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Spatial and temporal variation of nutrients in the Great Lakes-St. Lawrence basin: Implications for primary production in stream ecosystems

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Abstract

Growing human populations and associated land use activities has increased the amount of nutrients delivered to surface waters. Eutrophication from the over-enrichment of nitrogen and phosphorus has degraded ecosystem conditions in streams, lakes, and coastal areas worldwide. Thus, the management of anthropogenic nutrient loading is a global concern. This thesis employed a combination of field and experimental research to provide watershed managers with information on the spatial and temporal patterns in stream nutrient enrichment, and the associated ecological effects of anthropogenic nutrient loading in the Great Lakes-St. Lawrence basin. Four studies were completed to address this research goal. First, I evaluated spatial and temporal patterns in stream nutrient enrichment and the potential for stream nutrient limitation in the lower Great Lakes-St. Lawrence basin. Second, I assessed the nutrient mitigation benefits of agricultural best management practices promoted throughout the Great Lakes basin to reduce stream nutrient concentrations. Third, I examined patterns in ecosystem metabolism among three streams exposed to human activities that differed in nutrient exposure. Lastly, I conducted a stream mesocosm experiment to compare the response of benthic algae communities to temporal patterns of phosphorus loading associated with point and nonpoint source phosphorus enrichment. Findings from this thesis indicate that nutrient enrichment is widespread in streams of the lower Great Lakes-St. Lawrence basin and that the management of both nitrogen and phosphorus is needed to minimize the risk of stream degradation by eutrophication. Converting agriculture to natural land covers and some agricultural best management practices appear to be effective strategies to reduce stream nutrient concentrations. However, agricultural practices designed to improve farm drainage may counter the nutrient mitigation benefits of these best management strategies. Moreover, results from this thesis indicate that episodic and continuous nutrient enrichment can support stream primary production to a similar extent. Therefore, management strategies should consider remedial actions that provide the greatest return on investment in nutrient load reduction. However, variation in extraneous physical-chemical conditions of streams can influence the response of primary producer communities to nutrient enrichment, thus future research is needed to better understand the ecological implications of episodic nutrient loading in streams.

Keywords

Streams, nutrients, nitrogen, phosphorus, eutrophication, land cover, management, stream metabolism, algae

Summary for Lay Audience

Nutrients (i.e., nitrogen and phosphorus) are resources that are essential for the growth of plants (i.e., primary producers) in streams, lakes, and coastal areas. But too much nutrients can cause the excessive growth of primary producers and harm aquatic ecosystems. Nutrient pollution is common in streams, lakes, and coastal areas that are nearby agricultural and urban landscapes. Because agricultural and urban landscapes have increased with growing human populations, we need to better understand the effects of nutrient pollution to help protect aquatic ecosystems. The purpose of this thesis was to examine the extent of nutrient pollution and the effects of human activities on stream ecosystems in Great Lakes-St. Lawrence basin. Findings from this thesis were used to provide recommendations on how to better protect stream ecosystems from the effects of nutrient pollution. Four studies were conducted to address this research purpose. First, nutrients were measured in streams throughout southern Ontario to provide information on the extent of nutrient pollution. Results showed that both nitrogen and phosphorus pollution were widespread throughout the region. Second, common actions used to prevent nutrient pollution from agricultural landscapes were evaluated to determine their success. Findings suggested that natural vegetation nearby streams could reduce nutrient pollution, but actions to remove water from farm fields increased nutrient pollution. Next, this thesis assessed the effects of nutrient pollution from agricultural and urban activities on stream ecosystems with two studies. In real streams, nutrient pollution from urban sewage treatment facilities had a greater effect on stream primary producers than nutrient pollution from agricultural activities. But in a controlled environment, continuous nutrient pollution from urban sewage treatment facilities had an equal effect on stream primary producers as short-term nutrient pollution from agricultural activities. Actions to reduce nitrogen and phosphorus pollution from human landscapes are needed to help protect stream ecosystems, but future research is required to better understand the effects of short-term nutrient pollution to help target management actions. Re-establishing natural vegetation in agricultural and urban landscapes was identified in this thesis as one potential strategy to help protect stream ecosystems from nutrient pollution.

Co-Authorship Statement

Chapter 2 was published in *Journal of Great Lakes Research* (reprint permission in Appendix C). Nolan J. T. Pearce was the first author and Adam G. Yates was the only co-author. N. Pearce conceived the research objectives and designed the study, processed the geospatial and water chemistry data, analyzed the processed data, interpreted the statistical results, and drafted the manuscript for publication. A. Yates contributed to refining the manuscript for publication.

> Pearce, N. J. T., & Yates, A. G. (2020). Spatial and temporal patterns in macronutrient concentrations and stoichiometry of tributaries draining the lower Great Lakes-St. Lawrence basin. *Journal of Great Lakes Research, 46*(4). doi: 10.1016/j.jglr.2020.05.002

Chapter 3 was published in *Science of the Total Environment* (reprint permission in Appendix C). Nolan J. T. Pearce was the first author and Adam G. Yates was the only co-author. Chapter 3 expands on previous work by N. Pearce and A. Yates (Pearce and Yates, 2015; doi: 10.3390/w7126661). N. Pearce refined and conceived the research objectives, processed the geospatial and water chemistry data, analyzed the processed data, interpreted the statistical results, and drafted the manuscript for publication. A. Yates contributed to the study design, field data collection, and refining the manuscript for publication.

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Chapter 4 was published in *Hydrobiologia* (reprint permission in Appendix D). Nolan J. T. Pearce was the first author and the co-authors were Kathryn E. Thomas (Environment and Climate Change Canada), Patricia A. Chambers (Environment and Climate Change Canada), Jason J. Venkiteswaran (Wilfrid Laurier University), and Adam G. Yates. N. Pearce conceived the research objectives and designed the study, modelled stream ecosystem metabolism from continuous muti-parameter data, analyzed the processed data, interpreted the statistical results, and drafted the manuscript for publication. K. Thomas and P. Chambers contributed to field data collection. J. Venkiteswaran provided consultation for data analysis and interpretation. A. Yates contributed to field data collection and refining the manuscript for publication.

> Pearce, N. J. T., Thomas, K. E., Chambers, P. A., Venkiteswaran, J. J., & Yates, A. G. (2020). Metabolic regimes of three mid-order streams in southern Ontario, Canada exposed to contrasting sources of nutrients. *Hydrobiologia, 847*, 1925-1942. doi: 10.1007/s10750-020-04222-0

Chapter 5 has been accepted for publication in *Water Research*. Nolan J. T. Pearce was the first author and the co-authors were Kathryn E. Thomas, Isabelle Lavoie (Institut national de la recherche scientifique), Patricia A. Chambers, and Adam G. Yates. N. Pearce conceived the research objectives and designed the study, carried out the artificial stream experiment, collected and processed the ecological samples, analyzed the processed data, interpreted the statistical results, and drafted the manuscript for publication. K. Thomas and P. Chambers provided consultation on the conception and design of the study. I. Lavoie assisted in the taxonomic identification of diatom samples. A. Yates contributed to the conception and design of the study and refining the manuscript for publication.

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Chapter 1

1 Introduction

1.1 Nutrient enrichment of freshwater ecosystems

Nutrient enrichment of freshwater ecosystems is a global concern (Bennett et al., 2001; Smith et al., 2003; Elser et at., 2007). Growing human populations and associated land use activities (e.g., crop production, livestock husbandry, and sewage discharge) has increased the amount of nutrients delivered to surface waters (Carpenter et al., 1998). For example, about one third of lakes and streams in Europe are affected by nutrient enrichment (European Environment Agency, 2018). Similarly, greater than 50% of lakes and streams in the continental United States were reported to have enriched nutrient concentrations linked to the intensity of human activities occurring in their watersheds (Alexander & Smith, 2006; Dodds et al., 2009; Paulsen et al., 2008; Stoddard et al., 2016). Moreover, climate change is projected to increase the transport of nutrients from landscape sources to surface waters through more frequent and/or intense precipitation events in many regions worldwide (Kaushal et al., 2014; 2018; Sinha et al., 2017).

In North America, freshwater ecosystems in the Great Lakes-St. Lawrence basin experience nutrient pollution from land use activities (Robertson & Saad, 2011; Cornwell et al., 2015) and are particularly susceptible to future climate changes (d'Orgeville et al., 2014; Sinha et al., 2017). Furthermore, nutrient inputs from tributaries draining urban and agricultural watersheds has resulted in the degradation of water quality and ecological conditions in many nearshore areas of the lakes, notably Lake Erie (Stumpf et al., 2012; Kane et al., 2014; Ho & Michalak, 2017). Management of nutrient enrichment has therefore received increasing attention in the agricultural and built-up regions of the Great Lakes-St. Lawrence basin.

Carbon, nitrogen, and phosphorus are essential macronutrients required to support aquatic food webs. However, the overabundance of nutrients in surface waters can increase autotrophic (i.e., phytoplankton, cyanobacteria, benthic algae, and macrophytes) production and alter the balance of aquatic ecosystems. The process by which streams, lakes, and other waterbodies become over-enriched with nutrients (i.e., nitrogen and phosphorus) is defined as eutrophication. Increased autotrophic production from eutrophication can have ecological consequences including the degradation of water quality, dissolved oxygen depletion, and biodiversity loss (Chislock et al., 2013). Furthermore, eutrophication can result in socio-economic impacts (e.g., recreation, tourism, and human health risk) and alter the capacity of aquatic environments to provide ecosystem services (Dodds et al., 2009; Wurtsbaugh et al., 2019). Consequently, understanding the extent of nutrient enrichment in developed watersheds and the associated influence of nutrients on autotrophic production is crucial for managing the undesirable effects of eutrophication in surface waters.

To date, considerable attention has been placed on the management of eutrophication in waterbodies of societal importance (e.g., Lake Erie, Lake Winnipeg, Lake Okeechobee, Lake Champlain, and Lake Victoria); as a result, eutrophication science in lakes has largely informed nutrient management. Eutrophication research in experimental lakes was instrumental in concluding that of the primary macronutrients required for algal production (i.e., carbon, nitrogen, and phosphorus), phosphorus alone controls eutrophication in freshwater ecosystems (Schindler, 1974; 1977). Low natural phosphorus supplies and the ability of prokaryotic algae to fix atmospheric nitrogen has led to the general acceptance of the phosphorus limitation paradigm for eutrophication management (Schindler et al., 2008; 2016).

In the Great Lakes-St. Lawrence basin, phosphorus has been associated with eutrophication in many nearshore and open water areas of the lower Great Lakes (Munawar & Fitzpatrick, 2018; Mohamed et al., 2019). Regional nutrient management programs have therefore targeted the reduction of phosphorus to combat the effects of lake eutrophication (Mohamed et al., 2019). However, eutrophication is also a widespread problem in streams and rivers that drain developed watersheds (Dodds, 2006; Dodds & Smith, 2016). Indeed, past studies have reported that many routinely monitored streams in the Great Lakes-St. Lawrence basin have enriched concentrations of nitrogen and phosphorus (Dodds et al., 2009; Thomas et al., 2018). Yet, despite the occurrence of widespread nutrient enrichment, stream eutrophication science has lagged behind that for lakes and strategies proposed to mitigate lake eutrophication may not be fully transferable to stream ecosystems (Biggs, 2000; Hilton et al., 2006; Dodds & Smith, 2016).

To effectively manage eutrophication and guide nutrient reduction efforts, researchers must identify the specific macronutrients whose control will most effectively mitigate risk of ecosystem degradation by increased autotrophic production (Jarvie et al., 2018). In contrast to lakes, where there is considerable support for phosphorus limitation of autotrophic communities (Schindler et al., 2008; 2016), *in situ* nutrient limitation experiments in streams have shown more variability in the primary macronutrient limiting autotrophic production (Francoeur, 2001; Elser et al., 2007; Beck et al., 2017). Indeed, meta-analyses have reported approximately equal numbers of streams limited by either nitrogen or phosphorus alone, or co-limited by nitrogen and phosphorus together. However, the addition of both nutrients to streams has commonly facilitated the largest increase in autotrophic production (Francoeur, 2001; Elser et al., 2007; Beck et al., 2017).

Differences in the effects of increased nutrients between streams and lakes may be explained by the rapid water renewal rates and a prominent autotrophic community of attached benthic algae in addition to factors such as flow disturbance, herbivory, and light attenuation that characterize stream ecosystems. Moreover, spatial patterns and proximal differences in the dynamic physical environment have been shown to explain some differences observed in nutrient limitation among streams (Beck et al., 2017). Thus, while nitrogen and phosphorus are important drivers of autotrophic production, there may be some regional specificity in the nutrient limitation of stream ecosystems. In the Great Lakes-St. Lawrence basin, phosphorus remains the focus of regional nutrient management programs (Mohamed et al., 2019), however, additional research on stream nutrient enrichment is needed to provide managers with information necessary to guide basin-wide eutrophication management.

The risk of nutrient losses from agricultural lands in the Great Lakes-St. Lawrence basin are increasingly being addressed through the implementation of agricultural best management practices (BMPs) (Kerr et al., 2016). BMPs (e.g., riparian buffer areas, manure storage structures, and fertilizer management) are designed to lessen the application of nutrients to agricultural landscapes and mitigate the transport of nutrients to surface waters. However, agricultural BMPs are often promoted as lake-centric strategies whereby streams are perceived as nutrient conduits to downstream waterbodies (Kreiling et al., 2017). Many past studies have used watershed modeling approaches to investigate the adoption of agricultural BMPs and determine the level of implementation needed to achieve downstream water quality objectives (Bosch et al., 2013; Wilson et al., 2019). Moreover, most field studies on BMPs employ an edge-of-field design to measure the amount of nutrients transported to surface waters in association with different agricultural practices (Lam et al., 2016; King et al., 2018). Fewer studies, however, have evaluated the influence of agricultural BMPs on the concentration, composition, and form of nutrients in streams at the watershed scale (but see Melland et al., 2018; Kroll et al., 2019).

1.2 Nutrients in stream ecosystems

1.2.1 Nutrient fractionation

Nitrogen and phosphorus in stream water can vary in composition (i.e., inorganic and organic) and form (i.e., dissolved and particulate) (Table 1-1). Organic nutrients consist mainly of detrital particles and dissolved organic molecules, whereas inorganic nutrients can be attached to sediments or dissolved in water. Particulate and organic nutrients are largely inaccessible and require physicochemical (i.e., sorption/desorption and metal-phosphorus bonding) or biological (i.e., enzymatic) transformation prior to autotrophic uptake. In contrast, dissolved inorganic nutrients are directly bioavailable and often used to measure the immediate supply of nutrients to autotrophic communities (Dodds, 2003). However, nutrients in stream ecosystems are highly reactive and can readily cycle among dissolved, particulate, organic, and inorganic forms. Internal recycling of nutrients through decomposition and mineralization cycles can support autotrophic production in the absence of external inputs to streams (Paul et al., 1991; Mulholland et al., 1991; 1995). In addition, nitrogen can interact with atmospheric pools though biologically mediated transformations (i.e., nitrogen fixation and denitrification) and can become freely available to, or removed from, internal nutrient cycles. The composition and form of nutrients in streams can have direct implications for autotrophic production. Thus, consideration of which nutrients are measured to evaluate nutrient enrichment is required. For example, measurements of total nutrient concentrations are often more appropriate to evaluate the overall supply of nutrients and have been widely recommended to estimate nutrient limitation and stream nutrient status (Dodds, 2003). In contrast, concentrations of dissolved forms may prove useful in experimental settings and more detailed investigations of nutrient availability.

Table 1-1. Common parameters used to measure the composition and form nitrogen and phosphorus in stream water. Vertical nutrient measures encompass multiple fractionations as shown bound by the corresponding lines. Adapted from Allan & Castillo (2007).

			Nitrogen
Total Nitrogen	Total Dissolved Nitrogen		Dissolved inorganic nitrogen
			- *Nitrate (NO_3^-)*
			- *Nitrite (NO_2^-)*
			- *Ammonium (NH_4^+)*
			Dissolved organic nitrogen
			- *Amino compounds*
			- *Proteins*
			- *Nucleic acids*
			Particulate organic nitrogen
			- *Detritus*
			Phosphorus
Total Phosphorus	Total Dissolved Phosphorus		Dissolved inorganic phosphorus
			- *i.e., soluble reactive phosphorus*
			- *Orthophosphate (PO_4^{-3})*
		Total Organic Phosphorus	Dissolved organic phosphorus
			- *Nucleic acids*
			- *Lipids*
			Particulate organic phosphorus
			- *Detritus*
			Particulate inorganic phosphorus
			- *Sediment bound*

Total nutrient concentrations can be used to assess the degree of nitrogen and phosphorus enrichment in stream ecosystems. However, autotrophs require different quantities of macronutrients for optimal growth. Thus, in addition to total concentrations, the relative availability of nitrogen and phosphorus, as well as carbon supplies, can influence autotrophic production in streams. Past research on marine phytoplankton demonstrated that the molar ratio of 106 carbon : 16 nitrogen : 1 phosphorus corresponded to the macronutrient requirements necessary for optimal autotrophic production and was thereafter known as the Redfield ratio (Redfield, 1958). Although there are species specific differences in macronutrient requirements (Klasumeier et al., 2004), the Redfield ratio is often regarded as near optimal for autotrophic production in aquatic ecosystems, including in streams and rivers (Hillebrand & Sommer, 1999). Macronutrient stoichiometry or the molar ratio of carbon, nitrogen, and phosphorus in streams can be compared to the Redfield ratio to indicate if nutrient supplies are depleted relative to the autotrophic requirements for optimal production. Moreover, macronutrient stoichiometry can be combined with total nutrient concentrations to determine the potential for individual nutrients to limit autotrophic production (Dodds, 2003; Tank & Dodds, 2003; Keck & Lepori, 2012). Macronutrient stoichiometry can therefore be used to derive nuanced information from routine water quality data and inform managers on the specific nutrients that need to be controlled to minimize the risk of stream degradation by eutrophication (Jarvie et al., 2018).

1.2.2 Sources of nutrients

Nitrogen is abundant in the atmosphere (N_2), but gaseous nitrogen is mostly unavailable to primary producers. However, atmospheric nitrogen can be converted into reactive or bioavailable nitrogen through an energetically demanding process called nitrogen fixation. Nitrogen can be fixed abiotically through lightning strikes and industrial processes. In addition, specialized prokaryotic organisms found in terrestrial and aquatic environments can biotically transform atmospheric nitrogen into bioavailable forms. In stream ecosystems, cyanobacteria can fix atmospheric nitrogen to support their biotic nutrient requirements. Through cellular excretion and the decomposition and mineralization of organic matter, cyanobacteria can also increase the amount of reactive nitrogen available in streams. However, nitrogen fixation rates can differ substantially among streams and may therefore only be a substantial source of reactive nitrogen in streams with abundant cyanobacteria (Grimm & Petrone, 1997). In addition to nitrogen fixation, other natural sources of nitrogen can include atmospheric deposition and terrestrial organic matter inputs transported to streams through groundwater and overland runoff. Nitrogen inputs to streams that drain undisturbed watersheds are often low and in organic forms (Clark et al., 2000; Binkley et al., 2004). Moreover, instream nitrogen availability from natural sources can vary spatially with differences in the landscape and physiographic properties of stream catchments (e.g., soil type, hydrologic characteristics, and terrestrial vegetation) (Bernhardt et al., 2005; Creed & Beall, 2009).

Natural or background sources of phosphorus to streams are also often low and mostly in particulate and organic forms (Clark et al., 2000; Binkley et al., 2004). Phosphorus is largely confined in rocks and sediment, and natural sources of phosphorus include physical and chemical weathering of rocks, atmospheric deposition of soil and dust particles, and inputs of organic matter (Holtan et al., 1988). Instream phosphorus concentrations can vary among streams with natural variation in geology, soil, and vegetation in stream catchments (Dillon & Kirchner, 1975; Dillon & Molot, 1997). Moreover, phosphorus is often in high demand in undisturbed streams, thus in the absence of external inputs the decomposition and mineralization of organic matter can provide an important source of phosphorus to stream ecosystems (Mulholland et al., 1991; 1995).

Anthropogenic activities have greatly altered the global cycles of nitrogen and phosphorus (Vitousek et al., 1997; Mainstone & Parr, 2002; Withers & Jarvie, 2008) and as a result many streams have nutrient concentrations orders of magnitude above background levels (Dodds et al., 2009). Streams draining human-altered watersheds can receive nutrients from a range of sources that vary in amount (i.e., nutrient load), chemical composition, and pattern of delivery (Edwards & Withers, 2007; 2008; Withers & Jarvie, 2008). Nutrients from human activities can additively increase the total nutrient load delivered to streams (Seitz et al., 2011) and instream nutrient concentrations have been observed to vary spatially with the intensity of human activities in stream catchments (Allan, 2004). For example, Thomas et al., (2018) and others (e.g., Allan et al., 1997; Johnson et al., 1997; Chen & Driscoll, 2009) demonstrated that greater proportions of urban and agricultural land cover in stream catchments were positively associated with water nitrogen and phosphorus concentrations. However, it becomes increasingly difficult to identify where to target nutrient control practices in streams draining catchments that receive nutrients from many different human activities (Downes, 2010; Ormerod et al., 2010). Thus, in regions with mixed anthropogenic nutrient inputs, it is increasingly important to understand the effects that different nutrient sources have on stream ecosystem conditions.

Anthropogenic nutrient sources are broadly classified as either point (e.g., sewage effluent) or nonpoint (e.g., agricultural and urban runoff) based on their hydrologic and compositional attributes (Table 1-2). Point sources, or “end of pipe” inputs, directly release contaminants including nutrients into stream ecosystems. Growing human populations have created a greater demand on activities that contribute to point source nutrient loading, namely sewage treatment plants. Rivers receiving effluent from sewage treatment plants often have enriched concentrations of dissolved inorganic forms of nutrients (Mainstone & Parr, 2002; Withers & Jarvie, 2008; Carey & Migliaccio, 2009) and in some streams inputs from point sources can contribute up to 50% of the total nutrient load (Carpenter et al., 1998; David & Gentry, 2000). However, differences in sewage treatment plant operations (e.g., level of treatment, discharge volume, and population served) can strongly influence the nutrient mediated impacts of point sources (Carey & Migliaccio, 2009). For example, passive sewage treatment (e.g., wastewater stabilization ponds, sewage lagoons, and septic tanks) in rural municipalities can result in poorer effluent quality but of a lower volume compared to near continuous inputs of actively treated sewage from mechanical treatment plants in urban centers (e.g., tertiary sewage treatment plants). Past studies have shown increased algal biomass and autotrophic production downstream of sewage treatment plants (Masseret et al., 2000; Gücker et al., 2006), but the effects of effluent on autotrophic communities may differ with treatment level and physical controls on algal accrual (Welch et al., 1992). However, because of their distinguishable footprints, point sources are easily identified and have been an obvious target for nutrient management in regions with financial capital to support advanced sewage treatment.

Table 1-2. Hydrologic and compositional attributes of point and nonpoint sources of nutrients. Adapted from Edwards & Withers (2008).

Source	Delivery Pattern	Runoff Dependency	Amount	Composition
Point Source - *Sewage treatment facilities* - *Industrial effluent* - *Septic tanks*	Semi-continuous to continuous	Low	High load and concentration	Mostly dissolved inorganic
Nonpoint Source - *Agricultural runoff* - *Impervious surface runoff*	Episodic	High	Variable load and concentration	Mixed composition

Nutrients from nonpoint sources are derived from activities occurring on the landscape and compared to point sources are more difficult to monitor and manage. Surface and subsurface water runoff governed by precipitation events can intercept and transport land-based nutrients into waterways. Agricultural activities (i.e., fertilizer application and livestock husbandry) are a substantial nonpoint source of both dissolved and particulate forms of nutrients to streams (Allan, 2004). Moreover, agricultural activities that occur in hydrologically connected areas of stream catchments (e.g., proximal to streams and areas that generate water runoff) have been found to contribute disproportionally to stream nutrient loading (Pinoke et al., 2000; Walter et al., 2000; Kleinman et al., 2006). Catchments dominated by agricultural land cover often have streams with increased autotrophic production associated with enriched concentrations of nitrogen and phosphorus (Niyogi et al., 2007; Frankforter et al., 2010; Griffiths et al., 2013). However, as nutrient inputs from nonpoint sources are dependent on water runoff, streams may show substantial temporal variation in nutrient concentrations (Petry et al., 2002; Stutter et al., 2008; Bieroza et al., 2014). Past studies have shown that nutrient concentrations in agricultural streams can become temporarily elevated during discharge events and that the concentration and duration of nutrient inputs are associated with the amount of precipitation and antecedent conditions in stream catchments (Sharpley et al., 2008; Bowes et al., 2015; Lloyd et al., 2016; Blaen et al., 2017). Thus, although agricultural nonpoint sources have been identified as the leading source of nutrients in many regions throughout North America (Carpenter et al., 1998) including in the lower Great Lakes-St.

Lawrence basin (Maccoux et al., 2016), few studies have examined how short-duration high-concentration nutrient inputs influence autotrophic communities due to the complexity of loading events.

1.3 Temporal patterns of nutrient loading

1.3.1 Ecological relevance of point and nonpoint sources

Differences in the temporal pattern of nutrient delivery from point and nonpoint sources may result in different ecological relevancies of increased nutrient loadings to streams. For example, point sources often continuously input nutrients to streams and while nutrient concentrations may be diluted from increased discharge, nutrient loads are largely independent of hydrologic variation. In contrast, nonpoint sources require runoff events for nutrient transport and episodically increase the nutrient load delivered to streams whereby stream nutrient concentrations are temporarily enriched with increasing discharge (Figure 1-1). Nutrient loading patterns from point and nonpoint sources can therefore differ in continuity from near-continuous to highly episodic. However, because of the unidirectional flow of water, instream residence times of near-continuous and episodic nutrient loads differ substantially and can result in different exposures to enriched nutrient concentrations. For example, enriched nutrient concentrations from episodic loading can persist from several hours to multiple days, whereas streams that receive continuous nutrient loads can have chronically enriched concentrations. The potential for nutrient enrichment to augment autotrophic production may differ with the exposure time of nutrient enrichment as nutrients delivered episodically may be exported faster than can be utilized by autotrophic communities (Hilton et al., 2006; Stamm et al., 2014; Withers et al., 2014). Thus, to understand the implications of anthropogenic nutrient enrichment on stream eutrophication it is critical to consider the temporal attributes of nutrient loading (Edwards & Withers, 2007; 2008; Withers & Jarvie, 2008).

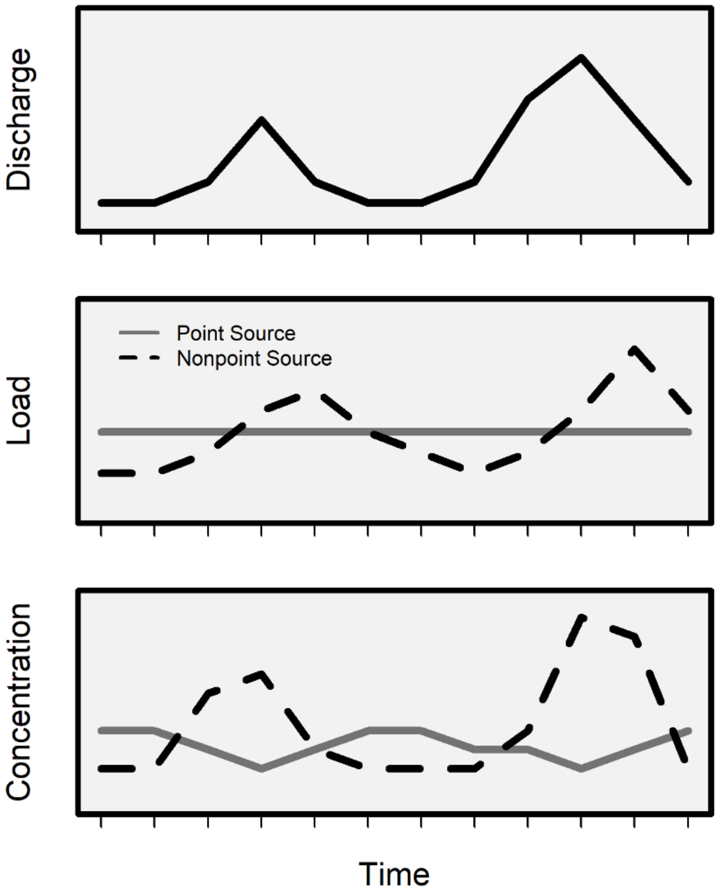

Figure 1-1. Contribution of generalized point and nonpoint sources to the load and concentration of nutrients in streams in relation to hydrologic variation in discharge (arbitrary scales). Adapted from Mainstone & Parr (2002).

Episodic nutrient loadings have not been considered ecologically relevant to eutrophication in stream ecosystems (Stamm et al., 2014; Withers et al., 2014). Low residence time of episodic nutrient loads and the potential for hydrologic events to scour benthic environment has led to the consensus, that in streams, the most relevant metric for eutrophication is baseflow nutrient concentrations. However, disturbance to stream autotrophic communities can vary with the hydrologic characteristics (e.g., flow magnitude, sediment size and stability, and stress) of flow events and differ spatially within a stream reach (Biggs & Close, 1989; Peterson & Stevenson, 1992; Biggs et al., 1998; Katz et al., 2018). For example, Katz et al., (2018) reported that the extent of disturbance to benthic algae biomass ranged from 8 to 70% across a gradient of stream discharge from elevated baseflow to bankfull. Many past studies have documented that benthic algae communities are resistant to disturbance from small to moderate increases in stream discharge (Biggs & Close, 1989; Biggs et al., 1998) and that sub-scouring flow events can stimulate algal production (Horner & Welch, 1981; Stevenson, 1990; Townsend et al., 2012). Moreover, small to moderate runoff events can contribute a substantial proportion of the total nutrient load delivered to streams draining agricultural catchments (Macrae et al., 2007). Thus, episodic nutrient loading has the potential to have largely unanticipated influence on benthic algae communities in streams.

Recognition of widespread nutrient enrichment in streams has prompted research on the influence of nutrients on benthic algal communities. However, most studies have evaluated the response of benthic algae to spatial differences in nutrient concentrations or under experimental conditions where communities are exposed to constant nutrient concentrations. Past field studies have commonly reported nonlinear associations between benthic algae structure (e.g., biomass and taxonomic composition) and function (e.g., gross primary production and extracellular enzyme activity), and nutrient concentrations among streams (Stevenson et al., 2008; Clapcott et al., 2010; Black et al., 2011; Rier et al., 2011; Taylor et al., 2014). Moreover, experimental studies in artificial streams have been used to help identify nutrient concentration thresholds associated with the undesirable growth of algal communities (Rier & Stevenson, 2006; Wagenhoff et al., 2013; Taylor et al., 2020). Nonlinear patterns in algae-nutrient relationships have supported the development of regional nutrient criteria to provide managers with information on stream conditions from passive water quality monitoring programs (Evans-White et al., 2013). However, the potential for algae to respond to short-duration nutrient inputs may confound existing algae-nutrient relationships and limit the ability of baseflow water quality monitoring to provide information on ecological conditions in some episodically enriched streams. Therefore, to better inform managers on where nutrient reductions can bring about stream improvement further research is needed to understand the response of algae communities to short-duration high-concentration nutrient inputs to streams.

1.3.2 Adaptations of algae to variable nutrient supplies

Many species of benthic algae have evolved strategies to survive and compete for resources in nutrient limited environments. For example, algal species may differ in nutrient uptake rates and growth kinetics (Grover, 1989), cellular nutrient requirements (Tilman, 1976; 1977), and in the secretion of extracellular enzymes to breakdown dissolved organic nutrients (Rengefors et al., 2001; 2003). Another well documented strategy employed by algal species is the storage of excess nutrients within cellular structures (Kromkamp, 1987; Grover, 1991). Specifically, algae can convert excess phosphorus into intracellular storage compounds (i.e., polyphosphate) for use as energy and structural synthesis under future conditions of low phosphorus availability (Rao et al., 2009; Solovchenko et al., 2019). The storage of excess phosphorus is defined as luxury or overplus consumption where the two are differentiated by enriched and limited background phosphorus concentrations, respectively. The overplus consumption of phosphorus may therefore support the continued growth of algae in streams that experience episodic nutrient loading (Rier et al., 2016). However, few studies have investigated the response of stream algae to temporal variation in phosphorus loading. Rier et al., (2016) observed an overplus response of benthic algae communities to episodic phosphorus loading during storm events in natural oligotrophic streams and to phosphorus pulses simulated in stream mesocosms. Moreover, two other past studies reported increased algal phosphorus content in response to phosphorus pulses in artificial stream experiments (Humphrey & Stevenson, 1992; Davies & Bothwell, 2012). However, past investigations on the effects of phosphorus pulses have occurred in conditions with low total phosphorus loads, thus the potential of episodic phosphorus enrichment to augment stream eutrophication remains understudied.

1.4 Rational and objectives

Widespread nutrient enrichment of stream ecosystems is a continuing concern among researchers and environmental authorities, including those in the Great Lakes-St. Lawrence basin. Human activities, such as agricultural land practices and sewage treatment plants, have been shown to greatly increase the amount of nutrients transported into surface waters and these activities are expected to intensify with demands of a growing population. Nutrient enrichment can cause the degradation of streams and downstream waterbodies by eutrophication; thus, it is important to understand the effects of anthropogenic nutrient loading on stream ecosystems to better manage nutrient pollution in developed regions. The goal of this thesis was to investigate how anthropogenic nutrient enrichment influences the structure (e.g., water quality and algal biomass and taxonomic composition) and function (e.g., gross primary production) of stream ecosystems and to provide recommendations for eutrophication management. Research objectives in the four chapters of this thesis were to:

1. Evaluate the potential for nutrient limitation in routinely monitored tributaries of the lower Great Lakes-St. Lawrence basin (Ontario, Canada) and determine the effects of catchment land cover on stream nutrient status.
2. Assess the mitigation effects of common agricultural best management practices and their spatial location on stream nutrient concentrations throughout a hydrologic year.
3. Describe the temporal patterns in autotrophic production and ecosystem respiration in streams exposed to anthropogenic activities with contrasting nutrient delivery attributes.
4. Compare the response of benthic algal assemblages to phosphorus enrichment associated with differing temporal patterns of phosphorus loading (i.e., continuous and episodic).

1.5 Thesis organization

In Chapter 2, I conducted a nutrient limitation assessment of streams in the Great Lakes-St. Lawrence basin of Ontario, Canada. Specifically, I summarized the prevailing stream macronutrient stoichiometry and concentrations and compared these observations to established values in the literature that correspond to abrupt changes in algal growth. Novel evaluation of stream monitoring data allowed me to provide managers with information on the extent of stream nutrient enrichment and potential for stream nutrient limitation in the Great Lakes-St. Lawrence basin. Moreover, monitoring data was used to evaluate the influence of land cover located in hydrologically connected areas on stream macronutrient concentrations and stoichiometry. Results of this study suggest that nitrogen and phosphorus need to be reduced to minimize the risk of stream degradation by eutrophication and that dual nutrient reductions may be achieved through land cover management in hydrologically connected areas of stream catchments.

In Chapter 3, I investigated the benefits of agricultural best management practices in reducing stream nutrient concentrations in Grand River watershed of southern Ontario, Canada. The abundance and spatial location of common structural best management practices were quantified in stream catchments and associated with nutrient concentrations collected over a hydrologic year. Results of this study suggest that vegetated riparian buffers and manure storage structures can reduce stream nutrient concentrations, but tile drainage systems may override potential benefits. Findings support results from Chapter 2 that the restoration and maintenance of natural vegetation in hydrologically connected areas of stream catchments can improve water quality.

In Chapter 4, I used continuous, high-resolution data to compare patterns in stream primary production among three streams exposed to anthropogenic nutrient sources with contrasting nutrient loading patterns. Results of this study show that increased nutrient availability can augment stream primary production, but temporal patterns were associated with light, temperature, and discharge regimes.

In Chapter 5, I conducted a stream mesocosm experiment to investigate the potential of episodic phosphorus loading to augment stream eutrophication. Two treatments were enriched with equal loads of phosphorus that differed in loading pattern from continuous to episodic; a third treatment had a continuous loading pattern with an unenriched phosphorus load. Results indicated that phosphorus loading pattern influenced species composition of algal assemblages, but not algal biomass. Findings from this study provide evidence on the potential of short-duration high-concentrations of phosphorus to structure stream algae communities.

In the final chapter (Chapter 6), I summarize key research findings from chapters 2-5 and provide context on how my results build on our understanding of stream eutrophication science. Moreover, I provide recommendations for the management of stream nutrient enrichment to lower the risk of stream degradation by eutrophication. Lastly, I outline potential avenues for future research that build on the novel findings from this thesis.

1.6 References

Alexander, R. B., & Smith, R. A. (2006). Trends in the nutrient enrichment of U.S. rivers during the late 20th century and their relation to changes in probable stream trophic conditions. *Limnology and Oceanography, 51*, 639–654. doi: 10.4319/lo.2006.51.1_part_2.0639

Allan, J. D. (2004). Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics, 35*(1), 257–284. doi: 10.1146/annurev.ecolsys.35.120202.110122

Allan, J. D., & Castillo, M. M. (2007). Stream Ecology: Structure and Function of Running Waters. 2nd Edition, Springer, Netherlands. doi: 10.1007/978-1-4020-5583-6

Allan, J. D., Erickson, D. L., & Fay, J. (1997). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology, 37*(1), 149–161. doi: 10.1046/j.1365-2427.1997.d01-546.x

Beck, W. S., Rugenski, A. T., & Poff, N. L. (2017). Influence of experimental, environmental, and geographic factors on nutrient-diffusing substrate experiments in running waters. *Freshwater Biology, 62*(10), 1667–1680. doi: 10.1111/fwb.12989

Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impact on erodable phosphorus and eutrophication: A global perspective. *BioScience, 51*(3), 227–234. doi: 10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Bernhardt, E. S., Likens, G. E., Hall, R. O., Buso, D. C., Fisher, S. G., Burton, T. M., … Lowe, W. H. (2005). Can’t see the forest for the stream? In-stream processing and terrestrial nitrogen exports. *BioScience, 55*(3), 219. doi: 10.1641/0006-3568(2005)055[0219:ACSTFF]2.0.CO;2

Bieroza, M. Z., Heathwaite, A. L., Mullinger, N. J., & Keenan, P. O. (2014). Understanding nutrient biogeochemistry in agricultural catchments: the challenge of appropriate monitoring frequencies. *Environmental Science: Processes and Impacts, 16*(7), 1676–1691. doi: 10.1039/c4em00100a

Biggs, B. J. F., & Close, M. E. (1989). Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology, 22*(2), 209–231. doi: 10.1111/j.1365-2427.1989.tb01096.x

Biggs, B. J. F. (2000). Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society, 19*(1), 17–31. doi: 10.2307/1468279

Biggs, B. J. F., Goring, D. G., & Nikora, V. I. (1998). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. *Journal of Phycology, 34*(4), 598–607. doi: 10.1046/j.1529-8817.1998.340598.x

Binkley, D., Ice, G. G., Kaye, J., & Williams, C. A. (2004). Nitrogen and phosphorus concentrations in forest streams of the United States. *Journal of the American Water Resources Association, 40*(5), 1277–1291. doi: 10.1111/j.1752-1688.2004.tb01586.x

Black, R. W., Moran, P. W., & Frankforter, J. D. (2011). Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams. *Environmental Monitoring and Assessment, 175*(1–4), 397–417. doi: 10.1007/s10661-010-1539-8

Blaen, P. J., Khamis, K., Lloyd, C., Comer-Warner, S., Ciocca, F., Thomas, R. M., … Krause, S. (2017). High-frequency monitoring of catchment nutrient exports reveals highly variable storm event responses and dynamic source zone activation. *Journal of Geophysical Research: Biogeosciences, 122*(9), 2265–2281. doi: 10.1002/2017JG003904

Bosch, N. S., Allan, J. D., Selegean, J. P., & Scavia, D. (2013). Scenario-testing of agricultural best management practices in Lake Erie watersheds. *Journal of Great Lakes Research, 39*(3), 429–436. doi: 10.1016/j.jglr.2013.06.004

Bowes, M. J., Jarvie, H. P., Halliday, S. J., Skeffington, R. A., Wade, A. J., Loewenthal, M., … Palmer-Felgate, E. J. (2015). Characterising phosphorus and nitrate inputs to a rural river using high-frequency concentration–flow relationships. *Science of The Total Environment, 511*, 608–620. doi: 10.1016/j.scitotenv.2014.12.086

Carey, R. O., & Migliaccio, K. W. (2009). Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. *Environmental Management, 44*(2), 205–217. doi: 10.1007/s00267-009-9309-5

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*(3), 559–568. doi: 10.2307/2641247

Chen, X., & Driscoll, C. T. (2009). Watershed land use controls on chemical inputs to Lake Ontario embayments. *Journal of Environmental Quality, 38*(5), 2084–2095. doi: 10.2134/jeq2007.0435

Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. (2013). Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge 4*(4), 1-10.

