limiting nutrients since the limiting nutrient constrains biomass accumulation and so subsequently nutrient uptake and utilization (Vitousek et al. 1997). The type of essential nutrient that is limiting will vary across sites and regions, as water column nutrient ratios determine which nutrient is least accessible. The optimal nutrient ratio for growth of algae typically assumed to follow the "Redfield ratio" of 106:16:1 moles of C:N:P based on bulk atomic ratios initially determined for marine phytoplankton and seawater (Redfield 1958), and later confirmed for freshwater systems (Hillebrand and Sommer 1999). While the freshwater limiting nutrient has long been considered to be P based on research primarily performed on lakes (Schindler 1974), lotic systems have since been shown to often be N or co-limited instead (Dodds and Welch 2000; Elser et al. 2007). Optimal stoichiometry for freshwater periphyton has been measured for N:P at molar ratios <13 indicating N limitation, 13-22 indicating co-limitation, and >22 indicating P limitation (Hillebrand and Sommer 1999). However, nutrient limitation can be confounded by other environmental factors, including light, temperature, and pH, as these also affect plant growth (Vitousek et al. 1997; Keck and Lepori 2012).

1.1.3. Structural and Functional Stream Assessment

The quantification of ecosystem structure and ecosystem function are two methods by which stream health can be assessed (Riipinen, Davy-Bowker, and Dobson 2009). When quantified along gradients of environmental stress, these two types of assessment may lead to different conclusions with respect to ecosystem response to stressors; assessments highlight different ecosystem responses, therefore it is imperative to select the assessment type that corresponds to the question of interest (Cibils-Martina et al. 2019; Timoner et al. 2020). An established structural metric for assessing ecosystem health in streams involves the measurement of algal community composition across gradients of disturbance, and the determination of threshold concentrations at which algal biomass or taxonomic composition undergoes rapid shifts (Baker and King 2010; Black, Moran, and Frankforter 2011). Thresholds are significant points or zones of rapid change between alternate ecological conditions in response to small, continuous changes in one or more causal variables (Chambers et al. 2012). Algal community composition thresholds can occur as a result of stressors such as eutrophication and affects community interaction and function (Irvine and Murphy 2009). Nutrient uptake and saturation dynamics are functional metrics that can be used to determine the limiting nutrient and ecosystem health of a stream, via an assessment of a stream's capacity to attenuate additional nutrients (Newbold et al. 1981; O'Brien et al. 2007). Measurement of nutrient uptake kinetics is typically undertaken by measuring the movement of a "slug" of added nutrient as it moves downstream, and includes the experimental calculation of the longitudinal distance inorganic nutrients travel in the water column before being taken up (the nutrient uptake length; Sw), the mass tranfer coefficient (uptake velocity; vf), and mass flux from the water column to benthic system components (areal uptake rate; U) (Stream Solute Workshop 1990; Mulholland et al. 2002; Hauer and Lamberti 2006).

1.1.4. Study Region

The prairie provinces of Canada were settled by European immigrants starting in the late 19th century, despite some cautioning that the prairie was dry and contained poor land (Spry 1963). Since then, anthropogenic land use has been a continuous pressure on the Grassland and Parkland ecoregion of Alberta; much of the land conversion in these regions has been for agricultural production, although municipal developments, surface mining, and transportation networks also have large areal impacts (Anderson and Trew 1998). Previous research has determined stream nutrient concentrations in these ecoregions to generally be high and indicative of eutrophic conditions (Lorenz, Depoe, and Phelan 2008), with human development and agricultural land use having a detrimental effect on fish assemblages in the region (Stevens, Council, and Sullivan 2010). While water quality guidelines have been set by the federal government, it is generally understood that characteristics of biotic communities can differ between regions (CCME 2004; Thomas, Hall, and Scrimgeour 2015), and thus assessments of stressors should be region-specific when possible to set relative guidelines. Despite this, there has been little research conducted specific to streams in Alberta regarding the consequences of nutrient enrichment on aquatic biodiversity or ecosystem sevices.

1.2. Research Goal and Objectives

The purpose of this thesis was to undertake a large-scale characterization of stream ecosystem health across a gradient of in-stream nutrient concentration and agricultural land use in Alberta, to inform sound land use management and policy decisions. This was achieved via the determination of shifts in benthic algal community structure and nutrient uptake dynamics along this agricultural gradient.

The thesis is presented as two manuscript-style chapters. Of these, Chapter 2 examines shifts in periphytic algal community structure across a series of 55 agriculturally-impacted streams in Alberta, with specific objectives to: (1) assess which physicochemical variables best predict community composition based on taxonomic analysis; (2) determine which physicochemical variables best explain variance in total algal biomass and community composition based on analysis of taxonomically diagnostic pigments; and (3) assess potential bioindicators and community threshold shifts in response to nutrient gradients across a gradient of land use intensity. Chapter 3 examines nutrient uptake kinetics within a subset of the streams examined in Chapter 2, with specific objectives to: (1) assess nutrient retention efficiency of inorganic N and P across a gradient of nutrient concentrations and additional physicochemical variables; (2) determine saturation mechanics to assess the relationship between increasing nutrient concentrations and nutrient uptake capacity; and (3) consider whether nutrient ratios predict nutrient limitation in agriculturally impacted streams in Alberta.

1.3. Significance

Anthropogenic perturbation of the Earth's system has set in motion levels of climate change which will unequivocably have widespread impacts on humans and natural ecosystems (Pachauri, Mayer, and IPCC 2015). Globally, increasing average temperatures and extreme weather events have been observed since the 1950s. In Alberta, temperatures are expected to continue to increase (Kienzle 2018). While changes in precipitation are more difficult to predict due to spatial variation and many interacting effects, increases in precipitation intensity are expected (Shepherd and McGinn 2003). However, surface waterflow might be shifted as the snowpack melt that feeds river systems shifts to earlier in the spring (MacDonald et al. 2012), while anthropogenic catchment modifications will further modify stream flow (Schindler and Donahue 2006). Changing discharge regimes and increasing temperature both have the capacity to affect algal growth and nutrient uptake (Tekwani et al. 2013), and therefore climate change will affect aquatic ecosystem health in a variety of ways. Current ecosystem functioning therefore needs to be understood to better prepare for the shifts predicted to occur with changing temperature and precipitation regimes, and understand dynamics at chronic high nutrient concentrations (Rockström et al. 2009). In addition to climate change, anthropogenic nutrient sources through urban growth and increasing agricultural intensity to meet growing food demand are a continued stressor (Cassman et al. 2003; Martellozzo et al. 2015).

This study increases our understanding of how the health and function of lower order streams are affected by nutrient enrichment in Alberta. Identifying appropriate bioindicators will increase sampling efficiency and capability for municipalities and watershed foundations as ecosystem health can be assessed through a proxy rather than via assessment of the whole algal community. Identifying nutrient uptake kinetics and saturation dynamics will indicate concentraitons at which ecosystem function of nutrient processing is likely to become impaired. Quantification of current algal community structure, thresholds, and nutrient uptake kinetics will guide watershed management programs in improving surface water quality and aquatic ecosystem health criteria in landscapes dominated by anthropogenic land use, and provide a baseline for future research.

Ch. 2: Periphytic Algal Communities and Physicochemical Characteristics of Small Streams in the Agricultural Region of Alberta

2.1. Introduction

Periphytic algal communities occupy a key position in stream ecosystems since they provide the necessary resources for higher trophic levels. These diverse, sessile communities have short life cycles, allowing them to respond sensitively and rapidly to local changes in environmental conditions (Mateo et al. 2015). Periphyton can therefore be used to assess environmental changes in aquatic conditions and ecosystem services resulting from anthropogenic activity such as increased nutrient loading, and rise in temperature due to climate change (Stevenson 2014). The taxonomic composition, biomass, metabolism, and chemical byproducts of stream periphyton communities can be used as bioindicators of stream function and response to stressors. Excess nutrients have long been identified as a primary stressor in aquatic ecosystems, where they lead to eutrophication and, in turn, to toxic algal blooms, hypoxic zones, and deterioration of ecosystem services (Vitousek et al. 1997; Chambers et al. 2006; Irvine and Murphy 2009). Once the quality of an ecosystem is degraded, it is often difficult to reverse the change through restoration efforts (Dodds et al. 2008). Given that controls on eutrophication can vary across sites and systems, it is imperative to develop a regional understanding of which environmental variables need to be maintained most closely to baseline conditions to prevent degradation (Dodds et al. 2008; Grizzetti et al. 2019).

While diatoms have long been used as bioindicators, the whole algal community is important for understanding a wider variety of effects on stream ecosystems, as a focus on one taxonomic group may not reflect subtle shifts that can alter community structure and function (Kelly 2013; Mateo et al. 2015). Microscope cell counts and pigment analyses are established methods for quantifying algal community composition and abundance. Biomass estimated through pigment analysis can provide information on relative importance of taxonomic units within a community, or a community's influence within an ecosystem (Steinman et al. 2017). High-performance liquid chromatography (HPLC) is a well-established method of pigment analysis (Thomas, Hall, and Scrimgeour 2013; Steinman et al. 2017). However, caution is required when interpreting results, as the amount of pigment within algal cells can change depending on species composition and environmental conditions, and extract concentrations can change depending on solvent type and analytical procedure. Therefore, HPLC is often a useful tool in combination with microscopy, as knowledge of finer taxonomic resolution is informative in understanding aggregate responses by entire algal communities (Lauridsen, Schlüter, and Johansson 2011; Kahlert and McKie 2014).

Environmental stressors can cause shifts in algal community structure, as well-adapted taxa outcompete sensitive taxa. Nutrient enrichment can result in sudden shifts in algal biomass and community structure, as growth is often nutrient limited while dependent on taxa specific thresholds (Hillebrand and Sommer 1999; Cross et al. 2005; Elser et al. 2007; Andersen et al. 2009). The response of algal communities to a single variable is also highly dependent on other environmental conditions such as light, temperature, and stream velocity, as the combined effect of these variables affects the growth and nutrient exchange of algal communities (Beck et al. 2019; Vinebrooke and Leavitt 1999). When such taxonomic shifts occur, they can be accompanied by changes in community dynamics, and lead to decreases in biodiversity if many sensitive species are lost or a few tolerant species outcompete other community members, which decreases community resilience to further disturbances (Irvine and Murphy 2009; Cardinale 2011). If resilience thresholds are surpassed, the community will undergo a rapid shift into a new, and often undesirable, stable state that is resistant to returning to its previous conditions even upon removal of a stressor (Scheffer et al. 1993; Chambers et al. 2012). The presence of diverse communities may help buffer ecosystems against the ecological impacts of nutrient pollution, as increasing diversity has been found to promote nutrient uptake; thus, community composition can infer functional ability (Jerney et al. 2016). One method to determine thresholds and identify bioindicators is through the use of Threshold Indicator Taxa ANalysis (TITAN) (Baker and King 2010). Determining thresholds will provide a basis for setting nutrient criteria specific to the region (Dodds and Welch 2000), while specific bioindicator species or groups allow for monitoring of shifts only in those specific taxa and so conserves resources in future studies (Parmar, Rawtani, and Agrawal 2016).

Shifts in both dominant algal groups and specific taxa have been identified in response to nutrient loading and other stressors (Kelly 2013). In the case of nutrient enrichment, there has been evidence of shifts in periphytic communities towards dominance of green algae and cyanobacteria (Mateo et al. 2015). These shifts occur as phosphorus (P) may favour cyanobacteria growth due to the ability of certain taxa to fix nitrogen (N) (Paerl and Otten 2013). This would allow the increase of cyanobacteria in both biomass and dominance to indicate a likely increase in P, making it a sentinel of eutrophication (Mateo et al. 2015). However, this is not always the case, as cyanobacteria are widely distributed and other taxa such as *Nostoc* spp. and *Calothrix* spp. can indicate low nutrient concentrations and have therefore been used as bioindicators of this condition (Mateo et al. 2015). Diatoms are also commonly used as bioindicators of nutrient enrichment as several taxa are nutrient sensitive (Potapova and Charles 2007). Understanding change at the community level is particularly relevant because the community-level response to specific stressors may differ from the overall biomass response. For example, one study in the agricultural South Nation River watershed in Eastern Ontario, reported that overall periphyton biomass increased with nitrate, but this increase was only associated with green algae and so biodiversity and resilience decreased (Dalton, Boutin, and Pick 2015).

With changing climate and continued anthropogenic pressure, a broad suite of environmental variables that can play key roles in regulating algal growth are changing. This includes increased nutrient loading resulting from intensified agriculture, increased velocity with channelization, and increasing temperatures with climate change (Anderson and Trew 1998; Kienzle 2018). In the agricultural region of Alberta, temperatures and precipitation are expected to increase, while interaction between environmental variables could drive unforeseen environmental shifts (Shepherd and McGinn 2003). There is also a long history of agriculture and has been experiencing an increase in urban pressure which are both anthropogenic nutrient sources (Lorenz, Depoe, and Phelan 2008). The region has previously been documented to have high concentrations of nutrients in streams, but very little work has been conducted to understand how nutrient pressure has affected algal biomass or community structure, or its effect on stream function by limiting nutrient uptake potential (Dodds and Welch 2000).

The goal of this study was to relate patterns in periphytic algal abundance and community composition to catchment and climate-related physicochemical factors in 55 agricultural streams along a gradient of anthropogenic land use in the Parkland and Grassland ecoregions of Alberta. To accomplish this, we examined shifts in periphytic algal community structure across a series of 55 agriculturally-impacted streams in Alberta, with specific objectives to: (1) assess which physicochemical variables best predict community composition based on taxonomic analysis; (2) determine which physicochemical variables best explain variance in total algal biomass and community composition based on analysis of taxonomically diagnostic pigments; and (3) assess potential bioindicators and community threshold shifts in response to nutrient gradients across a gradient of land use intensity. We expected to detect a shift in algal communities by genera and groups along nutrient gradients, with algal biomass expected to go up and diversity to decrease with eutrophication. The study was motivated by the need to provide insight into current Alberta stream health and algal community composition for reference for future monitoring.

2.2. Methods

2.2.1. Study Region and Project Overview

A series of 55 streams of third to fifth Strahler-order were selected in the prairie region of Alberta (Figure 2.1), of which 26 were located in the Grassland ecoregion, and 29 were located in the Parkland ecoregion. Of these ecoregions, the Grassland is characterized by a semi-arid climate with low precipitation. Winters are cold and dry, with an average temperature of −9.3 °C in January and an average monthly precipitation of 13.3 mm. In contrast, summers are relatively warm in the Grassland ecoregion, with an average temperature of 19.5 °C in July and an average precipitation of 33.7 mm (Suffield A weather station; ECCC 2010b). Annual daily average temperature is 5.4 °C, and annual average precipitation is 305.8 mm. The Parkland ecoregion is somewhat cooler and wetter than the Grassland, with average temperatures of −14.1 and 17.0 °C in January and July, respectively, and an average precipitation 19.7 mm and 72.5 mm in these two months (Fabyan weather station; ECCC 2010a). Annual daily average temperature is 2.2 °C, and annual average precipitation is 411.8 mm.

All selected study streams were wadeable at baseflow, but many were non-wadeable at high flows. During the spring season all streams had flowing water, but during the summer season some became stagnant or near stagnant. Peak flows typically occur during snowmelt in April or during heavy summer rainfall events, although streams close to the foothills are influenced by mountain snowmelt into June. Typically, the largest inflow of nutrients and nutrient export in the Canadian Prairies occurs during snowmelt runoff (Jensen et al. 2011; Corriveau, Chambers, and Culp 2013). Within each ecoregion, streams were selected to span the gradient of agricultural intensity that occurs in this region of Alberta. These regions do not have true reference streams available since all stream have been impacted to some degree by land use alteration. Sites were sampled in four sampling periods; in late spring (late April-early June; 2017 and 2018) and summer (July-August; 2016 and 2017) months.

2.2.2. Environmental Variables

Stream width, depth, velocity, and discharge were measured using an Acoustic Doppler Velocimeter (ADV; FlowTracker2, SonTek, San Diego, CA) or Acoustic Doppler Current Profiler (ADCP; StreamPro, Teledyne, Poway, CA) depending on flow conditions. A handheld multiparameter water quality sonde (SmarTroll, In Situ, Pittsburgh, PA) was used to measure water characteristics including temperature, pH, specific conductivity, and dissolved oxygen. Water chemistry samples were collected during periphyton substrate deployment and retrieval at mid-stream and mid-depth. Samples were immediately placed on ice and chemical analyses were performed by ALS Environmental Laboratory (Edmonton, AB) following methods adapted from the APHA Standard Methods and the US EPA Test Methods (Table A2.1; APHA 2017; US EPA 2003). Analyses of total suspended solids (TSS), water column chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), total, total dissolved, and soluble reactive phosphorus (TP, TDP, SRP), and total, total Kjeldahl, ammonia, and nitrate+nitrite nitrogen (TN, TKN, NH_3-N, NO_2+NO_3-N) were assessed. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_3-N and NO_2+NO_3-N. Anthropogenic (crop, pasture, fallow, and residential land) and natural (grassland, shrubland, wetland, and water cover) watershed land cover classes, were calculated from the Annual Crop Inventory Database (AAFC 2016), following watershed delineation using ArcGIS (ESRI 2011).

2.2.3. Periphyton Sampling

Wildco® periphyton samplers were deployed to enable the incubation of slides as periphyton substrates in each of the study streams. In each stream, a single sampler containing sixteen glass microscope (1” x 3”) slides was deployed just below the water surface in the middle of the stream and retrieved after four weeks, which is the time frame after which colony senescence begins to occur (Jerney et al. 2016). Slides were stored chilled and in the dark in foil-wrapped, microscope slide mailing jars (LockMailer^{TM}, Simport Scientific Inc., Saint-Mathieu-de-Beloeil, QC) filled with filtered stream water, and were employed to quantify both relative abundance of algal taxa (eight slides; refrigerated until use) and photosynthetic pigment concentrations (eight slides; frozen until use).

2.2.4. Taxonomic Analysis of Periphyton by Microscope Cell Counts and Pigment Analysis

Algal counts were performed to the genus level by MB Laboratories Ltd. (Victoria, BC). Samples were first observed as wet mounts and then identified and enumerated using a Sedgewick-Rafter Counting Cell after fixing and staining with Lugol's Iodine with use of a taxonomic library and culture collection. Algal taxa were not identified to a finer taxonomic resolution after a pilot study in a subset of streams determined that identification to the species level was only possible for approximately 10% of the community members, due to difficulty of identification without specific morphological features, such as reproductive features, present in algal cells. As a result, genus-level identification was committed to as a balance between operational cost and technical feasibility.