Clapcott, J. E., Young, R. G., Goodwin, E. O., & Leathwick, J. R. (2010). Exploring the response of functional indicators of stream health to land-use gradients. *Freshwater Biology, 55*(10), 2181–2199. doi: 10.1111/j.1365-2427.2010.02463.x

Clark, G. M., Mueller, D. K., & Mast, M. A. (2000). Nutrient concentrations and yields in undeveloped stream basins of the United States. *Journal of the American Water Resources Association, 36*(4), 849–860. doi: 10.1111/j.1752-1688.2000.tb04311.x

Cornwell, E. R., Goyette, J.-O., Sorichetti, R. J., Allan, D. J., Kashian, D. R., Sibley, P. K., … Trick, C. G. (2015). Biological and chemical contaminants as drivers of change in the Great Lakes–St. Lawrence river basin. *Journal of Great Lakes Research, 41*(S1), 119–130. doi: 10.1016/j.jglr.2014.11.003

Creed, I. F., & Beall, F. D. (2009). Distributed topographic indicators for predicting nitrogen export from headwater catchments. *Water Resources Research, 45*(10), 1–12. doi: 10.1029/2008WR007285

David, M. B., & Gentry, L. E. (2000). Anthropogenic inputs of nitrogen and phosphorus and riverine export for Illinois, USA. *Journal of Environmental Quality, 29*(2), 494–508. doi: 10.2134/jeq2000.00472425002900020018x

Davies, J., & Bothwell, M. L. (2012). Responses of lotic periphyton to pulses of phosphorus: P-flux controlled growth rate. *Freshwater Biology, 57*(12), 2602–2612. doi: 10.1111/fwb.12032

Dillon, P. J., & Molot, L. A. (1997). Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Resources Research, 33*(11), 2591–2600. doi: 10.1029/97WR01921

Dillon, P. J., & Kirchner, W. B. (1975). The effects of geology and land use on the export of phosphorus from watersheds. *Water Research, 9*(2), 135–148. doi: 10.1016/0043-1354(75)90002-0

Dodds, W. K. (2006). Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography, 51*, 671–680. doi: 10.4319/lo.2006.51.1_part_2.0671

Dodds, W. K. (2003). Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *Journal of the North American Benthological Society, 22*(2), 171–181. doi: 10.2307/1467990

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., … Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science & Technology, 43*(1), 12–19. doi: 10.1021/es801217q

Dodds, W. K., & Smith, V. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters, 6*(2), 155–164. doi: 10.5268/IW-6.2.909

d’Orgeville, M., Peltier, W. R., Erler, A. R., & Gula, J. (2014). Climate change impacts on Great Lakes Basin precipitation extremes. *Journal of Geophysical Research: Atmospheres, 119*(18), 10799–10812. doi: 10.1002/2014JD021855

Downes, B. J. (2010). Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. *Freshwater Biology, 55*(Suppl. 1), 60–79. doi: 10.1111/j.1365-2427.2009.02377.x

Edwards, A. C., & Withers, P. J. A. (2007). Linking phosphorus sources to impacts in different types of water body. *Soil Use and Management*, *23*(Suppl. 1), 133–143. doi: 10.1111/j.1475-2743.2007.00110.x

Edwards, A. C., & Withers, P. J. A. (2008). Transport and delivery of suspended solids, nitrogen and phosphorus from various sources to freshwaters in the UK. *Journal of Hydrology, 350*(3–4), 144–153. doi: 10.1016/j.jhydrol.2007.10.053

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

European Environment Agency (2018). European Waters Assessment of Status and Pressures. Luxembourg: Publications Office of the European Union, pp. 90. doi: 10.2800/303664

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality, 42*(4), 1002–1014. doi: 10.2134/jeq2012.0491

Francoeur, S. N. (2001). Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society, 20*(3), 358–368. doi: 10.2307/1468034

Frankforter, J. D., Weyers, H. S., Bales, J. D., Moran, P. W., & Calhoun, D. L. (2010). The relative influence of nutrients and habitat on stream metabolism in agricultural streams. *Environmental Monitoring and Assessment, 168*(1–4), 461–479. doi: 10.1007/s10661-009-1127-y

Griffiths, N. A., Tank, J. L., Royer, T. V, Roley, S. S., Rosi-Marshall, E. J., Whiles, M. R., … Johnson, L. T. (2013). Agricultural land use alters the seasonality and magnitude of stream metabolism. *Limnology and Oceanography, 58*(4), 1513–1529. doi: 10.4319/lo.2013.58.4.1513

Grimm, N. B., & Petrone, K. C. (1997). Nitrogen fixation in a desert stream ecosystem. *Biogeochemistry, 37*(1), 33–61. doi: 10.1023/A:1005798410819

Grover, J. P. (1991). Resource competition in a variable environment: Phytoplankton growing according to the variable-internal-stores model. *The American Naturalist, 138*(4), 811–835. doi: 10.1086/285254

Grover, J. P. (1989). Phosphorus-dependent growth kinetics of 11 species of freshwater algae. *Limnology and Oceanography, 34*(2), 341–348. doi: 10.4319/lo.1989.34.2.0341

Gücker, B., Brauns, M., & Pusch, M. T. (2006). Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. *Journal of the North American Benthological Society, 25*(2), 313–329. doi: 10.1899/0887-3593(2006)25[313:EOWTPD]2.0.CO;2

Hillebrand, H., & Sommer, U. (1999). The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography, 44*(2), 440–446. doi: 10.4319/lo.1999.44.2.0440

Hilton, J., O’Hare, M., Bowes, M. J., & Jones, J. I. (2006). How green is my river? A new paradigm of eutrophication in rivers. *Science of The Total Environment, 365*(1–3), 66–83. doi: 10.1016/j.scitotenv.2006.02.055

Ho, J. C., & Michalak, A. M. (2017). Phytoplankton blooms in Lake Erie impacted by both long-term and springtime phosphorus loading. *Journal of Great Lakes Research, 43*(3), 221–228. doi: 10.1016/j.jglr.2017.04.001

Holtan, H., Kamp-Nielsen, L., & Stuanes, A. O. (1988). Phosphorus in soil, water and sediment: An overview. In: Persson, G., & Jansson, M. (eds) Phosphorus in Freshwater Ecosystems. Developments in Hydrobiology, Springer, Dordrecht, pp. 19–34. doi: 10.1007/978-94-009-3109-1_3

Horner, R. R., & Welch, E. B. (1981). Stream periphyton development in relation to current velocity and nutrients. *Canadian Journal of Fisheries and Aquatic Sciences, 38*(4), 449–457. doi: 10.1139/f81-062

Humphrey, K. P., & Stevenson, R. J. (1992). Responses of benthic algae to pulses in current and nutrients during simulations of subscouring spates. J*ournal of the North American Benthological Society, 11*(1), 37–48. doi: 10.2307/1467880

Jarvie, H. P., Smith, D. R., Norton, L. R., Edwards, F. K., Bowes, M. J., King, S. M., … Bachiller-Jareno, N. (2018). Phosphorus and nitrogen limitation and impairment of headwater streams relative to rivers in Great Britain: A national perspective on eutrophication. *Science of The Total Environment, 621*, 849–862. doi: 10.1016/j.scitotenv.2017.11.128

Johnson, L., Richards, C., Host, G., & Arthur, J. (1997). Landscape influences on water chemistry in Midwestern stream ecosystems. *Freshwater Biology, 37*(1), 193–208. doi: doi:10.1046/j.1365-2427.1997.d01-539.x

Kane, D. D., Conroy, J. D., Peter Richards, R., Baker, D. B., & Culver, D. A. (2014). Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *Journal of Great Lakes Research*, *40*(3), 496–501. doi: 10.1016/j.jglr.2014.04.004

Katz, S. B., Segura, C., & Warren, D. R. (2018). The influence of channel bed disturbance on benthic Chlorophyll a: A high resolution perspective. *Geomorphology, 305*, 141–153. doi: 10.1016/j.geomorph.2017.11.010

Kaushal, S. S., Gold, A. J., Bernal, S., & Tank, J. L. (2018). Diverse water quality responses to extreme climate events: An introduction. *Biogeochemistry, 141*(3), 273–279. doi: 10.1007/s10533-018-0527-x

Kaushal, S. S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., Newcomer, T. A., … Belt, K. T. (2014). Land use and climate variability amplify carbon, nutrient, and contaminant pulses: A review with management implications. *Journal of the American Water Resources Association, 50*(3), 585–614. doi: 10.1111/jawr.12204

Keck, F., & Lepori, F. (2012). Can we predict nutrient limitation in streams and rivers? *Freshwater Biology, 57*(7), 1410–1421. doi: 10.1111/j.1365-2427.2012.02802.x

Kerr, J. M., DePinto, J. V., McGrath, D., Sowa, S. P., & Swinton, S. M. (2016). Sustainable management of Great Lakes watersheds dominated by agricultural land use. *Journal of Great Lakes Research, 42*(6), 1252–1259. doi: 10.1016/j.jglr.2016.10.001

King, K. W., Williams, M. R., LaBarge, G. A., Smith, D. R., Reutter, J. M., Duncan, E. W., & Pease, L. A. (2018). Addressing agricultural phosphorus loss in artificially drained landscapes with 4R nutrient management practices. *Journal of Soil and Water Conservation, 73*(1), 35–47. doi: 10.2489/jswc.73.1.35

Klausmeier, C. A., Litchman, E., Daufresne, T., & Levin, S. A. (2004). Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature, 429*(6988), 171–174. doi: 10.1038/nature02454

Kleinman, P. J. A., Srinivasan, M. S., Dell, C. J., Schmidt, J. P., Sharpley, A. N., & Bryant, R. B. (2006). Role of rainfall intensity and hydrology in nutrient transport via surface runoff. *Journal of Environmental Quality, 35*(4), 1248–1259. doi: 10.2134/jeq2006.0015

Kreiling, R. M., Thoms, M. C., & Richardson, W. B. (2018). Beyond the edge: Linking agricultural landscapes, stream networks, and best management practices. *Journal of Environmental Quality, 47*(1), 42–53. doi: 10.2134/jeq2017.08.0319

Kroll, S. A., & Oakland, H. C. (2019). A review of studies documenting the effects of agricultural best management practices on physiochemical and biological measures of stream ecosystem integrity. *Natural Areas Journal*, *39*(1), 58. doi: 10.3375/043.039.0105

Kromkamp, J. (1987). Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research, 21*(3), 457–465. doi: 10.1080/00288330.1987.9516241

Lam, W. V., Macrae, M. L., English, M. C., O'Halloran, I. P., & Wang, Y. T. (2016). Effects of tillage practices on phosphorus transport in tile drain effluent under sandy loam agricultural soils in Ontario, Canada. *Journal of Great Lakes Research, 42*(6), 1260–1270. doi: 10.1016/j.jglr.2015.12.015

Lloyd, C. E. M., Freer, J. E., Johnes, P. J., & Collins, A. L. (2016). Using hysteresis analysis of high-resolution water quality monitoring data, including uncertainty, to infer controls on nutrient and sediment transfer in catchments. *Science of The Total Environment, 543*, 388–404. doi: 10.1016/j.scitotenv.2015.11.028

Maccoux, M. J., Dove, A., Backus, S. M., & Dolan, D. M. (2016). Total and soluble reactive phosphorus loadings to Lake Erie. *Journal of Great Lakes Research, 42*(6), 1151–1165. doi: 10.1016/j.jglr.2016.08.005

Macrae, M. L., English, M. C., Schiff, S. L., & Stone, M. (2007). Capturing temporal variability for estimates of annual hydrochemical export from a first-order agricultural catchment in southern Ontario, Canada. *Hydrological Processes, 21*(13), 1651–1663. doi: 10.1002/hyp.6361

Mainstone, C. P., & Parr, W. (2002). Phosphorus in rivers – ecology and management. *Science of The Total Environment, 282–283*, 25–47. doi: 10.1016/S0048-9697(01)00937-8

Masseret, E., Amblard, C., Bourdier, G., & Sargos, D. (2000). Effects of a waste stabilization lagoon discharge on bacterial and phytoplanktonic communities of a stream. *Water Environment Research, 72*(3), 285–294. doi: 10.2175/106143000X137509

Melland, A. R., Fenton, O., & Jordan, P. (2018). Effects of agricultural land management changes on surface water quality: A review of meso-scale catchment research. *Environmental Science & Policy, 84*, 19–25. doi: 10.1016/j.envsci.2018.02.011

Mohamed, M. N., Wellen, C., Parsons, C. T., Taylor, W. D., Arhonditsis, G., Chomicki, K. M., … Haffner, D. G. (2019). Understanding and managing the re-eutrophication of Lake Erie: Knowledge gaps and research priorities. *Freshwater Science, 38*(4), 675–691. doi: 10.1086/705915

Mulholland, P. J., Marzolf, E. R., Hendricks, S. P., Wilkerson, R. V., & Baybayan, A. K. (1995). Longitudinal patterns of nutrient cycling and periphyton characteristics in streams: A test of upstream-downstream linkage. *Journal of the North American Benthological Society*, *14*(3), 357–370. doi: 10.2307/1467202

Mulholland, P. J., Steinman, A. D., Palumbo, A. V., Elwood, J. W., & Kirschtel, D. B. (1991). Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology*, *72*(3), 966–982. doi: 10.2307/1940597

Munawar, M., & Fitzpatrick, M. A. J. (2018). Eutrophication in three Canadian Areas of Concern: Phytoplankton and major nutrient interactions. *Aquatic Ecosystem Health & Management, 21*(4), 421–437. doi: 10.1080/14634988.2018.1530895

Niyogi, D. K., Koren, M., Arbuckle, C. J., & Townsend, C. R. (2007). Stream communities along a catchment land-use gradient: Subsidy-stress responses to pastoral development. *Environmental Management, 39*(2), 213–225. doi: 10.1007/s00267-005-0310-3

Ormerod, S. J., Dobson, M., Hildrew, A. G., & Townsend, C. R. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology, 55*(Suppl. 1), 1–4. doi: 10.1111/j.1365-2427.2009.02395.x

Paul, B. J., Duthie, H. C., & Taylor, W. D. (1991). Nutrient cycling by biofilms in running waters of differing nutrient status. *Journal of the North American Benthological Society, 10*(1), 31–41. doi: 10.2307/1467762

Paulsen, S. G., Mayio, A., Peck, D. V., Stoddard, J. L., Tarquinio, E., Holdsworth, S. M., … Olsen, A. R. (2008). Condition of stream ecosystems in the US: An overview of the first national assessment. *Journal of the North American Benthological Society, 27*(4), 812–821. doi: 10.1899/08-098.1

Peterson, C. G., & Stevenson, R. J. (1992). Resistance and resilience of lotic algal communities: Importance of disturbance timing and current. *Ecology, 73*(4), 1445–1461. doi: 10.2307/1940689

Petry, J., Soulsby, C., Malcolm, I. A., & Youngson, A. F. (2002). Hydrological controls on nutrient concentrations and fluxes in agricultural catchments. *Science of The Total Environment, 294*(1–3), 95–110. doi: 10.1016/S0048-9697(02)00058-X

Pionke, H. B., Gburek, W. J., & Sharpley, A. N. (2000). Critical source area controls on water quality in an agricultural watershed located in the Chesapeake Basin. *Ecological Engineering, 14*(4), 325–335. doi: 10.1016/S0925-8574(99)00059-2

Rao, N. N., Gómez-García, M. R., & Kornberg, A. (2009). Inorganic polyphosphate: Essential for growth and survival. *Annual Review of Biochemistry, 78*(1), 605–647. doi: 10.1146/annurev.biochem.77.083007.093039

Redfield, A. C. (1958). The biological control of chemical factors in the environment. *American Scientist, 46*(3), 205–221.

Rengefors, K., Pettersson, K., Blenckner, T., & Anderson, D. M. (2001). Species-specific alkaline phosphatase activity in freshwater spring phytoplankton: Application of a novel method. *Journal of Plankton Research, 23*(4), 435–443. doi: 10.1093/plankt/23.4.435

Rengefors, K., Ruttenberg, K. C., Haupert, C. L., Taylor, C., Howes, B. L., & Anderson, D. M. (2003). Experimental investigation of taxon-specific response of alkaline phosphatase activity in natural freshwater phytoplankton. *Limnology and Oceanography, 48*(3), 1167–1175. doi: 10.4319/lo.2003.48.3.1167

Rier, S. T., Nawrocki, K. S., & Whitley, J. C. (2011). Response of biofilm extracellular enzymes along a stream nutrient enrichment gradient in an agricultural region of north central Pennsylvania, USA. *Hydrobiologia, 669*(1), 119–131. doi: 10.1007/s10750-011-0654-z

Rier, S. T., & Stevenson, R. J. (2006). Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia, 561*(1), 131–147. doi: 10.1007/s10750-005-1610-6

Rier, S. T., Kinek, K. C., Hay, S. E., & Francoeur, S. N. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. *Freshwater Science, 35*(2), 490–502. doi: 10.1086/685859

Robertson, D. M., & Saad, D. A. (2011). Nutrient inputs to the Laurentian Great Lakes by source and watershed estimated esing SPARROW watershed models. *Journal of the American Water Resources Association, 47*(5), 1011–1033. doi: 10.1111/j.1752-1688.2011.00574.x

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science, 195*(4275), 260–262. doi: 10.1126/science.195.4275.260

Schindler, D. W. (1974). Eutrophication and recovery in experimental lakes: Implications for lake management. *Science, 184*(4139), 897–899. doi: 10.1126/science.184.4139.897

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science & Technology, 50*(17), 8923–8929. doi: 10.1021/acs.est.6b02204

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., … Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences, 105*(32), 11254–11258. doi: 10.1073/pnas.0805108105

Seitz, N. E., Westbrook, C. J., & Noble, B. F. (2011). Bringing science into river systems cumulative effects assessment practice. *Environmental Impact Assessment Review, 31*(3), 172–179. doi: 10.1016/j.eiar.2010.08.001

Sharpley, A. N., Kleinman, P. J. A., Heathwaite, A. L., Gburek, W. J., Folmar, G. J., & Schmidt, J. P. (2008). Phosphorus Loss from an agricultural watershed as a function of storm size. *Journal of Environmental Quality*, *37*(2), 362–368. doi: 10.2134/jeq2007.0366

Sinha, E., Michalak, A. M., & Balaji, V. (2017). Eutrophication will increase during the 21st century as a result of precipitation changes. *Science, 357*(6349), 405–408. doi: 10.1126/science.aan2409

Smith, R. A., Alexander, R. B., & Schwarz, G. E. (2003). Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology, 37*(14), 3039–3047. doi: 10.1021/es020663b

Solovchenko, A. E., Ismagulova, T. T., Lukyanov, A. A., Vasilieva, S. G., Konyukhov, I. V., Pogosyan, S. I., … Gorelova, O. A. (2019). Luxury phosphorus uptake in microalgae. *Journal of Applied Phycology, 31*(5), 2755–2770. doi: 10.1007/s10811-019-01831-8

Stamm, C., Jarvie, H. P., & Scott, T. (2014). What’s more important for managing phosphorus: Loads, concentrations or both? *Environmental Science & Technology, 48*(1), 23–24. doi: 10.1021/es405148c

Stevenson, R. J. (1990). Benthic algal community dynamics in a stream during and after a spate. *Journal of the North American Benthological Society, 9*(3), 277–288. doi: 10.2307/1467591

Stevenson, R. J., Hill, B. H., Herlihy, A. T., Yuan, L. L., & Norton, S. B. (2008). Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of the North American Benthological Society, 27*(3), 783–799. doi: 10.1899/07-077.1

Stoddard, J. L., Van Sickle, J., Herlihy, A. T., Brahney, J., Paulsen, S., Peck, D. V., … Pollard, A. I. (2016). Continental-scale increase in lake and stream phosphorus: Are oligotrophic systems disappearing in the United States? *Environmental Science & Technology, 50*(7), 3409–3415. doi: 10.1021/acs.est.5b05950

Stumpf, R. P., Wynne, T. T., Baker, D. B., & Fahnenstiel, G. L. (2012). Interannual variability of cyanobacterial blooms in Lake Erie. *PLoS ONE, 7*(8), e42444. doi: 10.1371/journal.pone.0042444

Stutter, M. I., Langan, S. J., & Cooper, R. J. (2008). Spatial contributions of diffuse inputs and within-channel processes to the form of stream water phosphorus over storm events. *Journal of Hydrology, 350*(3–4), 203–214. doi: 10.1016/j.jhydrol.2007.10.045

Tank, J. L., & Dodds, W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology, 48*(6), 1031–1049. doi: 10.1046/j.1365-2427.2003.01067.x

Taylor, J. M., King, R. S., Pease, A. A., & Winemiller, K. O. (2014). Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment. *Freshwater Biology, 59*(5), 969–984. doi: 10.1111/fwb.12320

Taylor, J. M., Rodman, A. R., & Scott, J. T. (2020). Stream algal biomass response to experimental phosphorus and nitrogen gradients: A case for dual nutrient management in agricultural watersheds. *Journal of Environmental Quality, 49*(1), 140–151. doi: 10.1002/jeq2.20039

Thomas, K. E., Lazor, R., Chambers, P. A., & Yates, A. G. (2018). Land-use practices influence nutrient concentrations of southwestern Ontario streams. *Canadian Water Resources Journal, 43*(1), 2–17. doi: 10.1080/07011784.2017.1411211

Tilman, D. (1977). Resource competition between plankton algae: An experimental and theoretical approach. *Ecology, 58*(2), 338–348. doi: 10.2307/1935608

Tilman, D. (1976). Ecological competition between algae: Experimental confirmation of resource-based competition theory. *Science, 192*(4238), 463–465. doi: 10.1126/science.192.4238.463

Townsend, S. A., Garcia, E. A., & Douglas, M. M. (2012). The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river. *Freshwater Science, 31*(4), 1233–1243. doi: 10.1899/11-163.1

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., … Tilman, D. G. (1997). Alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications*, *7*(3), 737. doi: 10.2307/2269431

Wagenhoff, A., Lange, K., Townsend, C. R., & Matthaei, C. D. (2013). Patterns of benthic algae and cyanobacteria along twin-stressor gradients of nutrients and fine sediment: a stream mesocosm experiment. *Freshwater Biology, 58*(9), 1849–1863. doi: 10.1111/fwb.12174

Walter, M. T., Walter, M. F., Brooks, E. S., Steenhuis, T. S., Boll, J., & Weiler, K. (2000). Hydrologically sensitive areas: Variable source area hydrology implications for water quality risk assessment. *Journal of Soil and Water Conservation, 55*(3), 277–284.

Welch, E. B., Quinn, J. M., & Hickey, C. W. (1992). Periphyton biomass related to point-source nutrient enrichment in seven New Zealand streams. *Water Research, 26*(5), 669–675. doi: 10.1016/0043-1354(92)90243-W

Wilson, R. S., Beetstra, M. A., Reutter, J. M., Hesse, G., Fussell, K. M. D. D., Johnson, L. T., … Winslow, C. (2019). Commentary: Achieving phosphorus reduction targets for Lake Erie. *Journal of Great Lakes Research, 45*(1), 4–11. doi: 10.1016/j.jglr.2018.11.004

Withers, P. J. A., & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of The Total Environment, 400*(1–3), 379–395. doi: 10.1016/j.scitotenv.2008.08.002

Withers, P., Neal, C., Jarvie, H., & Doody, D. (2014). Agriculture and eutrophication: Where do we go from here? *Sustainability, 6*(9), 5853–5875. doi: 10.3390/su6095853

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *Wiley Interdisciplinary Reviews: Water, 6*(5), 1–27. doi: 10.1002/wat2.1373

Chapter 2

2 Spatial and temporal patterns in macronutrient concentrations and stoichiometry of tributaries draining the lower Great Lakes-St. Lawrence basin

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2.1 Introduction

Eutrophication from widespread nutrient (i.e., nitrogen and phosphorus) enrichment has resulted in the impairment of water quality and ecosystem services in many rivers and downstream waterbodies (Wurtsbaugh et al., 2019), including the lower Laurentian Great Lakes (Munawar & Fitzpatrick, 2018; Mohamed et al., 2019). The extent of eutrophication in the Great Lakes is spatially variable from select areas of concern (e.g., Green Bay, Lake Michigan; Saginaw Bay, Lake Huron; Hamilton Harbour, Lake Ontario) to large open water areas (e.g., Western Basin, Lake Erie). Yet, for both local and wide-scale eutrophication, nutrient loading from tributaries that drain catchments altered by human activities has been identified as one of the leading sources of nutrients supporting increased primary production (Stumpf et al., 2012; Kane et al., 2014; Ho & Michalak, 2017). Managers have accepted that phosphorus is the main nutrient that limits production in the Great Lakes (Mohamed et al., 2019), and to date much of the research on Great Lakes tributaries has focused on the management of phosphorus loadings to control downstream eutrophication (Baker et al., 2019). However, apart from quantifying nutrient loads of large tributaries (Maccoux et al., 2016), there has been limited assessment of the nutrient status of streams to inform broad-scale eutrophication management in the Great Lakes-St. Lawrence basin.

Streams can provide important ecosystem services (e.g., nutrient processing and retention) to downstream waterbodies (Lowe & Likens, 2005) but are also of ecological and societal importance themselves (Stevenson, 2014). Concerns over stream eutrophication have led to the development of regional nutrient guidelines that provide concentration limits necessary to achieve or maintain desired ecological conditions in streams (Chambers et al., 2012; Evans-White et al., 2013; Poikane et al., 2019). Prevailing nitrogen and phosphorus concentrations with respect to regional nutrient guidelines (i.e., “nutrient status”) are often used to inform managers on the potential for stream eutrophication. However, primary producers require nitrogen and phosphorus in different quantities and along with other resources (e.g., carbon and light), the relative proportions of macronutrients in tandem can further regulate stream primary production.

Macronutrient stoichiometry and nutrient ratios have been widely used to predict nutrient limitation of primary producer communities (Dodds & Smith, 2016). Redfield (1958) identified that marine algae require a balanced supply of carbon (C), nitrogen (N), and phosphorus (P) that correspond to a molar ratio of 106C:16N:1P, thereafter known as the Redfield ratio. Although variability in the stoichiometric requirements of primary producers exists (Klausmeier et al., 2004), the Redfield ratio is often considered near optimal for primary production in aquatic ecosystems, including streams and rivers (Hillebrand & Sommer, 1999). Past studies have used macronutrient ratios to predict nutrient limitation in streams, whereby the depletion of a nutrient relative to the Redfield ratio indicates potential limitation for that nutrient (Keck & Lepori, 2012). However, macronutrient ratios have often failed to align with nutrient limitation experiments in streams except when large deviations from the Redfield ratio were observed (Keck & Lepori, 2012). Several confounding factors have been identified to provide a potential explanation for misalignments between macronutrient stoichiometry and *in situ* limitation experiments and include nutrient form (Dodds, 2003), species-specific requirements (Danger et al., 2008), and limitation of other resources (e.g., light; Harpole et al., 2011). However, past studies have suggested that regional assessments of stream nutrient limitation can be improved through the combination of macronutrient ratios and absolute nutrient concentrations (Dodds, 2003; Tank & Dodds, 2003; Keck & Lepori, 2012). Thus, there is growing interest to bring together traditional concentration-based monitoring criteria with macronutrient stoichiometry to better evaluate nutrient status and the limits imposed by nutrients on stream ecosystems (Dodds & Smith, 2016; Jarvie et al., 2018).

Human disturbance of biogeochemical cycles of carbon, nitrogen, and phosphorus has resulted in spatial and temporal variation in stream macronutrient concentrations (Paul & Meyer, 2001; Allan, 2004). Although numerous studies in the Great Lakes region have documented that human activities and landscape alteration can influence stream macronutrient concentrations (e.g., Allan et al., 1997; Johnson et al., 1997; Chen and Driscoll, 2009; Thomas et al., 2018), fewer studies have evaluated their influence on stream water C:N:P stoichiometry. Global patterns in stream macronutrient stoichiometry have shown increasing proportions of nitrogen relative to phosphorus because of human disturbance (Turner et al., 2003; Peñuelas et al., 2013). Moreover, distinct spatial variations in macronutrient stoichiometry also occur at smaller scales as a result of local human activities (Jarvie et al., 2008), catchment physiography (Green et al., 2007), and position along the river continuum (Jarvie et al., 2018). Thus, to inform nutrient management on the potential for nutrient limitation in human-altered watersheds, it is important to understand the implications that human activities have on patterns in stream macronutrient stoichiometry.

Temporal variation in macronutrient concentrations may also impose limits on stream ecosystems through intra-annual differences in C:N:P stoichiometry. Hydrologic events can influence stream macronutrient ratios (Rattan & Chambers, 2017; Smith and Jarvie, 2018) and may interact with spatial differences in catchment transport pathways to generate contrasting temporal patterns in C:N:P stoichiometry for different streams (Green et al., 2007; Green & Finlay, 2010). Moreover, seasonal variation in nutrient concentrations has been shown to alter stream macronutrient ratios across longer temporal scales (Dupas et al., 2017; Green & Finlay, 2010). Streams that experience temporal variation in the potential for nutrient limitation may thus require separate nutrient management actions to mitigate the degradation of stream environments at different times of the year.

The goal of our study was to provide knowledge on the potential for nutrient limitation in tributaries of the lower Great Lakes-St. Lawrence basin through the evaluation of spatial and temporal patterns in stream macronutrient concentrations and stoichiometry. To achieve our goal, we used a 10-year dataset (2007 to 2016) collected by the provincial stream water quality monitoring network for 127 streams located in central, eastern, and southwestern Ontario, Canada, to address three objectives: (1) describe the prevailing carbon, nitrogen, and phosphorus concentrations and stoichiometry within individual streams and quantify temporal variability therein; (2) assess the potential for nutrient limitation based on correspondence between absolute nutrient concentrations and C:N:P stoichiometry; and (3) evaluate the influence of land cover on spatial patterns of macronutrient concentrations and C:N:P stoichiometry.

2.2 Methods

2.2.1 Study area and data acquisition

Our study examined streams located in the lower Great Lakes-St. Lawrence basin of Ontario, Canada (Figure 2-1). Surficial geology in the study region is comprised primarily of exposed bedrock in the central and eastern sub-regions with mixed till covering the southwestern portion of the province (Baldwin et al., 2000). Land cover also varies across the study area with increased agricultural and urban cover in the southwestern and eastern regions (Crins et al., 2009). Climate across southern Ontario is temperate and humid with lower annual temperatures at greater latitudes (midpoint: 4.6 to 7.9 ^{o}C; Crins et al., 2009). Annual precipitation is similar across the region (midpoint: 897 to 953 mm; Crins et al., 2009).

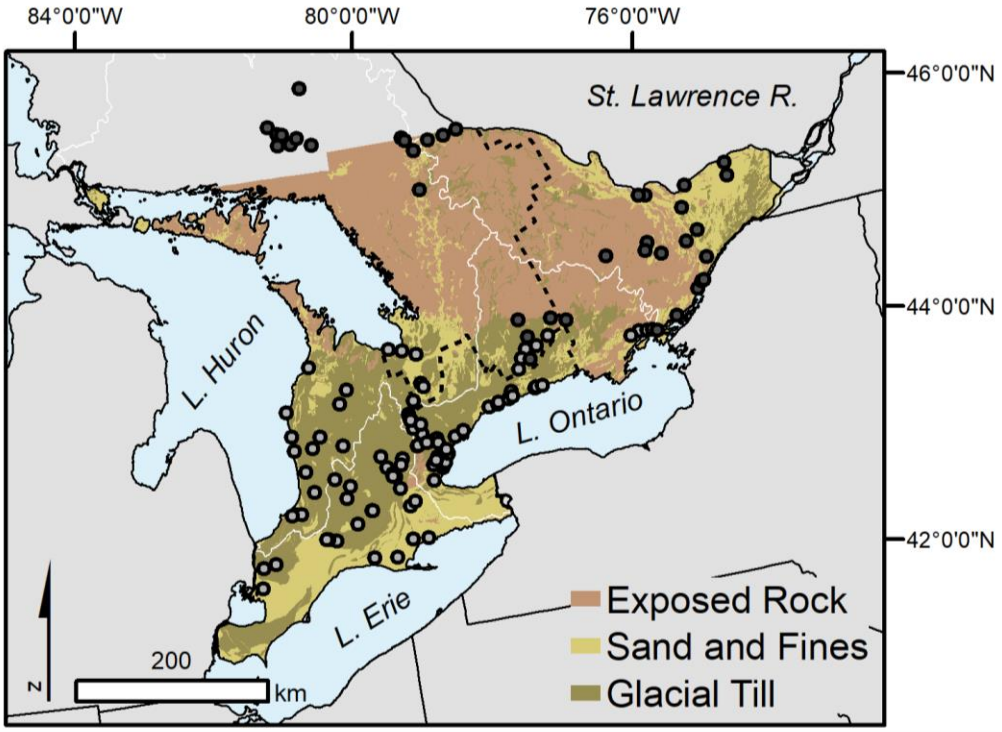

Figure 2-1. Selected study streams (n = 127) monitored by Ontario's Provincial Stream Water Quality Monitoring Network. A subset of sites (light grey; n = 89) draining glacial till catchments in southern Ontario was selected for land cover composition analyses. Dashed black lines delineate sub-regional boundaries for southwestern (including the golden horseshoe), central, and eastern Ontario. White lines represent watershed boundaries of the Great Lakes-St. Lawrence basin. Physiography data obtained from the Ontario Ministry of Energy, Northern Development and Mines (2017).

Nutrient data were obtained from Ontario's provincial water quality monitoring network (PWQMN), which measures water quality in streams throughout Ontario. Streams in the PWQMN are typically sampled monthly from April through November for various water quality parameters, including total and dissolved nutrients (i.e., nitrogen, phosphorus, and carbon). Monthly water quality monitoring by the PWQMN can capture variable hydrologic conditions (i.e., storms and event-flow) by chance, but water quality data largely represent baseflow conditions. Streams included in our study were restricted to those having monitoring sites with at least five water quality samples per year and data for at least 7 years of the 10-year period from 2007 to 2016. Study streams were further restricted to those with available total nitrogen, total phosphorus, and total dissolved carbon (dissolved organic carbon + dissolved inorganic carbon; *sensu* Smith et al., 2017) concentration data required to assess macronutrient stoichiometry. A total of 127 monitoring sites met these criteria (Figure 2-1). PWQMN datasets were downloaded from the Ontario data catalogue (OMECP, 2018).

Most of the 127 selected streams had sampling sites in southwestern Ontario (n = 75) with fewer sites in the central (n = 29) and eastern (n = 23) sub-regions (Figure 2-1). Moreover, of the selected streams 48 drained into Lake Ontario, 32 into Lake Huron, 26 into Lake Erie, and 21 into the St. Lawrence River. Selected streams were predominantly mid-order (Strahler order 4th to 6th) and drained catchment areas from near 10 to 8500 km^2 with a median of 192.8 km^2 (Table 2-1; Appendix A: Figure A-1). Nearly three quarters of the selected streams (n = 89) drained catchments within the glacial till physiographic region of southern Ontario.

Table 2-1. Descriptive statistic summary of watershed area and land cover composition for the 127 selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network.

	Mean	St. Deviation	Median	Minimum	1st Quartile	3rd Quartile	Maximum
Area (km^2)	820.1	1522.2	192.8	9.0	102.4	641.4	8501.8
Natural (%)	39.5	25.6	33.1	3.8	18.6	57.4	93.5
Wetland (%)	3.1	3.4	1.8	0.0	0.6	3.9	18.5
Agriculture (%)	42.7	27.9	45.6	0.0	17.3	65.4	90.1
Urban (%)	12.3	15.8	7.1	0.1	3.7	14.1	93.1
Water (%)	0.9	1.8	0.5	0.0	0.2	1.1	14.0

Catchment land cover of the selected streams was classified into five categories: natural (i.e., forests, shrublands, and grasslands), wetland, water (i.e., lakes and reservoirs), agriculture (i.e., row crops and pasture), and urban (i.e., settlements, roads, and built-up areas). Catchments varied from approximately 0 to 90% in agriculture, urban, and natural land covers, but agriculture (median = 45.6%) generally covered the largest proportion of catchment area in comparison to natural (median = 33.1%) and urban (median = 7.1%) lands. In contrast, wetland and water cover occupied smaller ranges (0 to 18.5% and 0 to 14.0%, respectively), and had a median cover of less than 2%.

Instream measurements of total phosphorus, dissolved reactive phosphorus, dissolved organic carbon, and dissolved inorganic carbon were collected throughout each year of the 10-year study period. However, forms of nitrogen measured by the PWQMN (i.e., total ammonium, total nitrates, nitrite, total kjeldahl nitrogen, and total nitrogen) varied such that total nitrogen and total kjeldahl nitrogen were only measured from 2007 to 2014 and 2013 to 2016, respectively. For years without direct measurements of total nitrogen (2015 and 2016), the sum of total ammonium, total nitrates, nitrite, and total kjeldahl nitrogen was used to calculate total nitrogen. In addition, analytical methods for the determination of total phosphorus changed in mid-2013 from an acid digestion method (E3367A) to persulfate, ultraviolet radiation digestion method (E3516) (OMECP, 2018). Although this change in measurement protocol has the potential to influence the temporal integrity of the dataset, given the summative nature of our study design and lack of observable difference in total phosphorus concentrations among study years, we treated total phosphorus measurements from differing methods as equivalent. Nutrient concentrations for all study years were analyzed colorimetrically (OMECP, 2018).

2.2.2 Nutrient concentrations

Descriptive statistics were used to summarize the average, interannual variability, and intra-annual variability of nutrient concentrations in the selected study streams from 2007 to 2016. Average nutrient concentrations for each stream were computed by taking the arithmetic mean ($n \geq 7$) of the mean annual water quality concentrations from monthly ($n \geq 5$) samples collected during each of the 10 study years. Correspondingly, the standard deviation of the mean annual concentrations over the 10-year study period was used to describe interannual variability in nutrient concentrations. Intra-annual variability in nutrient concentrations was quantified for each stream as the arithmetic mean of the annual standard deviations from the monthly samples collected within each study year.

Ten-year average total nitrogen (TN) and total phosphorus (TP) concentrations (Appendix A: Figure A-2) were strongly correlated with dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP) concentrations with Pearson correlation coefficients of 0.99 ($p < 0.001$) and 0.88 ($p < 0.001$), respectively (Appendix A: Figure A-3). Moreover, measurements of total nutrient concentrations are often more representative of the overall nutrient supply (e.g., sediment-bound, detrital, and dissolved) available to primary producers than dissolved forms (Dodds, 2003). Therefore, we used measures of total nutrient concentrations in analyses of macronutrient concentrations and stoichiometry. However, ten-year average total dissolved carbon

(TDC) concentrations (Appendix A: Figure A-2) were not strongly correlated with dissolved organic carbon (DOC) concentrations ($r = -0.24$, $p = 0.007$; Appendix A: Figure A-3), thus both measures were included in land cover analyses. To evaluate the nutrient status of each stream, 10-year averaged TN and TP concentrations were compared with regional nutrient guidelines (TN = 1.06 mg L^{-1}; TP = 0.024 mg L^{-1}) adopted by the Canadian Council of Ministers of the Environment (Chambers et al., 2008; CCME, 2016) whereby streams with nutrient concentrations above the guideline were considered to have enriched concentrations (Chambers et al., 2012).

2.2.3 Carbon : nitrogen : phosphorus stoichiometry

Macronutrient stoichiometry for each study stream was visualized with the use of ternary plots (*sensu* Smith et al., 2017) created with the *ternary* package in R (Smith, 2017). In brief, molar units of TN, TP, and TDC were calculated as the quotient of concentration and molar mass, then Redfield transformed, such that a balanced C:N:P composition (i.e., TDC_R = 33.3%, TN_R = 33.3%, TP_R = 33.3%) corresponded with the Redfield ratio of 106C:16N:1P (Redfield, 1958). Streams with balanced C:N:P compositions (i.e., center of ternary plot) thus represent conditions with near optimal ratios of carbon, nitrogen, and phosphorus for primary production (Hillebrand & Sommer, 1999). Deviations in C:N:P composition indicate the relative depletion of one or more nutrients in reference to the Redfield ratio and can be used to signify an imbalance in relative nutrient availability. Nutrient depletion has been operationalized as having a relative composition of TN_R, TP_R, or TDC_R of less than 20% (*sensu* Smith et al., 2017). As a result, there are seven zones of relative nutrient availability (Figure 2-2): balanced (TDC_R > 20%, TN_R > 20%, TP_R > 20%), phosphorus depleted (TDC_R > 20%, TN_R > 20%, TP_R < 20%), nitrogen depleted (TDC_R > 20%, TN_R < 20%, TP_R > 20%), carbon depleted (TDC_R < 20%, TN_R > 20%, TP_R > 20%), phosphorus and nitrogen co-depleted (TDC_R > 60%, TN_R < 20%, TP_R < 20%), phosphorus and carbon co-depleted (TDC_R < 20%, TN_R > 60%, TP_R < 20%), and nitrogen and carbon co-depleted (TDC_R < 20%, TN_R < 20%, TP_R > 60%). However, as stoichiometric compositions do not account for absolute nutrient concentrations, the correspondence between Redfield nutrient depletion and nutrient concentrations was evaluated to provide information on the potential for nutrient limitation (Jarvie et al., 2018). Streams were considered to have the potential for nutrient limitation if the nutrients that were depleted relative to the Redfield ratio had absolute concentrations below regional nutrient guidelines.

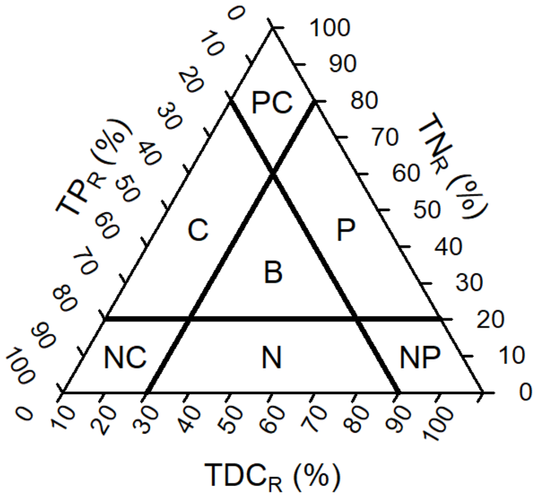

Figure 2-2. Ternary diagram of Redfield ratio total nitrogen (TN$_R$), total phosphorus (TP$_R$), and total dissolved carbon (TDC$_R$) composition depicting the seven zones of relative nitrogen (N), phosphorus (P), and carbon (C) availability. Nutrient acronyms indicate single or co-nutrient depletion. Center of the ternary diagram represents a balanced (B) Redfield ratio composition. Modified from Smith et al., (2017).

Redfield transformed C:N:P compositions were computed for every monthly sampling event from TN, TP, and TDC concentrations measured from 2007 to 2016 in each of the 127 study streams. For each study year, the geometric mean composition and geometric standard deviation were computed from the monthly samples ($n \geq 5$) recorded at each study stream. Subsequently, the geometric mean and standard deviation were computed from the mean annual compositions ($n \geq 7$) to represent the prevailing C:N:P stoichiometry and interannual variability in C:N:P stoichiometry for each stream. Intra-annual variability in C:N:P stoichiometry was calculated for each stream as the arithmetic mean of the annual geometric standard deviations. The percentage of observations that fell within each resource depletion zone was also calculated to provide context on the directionality of stoichiometric variability. Compositional data analyses were performed in R with the *compositions* package (van den Boogaart & Tolosana-Delgado, 2019).

To describe the correspondence in stream nutrient chemistry and C:N:P stoichiometry, Pearson correlations were computed between 10-year mean TN, TP, TDC concentrations, inter- and intra-variability in nutrient concentrations, and inter- and intra-variability in C:N:P stoichiometry with observations from all study streams ($\alpha = 0.05$). All variables were log transformed to improve normality. Correlations were computed in R with the *stats* package (R Core Team, 2020).

2.2.4 Influence of land cover on carbon : nitrogen : phosphorus stoichiometry

A subset of the 127 study streams was selected to investigate the influence of land cover (i.e., agriculture, urban, natural, and wetland) on Redfield C:N:P stoichiometry. Selected sites were restricted to streams draining catchments within the till physiographic region in southern Ontario (n = 89; Figure 2-1) to limit potential confounding effects of variation in physiography. Within our study period (2007 to 2016), annual land cover data for Ontario was available from 2011 onward and was obtained from Agriculture and Agri-Food Canada’s Annual Crop Inventory (AAFC, 2019). Change in the land cover composition of study catchments between 2011 and 2016 was spatially variable (agriculture: -3.7 ± 4.8; urban: 1.4 ± 3.1%; natural: 1.8 ± 4.2%; wetland: 0.5 ± 1.4). However, agricultural (r = 0.99), urban (r = 0.98), natural (0.99), and wetland (r = 0.91) cover were strongly correlated among catchments between the 2 years. Given the observed similarity in land cover composition and overall improvement in the classification accuracy of agricultural and non-agricultural land covers (AAFC, 2019), the 2016 dataset was used to quantify the land cover composition of our study catchments.

Although it is widely acknowledged that catchment land cover can affect stream water quality, areas of land in hydrologically active locations proximal to streams have been identified as more influential than more disconnected areas of the catchment (King et al., 2005; Walsh & Webb, 2014; Giri & Qiu, 2016). Thus, we employed an approach suggested by Peterson et al., (2011) and modified by Staponites et al., (2019), whereby spatial locations in the catchment were inversely weighted based on the Euclidean distance of each raster cell (30-m resolution) to the stream network multiplied by the natural log of the cell's associated flow accumulation. Raster values were then summed by land cover category, and the proportion (expressed as a percent) of spatial influence within each land cover category was used to describe the spatially weighted land cover composition (Figure 2-3). The land cover classification method applied in our study was found by Staponites et al., (2019) to be the most effective predictor of water quality in headwater catchments of central Europe. Spatially weighted land cover influence was quantified using the Ontario provincial digital elevation model (OMNRF, 2015) in ArcGIS version 10.7 (Esri inc., 2019).

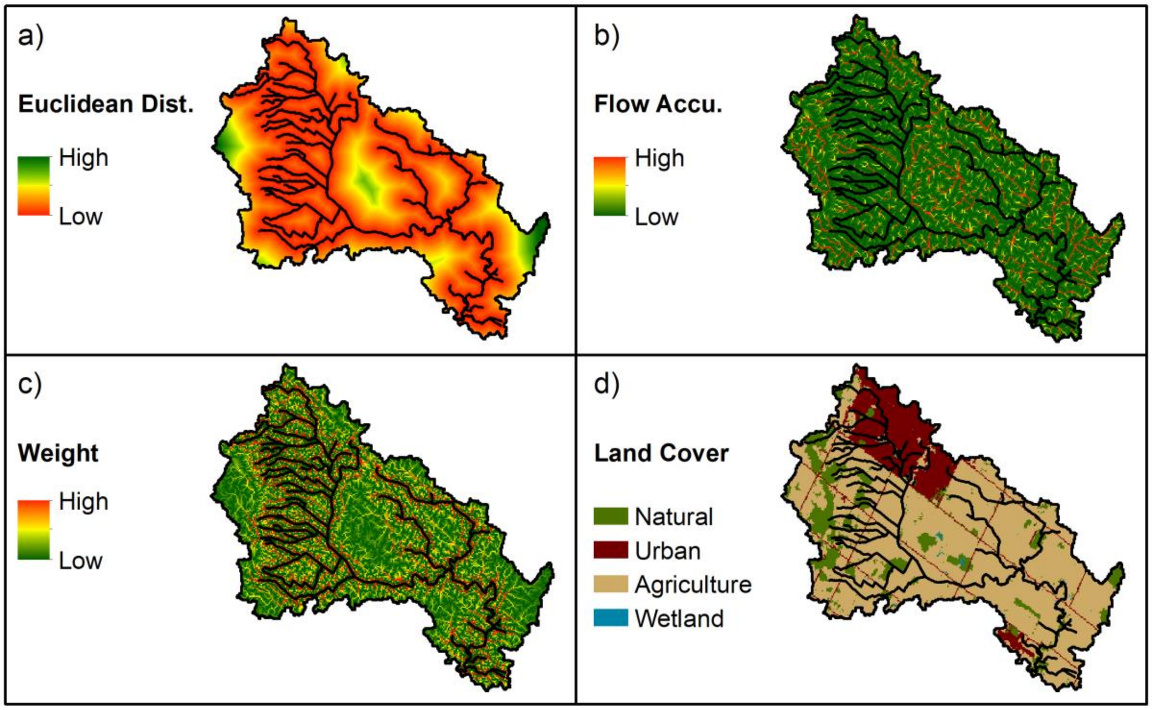

Figure 2-3. Weighting factors used to quantify the spatial influence of land cover categories within stream catchments. Increasingly red colours correspond to more influential spatial locations based on (a) proximity to the stream network (euclidian distance) and (b) the accumulated water flow (flow accumulation) in the catchment. Euclidian distance and flow accumulation were combined (c) to identify the most influential locations in the catchment, and the proportion of total catchment weight associated with each land cover category (d) was quantified.

The influence of land cover was first assessed with Pearson correlation analyses ($\alpha = 0.05$) to describe the collinearity and correlation structure between spatially weighted land cover categories and stream nutrient concentrations (TN, TP, TDC, and DOC) among the subset of study streams (n = 89). Land cover and nutrient concentrations were arcsine and log transformed, respectively, to improve data normality.

Associations between spatially weighted land cover (arcsine transformed) and C:N:P stoichiometry were evaluated with Dirichlet regression models using the *DirichletReg* package in R (Maier, 2015). Compositional stoichiometric data is restricted in that each component must have a value from 0 to 100% and the sum of n-components must equal 100% (Vermeesch, 2019). Traditional regression models (e.g., general linear models) between individual stoichiometric components and independent predictor variables fail to meet the unified data assumption and are therefore not appropriate to evaluate compositional data. Dirichlet regression models preserve the compositional nature of multi-component dependent variables (e.g., ternary stoichiometric compositions) and can be used to interpret the influence of one to multiple independent variables by applying a traditional regression model framework (Maier, 2014). Both simple and multiple Dirichlet regression models were used to evaluate the potential effects of spatially weighted land cover categories (independent variables) on Redfield C:N:P stoichiometry (dependent variables) to provide complementary information on the effects of spatially weighted land cover from simple and partial regression coefficients (King et al., 2005; Morrissey & Ruxton, 2018). Model performance was summarized using R^2 and log likelihood (LL) values and evaluated based on the comparison of AICc and BIC scores against the null Dirichlet regression model whereby a $\Delta AIC_c/BIC > 2$ per additional degree of freedom (df) constituted an improved model fit. For models that performed better than the null, the significance of regressors on each dependent variable was evaluated at $\alpha = 0.05$.

2.3 Results

2.3.1 Nutrient concentrations

Median 10-year average TN, TP, and TDC concentrations were 2.21 mg L^{-1}, 0.042 mg L^{-1}, and 54.4 mg L^{-1}, and varied by 29, 33, and 9 orders of magnitude among streams, respectively (Figure 2-4). Similarly, interannual variability in nutrient concentrations varied about 67-fold among streams for TN (0.05 to 3.29 mg L^{-1}) and TP (0.002 to 0.138

mg L^{-1}), and 23-fold for TDC (0.33 to 7.68 mg L^{-1}). Ten-year average concentrations and interannual variability in TN, TP, and TDC indicated that streams in southwestern Ontario had observations that were consistently in the upper half of the dataset, whereas streams in central and eastern Ontario had observations that were frequently in the lower half.

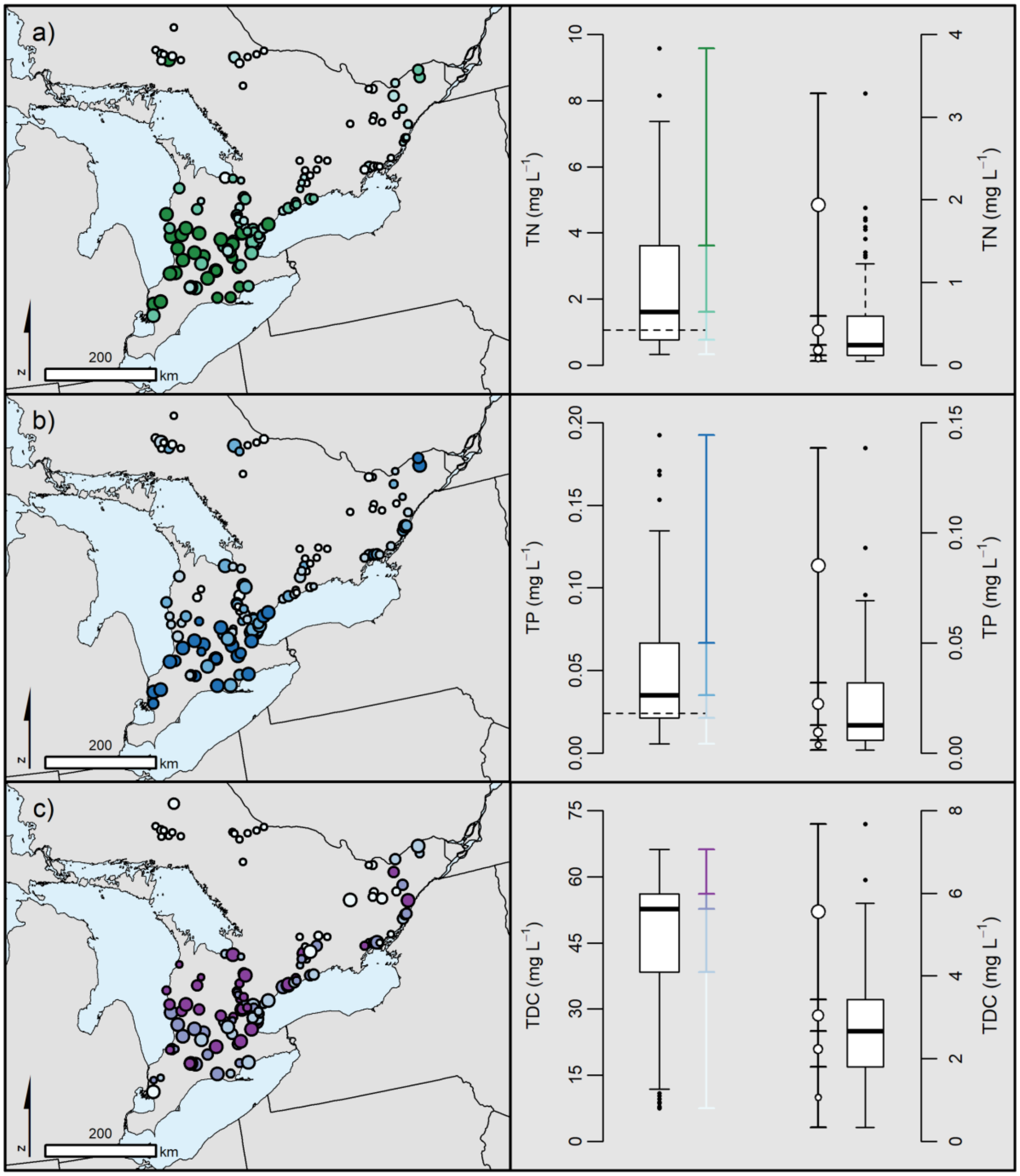

Figure 2-4. Spatial representation (left) of quantile plots (right) summarizing 10-year average (a) total nitrogen (TN; green), (b) total phosphorus (TP; blue), and (c) total dissolved carbon (TDC; purple) concentrations (colour ramp) and interannual variability in water quality concentrations (symbol size) of the 127 selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network. Dashed lines represent total phosphorus (0.026 mg L^{-1}) and total nitrogen (1.06 mg L^{-1}) nutrient guidelines for Ontario streams (CCME, 2016). Cartographic colour and symbol values are represented by the quartile ranges of the respective quantile plots.

Intra-annual variability in TN, TP, and TDC also occupied a large range among streams (TN: 0.06 to 4.35 mg L^{-1}; TP: 0.002 to 0.186 mg L^{-1}; TDC: 0.66 to 12.83 mg L^{-1}) and was highly correlated with interannual variability (TN: $r = 0.96$, $p < 0.001$; TP: $r = 0.95$, $p < 0.001$; TDC: $r = 0.83$, $p < 0.001$). Likewise, 10-year average TN, TP, and TDC concentrations were significantly correlated among study streams with Pearson correlation coefficients of 0.70 ($p < 0.001$), 0.65 ($p < 0.001$), and 0.50 ($p < 0.001$), for the pairwise comparisons of TN-TP, TN-TDC, and TP-TDC, respectively.

Comparison of 10-year average nutrient concentrations to regional nutrient guidelines indicated that 35% of streams were below guidelines for TN (guideline = 1.06 mg L^{-1}) and 32% for TP (guideline = 0.026 mg L^{-1}). Streams below the guidelines were located largely within the central and eastern regions of the province (~ 93% < TN guideline; ~ 68% < TP guideline). Jointly, 22% of streams were below TN and TP guidelines and 55% were above.

2.3.2 Carbon : nitrogen : phosphorus stoichiometry

Ternary plots of 10-year average Redfield C:N:P stoichiometry revealed variation among streams, primarily in the relative amount of nitrogen (Figure 2-5). Of the 127 study streams, 35% were phosphorus depleted and 65% were co-depleted for nitrogen and phosphorus. Streams located in southwestern Ontario were more frequently phosphorus depleted (~ 51%). In contrast, streams in the central and eastern regions were largely co-depleted for nitrogen and phosphorus (~ 90%). However, the correspondence between nutrient depletion and nutrient concentration indicated that 2% of streams had the potential for phosphorus limitation, whereas 21% of streams had the potential for nitrogen and phosphorus co-limitation. Streams with nutrient concentrations above regional guidelines (55%; n = 70) had a comparable frequency of phosphorus depletion (59%) and nitrogen and phosphorus co-depletion (41%).

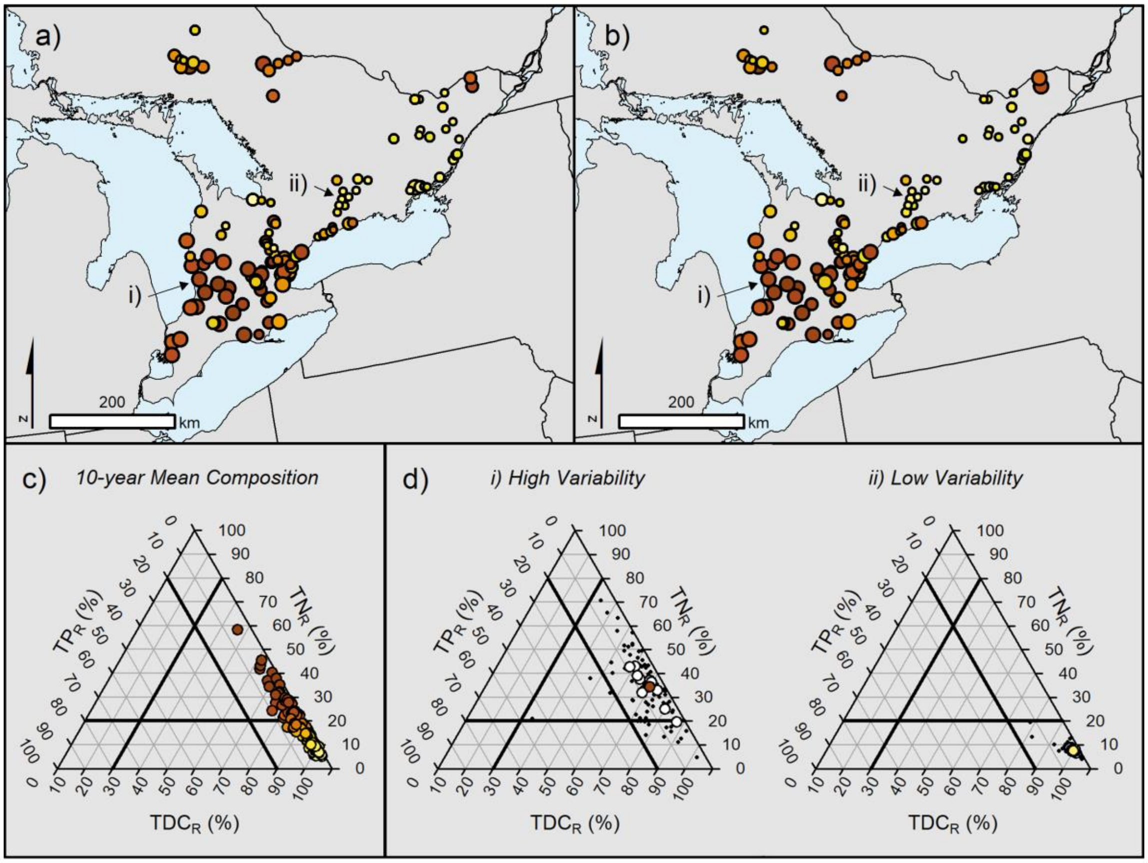

Figure 2-5. Spatial representation (colour ramp; a, b) of a ternary diagram (c) showing the 10-year geometric mean of the Redfield ratio total nitrogen (TN$_R$), total phosphorus (TP$_R$), and total dissolved carbon (TDC$_R$) composition in the 127 selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network. Interannual (a) and intra-annual (b) variability in Redfield ratio compositions from 2007 to 2016 are depicted by symbol size whereby ascending size corresponds to increasing variation based on the quartiles of each variability dataset. Two ternary diagrams (d) are shown as examples of Redfield ratio compositions in streams with high (i) and low (ii) temporal variability. Black points represent Redfield ratio composition of monthly water samples, white circles represent the annual geometric mean Redfield ratio composition, and coloured circles represent the 10-year geometric mean Redfield ratio composition.

Inter- and intra-annual variability in Redfield C:N:P stoichiometry varied 13 and 18-fold among streams, respectively. Streams in southwestern Ontario were observed to have greater inter- and intra-annual variation in C:N:P stoichiometry compared to those in central and eastern Ontario (Figure 2-5). Interannual variation of C:N:P stoichiometry for individual streams was observed to vary largely within individual Redfield depletion zones with 80% of streams having more than 90% of associated observations within a single zone. Interannual variation in C:N:P stoichiometry across zones was restricted to variation between phosphorus depletion and nitrogen and phosphorus co-depletion, whereby 32% and 68% of mean annual observations from all streams (n = 1188) fell within the two zones, respectively. Intra-annual variation in C:N:P stoichiometry varied more greatly across depletion zones. However, the majority of streams (56%) had over 90% of monthly observations within a single Redfield zone. Monthly C:N:P stoichiometry observations from all streams (n = 8872) indicated a tendency towards phosphorus depletion (36%) and nitrogen and phosphorus co-depletion (63%) than other zones of C:N:P depletion (< 1%).

Streams in central and eastern Ontario with lower total nutrient concentrations and variability were observed to have more temporally stable Redfield C:N:P compositions than the higher nutrient concentration streams of southwestern Ontario. Pearson correlations between interannual variation in C:N:P stoichiometry and interannual variation in nutrient concentrations revealed a greater association for TN ($r = 0.81$, $p < 0.001$) than for TP ($r = 0.65$, $p < 0.001$) and TDC ($r = 0.19$, $p = 0.036$). Interannual variation in C:N:P stoichiometry was also correlated with the average variation of C:N:P stoichiometry within study years ($r = 0.90$, $p < 0.001$). The relative strength of associations between TN ($r = 0.84$, $p < 0.001$), TP ($r = 0.77$, $p < 0.001$), and TDC ($r = 0.48$, $p < 0.001$) and intra-annual variability in C:N:P stoichiometry was similar to that observed for correlations between nutrient concentrations and interannual variability.

2.3.3 Influence of land cover on carbon : nitrogen : phosphorus stoichiometry

Southern Ontario streams selected to evaluate the influence of spatially weighted land cover composition on Redfield C:N:P stoichiometry occupied a gradient of weighted natural (3 – 69%), wetland (< 1 – 16%), agricultural (2 – 91%), and urban (2 – 96%) land cover compositions. Agriculture was negatively correlated with natural ($r = -0.52$, $p < 0.001$), wetland ($r = -0.49$, $p < 0.001$), and urban ($r = -0.63$, $p < 0.001$) land cover weights. Whereas wetland and natural were positively correlated ($r = 0.75$, $p < 0.001$), and urban had a negative correlation with natural ($r = -0.28$, $p = 0.007$) and wetland ($r = -0.22$, $p = 0.037$), respectively.

TN and TP concentrations were negatively correlated with natural (TN: r = -0.61, p < 0.001; TP: r = -0.66, p < 0.001) and wetland (TN: r = -0.67, p < 0.001; TP: r = -0.55, p < 0.001) covers, but positively correlated with agriculture (TN: r = 0.57, p < 0.001; TP: r = 0.34, p = 0.001). Urban land cover weights were not associated with TN (r = -0.04, p = 0.698) or TP (r = 0.21, p = 0.053) concentrations. No land cover variables were correlated with TDC (urban: r = -0.16, p = 0.142; natural: r = 0.04, p = 0.743; wetland: r = -0.07, p = 0.530; agriculture: r = 0.20, p = 0.054) or DOC (urban: r = -0.11, p = 0.296; natural: r = -0.18, p = 0.099; wetland: r = 0.18, p = 0.097; agriculture: r = 0.14, p = 0.178) concentrations.

Multiple Dirichlet regression between all spatially weighted land cover categories and Redfield C:N:P stoichiometry resulted in greater model performance (R^2 = 0.42; LL = 371.0; df = 15; AIC_c = -705.4; BIC = -674.6) in comparison to the null model (LL = 317.6; df = 3; AIC_c = -629.0; BIC = -621.8). However, there were no significant partial effects of land cover on individual stoichiometric components (Appendix A: Table A-1). Simple Dirichlet regression models of individual weighted land cover categories also resulted in improved model performance compared to null models. Direct effects of land cover on individual C:N:P stoichiometric components were observed for natural (R^2 = 0.31, LL = 353.0; df = 6; AIC_c = -693.0; BIC = -679.1), wetland (R^2 = 0.35, LL = 357.9; df = 6; AIC_c = -702.7; BIC = -688.8), and agriculture (R^2 = 0.29, LL = 344.1; df = 6; AIC_c = -675.2; BIC = -661.3) cover types but not for urban (R^2 = -0.02, LL = 321.6; df = 6; AIC_c = -630.2; BIC = -616.3) cover (Figure 2-6). Spatially weighted natural land cover had a significant positive association with TDC_R (coefficient = 2.127 ± 0.743, Z = 2.861, p = 0.004) but did not have significant associations with TN_R (coefficient = -0.209 ± 0.673, Z = -0.310, p = 0.756) or TP_R (coefficient = -0.432 ± 0.691, Z = -0.625, p = 0.532). Likewise, spatially weighted wetland cover was positively associated with TDC_R (coefficient = 5.071 ± 1.227, Z = 4.133, p < 0.001) but was not associated with either TN_R (coefficient = 0.324 ± 0.1.23, Z = 0.263, p = 0.792) or TP_R (coefficient = 1.183 ± 1.517, Z = 0.780, p = 0.435). In contrast, spatially weighted agricultural land cover had a significant positive association with TN_R (coefficient = 2.018 ± 0.474, Z = 4.254, p < 0.001) and TP_R (coefficient = 1.125 ± 0.523, Z = 2.152, p = 0.031) but no significant association with TDC_R (coefficient = 0.364 ± 0.415, Z = 0.877, p = 0.38).

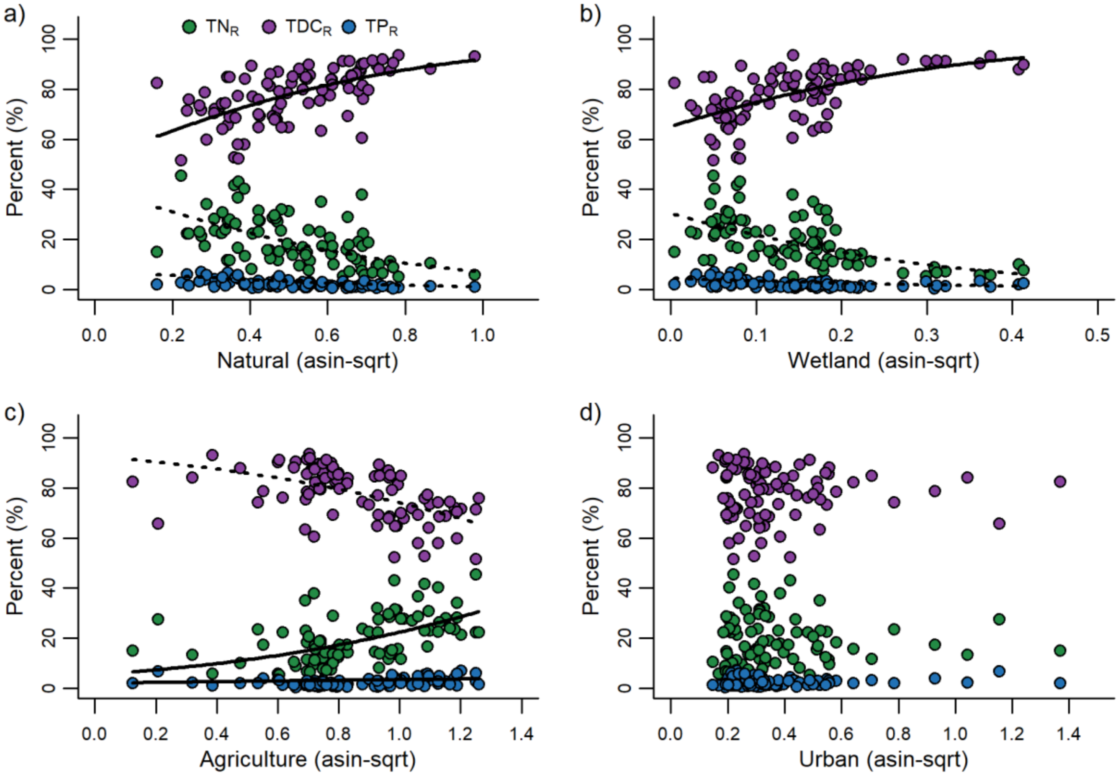

Figure 2-6. Simple Dirichlet regression models between Redfield transformed total nitrogen (TN$_R$; green), total phosphorus (TP$_R$; blue), and total dissolved carbon (TDC$_R$; purple) compositions and spatially weighted (a) natural, (b) wetland, (c) agriculture, and (d) urban land cover variables. Solid lines represent significant associations between regressors and individual stoichiometric components ($p < 0.05$). Dashed lines represent non-significant associations between regressors and individual stoichiometric components in Dirichlet regression models with improved model performance in comparison to the null.

2.4 Discussion

Spatial and temporal variation was observed in macronutrient concentrations and stoichiometry summarized from a 10-year dataset of stream nutrients throughout the southern Great Lakes-St. Lawrence basin of Ontario, Canada. Ten-year mean concentrations of TN and TP indicated that most of the routinely monitored streams had nutrient concentrations that exceeded regional nutrient guidelines. Streams exceeding nutrient guidelines were located largely in southwestern Ontario. Redfield C:N:P stoichiometry of streams in southwestern Ontario suggested that many of these enriched streams were relatively depleted of phosphorus, whereas nitrogen and phosphorus co-depletion was more common in the central and eastern regions. In addition, streams in southwestern Ontario had greater temporal variability in C:N:P stoichiometry among and within each of the 10 study years. Our results suggest that variation in C:N:P stoichiometry of Ontario streams is largely a function of spatial and temporal differences in the relative amount of nitrogen, which is associated with the amount of agricultural land cover in stream catchments.

2.4.1 Nutrient limitation assessment

Comparison of TN and TP concentrations to regional nutrient guidelines revealed that 78% of streams had concentrations of either nitrogen or phosphorus that have been associated with poor ecological conditions (Chambers et al., 2012). Median TN (2.21 mg L^{-1}) and to a lesser extent TP (0.042 mg L^{-1}) concentrations summarized across the lower Great Lakes region of Ontario were comparable to routinely monitored streams draining overlapping ecoregions (VI: TN = 3.37 mg L^{-1}, TP = 0.168 mg L^{-1}; VII: TN = 0.928 mg L^{-1}, TP = 0.080 mg L^{-1}) of the US mid-west and mid-Atlantic (Dodds et al., 2009). Moreover, our observation that most of the studied streams in Ontario were nutrient enriched is consistent with findings from Dodds et al., (2009) that more than 87% of streams in the US mid-west and mid-Atlantic ecoregions have nutrient concentrations exceeding regional reference conditions.

Southwestern Ontario is largely developed compared to the central and eastern regions of Ontario (Crins et al., 2009). Thus, our observation of widespread nutrient enrichment in streams draining the agriculturally intensive and built-up catchments of the Lake Erie and the southern Lake Huron and Ontario watersheds is consistent with the widely observed effects of land use on stream ecosystems (Allan, 2004). In addition, routine monitoring sites in the PWQMN are often biased to accessible locations (e.g., middle to large order streams with road access) that frequently co-occur with human development. As such, our dataset was positively skewed to catchments with low natural land cover, which likely resulted in a lower frequency of streams below TN and TP guidelines. Nevertheless, our observation of pervasive nutrient enrichment in tributaries of the lower Great Lakes-St. Lawrence basin demonstrates the need for nutrient management to lower the potential for stream eutrophication and improve ecological conditions throughout the region.

Redfield C:N:P stoichiometry indicated that phosphorus depletion and nitrogen and phosphorus co-depletion occurred proportionately in streams with nitrogen and phosphorus concentrations that exceeded nutrient guidelines. However, Prater et al., (2017) reported that seston in Lake Erie tributaries of southwestern Ontario had a balanced C:N:P stoichiometry with low C:P and N:P ratios and little temporal variability. Thus, while relatively depleted of phosphorus or nitrogen and phosphorus, the enriched nutrient concentrations observed in many of our study streams may be saturating autotrophic uptake and production.

In contrast, C:N:P stoichiometry in streams with nutrient concentrations below regional guidelines indicated that nearly all were co-depleted for nitrogen and phosphorus. Our finding that most streams had a stronger potential for nutrient co-limitation is consistent with the disproportional occurrence of N and P co-limitation in meta-analyses of stream nutrient limitation experiments (Francoeur, 2001; Elser et al., 2007; Beck et al., 2017). Nutrient management in the Great Lakes-St. Lawrence basin, however, has emphasized phosphorus limitation, which has been widely linked to freshwater eutrophication (Schindler et al., 2016) and specifically to eutrophication in the lower Great Lakes (Mohamed et al., 2019). Although prioritizing phosphorus reductions for the protection of downstream ecosystems can have water quality benefits upstream (Keitzer et al., 2016), our observation of elevated nitrogen and phosphorus concentrations and nutrient co-limitation in tributaries of the Great Lakes suggests that dual nutrient management may be required to further improve ecological conditions in streams impaired by nutrient enrichment and minimize the risk of stream degradation by eutrophication in streams that drain less disturbed watersheds.

Temporal variation in Redfield C:N:P stoichiometry corresponded to nutrient status in that streams with lower nutrient concentrations had temporally stable C:N:P stoichiometries. Streams with lower nutrient concentrations in the United Kingdom were also shown to have less variation in C:N:P stoichiometry than nearby streams with higher concentrations (Smith et al., 2017). Stoichiometric stability in stream water macronutrients may therefore be associated with allochthonous nutrient inputs from stream catchments as we observed that less disturbed streams in central and eastern

Ontario had lower temporal variability in C:N:P stoichiometry than streams draining agricultural and built up catchments in southwestern Ontario. Moreover, we found that the temporal variation observed in C:N:P stoichiometry was most associated with temporal variation in nitrogen concentrations among our study streams. Dominant hydrologic flow paths (e.g., overland, subsurface, groundwater, and artificial drainage) have been shown to dictate the directionality of temporal variation in C:N:P stoichiometry due to the respective mobility of nitrogen and phosphorus in stream catchments (Green et al., 2007). For example, Green and Finlay (2010) found that the direction of association between seasonal variation in discharge and N:P ratios differed with catchment hydrology in streams located in humid climates compared to semiarid climates of the contiguous United States. Although we observed that temporal variation in C:N:P stoichiometry varied 13 to 18-fold among streams, temporal variation in all study streams tended to occur within the bounds of a single Redfield depletion zone. Thus, while temporal variation in C:N:P stoichiometry of streams in the Great Lakes-St. Lawrence basin appears to increase alongside nutrient enrichment, seasonal and intra-annual variation in stream water chemistry had little repercussion on changing which nutrient was primarily depleted.

In addition to seasonal and intra-annual variation, high flow events can temporarily alter stream macronutrient concentrations and interact with preferential flow paths to influence stream macronutrient ratios (Green et al., 2007; Rattan & Chambers, 2017). For example, Green and Finlay (2010) found that stream water N:P ratios decreased with high flow events in less disturbed streams of the contiguous United States. In contrast, Smith and Jarvie (2018) observed that high flow events in the Maumee River watershed (Lake Erie) altered the C:N:P stoichiometry along an agricultural tributary through an increase in the proportion of instream nitrogen relative to carbon and phosphorus. However, the low monitoring frequencies of the PWQMN (i.e., monthly) likely resulted in a significant underrepresentation of high flow events in the data used for our study. As a result, we were unable to identify the role hydrologic conditions may have played in influencing the observed patterns in macronutrient concentrations and stoichiometry. Therefore, our results best represent patterns in macronutrient concentrations and stoichiometry associated with baseflows and temporal variation over the growing season. Future high-resolution studies would be required to investigate the effects of high flow events and further evaluate spatial differences in the temporal variation of stream water macronutrient stoichiometry in the Great Lakes-St. Lawrence basin.