Taxonomically diagnostic photosynthetic pigments were analyzed using HPLC at the University of Alberta for spring 2017, summer 2017, and spring 2018. Samples for HPLC were not obtained during the 2016 summer season. Frozen slides were freeze-dried to negate any confounding influence of variation in water content affecting pigment extraction efficiencies across the samples (Hansson 1988; Vinebrooke and Leavitt 1999). The slides were then immersed in a pigment extraction solution (80:20 methanol:acetone) for 24 hours. Extracts were filtered using a 0.22 µm syringe filter, and completely dried down under N gas to offset oxidative degradation of the pigments. Dried pigments were reconstituted using a solution injection (500-1000 µL) and transferred to injection vials prior to HPLC analysis (Model 1100, Agilent Technologies, Waldbronn, Germany). The instrument was calibrated and identified pigments using commercial standards purchased from DHI Water and Environment Institute (Hørsholm, Denmark). Concentrations of all pigments are expressed as µg cm^{-2}.

The pigments chlorophyll *a* and beta-carotene were considered as reliable proxies of total algal biomass, fucoxanthin and diadinoxanthin were used to indicate the presence of diatoms, and violaxanthin and chlorophyll *b* were used as indicators of green algae (Hauer and Lamberti 2006). Lutein and zeaxanthin are pigments indicative of green and blue-green algae, respectively, but can be difficult to distinguish on the output chromatogram, so concentrations of these two pigments were combined and used to indicate either taxonomic group (Vinebrooke and Leavitt 1999; Hauer and Lamberti 2006). Phaeophytin is a degradation product that represents senesced algae in the sample. It can be difficult to distinguish phaeophytin produced due to death of algal cells within the stream or due to the process of extraction. However, as samples were frozen after delivery to the lab, the pigment was included in the analysis.

2.2.5. Community Analyses

Chemical variables and stream flow characteristics were averaged between deployment and retrieval, and displayed in box plots to assess variation by ecoregion and season. Significance of differences and interaction between ecoregion and season was determined through a two-way ANOVA with interaction. Total biomass was calculated from water column samples for planktonic biomass and HPLC for periphytic samples. Species richness and the Shannon-Weiner Index were calculated from algal cell counts. The response of each of these four metrics to concentrations of TN and TP was analysed using linear regressions with base R 3.6.1 (R Core Team 2013). For the following analyses environmental variables were screened for redundancy and non-significance using forward selection with the 'vegan' package (v 2.5-6; $p<0.05$; Oksanen et al. 2019). An initial forward selection model indicated that the most significant explanatory variables were the year and season of sample collection for both taxonomic- and pigment-specific analyses. These temporal variables capture variation in physicochemical parameters rather than directly driving differences between communities through bottom-up and/or top-down processes (Beck et al. 2019). As a result, the data were divided into four temporal subgroups and reanalyzed by individual year-seasons, with forward selection and Reduncancy Analeyeses (RDAs) performed separately on taxonomic and pigment community data ('vegan' v 2.5-6; Oksanen et al. 2019). RDAs were determined to be appropriate after preliminary detrended correspondence analysis (DCA) determined a linear species response model to better characterize the data set compared to a unimodal species response model. Prior to analysis, the algal taxonomic data were Hellinger transformed; pigment data was ln(x+1) transformed; and watershed, stream characteristics, and chemistry variables were $\log_{10}(x)$ transformed to normalize data. A constant as small as possible was added to variables with 0 and negative values to enable log transformation. Variables included were watershed land use type, stream water temperature, pH, specific conductivity, dissolved oxygen, width, depth, velocity, TSS, chl *a*, NH3-N, TN, DIN, SRP, TP, DOC, and ecoregion. Permutation testing was performed to verify the significance of the models. All figures were created with package 'ggplot2' (v 3.2.1; Wickham 2016).

2.2.6. Indicator Species

The package ‘indicspecies’ (v 1.7.6; De Cáceres and Legendre 2009) was used to determine indicator species across gradients of the key nutrients N and P. The ‘indicspecies’ approach requires that sites be grouped into a series of concentration bins containing a minimum of 10 sites, and then the existance of unique indicator taxa within each bin is determined (De Cáceres, Legendre, and Moretti 2010). For our analysis, the nutrient gradients were split into three bins within each sampling period, with the same concentration cutoff for the bins in each period. TITAN (Threshold Identification Taxa ANalysis) is a multivariate threshold and indicator species approach that builds on the ‘indicspecies’ package to determine whether individual indicator species respond positively or negatively across environmental gradients, rather than within bins (Baker and King 2010). We therefore also used TITAN (v. 2.4; Baker, King, and Kahle 2019) to further explore whether species indicative of thresholds in algal community composition existed. TITAN identifies whether a taxa consistently displays a rapid decrease (z- score) or increase (z+ score) across an environmental gradient of given sites. Following our forward selection models and RDA analyses, our indicspecies and TITAN analyses were split by year and season.

2.3. Results

2.3.1. Variation in Stream Characteristics

Basic watershed and physicochemical characteristics of the study sites are presented in Figure 2.2 and Table A2.1. The two-way ANOVA analyses indicated broad geographic and temporal differences in several key parameters. Overall, the percentage of anthropogenic land use was higher for the Parkland ecoregion sites compared to the Grassland sites. Stream velocity was similar between the ecoregions, while water temperature was slightly lower in the Parkland ecoregion, similar to average air temperatures. Both velocity and water temperature showed significant seasonal differences with only water temperature showing a slight interaction effect, owing to snowmelt after the winter and higher overall temperatures during the summer. TSS was similar between ecoregions, while specific conductivity was slightly higher in the Grassland, and neither differed between the seasons. Mean pH values show both a geographic and seasonal difference, and was higher in the Grassland and summer.

The Parkland ecoregion had higher in-stream concentrations of both TN and TP, and TP had a significant seasonal effect, while neither showed a significant interaction effect (Figure 2.2; Table A2.2). Streams were mostly classified as mesotrophic or eutrohpic according to TN and eutrophic according to TP concentrations (Dodds, Jones, and Welch 1998). The molar TN:TP ratio was similar between ecoregions and seasons, and mean nutrient ratios indicate that streams in this regions are typically N limited. Taken together, the major observed differences between ecoregions consist of variables that are commonly affected by anthropogenic land use such as nutrients, specific conductivity, and pH, although these variables can also be influenced by geology (Lavoie et al. 2004). In comparison to available guidelines, both ecoregions could be considered high in nutrients despite lower anthropogenic land use in the Grassland.

2.3.2. Algal Communities

A total of 98 algal genera were detected across all sampled sites and sampling periods. Diatoms were the dominant taxonomic group, accounting for 49 - 57% of all genera identified. This was followed by green algae (23-27% of all taxa), Cyanobacteria (11-15% of all taxa), and chrysophytes, dinoflagellates, euglenoids, yellow-greens, and synorophytes (together, less than 10% of all taxa). Of the individual genera detected, 43 were present in at least 10% of the streams (Figure 2.3). Severl diatom genera were present across the majority of sites, including *Synedra* (96% detection), *Navicula* (94% detection), *Fragilaria* (90% detection), *Gomphonema* (83% detection) and *Cocconeis* (74% detection). Of the green algae, *Ankistrodesmus* (75% detection), *Scenedesmus* (70% detection), and *Stigeoclonium* (62% detection) were most common. The most commonly detected Cyanobacterium was *Anabaena* (67% detection). While planktonic chlorophyll *a* (measured from mid-channel grab samples) increased with increasing TN and TP concentration within each sampling period, slide-derived periphytic chlorophyll *a* did not (Figure 2.4; Table A2.2). Similarly, there were no clear changes in periphytic algal species diversity or richness with increasing nutrient concentrations.

2.3.3. Drivers of Algal Community Composition

Forward selection models and RDA outputs showed that season and year were the strongest variables explaining taxonomic variation among the sampled sites. Therefore, analyses were performed for each sampling period to better elucidate landscape and physicochemical drivers of community structure (Figure 2.5). Using forward selection permutation models, variation in algal community composition was best explained by velocity (3 of 4 sampling periods), nitrogen (DIN and TN; 2 of 4 sampling periods), watershed land use type (residential and grassland; 2 of 4 sampling periods), ecoregion (2 of 4 sampling periods), temperature (1 of 4 sampling periods), TSS (1 of 4 sampling periods), and chlorophyll *a* (1 of 4 sampling periods) in significant models. Ecoregion likely accounted for variation that was not captured by other measured environmental variables, while the models not including ecoregion did visually separate by ecoregion as well along land use type (Figure 2.5). Taxonomic groups did not clearly separate out in the RDAs (Figure A2.1), suggesting that individual taxa within broader taxonomic groups do not all respond similarly to the environmental variations that were observed.

Variables explaining variation in pigment-inferred community composition (Figure 2.6) differed slightly from those captured by RDAs conducted using genus-level data. Across all sampling periods, variation in pigment composition was best explained by nitrogen as DIN (all sampling periods). Other drivers included temperature (2 of 3 sampling periods), and wetland land use, stream width, and SRP (1 of 3 sampling periods each). In contrast to the genera-based assessment, there was no clear separation of sites by ecoregion in the pigment-based analysis (Figure 2.6). Similar to the genera-based assessment, however, there was also no separation of pigment groupings within ordianation space. Instead, all pigments clustered together, indicating no specific pigment responsed differently to the identified explanatory variables, which suggests synchronous changes in biomass and the taxonomic groups diagnosed through pigments (Figure 2.6).

2.3.4. Indicator Species

There were no indicator taxa that were common to the low, medium, or high TN concentration bins across any of the sampling periods, and none of the selected indicator taxa for TN were found across more than one sampling period or concentration bin (Table 2.1). TN indicator taxa that were identified varied widely in their characteristics, from *Spirogyra*, a filamentous green algae that was indicative of medium TN concentrations in summer 2016 to *Euglena*, a flagellated euglenoid, that was indicative of high TN in summer 2017. Similarly, the TITAN method also did not identify any taxa as potential indicator genus across the sampling periods. *Phacus*, a flagellated euglenoid, did show a positive TN threshold response in both spring and summer 2017, but not summer 2016 or spring 2018 (Table 2.2).

Similar as with TN, there was also no common indicator taxa that was identified for TP across sampling periods, for either the 'indicspecies' or TITAN aproach (Table 2.1 and 2.2). Only *Tetraedron*, a unicellular green alga, was identified as being a potential indicator species twice. However, it indicated medium levels of TP in spring 2017, indicating low levels of TP in summer 2017. TITAN identified *Phacus* as exhibiting a positive threshold response to TP in spring and summer 2017, and also identified *Characiopsis*, an attached yellow-green algae, as a positive threshold indicator of TP in the same time periods (Table 2.2).

2.4. Discussion

In general, the analyses highlighted the importance of temporal and regional factors affecting periphytic algal communities in streams located in the Parkland and Grassland ecoregions of Alberta. Stream velocity, N, land use, ecoregion, and temperature were identified as key drivers of community composition diversity in genus-level analyses. N also best explained diversity in community composition using pigment-based analyses. Although N and P concentrations did not explain changes in chlorophyll-inferred periphytic algal biomass or taxonomic diversity, they were correlated positively with phytoplankton biomass. Variance within taxa and community thresholds across the study sites precluded definitions of criteria for nutrient concentrations. Nevertheless, these findings collectively highlight the role of N in driving algal dynamics in these streams.

2.4.1. Physicochemical and Periphytic Algal Community Comparison

The few studies that have examined the physicochemical and periphtic algal properties of streams in the Grassland and Parkland ecoregions of Alberta allow for comparisons with our current assessment. Abiotic variables from pH to temperature were similar in magnitude to streams surveyed along the Oldman-South Saskatchewan river system in the 1970s, indicating environmental characteristics have stayed relatively consistent (Green and Davies 1980). While maximum TN and TP concentrations were higher in our study sites, the Oldman-South Saskatchewan River system lies in the Grassland ecoregion, which in our study had significantly lower concentrations of nutrients (Figure 2.2). Agricultural land cover has remained relatively constant in Alberta over the last few decades, which is reflected in the similarity between nutrient concentrations in this study compared to a recent study spanning the agricultural Grassland, Parkland, and Boreal Transition ecoregions (Lorenz, Depoe, and Phelan 2008). The relationship between increasing nutrient concentrations and anthropogenic land use in the watershed did not have strong relatinships, which would be expected in this region (Anderson and Trew 1998). This is potentially due to intensity not being accounted for within land cover, and despite this the study sites did provided a gradient of nutrient concentrations across sample sites (Figure 2.4).

Periphytic algal community composition was also similar to the benthic communities reported in the Oldman-South Saskatchewan survey (Green and Davies 1980), which reported a total of 201 species and varieties across fewer sites than our assessment. For both surveys, diatoms were the most common taxonomic group, but represented a smaller proportion of groups in our study (49-57% in our survey compared to 58.2% by Green and Davies 1980) (Figure 2.3). Green algae (23-27% compared to 22.4%) and cyanobacteria (11-15% compared to 12.9 %) also represented a similar proportion between the two surveys. Chrysophyta were not found to be dominant at any point in our study, but did represent a similar proportion between the surveys. Both surveys found planktonic species to be common in the periphytic assemblage, likely due to settling during low water flow, indicating a general connection between the planktonic assemblage and periphytic community (Tekwani et al. 2013). Overall, there were no notable differences found between the two surveys, indicating that more than 40 years of ongoing anthropogenic disturbance has not shifted the periphytic community composition from the surveyed state. This could indicate that communities had already shifted from pre-anthropogenic influence conditions, but there is no survey data for this region dating back to before settlement, making reference conditions difficult to determine.

2.4.2. Community Biomass, Composition, and Drivers

Along the measured environmental gradients there were also no significant linear trends in the periphytic community properties of total biomass, taxonomic diversity, and richness (Figure 2.4). In part, these results may be attributed to the reported lack of concordance between benthic algal chlorophyll *a* and biovolume estimates (Baulch et al. 2009). On the other hand, this may indicate some level of resilience against community shifts within the range of mesotrohpic to eutrophic systems sampled here (Dodds, Jones, and Welch 1998). In which case community shifts might have a threshold we did not yet observe, or shifts have already happened at lower nutrient concentrations, as sudden changes in environmental variables due to anthropogenic activity are expected to shift communities at some point (Dodds 2007; Jankowski and Schindler 2019).

There were likely different dynamics at play in each stream that drove community composition and overall algal biomass (Hicks and Taylor 2019). N was a stronger driver compared to P, which would indicate an N limited system, in accordance with the water column nutrient ratios. Velocity could indicate the importance of shear stress as a driver of algal community composition, as less prostrate taxa are more susceptible to scouring under high velocity conditions (Tekwani et al. 2013). The importance of land use variables within the genera-based RDA was associated with the separation of Grassland and Parkland sites in ordination space, given that agricultural land use is overall more intense in the Parkland ecoregion than the Grassland ecoregion (Figure 2.5). This likely represents an aggregate of variables such as pH and specific conductivity, which are affected by a combination of agricultural practices and innate abiotic factors separating ecoregions (Lavoie et al. 2004). Temperature generally stimulates algal growth, shifting commmunities towards erected growth forms during colder periods and prostrated growth forms in warmer periods as genera differ in their temperature-dependent growth rates (Butterwick, Heaney, and Talling 2004; Tekwani et al. 2013).

Pigment-based variance was strongly driven by nitrogen, and similarly to the count-based method identified temperature and land use cover. Stream velocity was not identified as a strong driver, but width is connected to both stream velocity and TSS. Therefore, there is some agreement between drivers identified by the two enumeration methods. Despite pigment composition within algae being altered due to light, growth phase, and nutrient regimes (Higgins, Wright, and Schlüter 2011), it is a useful method for broader interpretation of community shifts between sites, but not as accurate in identifying physicochemical drivers in these systems. Neither the genera- nor pigment-based RDAs showed clear taxonomic grouping driven by physicochemical variables, therefore displaying no community shift (Figures 2.6; A2.1).

2.4.3. Indicator Taxa

A few genera were identified as potential indicator taxa using ‘indicspeices’, but none were consistently identified across all sampling periods for either the TN or TP concentrations in our study. Using TITAN, *Phacus* and *Characiopsis* had positive thresholds in spring and summer 2017 for both TN and TP. However, the overlap between these two time periods is likely due more to similarity within sampling year than to these taxa being appropriate choices for indicator species in these systems, as there is no overlap with the other sampling periods. No previous literature has identified *Characiopsis* as an indicator taxa, while *Phacus* has been reported as a potential indicator for pollution (Li et al. 2017). This aligns with our finding as *Phacus* displayed a positive threshold, and so drastically increase in number of individual cells along TN and TP gradients as evidenced by being present in the high bin. However, as there is no evidence across all sampling periods for this response, we cannot recommend any taxa to be used as a bioindicator in this region.

Unlike in previous studies, TITAN did not identify strong threshold reponses in enough taxa to identify a consistent community-based stream nutrient threshold (Smucker, Detenbeck, and Morrison 2013; Porter-Goff, Frost, and Xenopoulos 2013). Due to the low level of threshold taxa, TITAN analysis cannot accurately identify a threshold level of community change along the sampled gradient of TN or TP. In this study, 40-52 genera were included in the analysis depending on sampling period, while only 1-5 genera were identified as having a reliable and pure threshold response. Therefore, algal communities do not appear to display threshold responses at the genera level across the nutrient gradient sampled. This finding aligns with a lack of relationship between taxon richness or diversity and increasing TN or TP, and with no specific taxa or group being affected strongly by an environmental driver. The lack of threshold taxa is potentially due to taxonomic identification only providing information to genus level, however, no support for community shifts was found using the HPLC method either. Thus, algal communities likely do not experience major shifts along the sampled gradient in nutrients.

2.4.4. Nutrient Limitation and Criteria

Climate change will bring about distinct effects in different regions. Models predict increases in phytoplankton biomass and upstream shifts in phytoplankton maxima in cases of reduced flow and higher temperatures, while regions with increased flow will see shifts of phytoplankton maxima downstream (Quiel et al. 2011). Continued nutrient loading to surface water systems will interact with the shift in maxima driven by climate change, and it will be imperative to predict the increase of biomass in locations along the aquatic network to be prepared for eutrophication consequences (Vitousek et al. 1997). As nutrients and velocity are some of the strongest drivers of algal community structure in this region, we expect climate change to affect community structure, and therefore ecosystem function, despite the current periphytic communities observed here indicating a level of resilience across sampled nutrient gradients.