2.4.2 Influence of land cover on carbon : nitrogen : phosphorus stoichiometry

Spatially weighted land cover (i.e., agriculture, urban, natural, and wetland) was found to be associated with stream nutrient concentrations among a subset of streams in southern Ontario. Ten-year mean TN and TP concentrations were found to be positively associated with the spatial influence of agricultural land cover but were negatively associated with natural and wetland cover in stream catchments. The direction of the land cover to nutrient associations was consistent with results from many past studies in the Great Lakes-St. Lawrence basin (e.g., Johnson et al., 1997; Chen & Driscoll, 2009; Thomas et al., 2018). However, unlike some past studies (e.g., Brett et al., 2005; Miller et al., 2011; Raney & Eimers, 2014), we did not observe an association between urban land cover and measured TN and TP concentrations. Measures of urban land cover, however, may not adequately incorporate the influence of point source inputs (e.g., effluent quality and dilution factor), which are known to affect downstream nutrient concentrations (Carey & Migliaccio, 2009). For example, Thomas et al., (2018) found that independent variables describing sewage discharge in southwestern Ontario streams were stronger predictors of nutrient concentrations than the proportion of built-up area in the catchment. Thus, future studies in urban catchments may need to consider the incorporation of more complex criteria to account for point source impacts.

Spatially weighted natural and wetland cover were found to be positively associated with the stoichiometric proportion of carbon (TDC_R) available in stream water, whereas agriculture was positively associated with the stoichiometric proportion of nitrogen (TN_R) and phosphorus (TP_R). However, while we observed direct effects of land cover on Redfield C:N:P stoichiometry in simple regression models, no additional partial effects were observed in the multiple regression model. Our observation that human and natural land covers were inversely collinear restricts the independent interpretation of individual land cover effects. Therefore, our findings are more suited to address spatial differences in land cover composition altogether and the direct effects of human and natural land covers reported should be considered bilaterally.

Simple regression coefficients indicated that the spatial influence of wetland cover had a 2.4-fold greater effect on the stoichiometric proportion of carbon than natural land cover. Indeed, terrestrial vegetation and wetlands are considered allochthonous sources of carbon to streams (Tank et al., 2010), and Wilson and Xenopoulos (2008) reported the proportion of catchment area with wetland cover was the best predictor of DOC concentrations in streams of south-central Ontario. However, while we did not find evidence that the spatial influence of wetland or natural cover increased the instream concentrations of TDC or DOC, streams with a greater influence of wetland and natural land covers had lower concentrations of TN and TP. Thus, the observed increase in the stoichiometric proportion of carbon may be a result of lower TN and TP concentrations in streams with more natural land covers in their catchments.

Spatially weighted agricultural land cover was associated with an increase in TN and TP concentrations and with stoichiometric proportions of nitrogen and phosphorus. Moreover, regression coefficients differed in that agricultural land cover was 1.8-fold more influential on the stoichiometric proportion of nitrogen than phosphorus. Thus, our findings suggest that the Redfield C:N:P stoichiometry of streams influenced by agricultural inputs would be disposed to phosphorus depletion and increased N:P ratios. Although the influence of natural, wetland, and agricultural land cover on stream nutrient concentrations has been well documented (e.g., Chen & Driscoll, 2009; Thomas et al., 2018), fewer studies have evaluated their influence on stream water nutrient stoichiometry. Although standalone partial effects of land cover were not identified, our results show that concurrent human modification of natural landscapes can alter the C:N:P stoichiometry in streams of the Great Lakes-St. Lawrence basin.

Our finding that catchment land cover was associated with stream water C:N:P stoichiometry may have implications for community dynamics in streams of the Great Lakes-St. Lawrence basin. Differences in the stoichiometric proportions of carbon, nitrogen, and phosphorus can influence the elemental composition of primary producers and have cascading effects on higher trophic levels (Frost et al., 2002; Evans-White et al., 2009). For example, periphyton nutrient ratios have been shown to track the nutrient ratios of stream water in artificial streams (Stelzer & Lamberti, 2001), and O'Brien and Wehr (2010) found that periphyton N:P ratios in streams of the US mid-Atlantic increased in association with nutrient concentrations over an urban to agricultural land use gradient. Moreover, in the tributaries of the lower Great Lakes basin, wetland cover was positively associated with C:N and C:P ratios in stream seston (Prater et al., 2017) which coincides with our observation that catchments with greater wetland cover had increased stoichiometric proportions of carbon in stream water. Liess et al., (2012) reported that increased nutrient concentrations and stream water N:P ratios along a gradient of agricultural intensity altered periphyton C:N:P stoichiometry and resulted in decreased invertebrate taxon richness in streams of southern New Zealand. Thus, bottom up effects produced by spatial differences in stream water C:N:P stoichiometry may contribute to spatial patterns in the structure of stream communities. However, further studies are needed to understand the constraints imposed by water stoichiometry on the biotic integrity of higher trophic levels in southern Ontario streams.

We found that landscape alteration from natural to agricultural land covers could shift the Redfield C:N:P stoichiometry of streams from nitrogen and phosphorus co-depleted to phosphorus depleted. Depletion corresponds to the relative biotic demand for individual nutrients, and changes to which nutrient is depleted can influence uptake lengths in streams (Frost et al., 2002). For example, Drake et al., (2012) found that phosphorus-depleted stream periphyton with high C:P ratios increased phosphorus immobilization in a microcosm experiment. Likewise, stream water N:P ratios were negatively correlated with nitrogen uptake velocities in forested headwater streams of the US mid-Atlantic (Gibson et al., 2015). Even though variation abiotic sorption and release of phosphorus can have ramifications for biotic phosphorus uptake and also influence nutrient transport in streams (Lottig & Stanley, 2007; Jarvie et al., 2012), the increase we observed in the stoichiometric proportion of nitrogen in agricultural streams may result in greater nitrogen transport with little to no change in the biotic demand for phosphorus. Although C:N:P ratios in stream water have been shown to influence primary producers and their associated stream processes (Stelzer & Lamberti, 2001; Dodds et al., 2004), the effects of stoichiometry on nutrient spiralling may be further diminished in productive streams with enriched nutrient concentrations (Schade et al., 2011). Prioritizing nutrient mitigation through stoichiometric ratios that increase the capability of streams to retain nitrogen and phosphorus has the potential to improve eutrophication management in developed watersheds (Stutter et al., 2018). However, further research is required to understand the ecosystem level implications of varying stream water C:N:P stoichiometry on nutrient transport in enriched streams.

2.4.3 Conclusions

Increasing disturbance in human-altered watersheds can exacerbate impairment of streams and downstream ecosystems from nutrient enrichment. Overall, we found that 55% of routinely monitored streams in the lower Great Lakes-St. Lawrence basin of Ontario fail to meet regional TN and TP concentration guidelines (TN = 1.06 mg L^{-1}; TP = 0.026 mg L^{-1}) and that streams with nutrient concentrations below regional guidelines were primarily co-limited for nitrogen and phosphorus. Moreover, the depletion of individual nutrients within streams was temporally stable over the 10-year study period and did not reveal seasonal variation in the potential for nutrient limitation. Catchments influenced by agricultural land cover were associated with increasing phosphorus depletion through the larger increase in stream nitrogen relative to phosphorus. However, enriched nutrient concentrations in these agricultural streams suggests that autotrophic nutrient uptake is likely saturated. Therefore, to reduce downstream nutrient transport from developed watersheds, absolute nutrient concentrations need to be reduced to increase nutrient demand by stream biota and balance nutrient inputs with instream processing. Moreover, our assessment shows that nitrogen enrichment is widespread in tributaries of the lower Great Lakes, and that nitrogen may be an important regulator of stream production in less disturbed watersheds. Thus, while phosphorus remains the primary nutrient of concern in the Great Lakes-St. Lawrence basin (Munawar & Fitzpatrick, 2018; Mohamed et al., 2019), our study supports the need for simultaneous management of nitrogen and phosphorus. Indeed, we recommend that in human-altered streams currently experiencing eutrophication that management practices to reduce both nitrogen and phosphorus are needed to bring about ecosystem improvements. We also recommend that in less disturbed streams controls be put in place that limit future inputs of both nitrogen and phosphorus to most effectively minimize the risk of stream degradation by eutrophication.

Our results indicate that the management of land cover in hydrologically connected locations could be an effective approach to reduce stream nutrient concentrations. Past studies have recommended that the implementation of agricultural best management practices, particularly vegetated riparian buffers, in these critical source areas can reduce nutrient loading to streams (Pearce & Yates, 2017). Our findings further suggest that the reestablishment of nutrient retentive natural and wetland cover in hydrologically active agricultural areas of stream catchments can provide added benefits for nutrient management in the Great Lakes-St. Lawrence basin. Moreover, other nutrient management strategies not examined in this study may represent alternative measures to reduce nutrient concentrations in streams that drain agricultural and urban catchments. For example, the adoption of structural (e.g., tile drainage controls and livestock exclusion fences) and managerial (e.g., nutrient stewardship and conservation tillage) best management practices have been shown to reduce nutrient losses from agricultural landscapes (Kerr et al., 2016). In addition, managers may consider sewage treatment upgrades and infrastructure maintenance in combination with other structural practices that intercept overland runoff (e.g., storm water management and bioswales) to reduce nutrient loading from urban catchments (McFarland et al., 2019). Management practices can reduce the total nutrient load exported from watersheds, thus investing in strategies to reduce stream nutrient concentrations has the potential to improve stream ecosystem conditions while reducing eutrophication risk in downstream lakes and nearshore areas affected by nutrient loading from human-altered tributaries (Dodds & Smith, 2016; Paerl et al., 2016).

2.5 References

Agriculture and Agri-Food Canada (AAFC). (2019). Annual Crop Inventory 2009-2016 [Raster Dataset]. Government of Canada; Agriculture and Agri-Food Canada; Science and Technology Branch. Available from: https://open.canada.ca/data/en/dataset/ba2645d5-4458-414d-b196-6303ac06c1c9

Allan, J. D. (2004). Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics, 35*(1), 257–284. doi: 10.1146/annurev.ecolsys.35.120202.110122

Allan, J. D., Erickson, D. L., & Fay, J. (1997). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology, 37*(1), 149–161. doi: 10.1046/j.1365-2427.1997.d01-546.x

Baker, D. B., Johnson, L. T., Confesor, R. B., Crumrine, J. P., Guo, T., & Manning, N. F. (2019). Needed: Early-term adjustments for Lake Erie phosphorus target loads to address western basin cyanobacterial blooms. *Journal of Great Lakes Research, 45*(2), 203–211. doi: 10.1016/j.jglr.2019.01.011

Baldwin, D. J. B., Desloges, J. R., & Band, L. E. (2000). Physical Geography of Ontario. In: Perera A. H., Euler, D. L., & Thompson, I. D. (Ed.) *Ecology of a Managed Terrestrial Landscape: Patterns and Processes of Forest Landscapes in Ontario* (pp. 12-29). Vancouver, BC: UBC Press.

Beck, W. S., Rugenski, A. T., & Poff, N. L. R. (2017). Influence of experimental, environmental, and geographic factors on nutrient-diffusing substrate experiments in running waters. *Freshwater Biology, 62*(100), 1667–1680. doi: 10.1111/fwb.12989

Brett, M. T., Arhonditsis, G. B., Mueller, S. E., Hartley, D. M., Frodge, J. D., & Funke, D. E. (2005). Non-point-source impacts on stream nutrient concentrations along a forest to urban gradient. *Environmental Management, 35*(3), 330–342. doi: 10.1007/s00267-003-0311-z

Canadian Council of Ministers of the Environment (CCME). (2016). Guidance manual for developing nutrient guidelines for rivers and streams. ISBN 978-1-77202-022-9.

Carey, R. O., & Migliaccio, K. W. (2009). Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. *Environmental Management, 44*(2), 205–217. doi: 10.1007/s00267-009-9309-5

Chambers, P. A., Brua, R. B., McGoldrick, D. J., Upsdell, B. L., Vis, C., Culp, J. M., & Benoy, G. A. (2008). Nitrogen and Phosphorus Standards to Protect Ecological Condition of Canadian Streams in Agricultural Watersheds. National Agri-Environmental Standards Initiative Technical Series Report No. 4-56. 101 p.

Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A. (2012). Development of environmental thresholds for nitrogen and phosphorus in streams. *Journal of Environmental Quality*, *41*(1), 7–20. doi: 10.2134/jeq2010.0273

Chen, X., & Driscoll, C. T. (2009). Watershed land use controls on chemical inputs to Lake Ontario embayments. *Journal of Environmental Quality*, *38*(5), 2084–2095. doi: 10.2134/jeq2007.0435

Crins, W. J., Gray, P. A., Uhlig, P. W., & Wester, M. C. (2009). The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions. Ontario Ministry of Natural Resources; Science and Information Branch; Inventory, Monitoring and Assessment Section. Technical Report SIB TER IMA TR-01 1–76.

Danger, M., Daufresne, T., Lucas, F., Pissard, S., & Lacroix, G. (2008). Does Liebig's law of the minimum scale up from species to communities? *Oikos*, *117*(11), 1741–1751. doi: 10.1111/j.1600-0706.2008.16793.x

Dodds, W. K. (2003). Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *Journal of the North American Benthological Society*, *22*(2), 171–181. doi: 10.2307/1467990

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., … Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science and Technology*, *43*(1), 12–19. doi: 10.1021/es801217q

Dodds, W. K., Martí, E., Tank, J. L., Pontius, J., Hamilton, S. K., Grimm, N. B., … Gregory, S. (2004). Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. *Oecologia*, *140*(3), 458–467. doi: 10.1007/s00442-004-1599-y

Dodds, W. K., & Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters, 6*(2), 155–164. doi: 10.5268/IW-6.2.909

Drake, W. M., Scott, J. T., Evans-White, M., Haggard, B., Sharpley, A., Rogers, C. W., & Grantz, E. M. (2012). The effect of periphyton stoichiometry and light on biological phosphorus immobilization and release in streams. *Limnology, 13*(1), 97–106. doi: 10.1007/s10201-011-0359-z

Dupas, R., Musolff, A., Jawitz, J. W., Rao, P. S. C., Jäger, C. G., Fleckenstein, J. H., … Borchardt, D. (2017). Carbon and nutrient export regimes from headwater catchments to downstream reaches. *Biogeosciences, 14*(18), 4391–4407. doi: 10.5194/bg-14-4391-2017.

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

Esri inc., 2019. ArcGIS Desktop. Version 10.7. Environmental Systems Research Institute, Redlands, California, USA.

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality, 42*(4), 1002–1014. doi: 10.2134/jeq2012.0491

Evans-White, M. A., Dodds, W. K., Huggins, D. G., & Baker, D. S. (2009). Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society, 28*(4), 855–868. doi: 10.1899/08-113.1

Francoeur, S. N. (2001). Meta-analysis of lotic nutrient amendment experiments: Detecting and quantifying subtle responses. *Journal of the North American Benthological Society, 20*(3), 358–368. doi: 10.2307/1468034

Frost, P. C., Stelzer, R. S., Lamberti, G. A., & Elser, J. J. (2002). Ecological stoichiometry of trophic interactions in the benthos: Understanding the role of C:N:P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society, 21*(4), 515–528. doi: 10.2307/1468427

Gibson, C. A., Reilly, C. M. O., Conine, A. L., & Lipshutz, S. M. (2015). Nutrient uptake dynamics across a gradient of nutrient concentrations and ratios at the landscape scale. *Journal of Geophysical Research Biogeosciences, 120*(2), 326–340. doi: 10.1002/2014JG002747

Giri, S., & Qiu, Z. (2016). Understanding the relationship of land uses and water quality in Twenty First Century: A review. *Journal of Environmental Management, 173*, 41–48. doi: 10.1016/j.jenvman.2016.02.029

Green, M.B., & Finlay, J.C. (2010). Patterns of hydrologic control over stream water total nitrogen to total phosphorus ratios. *Biogeochemistry, 99*(1-3), 15–30. doi: 10.1007/s10533-009-9394-9

Green, M. B., Nieber, J. L., Johnson, G., Magner, J., & Schaefer, B. (2007). Flow path influence on an N:P ratio in two headwater streams: A paired watershed study. *Journal of Geophysical Research Biogeosciences, 112*(G3), 1–11. doi: 10.1029/2007JG000403

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E., … Smith, J. E. (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters, 14*(9), 852–862. doi: 10.1111/j.1461-0248.2011.01651.x

Hillebrand, H., & Sommer, U. (1999). The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography, 44*(2), 440–446. doi: 10.4319/lo.1999.44.2.0440

Ho, J. C., & Michalak, A. M. (2017). Phytoplankton blooms in Lake Erie impacted by both long-term and springtime phosphorus loading. *Journal of Great Lakes Research, 43*(3), 221–228. doi: 10.1016/j.jglr.2017.04.001

Jarvie, H. P., Sharpley, A. N., Scott, J. T., Haggard, B. E., Bowes, M. J., & Massey, L. B. (2012). Within-river phosphorus retention: Accounting for a missing piece in the watershed phosphorus puzzle. *Environmental Science and Technology*, *46*, 13284–13292. doi: 10.1021/es303562y

Jarvie, H. P., Smith, D. R., Norton, L. R., Edwards, F. K., Bowes, M. J., King, S. M., … Bachiller-Jareno, N. (2018). Phosphorus and nitrogen limitation and impairment of headwater streams relative to rivers in Great Britain: A national perspective on eutrophication. *Science of the Total Environment*, *621*, 849–862. doi: 10.1016/j.scitotenv.2017.11.128

Jarvie, H. P., Withers, P. J. A., Hodgkinson, R., Bates, A., Neal, M., Wickham, H. D., … Armstrong, L. (2008). Influence of rural land use on streamwater nutrients and their ecological significance. *Journal of Hydrology*, *350*(3-4), 166–186. doi: 10.1016/j.jhydrol.2007.10.042

Johnson, L. B., Richards, C., Host, G. E., & Arthur, J. W. (1997). Landscape influences on water chemistry in Midwestern stream ecosystems. *Freshwater Biology*, *37*(1), 193–208. doi: 10.1046/j.1365-2427.1997.d01-539.x

Kane, D. D., Conroy, J. D., Peter Richards, R., Baker, D. B., & Culver, D. A. (2014). Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *Journal of Great Lakes Research*, *40*(3), 496–501. doi: 10.1016/j.jglr.2014.04.004

Keck, F., & Lepori, F. (2012). Can we predict nutrient limitation in streams and rivers? *Freshwater Biology*, *57*(7), 1410–1421. doi: 10.1111/j.1365-2427.2012.02802.x

Keitzer, S. C., Ludsin, S. A., Sowa, S. P., Annis, G., Arnold, J. G., Daggupati, P., … Rewa, C. A. (2016). Thinking outside of the lake: Can controls on nutrient inputs into Lake Erie benefit stream conservation in its watershed? *Journal of Great Lakes Research*, *42*(6), 1322–1331. doi: 10.1016/j.jglr.2016.05.012

Kerr, J. M., Depinto, J. V., McGrath, D., Sowa, S. P., & Swinton, S. M. (2016). Sustainable management of Great Lakes watersheds dominated by agricultural land use. *Journal of Great Lakes Research 42*(6), 1252–1259. doi: 10.1016/j.jglr.2016.10.001

King, R. S., Baker, M. E., Whigham, D. F., Weller, D. E., Jordan, T. E., Kazyak, P. F., & Hurd, M. K. (2005). Spatial considerations for linking watershed land cover to ecological indicators in streams. *Ecological Applications*, *15*(1), 137–153. doi: 10.1890/04-0481

Klausmeier, C. A., Litchman, E., Daufresne, T., & Levin, S. A. (2004). Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature*, *429*(6988), 171–174. doi: 10.1038/nature02454

Liess, A., Le Gros, A., Wagenhoff, A., Townsend, C. R., & Matthaei, C. D. (2012). Landuse intensity in stream catchments affects the benthic food web: Cconsequences for nutrient supply, periphyton C:nutrient ratios, and invertebrate richness and abundance. *Freshwater Science*, *31*(3), 813–824. doi: 10.1899/11-019.1

Lottig, N.R., & Stanley, E.H. (2007). Benthic sediment influence on dissolved phosphorus concentrations in a headwater stream. *Biogeochemistry*, *84*, 297–309. doi: 10.1007/s10533-007-9116-0

Lowe, R. L., & Likens, G. E. (2005). Moving headwater streams to the head of the class. *BioScience*, *55*(3), 196–197. doi: 10.1641/0006-3568(2005)055[0196:MHSTTH]2.0.CO;2

Maccoux, M. J., Dove, A., Backus, S. M., & Dolan, D. M. (2016). Total and soluble reactive phosphorus loadings to Lake Erie a detailed accounting by year, basin, country, and tributary. *Journal of Great Lakes Research*, *42*(6), 1151–1165. doi: 10.1016/j.jglr.2016.08.005

Maier, M. J. (2015). DirichletReg: Dirichlet regression in R. Version 0.6-3.1.

Maier, M. J. (2014). DirichletReg: Dirichlet Regression for Compositional Data in R. Research Report Series / Department of Statistics and Mathematics, 125. WU Vienna

University of Economics and Business, Vienna. Available from: https://epub.wu.ac.at/4077/

McFarland, A. R., Larsen, L., Yeshitela, K., Engida, A. N., & Love, N. G. (2019). Guide for using green infrastructure in urban environments for stormwater management. *Environmental Science: Water Research and Technology, 5,* 643–659. doi: 10.1039/c8ew00498f

Miller, J. D., Schoonover, J. E., Williard, K. W. J., & Hwang, C. R. (2011). Whole catchment land cover effects on water quality in the Lower Kaskaskia River Watershed. *Water, Air, and Soil Pollution, 221*(1-4), 337–350. doi: 10.1007/s11270-011-0794-9

Mohamed, M. N., Wellen, C., Parsons, C. T., Taylor, W. D., Arhonditsis, G., Chomicki, K. M., … Haffner, D. G. (2019). Understanding and managing the re-eutrophication of Lake Erie: Knowledge gaps and research priorities. *Freshwater Science, 38*(4), 675–691. doi: 10.1086/705915

Morrissey, M. B., & Ruxton, G. D. (2018). Multiple regression is not multiple regressions: The meaning of multiple regression and the non-problem of collinearity. *Philosophy Theory and Practice in Biology, 10*(3), 1–24. doi: 10.3998/ptpbio.16039257.0010.003

Munawar, M., & Fitzpatrick, M. A. J. (2018). Eutrophication in three Canadian Areas of Concern: Phytoplankton and major nutrient interactions. *Aquatic Ecosystem Health and Management, 21*(4), 421–437. doi: 10.1080/14634988.2018.1530895

O’Brien, P.J., & Wehr, J.D. (2010). Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. *Hydrobiologia, 657*(1), 89–105. doi: 10.1007/s10750-009-9984-5

Ontario Ministry of Energy, Northern Development, and Mines (OMENDM). (2017). Physiography of Southern Ontario [Digital Map File]. Available from: https://www.mndm.gov.on.ca/en/mines-and-minerals/applications/ogsearth/physiography

Ontario Ministry of Natural Resources and Forestry (OMNRF). (2015). Provincial digital elevation model [Raster Dataset]. Available from: https://data.ontario.ca/dataset/provincial-digital-elevation-model

Ontario Ministry of the Environment Conservation and Parks (OMECP). (2018). Provincial (Stream) Water Quality Monitoring Network [2000-2016 MDB dataset]. Available from: https://data.ontario.ca/dataset/provincial-stream-water-quality-monitoring-network

Paerl, H. W., Scott, J. T., McCarthy, M. J., Newell, S. E., Gardner, W. S., Havens, K. E., … Wurtsbaugh, W. A. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environmental Science and Technology, 50*(20), 10805–10813. doi: 10.1021/acs.est.6b02575

Paul, M. J., & Meyer, J. L. (2001). Streams in the urban landscape. *Annual Review of Ecology, Evolution, and Systematics, 32*(1), 333–365. doi: 10.1146/annurev.ecolsys.32.081501.114040

Pearce, N. J. T., & Yates, A. G. (2017). Intra-annual variation of the association between agricultural best management practices and stream nutrient concentrations. *Science of the Total Environment, 586*, 1124–1134. doi: 10.1016/j.scitotenv.2017.02.102

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., … Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications, 4*(2394), 1–10. doi: 10.1038/ncomms3934

Peterson, E. E., Sheldon, F., Darnell, R., Bunn, S. E., & Harch, B. D. (2011). A comparison of spatially explicit landscape representation methods and their relationship to stream condition. *Freshwater Biology, 56*(3), 590–610. doi: 10.1111/j.1365-2427.2010.02507.x

Poikane, S., Kelly, M. G., Salas Herrero, F., Pitt, J., Jarvie, H. P., Claussen, U., … Phillips, G. (2019). Nutrient criteria for surface waters under the European Water Framework Directive: Current state-of-the-art, challenges and future outlook. *Science of the Total Environment, 695*, 1–14. doi: 10.1016/j.scitotenv.2019.133888

Prater, C., Frost, P. C., Howell, E. T., Watson, S. B., Zastepa, A., King, S. S. E., … Xenopoulos, M. A. (2017). Variation in particulate C : N : P stoichiometry across the Lake Erie watershed from tributaries to its outflow. *Limnology and Oceanography, 62*(S1), S194–S206. doi: 10.1002/lno.10628

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.6.1.

Raney, S. M., & Eimers, M. C. (2014). A comparison of nutrient export at two agricultural catchments: Insight into the effect of increasing urban land cover in southern Ontario. *Hydrological Processes, 28*(14), 4328–4339. doi: 10.1002/hyp.10215

Rattan, K. J., & Chambers, P. A. (2017). Total, dissolved and particulate N:P stoichiometry in Canadian prairie streams in relation to land cover and hydrologic variability. *Proceedings, 2*(5), 183. doi: 10.3390/ecws-2-04952

Redfield, A. C. (1958). The biological control of chemical factors in the environment. *American Scientist, 46*, 205–221.

Schade, J. D., Macneill, K., Thomas, S. A., Camille Mcneely, F., Welter, J. R., Hood, J., … Finlay, J. C. (2011). The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. *Freshwater Biology, 56*(3), 424–436. doi: 10.1111/j.1365-2427.2010.02509.x

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science and Technology, 50*(17), 8923–8929. doi: 10.1021/acs.est.6b02204

Smith, D. R., & Jarvie, H. P. (2018). Carbon, nitrogen, and phosphorus stoichiometric response to hydrologic extremes in a tributary to Lake Erie, USA. *Agricultural and Environmental Letters, 3*(1), 1–4. doi: 10.2134/ael2018.08.0043

Smith, D. R., Jarvie, H. P., & Bowes, M. J. (2017). Carbon, nitrogen, and phosphorus stoichiometry and eutrophication in River Thames tributaries, UK. *Agricultural and Environmental Letters, 2*(1), 1–4. doi: 10.2134/ael2017.06.0020

Smith, M. R. (2017). Ternary: An R package for creating ternary plots. Version 1.1.3.

Staponites, L. R., Barták, V., Bílý, M., & Simon, O. P. (2019). Performance of landscape composition metrics for predicting water quality in headwater catchments. *Scientific Reports, 9*(14405), 1–10. doi: 10.1038/s41598-019-50895-6

Stelzer, R. S., & Lamberti, G. A. (2001). Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography, 46*(2), 356–367. doi: 10.4319/lo.2001.46.2.0356

Stevenson, J. (2014). Ecological assessments with algae: a review and synthesis. *Journal of Phycology, 50*(3), 437–461. doi: 10.1111/jpy.12189

Stumpf, R. P., Wynne, T. T., Baker, D. B., & Fahnenstiel, G. L. (2012). Interannual variability of cyanobacterial blooms in Lake Erie. *PLoS One, 7*(8), 1–11. doi: 10.1371/journal.pone.0042444

Stutter, M. I., Graeber, D., Evans, C. D., Wade, A. J., & Withers, P. J. A. (2018). Balancing macronutrient stoichiometry to alleviate eutrophication. *Science of the Total Environment, 634*, 439–447. doi: 10.1016/j.scitotenv.2018.03.298

Tank, J. L., & Dodds, W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology, 48*(6), 1031–1049. doi: 10.1046/j.1365-2427.2003.01067.x

Tank, J. L., Rosi-Marshall, E. J., Griffiths, N. A., Entrekin, S. A., Stephen, M. L. (2010). A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society, 29*(1), 118–146. doi: 10.1899/08-170.1

Thomas, K. E., Lazor, R., Chambers, P. A., & Yates, A. G. (2018). Land-use practices influence nutrient concentrations of southwestern Ontario streams. *Canadian Water Resource Journal, 43*(1), 2–17. doi: 10.1080/07011784.2017.1411211

Turner, R. E., Rabalais, N. N., Justic, D., & Dortch, Q. (2003). Global patterns of dissolved N, P and Si in large rivers. *Biogeochemistry, 64*, 297–317. doi: 10.1023/A:1024960007569

van den Boogaart, K. G., & Tolosana-Delgado, M. B. (2019). compositions: Compositional data analysis. Version 1.40-3.

Vermeesch, P. (2019). Exploratory analysis of provenance data using R and the provenance package. *Minerals, 9*(3), 193. doi: 10.3390/min9030005

Walsh, C. J., & Webb, J. A. (2014). Spatial weighting of land use and temporal weighting of antecedent discharge improves prediction of stream condition. *Landscape Ecology, 29*(7), 1171–1185. doi: 10.1007/s10980-014-0050-y

Wilson, H. F., & Xenopoulos, M. A. (2008). Ecosystem and seasonal control of stream dissolved organic carbon along a gradient of land use. *Ecosystems, 11*(4), 555–568. doi: 10.1007/s10021-008-9142-3

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *Wiley Interdisciplinary Reviews Water, 6*(5), 1–27. doi: 10.1002/wat2.1373

Chapter 3

3 Intra-annual variation of the association between agricultural best management practices and stream nutrient concentrations

A version of this chapter has been published (Pearce & Yates, 2017. Science of the Total Environment. doi: 10.1016/j.scitotenv.2017.02.102).

3.1 Introduction

Nutrient enrichment is a global cause of surface water impairment (Smith, 2003). Increased nitrogen and phosphorus loadings can increase primary production and result in the eutrophication of freshwater ecosystems (Anderson et al., 2002). Eutrophication can degrade water quality, reduce aquatic biodiversity, and impair the delivery of freshwater ecosystem services. The recent recurrence of eutrophic conditions and algal blooms in Lake Erie has led Canada and the United States to jointly adopt phosphorus management objectives to reduce phosphorus loading from tributaries draining into the western and central basins of Lake Erie (Objectives and Targets Task Team, 2015). Management objectives are primarily focused on reducing phosphorus loading from nonpoint sources, which have been shown to contribute approximately 50% of the soluble reactive phosphorus load and 70% of the total phosphorus load to Lake Erie (Maccoux et al., 2016). Identifying effective strategies for management of landscape derived nutrients is thus a priority for achieving phosphorus reduction objectives.

Mitigation of nonpoint source pollution from agricultural landscapes has increasingly been pursued through the implementation of various managerial (e.g., nutrient management plans, conservation tillage, and livestock grazing management) and structural (e.g., manure storage facilities, vegetated buffer areas, and livestock exclusion fences) best management practices (BMPs) (Kleinman et al., 2015). Managerial BMPs target on-farm procedures and aim to control the addition of pollutants onto the landscape. In contrast, structural BMPs are designed to impede and intercept pollutants in agricultural runoff through the filtration, cementation, and uptake of contaminants before they are lost to aquatic systems. In addition, structural BMPs can be strategically located on the landscape in areas that contribute to or receive large amounts of water runoff.

Evaluating the effectiveness of agricultural BMPs at basin-wide scales is often impractical and becomes increasingly difficult without widespread implementation and time for mitigation effects to be realized (Makarewicz, 2009; Meals et al., 2010). Thus, the majority of studies evaluating the large scale, cumulative benefits of agricultural BMPs have taken a modeling approach (e.g., Bracmort et al., 2006; Rao et al., 2009; Zhang & Zhang, 2011; Bosch et al., 2013; Bosch et al., 2014), although exceptions, such as the U.S. Environmental Protection Agency's National Nonpoint Source Monitoring Program (Spooner et al., 2011), exist. However, small, headwater streams can act as early indicators of landscape effects on larger downstream ecosystems and provide an opportunity to assess the influence of BMPs in the field (Yates & Bailey, 2006). Past field studies, conducted at small spatial and temporal scales, have found that BMPs are effective conservation strategies and in many cases agricultural catchments with abundant BMPs have shown reductions in stream nutrient concentrations (Brannan et al., 2000; Inamdar et al., 2002; Bishop et al., 2005; Li et al., 2011). For example, Makarewicz et al., (2009) found that BMPs were able to reduce instream total phosphorus concentrations by up to 47% five years post implementation in catchments of the Finger Lakes, New York. Likewise, Richards et al., (2009) attributed observed reductions in suspended sediments and particulate phosphorus concentrations in two major tributaries of Lake Erie to continual implementation of agricultural management practices between 1975 and 2004. However, nutrient load reductions in Lake Erie tributaries appeared to be the smallest during high flow conditions in spring and winter outlining opportunities for further management (Richards et al., 2009). Additional past monitoring studies that have evaluated BMP implementation in agricultural catchments have also outlined variable results in regard to nutrient load reductions among seasons (e.g., Bishop et al., 2005; Edwards et al., 1997). These results are likely attributable to intra-annual variability in catchment hydrology, however, there is a lack of empirical, field assessments of intra-annual variation in BMP mitigation effects.

The potential for contaminants to be transported from agricultural landscapes into aquatic ecosystems is associated with climatic conditions and catchment scale characteristics that control overland and subsurface water movement. Numerous factors can influence hydrologic flow patterns including slope, soil type, vegetated cover, and location on the landscape (Jones et al., 2001). Based on these factors, targeting hydrologically connected locations prone to generating runoff and transporting contaminants has been recognized as an important consideration in BMP implementation (Walter et al., 2000) and is predicted to enhance BMP effectiveness (Veith et al., 2003; Tomer et al., 2009; Kaini et al., 2012). Indeed, many modeling studies have shown that targeted BMP placement can more effectively reduce nutrient losses than randomized BMP placement (Strauss et al., 2007; Giri et al., 2012; Rittenburg et al., 2015), however, few empirical field studies exist to support these findings.

BMPs designed to enhance the subsurface flow of water into streams for optimal crop growth and to reduce surface water erosion can also influence hydrologic flow patterns. Tile drainage systems can be implemented systematically throughout an agricultural field or targeted to poorly drained areas to facilitate the removal of surplus water. However, tile drains may counter the benefits realized by nutrient management BMPs by increasing the subsurface flow of nutrient rich water and limiting the filtration of runoff by riparian buffers (Osborne and Kovacic, 1993; Lemke et al., 2011). Tile drainage systems are therefore an important management strategy to investigate in tandem with other structural BMPs in order to understand the influence that common agricultural management practices have on instream nutrient concentrations at catchment scale.

The goal of our study was to assess temporal variation in cumulative effects of four common, structural BMPs (i.e. manure storage facilities (MS), livestock access restriction fences (LAR), riparian buffer area (RBA), and tile drainage (TD)) on instream nutrient concentrations at the catchment scale. We achieved this goal by quantifying the relationship between stream nutrient concentrations and metrics of BMP abundance and spatial location in 15 headwater catchments for each month over a hydrologic year. We predicted that for MS, LAR and RBA a greater abundance and greater degree of hydrologic connectivity would be associated with lower instream nutrient concentrations.

In contrast, we expected that increased use of TD systems would be associated with greater stream nutrient concentrations. Findings outline the influence that individual agricultural management practices have on instream nutrient concentrations throughout a hydrologic year and can be used to inform future BMP implementation strategies.

3.2 Methods

3.2.1 Study area

Our study area was located in the Lake Erie basin within the headwaters of the Grand River watershed (GRW), Ontario, Canada (Figure 3-1). Climate in the GRW is temperate with an average (± standard deviation) annual temperature of 7.3^{o}C (± 0.8^{o}C), total precipitation of 844.8 mm (± 140.5 mm), and snowfall of 146.9 cm (± 60.4 cm) (Government of Canada, 2016). At the regional scale, our study period (November 2013 – October 2014) was comparable and within one standard deviation of climate normals (1994 – 2013) in terms of annual precipitation (769.7 mm) and snowfall (192.2 cm), however, the average temperature (5.2^{o}C) throughout the hydrologic year was substantially colder and greater than two standard deviations of the 20-year average (Government of Canada, 2016). Land use in the GRW is dominated by agriculture, which covers approximately 75% of the 6,800 km^2 watershed (GRCA, 2008). Agricultural activities are predominantly a combination of row crops, including soybeans and corn, and various livestock operations of dairy cattle, beef, hog, and poultry (Yates & Bailey, 2010). Our study sites were established on 15, second or third order, agricultural streams in headwater catchments of the Nith and Conestogo subwatersheds (Figure 3-1 b.).

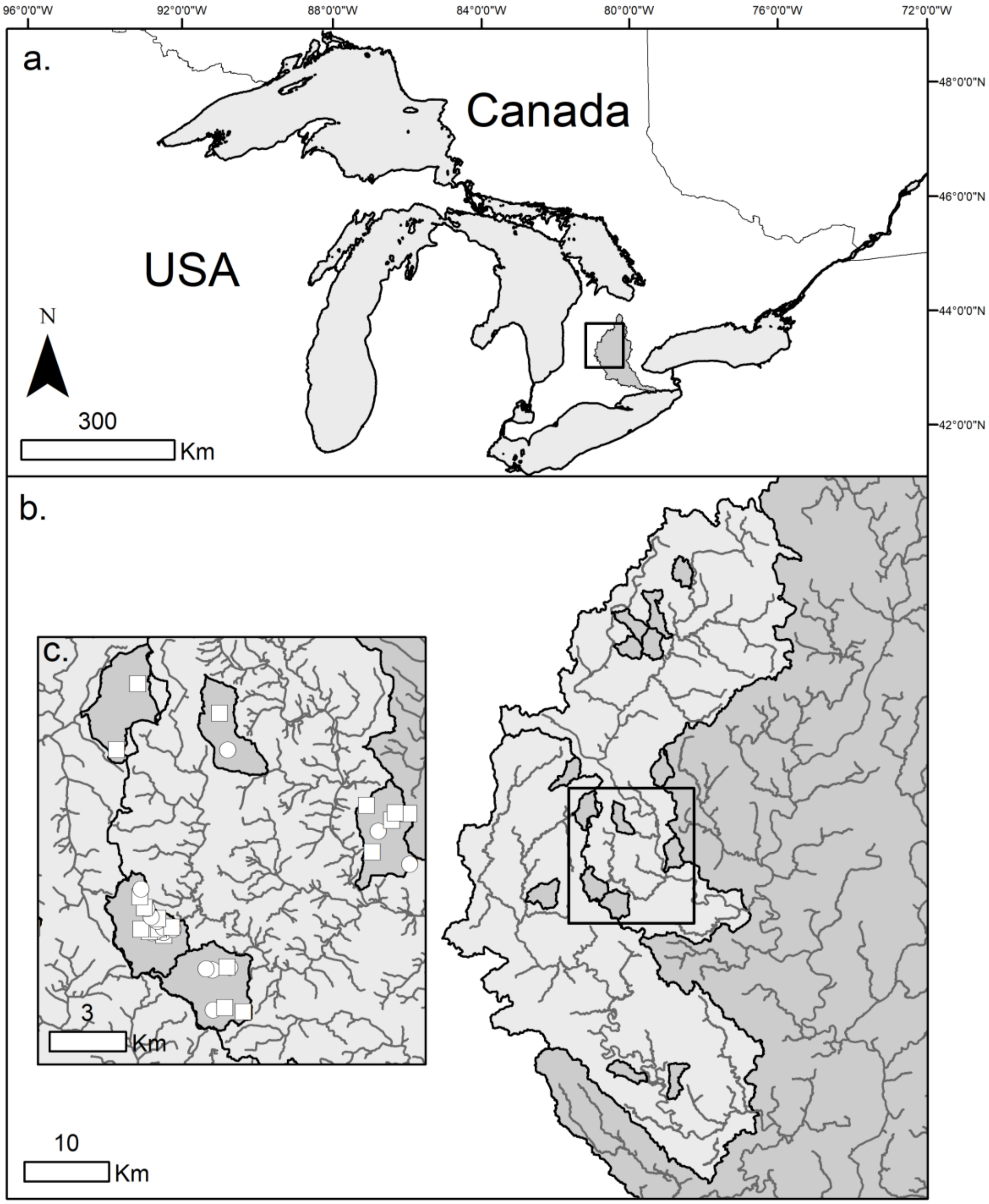

Figure 3-1. (a) Map showing the location of the Grand River Watershed (dark grey) in southern Ontario, Canada. (b) 15 study catchments were located throughout the Conestogo (n = 10) and Nith (n = 5) subwatersheds (light grey) and (c) represented a gradient of BMP implementation (e.g., circle = livestock access restriction fence, square = manure storage structure).

The Nith and Conestogo subwatersheds are characterized by intensive agricultural land use (>80%), glacial till soils, and a rolling to flat topography (Loomer & Cooke, 2011). Catchments of candidate study streams were selected to encompass a gradient of BMP implementation, while minimizing variation in physiography, land cover characteristics and dominant crop types using spatial information obtained from the Ontario Geological Survey and Agriculture and Agri-Food Canada (Table 3-1). Study catchment areas varied between 5 – 12 km^2 and were composed primarily of till (diamicton) soils (>55%). Study catchments had an average of 1.58 farms per km^2 and were dominated by agriculture (>75%), with low urban (<5%) and natural (<25%) land covers. Livestock, primarily cattle, were present in all catchments but specific numbers were not publicly available. During base flow conditions, study streams had an average (± standard deviation) width, depth, and velocity of 1.65 m (± 0.55), 0.14 m (± 0.06), and 0.05 m/s (± 0.05), respectively.

Table 3-1. Descriptive statistics of catchment land cover, physiography, and agricultural attributes for the 15 selected headwater catchments studied in the Grand River Watershed.

Catchment Attributes	**Mean**	**St. Dev.**	**CV**	**Med.**	**Max.**	**Min.**
Area (km^2)	7.06	1.25	0.18	6.90	9.61	5.43
# of farms / km^2	1.58	0.60	0.38	1.49	2.42	0.45
Land Cover						
% Agriculture	86.5	5.1	0.06	86.6	96.9	76.8
% Natural	11.0	5.1	0.46	11.3	21.5	1.1
% Urban	2.4	1.1	0.44	2.1	5.8	1.4
Surficial Geology						
% Silt	3.2	6.2	1.94	0.4	19.4	0.0
% Sand	11.2	8.9	0.79	10.0	28.5	0.0
% Organic	1.9	2.7	1.41	0.4	7.9	0.0
% Gravel	1.3	1.7	1.34	0.0	5.1	0.0
% Diamicton	82.4	12.0	0.15	82.6	99.2	56.6
Dominant Agricultural Activity						
% Pasture and Forage	26.3	9.9	0.38	29.9	41.5	11.3
% Wheat	11.7	5.4	0.46	11.4	22.2	3.5
% Corn	27.4	6.8	0.25	26.1	40.7	14.1
% Soybeans	18.2	8.7	0.48	19.3	37.0	7.1

3.2.2 Field sampling

Instream nutrient concentrations were measured in our 15 study streams by collecting grab water samples during near-baseflow conditions the first week of each month over the course of a hydrologic year (November 2013 – October 2014). Samples were collected in all months except February and March when the study streams were frozen. An additional sample was collected from each stream in the months of April and June accounting for seasonal variation in snowmelt and storm events for a total of 12 sampling events over the study period. Grab samples were collected according to the Canadian Aquatic Biomonitoring Network (CABIN) protocols by sampling in a well-mixed area of the stream at 60% depth (Reynoldson et al., 2012). Water samples were analyzed colorimetrically for major nutrient forms of nitrogen (total nitrogen (TN), ammonia + ammonium (NH_3-N), nitrite-nitrate (NO_3^-+NO_2^-)) and phosphorus (total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP)) using standard methods (Rice et al., 2012). Two samples at one individual site (Cons-02) collected in the months of April and September were compromised during transport to the lab and were excluded from the study.

3.2.3 Best management practice metrics

Study streams had catchments with varying abundances and locations of four common, structural BMPs: 1) manure storage structures (MS); 2) livestock access restriction fences (LAR); 3) riparian buffer areas (RBA), and; 4) tile drainage systems (TD) (Table 3-2). The majority of MS and LAR BMPs in the study catchments were implemented through the Grand River Conservation Authority's (GRCA) Rural Water Quality Program (RWQP). The RWQP offers financial assistance to agricultural landowners for the implementation of conservation practices. MS and LAR BMPs implemented through the RWQP ranged from 3 to 16 years old with the average BMP having been constructed 10 years prior to the end of our sampling period (October 2014). Structural BMPs implemented through the GRCA were located in our study catchments using the RWQP project database. Additional BMPs constructed through independent sources and the amount of intact RBA in each catchment were identified using high-resolution (0.5 m) orthoimagery from the Ontario Ministry of Natural Resources. The relative extent of TD in each catchment was calculated using tile drainage data from the Ontario Ministry of Agriculture, Food and Rural Affairs. Tile drainage was classified as either systematic, defined as tile drainage implemented at fixed intervals across entire agricultural fields, or targeted, defined as tile drainage implemented in specific low-lying poorly drained areas of the landscape. A total of nine metrics describing the abundance and location of specific BMP types present in the catchment were selected from two previous studies in the region (Pearce & Yates, 2015; Holmes et al., 2016; Table 3-3). Metrics were selected to maximize the representation of BMPs on the landscape and minimize metric redundancy and collinearity. Of the nine selected metrics, five described BMP abundance. Abundance metrics were the number of MS per farm (MS per farm), the percentage of river with adjacent LAR (% river w/LAR), the percentage of RBA within a 30 m buffer of the stream network in each catchment (%RBA), the percentage of catchment area with systematic subsurface tile drainage systems (%TD sys.), and the percentage of catchment area with targeted tile drainage systems (%TD targ.). The remaining four metrics described BMP location. Location metrics were the percentage of catchment area expected to contribute runoff to MS structures (MS % flow acc.) and to LAR fences (LAR % flow acc.), as well as the inverse of the hydrologic flow distance from MS structures (Inv. MS dist. to site) and LAR fences (Inv. LAR dist. to site) to the instream point of water sampling. Hydrologic distances for sites without LAR were given values 10-fold greater than the maximum (46 km) to emulate a negligible influence. One additional location metric was developed for use in our study, the percent flow accumulation of RBAs.

Table 3-2. List of the four structural best management practice (BMP) types examined in the 15 headwater catchments of the Grand River watershed. Associated descriptions and intended purpose of each BMP were provided (modified from Holmes et al., 2016).

BMP Type	Acronym	Description	Purpose
Manure Storage Structures	MS	Impermeable containment structures designed to hold livestock waste and prevent contaminants from being incorporated into surface and subsurface water runoff	Reduce nutrient losses from livestock waste in surface and subsurface runoff
Livestock Access Restriction Fences	LAR	Fences located adjacent to waterways that are designed to prevent livestock from accessing and disturbing stream channels	Reduce stream bank erosion and nutrient loads from livestock activity in stream channels
Riparian Buffer Areas	RBA	Areas of intact vegetation located within a 30 m buffer zone of a watercourse	Reduce the transfer of nutrients, sediments, and other contaminants to stream networks
Tile Drainage	TD	Subsurface drainage system designed to remove excess water from the soil below the surface	Optimize soil moisture levels for crop growth and reduce erosion

Table 3-3. List and description of metrics used to quantify the abundance and location of manure storage structures (MS), livestock access restriction fences (LAR), riparian buffer areas (RBA), and tile drainage (TD) in the 15 headwater catchments of the Grand River watershed. Predicted associations with stream nutrient concentrations are indicated.

Metric	Description	Prediction
MS per farm	Ratio of the number of manure storage structures per farm located within the study catchment	–
MS % flow acc.	Percentage of catchment area expected to contribute runoff to manure storage structures	–
Inv. MS dist. to site	Inverse of the hydrologic flow distance from manure storage structures to the instream point of water sampling	–
LAR % flow acc.	Percentage of catchment area expected to contribute runoff to livestock access restriction fences	–
Inv. LAR dist. to site	Inverse of the hydrologic flow distance from livestock access restriction fences to the instream point of water sampling	–
% river w/ LAR	Percentage of stream network with adjacent livestock access restriction fences	–
% RBA	Percentage of intact vegetation within a 30 m buffer of the stream network	–
RBA % flow acc.	Percentage of catchment area expected to contribute runoff to riparian buffer areas	–
% TD sys.	Percentage of catchment area with systematic subsurface drainage systems	+
% TD targ.	Percentage of catchment area with targeted subsurface drainage systems	+

Flow accumulation was quantified as the area of land upslope of a BMPs location expected to contribute overland runoff through that BMP. For example, a MS structure with a greater flow accumulation area should receive more water runoff and therefore prevent the loss of a greater amount of nutrients that otherwise would have been transferred into waterways. Likewise, RBAs with greater flow accumulation areas should intercept greater volumes of water runoff and have the potential to filter out greater amounts of nutrients. Flow accumulation was calculated in ArcGIS (Esri inc., 2010) using a 10 m resolution digital elevation model (DEM) of the region. Three difficulties were encountered when quantifying the RBA flow accumulation: 1) RBAs were represented as areas composed of multiple cells, therefore the amount of flow accumulation area would be accounted for multiple times for each downslope cell; 2) RBAs were haphazardly distributed throughout the riparian zones; and 3) small stream channel widths (< 2 m) and a coarse DEM (10 m) complicated the differentiation of stream channel from riparian zone. As a result, flow accumulation area was only calculated for individual cells with RBA vegetation present that were within a 15 – 30 m buffer of the stream network (Figure 3-2). Additionally, a representative flow accumulation area value was calculated as an average from all individual flow accumulation values that corresponded to cells with riparian vegetation present in the 15 – 30 m buffer. The average flow accumulation value was then used to determine the percentage of RBA flow accumulation area for the entire catchment. Flow accumulation metrics for TD were not calculated because of the lack of information on the locations of individual tiles and drainage outfalls.

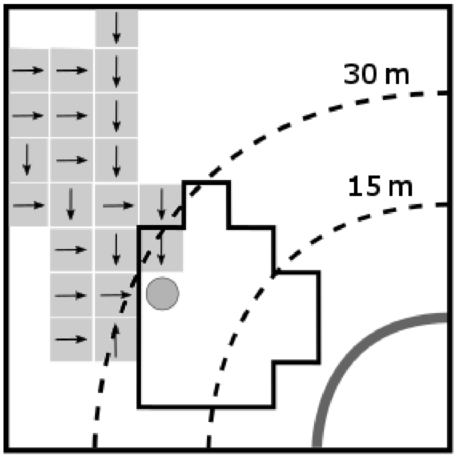

Figure 3-2. Schematic diagram showing the estimation of the flow accumulation area (squares) of a riparian buffer area (RBA) cell (circle) within a 15-30m buffer (dashed line) of a stream network (dark grey line). RBA is represented by the solid black outline.

3.2.4 Statistical analysis

Hydrologic date and antecedent precipitation metrics were established for each sampling event to account for the variation in time of year and antecedent hydrologic conditions prior to sampling. Hydrologic date corresponded to the specific date that each stream was sampled during the study period. Hydrologic date was quantified by assigning each day an ordinal value from 1 on the first day of the hydrologic year (November 1) to 365 on the last day of the hydrologic year (October 31). Antecedent precipitation referred to the total amount of precipitation a catchment received prior to sampling. To estimate antecedent precipitation, daily rainfall data were acquired from tipping bucket rain gauge stations maintained and monitored by the GRCA (GRCA, 2016). Rainfall data were collected and summarized for each individual study catchment from the rain gauge station that was closest to the center of the catchment area. Six rain gauge stations were used to estimate antecedent precipitation and were on average 5.95 km (± 1.97 km) from the center of the catchments. Estimations of antecedent precipitation for each study catchment were calculated by summing the total amount of rainfall (mm) over a 48-hour period prior to noon for the sampling day of each stream. A 48-hour antecedent period was chosen because study catchments had small mean areas (6.8 ± 1.0 km^2) and precipitation was expected to quickly drain through the basins.

Descriptive statistics were used to summarize BMP metrics in the 15 study catchments. Each site was assigned a set of ranks according to the value of each BMP metric. Ranks were assigned such that the BMP metric values predicted to be associated with the lowest concentration of nutrients were represented by a rank of one. A spearman rank correlation matrix was then performed among BMP metric rankings to examine any patterns in BMP implementation. Spearman rank correlations were completed using the *Hmisc* package (Harrell & Dupont, 2015) in R (R Core Team, 2015).

A principal component analysis (PCA) was performed on log-transformed nutrient data collected over the hydrologic year to identify if nutrient parameters were projected independently or in distinct groups. Loadings on PCA components were used to distinguish groups of nutrient parameters that were correlated over the study period. Identified groups were then used as individual sets of response variables in a partial least squared (PLS) regression analysis (Wold et al., 2001). PLS regression is a multivariate tool that analyzes the association between linear combinations of multiple predictor variables and one or more response variables (Carrascal et al., 2009). PLS regression models the association between variables by extracting orthogonal latent factors from the dataset that maximize the explained covariance between response and predictor variables. The model is then validated based on the ability of the model to accurately predict the values of response variables. Although PLS regression has the ability to analyze several response variables together, a single model with multiple independent response variables may have many latent factors and become difficult to interpret. Thus, the separate modeling of correlated response variables identified through a PCA will provide more straightforward results (Eriksson et al., 1995; Word et al., 2001). PLS regression is particularly useful where: 1) there are a large number of predictor variables relative to observations, and 2) predictor variables exhibit multicollinearity.

PLS regression was used to determine the association between response variables (i.e., log-transformed nutrient concentrations) and predictor variables (i.e., log or arcsine-transformed BMP metrics, hydrologic date, and antecedent precipitation) for all samples over the hydrologic year (n = 178). Separate PLS regression models were performed for nutrient parameter groups identified by the PCA. Selection of latent factors in the PLS model was based on the smallest number of latent factors that would maximize the predictive power (Q^2) of the model. A *k*-fold cross validation method was used to evaluate the structure of the model by randomly sub-setting the data into 10 equal segments. To complete the cross validation, one of the random subsets was used as validation data to test the model and the remaining nine subsets were used as training data. The validation process was repeated until each of the 10 data subsets were used to validate the model. Cross validation analyses were iterated 999 times to ensure reliability in determining the model structure. PLS models were considered predictive if $Q^2 > 0.0975$ as recommended by Abdi, (2010). Within predictive models (i.e., $Q^2 > 0.0975$), the variable influence on projection (VIP) scores and standardized coefficients were used to describe the influence of each predictor variable on the PLS model of response variables. Predictor variables were considered influential if VIP scores were greater than 1 and standardized coefficients corresponded to the direction of the predicted association between individual predictor variables and collinear response variables. PCA was conducted using the *stats* package (R Core Team, 2015) and PLS regression analyses were carried out using the *plsdepot* package (Sanchez, 2012) in R (R Core Team, 2015).

3.3 Results

3.3.1 Best management practice abundance and location

A total of 46 MS structures and 21 LAR fences were identified in the 15 study catchments. MS structures were present in all study catchments and had an average of 0.28 (± 0.15) structures per farm (Table 3-4). In contrast, LAR fences were present in 8 of the 15 catchments. The percentage of stream length with adjacent LAR fences varied from 0% to 19.8%. Intact RBAs were present in all catchments and ranged from 10.0% to 43.4% buffer coverage with an average of 29.2% (± 11.4%) cover. Tile drainage was also present in all catchments with systematic TD being implemented on average in 32.6% (± 18.0%) of the catchment area and targeted TD in 17.7% (± 17.6%) of the catchment area. Hydrologic distance from MS and LAR structures to sampling locations were the least variable metrics (CVs < 0.34). MS and LAR structures were, on average, 3.5 km (± 1.1 km) and 2.8 km (± 0.9 km) away from sampling locations, respectively. The percentage of catchment area predicted to contribute runoff through BMPs was minimal with the maximum flow accumulation area captured being 4.32% for a RBA. Overall, RBA percent flow accumulation area was the greatest with an average of 0.53% (± 1.29%), followed by LAR with 0.16% (± 0.33%) and MS with 0.04% (± 0.06%).

Correlations of BMP metric rankings among study catchment revealed patterns of BMP implementation within individual BMP types. A spearman rank correlation matrix showed that all LAR metrics had a significant ($p < 0.05$) positive correlation with each other ($r > 0.80$). Additionally, the association between MS per farm and MS percent flow accumulation revealed a significant positive correlation ($r = 0.67$). No significant correlations were found between other metrics or metric ranks of different BMP types.

Table 3-4. Raw values for each best management practice (BMP) metric used to describe the gradient of BMP implmentation in 15 headwater catchments of the Grand River watershed. Catchment ranking corresponds to the BMP metric value predicted to be associated with the lowest nutrient concentartions (i.e., 1 = low nutrient and 15 = high nutrient) are indicated by italicies. Descriptive statistics of each BMP metric for the 15 study cathments are summarized at the bottom of the table. Null values for the distance of livestock access restriction fences to the sampling site were calculated as 10x the maximum value (46 km) for statistical analyses. Refer to Table 3 for metic descriptions.

Site	MS per farm	MS % flow acc.	Inv. MS dist. to site (km)	% river w/ LAR	LAR % flow acc.	Inv. LAR dist. to site (km)	% RBA	RBA % flow acc.	% TD sys.	% TD targ.
C-01	0.29 *7*	0.01 *9*	2.9 *10*	1.6 *7*	0.01 *8*	2.1 *7*	27.2 *8*	0.07 *4*	26.9 *9*	0.0 *1*
C-02	0.40 *3*	0.04 *6*	4.5 *4*	0 *9*	0 *9*	- *9*	41.6 *3*	0.03 *11*	24.1 *7*	2.2 *2*
C-03	0.17 *11*	<0.01 *12*	5.0 *2*	0 *9*	0 *9*	- *9*	39.0 *6*	0.06 *6*	41.7 *11*	0.0 *1*
C-04	0.33 *5*	<0.01 *11*	4.8 *3*	0 *9*	0 *9*	- *9*	43.3 *2*	0.05 *9*	23.2 *6*	5.7 *4*
C-05	0.29 *7*	0.01 *10*	2.8 *12*	0 *9*	0 *9*	- *9*	26.4 *9*	0.14 *3*	15.7 *2*	2.7 *3*
C-07	0.40 *3*	0.17 *1*	3.0 *9*	5.2 *5*	0.16 *3*	4.7 *1*	10.1 *15*	0.03 *10*	74.0 *15*	0.0 *1*
C-08	0.08 *15*	<0.01 *15*	4.1 *6*	1.0 *8*	0.03 *7*	2.2 *5*	16.6 *14*	0.05 *8*	24.9 *8*	56.9 *13*
C-09	0.33 *5*	0.13 *3*	4.2 *5*	12.1 *2*	0.10 *5*	3.0 *4*	18.9 *12*	0.02 *13*	15.5 *1*	30.3 *10*
C-10	0.47 *2*	0.14 *2*	1.9 *14*	19.8 *1*	0.97 *1*	1.8 *8*	39.9 *5*	0.02 *15*	17.4 *3*	45.1 *12*
C-11	0.15 *12*	<0.01 *14*	2.4 *13*	10.7 *3*	0.96 *2*	2.1 *6*	32.1 *7*	0.02 *14*	18.9 *5*	13.9 *5*
N-01	0.22 *10*	<0.01 *13*	5.2 *1*	0 *9*	0 *9*	- *9*	22.9 *10*	0.03 *12*	56.7 *14*	20.7 *8*
N-02	0.12 *13*	0.03 *8*	3.5 *8*	0 *9*	0 *9*	- *9*	17.1 *13*	0.07 *5*	50.3 *12*	24.3 *9*
N-03	0.62 *1*	0.04 *5*	3.5 *7*	10.6 *4*	0.14 *4*	3.4 *2*	18.9 *11*	0.06 *7*	51.4 *13*	14.9 *7*
N-04	0.25 *9*	0.05 *4*	2.8 *11*	0 *9*	0 *9*	- *9*	43.4 *1*	4.32 *1*	29.9 *10*	14.6 *6*
N-05	0.11 *14*	0.04 *7*	1.9 *15*	2.3 *6*	0.03 *6*	3.1 *3*	40.0 *4*	2.95 *2*	18.0 *4*	34.0 *11*
Mean	0.28	0.04%	3.5	4.2%	0.16%	2.8	29.2%	0.53%	32.6%	17.7%
Med.	0.29	0.03%	3.5	1.0%	0.01%	2.6	27.2%	0.05%	24.9%	14.6%
Max.	0.62	0.17%	5.2	19.8%	0.97%	4.6	43.4%	4.32%	74.0%	56.9%
Min.	0.08	<0.01%	1.9	0%	0%	1.8	10.0%	0.02%	15.5%	0.0%
SD	0.15	0.06%	1.1	6.2%	0.33%	0.9	11.4%	1.29%	18.0%	17.6%
CV	0.53	1.25	0.31	1.46	2.07	0.34	0.39	2.44	0.55	1.00

3.3.2 Intra-annual variation of instream nutrients

Instream nitrogen concentrations summarized from all 15 study streams for each sampling event (i.e., November – October) revealed similar intra-annual trends between concentrations of TN and NO_3^-+NO_2^-, but not NH_3-N (Figure 3-3). Median TN and NO_3+NO_2^- concentrations were comparable and greatest in the colder months (October – April). Concentrations of TN and NO_3^-+NO_2^- decreased in the late spring (May and June) and summer (Late June and August), however, instream nitrogen concentrations showed a larger peak in the month of July corresponding to a large rain event (31.9 ± 21.4 mm). The interquartile range of TN and NO_3^-+NO_2^- concentrations for all study streams was also greatest in July, yet remained consistent throughout the remainder of the study period. Median TN and NO_3^-+NO_2^- concentrations summarizing the annual average nutrient concentrations in each study stream were 4.72 mg L^{-1} and 3.52 mg L^{-1}, respectively.

Concentrations of NH_3-N were the least variable of all nutrient parameters sampled and did not respond to antecedent precipitation events. NH_3-N concentrations showed minimal intra-annual variation exhibiting only one peak of greater concentrations in April, with the remainder of sampling events having lower instream NH_3-N concentrations. Likewise, the greatest interquartile range was present in April and to a lesser extent in January and August. The median NH_3-N concentration summarizing the annual average nutrient concentrations in each study stream was 0.13 mg L^{-1}.

Intra-annual (November – October) trends in phosphorus concentrations (i.e., TP, TDP, and SRP) summarized from all 15 study sites were similar throughout the hydrologic year (Figure 3-3). Median concentrations for all phosphorus forms were consistent throughout the study period with the exception of two peaks, one in April and one in the late summer (August). The interquartile range of phosphorus concentrations also increased alongside phosphorus concentrations and was greatest throughout the summer months (June – August). Peak instream phosphorus concentrations were not visually related to antecedent precipitation events over the study period. Annual average phosphorus concentrations from each study stream had median concentrations of 0.14 mg L^{-1}, 0.10 mg L^{-1}, and 0.09 mg L^{-1} for TP, TDP, and SRP, respectively.

Assessment of median nutrient concentrations throughout the year revealed substantial differences among streams (Figure 3-4). Streams with greater median phosphorus concentrations (e.g., Cons-07 and Nith-02) often revealed greater median nitrogen concentrations. Likewise, streams with lower median nutrient concentrations were consistent across all nutrient forms sampled (e.g., Cons-02 and Nith-04). However, two study streams (i.e., Cons-04 and Nith-03) with low median phosphorus concentrations had comparatively greater concentrations of TN and NO_3^-+NO_2^-. Streams characterized by low median nutrient concentrations also had less intra-annual variation than streams with higher median concentrations.

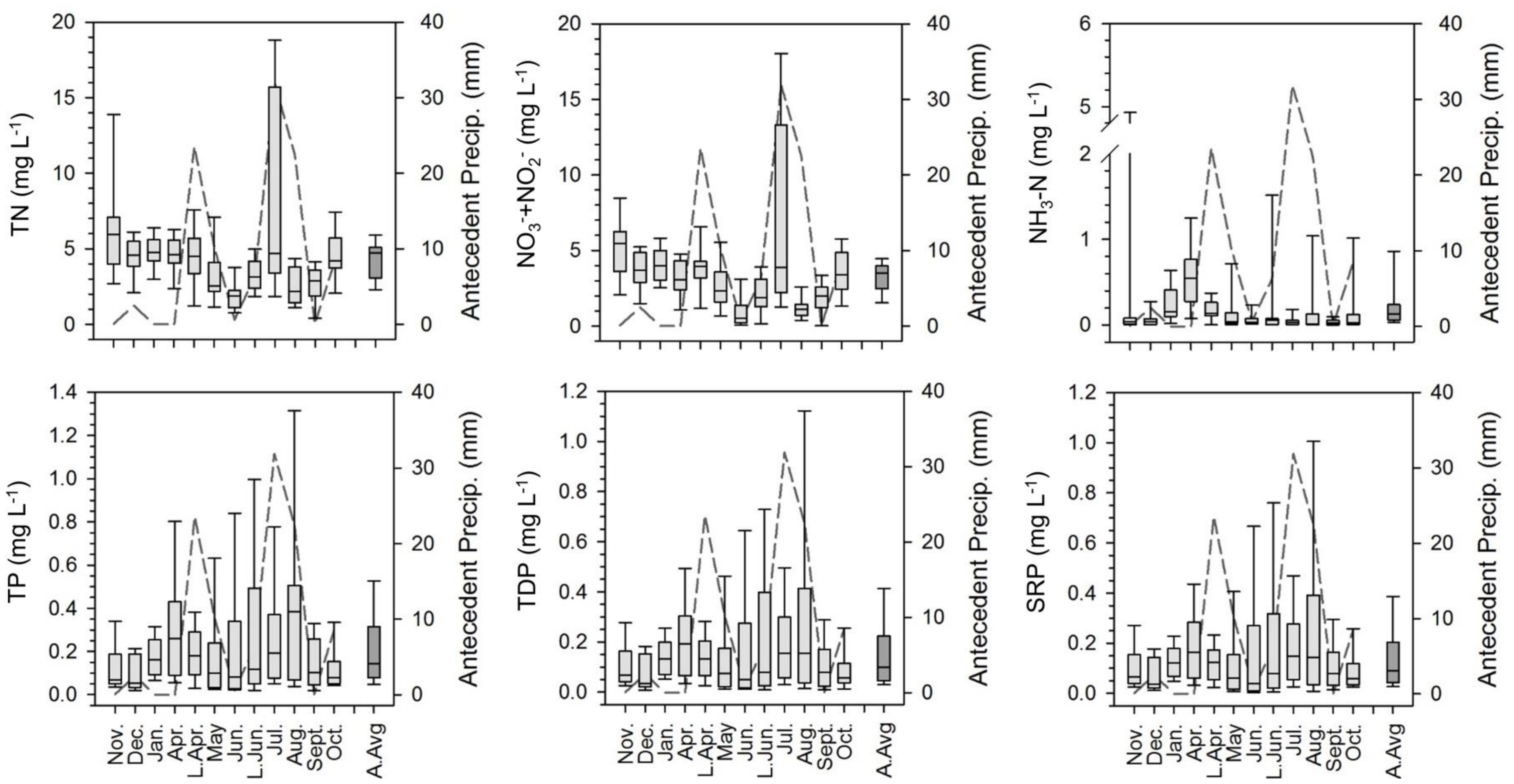

Figure 3-3. Summary of total nitrogen (TN), nitrate-nitrite ($NO_3^-+NO_2^-$), ammonium (NH_3-N), total phosphorus (TP), total dissolved phosphorus (TDP) and soluable reactive phosporus (SRP) concentrations measured during each sampling event in our 15 study streams within the Grand River watershed. Annual average nutrient concentrations from each study site are also summarized as indicated by the dark grey box. Box plots show the median nutrient concentration, interquartile range, and the 5th and 95th percentiales of the sampling event. Average antecedent (48 hours) precipitation among six rain gauge stations throughout the study period is represented by the secondary axis and dashed line.

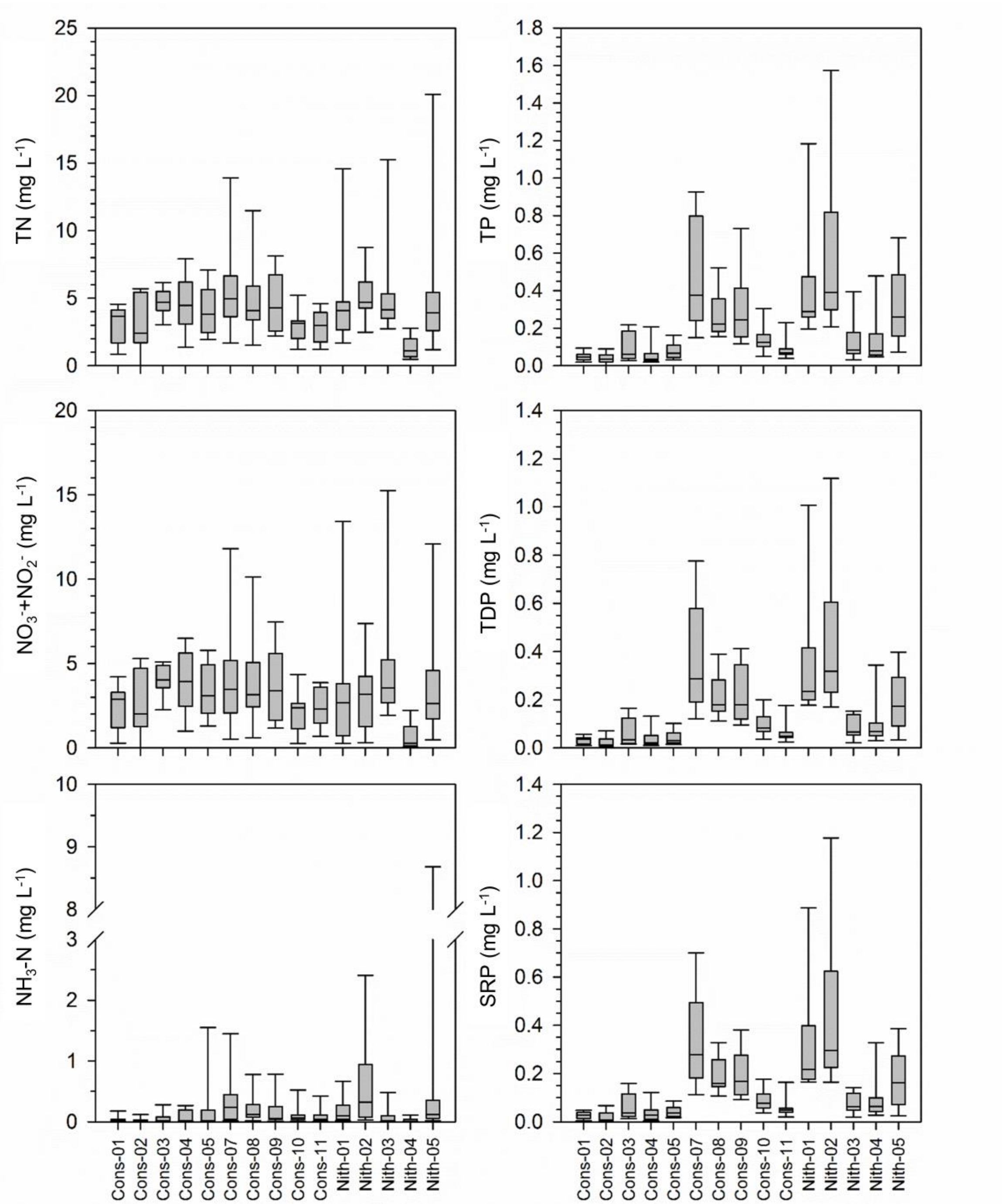

Figure 3-4. Summary of total nitrogen (TN), nitrate-nitrite (NO_3^-+NO_2^-), ammonium (NH_3-N), total phosphorus (TP), total dissolved phosphorus (TDP) and soluable reactive phosporus (SRP) concentrations measured throughout the study period in each of our 15 study streams within the Grand River watershed. Box plots show the median nutrient concentration, interquartile range, and the 5th and 95th percentiales of the sampling event.

3.3.3 Best management practice mitigation effects

Results of the PCA of monthly instream nutrient concentrations showed that the first two principal components explained 88.7% of the total variance. The first principal component (PC1) explained 59.3% of the variance and the second principal component (PC2) explained 29.4%. Loadings (PC1, PC2) indicated that PC1 was strongly associated with TP (-0.50, -0.18), TDP (-0.51, -0.17), SRP (-0.50, -0.15), and NH_3-N (-0.41, 0.07). TN (-0.25, 0.64) and NO_3^-+NO_2^- (-0.09, 0.71) were associated with PC2. Based on the findings of the PCA nutrient parameters were organized into two groups (i.e., Group 1: TP, TDP, SRP, and NH_3-N; Group 2: TN and NO_3^-+NO_2^-) for subsequent analyses.

The PLS regression model for the Group 1 nutrient parameters (i.e., TP, TDP, SRP, and NH_3-N) was considered predictive (Q^2 cumulative = 0.447) as explained by two latent factors (Figure 3-5). The PLS model explained 38.4% of the variation in predictor variables (R^2X) and 50.0% of the variation in response variables (R^2Y). All individual response variables were able to be predicted by the model with TP, TDP, SRP, and NH_3-N, having Q^2 values of 0.539, 0.564, 0.537, and 0.150, respectively. Predictor variables that were important (i.e., VIP > 1) in the model projection were %RBA (1.97), %TD targ. (1.54), %TD sys. (1.40), and MS/farm (1.09). Standardized model coefficients of predictor variables indicated that %RBA and MS/farm were negatively associated with Group 1 nutrient concentrations, whereas %TD targ. and %TD sys. were positively associated with nutrient concentrations.

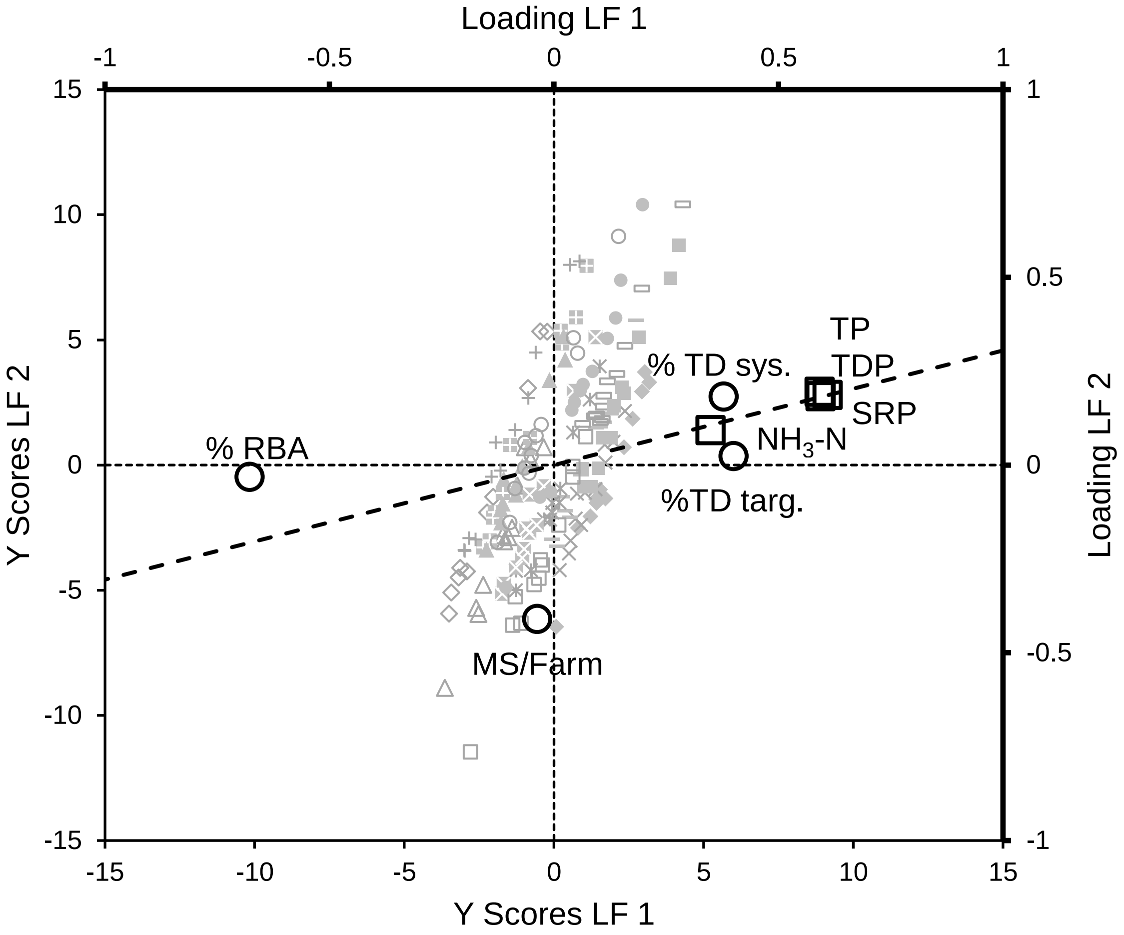

Figure 3-5. Scores and loadings biplot for the partial least squares (PLS) regression analysis of total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP) and ammonium (NH_3-N) concentrations as influenced by best management practice (BMP) metrics considered to be important in the model (VIP > 1). Y-scores of individual sites per sampling event on both latent factors (LF) are represented on the primary axes and are denoted by grey shapes, where individual shapes represent a single study site. Variable loadings are represented on the secondary axes and show the association between predictor variables (i.e, BMP metrics: circles), and response variables (i.e., nutrient concentrations: squares) via proximity to an assumed trendline (dashed line) spanning through the response variables and the origin. Predictor variables situated closer to the trendline and further from the origin are considered more influential in the model. Likewise, the position of predictor variables in reference to the response variables indicates the direction of association. VIP = variable influence on projection.

PLS regression of Group 2 nutrient parameters (i.e., TN and NO_3^-+NO_2^-) was also considered predictive with a cumulative Q^2 value of 0.140. The PLS model explained 16.3% of the variation in predictor variables (R^2X) and 20.4% of the variation in response variables (R^2Y) using one latent factor. Both response variables, TN ($Q^2 = 0.138$) and NO_3^-+NO_2^- ($Q^2 = 0.147$), were considered predictive in the PLS model. Important predictor variables were RBA % flow accumulation (2.13), hydrologic date (1.61), %RBA (1.48), and inverse MS distance to sampling point (1.25). All important predictor variables (VIP > 1) were negatively associated with TN and NO_3^-+NO_2^-. As hydrologic date was considered important in the Group 2 PLS model, individual PLS analyses were performed for each individual sampling event. Of the 12 sampling events only the Late April (Q^2 cumulative = 0.241) model was considered to be predictive. The Group 2 PLS model for the Late April sampling event explained 23.3% of the variation in predictor variables (R^2X) and 62.9% of the variation in response variables (R^2Y) on one latent factor. TN ($Q^2 = 0.215$) and NO_3^-+NO_2^- ($Q^2 = 0.267$) were both considered predictive. Four predictor variables were considered important (i.e., VIP > 1) in the model. %RBA (1.88), RBA % flow accumulation (1.54), and inverse MS distance to sampling point (1.12) metrics had negative associations with Group 2 nutrient concentrations. %TD sys. (1.19) had a positive association.