Across our study sites the observed water column TN:TP ratios indicated N to co-limitation (Hillebrand and Sommer 1999; Elser et al. 2007), which validates our finding that N is most likely to structure algal community composition. Nutrient limitation and algal productivity have also been found to relate to ecosystem function. Chen et al. (2015) found that N and P removal rates were positively correlated with algal productivity due to changes in community composition with species more efficient at absorbing nutrients. While we do not see community shifts within this region, there was no limiting threshold identified either, indicating the communities are potentially resilient along greater nutrient ratios than represented in the collected samples. Therefore, nutrient uptake and removal is also likely similar across the sampled nutrient concentration gradient, but driven by N.

2.4.5. Considerations

This work represents a snapshot of periphytic algal communities in streams of Alberta, and these communities represent only a small part of the overall ecosystem. The substrates used for sampling were artificial substrates deployed at the top of the water column, as diversity in streambed substrate required a common artificial substrates withough streambed sediment interference. However, this might have lead to colonization on the artificial substrate that differs from actual benthic communities at the streambed surface due to higher light levels near the stream surface (Lavoie et al. 2004; Wu et al. 2017). To fully understand the health of these aquatic ecosystems we need to take a holistic appraoch, as ecosystem functioning requires the understanding of the state of the interaction between multiple biological quality elements and the abiotic aspects of the system (Kelly 2013). Therefore, other important biological components of the ecosystems we should consider include microbial communities (Zeglin et al. 2019), and invertebrate grazers (Beck et al. 2019). In the future, additional and continued basic aquatic information should be gatherered within Alberta to add to the growing body of literature and to form specific nutrient criteria.

2.4.6. Conclusions

The structure of periphytic algal communities reflects an integrated response to a variety of environmental variables that affect stream ecosystems, and will show responses to changes in the environment due to anthropogenic land use and climate change. N was the more important driver compared to P in these systems indicating general N limitation, but because both nutrients affect algal growth they should both be simultaneously managed to ensure aquatic ecosystem health. Both velocity and temperature are environmental variables which are predicted to be affected by climate change and, due to their capacity to shape algal communities, will result in cascading changes to aquatic ecosystem structure and function (Jankowski and Schindler 2019). Therefore, we need to perform routine algal assessments to monitor algal communities and integrate these assessments into the decision-making process of aquatic management. Algal identification and pigment analysis methods revealed similar drivers of community composition, and for the purpose of identifying nutrient drivers, pigment analysis would be adequate. No specific taxa or group responded differently compared to the rest of the community, so community thresholds were not observed along measured nutrient concentration gradients.

Table 2.1. Genera indicative of low, medium, and high concentrations of total nitrogen (TN) and total phosphorus (TP) within temporal sampling groups, where TN groupings (low=<1.10, medium=1.10-2.00, high=>2.00 mg L^{-1}) and TP groupings (low=<0.1485, medium=0.1485-0.2974, high=>0.2974 mg L^{-1}) were chosen to ensure at least 10 sites within each grouping. For each of TN and TP, indicator taxa that were identified across more than one sampling period are indicated in bold.

Sampling period	Nutrient Level	TN Indicator Taxa	TP Indicator Taxa
Summer 2016	Low	None	*Achnanthes*
	Medium	*Spirogyra*	None
	High	None	None
Spring 2017	Low	*Melosira* *Pediastrum*	*Frustulia* *Meridion* *Euglena*
	Medium	None	***Tetraedron***
	High	*Characiopsis*	*Amphiprora* *Pteromonas*
Summer 2017	Low	*Spirulina* *Staurastrum*	***Tetraedron*** *Staurastrum*
	Medium	None	None
	High	*Euglena*	*Characiopsis* *Phacus*
Spring 2018	Low	None	None
	Medium	None	None
	High	None	*Actinastrum*

Table 2.2. Indicator taxa across a gradient of total nitrogen (TN) and total phosphorus (TP) based on threshold responses detected by TITAN. For each of TN and TP, indicator taxa that were identified across more than one sampling period are indicated in bold.

Sampling period	Z-score	TN Indicator Taxa	TP Indicator Taxa
Summer 2016	Z+	None	None
	Z-	*Cocconeis*	None
Spring 2017	Z+	*Lyngbya* ***Phacus*** *Trachelomonas*	***Characiopsis*** ***Phacus***
	Z-	None	*Melosira* *Frustulia*
Summer 2017	Z+	*Anabaena* *Characiopsis* *Fragilaria* ***Phacus*** *Synedra*	*Anabaena* ***Characiopsis*** *Nitaschia* ***Phacus***
	Z-	*Pinnularia*	None
Spring 2018	Z+	*Fragilaria*	None
	Z-	None	None

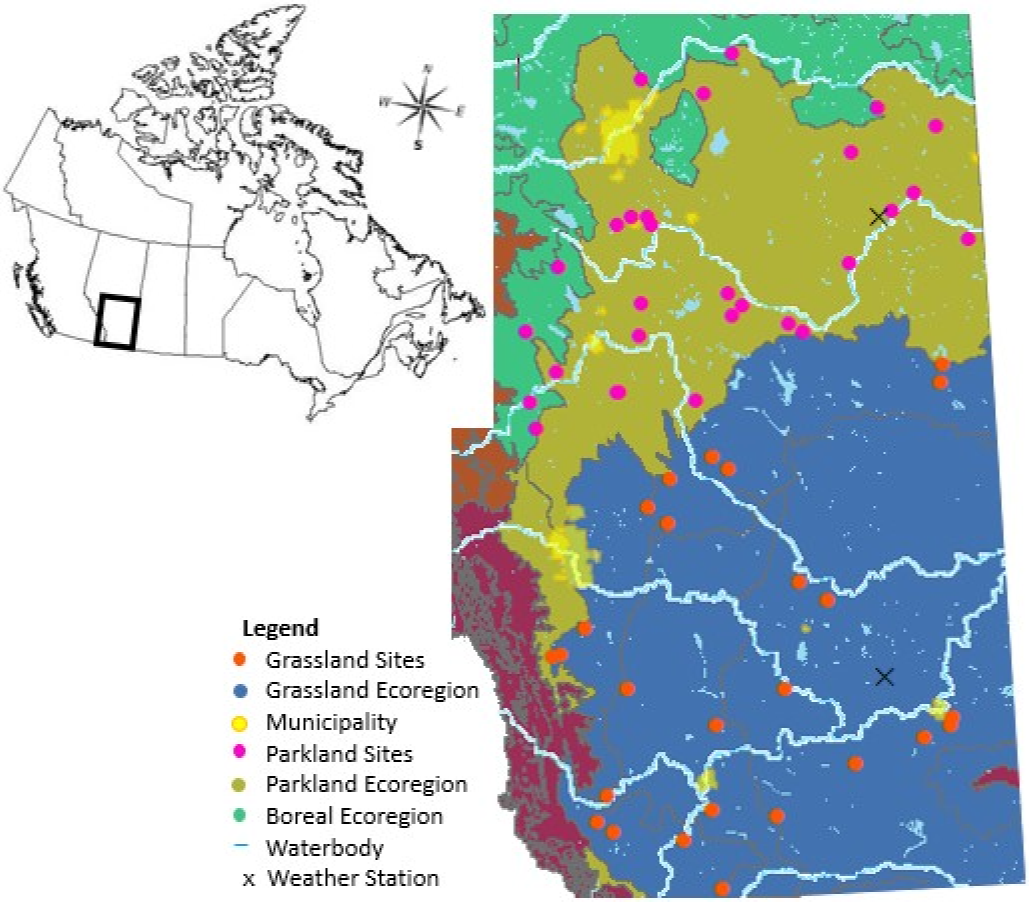

Figure 2.1. Map of the Parkland and Grassland natural regions of Alberta with sample sites indicated. The indicated weather stations were used to determine the climate data presented in the site description.

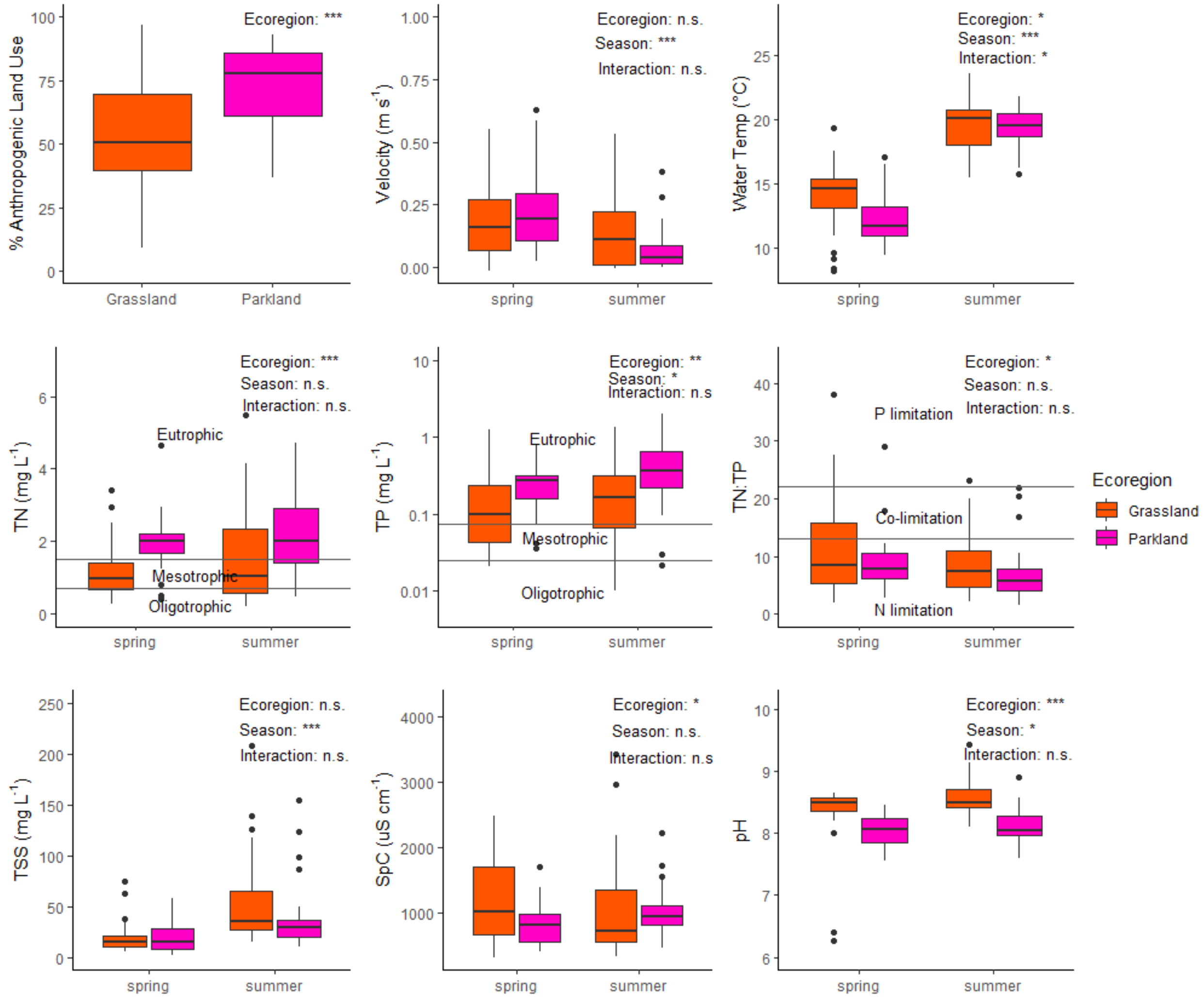

Figure 2.2. Boxplots to show watershed and stream physicochemal characteristics by ecoregion and season measured between 2016 and 2018. Within each metric, two-way ANOVA was used to determine differences between season and ecoregion, and their interaction. T-test was used to determine difference between ecoregion. Significance levels are indicated by stars (<0.001=***, <0.01=**, <0.05=*). Abbreviated variables represent total nitrogen (TN), total phosphorus (TP), total nitrogen to total phosphorus molar ratio (TN:TP), total suspended solids (TSS), specific conductivity (SpC). Grassland spring n=26, Grassland summer n=27, Parkland spring n=29, Parkland summer n=29.

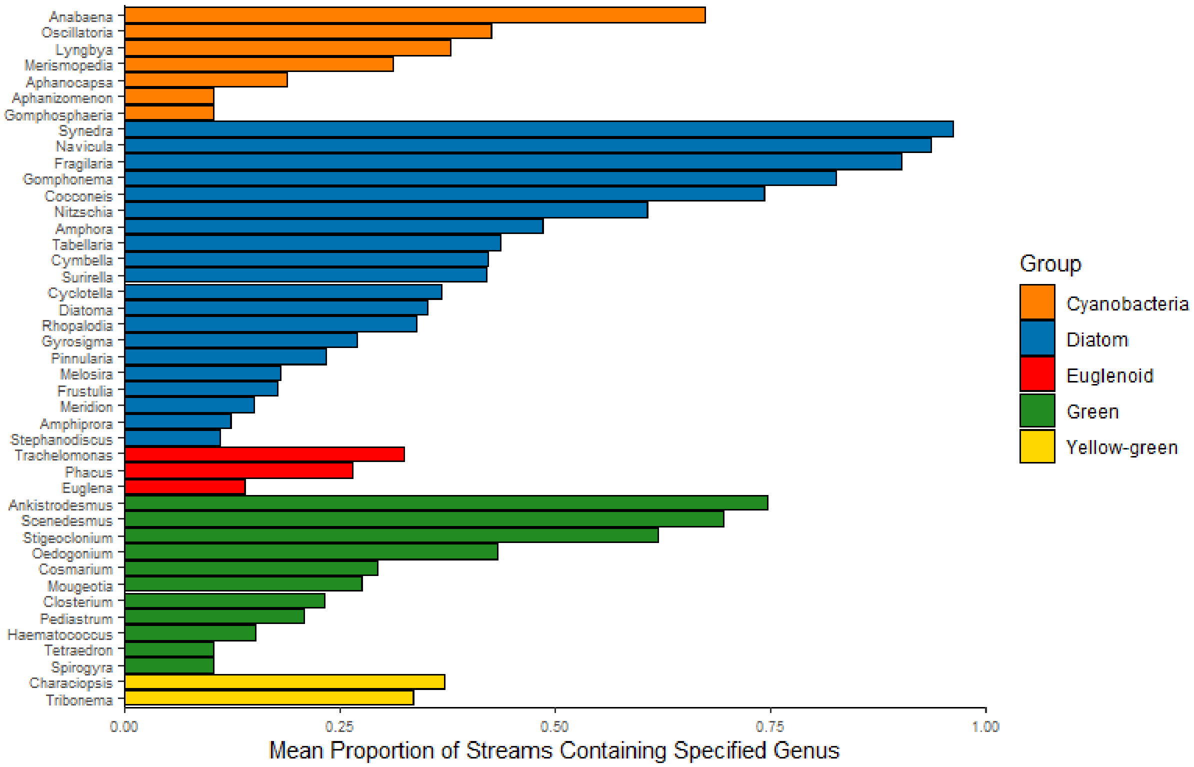

Figure 2.3. Mean proportion of sampled streams containing specified genus, after proportion was averaged between sampling periods. Only genera present in at least 10% of streams are included.

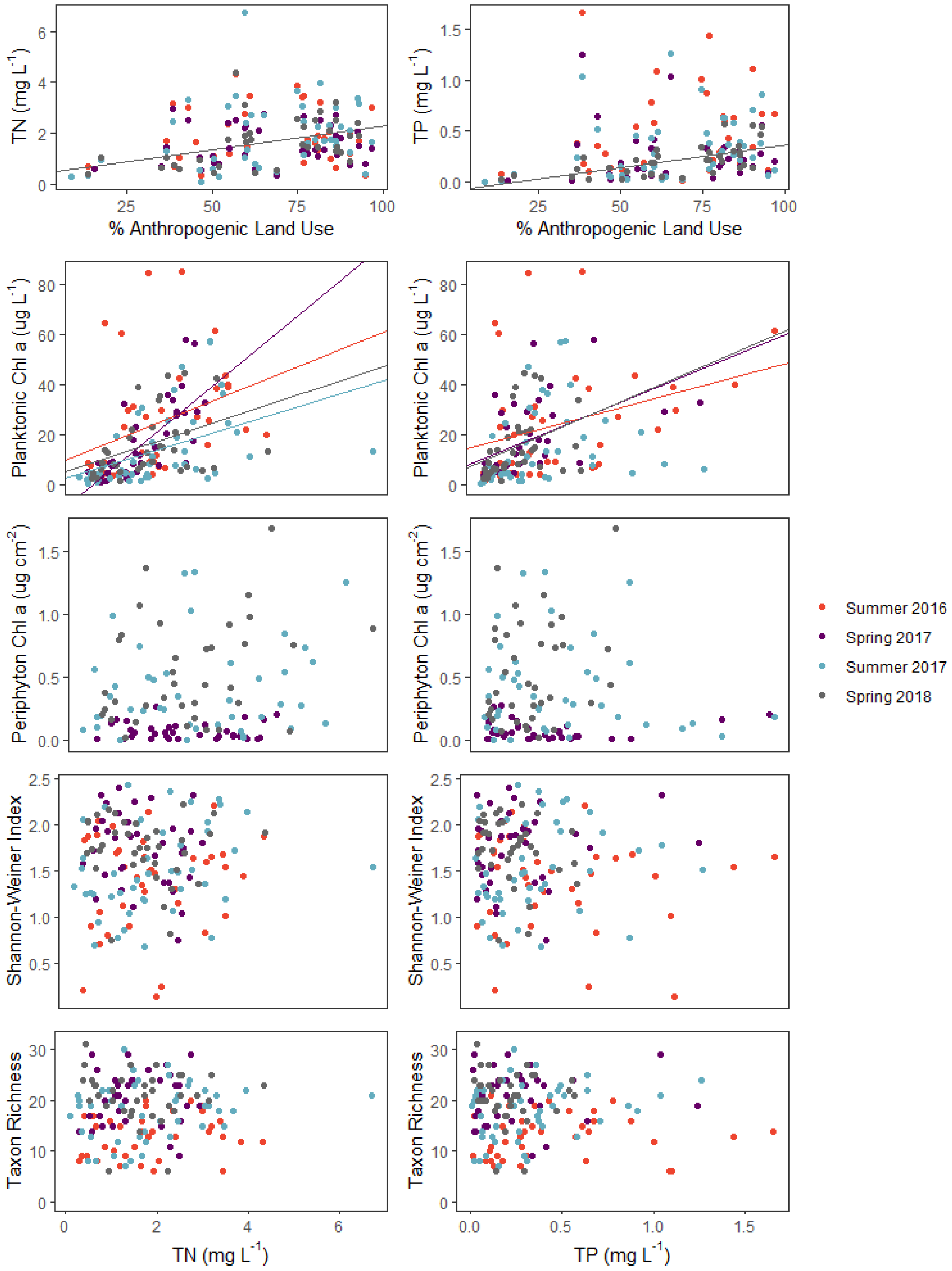

Figure 2.4. Season- and year-specific relationships between total nitrogen (TN) and total phosphorus (TP) concentrations and watershed and algal community characteristics. Significant linear regressions ($p < 0.05$) are indicated.

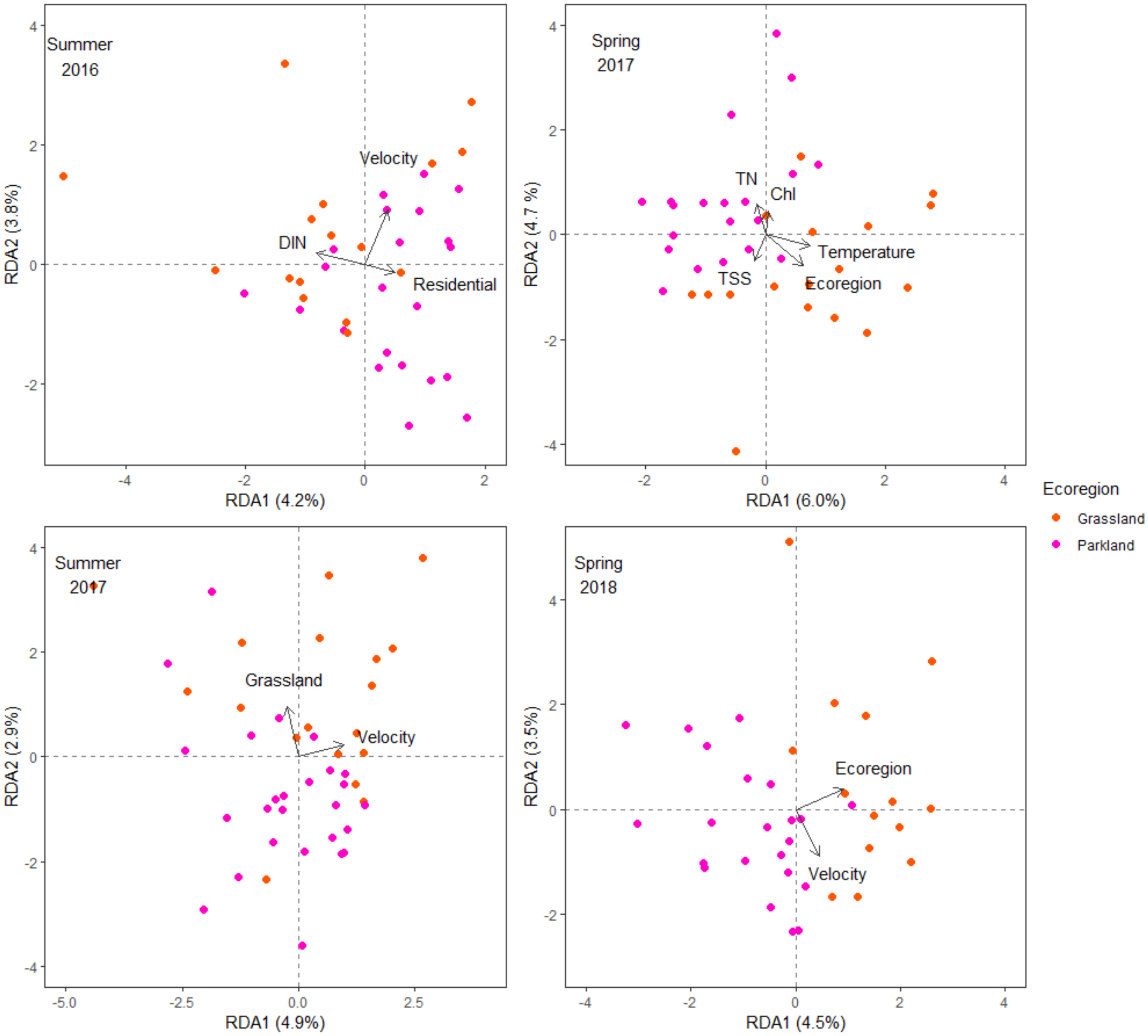

Figure 2.5. Redundancy analysis (RDA) of genus-level count data showing sites by the first two RDA axes and the percentage of the dataset variance explained by RDA1 and RDA2.Vectors represent significant variables driving community structure response.

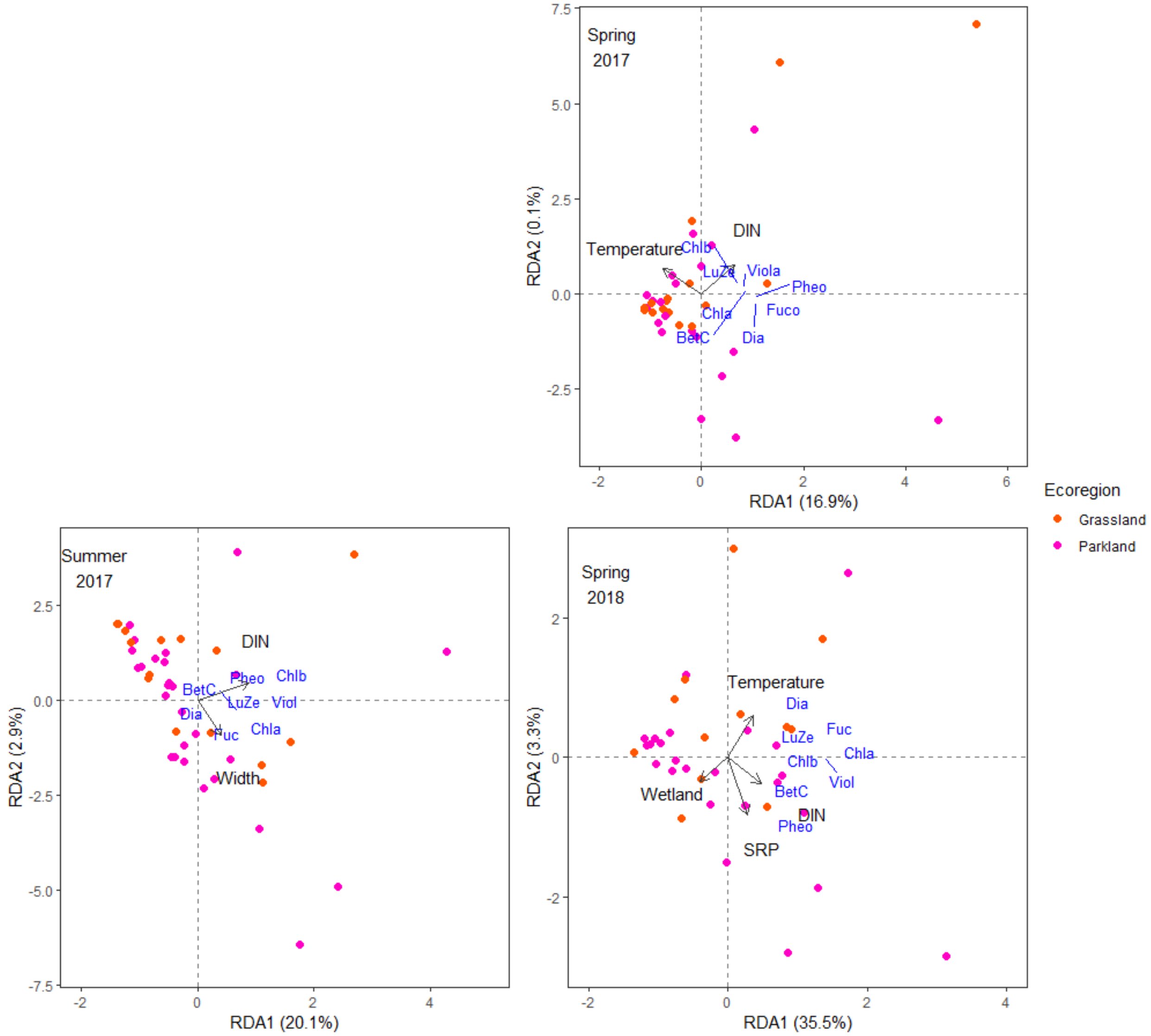

Figure 2.6. Redundancy analysis (RDA) of pigment data showing the first two RDA axes and the percentage of the dataset variance explained by RDA1 and RDA2. Vectors represent significant variables driving community data variance. Blue features indicate pigment scores.

Ch. 3: Nutrient Uptake Dynamics Across a Gradient of Nutrient Concentration and Anthropogenic Land Use in Albertan Streams

3.1. Introduction

Streams are an important feature of the landscape that transport water from headwaters to larger receiving waterbodies downstream. During this downstream transport, streams provide ecosystem services that contribute to the welfare of human populations, including water filtration, flood protection, and cultural use (Jackson et al. 2001; Grizzetti et al. 2019). Another ecosystem service provided by streams is nutrient uptake and release, which controls the rate of nutrient transport downstream, and thus autotrophic and heterotrophic processes across broad spatial scales (Webster and Patten 1979; Newbold et al. 1981). Anthropogenic additions of nutrients through point and non-point sources can disturb stream systems, as elevated concentrations can decrease nutrient uptake by impairing nutrient cycling processes. Resultant downstream eutrophication can have multiple deleterious effects, including algal blooms, oxygen depletion, and fish death (Bormann and Likens 1967; Vitousek et al. 1997; Carpenter et al. 1998; Chambers et al. 2012). A broad-scale understanding of current ecosystem functioning and the potential effects of future nutrient loading is therefore critical for water quality management and the protection of aquatic health, particularly in anthropogenically-altered watersheds.

Nutrients "spiral" during downstream transport as they are taken up via biotic pathways and adsorption, and subsequently released through various mechanisms including exudation and desorption (Newbold et al. 1981). Nutrient addition experiments can therefore be performed to calculate rates of nutrient uptake, and these rates can be compared to assess aquatic ecosystem function (Stream Solute Workshop 1990; Mulholland et al. 2002; Ensign and Doyle 2006). Commonly calculated spiraling metrics include uptake length (Sw; m), which is the stream length over which a solute is transported before being taken up; uptake velocity (vf; mm min^{-1}), which is the mass transfer coefficient of uptake that is standardized by stream flow conditions; and areal uptake rate (U; mg mm^{-2} min^{-1}), which additionally considers ambient concentration, and therefore represents the mass flux (Stream Solute Workshop 1990; Hauer and Lamberti 2006).

Stream nutrient uptake and limitation are interconnected through relationships between nutrient supply and demand in addition to stream flow, and therefore vary along the river network (Wollheim et al. 2018). In stream environments light often penetrates to sediments, and so the benthic component is the predominant zone of biogeochemical nutrient transformations (Cross et al. 2005; Welti et al. 2017). Variation in nutrient ratios (following Redfield dynamics) can also regulate uptake rates (Redfield 1958; Hillebrand and Sommer 1999), whereby saturation of biological nutrient uptake can occur in cases where one nutrient is in excess, or all nutrients become readily available which results in other variables such as light become limiting (Bernot and Dodds 2005). Variation in uptake kinetics along stream networks can therefore be described using various saturation models.

The most basic of these saturation models describes first order uptake kinetics, whereby uptake is tightly coupled to available nutrients and uptake rate is therefore directly proportional to concentration (Stream Solute Workshop 1990; Hauer and Lamberti 2006; O'Brien et al. 2007). In contrast, the Efficiency Loss model assumes declining uptake efficiency with increasing nutrient concentrations, but not to the level where saturation occurs. Finally, the Michaelis-Menten model describes uptake kinetics under conditions where nutrient supply increases to levels that exceed demand, which leads to uptake saturation at higher concentrations (Dodds et al. 2002; Earl, Valett, and Webster 2006; O'Brien et al. 2007). Due to the number of variables affecting uptake, not all streams will experience equivalent saturation dynamics, and it is also possible for saturation dynamics to shift over time according to long-term increases in nutrients through ecosystem change and adaptation (Gibson et al. 2015; Acuña et al. 2019).

As nutrient uptake is largely biotic, variables such as temperature, discharge, and light are expected to affect uptake rates and eventually contribute to saturation through their effects on biotic standing stock, metabolism, and productivity (Bernot and Dodds 2005). In addition to biotic uptake, adsorption and precipitation also contribute to the apparent uptake rate, so variables such as the proportion of silt and organic matter in the streambed can be important for understanding saturation dynamics (Patil et al. 2013). Long uptake lengths can be expected where nutrient concentrations are high (Ensign and Doyle 2006), while other variables such as sinuosity and greater interaction between the streambed and the water column increases uptake, hence, shortens uptake lengths (Dodds et al. 2002; Mulholland et al. 2002). As such, understanding saturation dynamics and providing baseline data of ecosystem function within specific regions is critical for predicting current and future ecosystem health and nutrient uptake capacity.

Agriculture is an important part of the economy in Alberta, with large areas devoted to cropland and cattle grazing. This has the potential to contribute anthropogenic nutrients to streams, alongside non-point sources such as overland runoff from residential areas. Although past research has established a cursory understanding of nutrient loading to Albertan streams, the effect of increasing anthropogenic nutrient contributions on ecosystem function in this region is poorly understood. With phosphorus (P) levels more commonly exceeding federal aquatic nutrient guidelines (Lorenz, Depoe, and Phelan 2008), nutrient control efforts have generally focused on P control, despite the role of nitrogen (N) as a potential limiting nutrient in many stream systems (Hillebrand and Sommer 1999; Taube et al. 2019). Therefore, because efforts to maintain ecosystem health through nutrient criteria should focus on the limiting nutrient to prevent immediate shifts in biomass or ecosystem function, increased focus on N might need to be considered in this region (Dodds and Welch 2000).

To better understand ecosystem health and recommend water quality guidelines, watershed managers require knowledge of current stream function in Alberta. In this study, we (1) assess nutrient retention efficiency of inorganic N and P across a gradient of respective ambient nutrient concentrations and additional physicochemical variables; (2) determine saturation mechanics to assess the relationship between increasing nutrient concentrations and nutrient uptake capacity; and (3) consider whether nutrient ratios predict nutrient limitation in agriculturally impacted streams in Alberta. These objectives were addressed using a series of nutrient tracer injection experiments conducted across 21 steams in the Parkland and Grassland ecoregions of Alberta. Injections were used to calculate Sw, vf, and U, for ammonia (NH_3-N) and soluble reactive phosphorus (SRP), which were compared to physicochemical parameters to explore controls on nutrient uptake and saturation dynamics. In general, we expected higher demand for N compared to P based on previously measured nutrient ratios. We also anticipated that saturation would be exhibited in the streams with the highest nutrient concentrations as biological demand was satisfied.

3.2. Methods

3.2.1. Study Region and Project Overview

A set of 21 third to fifth Strahler-order streams were selected in the agricultural zone of Alberta: of these, nine were located in the Grassland ecoregion, and 12 were located in the Parkland ecoregion (Figure 3.1). Anthropogenic land use is nearly ubiquitous within these major ecoregions of Alberta, which are part of the Great Plains, and so are similar in their climate, dominant soil characteristics, and flora while also exhibiting notable differences. Overall, the Parkland ecoregion experiences higher agricultural intensity than the Grassland ecoregion, with streams consequently showing lower levels of compliance with federal aquatic nutrient guidelines (Lorenz, Depoe, and Phelan 2008). Generally, P concentrations were often found to exceed federal guidelines, while N species were typically in compliance.

The Grassland ecoregion is characterized by a semi-arid climate with low precipitation (Suffield A weather station; ECCC 2010b). Soils are mainly Brown and Dark Brown Chernozemics (AAFC 2011). The Parkland ecoregion is cooler and wetter than the Grassland, with almost double the rainfall in the summer (Fabyan weather station; ECCC 2010a). Parkland soils are predominantly Black Chernozemics and thus typically have a higher percent organic matter and lower water deficit compared to Grassland soils (AAFC 2011). Chernozemic soils are well to imperfectly drained, with parent materials ranging from coarse sands through to fine-textured silts and clay loam (Soil Classification Working Group 1998). Due to the dry climate and reasonable drainage there is potential for high amounts of groundwater interaction and input, which would impact fluvial processes and therefore nutrient uptake rates, as geochemical processes and flow rates can be modified via these surface-subsurface interactions (Mulholland, Steinman, and Elwood 1990). However, while the importance of the hyporheic zone for nutrient uptake has been demonstrated, it is not usually actively considered in nutrient injection studies.

All selected study streams were wadable and flowing during the time of the injection experiment. Streams were selected to span a range of anthropogenic land use intensity, and thus nutrient concentrations, typical for this region of Alberta, while ensuring accessibility and suitability of the experimental sites. The selection of true reference catchments was not possible due to pervasive anthropogenic land use throughout both of the ecoregions. The experimental stream reaches did not contain major visible surface inflow or outflow. Sites vary in their riparian characteristics as a result of private landowners allocating different areas of land to buffer strips, and because native Parkland riparian areas include more trees and reeds compared to native Grassland riparian areas, which tend to be populated by riparian grasses and low-lying shrubs.

3.2.2. Nutrient Injections

Injections were performed based on procedures detailed by the Stream Solute Workshop (1990) and Hauer and Lamberti (2006). Stream width, depth, and velocity were measured at the injection site with a handheld Acoustic Doppler Velocimeter (ADV; FlowTracker2, SonTek, San Diego, CA), while discharge was calculated using velocity and stream cross-sectional area using imbedded ADV protocols. Discharge on the experimental day was used in conjunction with previous water flow measurements to calculate the experimental stream reach (experimental length) and the expected mixing zone length. The length of the mixing zone was calculated using formulas presented in the Code of Practice for Hydrologic Tracing Analysis Studies (Government of Alberta 1996), and the total experimental length was determined as the estimated end point of a 3-hour continuous solute injection. The experimental stream reach varied from 109 to 1831 m (median 822.5 m), with longer reaches required for streams with higher velocities. After subtracting the length of the mixing zone, the remaining reach length was divided into equal parts to create five downstream sampling sites. The first sampling site was positioned at the estimated mixing zone length plus 10% or 20 m, whichever was greatest. Pre-injection samples (see below) were then collected and stream flow characteristics (velocity, depth, and discharge) were measured at the injection site and the five downstream collection locations.

Chloride (NaCl) was used as a conservative tracer while phosphate (NaH_2PO_4) and ammonium (NH_4Cl) salts were used to assess SRP and NH_3-N uptake, respectively. The amount of nutrient salts and water required for an approximately 3-hour injection was calculated based on injection site stream flow characteristics, bellows pump rate, tank capacity (500 L max), and previous nutrient measurements; with an intended concentration increase of 4 mg L^{-1} for Cl, and 0.2-0.4 mg L^{-1} for SRP and NH_3-N (Stream Solute Workshop 1990). Specific conductance, temperature, dissolved oxygen (DO), and pH were measured continuously through the injection using a deployable multiparameter water quality sonde (YSI EXO2, Yellow Springs, OH) at sites 1 and 4. Post-injection samples were taken starting at site 1 and working downstream once conductivity plateaued at site 4, indicating conservative-tracer steady state was achieved. Injections were conducted over a single day at each site between June 15 and August 31 in 2017 (mostly Grassland sites) and between June 18 and June 28 in 2018 (mostly Parkland sites; Table

3-1) with three sites in each of the Grassland and Parkland ecoregions having experiments repeated in each year.