3.4 Discussion

Agricultural BMPs are increasingly being advocated as effective strategies to reduce nonpoint source inputs of nutrients to waterways (Kleinman et al., 2015). Yet, the majority of studies that have examined BMP effectiveness have taken either a modeling approach (e.g., Bracmort et al., 2006; Rao et al., 2009; Zhang & Zhang, 2011; Bosch et al., 2013; Bosch et al., 2014) or performed small scale, field assessments (e.g., Brannan et al., 2000; Bishop et al., 2005; King et al., 2008; Li et al., 2011). Our study provides a field-based assessment of the cumulative influence of agricultural BMPs on stream nutrient concentrations at large spatial scales, while accounting for effects of seasonal variation. Overall, structural BMPs implemented in our study region (i.e., manure storage structures, livestock access restriction fences, riparian buffer areas, and tile drainage) appeared to be influencing instream concentrations of phosphorus (i.e., TP, TDP, and SRP) and NH_3-N throughout the year. Mitigation effects on TN and NO_3^-+NO_2^-, however, appeared to be restricted to the snowmelt period.

Our study found that riparian buffers and manure storage systems were associated with reduced phosphorus and NH_3-N concentrations throughout the hydrologic year. Results further indicated that riparian buffers were the most effective of the studied BMPs at reducing nutrient concentrations, followed by MS structures. Several other past studies have also found that greater amounts of natural vegetation in riparian zones were associated with reduced instream nutrient concentrations (e.g., Osborne & Kovacic, 1993; Lee et al., 2003; Hoffmann et al., 2009) and have outlined the effectiveness of MS structures (Brannan et al., 2000; Inamdar et al., 2002). However, unlike some previous studies that have suggested that the positioning of structural BMPs on preferential flow routes reduces nutrient losses to streams (Walter et al., 2000; Tomer et al., 2009; Rittenburg et al., 2015), our results showed no association between phosphorus and NH_3-N concentrations and the area of flow accumulation associated with buffered riparian areas or MS structures. The lack of association highlights the possibility that BMPs currently located in areas of greater hydrological connectivity may be inadequate to deal with the large volumes of water runoff potentially routed through these locations. Increasing the number of BMPs in hydrologically connected areas as well as co-locating different types of BMPs along flow paths that collect significant amounts of runoff may thus be needed to effectively retain phosphorus and ammonium on the land. For example, riparian buffer strips may need to be wider or augmented by vegetated filter strips further upslope on the flow path to enhance mitigation effects.

Similar to previous studies, our results indicate increased nutrient losses in agricultural regions with extensive tile drainage systems (Sims et al., 1998; Smith et al., 2015). Both systematic and targeted tile drainage systems were positively associated with the concentrations of phosphorus and NH_3-N in headwater streams throughout the hydrologic year. The extensive tile drainage systems in these regions may thus undermine the potential water quality benefits of other BMPs by rerouting water runoff directly into headwater streams before nutrients can be intercepted and filtered by riparian vegetation (Osborne & Kovacic, 1993). Likewise, the strong positive influence of tile drainage systems may mask the cumulative benefits of structural BMPs that are unable to override the increased nutrient losses from tile drains (Lemke et al., 2011). To achieve nutrient reduction goals and enhance the cumulative benefits of existing BMPs, management authorities may need to consider investing in the conversion of current tile drainage systems to controlled drainage systems that will allow farmers to actively manage the water table to meet crop needs and reduce nutrient losses (Drury et al., 1996; Ng et al., 2002).

Agricultural BMPs were only associated with nitrogen (i.e., TN and NO_3^-+NO_2^-) during late April. The late April sampling event corresponded to the snowmelt period in the study region and captured high flow conditions. During the snowmelt period riparian buffer areas were the primary BMP responsible for the significant association observed. Both the percentage of RBA and flow accumulation of RBAs were negatively associated with the concentrations of TN and NO_3^-+NO_2^- in headwater streams. Our results thus suggest that riparian vegetation in hydrologically connected areas may receive and capture more water runoff and provide greater cumulative benefits during high flow periods supporting the importance of RBA location on the landscape (Tomer et al., 2003; Tomer et al., 2009). However, the positive association between systematic tile drainage and TN and NO_3^-+NO_2^- indicates tile drainage may limit the nitrogen capture benefits provided by RBAs in our study area. Indeed, enhanced transport of subsurface water to streams through tile drainage has been linked to reduced capacity of riparian vegetation to filter available nitrogen (David et al., 1997). Furthermore, our finding that tile drainage was strongly associated with nitrogen post-snowmelt is consistent with findings that nutrient losses through tile drains may be particularly important in early spring when nutrients from fall and winter fertilizer and manure applications are released from frozen soils and leached to tile drain networks (Randall et al., 2003). Thus, although our results provide evidence that riparian buffer areas may decrease the amount of nitrogen transferred into streams following snowmelt and phosphorus throughout the year, our results also indicate that tile drainage may increase nutrient losses to streams and counter the benefits of other BMPs. Given the apparent trade-off, watershed managers may need to evaluate which management priorities are of greater concern in the respective region and plan the implementation of tile drainage systems accordingly.

The lack of association between BMPs and major nitrogen forms during periods other than snowmelt may be explained by previous studies that have indicated that NO_3^-+NO_2^-

is readily leached into the soil and transported to river systems through subsurface and groundwater flow (Pärn et al., 2012). NO_3^-+NO_2^- in groundwater is often influenced by legacy effects from past agricultural activities as groundwater movement typically occurs at slow rates (Tomer & Burkart, 2003; Meals et al., 2010). Indeed, a study by Rudolph et al., (2015) in the Grand River watershed found that although recent managerial BMPs had reduced nitrate concentrations in the shallow vadose zone, significant time lags would exist before reductions in groundwater nitrogen loadings to surface waters would be observed. Mitigation benefits of the structural BMPs included in our study may thus have been masked by legacy effects of nitrogen in groundwater. Consequently, recently implemented nutrient management practices, such as many of the MS structures examined in our study, may require further time to reveal the expected benefits in the form of reduced stream nitrogen concentrations.

Nutrient concentrations from our study of 15 agricultural streams in southern Ontario were comparable to the results of studies on BMP implementation in other agricultural catchments located in temperate biomes. For example, Gabel et al., (2012) reported autumn and spring TDP (0.05 mg L^{-1}, 0.09 mg L^{-1}), NH_3-N (0.21 mg L^{-1}, 0.18 mg L^{-1}), and NO_3^- (0.96 mg L^{-1}, 1.10 mg L^{-1}) concentrations that fell within the average annual range of concentrations measured in our streams. Likewise, Makarewicz et al., (2009) reported comparable annual average concentrations of TP (0.19 mg L^{-1}) and NO_3^-+NO_2^- (4.20 mg L^{-1}) in an agricultural stream with a catchment containing a variety of structural BMPs and operational conservation practices (e.g., fertilizer application management). Similar to past studies (e.g., Vanni et al., 2001), the concentration of nutrients sampled in our study streams varied considerably (4 to 11-fold) among sampling events throughout the hydrologic year. Intra-annual patterns of nutrient concentrations observed in our study were also consistent with trends in agricultural regions of the upper Mississippi river basin, where maximum phosphorus and minimum nitrogen concentrations were generally measured in late summer through early fall (Houser & Richardson, 2010). However, the seasonal variation of nutrient concentrations observed in our study was associated with the abundance of BMPs such that catchments with greater amounts of RBA (e.g., Nith-04, Cons-04, and Cons-02) were characterized by consistently lower nutrient concentrations with minimal seasonal variability, whereas low RBA catchments (e.g., Cons-07, Cons-08, and Nith-02) had greater nutrient concentrations that varied substantially with season. The observation of catchment specific patterns in nutrient concentrations further suggests that BMP implementation may influence the nutrient status of headwater streams throughout the year.

Variability in nutrient concentrations within study streams was not strictly associated with time of year as large peaks in nutrient concentrations following precipitation events were also observed. The co-occurrence of the largest antecedent precipitation event (i.e., July) and increased nitrogen and phosphorus concentrations is not surprising given the known linkages between storm events and overland nutrient transport (Pionke et al., 1996; Kleinman et al., 2006; Royer et al., 2006). For example, in agricultural regions of the Chesapeake Bay basin, Sharpley et al., (2008) found that storm events could produce instream TP concentrations ranging from 0.372 mg L^{-1} to 0.943 mg L^{-1} depending on storm size, which was comparable to the increased TP concentrations following rain events in our study. Studies have shown that implementation of BMPs can substantially reduce nutrient losses associated with storm events (Zollweg & Makarewicz, 2009). Our finding that nutrient concentrations in streams with greater numbers of BMPs (e.g., Cons-2, Cons-4, and Nith-4) appeared less affected by storm events relative to the 4.5-fold increase in nitrogen concentrations in streams with few or no BMPs (e.g., Cons-7, Nith-1, and Nith-3) is consistent with these past studies. However, because our study did not explicitly target storm events, periods of high flow are likely underrepresented in our assessment precluding us from making strong conclusions regarding BMP effects on nutrient losses associated with storms.

The temporal resolution of watershed assessments can present limitations in establishing the intra-annual patterns of nutrient concentrations (Sharpley et al., 2009; Cassidy & Jordan, 2011). High resolution monitoring of TP concentrations in agricultural catchments of Ireland showed that traditional monthly sampling regimes underestimated TP loads by up to 60% (Cassidy & Jordan, 2011). Coarse sampling programs, even at a weekly resolution, can miss or capture events contributing to unbalanced nutrient loadings (Sharpley et al., 2009). Although our study employed a monthly sampling regime and undoubtedly missed storm driven runoff events, our goal was to provide an associative study between BMP implementation and the concentration of instream nutrients, not to quantify changes in nutrient loadings. Given that structural BMPs are designed to capture surface runoff contaminants, the association between BMPs and instream nutrient concentrations during baseflow conditions is likely underestimated assuming BMPs remain effective during high flow events. To improve our understanding of the relationship between flow variation and the realized cumulative benefits of agricultural BMPs at a landscape scale, high resolution monitoring studies targeting storm flows at different times of the year are required.

Numerous studies have highlighted the importance of targeted BMP location (Walter et al., 2000; Tomer et al., 2009; Rittenburg et al., 2015), however, in our study the cumulative benefits of BMP placement were only realized during the spring following snowmelt. The limited importance of BMP location observed in our study may be in part due to the underrepresentation of high flow events in our dataset as location is likely to be disproportionately important during the largest runoff events. A second limitation of our study was the availability of BMPs that were located in areas predicted to be receiving concentrated overland flows. Our finding that the flow accumulation area of the studied BMPs ranged from less than 0.01% to 4.32% of the catchment area outlines the limited hydrologic connectivity achieved by voluntary BMP implementation in this area of the Grand River watershed. The lack of BMPs in hydrologically connected areas throughout our study catchments may not surpass the level of implementation necessary to measurably reduce losses of nutrients to receiving streams. Although past research has indicated that targeting source locations of nutrient loss appears to be a promising strategy (Rittenburg et al., 2015), further field studies are required to assess the potential trade-offs between the abundance and spatial location of structural BMPs required to meet water quality objectives.

3.4.1 Applications and recommendations

Overall, our study indicates that increasing the abundance of structural BMPs would be an effective strategy to assist management authorities in meeting phosphorus reduction goals in small agricultural streams. Locally, this finding supports proposals to address nonpoint sources of phosphorus in the Lake Erie basin through increased investment into

BMP implementation programs. Broader implementation of RBAs and MS structures should reveal phosphorus reductions at the watershed scale and ultimately reduce phosphorus loadings to downstream ecosystems. However, RBAs only appear effective at reducing nitrogen concentrations during snowmelt. Additionally, nutrient losses through tile drainage systems requires further consideration as their influence appears to counter the mitigation effects of other BMPs on the landscape. Farmer participation in nutrient stewardship through the installation of controlled tile drainage systems may yield greater cumulative benefits by limiting the subsurface transport of nutrients. Alternative management strategies such as rerouting tile drainage outputs through riparian wetlands may also allow for greater nutrient mitigation through uptake by riparian vegetation (Jaynes & Isenhart, 2014). The combined use of additional structural BMPs and management strategies addressing nutrient export from tile drainage systems may therefore be an effective approach to limit nutrient losses in agricultural catchments.

3.5 References

Abdi, H. (2010). Partial least squares regression and projection on latent structure regression (PLS Regression). *Wiley Interdisciplinary Reviews: Computational Statistics, 2*(1), 97–106. doi: 10.1002/wics.51

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, compositions, and consequences. *Estuaries*, *25*, 704–726. doi: 10.1007/BF02804901

Bishop, P., Hively, W., Stedinger, J., Rafferty, M., Lojpersberger, J., & Bloomfield, J. (2005). Multivariate analysis of paired watershed data to evaluate agricultural best management practice effects on stream water phosphorus. *Journal of Environmental Quality, 34*(3), 1087–1101. doi: 10.2134/jeq2004.0194

Bosch, N. S., Allan, J. D., Selegean, J. P., & Scavia, D. (2013). Scenario-testing of agricultural best management practices in Lake Erie watersheds. *Journal of Great Lakes Research, 39*(3), 429–436. doi: 10.1016/j.jglr.2013.06.004

Bosch, N. S., Evans, M. A., Scavia, D., & Allan, J. D. (2014). Interacting effects of climate change and agricultural BMPs on nutrient runoff entering Lake Erie. *Journal of Great Lakes Research, 40*(3), 581–589. doi: 10.1016/j.jglr.2014.04.011

Bracmort, K. S., Arabi, M., Frankenberger, J. R., Engel, B. A., & Arnold, J. G. (2006). Modeling long-term water quality impact of structural BMPs. *Transactions of the American Society of Agricultural and Biological Engineers, 49*(2), 367–374. doi: 10.13031/2013.20411

Brannan, K. M., Mostaghimi, S., McClellan, P. W., & Inamdar, S. (2000). Animal waste BMP impacts on sediment and nutrient losses in runoff from the Owl Run watershed. *Transactions of the American Society of Agricultural Engineers, 43*, 1155–1166. doi: 10.13031/2013.3008

Carrascal, L. M., Galván, I., & Gordo, O. (2009). Partial least squares regression as an alternative to current regression methods used in ecology. *Oikos, 118*(5), 681–690. doi: 10.1111/j.1600-0706.2008.16881.x

Cassidy, R., & Jordan, P. (2011). Limitations of instantaneous water quality sampling in surface-water catchments: Comparison with near-continuous phosphorus time-series data. *Journal of Hydrology, 405*(1-2), 182–193. doi: 10.1016/j.jhydrol.2011.05.020

David, M. B., Gentry, L. E., Kovacic, D. A., & Smith, K. M. (1997). Nitrogen balance in and export from an agricultural watershed. *Journal of Environmental Quality, 26*(4), 1038–1048. doi: 10.2134/jeq1997.00472425002600040015x

Drury, C. F., Tan, C. S., Gaynor, J. D., Oloya, T. O., & Welacky, T. W. (1996). Influence of controlled drainage-subirrigation on surface and tile drainage nitrate loss. *Journal of Environmental Quality, 25*(2), 317–324. doi: 10.2134/jeq1996.00472425002500020016x

Edwards, D. R., Daniel, T. C., Scott, H. D., Murdoch, J. F., & Vendrell, P. F. (1997). Effect of BMP implementation on storm flow quality of two northwestern Arkansas streams. *Transactions of the American Society of Agricultural Engineers, 40*(5), 1311–1319. doi: 10.13031/2013.21389

Eriksson, L., Hermens, J. L. M., Johansson, E., Verhaar, H. J. M., & Wold, S. (1995). Multivariate analysis of aquatic toxicity data with PLS. *Aquatic Science, 57*, 217–241. doi: 10.1007/BF00877428

Esri inc., 2010. ArcGIS Desktop. Version 10.0. Environmental Systems Research Institute, Redlands, California, USA.

Gabel, K. W., Wehr, J. D., & Truhn, K. M. (2012). Assessment of the effectiveness of best management practices for streams draining agricultural landscapes using diatoms and macroinvertebrates. *Hydrobiologia, 680*(1), 247–264. doi: 10.1007/s10750-011-0933-8

Giri, S., Nejadhashemi, A. P. & Woznicki, S. A. (2012). Evaluation of targeting methods for implementation of best management practices in the Saginaw River Watershed. *Journal of Environmental Management, 103*, 24–40. doi: 10.1016/j.jenvman.2012.02.033

Government of Canada. (2016). Canadian Climate Normals 1981–2010 Station Data. Normals Data, Waterloo Wellington-A Ontario Canada. Available from: https://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?searchType=stnName&txtStationName=Waterloo&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=4832&dispBack=1

Grand River Conservation Authority. 2008. Lake Erie Source Protection Region Technical Team: Grand river watershed characterization report. Available from: https://www.sourcewater.ca/en/source-protection-areas/Grand-River-Background-reports.aspx

Grand River Conservation Authority (GRCA). (2016). Monitoring Data. Grand River Information Network. Available from: https://data.grandriver.ca/downloads-monitoring.html

Harrell, F.E., & Dupont, C. (2015). Hmisc: Harrell miscellaneous. Version 3.17-1.

Hoffmann, C. C., Kjaergaard, C., Uusi-Kämppä, J., Hansen, H. C. B., & Kronvang, B. (2009). Phosphorus retention in riparian buffers: Review of their efficiency. *Journal of Environmental Quality, 38*(5), 1942–1955. doi: 10.2134/jeq2008.0087

Holmes, R., Armanini, D. G., & Yates, A. G. (2016). Effects of best management practice on ecological condition: Does location matter? *Environmental Management, 57*(5), 1062–1076. doi: 10.1007/s00267-016-0662-x

Houser, J. N., & Richardson, W. (2010). Nitrogen and phosphorus in the Upper Mississippi River: Transport, processing, and effects on the river ecosystem. *Hydrobiologia, 640*(1), 71–88. doi: 10.1007/s10750-009-0067-4

Inamdar, S. P., Mostaghimi, S., Cook, M. N., Brannan, K. M., & McClellen, P. W. (2002). A long-term, watershed-scale, evaluation of the impacts of animal waste BMPs on indicator bacteria concentrations. *Journal of the American Water Resource Association, 38*(3), 819–833. doi: 10.1111/j.1752-1688.2002.tb00999.x

Jaynes, D. B., & Isenhart, T. M. (2014). Reconnecting tile drainage to riparian buffer hydrology for enhanced nitrate removal. *Journal of Environmental Quality, 43*(2), 631–638. doi: 10.2134/jeq2013.08.0331

Jones, K. B., Neale, A. C., Nash, M. S., Van Remortel, R. D., Wickham, J. D., Riitters, K. H., & O’neill, R. V. (2001). Predicting nutrient and sediment loadings to streams from landscape metrics: A multiple watershed study from the United States mid-Atlantic region. *Landscape Ecology, 16*(4), 301–312. doi: 10.1023/A:1011175013278

Kaini, P., Artita, K., & Nicklow, J. (2012). Optimizing structural best management practices using SWAT and genetic algorithm to improve water quality goals. *Water Resource Management, 26*(7), 1827–1845. doi: 10.1007/s11269-012-9989-0

King, K. W., Smiley, P. C., Baker, B. J., & Fausey, N. R. 2008. Validation of paired watersheds for assessing conservation practices in the Upper Big Walnut Creek watershed, Ohio. *Journal of Soil and Water Conservation, 63*(6), 380–395. doi: 10.2489/jswc.63.6.380

Kleinman, P. J. A., Sharpley, A. N., Withers, P. J. A., Bergström, L., Johnson, L. T., & Doody, D. G. (2015). Implementing agricultural phosphorus science and management to combat eutrophication. *Ambio, 44*(Suppl 2), 297–310. doi: 10.1007/s13280-015-0631-2

Kleinman, P. J. A., Srinivasan, M. S., Dell, C. J., Schmidt, J. P., Sharpley, A. N., & Bryant, R. B. (2006). Role of rainfall intensity and hydrology in nutrient transport via surface runoff. *Journal of Environmental Quality, 35*(4), 1248–1259. doi: 10.2134/jeq2006.0015

Lee, K. H., Isenhart, T. M., & Schultz, R. C. (2003). Sediment and nutrient removal in an established multi-species riparian buffer. *Journal of Soil and Water Conservation, 58*(1), 1–8.

Lemke, A. M., Kirkham, K. G., Lindenbaum, T. T., Herbert, M. E., Tear, T. H., Perry, W. L., & Herkert, J. R. (2011). Evaluating agricultural best management practices in tile-drained subwatersheds. *Journal of Environmental Quality, 40*(4), 1215–1228. doi: 10.2134/jeq2010.0119

Li, S., Elliott, J. A., Tiessen, K. H. D., Yarotski, J., Lobb, D. A., & Flaten, D. N. (2011). The effects of multiple beneficial management practices on hydrology and nutrient losses in a small watershed in the Canadian Prairies. *Journal of Environmental Quality, 40*(5), 1627–1642. doi: 10.2134/jeq2011.0054

Loomer, H. A., & Cooke, S. E. (2011). Water quality in the Grand River Watershed: Current conditions and trends (2003 – 2008). Grand River Conservation Authority. Available from: https://www.sourcewater.ca/en/source-protection-areas/Grand-River-Background-reports.aspx

Maccoux, M. J., Dove, A., Backus, S. M., & Dolan, D. M. (2016). Total and soluble reactive phosphorus loadings to Lake Erie a detailed accounting by year, basin, country, and tributary. *Journal of Great Lakes Research, 42*(6), 1151–1165. doi: 10.1016/j.jglr.2016.08.005

Makarewicz, J. C. (2009). Nonpoint source reduction to the nearshore zone via watershed management practices: Nutrient fluxes, fate, transport and biotic responses - Background and objectives. *Journal of Great Lakes Research, 35*(Suppl 1), 3–9. doi: 10.1016/j.jglr.2008.08.002

Makarewicz, J. C., Lewis, T. W., Bosch, I., Noll, M. R., Herendeen, N., Simon, R. D., … Vodacek, A. (2009). The impact of agricultural best management practices on downstream systems: Soil loss and nutrient chemistry and flux to Conesus Lake, New York, USA. *Journal of Great Lakes Research, 35*(Suppl 1), 23–36. doi: 10.1016/j.jglr.2008.10.006

Meals, D. W., Dressing, S. A., & Davenport, T. E. (2010). Lag time in water quality response to best management practices: A review. *Journal of Environmental Quality, 39*(1), 85–96. doi: 10.2134/jeq2009.0108

Ng, H. Y. F., Tan, C. S., Drury, C. F., & Gaynor, J. D. (2002). Controlled drainage and subirrigation influences tile nitrate loss and corn yields in a sandy loam soil in Southwestern Ontario. *Agriculture Ecosystems and Environment, 90*(1), 81–88. doi: 10.1016/S0167-8809(01)00172-4

Objectives and Targets Task Team. (2015). Recommended phosphorus loading targets for Lake Erie. Annex 4 Objectives and Targets Task Team Final Report to the Nutrients Annex Subcommittee. May 11, 2015. http://graham.umich.edu/scavia/wp-content/uploads/2015/06/FINAL-Objectives-TT-report-en-150624-3.pdf.

Osborne, L. L., & Kovacic, D. A. (1993). Riparian vegetated buffer strips in water-quality restoration and stream management. *Freshwater Biology, 29*(2), 243–258. doi: 10.1111/j.1365-2427.1993.tb00761.x

Pärn, J., Pinay, G., & Mander, Ü. (2012). Indicators of nutrients transport from agricultural catchments under temperate climate: A review. *Ecological Indicators, 22*, 4–15. doi: 10.1016/j.ecolind.2011.10.002

Pearce, N. J. T., & Yates, A. G. (2015). Agricultural best management practice abundance and location does not influence stream ecosystem function or water quality in the summer season. *Water*, *7*(12), 6861–6876. doi: 10.3390/w7126661

Pionke, H. B., Gburek, W. J., Sharpley, A. N., & Schnabel, R. R. (1996). Flow and nutrient export patterns for an agricultural hill-land watershed. *Water Resources Research, 32*(6), 1795–1804. doi: 10.1029/96WR00637

R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.2.2.

Randall, G. W., Vetsch, J. A., & Huffman, J. R. (2003). Nitrate losses in subsurface drainage from a corn-soybean rotation as affected by time of nitrogen application and use of nitrapyrin. J. Environ. Qual. 32(5), 1763–1772. doi: 10.2134/jeq2003.1764

Rao, N. S., Easton, Z. M., Schneiderman, E. M., Zion, M. S., Lee, D. R., & Steenhuis, T. S. (2009). Modeling watershed-scale effectiveness of agricultural best management practices to reduce phosphorus loading. Journal of Environmental Management, 90(3), 1385–1395. doi: 10.1016/j.jenvman.2008.08.011

Reynoldson, T., Logan, C., Pascoe, T., Thompson, S., Strachan, S., Mackinlay, C., … Paull, T. (2012). Canadian Aquatic Biomonitoring Network (CABIN) Field Manual: Wadeable Streams; Environment Canada: Dartmouth, NS, Canada.

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). Standard Methods for the Examination of Water and Wastewater, 22nd ed.; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, USA.

Richards, R. P., Baker, D. B., & Crumrine, J. P. (2009). Improved water quality in Ohio tributaries to Lake Erie: A consequence of conservation practices. *Journal of Soil and Water Conservation, 64*(3), 200–211. doi: 10.2489/jswc.64.3.200

Rittenburg, R. A., Squires, A. L., Boll, J., Brooks, E. S., Easton, Z. M., & Steenhuis, T. S. (2015). Agricultural BMP effectiveness and dominant hydrological flow paths: Concepts and a review. *Journal of the American Water Resource Association, 51*(2), 305–329. doi: 10.1111/1752-1688.12293

Royer, T. V, David, M. B., & Gentry, L. E. (2006). Timing of riverine export of nitrate and phosphorus from agricultural watersheds in Illinois: Implications for reducing nutrient loading to the Mississippi River. *Environmental Science and Technology, 40*(13), 4126–4131. doi: 10.1021/es052573n

Rudolph, D. L., Devlin, J. F., & Bekeris, L. (2015). Challenges and a strategy for agricultural BMP monitoring and remediation of nitrate contamination in unconsolidated aquifers. *Groundwater Monitoring and Remediation*, *35*(1), 97–109. doi: 10.1111/gwmr.12103

Sanchez, G. (2012). plsdepot: Partial Least Squares (PLS) Data Analysis Methods. Version 0.1.17.

Sharpley, A. N., Kleinman, P. J. A., Heathwaite, A. L., Gburek, W. J., Folmar, G. J., & Schmidt, J. P. (2008). Phosphorus loss from an agricultural watershed as a function of storm size. *Journal of Environmental Quality, 37*(2), 362–368. doi: 10.2134/jeq2007.0366

Sharpley, A. N., Kleinman, P. J. A., Jordan, P., Bergström, L., & Allen, A. L. (2009). Evaluating the success of phosphorus management from field to watershed. *Journal of Environmental Quality, 38*(5), 1981–1988. doi: 10.2134/jeq2008.0056

Sims, J. T., Simard, R. R., & Joern, B. C. (1998). Phosphorus loss in agricultural drainage: Historical perspective and current research. *Journal of Environmental Quality, 27*(2), 277–293. doi: 10.2134/jeq1998.00472425002700020006x

Smith, D. R., King, K. W., Johnson, L., Francesconi, W., Richards, P., Baker, D., & Sharpley, A. N. (2015). Surface runoff and tile drainage transport of phosphorus in the midwestern United States. *Journal of Environmental Quality, 44*(2), 495–502. doi: 10.2134/jeq2014.04.0176

Smith, V. (2003). Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research, 10*(2), 126–139. doi: 10.1065/espr2002.12.142

Spooner, J., Szpir, L. A., Line, D. E., Osmond, D. L., Meals, D. W., & Grabow, G. L. (2011). 2011 Summary Report: Section 319 National Nonpoint Source Monitoring Program (NNPSMP) Projects. NSCU Water Quality Group, Biological and Agricultural Engineering Department, North Carolina State University, Raleigh, NC. 456p.

Strauss, P., Leone, A., Ripa, M. N., Turpin, N., Lescot, J. M., & Laplana, R. (2007). Using critical source areas for targeting cost-effective best management practices to mitigate phosphorus and sediment transfer at the watershed scale. *Soil Use and Management, 23*(S1), 144–153. doi: 10.1111/j.1475-2743.2007.00118.x

Tomer, M. D., & Burkart, M. R. (2003). Long-term effects of nitrogen fertilizer use on ground water nitrate in two small watersheds. *Journal of Environmental Quality, 32*(6), 2158–2171. doi: 10.2134/jeq2003.2158

Tomer, M. D., Dosskey, M. G., Burkart, M. R., James, D. E., Helmers, M. J., Eisenhauer, D. E. (2009). Methods to prioritize placement of riparian buffers for improved water quality. *Agroforestry Systems, 75*(1), 17–25. doi: 10.1007/s10457-008-9134-5

Tomer, M. D., James, D. E., & Isenhart, T. M. (2003). Optimizing the placement of riparian practices in a watershed using terrain analysis. *Journal of Soil and Water Conservation, 58,* 198–206.

Vanni, M. J., Renwick, W. H., Jenifer, L., Auch, J. D., & Schaus, M. H. (2001). Dissolved and particulate nutrient flux from three adjacent agricultural watersheds: A five-year study. *Biogeochemistry, 54*(1), 85–114. doi: 10.1023/A:1010681229460

Veith, T. L., Wolfe, M. L., & Heatwole, C. D. (2003). Optimization procedure for cost effective BMP placement at a watershed scale. *Journal of the American Water Resource Association, 39*(6), 1331–1343. doi: 10.1111/j.1752-1688.2003.tb04421.x

Walter, M. T., Walter, M. F., Brooks, E. S., Steenhuis, T. S., Boll, J., & Weiler, K. (2000). Hydrologically sensitive areas: Variable source area hydrology implications for water quality risk assessment. *Journal of Soil and Water Conservation, 55*(3), 277–284.

Wold, S., Sjostrom, M., & Eriksson, L. (2001). PLS-regression: A basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems, 58*(2), 109–130. doi: 10.1016/S0169-7439(01)00155-1

Yates, A. G., & Bailey, R. C. (2006). The stream and its altered valley: Integrating landscape ecology into environmental assessments of agro-ecosystems. *Environmental Monitoring and Assessment, 114*(1-3), 257–271. doi: 10.1007/s10661-006-4779-x

Yates, A. G., & Bailey, R. C. (2010). Improving the description of human activities potentially affecting rural stream ecosystems. *Landscape Ecology, 25*, 371–382. doi: 10.1007/s10980-009-9413-1

Zhang, X., & Zhang, M. (2011). Modeling effectiveness of agricultural BMPs to reduce sediment load and organophosphate pesticides in surface runoff. *Science of the Total Environment, 409*(10), 1949–1958. doi: 10.1016/j.scitotenv.2011.02.012

Zollweg, J., & Makarewicz, J. C. (2009). Detecting effects of best management practices on rain events generating nonpoint source pollution in agricultural watersheds using a physically-based stratagem. *Journal of Great Lakes Research, 35*(Suppl 1), 37–42. doi: 10.1016/j.jglr.2008.10.005

Chapter 4

4 Metabolic regimes of three mid-order streams in southern Ontario, Canada exposed to contrasting sources of nutrients

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4.1 Introduction

Stream ecosystem metabolism describes the process of carbon fixation (gross primary production; GPP) and mineralization (ecosystem respiration; ER) in streams and encompasses the activities of all autotrophic and heterotrophic organisms. GPP and ER can provide insight into trophic structure and energy pathways of aquatic food webs (Rosenfeld & Mackay, 1987; Meyer, 1989), and have been linked to important biogeochemical processes, including organic matter dynamics (Tank et al., 2010) and nutrient retention (Hall & Tank, 2003; Roberts & Mulholland 2007; Rode et al., 2016). While stream metabolism can have applications for monitoring and assessment (von Schiller et al., 2017), spatial and temporal variation in the physical environment of stream ecosystems presents a challenge in linking GPP and ER with environmental conditions (Bernhardt et al., 2018).

Physical conditions (e.g., light, temperature, and discharge) have been associated with spatial variability in stream metabolism at the reach scale (Beaulieu et al., 2013; Siders et al., 2017). However, studies undertaken at large spatial extents have observed more limited predictability of GPP and ER with catchment-scale and local drivers (Mulholland et al., 2001; Bernot et al., 2010; Yates et al., 2013; Pastor et al., 2017). For example, while nutrient supply has been shown to stimulate primary production within individual streams (Rosa et al., 2013; Gough et al., 2016), a consistent effect of nutrients on GPP and ER has not been observed among streams (Lamberti & Steinman, 1997; Morgan et al., 2006; Hoellein et al., 2013; Frankforter et al., 2010).

Inter- and intra-annual variability in stream metabolism (Roberts et al., 2007; Clapcott et al., 2016) may explain inconsistencies in the identification of large-scale spatial patterns. Short duration studies (e.g., < 2 weeks), which typically occur under optimal conditions for logger deployment (i.e., hydrological stability), may not give representative estimates of metabolic rates and be insufficient to capture the effects of drivers that vary through time (Lamberti & Steinman, 1997; Finlay, 2011; Bernhardt et al., 2018). Although short-term studies have pragmatic advantages (Clapcott et al., 2016), their short duration may limit generalizations regarding the patterns and drivers of GPP and ER. As such, it has been recommended that researchers examine the characteristic temporal pattern (i.e., metabolic regime, *sensu* Bernhardt et al., 2018), as well as the typical rate and variability (i.e., metabolic status), of GPP and ER in stream ecosystems.

Nutrients from human activities have been predicted to alter the metabolic regime and status of streams (Bernhardt et al., 2018). Elevated nutrients may stimulate GPP, and to a lesser extent ER, thereby altering the metabolic balance of streams (Young et al., 2008). However, human activities differ in the amount, composition, and delivery of nutrients. Point sources (e.g., sewage treatment plants) typically release nutrients continuously and have a press effect on stream ecosystems. In contrast, nonpoint sources (e.g., agricultural activities) deliver high concentrations of nutrients over short durations following runoff events and have a pulse effect on streams. Differences in nutrient source may therefore affect a stream’s metabolic regime and status through an algal-mediated response to the amount and pattern of nutrient availability (Young et al., 2008; Finlay, 2011; Bernhardt et al., 2018).

Yet, while nutrients have been shown to control algal production, a nutrient-mediated response may be overshadowed by stronger physical controls, such as light and flow-related disturbances (Hill et al., 2009). For example, streams with dissimilar light conditions may not share an analogous response to elevated nutrient concentrations through resource colimitation or synergistic effects (Hill et al., 2011; Alberts et al., 2017; Warren et al., 2017). Limited research has been conducted on the potential for nutrient source to alter the associations between stream metabolism and temporal variation of other proximate metabolic drivers (e.g., light, temperature, and discharge). Investigations into the patterns and physicochemical controls of stream metabolism under differing nutrient conditions may improve our understanding of the functional capacity of streams exposed to common human activities.

The objectives of our study were three-fold. First, to describe the metabolic regime and metabolic status of three mid-order streams exposed to contrasting human nutrient sources (i.e., sewage treatment plant effluent, sewage lagoon effluent, and agricultural runoff). Second, to determine the associations between a river’s climate (i.e., light, temperature, and discharge; *sensu* Bernhardt et al., 2018) on each stream’s metabolic regime throughout the growing season (June to November). Third, to assess if the responses of GPP and ER to these physical drivers were comparable among streams exposed to different nutrient sources.

4.2 Methods

4.2.1 Study area

Our study was undertaken in the mixed-wood plains ecozone (Crins et al., 2009) of southern Ontario, Canada (Figure 4-1). Regional climate is temperate with an average annual temperature between 4.9 to 7.8 ^{o}C, and total precipitation from 759 to 1087 mm (1968-1988; Crins et al., 2009). The southern Ontario landscape is characterized by a post-glacial physiography with an underlying geology composed predominantly of calcareous bedrock. Land use in the region is dominated by agriculture, consisting mainly of row crop and livestock operations, with remnant forest patches and urban centers dispersed throughout the landscape (Yates & Bailey, 2010).

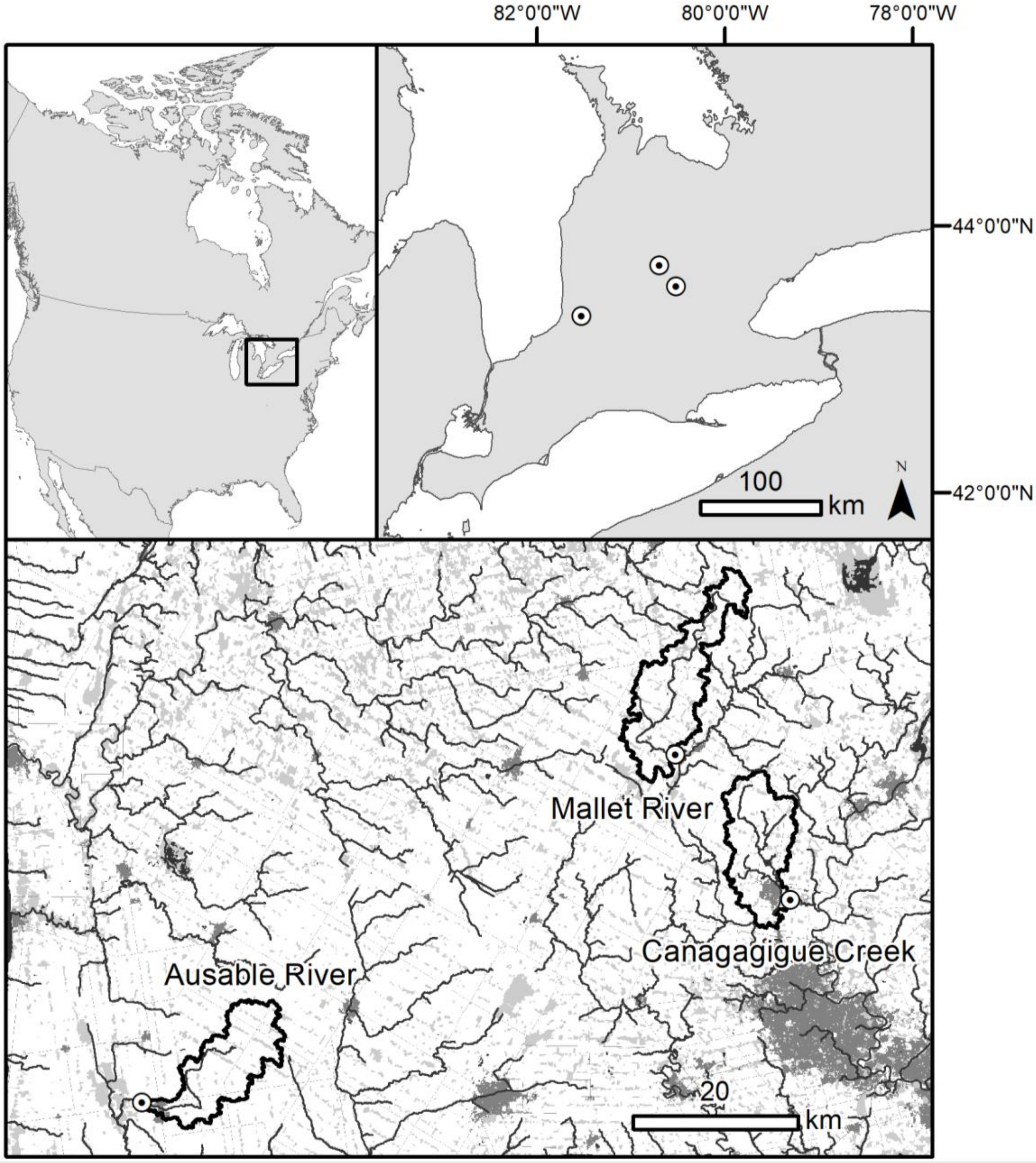

Figure 4-1. Location of study streams in southern Ontario, Canada (top). Delineated catchments of study streams are outlined in black (bottom). Land cover for the region is categorized as agriculture (white), natural (light grey), urban (medium grey), and water (dark grey).