Water samples were placed on ice and submitted to ALS Environmental Laboratory in Edmonton, AB, for analysis as soon as possible. Pre-injection samples were analyzed for total nitrogen (TN), total Kjedahl nitrogen (TKN), ammonia (NH_3-N), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC) following standard methods by (Appendix Table 3-1). Post-injection samples were only analyzed for NH_3-N, NO_3-N, and SRP.

Uptake, or spiraling, lengths (Sw), were calculated from the difference in nutrient concentrations between pre- and post-injection water samples, corrected for dilution by the difference in concentration of the conservative tracers (equation 3.1), following methods reported by the Stream Solute Workshop (1990). Using the calculated Sw, the nutrient uptake velocity (vf) and areal uptake rate (U) were calculated for both NH_3-N and SRP using equations 3.2 and 3.3, respectively (Stream Solute Workshop 1990). These metrics are related to each other as follows:

$$Sw = \frac{1}{|k_{w|}} \tag{3.1}$$

$$vf = \frac{uh}{\mathrm{Sw}} \tag{3.2}$$

$$U = vf\ C \tag{3.3}$$

Where kw is the slope of decreasing nutrient concentration from site 1 to 5, corrected for the conservative tracer and ln-transformed, along the stream reach (m); u represents stream velocity; h represents stream depth, which was calculated as the average within-stream depth across the experimental stream reach; and *C* is the ambient nutrient concentration of the tracer in question.

3.2.3. Streambed Characteristics and Loss on Ignition (LOI)

Sediment samples were collected in July 2018 from the Parkland streams by driving a 7.5 cm diameter PVC pipe into the streambed mid-stream and capping the other end to create suction (McDaniel, David, and Royer 2009; Giannopoulos et al. 2019). Three 10 cm deep cores were taken at random locations upstream of the sampling site before combining in a bucket. Samples were allowed to settle, supernatant water was skimmed off, and the resulting sediment samples were put in a Whirl-Pak® bag and stored on ice before being dried for 24 h at 60°C. Samples were sieved (2 mm mesh size) and 5 g of sieved sample was oven-dried overnight at 105°C before being put into prepared porcelain crucibles. Samples were placed in a muffle furnace for 4 h at 550°C, and weighed after cooling in a desiccator. Post-ignition weights were then used to calculate percent organic matter (Heiri, Lotter, and Lemcke 2001). Particle size distributions were calculated from the muffled samples of particles <2 mm using a mechanical sieve shaker with sieve sizes separating size classes into 2 mm-500 µm, 500 µm-250 µm, 250 µm-63 µm, and <63 µm fractions (McDaniel, David, and Royer 2009).

We also estimated percent macrophyte and periphyton streambed cover within two weeks of the injection experiment. At each site, a location in the middle of the stream channel was randomly selected, and at four locations randomly upstream of the initial site, and a 1 m^2 gridded PVC sampling quadrat was used to estimate percent biotic streambed cover, combining macrophytes and periphyton. At this time water samples were collected for chlorophyll *a* and nutrient analyses, and placed on ice before being analyzed by ALS Environmental Laboratory according to standard methods (Table S3.1).

3.2.4. Data Analyses and Statistics

We used the ArcHydro toolbox in ArcGIS (ESRI 2011) to calculate Strahler order and regional stream channel length. Watershed land use percentage was calculated based on the Annual Crop Inventory Database (AAFC 2016) also using ArcGIS (ESRI 2011). We examined the relationship between U and ambient NH_3-N and SRP to determine evidence of first order uptake, Efficiency Loss, or Michaelis-Menten saturation dynamics, via fit testing with linear regression, power functions, and Michaelis-Menten curves, respectively (O'Brien et al. 2007). An Information Theoretic Approach, using Akaike Information Criterion adjusted for small sample size (AICc), was used to select the physicochemical parameters and land cover that best describe variation in U for NH_3-N and SRP from a global model (Burnham and Anderson 2002). Physicochemical variables included mean temperature, pH, and DO collected at the first sampling site of the experimental reach at each stream, while depth and velocity represented stream flow and discharge was excluded to avoid model redundancy. TN and TP were the only nutrients included to ensure variance inflation factor (VIF) scores below 5. This makes sure no co-variables artificially inflate the ability of the model to describe variance. Land use variables included residential cover and a composite measure of agriculture (sum of crop, pasture, and fallow lands), calculated as proportional coverage within each watershed. We considered all possible linear model combinations, and present models with a substantial level of empirical support (within 2 Δ AICc; Burnham and Anderson 2002). We additionally calculate the Akaike weight (ω_i) as a measure of the likelihood that model *i* is the 'best' model within the suite of models considered. We estimate the relative importance of variables as the sum of Akaike weights across all models within which a given variable occurs (Burnham and Anderson 2002). AICc calculations were performed using package 'MuMIn' (v 1.43.17; Bartoń 2020). All figures were created with package 'ggplot2' (v 3.2.1; Wickham 2016) using R 3.6.1 (R Core Team 2013).

3.3. Results

3.3.1. Variation in Stream Characteristics

Anthropogenic land use as watershed land cover ranged from 14 to 95% (median 76%) (Figure 3.2). TN ranged from 0.354 to 3.919 mg L^{-1} (median 0.914 mg L^{-1}), while NH_3-N ranged from 0.011 to 0.335 mg L^{-1} (median 0.035 mg L^{-1}), and was not closely related to TN (Figure 3.2, r^2= 0.006, p=0.717). Notably none of the NH_3-N measurements exceeded federal guidelines (calculated considering stream pH and temperature; CCME 2010). TP ranged from 0.010 to 0.605 mg L^{-1} (median 0.190 mg L^{-1}), while SRP ranged from 0.004 to 0.423 mg L^{-1} (median 0.101 mg L^{-1}), and showed a strong positive relationship with TP (Figure 3.2, r^2=0.780, $p<0.001$). Molar TN:TP ratios ranged from 2.00 to 35.4 (median 7.55) with 19 sites falling in the range of N limitation, 4 sites falling within the co-limited range, and one site indicating P limitation (assuming values <13, 13-22, and > 22 for N, co-, and P limitation of stream benthos; Hillebrand and Sommer 1999). Other key physicochemical variables are provided in Table 3.1.

3.3.2. Nutrient Cycling and Uptake Relative to Ambient Nutrient Availability

NH_3-N uptake lengths were on average 1512 m and ranged between 49 and 4811 m (Table 3.1 and Figure 3.3). Both ammonia uptake length (Sw NH_3-N) and uptake velocity (vf NH_3-N) did not exhibit any significant relationship with ambient nutrient concentrations, while areal uptake rate (U NH_3-N) increased linearly with NH_3-N (Figure 3.3, r^2=0.949, $p<0.001$), indicating first order uptake. Efficiency Loss (r^2=0.937, $p<0.001$) and Michaelis-Menten

($r^2=0.254$, p=0.024) models also showed a statistically significant fit for the relationship between U NH_3-N and ambient N concentrations, indicating muddled saturation dynamics. P uptake lengths (Sw) were longer than the equivalent lengths for NH_3-N, with a mean length of 5866m and a range of 60-111,905 m (Table 3-1 and Figure 3.4). As with NH_3-N, SRP uptake length (Sw SRP) and uptake velocity (vf SRP) did not exhibit any significant relationship with ambient nutrient concentrations indicating first order uptake kinetics, while linear (Figure 3.4, $r^2=0.344$, p=0.004) and Efficiency Loss ($r^2=0.555$, $p<0.001$) models were both supported for the relationship between uptake rate (U SRP) and increasing ambient SRP confusing saturation dynamics. The Michaelis-Menten model, in contrast, was not supported with $p>0.10$.

3.3.3. Nutrient Cycling and Uptake Models

The AICc analysis to examine controls on U NH_3-N resulted in four models that fell within 2 Δ AICc of the top model (Table 3.2). U NH_3-N was positively related to velocity (weighted importance of 0.74) and TN (0.16), and negatively related to agriculture (0.84) and temperature (0.33). The best model for vf NH_3-N was identified as the null model, while the other twelve models included positive variables DO (0.42), depth (0.29), and velocity (0.26), and negative variables temperature (0.37) and TP (0.05).

The AICc analysis for controls on U SRP resulted in four models within 2 Δ AICc of the top model (Table 3.2). U SRP was positively related to TP (0.73) and TN (0.50), and negatively related to depth (1.00), pH (1.00), and residential land use (0.23). Four models fell within 2 Δ AICc for vf SRP as well. In this model, vf SRP was positively related to temperature (0.40) and negatively related to agriculture (1.00), pH (1.00), and depth (0.37).

3.3.4. Relative Nutrient Uptake Rates

In general, uptake lengths for NH_3-N (Sw NH_3-N) increase with those for SRP (Sw SRP), with Sw NH_3-N typically shorter than Sw SRP within individual catchments (Figure 3.5). However, due to the long uptake length for SRP at Unnamed Creek, a significant linear relationship between these variables did not exist. U NH_3-N and U SRP were also not correlated to one another (Figure 3.5), but did indicated faster uptake for NH_3-N compared to SRP (Gibson et al. 2015). Unnamed Creek has a unique hydrological regime which influences this uptake length, as it is an irrigation return site and conveys relatively high volumes of water from the irrigation network during the growing season. Symbol colour indicates predicted N or co-limitation based on the respective water column TN:TP, and shows how predicted limitation does not always align with the limitation based on experimental uptake length and rate in these systems. P limited sites were not predicted within this data subset, as we were not able to calculate uptake for NH_3-N for the only P limited site in our full dataset. Of the 18 sites where there were paired uptake measurements, 16 appeared to be N limited based on relative uptake lengths (Sw; Figure 3.5a), and 15 appeared to be N limited based on relative areal uptake rates (U; Figure 3.5b).

3.3.5. Stream Bed Substrate and Biotic Cover

Sand-sized grains comprised 73.9% of Parkland streambed sediment on average, with silt and clay-sized grains comprising 7.8% (Table 3.3). Substrate information could not be included in the AICc models described above, as samples were only collected for the 2018 sites. Biotic streambed cover ranged from 0% to 70% (median 0.82%). Biotic cover also did not significantly increase with ambient NH_3-N or SRP concentrations (Figure 3.1). In contrast, phytoplankton chlorophyll *a* increased linearly with both NH_3-N ($r^2=0.272$, $p=0.009$) and SRP ($r^2=0.155$, $p=0.057$), with these relationships indicating a stronger relationship with N, relative to P. Given that samples for biotic streambed cover and chlorophyll *a* were collected on different dates than injection experiments, and that the short growing season in this region causes standing stock to vary drastically over the summer months, these variables were also not included in the descriptor models described above.

3.4. Discussion

3.4.1. Nutrient Cycling, Uptake, and Saturation

The goal of this study was to investigate nutrient uptake kinetics in agricultural streams in Alberta, and assess the influence of variation in ambient nutrient concentrations and other physicochemical characteristics on nutrient uptake rates. We did this to better understand variation in stream health and function across streams in Alberta's agricultural area, given the poor understanding of nutrient saturation and limitation dynamics in this region, and potentially adverse influence of pervasive residential and agricultural land use. Uptake lengths and velocity for both NH_3-N and SRP were similar compared to studies using similar methodologies across a range of stream orders (Ensign and Doyle 2006), indicating uptake rates in Alberta are relative to those in streams across different ecoregions. However, most uptake studies are done in smaller streams, and compared to average uptake length and velocity of only third and fourth order streams as were used in this study, uptake in Alberta took longer (Ensign and Doyle 2006). When considering Sw and vf, we found that both NH_3-N and SRP exhibited first order uptake kinetics, as there was no relationship along ambient nutrient gradient for Sw and vf (O'Brien et al. 2007). U displayed both first order uptake and saturation dynamics (Michaelis-Menten; Figures 3.3, 3.4), thus indicating some potential for nutrient saturation when Sw, vf, and U are considered as a whole (O'Brien et al. 2007).

These results indicate that, while our study streams continue to take up both NH_3-N and SRP in watersheds that include up to 95% anthropogenic land use (crop, pasture and grazing, and fallow, and residential land use), there is potential for a sudden increase in nutrient concentration to cause saturation if the rate of adaptation by the biotic communities is overwhelmed (Stutter, Demars, and Langan 2010; Acuña et al. 2019). Although biological communities can increase in abundance under a stable increase in nutrient loading, sudden increases in nutrient concentration can exceed biotic growth. This could indicate that some streams, including those studied here, have the potential to shift in saturation kinetics, as biotic communities fail to continue to grow and take up nutrients, and so become suddenly saturated (Acuña et al. 2019). In addition, streams can saturate at different concentration points through a variety of differences in variables such as streambed geology leading to different sorption kinetics (Mulholland et al. 2009), and variety in hyporheic flow affecting nitrification and denitrification rates, and subsurface adsorption (Dodds et al. 2002). Hyporheic inflow and nutrient storage in sediment is likely to play a role in stream nutrient kinetics in Alberta, as both have been reported in a larger river in the Grassland ecoregion (Taube et al. 2019). However, the relative influence of hyporheic exchange on nutrient processing in small prairie streams is unknown and cannot be inferred based on studies on larger river systems, and therefore should be explored further.

While first order uptake kinetics are generally expected for oligotrophic systems (Gibson et al. 2015), first order uptake has also been demonstrated at higher nutrient concentrations, where mass transfer components were not yet saturated due to high-saturation sorption kinetics or dissimilatory processes such as nitrification and denitrification (Dodds et al. 2002). In other regions, a lack of clear saturation even at high nutrient concentrations has been shown for NH_3-N and SRP, due to adaptation of biotic communities and increasing biotic biomass along with chronically increased nutrient loading (Simon et al. 2005; Acuña et al. 2019). Michaelis-Menten saturation has also been observed for NH_3-N, NO_3^--N, and SRP, in agricultural and urban streams due to anthropogenic impacts (Newbold et al. 2006), and elsewhere due to redox conditions driving saturation (Arango et al. 2008). Efficiency Loss has been recorded for NO_3-N when comparing a large variety of streams, from mountain headwaters to agricultural streams, due to a decrease in efficiency of denitrification (Mulholland et al. 2009). This shows that streams in the Alberta region show similar dynamics compared to some streams, but not others, and therefore assessing region-specific functioning is imperative to understanding the ecosystem health in a specific location.

3.4.2. N vs. P Limitation in Albertan Streams

Across our study streams NH_3-N and SRP uptake rates increased together, but SRP uptake lengths were consistently longer than for NH_3-N, and NH_3-N was taken up at a faster rate (Figure 3.5). This indicates N limitation in these systems, in line with previously measured nutrient concentrations (Lorenz, Depoe, and Phelan 2008), and our ambient measurements of nutrient concentration. Despite residential development and fertilized croplands being common to all watersheds in the study, NH_3-N concentrations were generally below federal guidelines (CCME 2010). In contrast, TP was generally above these guidelines, with all but three sites classified as eutrophic based on their TP concentrations (CCME 2004). There are no SRP specific guidelines set, as up to 95% of TP is expected to be phosphates, so guidelines will be similar (CCME 2004). Therefore, management measures should particularly keep N additions in mind, as this nutrient is most likely to be limiting for biotic growth and thus nutrient cycling. In addition, in light of excess TP concentrations, and given the historic loading to the system due to anthropogenic activity, P is likely to be in oversupply for some time due to retention in the sediment bed and internal cycling of retained P (Hamilton 2012). Therefore, legacy P in the system will be present and N will long be limiting in comparison. So, in the near term, N management of external loading should be encouraged in conjunction with P legacy and loading management for the preservation of aquatic stream health. In addition, conserving the ability of streams to attenuate and cycle nutrients will prevent added nutrients from flowing downstream to receiving waterbodies quickly and driving eutrophication pressure in those systems.

3.4.3. Physiochemical Variables Influencing Uptake

The linear models describing controls on U NH_3-N were not very strong (Table 3.3). Velocity and TN both showed positive relationships to U NH_3-N, indicating an increase in areal uptake rate with increasing stream flow and nutrient concentration. These findings are similar to previous studies across streams from reference conditions to urban streams, where NH_3-N, depth, and velocity were positively correlated with N uptake length (Simon et al. 2005; Newbold et al. 2006; Mulholland et al. 2009). In the current study, temperature and agricultural land use both had a negative effect on U NH_3-N, which is counter some expectations, as temperature generally have a positive effect on algal biomass, which should increase uptake rate (Chen et al. 2015). Instead, deeper and faster streams often experience lower temperatures compared to shallow and slow streams and so there is an expected negative relationship between temperature and velocity. Therefore, temperature is potentially more strongly related to other variables compared to nutrient uptake. Agricultural land use was negatively related to U NH_3-N, and might be connected to the negative relationship of TP with vf NH_3-N. The vf NH_3-N models are less strong compared to the U NH_3-N models due to the removal of the relationship between uptake rate and nutrients, which highlights the importance of ambient nutrient concentration when comparing uptake rate between streams. DO had a positive relationship with vf NH3-N, and similarly to velocity might be connected to temperature, as more DO can be taken up at lower temperatures (Montgomery, Thom, and Cockburn 1964). Low relationship strength between the models and uptake rate can indicate uptake rate is sensitive to a variety of anthropogenically influenced variables, perhaps beyond those measured in this study (Newbold et al. 2006).

Linear models describing controls on U SRP were stronger than models for U NH_3-N (Table 3.3). Similar to U NH_3-N, land use also played a role, but in the form of residential land cover, indicating a negative effect of anthropogenic related variables on uptake rate. This is despite the positive relationship between anthropogenic land use and nutrients, which are positively related (Figure 3.2). pH was negatively related to U SRP, potentially due to the connection between pH and anthropogenic land use or geology (Lavoie et al. 2014). Increased weathering with agricultural land use can increase mineral additions affecting pH into streams. The inclusion of both TN and TP variables highlight the importance of ambient nutrients uptake rate within streams. TP is closely related to SRP in these systems, and this is why TP has a stronger effect on U SRP compared to TN and U NH_3-N. The negative relationship between SRP uptake and depth may support the importance of benthic interactions in the uptake mechanism, as shallower streams have a larger stream water to streambed interface, and thus greater interaction between the streambed and overlying water column. The vf SRP models were similar to U SRP, although depth explained less variance, anthropogenic land use in the form of agriculture explained more, and nutrients did not explain any variance.

3.4.4. Considerations

There are many additional variables that have the potential to influence nutrient uptake that were not included in these models. To start, biotic variables such as microbe community composition and their contribution to nutrient processing is an important component of the ecosystem not included (Zeglin et al. 2019). Phytoplankton algal biomass increases with both ambient TN (r^2=0.239, p=0.009) and TP concentrations (r^2=0.117, p=0.057) in these streams (Figure A3.1). In contrast, benthic biotic biomass as defined by macrophyte and periphyton substrate cover estimates did not increase with either TN (r^2=0.026, p=0.470) or TP (r^2=0.035, p=0.401). The predominantly inorganic sandy bottoms potentially make it difficult to sustain substantial biotic streambed cover due to suboptimal sediment cohesiveness restricting root growth (Barko and Smart 1986), and therefore benthic organisms might be unable to contribute proportionally to uptake with increased levels of nutrients. Next, due to the method chosen for streambed sediment testing, we might have underestimated clay content since clay particles may not have settled before decanting the water and thus streambed sediment is still not well defined. This knowledge is required as abiotic retention processes are likely to influence P nutrient uptake in Alberta when considering nutrient retention in sediments, thereby influencing nutrient uptake kinetics in this region (Taube et al. 2019). Lastly, differences in the degree of hyporheic exchange between streams may also have contributed to the observed variation in saturation dynamics between streams.

In review of the nutrient tracer injection methods, although non-isotopic nutrient injections are reasonably common (Ensign and Doyle 2006), they typically overestimate nutrient uptake length and therefore underestimate uptake rate (Mulholland et al. 2002). In 6 of 26 (23%) original experimental nutrient additions, uptake rates could not be adequately calculated due to limited uptake occurring within the span of the experimental stream reach, leading to no estimates of the slope of the uptake line (kw) and exclusion from our analyses. In addition, when more than 10% of the experimentally added nutrients are not taken up before the terminal measurement point, any calculations that are based on the concentration relationships are from the initial part of the uptake slope, especially if saturation dynamics are not first order uptake (Mulholland et al. 2002). In that case the slope of the uptake regression is different in the tail, and when not included, slower uptake rate is not accounted for in the overall calculation. When no significant decline in the concentration of added nutrients can be detected over the stream reach during the experiment, it suggests the uptake length is long or even saturated (Mulholland et al. 2002; Simon et al. 2005). This occurred across nutrient concentrations although particularly at high ambient P concentrations, so saturation is likely tied to other variables as well.

3.3.5. Conclusions

This study demonstrated that nutrient uptake kinetics did not shift across a range of nutrient concentrations in Alberta. Uptake length and uptake velocity of NH_3-N and SRP stayed consistent along respective ambient nutrient concentrations, meaning the nutrients do not stay in the water column much longer when concentrations were higher, and stream ecosystems have adapted to taking up the nutrient at respective ambient concentrations for each stream. Despite this, future nutrient additions at high concentrations could outpace biotic adaptation speed and lead to saturation. N is the limiting nutrient for biotic growth in these systems, and is also taken up at a quicker rate compared to P. Considering both N as the limiting nutrient and the legacy P concentrations present, both nutrients should be simultaneously monitored and managed in these systems to prevent rapid funneling of nutrients to downstream receiving waterbodies. To better understand the role of watersheds in agricultural Alberta, in the future there needs to be more intensive watershed research along an aquatic system from headwater stream to receiving eutrophic lakes, as uptake shifts with stream flow characteristics (Ensign and Doyle 2006; Wollheim et al. 2018), and needs to be combined with long term monitoring of other variables which affect nutrient uptake (Abbott et al. 2018). Long term monitoring will aid in determining how biogeochemical processes continue to function with continued nutrient loading by anthropogenic land use and during future climate change.

Table 3.1. Select physicochemical attributes of all sites, with ecoregion and data of the injection experiment specified. Provided are measured data for total nitrogen (TN), ammonia (NH_3-N), total phosphorus (TP), and soluble reactive phosphorus (SRP). Uptake length (Sw), uptake velocity (vf), and uptake rate (U) are presented for both ammonia (e.g. Sw N) and SRP (e.g. Sw P). Percent macrophyte cover (Macro % cover) of streambed and chlorophyll *a* (Chl a) were each collected within two weeks of the injection date.

Stream name	Ecoregion	Injection Date	TN mg L^{-1}	NH_3-N mg L^{-1}	TP mg L^{-1}	SRP mg L^{-1}	Depth m	Velocity m s^{-1}	Sw N m	vf N mm^{-1} min	U N mg mm^{-2} min^{-1}	Sw P m	vf P mm^{-1} min	U P mg mm^{-2} min^{-1}	Macro % cover	Chla ug L^{-1}
Buffalo Ck	Parkland	20/6/18	1.720	0.057	0.211	0.148	0.438	0.084	1310	1.69	0.083	9714	0.23	0.034	16.00	4.02
Bullshead Ck	Grassland	23/6/17	0.891	0.335	0.073	0.007	0.290	0.533	1123	8.28	3.432	5061	1.83	0.011	0.00	15.3
Beaver Ck	Grassland	4/7/17	0.354	0.023	0.01	0.006	0.200	0.139				285	5.78	0.032		1.58
Dogpound Ck	Parkland	28/8/17	0.451	0.017	0.025	0.084	0.315	0.123	247	9.42	0.162	1057	2.15	0.015	37.27	1.46
Dogpound Ck	Parkland	25/6/18	0.560	0.022	0.03	0.005	0.461	0.228	1922	3.28	0.058	4216	1.50	0.012	0.43	2.13
Eagle Ck	Parkland	26/6/18	0.757	0.026	0.04	0.008	0.317	0.035	236	2.78	0.071	807	0.82	0.006	1.30	2.69
Grizzlybear Ck	Parkland	21/6/18	3.919	0.044	0.605	0.423	0.429	0.025	333	1.93	0.089	442	1.45	0.606	0.00	20.6
Iron Ck	Parkland	27/6/18	1.970	0.04	0.262	0.184	0.461	0.030	451	1.84	0.061	19941	0.04	0.008	24.06	5.02
Kneehill Ck	Grassland	28/6/17	2.410	0.011	0.317	0.117	0.320	0.169	935	3.45	0.038	3874	0.83	0.094	0.00	31.1
Kneehill Ck	Grassland	28/6/18	0.937	0.037	0.098	0.004	0.403	0.054	782	1.98	0.080	12111	0.13	0.001	0.00	27.8
Lasthill Ck	Parkland	28/6/18	0.632	0.027	0.067	0.011	0.433	0.040	4811	0.22	0.005	1004	1.04	0.012	0.00	10.7
Lloyd Crk	Parkland	25/6/18	1.403	0.034	0.243	0.166	0.205	0.010				60	2.02	0.334	0.00	6.24
Matzhiwin Ck	Grassland	28/6/17	0.659	0.042	0.223	0.198	0.330	0.171	1319	2.56	0.097	15866	0.21	0.042	0.00	4.67
Onehill Ck	Grassland	29/6/17	0.633	0.036	0.131	0.097	0.440	0.440	4241	2.73	0.096				21.67	5.65
Pipestone Ck	Parkland	21/6/18	0.473	0.074	0.073	0.050	0.727	0.027	2994	0.39	0.012	6658	0.18	0.019	12.4	0.01
Pothole Ck	Grassland	6/7/17	1.939	0.032	0.248	0.110	0.198	0.337	3763	1.30	0.029				0.11	11.4
Ray Ck	Parkland	19/6/18	0.434	0.032	0.144	0.017	0.377	0.025	274	2.08	0.089	1671	0.34	0.051	70.1	2.05
Rosebud Ck	Grassland	29/6/17	1.101	0.046	0.201	0.145	0.440	0.094	320	7.68	0.143	6009	0.41	0.088	17.79	40.6
Rosebud Ck	Grassland	29/6/18	1.679	0.037	0.298	0.216	0.675	0.142	361	15.90	0.541				24.09	34
Seven Persons Ck	Grassland	15/6/17	1.199	0.034	0.183	0.104	0.492	0.113	293	8.28	0.642	322	7.56	0.381	41.07	30.2
Sturgeon R	Parkland	31/8/17	1.416	0.034	0.187	0.098	0.526	0.137	665	6.48	0.208				0.40	1.9
Threehills Ck	Parkland	19/6/18	1.613	0.019	0.193	0.129	0.371	0.021	48	9.84	0.152	1034	0.46	0.059	0.00	4.880

Unnamed Ck	Grassland	11/7/17	0.811	0.060	0.303	0.062	0.630	0.719	2909	9.30	0.559	111905	0.24	0.015		4.71
Weiller Ck	Parkland	18/6/18	0.824	0.035	0.413	0.218	0.485	0.029	3925	0.20	0.009	2157	0.36	0.078	0.00	5.77

Table 3.2. Sediment characteristics for 12 Parkland sites based on Loss on Ignition and particle size analyses.

Stream name	Sampling Date	Gravel %	Sand %	Silt + clay %	Organics >2mm %	Organics <2 mm %
Buffalo Ck	20/6/18	0.0	81.4	18.5	0.0	0.03
Dogpound Ck	25/6/18	1.1	98.8	0.0	0.0	0.01
Eagle Ck	26/6/18	90.0	8.8	0.9	0.0	0.31
Grizzlybear Ck	21/6/18	0.3	76.7	22.8	0.1	0.04
Iron Ck	27/6/18	41.3	57.7	0.8	0.1	0.01
Kneehill Ck	28/6/18	4.5	94.5	1.0	0.0	0.01
Lasthill Ck	28/6/18	0.0	98.3	1.6	0.0	0.01
Pipestone Ck	21/6/18	23.7	75.9	0.3	0.1	0.01
Ray Ck	19/6/18	0.8	75.6	23.6	0.0	0.04
Rosebud Ck	29/6/18	56.2	43.7	0.1	0.0	0.01
Threehills Ck	19/6/18	0.0	78.0	21.8	0.2	0.06
Weiller Ck	18/6/18	0.1	97.5	2.3	0.1	0.01

Table 3.3. Best AICc models (Δ AICc < 2.0) predicting uptake rate (U) and uptake velocity (vf) for NH_3-N and SRP. The sign of model coefficients is indicated for each variable. Variables that were available for global model selection were: water temperature, pH, dissolved oxygen (DO), depth, discharge, ammonia (NH_3-N), soluble reactive phosphorus (SRP), agricultural land use in watershed, and residential land use in watershed.

Interest variable	Model	AICc	Δ AICc	Weight	Adjusted R^2
U NH_3-N	-agriculture, +velocity	59.77	0.00	0.41	0.3593
	-agriculture	60.62	0.86	0.27	0.2741
	-temperature, +velocity	61.52	1.76	0.17	0.3060
	-agriculture, +velocity, -temperature, +TN	61.70	1.94	0.16	0.4375
vf NH_3-N	Null model – random error	66.04	0.00	0.14	-
	-temperature	66.19	0.15	0.13	0.0651
	+DO	66.64	0.60	0.10	0.0458
	+velocity	66.83	0.79	0.09	0.0378
	+DO, +velocity	67.27	1.23	0.07	0.0988
	+DO, +depth	67.29	1.25	0.07	0.0981
	+DO, -temperature	67.51	1.47	0.07	0.0891
	+DO, -temperature, +depth	67.53	1.49	0.06	0.1752
	-temperature, +depth	67.62	1.58	0.06	0.0844
	+depth	67.91	1.86	0.05	0
	-temperature, +velocity	67.92	1.88	0.05	0.0719
	+DO, +depth, +velocity	67.93	1.89	0.05	0.1599
	-TP	68.01	1.97	0.05	0
U SRP	-depth, -pH, +TN	49.00	0.00	0.27	0.5848
	-depth, -pH, +TP	49.01	0.01	0.27	0.5846
	-depth, -pH, +TP, -residential	49.27	0.27	0.23	0.6357
	-depth, -pH, +TP, +TN	49.29	0.29	0.23	0.6353
vf SRP	-agriculture, -pH	38.41	0.00	0.37	0.7242
	-agriculture, -pH, +temperature	39.16	0.75	0.26	0.7461
	-agriculture, -pH, -depth	39.37	0.95	0.23	0.7435
	-agriculture, -pH, +temperature, -depth	40.38	1.96	0.14	0.7665

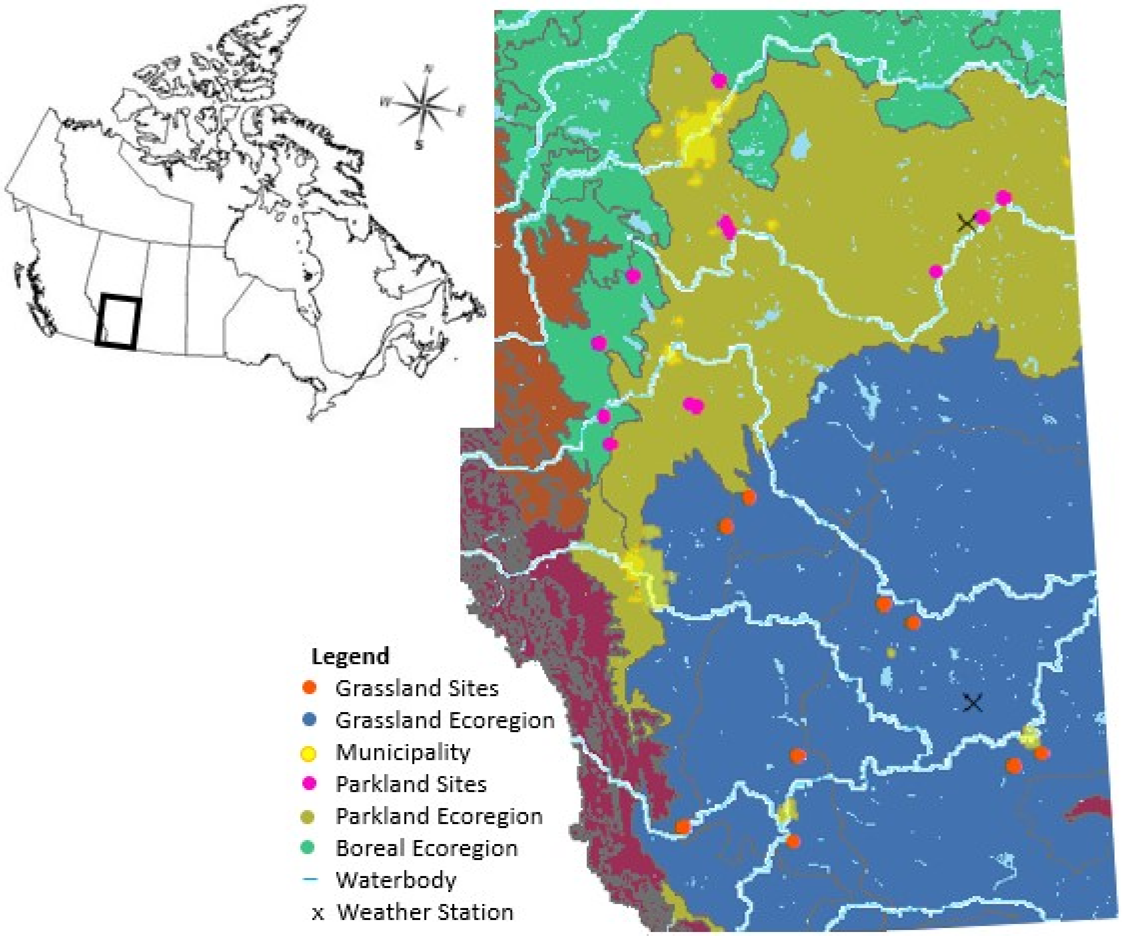

Figure 3.1. Map of the Parkland and Grassland natural regions of Alberta with sample sites identified. The indicated weather stations were used to determine the climate data presented in the site description.

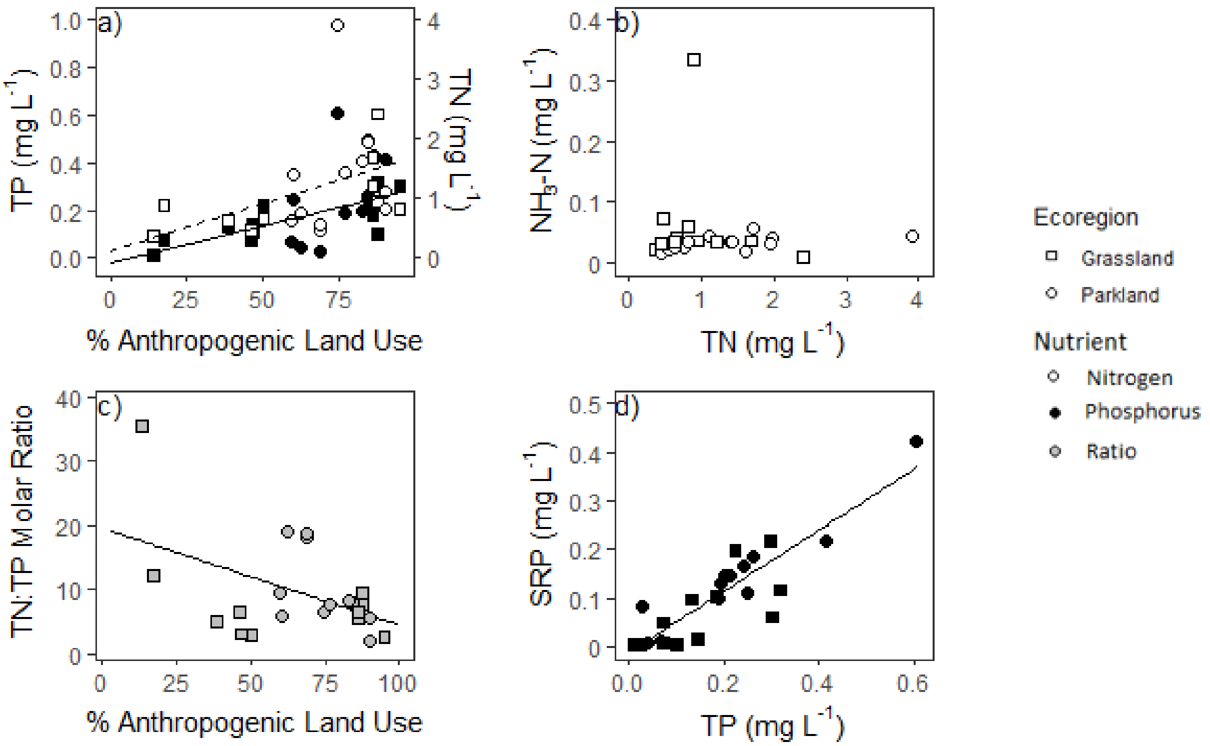

Figure 3.2. Nutrient concentration regressions. a) Concentrations of total phosphorus (TP) and total nitrogen (TN) according to the percentage of anthropogenic land use calculated as the combined area of crop, pasture, fallow, and residential lands. b) The relationship between ammonia (NH_3-N) and TN. c) The relationship between the TN:TP molar ratio and anthropogenic land use with significant regression. d) The relationship between soluble reactive phosphorus (SRP) and TP. Linear regressions significant at $p<0.05$ are indicated.