Our study assessed three mid-order streams: Mallet River, Canagagigue Creek, and Ausable River (Figure 4-1). Study streams were selected to have comparable catchment physiography and reach characteristics, but differ in anthropogenic nutrient sources (Table 4-1). Sampling locations on each stream were restricted to segments with similar amounts of riparian canopy cover (~ 30%) within a ~ 650 m upstream reach, based on field and orthophotography observations. Substrate in our three study reaches ranged from a mixture of large cobble to fine sand characteristic of a till geology, and the primary producer community consisted mainly of benthic periphyton with dispersed submergent macrophytes.

Table 4-1. Stream and catchment attributes for the three streams studied in southern Ontario, Canada.

	Canagagigue Creek	Ausable River	Mallet River
Nutrient Exposure	Continuous	Semi-Continuous	Pulse
Nutrient Source	Tertiary STP	Lagoon STP	Agriculture
Med. (± mad) TN	^{ab}5.15 (± 2.24) mg L^{-1}	^{a}6.98 (± 2.92) mg L^{-1}	^{b}4.05 (± 2.12) mg L^{-1}
Med. (± mad) TP	^{a}0.11 (± 0.06) mg L^{-1}	^{a}0.15 (± 0.10) mg L^{-1}	^{b}0.03 (± 0.02) mg L^{-1}
Strahler Order	5	3	4
Ave. Stream Width	10.03 m	8.25 m	9.28 m
Ave. Stream Depth	0.38 m	0.33 m	0.41 m
Ave. Discharge (min.-max.)	1.1 (0.4-4.3) $m^3 s^{-1}$	1.4 (< 0.1-17.8) $m^3 s^{-1}$	1.1 (0.2-19.3) $m^3 s^{-1}$
Catchment Area	115.92 km^2	115.83 km^2	143.97 km^2
Surficial Geology	Till	Till	Till
Precipitation (Jun. – Nov.)	306.0 mm	429.9 mm	405.4 mm
Agriculture	83.96 %	88.34 %	85.30 %
Urban	5.93 %	2.60%	1.17 %
Natural	9.24 %	8.87%	13.31 %

Land cover was obtained from the Agriculture and Agri-food Canada 2012 Annual Crop Inventory Data Product. Physiography was determined from the Ontario Geological Survey 2007 Physiography of southern Ontario Data Product. Median (± median absolute deviation) total nitrogen (TN) and total phosphorus (TP) concentrations were summarized from the Ontario Provincial Stream Water Quality Monitoring Network from 2008 to 2012. Significant differences in medians were evaluated with a Kruskal-Wallis and Dunn’s post-hoc test with a Holm’s p-value adjustment ($p < 0.05$) and signified by corresponding letters (a, b, c) that are in descending order based on the median. STP: sewage treatment plant.

Study streams drain predominantly agricultural watersheds and were exposed to nutrient loading associated with runoff from crop and livestock production. In addition to diffuse agricultural inputs, Canagagigue Creek and Ausable River received nutrients from municipal sewage effluent, while Mallet River had no point sources of sewage effluent. Sampling locations were approximately 1.5 km and 1.3 km downstream from effluent outfalls on Canagagigue Creek and Ausable River, respectively.

Effluent discharged into Canagagigue Creek and Ausable River differed in level and mode of treatment. Canagagigue Creek flows through the town of Elmira, Ontario and is exposed to nutrient loading from the year-round discharge of sewage effluent from a tertiary sewage treatment plant (population served: 7,090; capacity: 7,800 m^3 day^{-1}). In contrast, Ausable River flows through the town of Exeter, Ontario and receives effluent from a multi-celled sewage lagoon (also known as a wastewater stabilization pond) with secondary effluent treatment (population served: 5,000; capacity: 7,051 m^3 day^{-1}).

Operations also differed between the Elmira sewage treatment plant (Canagagigue Creek) and the Exeter sewage lagoon (Ausable River). The former discharged continuously throughout the year, whereas the lagoon discharged periodically during the spring and summer when effluent in the final holding cell was at capacity and in compliance with water quality guidelines. Under this release regime, the Ausable River received effluent for 128 days of the 148-day discharge period (April 16 – September 10, 2013) during the study year. In addition, sewage lagoon effluent was gradually discharged to the Ausable River through a sand filtration system to remove particulate matter which in combination with irregular mixing in the lagoon resulted in variable nutrient concentrations throughout the discharge period. Thus, although Canagagigue Creek and Ausable River were both exposed to point sources of nutrients, effluent discharges from the lagoon outfall were more variable and therefore described as having a semi-continuous input of nutrients. In contrast, Mallet River was considered to receive pulsed nutrient inputs, as nutrients from agricultural nonpoint sources are primarily delivered episodically during runoff events (Withers & Jarvie, 2008).

Study streams were further differentiated based on concentrations of major forms of nitrogen (total nitrogen, TN; nitrate+nitrite nitrogen, NO_3^-+NO_2^-; total ammonia nitrogen, NH_3-N) and phosphorus (total phosphorus, TP; total dissolved phosphorus, TDP; soluble reactive phosphorus, SRP). Our three study streams (ID: 08002201602, 16018401602, 16018409102) are sampled (monthly; April to November) by the Ontario Provincial Stream Water Quality Monitoring Network’s (PWQMN) long-term monitoring program (OMECP, 2018). Monitoring data from 2008 to 2012 indicated that TN and TP concentrations differed among study streams and were greatest in Ausable River (sewage lagoon effluent) and Canagagigue Creek (sewage treatment plant effluent), followed by Mallet River (agricultural activities; Table 4-1).

We collected additional water samples every third week alongside the PQWMN in 2013 to further describe nutrient concentrations that were representative to our study period (June to November 2013). All nutrient concentrations were analyzed colorimetrically and detection limits for NH_3-N and SRP were 0.005 mg L^{-1} and 0.0002 mg L^{-1}, respectively. Nutrient samples collected from our study period also supports the differentiation of streams but revealed greater contrast between Ausable River and Canagagigue Creek (Figure 4-2). To encompass operational differences between sewage treatment plants, three sampling events occurred when no lagoon effluent was being discharged to the Ausable River: two in October when operations had ceased and one in July in between holding cell discharges (Figure 4-2).

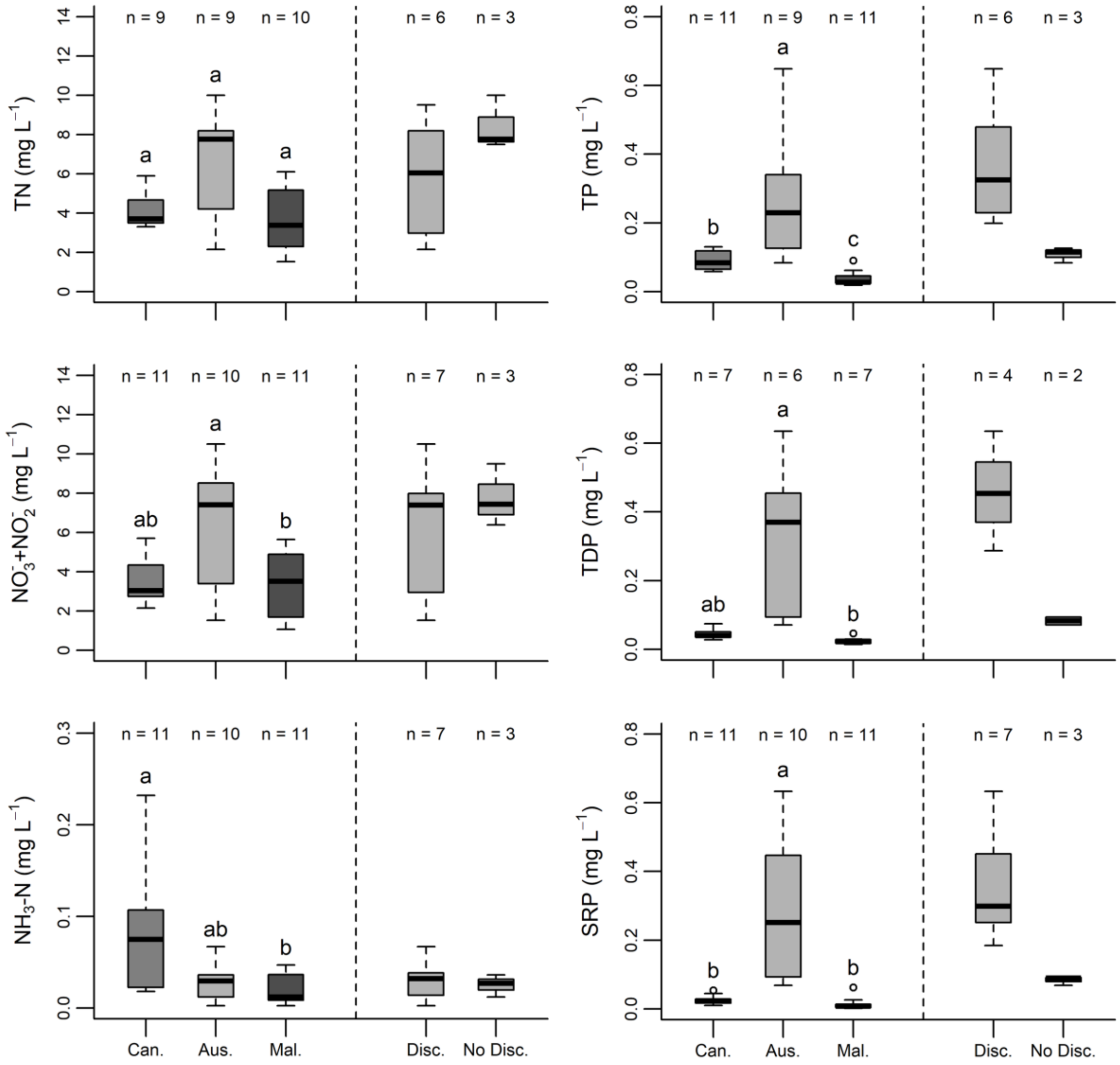

Figure 4-2. Boxplots (left) represent central tendencies (25th, 50th, and 75th percentiles; whiskers denote ± 1.5 x interquartile range) of nutrient concentrations in Canagagigue Creek (Can.), Ausable River (Aus.), and Mallet River (Mal.) from June to November 2013. Sample size for each box is noted by n, and differences in pairwise comparisons are signified by corresponding letters (a, b, c) that are in descending order based on the median. Significant differences in medians were evaluated with a Kruskal-Wallis and Dunn's post-hoc test with a Holm's p-value adjustment ($p < 0.05$). Boxplots (right) represent nutrient concentrations sampled in Ausable River with (Disc.) and without (No Disc.) the discharge of sewage lagoon effluent.

4.2.2 Stream metabolism

Stream metabolism was estimated from June to November 2013, using the open system, single station method in each of the study streams (Grace & Imberger, 2006). The open system method is reliable in large, well-mixed streams and provides an ecosystem level estimate of GPP and ER. To estimate GPP and ER, the reaeration of oxygen (*k*) throughout the stream reach must be accurately determined (Raymond et al., 2012). Dissolved oxygen modeling can provide a reasonable approximation of *k* in large, productive streams, and is less demanding in terms of cost and sampling effort compared to direct measurements (von Schiller et al., 2017). Daily volumetric rates of GPP, ER, and *k* were estimated from continuous dissolved oxygen, temperature, light, and discharge records with the *streamMetabolizer* package (Appling et al., 2018a). Within the package, a Bayesian hierarchical model was used with a trapezoidal ordinary differential equation method to estimate stream metabolism parameters. Reaeration was pooled in six bins corresponding to discharge to achieve more consistent estimates of *k* (Appling et al., 2018b). Daily estimates of stream metabolism and *k* were considered reliable by inspecting the model output for adequate convergence, fit of predicted DO measures, model errors and warnings, and variability in the daily estimations of *k*, GPP, and ER. Estimations from study days that failed to satisfy model validation requirements were not included in further analyses. GPP and ER were standardized to stream size by multiplying volumetric rates by daily estimates of depth.

Continuous data used to estimate stream metabolism were collected by multiple data loggers deployed at each monitoring site. Multi-parameter sondes (YSI Sonde #6600) were used to continuously (15 min intervals) measure dissolved oxygen (DO) concentration and temperature over the study period. In addition, a photosynthetically active radiation (PAR) logger was deployed in a location representative of the local canopy cover within each study reach to continuously (15 min intervals) measure light reaching the stream. Discharge and precipitation were summarized to describe daily hydrologic conditions at each study site. Hydrologic data were obtained from river flow (ID: 02FF009, 02GA023, 14918) and tipping bucket rain gauges (ID: 61HKLR, 14929, 15171) operated by Environment and Climate Change Canada and the Grand River

Conservation Authority (ECCC, 2018; GRIN, 2018). Stream depth was estimated throughout the study period from the linear equation between stage level and three separate measures of stream depth collected over differing hydrologic conditions ($R^2 > 0.9$). Stream depth was measured as the average depth of 10 equally spaced points across five evenly spaced transects over an approximately 200 m reach upstream of the multi-parameter sonde.

4.2.3 Data analysis

Metabolic status was described as the magnitude and variability of GPP and ER over the sampling period. To provide an equitable comparison of metabolic rates, only sampling days that were reliable for all sites were included in analyses (n = 113 of a possible 140 days). A bivariate kernel density analysis was used to visualize the paired daily rates of GPP and ER throughout the study period and was computed with the use of the *ks* package in R (Duong, 2018). Bivariate metabolism distributions were compared among study streams with a multivariate two sample Cramer test (Baringhaus & Franz, 2004). In brief, the equality of the underlying distributions was evaluated through replicated (n = 1000) Monte-Carlo bootstrap methods on the Euclidian interpoint distances between two samples (Baringhaus & Franz, 2004). GPP and ER were further compared with the use of a Levene's test to compare variability about the median among study streams. GPP and ER failed to meet homogeneous variance and normality assumptions. Therefore, a Kruskal-Wallis and Dunn's post-hoc test with a Holm's p-value adjustment was used to conduct pairwise comparisons of median GPP and ER among streams. Analyses were computed with the *cramer*, *stats*, *car*, and *dunn.test* packages in R (Franz, 2015; Dinno, 2017; R Core Team, 2017; Fox et al., 2018). Results were considered significant at $p < 0.01$ to reduce the probability of type one error owing to the increased sensitivity of our large sample size.

To examine the metabolic regime (i.e., temporal dynamics) of each stream, daily time series were developed for GPP and ER. Data gaps existed within each time series as estimates of GPP and ER could not be reliably determined for all 140 study days (Canagagigue = 135; Ausable = 133; Mallet = 118). To obtain a complete data set, missing values were interpolated with an autoregressive integrated moving average model for each time series with the use of the *imputeTS* and *forecast* packages in R (Moritz, 2017; Hyndman et al., 2018). Interpolated values were only used in time series analyses that aimed to investigate temporal patterns of stream metabolism. Interpolated rates were not included in comparative analyses of metabolic status as continuous datasets were unnecessary and outcomes of comparative statistics have a greater dependency on absolute values. Temporal patterns of GPP and ER were compared among study streams with the use of a Spearman's rank correlation analysis from the *Hmisc* package in R and were considered significant at $p < 0.01$ (Harrell & Dupont, 2018).

PAR, temperature, and discharge were compared among streams to assess differences in physical drivers known to influence stream metabolism. All physical drivers failed to meet parametric assumptions, therefore a Kruskal-Wallis and Dunn's post-hoc test with a Holm's p-value adjustment was used to compare medians of PAR, temperature, and discharge among streams. In addition, Spearman's correlation analyses were used to conduct pairwise comparisons of the temporal patterns in physical drivers between streams. Analyses were conducted in R with the *stats*, *dunn.test*, and *Hmisc* packages and results were considered significant at $p < 0.01$ (Dinno, 2017; R Core Team, 2017; Harrell & Dupont, 2018).

Two approaches were used to evaluate the influence of physical drivers on stream metabolism. First, generalized additive models (GAM) were used to determine the associations of light, temperature, and discharge with GPP and ER in each study stream. Relationships between independent and dependent variables were modeled based on a GAM smoothing function to identify significant linear or non-linear associations. The estimated degrees of freedom (edf) of the model can be interpreted as the complexity of the non-linear association between variables, where 1 is completely linear and larger values represent further non-linearity. GAM models were used to simultaneously examine the influence of the multiple physical variables (independent) on individual metabolism (i.e., GPP and ER) variables (dependent) with a multiple regression framework. An autocorrelation function was added to each GAM to take into account the structure of model residuals and reduce the effects of autocorrelation on the model output. GAMs were performed in R with the use of the *mgcv* package (Wood, 2018).

Thin plate regression splines were used to smooth the data and a conservative number of degrees of freedom was chosen to prevent overfitting of the data ($k < 8$). PAR (total: mol m^{-2} day^{-1}), temperature (average: ^{o}C), and discharge (average: m^3 s^{-1}) were summarized at a daily resolution to evaluate associations with stream metabolism ($p < 0.01$).

Associations between day-to-day variability in physical drivers and stream metabolism were determined for each stream using multiple Spearman's cross-correlation analyses with the interpolated metabolism time series. Cross-correlation computes the correlation between specified variables for the matching time interval, as well as correlations with a defined time lag. To interpret the temporal structure of cross-correlation analyses, Spearman's autocorrelation was also computed to for each individual physical driver and metabolic parameter. Prior to analyses, physical drivers and metabolic rates that showed seasonal trends over the study period (i.e., GPP, ER, PAR, and temperature) were decomposed to remove the long-term progression of each time series and isolate variability associated with day-to-day fluctuations. Discharge patterns were episodic and undifferentiated among study streams, and therefore not subject to trend decomposition analyses. Seasonal trends were evaluated by fitting a GAM between the individual physical drivers and time. Thin plate regression splines were used to smooth the data, and the degrees of freedom in the models were minimized to adequately capture the seasonal trend throughout the study period ($k < 5$). Seasonal trends were removed from the data by calculating the residuals between the raw data and respective GAM. Spearman's cross-correlation and autocorrelation analyses were then performed on the decomposed GPP and ER, and individual physical drivers for each study stream. Cross and autocorrelations were considered significant at $p < 0.01$ and computed with the *stats* package in R (R Core Team, 2017).

4.3 Results

4.3.1 Stream metabolic status

Bivariate distributions of GPP and ER were different among all study streams (Canagagigue-Ausable: $T = 7.7$, $p < 0.001$; Canagagigue-Mallet: $T = 71.0$, $p < 0.001$;

Ausable-Mallet: $T = 48.2$, $p < 0.001$; Figure 4-3). The distribution of paired metabolic rates from Canagagigue Creek and Ausable River were observed to overlap to a greater extent than the distribution from Mallet River, which was approximately 2-fold smaller than the other two streams. Net metabolism over the 113-day study period was heterotrophic in both Canagagigue Creek (-160.2 g O_2 m^{-2} study $period^{-1}$) and Ausable River (-40.4 g O_2 m^{-2} study $period^{-1}$), but autotrophic in Mallet River (30.2 g O_2 m^{-2} study $period^{-1}$). Daily metabolic balance, however, was typically neutral ($0.5 < GPP:ER < 1$) among streams (Canagagigue = 71 days; Ausable = 52 days; Mallet = 42 days) with a greater number of days classified as autotrophic ($GPP:ER > 1$; Canagagigue = 24 days; Ausable = 56 days; Mallet = 69 days) than heterotrophic ($GPP:ER < 0.5$; Canagagigue = 18 days; Ausable = 5 days; Mallet = 2 days). Average (± standard deviation) reaeration rates ($k600$) were 7.1 ± 2.2, 6.7 ± 2.0, and 4.3 ± 1.4 day^{-1} for Canagagigue Creek, Ausable River, and Mallet River, respectively.

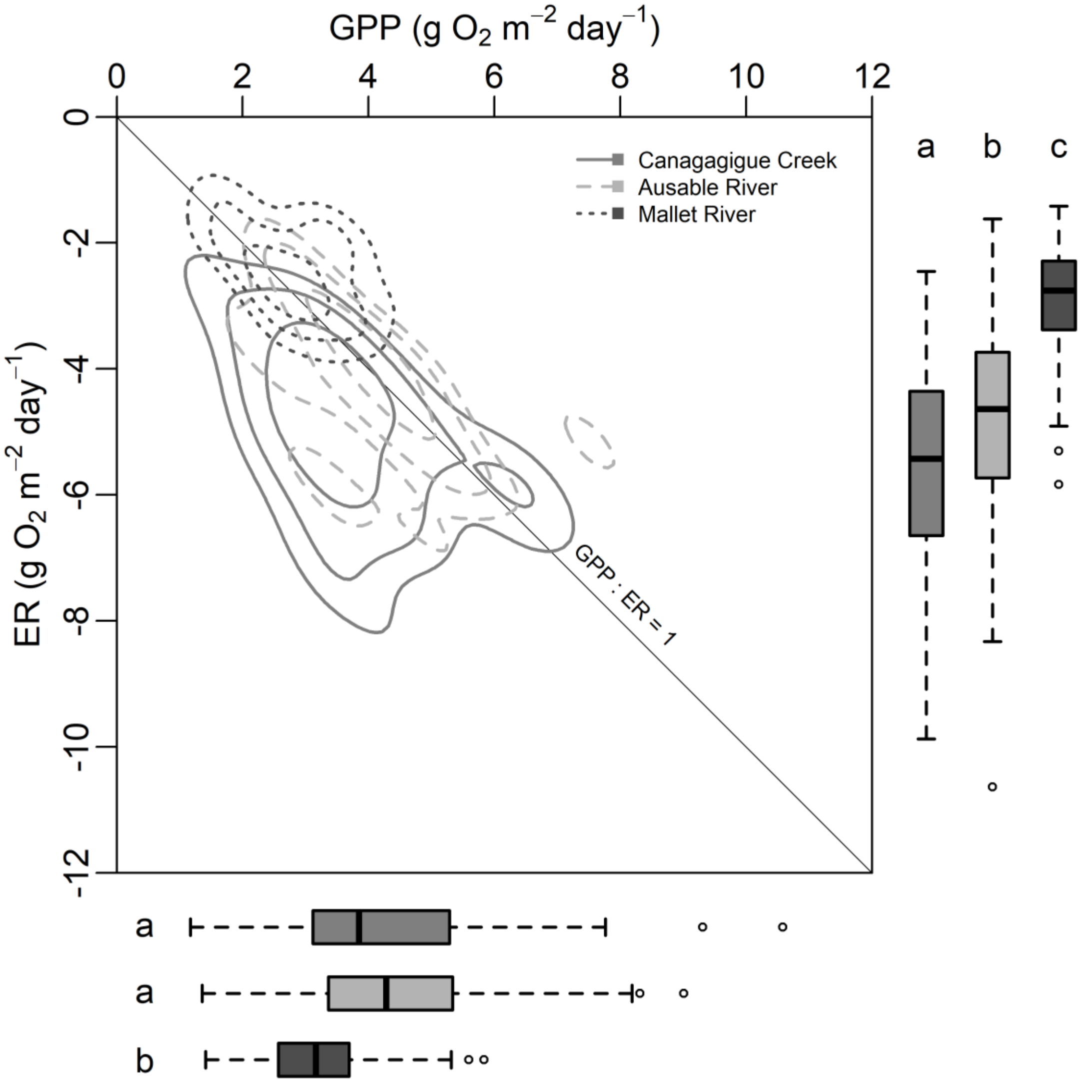

Figure 4-3. Kernel density plot showing the paired rates of gross primary production (GPP) and ecosystem respiration (ER) over the same 113 reliable sampling days for Canagagigue Creek (solid line, medium grey), the Ausable River (long dash, light grey), and the Mallet River (small dash, dark grey). Density contours in an outward direction represent 25%, 50%, and 75% of the data distribution. Boxplots represent central tendencies (25th, 50th, and 75th percentiles; whiskers denote ± 1.5x interquartile range) of GPP and ER. Pairwise comparisons are signified ($p < 0.01$) by corresponding letters (a, b, c) that are in descending order based on the median.

GPP was found to differ among study streams ($\chi^2 = 48.2$, df = 2, $p < 0.001$; Figure 4-3). Pairwise comparisons between streams (Canagagigue-Ausable: $|Z| = 1.4$, $p = 0.158$; Canagagigue-Mallet: $|Z| = 5.2$, $p < 0.001$; Ausable-Mallet: $|Z| = 6.6$, $p < 0.001$) showed there was no difference in median GPP between Canagagigue Creek and Ausable River, but both had greater GPP than Mallet River. Median ER also differed among streams (χ^2 = 149.2, df = 2, p < 0.001; Canagagigue-Ausable: |Z| = 2.7, p = 0.007; Canagagigue-Mallet: |Z| = 11.7, p < 0.001; Ausable-Mallet: |Z| = 9.0, p < 0.001) and was greatest in Canagagigue Creek followed by Ausable River and Mallet River. Likewise, variability of GPP and ER around the median revealed pairwise differences among streams. The median absolute deviation of GPP and ER in Canagagigue Creek (GPP: 1.5 g O_2 m^{-2} day^{-1}; ER: 1.9 g O_2 m^{-2} day^{-1}) and Ausable River (GPP: 1.6 g O_2 m^{-2} day^{-1}; ER: 1.5 g O_2 m^{-2} day^{-1}) were not different (GPP: p = 0.460; ER: p = 0.099), but GPP and ER in both sewage receiving streams varied more (p < 0.001) than Mallet River (GPP: 0.9 g O_2 m^{-2} day^{-1}; ER: 0.9 g O_2 m^{-2} day^{-1}).

4.3.2 Stream metabolic regime

Stream metabolism time series revealed significant pairwise correlations between all streams (n = 140; p < 0.001; Figure 4-4). Spearman correlation values for temporal patterns of GPP were 0.70, 0.67, and 0.66 for Canagagigue-Ausable, Canagagigue-Mallet, and Ausable-Mallet, respectively. Spearman correlations for ER were 0.54, 0.50, and 0.40 for the aforementioned pairs.

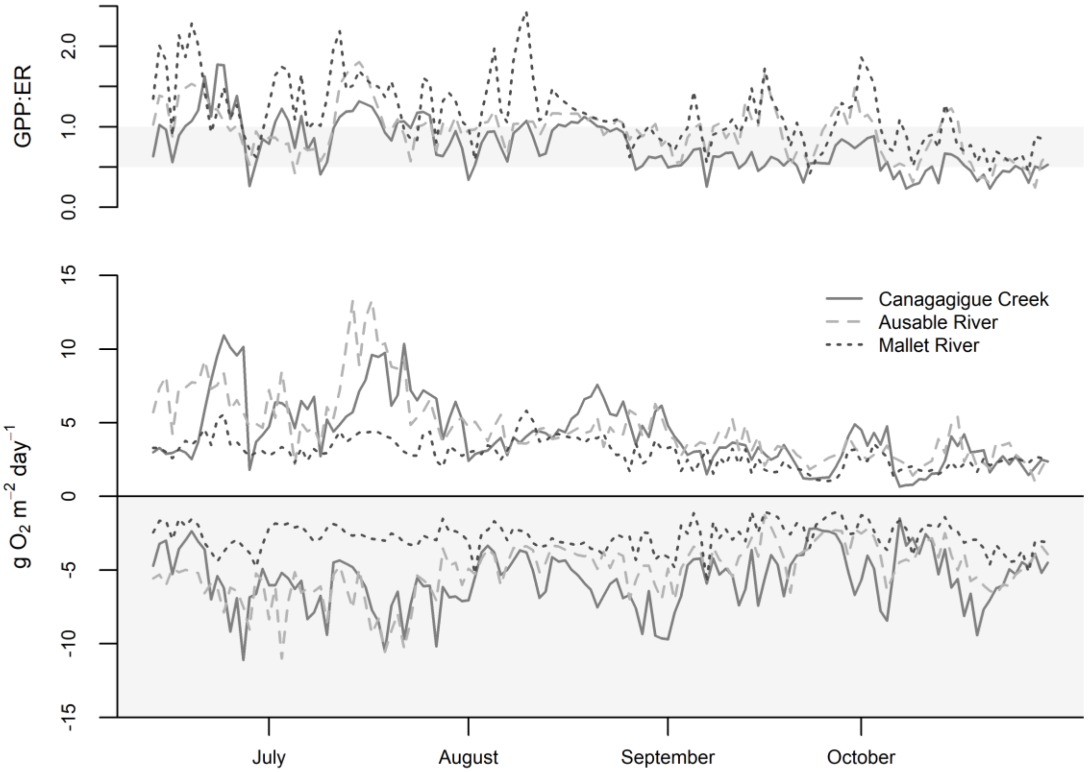

Figure 4-4. Time series plots of gross primary production (GPP) to ecosystem respiration (ER) ratio (top), GPP, and ER (bottom) over 140 sampling days for Canagagigue Creek (solid line, medium grey), Ausable River (long dash, light grey), and Mallet River (small dash, dark grey).

4.3.3 Physical drivers of stream metabolism

Comparisons of physical drivers revealed among stream differences in median stream temperature ($\chi^2 = 11.4$, df = 2, p = 0.003) and discharge ($\chi^2 = 40.0$, df = 2, $p < 0.001$), but not PAR ($\chi^2 = 5.2$, df = 2, p = 0.076; Figure 4-5). Temperature was greater in Ausable River than Canagagigue Creek ($|Z| = 3.3$, p = 0.003), but neither site was different from Mallet River. Likewise, discharge was greater in Canagagigue Creek ($|Z| = 6.2$, $p < 0.001$) and Mallet River ($|Z| = 4.2$, $p < 0.001$) when compared to Ausable River. Maximum differences detected in the median values of temperature and discharge among streams were 1.3 ^{o}C and 0.35 $m^3 s^{-1}$, respectively (n = 140). Spearman correlation analyses revealed that PAR and temperature had correlation coefficients greater than 0.90 for all pairwise combinations ($p < 0.001$). Discharge was also correlated among streams with correlation coefficients of 0.83, 0.67, and 0.73 ($p < 0.001$) for Canagagigue-Ausable, Canagagigue-Mallet, and Mallet-Ausable, respectively (Figure 4-5).

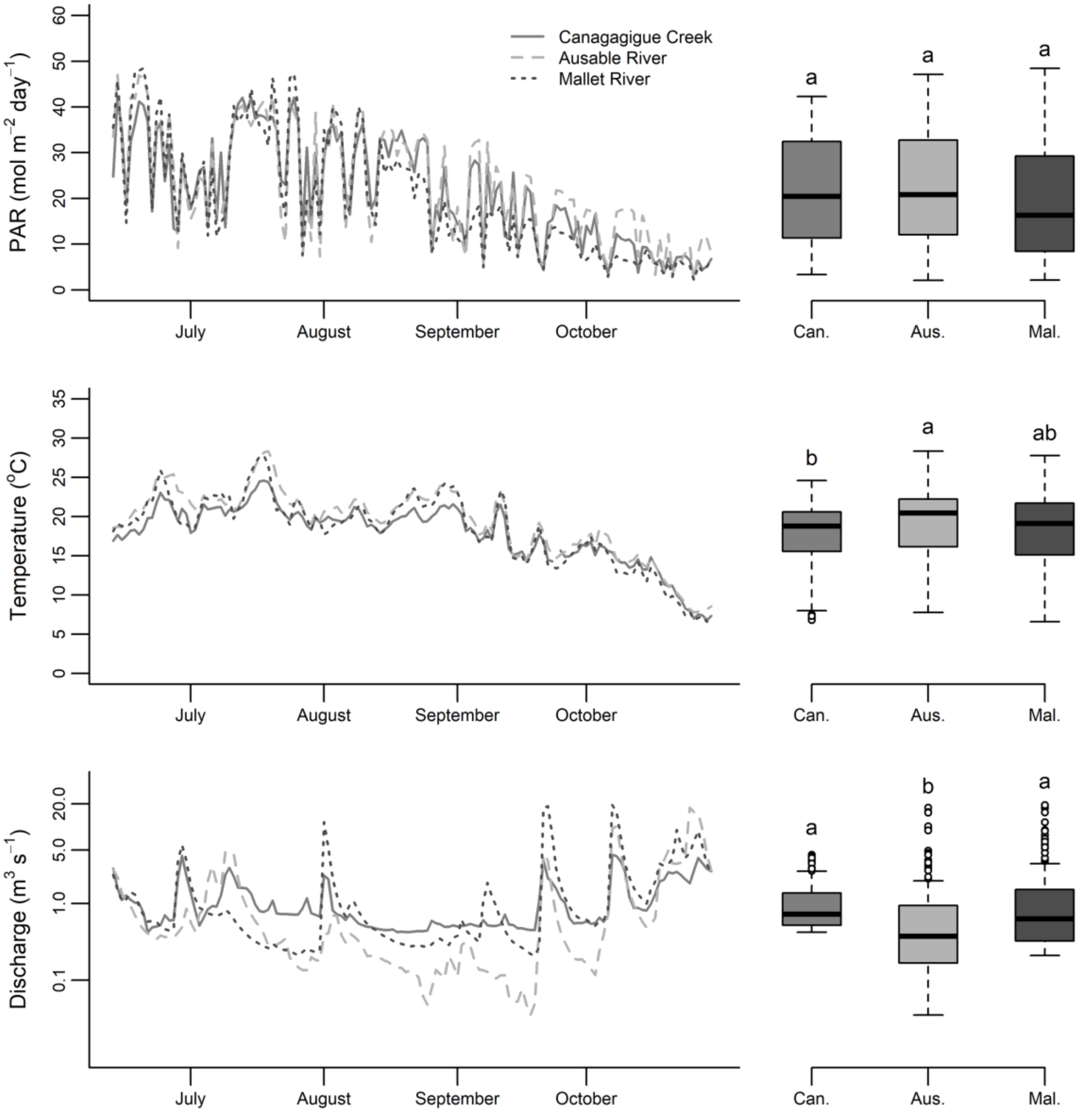

Figure 4-5. Time series and boxplots (25th, 50th, and 75th percentiles; whiskers denote ± 1.5 x interquartile range) of photosynthetically active radiation (PAR), temperature, and discharge (log scale) for Canagagigue Creek (solid line, medium grey; Can.), Ausable River (long dash, light grey; Aus.), and Mallet River (small dash, dark grey; Mal.) over 140 sampling days from June to November 2013. Differences in pairwise comparisons are signified ($p < 0.01$) by corresponding letters (a, b, c) that are in descending order based on the median.

GAMs revealed significant associations between stream metabolism and physical drivers for all three study streams and explained 55.2%, 46.4%, and 57.6% of the variation in GPP and 48.0%, 60.5%, and 25.4% in ER, for Canagagigue Creek, Ausable River, and Mallet River, respectively (Figure 4-6). GPP revealed a positive association with PAR in Mallet River (edf = 2.50, F = 14.12, $p < 0.001$), but not in Canagagigue Creek (edf = 1.00, F = 0.13, p = 0.723) or Ausable River (edf = 1.60, F = 3.68, p = 0.119). However, the trend observed for GPP in Mallet River varied only 1.4-fold over the range of PAR. In contrast, PAR was negatively associated with ER and had an approximately linear relationship in all streams (Canagagigue: edf = 1.00, F = 68.61, $p < 0.001$; Ausable: edf = 2.64, F = 14.25, $p < 0.001$; Mallet: edf = 1.00, F = 9.71, p = 0.002).

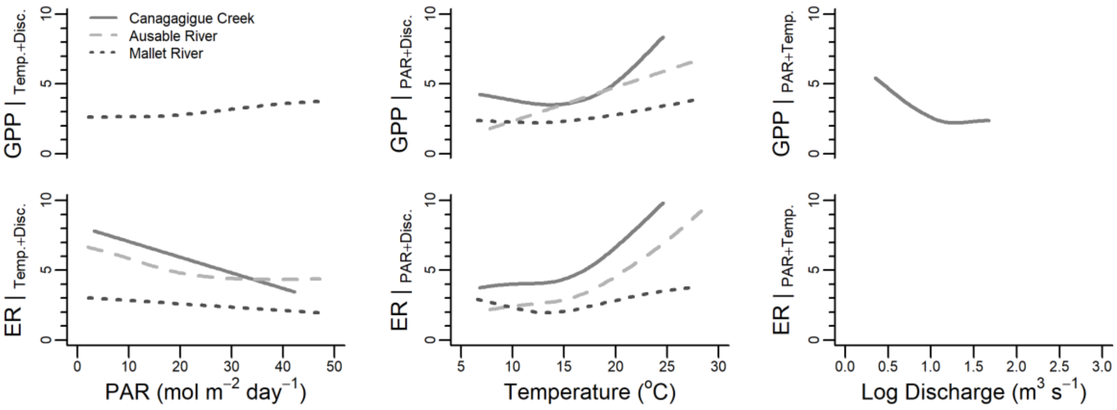

Figure 4-6. Partial effects plots of generalized additive models (GAM) between physical drivers and rates of gross primary production (GPP) and ecosystem respiration (ER) for Canagagigue Creek (solid line, medium grey), Ausable River (long dash, light grey), and Mallet River (small dash, dark grey). Non-significant ($p > 0.01$) associations not shown.

Temperature had a positive association with GPP (Canagagigue: edf = 3.00, F = 9.54, $p < 0.001$; Ausable: edf = 1.00, F = 12.90, $p < 0.001$; Mallet: edf = 2.40, F = 6.86, $p < 0.001$) and the rate of ER (Canagagigue: edf = 2.77, F = 17.51, $p < 0.001$; Ausable: edf = 2.94, F = 33.24, $p < 0.001$; Mallet: edf = 3.35, F = 6.80, $p < 0.001$) in all streams. All associations with temperature were non-linear apart from GPP in the Ausable River. The trajectory of the non-linear associations for GPP and ER changed around a stream water temperature of ~15 ^{o}C and began to increase more rapidly.

Discharge had a negative, nonlinear association with GPP in Canagagugie Creek (edf = 2.72, F = 15.91, $p < 0.001$), but was not associated with ER (edf = 1.00, F = 2.74, p = 0.100). In Ausable River and Mallet River discharge was not associated with GPP (Ausable: edf = 1.00, F = 0.14, p = 0.709; Mallet: edf = 1.00, F = 0.30, p = 0.585) or with ER (Ausable: edf = 1.64, F = 4.14, p = 0.014; Mallet: edf = 1.70, F = 3.92, p = 0.050).

Decomposed metabolism, PAR, and temperature, as well as untransformed discharge, were autocorrelated throughout the study period. GPP revealed a significant autocorrelation ($p < 0.01$) of 3 days for Canagagigue Creek, Ausable River, and Mallet River. Autocorrelation of ER was slightly lower at 2 days for Canagagigue Creek and 1 day for the Ausable and Mallet Rivers. PAR had the lowest degree of autocorrelation of 1 day for all study sites, whereas temperature had an autocorrelation of 2-3 days, and discharge plateaued at approximately 8 days.

Cross-correlation analyses between stream metabolism and physical drivers revealed comparable magnitudes and lag periods among sites. Likewise, correlations existed over a lag period of multiple days for most physical drivers. Therefore, peak correlations between physical drivers and metabolism were used to examine the influence of day-to-day variability in preceding environmental conditions on GPP and ER.