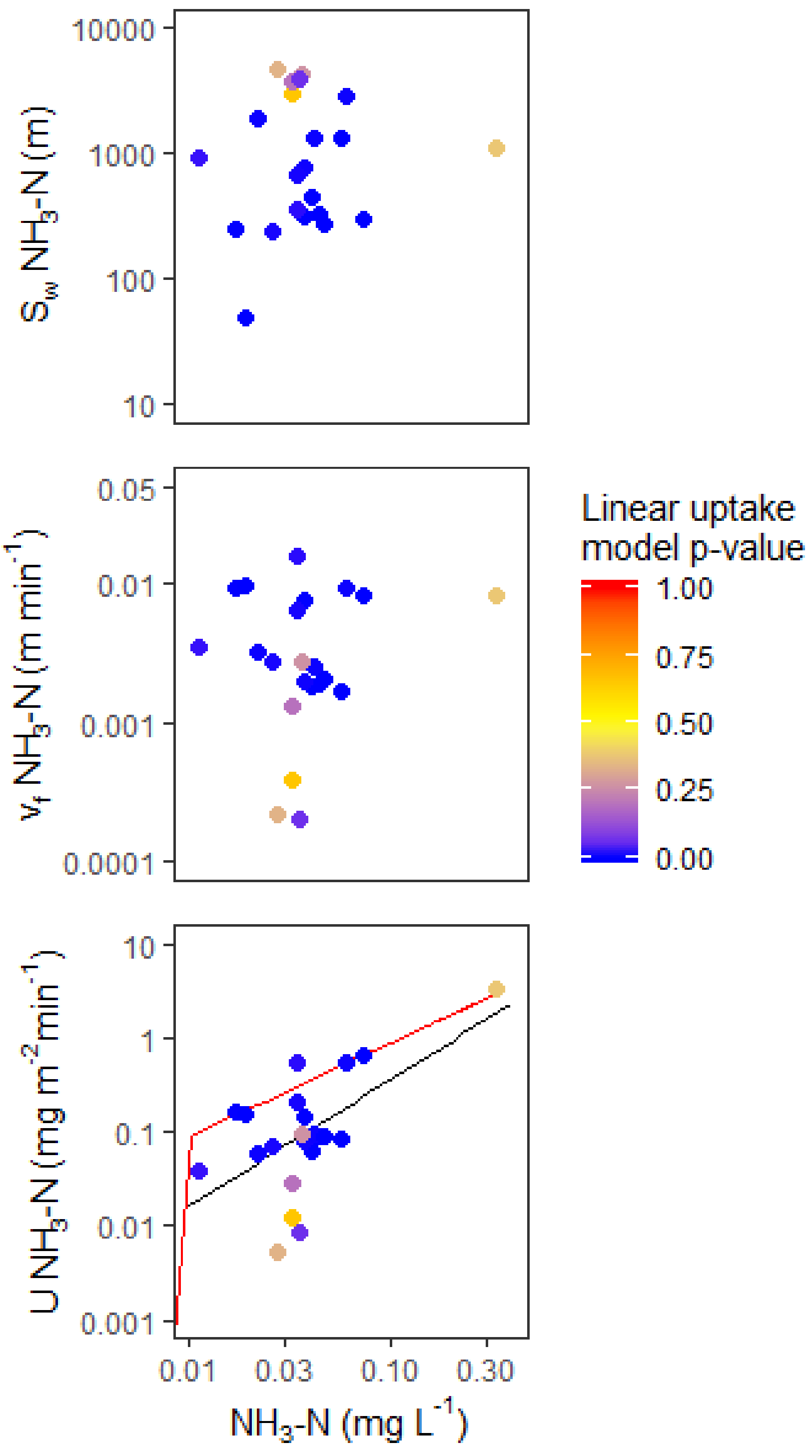

Figure 3.3. Uptake length (Sw NH_3-N), uptake velocity (vf NH_3-N), and areal uptake rate (U NH_3-N) of NH_3-N after experimental nutrient injections in streams relative to ambient levels of NH_3-N at the time of injection (n=22). Axes are presented on logarithmic scale. Shading is based on the numeric p-value of the uptake slope (kw). Significant relationship for first order (black line) and Michalis-Menten (red line) saturation are shown.

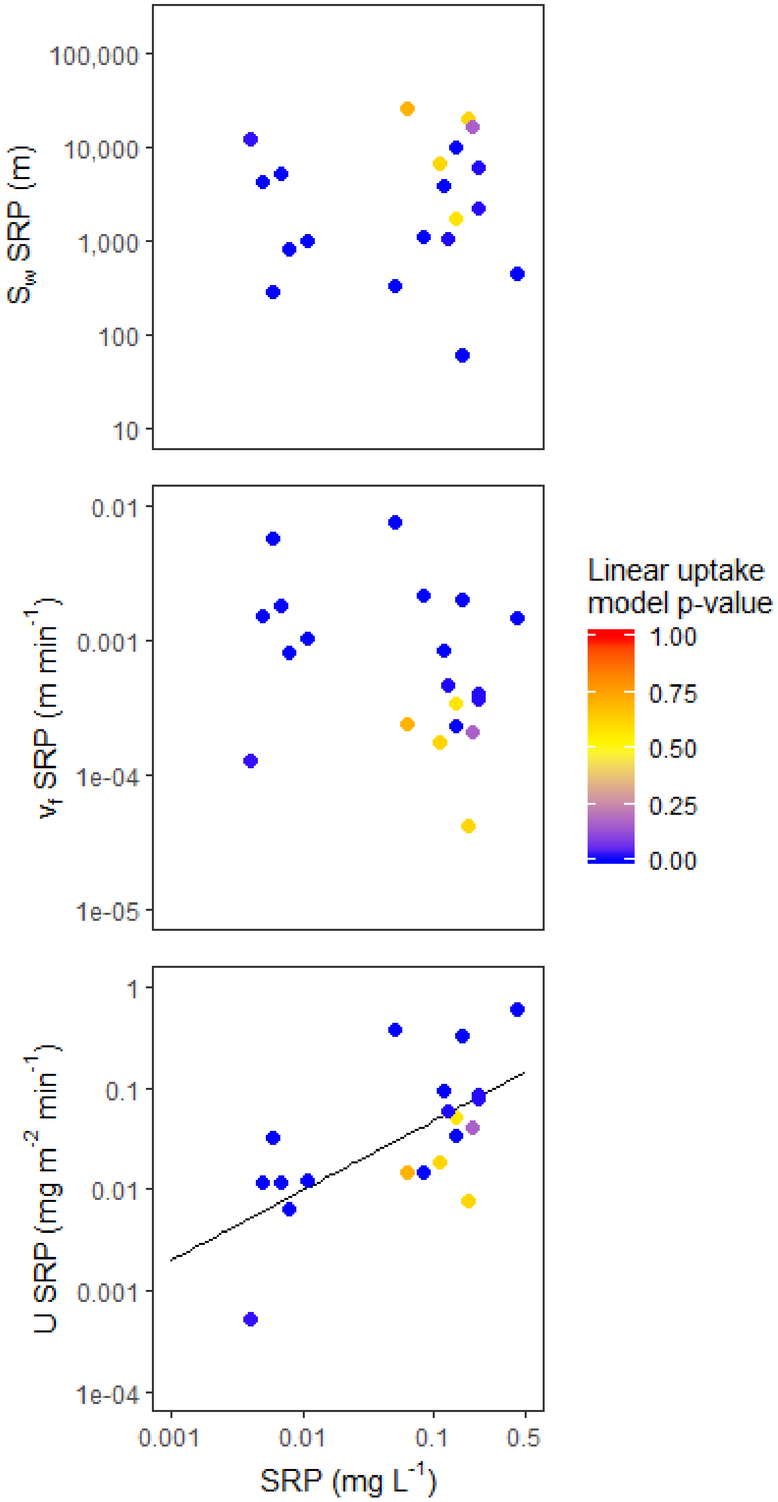

Figure 3.4. Uptake length (Sw SRP), uptake velocity (vf SRP), and areal uptake rate (U SRP) of NH_3-N after experimental nutrient injections in streams relative to ambient levels of NH_3-N at the time of injection (n=20). Axes are presented on logarithmic scale. Shading is based on the numeric p-value of the uptake slope (kw). Significant relationship for first order (black line) is shown.

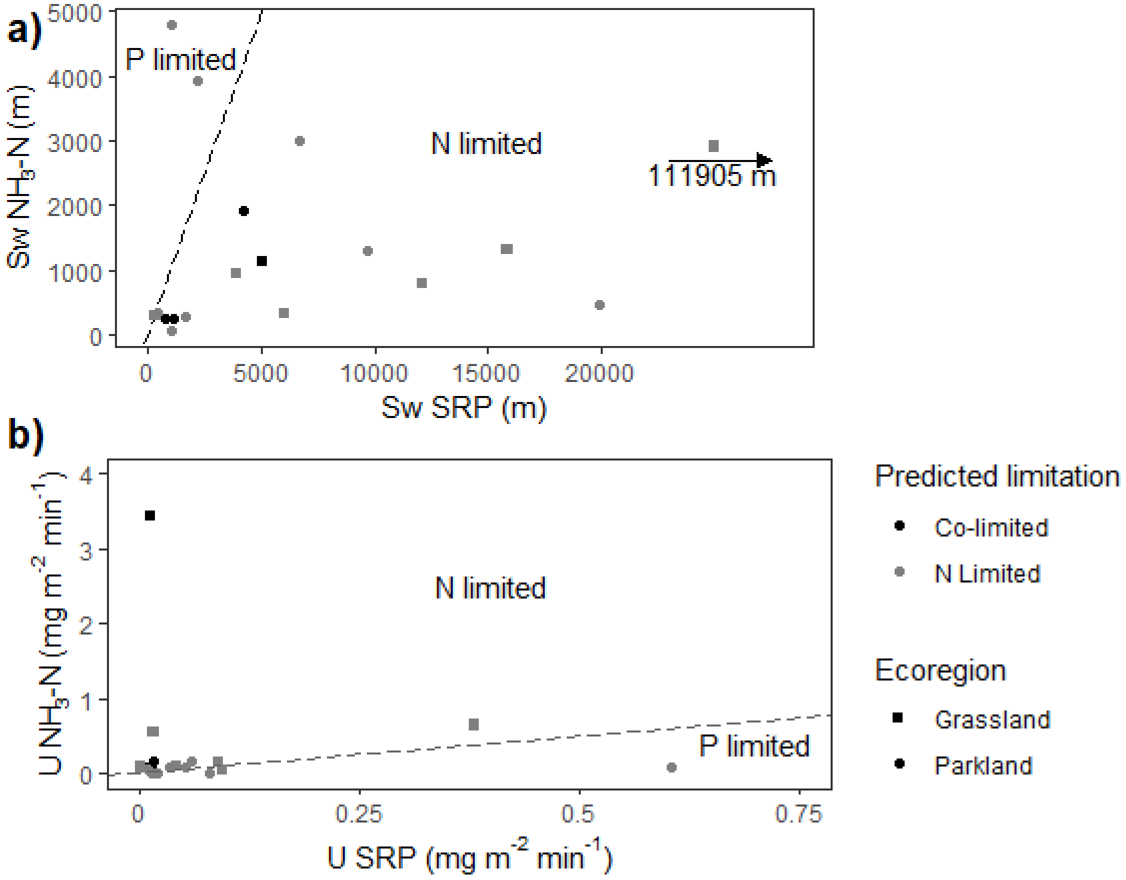

Figure 3.5. Uptake comparisons for n=18 sites where uptake dynamics were calculated for both NH_3-N and SRP. a) Uptake length (Sw) of NH_3-N compared to uptake length of SRP based on experimental nutrient additions. b) Uptake rate (U) of NH_3-N compared to uptake rate for SRP. Dashed line divides N from P limitation. Shading indicates predicted limitation based on TN:TP ratios on the injection day, following the ratios described in Hillebrand and Sommer (1999).

Ch. 4: General Conclusions

4.1. Summary of Findings

This thesis characterized ecosystem health of Albertan streams across nutrient concentration gradients in the Grassland and Parkland ecoregions, by assessing periphytic algal community structure, thresholds, and nutrient uptake kinetics and saturation dynamics. The results broadly revealed streams to be N limited across a large geographic extent in Alberta, with nitrogen (N) being the driving nutrient in algal community structure and being taken up at a faster rate compared to phosphorus (P) along stream reaches.

In addition to the over-riding importance of N, the results in Chapter 2 highlight the importance of seasonality and regional factors for structuring periphytic algal communities. Other important drivers of community composition were watershed land use, stream velocity, and temperature. Bioindicator and threshold analyses could not be used to identify nutrient criteria as there were no major shifts along the sampled nutrient gradient when taxa were assessed at the genera level. This work is the first follow-up investigation designed to quantify algal communities in the region since a survey completed decades ago (Green and Davies 1980), which allowed for the comparisons of community snapshots, in which no major differences were found. The results in Chapter 3 reveal first order uptake kinetics to be the dominant uptake dynamic displayed in streams in this study. This indicates a close connection between nutrient concentration and biotic uptake, for both N and P, and suggests that algal communities may largely be adapted to current nutrient concentrations. N was generally taken up at a more rapid rate than P, identifying broad-scale N limitation.

Considering the structural and functional assessment methods of stream health together, streams in the Grassland and Parkland ecoregion of Alberta appear to display a resilience to change along the gradient of nutrient concentrations present, as there is no evidence of community thresholds or uptake saturation. However, sudden increases in nutrient concentration could change dynamics as biotic communities do need time to adapt (Stutter, Demars, and Langan 2010; Acuña et al. 2019), so efforts should be made to maintain current nutrient status and thus ecosystem health. Given the lack of reference watersheds with no anthropogenic influence in either of the Grassland or Parkland ecoregions on which nutrient criteria can be based (Dodds and Oakes 2004), it is possible that nutrient concentrations are already elevated well above baseline conditions, and therefore communities have already shifted in composition and functional capacity from conditions before anthropogenic influence.

4.2. Future Research

Additional types of functional assessments such as metabolism would provide a greater understanding of how different components of the ecosystem interact (Bernot et al. 2010), and the range of physicochemical factors that are important for governing biogeochemical functioning in Albertan streams (Wollheim et al. 2018). Another important component that was missing from this research is an understanding of the hyporheic flow patterns and groundwater inflow to streams in this region, which is important for interpreting nutrient uptake experimental results (Dodds et al. 2002). Chernozemic geology and landscape morphology indicates well to imperfectly drained soils (AAFC 2011), and hyporheic zones are a known sink of nutrients in larger rivers in Alberta (Taube et al. 2019). Combined with long-term continued monitoring, understanding hyporheic flow patterns would allow for better predictions of changes in essential ecosystem services as changes in stream velocity due to climate change are expected to affect nutrient uptake (Martí et al. 2009). A drop in specific conductivity was observed in the data before – and during – arrival of the injection plateau in most injection experiments. This could point to important hydrological characteristics in the region, including diel trends in water source affecting nutrient concentration within the streams which would lead to shifts in nutrient uptake rates on a daily basis (Chamberlin et al. 2019). The data collected in this study, and in the future, should also be used for large-scale modeling to assist in the prediction of nutrient concentrations in areas where sampling is not logistically possible, and to predict ecosystem health across the ecoregions to set comprehensive nutrient criteria (Dodds and Welch 2000; Morales-Marín et al. 2015).

4.3. Research Improvements

The research described here provides some areas of improvement for future projects in this region and on these topics. To better be able to identify thresholds in algal communities along a nutrient gradient, species-level identification is likely required instead of genus-level identification (Smucker et al. 2013; Porter-Goff et al. 2013; Thomas et al. 2015). The identification could additionally be done on phytoplankton instead, as phytoplankton biomass had a stronger relationship with nutrient concentrations in these systems compared to periphyton biomass, and this might enable the identification of nutrient thresholds. Species-level information would also enable an assessment of algal functional traits, which are an indication of functional shifts within the algal community (Mateo et al. 2015; Tapolczai et al. 2016). Since the spring sampling period in this research was likely not representative of the snowmelt period, a comprehensive survey of early-season succession communities would supplement information on seasonal variety in algal communities in this region (Beck et al. 2019). Nutrient injection experiments can also be performed using isotopes, which would result in more representative nutrient uptake rates by avoiding large raises in water column nutrient concentration (Ensign and Doyle 2006). However, this is a more expensive and logistically intense method compared to the nutrient salt injections performed in this study, which is why the current method was selected. Alternatively, other methods to explore nutrient uptake kinetics include multiple nutrient addition (Payn et al. 2005) and Tracer Additions for Spiraling Curve Characterization (TASCC; Covino, McGlynn, and McNamara 2010). In addition, nutrient uptake was explored only across different watersheds in this study, and performing nutrient amendment experiments within the same watershed from headwaters to outlet will enable a better understanding of how uptake shifts across the river continuum.

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APPENDICES

Appendix 1. Supporting Information for Chapter 2

Table A2.1. Aquatic parameters, with abbreviations, measured during this study. Analyses followed methods adapted from the APHA Standard Methods and the US EPA Test Methods (APHA 2017; US EPA 2003), as described below.

Parameter	Abbreviation	Method Reference
Total Suspended Solids	TSS	APHA 2540 D-Gravimetric
Total Nitrogen	TN	APHA 4500 N-Calculated
Total Kjeldahl Nitrogen	TKN	APHA 4500 NorgD (modified)
Ammonia in Water by Colour	NH_3-N	APHA 4500 NH3-NITROGEN (AMMONIA)
Nitrite in Water by IC	NO_2-N	EPA 300.1 (modified)
Nitrate in Water by IC	NO_3-N	EPA 300.1 (modified)
Total P in Water by Colour	TP	APHA 4500-P PHOSPHORUS
Total Dissolved P in Water by Colour	TDP	APHA 4500-P PHOSPHORUS
Diss. Orthophosphate in Water by Colour (Soluble Reactive Phosphorus)	SRP	APHA 4500-P PHOSPHORUS
Dissolved Organic Carbon by Combustion	DOC	APHA 5310 B-WP
Chlorophyll *a*	Chla	EPA 445.0 ACET

Table A2.2. Summary statistics for Figure 2.2.

Variable	Ecoregion	Season	Statistics				
			Mean	Median	Ecoregion	Season	Interaction
% Anthropogenic Land Use	Grassland	-	54.27	50.58	$p<0.001$	-	-
	Parkland	-	74.07	77.93			
Velocity (m s^{-1})	Grassland	Spring	0.190	0.159	$p=0.706$	$p<0.001$	$p=0.085$
		Summer	0.228	0.110			
	Parkland	Spring	0.139	0.193			
		Summer	0.080	0.038			
Water Temperature (°C)	Grassland	Spring	13.93	14.60	$p=0.0150$	$p<0.001$	$p=0.0401$
		Summer	19.48	20.13			
	Parkland	Spring	12.12	11.68			
		Summer	19.38	19.55			
TN (mg L^{-1})	Grassland	Spring	1.20	0.98	$p<0.001$	$p=0.204$	$p=0.691$
		Summer	1.54	1.05			
	Parkland	Spring	1.98	2.02			
		Summer	2.15	2.01			
TP (mg L^{-1})	Grassland	Spring	0.21	0.10	$p=0.007$	$p=0.028$	$p=0.180$
		Summer	0.25	0.17			
	Parkland	Spring	0.28	0.28			
		Summer	0.48	0.37			
TN:TP	Grassland	Spring	11.2	8.39	$p=0.081$	$p=0.053$	$p=0.774$
		Summer	8.80	7.31			
	Parkland	Spring	8.59	7.86			
		Summer	6.87	5.63			
TSS (mg L^{-1})	Grassland	Spring	19.77	15.50	$p=0.085$	$p<0.001$	$p=0.135$
		Summer	57.37	35.50			
	Parkland	Spring	18.79	15.50			
		Summer	38.84	29.18			
Specific Conductivity (µS cm^{-1})	Grassland	Spring	1217	1011	$p=0.038$	$p=0.840$	$p=0.162$
		Summer	1078	720			
	Parkland	Spring	832	805			
		Summer	1001	950			
pH	Grassland	Spring	8.30	8.50	$p<0.001$	$p=0.014$	$p=0.118$
		Summer	8.60	8.50			
	Parkland	Spring	8.05	8.08			
		Summer	8.13	8.05			

Table A2.3. Regression statistics for Figure 2.4.

Nutrient	Variable	Sampling period	Adjusted r^2	p-value
TN	% Anthropogenic Land Use	Summer 2016	-0.01666	0.5343
		Spring 2017	-0.01479	0.4952
		Summer 2017	0.04691	0.08228
		Spring 2018	0.1719	**0.006927**
	Planktonic Chlorophyll *a*	Summer 2016	0.0904	**0.03728**
		Spring 2017	0.5315	**<0.001**
		Summer 2017	0.1892	**0.001659**
		Spring 2018	0.1107	**0.02684**
	Benthic Chlorophyll *a*	Spring 2017	-0.02023	0.5838
		Summer 2017	-0.006501	0.3963
		Spring 2018	0.06018	0.08068
	Shannon-Weiner Index	Summer 2016	-0.008549	0.4129
		Spring 2017	0.01384	0.2281
		Summer 2017	0.04626	0.08376
		Spring 2018	-0.02133	0.6073
	Taxon Richness	Summer 2016	0.005604	0.2789
		Spring 2017	-0.02309	0.6677
		Summer 2017	0.03271	0.1221
		Spring 2018	-0.02331	0.6555
TP	% Anthropogenic Land Use	Summer 2016	0.006424	0.273
		Spring 2017	-0.02523	0.7377
		Summer 2017	0.04306	0.09148
		Spring 2018	0.3731	**<0.001**
	Planktonic Chlorophyll *a*	Summer 2016	0.0854	**0.04181**
		Spring 2017	0.2501	**<0.001**
		Summer 2017	0.04247	**0.093**
		Spring 2018	0.1095	**0.02753**
	Benthic Chlorophyll *a*	Spring 2017	-0.02468	0.6946
		Summer 2017	-0.01195	0.4768
		Spring 2018	0.03358	0.1458
	Shannon-Weiner Index	Summer 2016	-0.01606	0.5235
		Spring 2017	-0.008506	0.4097
		Summer 2017	0.01069	0.2311
		Spring 2018	0.02154	0.1922
	Taxon Richness	Summer 2016	-0.02759	0.9354
		Spring 2017	-0.02046	0.6011
		Summer 2017	0.06143	0.05534
		Spring 2018	-0.01614	0.5097

Table A2.4. RDA significance reporting. RDA1 and RDA2 represent the significance for each constrained axis. Significance of variables selected through forward selection was sequentially assessed for each term.

Enumeration method	Sampling period	Assessing	p-value
Count data	Summer 2016	Model	0.005
		RDA1 RDA2	0.079 0.082
		Velocity DIN Residential	0.021 0.028 0.168
	Spring 2017	Model	0.001
		RDA1 RDA2	0.001 0.022
		Temperature TN TSS Ecoregion Chl	0.001 0.034 0.005 0.028 0.063
	Summer 2017	Model	0.001
		RDA1 RDA2	0.002 0.072
		Velocity Grassland	0.001 0.067
	Spring 2018	Model	0.003
		RDA1 RDA2	0.030 0.135
		Velocity Ecoregion	0.061 0.007
Pigment data	Spring 2017	Model	0.02
		RDA1 RDA2	0.025 0.696
		Temperature DIN	0.025 0.068
	Summer 2017	Model	0.002
		RDA1 RDA2	0.004 0.342
		Width DIN	0.077 0.002
	Spring 2018	Model	0.001
		RDA1 RDA2	0.002 0.616
		Temperature DIN Wetland SRP	0.051 0.002 0.035 0.035

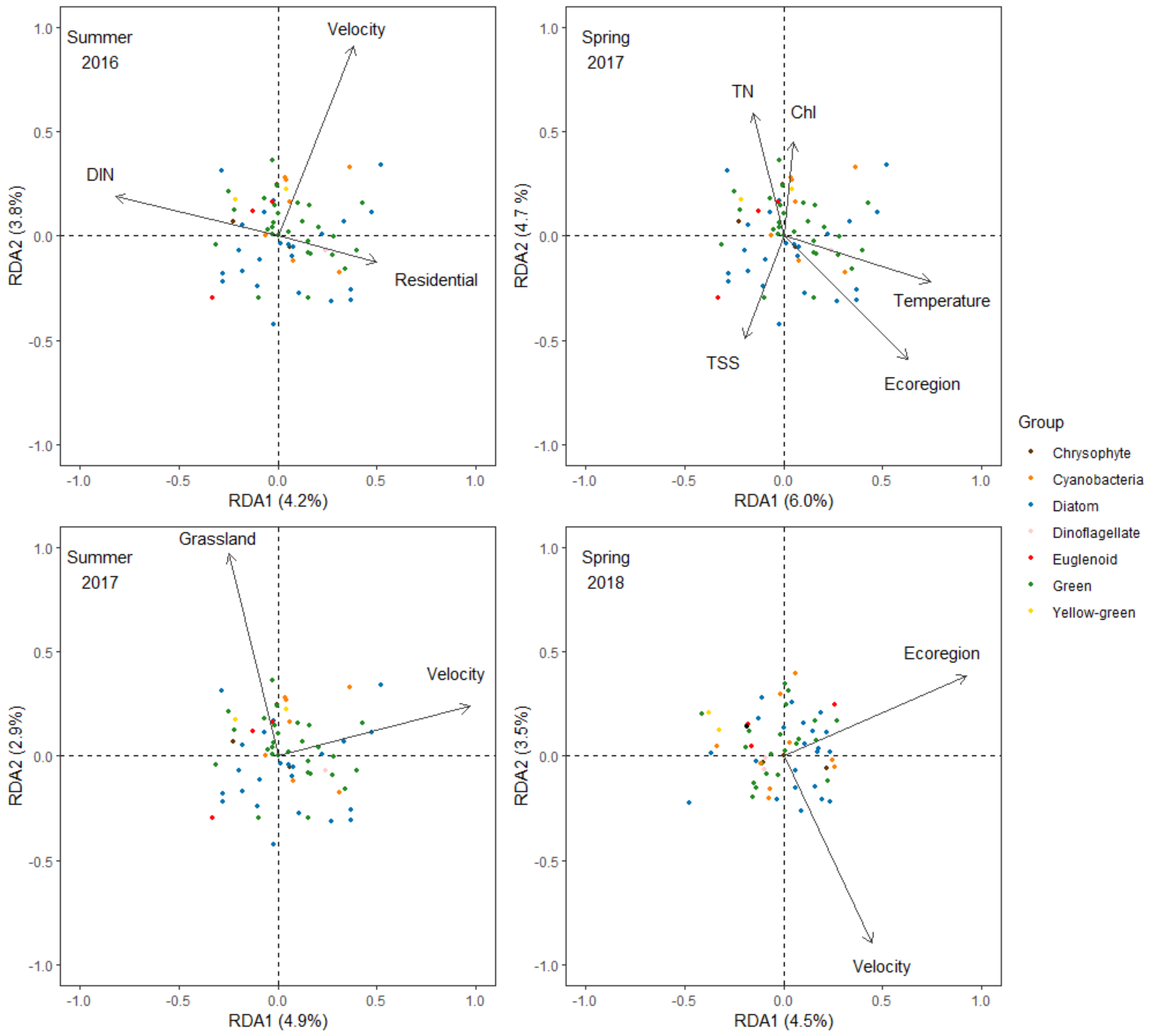

Figure A2.1. Redundancy analysis (RDA) of genus-level count data showing taxa, by the first two RDA axes and the percentage of the dataset variance explained by RDA1 and RDA2. Vectors represent significant physicochemical variables driving community data variance.

Appendix 2. Supporting Information for Chapter 3

Table A3.1. Aquatic parameters, with abbreviations, measured during this study. Analyses followed methods adapted from the APHA Standard Methods and the US EPA Test Methods (APHA 2017; US EPA), as described below.

Parameter	**Abbreviation**	**Method Reference**
Total Suspended Solids	TSS	APHA 2540 D-Gravimetric
Total Nitrogen	TN	APHA 4500 N-Calculated
Total Kjeldahl Nitrogen	TKN	APHA 4500 NorgD (modified)
Ammonia in Water by Colour	NH3N	APHA 4500 NH3-NITROGEN (AMMONIA)
Nitrite in Water by IC	NO_2-N	EPA 300.1 (modified)
Nitrate in Water by IC	NO_3-N	EPA 300.1 (modified)
Total P in Water by Colour	TP	APHA 4500-P PHOSPHORUS
Total Dissolved P in Water by Colour	TDP	APHA 4500-P PHOSPHORUS
Diss. Orthophosphate in Water by Colour (Soluble Reactive Phosphorus)	SRP	APHA 4500-P PHOSPHORUS
Dissolved Organic Carbon by Combustion	DOC	APHA 5310 B-WP
Chlorophyll *a*	Chla	EPA 445.0 ACET

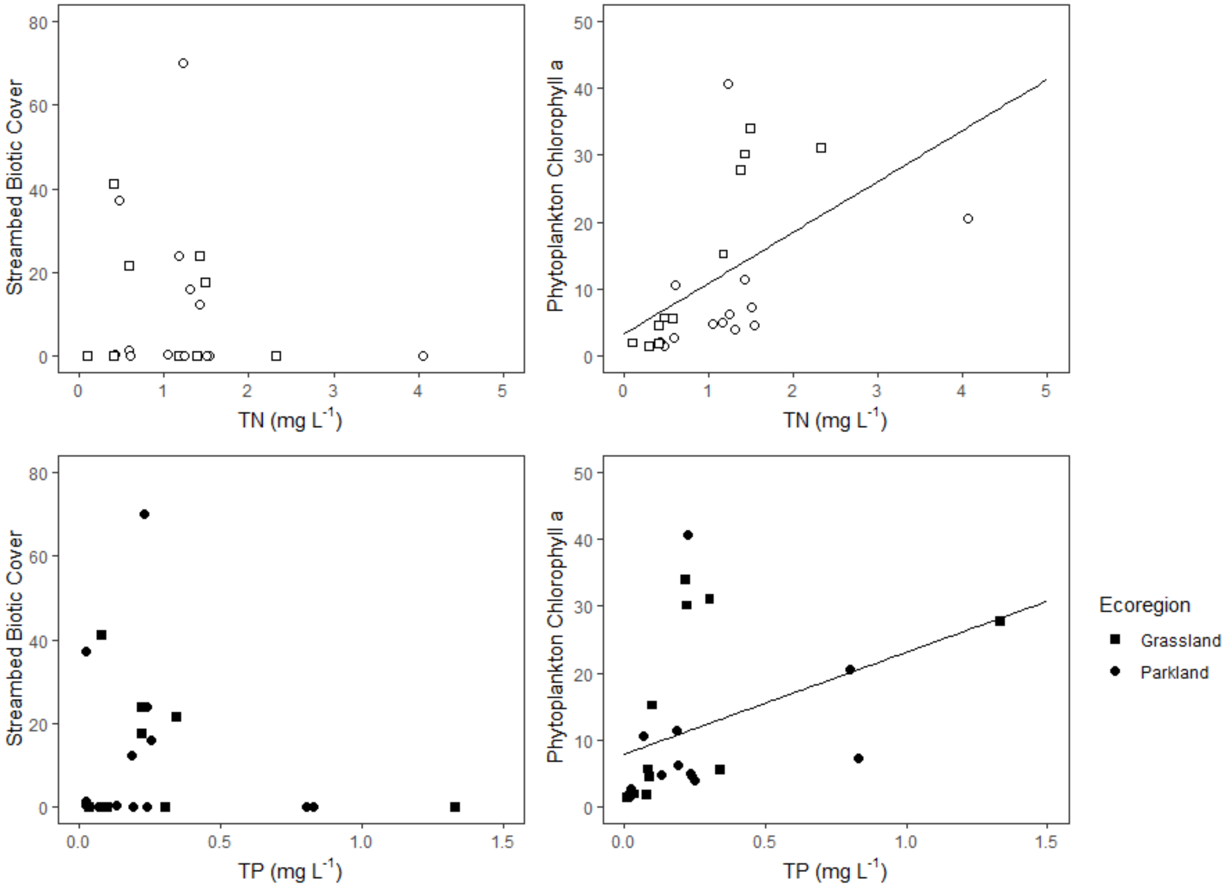

Figure A3.1. Streambed biotic cover and phytoplankton chlorophyll *a* along total nitrogen (TN) and total phosphorus (TP) gradients.

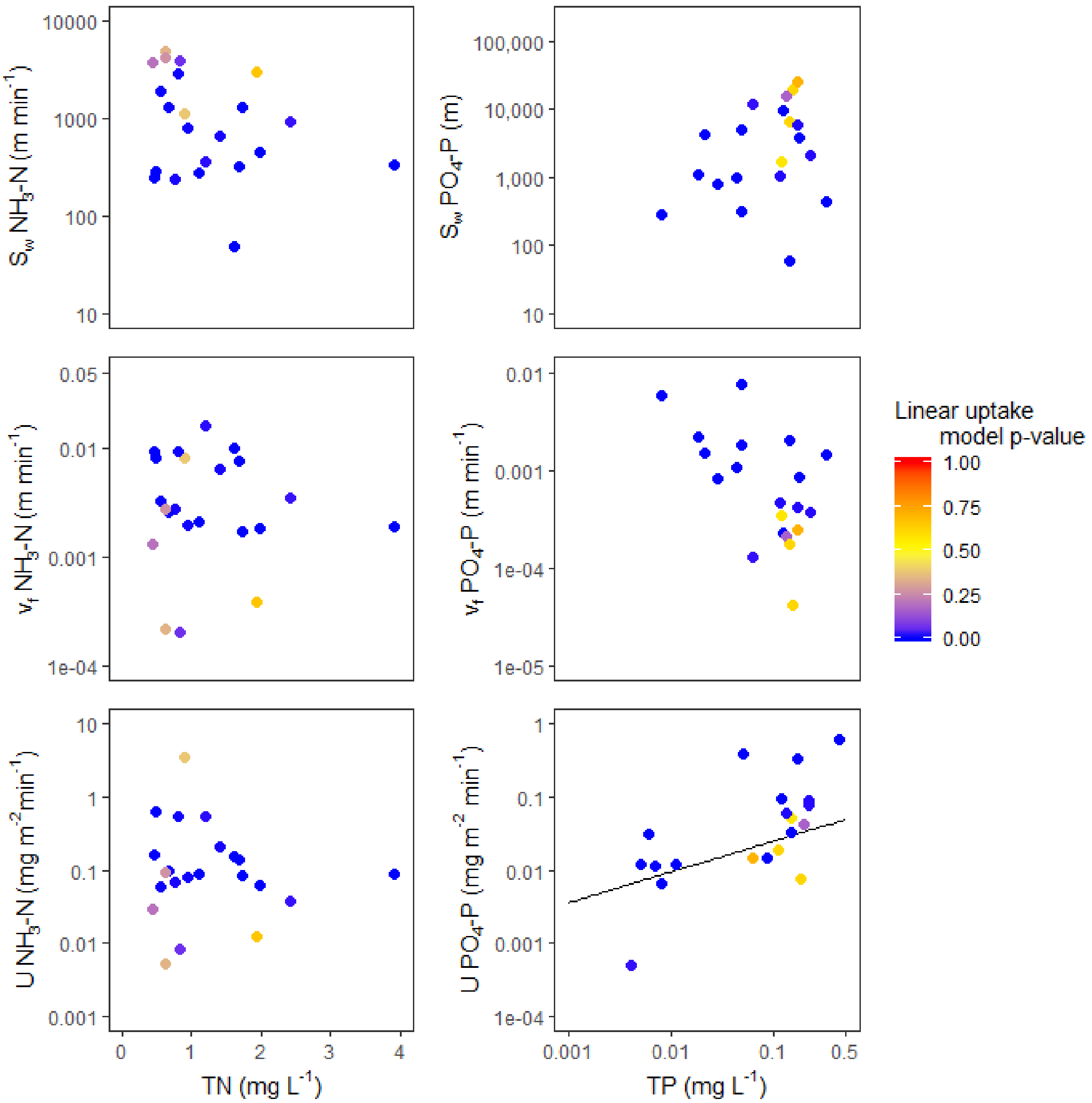

Figure A3.2. First column displays uptake length (Sw NH3-N), uptake velocity (vf NH3-N), and areal uptake rate (U NH3-N) of NH3-N along concentrations of TN (n=22). Second column displays uptake length (Sw SRP), uptake velocity (vf SRP), and areal uptake rate (U SRP) along SRP along concentrations of TP (n=20). Y-axes are log-transformed. Shading is based on numeric p-value of uptake slope (kw) on which uptake lengths, and associated velocity and rates, are based.