PAR was found to have a positive antecedent forcing relationship with GPP in all study streams. Cross-correlations between PAR and GPP peaked with a lag time of approximately 1 to 4 days. Peak correlations were 0.32 ($p < 0.001$), 0.39 ($p < 0.001$), and 0.36 ($p < 0.001$) for Canagagigue Creek, Ausable River, and Mallet River, respectively. PAR revealed no discernible peak correlation pattern with magnitude of ER, however individual negative correlations appeared in Canagagigue Creek ($r = -0.55$, $p < 0.001$) and Ausable River ($r = -0.36$, $p < 0.001$) at a lag of 0.

Temperature had a positive association with GPP and the magnitude of ER in all study streams. Correlations between GPP and temperature peaked at a lag of 0 and showed the strongest correlation of all physical drivers (Canagagigue: $r = 0.43$, $p < 0.001$; Ausable: $r = 0.40$, $p < 0.001$; Mallet: $r = 0.47$, $p < 0.001$). The association between ER and temperature was also significant at a time lag of 0 for all streams, however, correlations peaked at a time lag of 1 day for Canagagigue Creek ($r = 0.35$, $p < 0.001$) and Ausable River ($r = 0.37$, $p < 0.001$), and was greatest at a lag of 3 days in Mallet River ($r = 0.28$, $p = 0.001$).

Discharge was only correlated with GPP and ER in Canagagigue Creek and Mallet River. Discharge was found to have a negative association with GPP that peaked at a time lag of 0-1 days (Canagagigue: $r = -0.44$, $p < 0.001$; Mallet: $r = -0.23$, $p = 0.006$), whereas the association between discharge and ER had a lag time of 2-3 days (Canagagigue: $r = -0.30$, $p < 0.001$; Mallet: $r = -0.23$, $p = 0.007$).

4.4 Discussion

Assessment of ecosystem metabolism for three mid-order streams in southern Ontario, Canada showed that metabolic status (i.e., magnitude and variability) differed among streams, whereas metabolic regime (i.e., temporal patterns) was most associated with seasonal changes in temperature. Physical drivers (e.g., light, temperature, and discharge) demonstrated spatial and temporal comparability among streams. Thus, observed differences in metabolic status appear to be linked to differences in the types of nutrient sources in the catchment (i.e., agricultural runoff versus sewage effluent). The observation that our two streams exposed to sewage effluent had greater rates of GPP and ER than the stream influenced solely by agricultural runoff is consistent with predicted ecological response of stream metabolism to poorer ecosystem condition (Young et al., 2008; Finlay, 2011).

4.4.1 Stream metabolic status

Metabolic status differed among the three study streams in accordance with the predominant human activity. Median metabolic rates increased from 2.9 to 4.1 g O_2 m^{-2} day^{-1} GPP and 2.5 to 5.4 g O_2 m^{-2} day^{-1} ER in association with sewage enrichment. While among stream differences in median rates of GPP and ER were small (0.8 – 1.2 g O_2 m^{-2} day^{-1} GPP and 0.9 – 2.9 g O_2 m^{-2} day^{-1} ER), the large number of monitoring days ($n = 113$) in our study allowed for detection of smaller effect sizes in GPP and ER among sites, increasing the potential for type I errors. However, our finding of increased GPP and ER with increased exposure to anthropogenic activities (i.e., agriculture and sewage effluent versus agriculture alone) is consistent with past studies that reported increases in stream metabolism associated with human land use gradients (Gücker et al., 2009; Griffiths et al., 2013; Fuß et al., 2017). Likewise, greater rates of GPP and ER have been observed in studies of streams receiving treated sewage effluent compared to reaches receiving no effluent (Gücker et al., 2006; Aristi et al., 2015; Rodriguez-Castillo et al., 2017). Furthermore, our finding of a wider density distribution of paired daily metabolic rates and greater variability of GPP and ER in effluent receiving streams supports Bernhardt et al.,'s (2018) hypothesis that a greater availability of resources can expand the distribution of GPP and ER relative to the dominant metabolic rates for a stream. Thus, the greater metabolic rates in the effluent-receiving streams, together with the longer duration of higher metabolic rates, supports our hypothesis of a meaningful difference in metabolic status between effluent versus agriculturally influenced streams.

Assessment of metabolic balance revealed that the streams receiving sewage effluent were more heterotrophic (sewage treatment plant: -160.2 g O_2 m^{-2} study period^{-1}; sewage lagoon: -40.4 g O_2 m^{-2} study period^{-1}) than the agricultural stream (30.2 g O_2 m^{-2} study period^{-1}). Past studies have also reported an increase in heterotrophy with the input of sewage effluent (Gücker et al., 2006; Aristi et al., 2015; Arroita et al., 2019). However, we also observed differences between effluent receiving streams as the stream with the tertiary sewage treatment plant had a wider distribution of daily metabolic rates associated with greater ER and a net metabolism 4 times more negative than the stream receiving sewage lagoon effluent.

Sewage effluents can contain variable combinations of nutrients, organic matter, and other contaminants (e.g., pharmaceuticals, personal care products, and industrial chemicals) that may have mixed effects on stream ecosystems, and metabolism in particular (Aristi et al., 2015). Our finding of equal GPP between effluent receiving streams, but lower ER in the stream receiving sewage lagoon effluent suggests that an organic matter subsidy from the tertiary sewage effluent may be responsible for differences observed in metabolic balance. Indeed, organic matter is a fundamental driver of ER (Tank et al., 2010). Moreover, the observation of increased heterotrophy coincided with greater concentrations of dissolved organic carbon (n = 7) in the stream receiving sewage treatment plant effluent (5.30 ± 0.45 mg L^{-1}) than the stream receiving sewage lagoon effluent (3.80 ± 0.59 mg L^{-1}).

NH_3-N (ammonia + ammonium) concentrations were also greater downstream of the tertiary sewage treatment plant outfall (sewage treatment plant: 0.075 ± 0.077 mg L^{-1}; sewage lagoon: 0.030 ± 0.014 mg L^{-1}), which may have stimulated microbial activity (Webster et al., 2003). In addition, nitrification of ammonium consumes dissolved oxygen through oxidation and thus can be incorporated into estimates of ER (Cooper, 1984). However, based on ecosystem-scale nitrification models by Kemp & Dodds, (2002) the oxygen consumption by nitrifiers would account for less than 5% of ER in our study streams. Thus, increased concentrations of organic matter and NH_3-N most likely contributed to the differences observed in heterotopic status of sewage receiving streams. However, because our study did not measure additional contaminants often contained in wastewater, we cannot account for the possible role that the different treatment methods may have had on the quantities and potential effects of emerging contaminants on GPP and ER.

Although each of the human activities examined in our study have the potential to influence stream ecosystems through multiple co-occurring stressors, our results suggest that, in addition to organic matter, increased nutrient availability likely contributed to differences observed in metabolic status. Effluent receiving streams had concentrations of total phosphorus 2 to 16 times greater than the agriculturally influenced stream, which corresponded with increased GPP and ER. The apparent demand for phosphorus across our study streams was further supported by large nitrogen to phosphorus ratios (N:P > 46:1). However, despite or finding that that total phosphorus concentrations were 9-fold greater downstream of the sewage lagoon than the tertiary sewage treatment plant, no difference was observed in GPP between the two sewage receiving streams. Additional controls that govern stream productivity may therefore determine the capacity of a stream ecosystem to respond to increased resource availability.

4.4.2 Stream metabolic regime

No differences were observed in metabolic regime among our study streams. Temporal patterns of GPP and ER were correlated between all streams throughout the growing season, suggesting that external factors may have a more important influence on the temporal variation in stream metabolism than resource delivery associated with runoff and sewage effluent. Indeed, past studies have shown that climatic and hydrologic regimes are often dominant controls of temporal variability in stream metabolism (Young & Huryn, 1996; Acuña et al., 2004; Uehlinger, 2006). For example, dams that reduce the frequency and severity of flood events have been shown to enhance GPP and ER (Munn & Brusven, 2004; Aristi et al., 2014). Moreover, Savoy et al., (2019) identified characteristic patterns in stream productivity regimes that could be differentiated based solely on site conditions and environmental variables (e.g., PAR, temperature, and discharge). Our finding that physical drivers were associated with temporal variation in GPP and ER for all three study streams thus suggests that seasonality may be more important in setting the domain of a stream's metabolic regime than differences in exposure to human activities.

Day-to-day variation in PAR was correlated with temporal variability in GPP within all study streams. Past studies have shown that light availability can control day-to-day and seasonal variation in GPP (Uehlinger, 2006; Roberts et al., 2007; Beaulieu et al., 2013). Although we did not observe a strong seasonal effect of light within streams, our study identified a time lag (1 to 4 days) whereby short periods of high light availability were associated with increased GPP. Given the short autocorrelation structure of PAR (1 day), the delayed response observed in GPP may be a result of biofilm accrual from periods of stimulated photosynthesis. In contrast, PAR was negatively associated with ER within study streams. Roberts et al., (2007) found that ER was decoupled from temperature in a first-order deciduous forest stream due to increased allochthonous carbon inputs during the colder autumn seasons. Increased ER on days with low total light availability may therefore be related to seasonal factors independent of physical drivers such as autumn leaf litter inputs during periods of little daylight.

Temperature revealed an escalating association with GPP and ER over the study period. While temperature is known to regulate metabolic activity (Demars et al., 2011), factors such as resource availability can alter the ecosystem-level response to increases in temperature (Yvon-Durocher et al., 2012; Song et al., 2018). Our finding that the rate of increase in GPP and ER was greater for the two effluent receiving streams than the agricultural stream suggests that the metabolism of streams with greater nutrient availabilities may respond more rapidly to seasonal changes in temperature. Moreover, of the physical drivers of a river's climate (i.e., light, temperature, and discharge; *sensu* Bernhardt et al., 2018), stream metabolism appeared most sensitive to seasonal variation in water temperature, suggesting that changes to the thermal regime of streams associated with forecasted global warming or other anthropogenic impacts (Kaushal et al., 2010) may have a substantial influence on the functional capacity of streams to support ecosystem processes.

Discharge events throughout the study period were observed to coincide with decreased GPP and ER in all streams, however, discharge was only found to be associated with GPP in the stream that received tertiary sewage effluent. Past research has demonstrated the importance of high flow disturbances to the ecology of streams (Bernhardt et al., 2018; Woodward et al., 2016). For example, O'Connor et al., (2012) observed a threshold-type relationship over a 4-year record of discharge and diel dissolved oxygen fluctuations in an agricultural river located in the U.S. mid-west. We also observed one threshold association between discharge and GPP. However, the low frequency of events that differed in flow magnitude captured in our dataset may have resulted in the inability of regression models to detect a significant effect of discharge in the other two streams.

Past research suggests that confounding factors from the physical environment, such as light availability or hydrologic disturbances, can override the response of stream metabolism to additional metabolic drivers (Alberts et al., 2017; Siders et al., 2017; Warren et al., 2017; Bernhardt et al., 2018). Among stream variation in physical drivers measured in our study appear to show sufficient spatial and temporal comparability to permit the comparison of GPP and ER among streams with contrasting anthropogenic nutrient sources. Although median discharge and temperature were found to differ spatially, among stream differences detected in the median of temperature (1.3 ^{o}C) and discharge (0.35 $m^3 s^{-1}$) were minimal and may have limited ecological relevance. Moreover, temporal patterns of discharge and temperature were correlated among all study streams, which may be more important to a stream's metabolic regime than differences in central tendency. For example, our study streams experienced similar regional hydrologic variation in precipitation, thus disturbances to stream metabolism occurred concurrently in all streams.

Temperature can have a more direct control on metabolism (Demars et al., 2011; Rasmussen et al., 2011). However, the effects of temperature can be confounded by light availability (Sabater et al., 2000; Hoellein et al., 2012; Huryn et al., 2014), which is often regarded as the strongest physical control of stream metabolism among streams (Mulholland et al., 2001; Bernot et al., 2010; Beaulieu et al., 2013). PAR was equivalent and highly correlated among all streams. In addition, turbidity from nominal baseflow sampling (n = 4) was less than 10 NTU for all streams; therefore, among stream differences in light availability were effectively minimized. Thus, differences in the dominant human activities influencing our study streams appear to be the most likely explanation for the differences observed in stream metabolism. Our study further demonstrates the importance of selecting reaches with adequate spatial comparability in physical drivers of metabolism to enable effective assessment of environmental stressors.

The ability of our study to make clear linkages between the observed patterns of stream metabolism and human activities was limited by a lack of high-resolution water quality data. Day-to-day variations in nutrients can have an important influence on the temporal patterns of GPP and ER, and field experiments have observed changes in stream metabolism following nutrient additions (Rosemond et al., 2015; Gough et al., 2016; Kominoski et al., 2018). Further research using high resolution nutrient data is needed to fully understand the temporal importance of nutrients on the metabolic regime of human-influenced streams. In addition, a more robust water quality monitoring program would be required to better diagnose the potential mixed effects of human activities on stream metabolism.

4.4.3 Conclusions

The range of metabolic conditions exhibited by our study streams appeared to be constrained by region-wide variations in physical environmental factors (i.e., light, temperature and discharge), but the prevailing condition of each stream within the regional metabolic range appeared to be dictated by the dominant source of nutrients to each stream. The observed interaction between the regional environmental template and catchment-scaled human activity on the metabolic regime and status of streams has potential implications for stream management aimed at mitigating nutrient loading and associated eutrophication. Our findings suggest that managers may need to balance effects of human activities with regional environmental constraints on a stream's metabolic potential in setting regional nutrient targets in order to achieve and maintain the desired ecological condition of individual streams and associated ecosystem services (e.g., waste assimilation and recreation). Moreover, understanding the metabolic potential of stream ecosystems should enable more effective management of downstream ecosystems by ensuring that nutrient loadings do not exceed the assimilative capacity of stream ecosystems draining to important downstream waterbodies.

4.5 References

Acuña, V., Giorgi, A., Muñoz, I., Uehlinger, U., & Sabater, S. (2004). Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. *Freshwater Biology, 49*(7), 960–971. doi: 10.1111/j.1365-2427.2004.01239.x

Alberts, J. M., Beaulieu, J. J., & Buffam, I. (2017). Watershed land use and seasonal variation constrain the influence of riparian canopy cover on stream ecosystem metabolism. *Ecosystems, 20*(3), 553–567. doi: 10.1007/s10021-016-0040-9

Appling, A. P., Hall, R. O., Arroita, M., & Yackulic, C. B. (2018a). streamMetabolizer: Models for Estimating Aquatic Photosynthesis and Respiration. Version 0.10.9.

Appling, A. P., Hall, R. O., Yackulic, C. B., & Arroita, M. (2018b). Overcoming equifinality: Leveraging long time series for stream metabolism estimation. *Journal of Geophysical Research Biogeosciences, 123*(2), 624–645. doi: 10.1002/2017JG004140

Aristi, I., Arroita, M., Larrañaga, A., Ponsatí, L., Sabater, S., von Schiller, D., … Acuña, V. (2014). Flow regulation by dams affects ecosystem metabolism in Mediterranean rivers. *Freshwater Biology, 59*(9), 1816–1829. doi: 10.1111/fwb.12385

Aristi, I., von Schiller, D., Arroita, M., Barceló, D., Ponsatí, L., García-Galán, M. J., … Acuña, V. (2015). Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: Subsidy or stress? *Freshwater Biology 60*(7), 1398–1410. doi: 10.1111/fwb.12576

Arroita, M., Elosegi, A., & Hall, R. O. (2019). Twenty years of daily metabolism show riverine recovery following sewage abatement. *Limnology and Oceanography, 64*(S1), S77–S92. doi: 10.1002/lno.11053

Baringhaus, L., & Franz, C. (2004). On a new multivariate two-sample test. *Journal of Multivariate Analysis, 88*(1), 190–206. doi: 10.1016/S0047-259X(03)00079-4

Beaulieu, J. J., Arango, C. P., Balz, D. A., & Shuster, W. D. (2013). Continuous monitoring reveals multiple controls on ecosystem metabolism in a suburban stream. *Freshwater Biology, 58*(5), 918–937. doi: 10.1111/fwb.12097

Bernhardt, E. S., Heffernan, J. B., Grimm, N. B., Stanley, E. H., Harvey, J. W., Arroita, M., … Yackulic, C. B. (2018). The metabolic regimes of flowing waters. *Limnology Oceanography, 63*(S1), 99–118. doi: 10.1002/lno.10726

Bernot, M. J., Sobota, D. J., Hall, R. O., Mulholland, P. J., Dodds, W. K., Webster, J. R., … K. Wilson. (2010). Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology, 55*(9), 1874–1890. doi: 10.1111/j.1365-2427.2010.02422.x

Clapcott, J. E., Young, R. G., Neale, M. W., Doehring, K., & Barmuta, L. A. (2016). Land use affects temporal variation in stream metabolism. *Freshwater Science, 35*(4), 1164–1175. doi: 10.1086/688872

Cooper, A. B. (1984). Activities of benthic nitrifiers in streams and their role in oxygen consumption. *Microbial Ecology, 10*(4), 317–334. doi: 10.1007/BF02015557

Crins, W. J., Gray, P. A., Uhlig, P. W., & Wester, M. C. (2009). The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions. Ministry of Natural Resources Science and Information Branch, Inventory, Monitoring and Assessment Section. Technical Report SIB TER IMA TR-01 1–76.

Demars, B. O. L., Manson, J. R., Ólafsson, J. S., Gíslason, G. M., Gudmundsdóttir, R., Woodward, G., … Friberg, N. (2011). Temperature and the metabolic balance of streams. *Freshwater Biology, 56*(6), 1106–1121. doi: 10.1111/j.1365-2427.2010.02554.x

Dinno, A. (2017). dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. Version 1.3.5.

Duong, T. (2018). ks: Kernel Smoothing. Version 1.11.0.

Environment and Climate Change Canada (ECCC). (2018). Water Level and Flow, Historical Hydrometric Data. Available from: https://wateroffice.ec.gc.ca/mainmenu/historical_data_index_e.html

Finlay, J. C. (2011). Stream size and human influences on ecosystem production in river networks. *Ecosphere, 2*(8), 1–21. doi: 10.1890/ES11-00071.1

Fox, J., Weisberg, S., & Price, B. (2018). car: Companion to Applied Regression. Version 3.0.0.

Frankforter, J. D., Weyers, H. S., Bales, J. D., Moran P. W., & Calhoun, D. L. (2010). The relative influence of nutrients and habitat on stream metabolism in agricultural streams. *Environmental Monitoring and Assessment, 168*(1-4), 461–479. doi: 10.1007/s10661-009-1127-y

Franz, C. (2015). cramer: multivariate nonparametric Cramer-Test for the two-sample-problem. Version 0.9-1.

Fuß, T., Behounek, B., Ulseth, A. J., & Singer, G. A. (2017). Land use controls stream ecosystem metabolism by shifting dissolved organic matter and nutrient regimes. *Freshwater Biology, 62*(3), 582–599. doi: 10.1111/fwb.12887

Gough, L., Bettez, N. D. Slavik, K. A. Bowden, W. B. Giblin, A. E. Kling, G. W., … Shaver, G. R. (2016). Effects of long-term nutrient additions on arctic tundra, stream, and lake ecosystems: Beyond NPP. *Oecologia, 182*(3), 653–665. doi: 10.1007/s00442-016-3716-0

Grace, M. R., & Imberger, S. J. (2006). Stream Metabolism: Performing & Interpreting Measurements. Water Studies Center Monash University, Murray Darling Basin Commission and New South Wales Department of Environment and Climate Change.

Grand River Information Network (GRNI). (2018). Grand River Information Network, Monitoring Data Downloads. Available from: https://data.grandriver.ca/downloads-monitoring.html

Griffiths, N. A., Tank, J. L., Royer, T. V., Roley, S. S., Rosi-Marshall, E. J., Whiles, R. R., … Johnson, L. T. (2013). Agricultural land use alters the seasonality and magnitude of stream metabolism. *Limnology and Oceanography, 58*(4), 1513–1529. doi: 10.4319/lo.2013.58.4.1513y

Gücker, B., Boechat, I. G., & Giani, A. (2009). Impacts of agricultural land use on ecosystem structure and whole-stream metabolism of tropical Cerrado streams. *Freshwater Biology, 54*(10), 2069–2085. doi: 10.1111/j.1365-2427.2008.02069.x

Gücker, B., Brauns, M., & Pusch, M. T. (2006). Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. Journal of the North American Benthological Society, 25(2) 313–329. doi: 10.1899/0887-3593(2006)25[313:EOWTPD]2.0.CO;2

Hall, R. O., & Tank, J. L. (2003). Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography, 48*(3), 1120–1128. doi: 10.4319/lo.2003.48.3.1120

Harrell, F. E., & Dupont, C. (2018). Hmisc: Harrell Miscellaneous. Version 4.1-1.

Hill, W. R., Fanta, S. E., & Roberts, B. J. (2009). Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography, 54*(1), 368–380. doi: 10.4319/lo.2009.54.1.0368

Hill, W. R., Roberts, B. J., Francoeur, S. N., & Fanta, S. E. (2011). Resource synergy in stream periphyton communities. *Journal of Ecology, 99*(2), 454–463. doi: 10.1111/j.1365-2745.2010.01785.x

Hoellein, T. J., Bruesewitz, D. A., & Hamilton, D. P. (2012). Are geothermal streams important sites of nutrient uptake in an agricultural and urbanising landscape (Rotorua, New Zealand)? *Freshwater Biology, 57*(1), 116–128. doi: 10.1111/j.1365-2427.2011.02702.x

Hoellein, T. J., Bruesewitz, D. A., & Richardson, D. C. (2013). Revisiting Odum (1956): A synthesis of aquatic ecosystem metabolism. *Limnology and Oceanography, 58*(6), 2089–2100. doi: 10.4319/lo.2013.58.6.2089

Huryn, A. D., Benstead, J. P., & Parker, S. M. (2014). Seasonal changes in light availability modify the temperature dependence of ecosystem metabolism in an arctic stream. *Ecology, 95*(10), 2826–2839. doi: 10.1890/13-1963.1

Hyndman, R. (2018). forecast: forecasting functions for time series and linear models. Version 8.4.

Kaushal, S. S., Likens, G. E., Jaworski, N. A., Pace, M. L., Sides, A. M., Seekell, D. … Wingate, R. L. (2010). Rising stream and river temperatures in the United States. *Frontiers in Ecology and the Environment, 8*(9), 461–446. doi: 10.1890/090037

Kemp M. J., & Dodds, W. K. (2002). The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. *Limnology and Oceanography, 47*(5), 1380–1393. doi: 10.4319/lo.2002.47.5.1380

Kominoski, J. S., Rosemond, A. D., Benstead, J. P., Gulis, V., & Manning, D. W. P. (2018). Experimental nitrogen and phosphorus additions increase rates of stream ecosystem respiration and carbon loss. *Limnology and Oceanography, 63*(1), 22–36. doi: 10.1002/lno.10610

Lamberti, G. A. & Steinman, A. D. (1997). A comparison of primary production in stream ecosystems. *Journal of the North American Benthological Society 16*(1), 95–103. doi: 10.2307/1468241

Meyer, J. L. (1989). Can P / R ratio be used to assess the food base of stream ecosystems? A comment on Rosenfeld and Mackay (1987). *Oikos, 54*(1), 119–121. doi: 10.2307/3565905

Morgan, A. M., Royer, T. V., David, M. B., & Gentry, L. E. (2006). Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. *Journal of Environmental Quality, 35*(4), 1110–1117. doi: 10.2134/jeq2005.0433

Moritz, S. (2017). imputeTS: Time Series Missing Value Imputation. Version 2.5.0.

Mulholland, P. J., Fellows, C. S., Tank, J. L., Grimm, N. B., Webster, J. R., Hamilton, S. K., … Peterson, B.J. (2001). Inter-biome comparison of factors controlling stream metabolism. *Freshwater Biology, 46*(11), 1503–1517. doi: 10.1046/j.1365-2427.2001.00773.x

Munn, M. D., & Brusven, M. A. (2004). The influence of Dworshak Dam on epilithic community metabolism in the Clearwater River, U.S.A. *Hydrobiologia, 513*(1), 121–127. doi: 10.1023/B:hydr.0000018177.78841.08

O’Connor, B. L., Harvey, J. W., & McPhillips, L. E. (2012). Thresholds of flow-induced bed disturbances and their effects on stream metabolism in an agricultural river. *Water Resources Research*, *48*(8), 1–18. doi: 10.1029/2011WR011488

Ontario Ministry of the Environment, Conservation and Parks (OMECP). (2018). Provincial (Stream) Water Quality Monitoring Network. Available from: https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network

Pastor, A., Lupon, A., Gómez-Gener, L., Rodríguez-Castillo, T., Abril, M., Arce, M. I., … N. Catalán. (2017). Local and regional drivers of headwater streams metabolism: Insights from the first AIL collaborative project. *Limnetica*, *36*(1), 67–85. doi: 10.23818/limn.36.06

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.4.2.

Rasmussen, J. J., Baattrup-Pedersen, A., Riis, T., & Friberg, N. (2011). Stream ecosystem properties and processes along a temperature gradient. *Aquatic Ecology*, *45*(2), 231–242. doi: 10.1007/s10452-010-9349-1

Raymond, P. A., Zappa, C. J., Butman, D., Bott, T. L., Potter, J., Mulholland, P. J., … Newbold, D. (2012). Scaling the gas transfer velocity and hydraulic geometry in streams and small rivers. *Limnology and Oceanography: Fluids and Environments 2*(1): 41–53. doi: 10.1215/21573689-1597669

Roberts, B. J., & Mulholland, P. J. (2007). In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. *Journal of Geophysical Research Biogeosciences*, *112*(G4), 1–11. doi: 10.1029/2007JG000422

Roberts, B. J., Mulholland, P. J., & Hill, W. R. (2007). Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems*, *10*(4), 588–606. doi: 10.1007/s10021-007-9059-2

Rode, M., Halbedel (née Angelstein), S., Anis, M. R., & Borchardt, D. (2016). Continuous in-stream assimilatory nitrate uptake from high-frequency sensor measurements. *Environmental Science and Technology, 50*(11), 5685–5694. doi: 10.1021/acs.est.6b00943

Rodríguez-castillo, T., Barquín, J., Álvarez-cabria, M., Peñas, F. J., & Álvarez, C. (2017). Effects of sewage effluents and seasonal changes on the metabolism of three Atlantic rivers. *Science of the Total Environment, 599–600*, 1108–1118. doi: 10.1016/j.scitotenv.2017.05.067

Rosa, R. D. S., Aguiar, A. C. F., Boëchat, I. G., & Gücker, B. (2013). Impacts of fish farm pollution on ecosystem structure and function of tropical headwater streams. *Environmental Pollution*, *174*, 204–213. doi: 10.1016/j.envpol.2012.11.034

Rosemond, A. D., Benstead, J. P., Bumpers, P. M., Gulis, V., Kominoski, J. S., Manning, D. W. P., … Wallace, J. B. (2015). Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science*, *347*(6226), 1142–1144. doi: 10.1126/science.aaa1958

Rosenfeld, J. S., & Mackay, R. J. (1987). Assessing the food base of stream ecosystems: Alternatives to the P/R ratio. *Oikos*, *50*(1), 141–147. doi: 10.2307/3565412

Sabater, F., Butturini, A., Marti, E., Munoz, I., Romaní, A. M., Wray, J., & Sabater, S. (2000). Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. *Journal of the North American Benthological Society*, *19*(4), 609–620. doi: 10.2307/1468120

Savoy, P., Appling, A. P., Heffernan, J. B., Stets, E. G., Read, J. S., Harvey, J. W., & Bernhardt, E. S. (2019). Metabolic rhythms in flowing waters: An approach for classifying river productivity regimes. *Limnology and Oceanography*, *64*(5), 1835–1851. doi: 10.1002/lno.11154

Siders, A. C., Larson, D. M., Ruegg, J., & Dodds, W. K. (2017). Probing whole-stream metabolism: Influence of spatial heterogeneity on rate estimates. *Freshwater Biology, 62*(4), 711–723. doi: 10.1111/fwb.12896

Song, C., Dodds, W. K., Rüegg, J., Argerich, A., Baker, C. L., Bowden, W. B., … Ballantyne IV, F. (2018). Continental-scale decrease in net primary productivity in streams due to climate warming. *Nature Geoscience, 11*(6), 415–420. doi: 10.1038/s41561-018-0125-5

Tank, J. L., Rosi-Marshall, E. J., Griffiths, N. A., Entrekin, S. A., & Stephen, M. L. (2010). A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society, 29*(1), 118–146. doi: 10.1899/08-170.1

Uehlinger, U. (2006). Annual cycle and inter-annual variability of gross primary production and ecosystem respiration in a floodprone river during a 15-year period. *Freshwater Biology, 51*(5), 938–950. doi: 10.1111/j.1365-2427.2006.01551.x

von Schiller, D., Acuña, V., Aristi, I., Arroita, M., Basaguren, A., Bellin, A., … Elosegi, A. (2017). River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors. *Science of the Total Environment, 596–597*, 465–480. doi: 10.1016/j.scitotenv.2017.04.081

Warren, D. R., Collins, S. M., Purvis, E. M., Kaylor, M. J., & Bechtold, H. A. (2017). Spatial variability in light yields colimitation of primary production by both light and nutrients in a forested stream ecosystem. *Ecosystems, 20*(1), 198–210. doi: 10.1007/s10021-016-0024-9

Webster, J. R., Mulholland, P. J., Tank, J. L., Valett, H. M., Dodds, W. K., Peterson, B. J., … Wollhem, W. M. (2003). Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology, 48*(8), 1329–1352. doi: 10.1046/j.1365-2427.2003.01094.x

Withers, P. J. A., & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of the Total Environment, 400*(1-3), 379–395. doi: 10.1016/j.scitotenv.2008.08.002

Wood, S. N. (2018). mgcv: Mixed GAM Computation Vehicle with Automatic Smoothness Estimation. Version 1.8.23.

Woodward, G., Bonada, N., Brown, L. E., Death, R. G., Durance, I., Gray, C., … Pawar, S. (2016). The effects of climatic fluctuations and extreme events on running water ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences 371*(1694), 20150274. doi: 10.1098/rstb.2015.0274

Yates, A. G., & Bailey, R. C. (2010). Improving the description of human activities potentially affecting rural stream ecosystems. *Landscape Ecology, 25*(3), 371–382. doi: 10.1007/s10980-009-9413-1

Yates, A. G., Brua, R. B., Culp, J. M., & Chambers, P. A. (2013). Multi-scaled drivers of rural prairie stream metabolism along human activity gradients. *Freshwater Biology 58*(4), 675–689. doi: 10.1111/fwb.12072

Young, R. G., & Huryn, A. D. (1996). Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. *Canadian Journal of Fisheries and Aquatic Sciences, 53*(10), 2199–2211. doi: 10.1139/f96-186

Young, R. G., Matthaei, C. D., & Townsend, C. R. (2008). Organic matter breakdown and ecosystem metabolism: Functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society, 27*(3), 605–625. doi: 10.1899/07-121.1

Yvon-Durocher, G., Caffrey, J. M., Cescatti, A., Dossena, M., del Giorgio, P., Gasol, J. M., … Allen, A. P. (2012). Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature, 487*(7408), 472–476. doi: 10.1038/nature11205

Chapter 5

5 Episodic loadings of phosphorus influence growth and composition of benthic algae communities in artificial stream mesocosms

A version of this chapter has been accepted for publication (Pearce et al., In Press. Water Research)

5.1 Introduction

Phosphorus (P) enrichment is regarded as a common cause of freshwater eutrophication (Correll, 1998), and many past studies have reported P limitation or co-limitation of benthic algae in streams (Francoeur, 2001; Elser et al., 2007). In P limited systems, increased biomass (Chételat et al., 1999; Biggs, 2000; Bowman et al., 2005) and growth rates (Fellows et al., 2006; Rier & Stevenson, 2006) of benthic algal communities have been positively associated with P concentrations and bioavailability. Similarly, the relative abundance of algal species can differ across stream P gradients (Stevenson et al., 2008; Black et al., 2011; Taylor et al., 2018). For example, Stevenson et al., (2012) reported an increase in nuisance green algae with greater total P concentrations among 70 streams in the US Midwest. In response to findings that benthic algal communities respond readily to changing P concentrations, watershed managers have regularly adopted P concentration guidelines to mitigate excessive algal growth in streams (Evans-White et al., 2013). However, temporal variation in stream P loading from human activities has the potential to confound benthic algae-P relationships and result in unanticipated ecological consequences (Jarvie et al., 2013).

Temporal variability in P loading to streams can depend on the type and intensity of human land use (Edwards & Withers, 2008). In undisturbed catchments, P loadings to streams are low and primarily associated with precipitation events that result in soil erosion and mineral weathering. Runoff events can transport particulate P to streams and cause temporal variation in the total P load, although the bioavailable fraction of P often varies minimally despite changes in discharge (Meyer & Likens, 1979). In contrast, anthropogenic P loadings can substantially increase the quantity of P in streams

(Mainstone & Parr, 2002; Withers & Jarvie, 2008). Point sources (e.g., wastewater discharge) typically input enriched loads of bioavailable P from discrete locations with minimal temporal variability (Jarvie et al., 2006). Conversely, P loads from nonpoint sources (e.g., urban and agricultural lands) are derived from the landscape and transported to streams through episodic runoff events (Mainstone & Parr, 2002; Withers & Jarvie, 2008). Consequently, inputs of P from nonpoint sources exhibit substantial temporal variation in particulate and bioavailable P loads (Sharpley et al., 2001). Streams draining human-modified catchments can therefore receive enriched loads of P from two sources each with a distinct loading pattern: continuous inputs from point sources or episodic inputs from nonpoint sources. Effective management of stream eutrophication in catchments with mixed anthropogenic land uses thus requires information on the relative contributions that point and nonpoint sources have on stream P enrichment. However, few studies have considered how the loading pattern of P impacts stream eutrophication.

Traditionally, loads associated with episodic P enrichment have not been considered ecologically relevant to stream ecosystems (Stamm et al., 2014; Withers et al., 2014). However, many algae taxa are capable of assimilating excess P during periods of P surplus (i.e., “luxury consumption”) for use under P-limiting conditions (Solovchenko et al., 2019). Past studies have demonstrated that algae can increase intracellular P storage in response to episodic P additions (Humphrey & Stevenson, 1992; Rier et al., 2016) and microscale P pulses have been shown to influence the community structure and function of algal biofilms (Davies & Bothwell, 2012). Moreover, the potential for algal communities to utilize short duration increases in P has been demonstrated through increased P retention in stream nutrient addition experiments (Meals et al., 1999; Weigelhofer et al., 2018). However, past investigations on the effects of P pulses have occurred in conditions with low total P loads or have concentrated on stream biogeochemistry, thus the potential of episodic P enrichment to augment stream eutrophication remains unclear. Understanding the capacity of benthic algal communities to respond to contrasting temporal patterns of P enrichment associated with point and nonpoint source P loading is thus required to inform stream eutrophication monitoring and management in developed watersheds.

The goal of our study was to determine the effect of temporal patterns in P enrichment, consistent with point source and nonpoint source inputs, on the growth and taxonomic composition of benthic algal communities. To accomplish this goal we conducted a mesocosm experiment in artificial streams where algal communities were exposed to P loadings that simulated unenriched, continuously enriched, or episodically enriched conditions, with P loads over the duration of the experiment being the same for the two P enriched treatments. Mesocosm experiments provide the opportunity to examine more nuanced effects of P enrichment by balancing ecological realism with added control in environmental conditions and replicability (Menczelesz et al., 2019). Thus, our experimental research can provide unambiguous evidence on the potential for algal communities to respond to contrasting patterns of P enrichment and help inform future field studies. Findings from our experiment will also directly inform stream eutrophication management and the refinement of ecological indicators for monitoring in streams draining mixed land use catchments.

5.2 Methods

5.2.1 Experimental design and set-up

We used a single factor research design to investigate the response of algal communities to simulated P enrichment patterns associated with three different land use scenarios in a 29-day artificial stream mesocosm experiment. Nine artificial streams were randomly assigned to one of three different P treatments: 1) an unenriched P load with a continuous concentration of 10 µg L^{-1} SRP (unenriched); 2) an enriched P load with a continuous P concentration of 50 µg L^{-1} SRP (continuously enriched), and; 3) an enriched P load with a continuous concentration of 10 µg L^{-1} SRP that received two separate 48-hour additions (“pulses”) of P at 300 µg L^{-1} SRP on days 10 and 19 (episodically enriched). P enriched treatments were selected such that total P loads at the end of the 29-day experiment were the same for continuously and episodically enriched treatments (Figure 5-1). Total nitrogen was constant for all treatments at a continuous concentration of 1500 µg L^{-1}. Experimental P concentrations were chosen with regard to regional nutrient criteria

(Chambers et al., 2012) and reflected concentrations observed in human influenced streams of southern Ontario, Canada (Thomas et al., 2018).

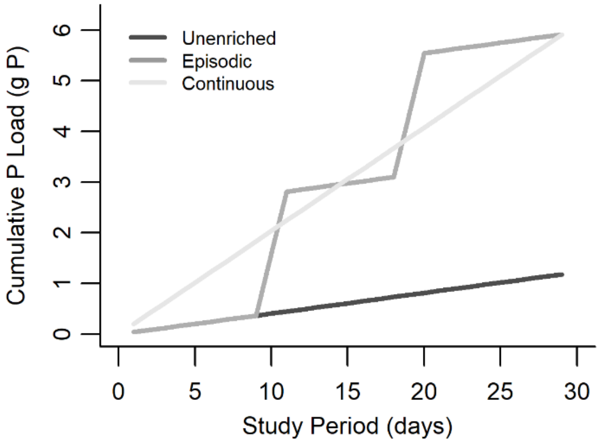

Figure 5-1. Cumulative experimental phosphorus load for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) over the 29-day experiment.

Our artificial stream mesocosm experiment was conducted at the Thames River Experimental Stream Sciences (TRESS) center in London, Ontario, Canada, in the early summer (June 19 – July 18) of 2016 (Figure 5-2). Artificial streams were comprised of sinuous flumes (0.15 m deep by 0.20 m wide by 7.0 m long) that received a continuous supply of low nutrient (TN = 406 µg L^{-1}; TP < 1.0 µg L^{-1}) carbon filtered water from the Lake Huron Water Supply System through individual diaphragm pumps. Concentrated P (KH_2PO_4) stored in 1000 L carboys was delivered to each artificial stream through individual dosing pumps connected via chemical injection fittings to the outflow of each diaphragm pump. Similarly, a single dosing pump was used to add concentrated nitrogen (NH_4NO_3) to the common water supply of the facility. Flow rates of dosing and diaphragm pumps were calibrated daily to ensure concentrated nutrients were continuously delivered at the appropriate rates to achieve and maintain target artificial stream nutrient concentrations. Water and nutrients delivered to each artificial stream were circulated throughout the flume with an impeller pump and were partially recycled with the use of a reservoir (2.5 hr residence time). Because of the continuous and controlled delivery of water and nutrients, concentrations in the artificial streams remained temporally stable and were largely calculated based on the amount of P added to the nutrient carboys and the calibrated flow rates of each dosing and diaphragm pump. Water samples were collected on four occasions to confirm treatment P concentrations and were found to be within an acceptable margin of error of calculated concentrations.

Light availability (60% shade) and water temperature were controlled and equal among artificial streams and reflected diurnal cycles and day-to-day variability in ambient climatic conditions (Appendix B: Figure B-1). Mean (± standard deviation) daily photosynthetically active radiation and temperature over the experimental period were 27.4 ± 6.2 mol m^{-2} day^{-1} and 20 ± 1.8 ^{o}C, respectively. Flow velocity was set at 0.1 m s^{-1} in all artificial streams and was measured daily to ensure the selected velocity was maintained for the duration of the experiment. Substrate in all artificial streams consisted of cobble (D50 = 46 mm).

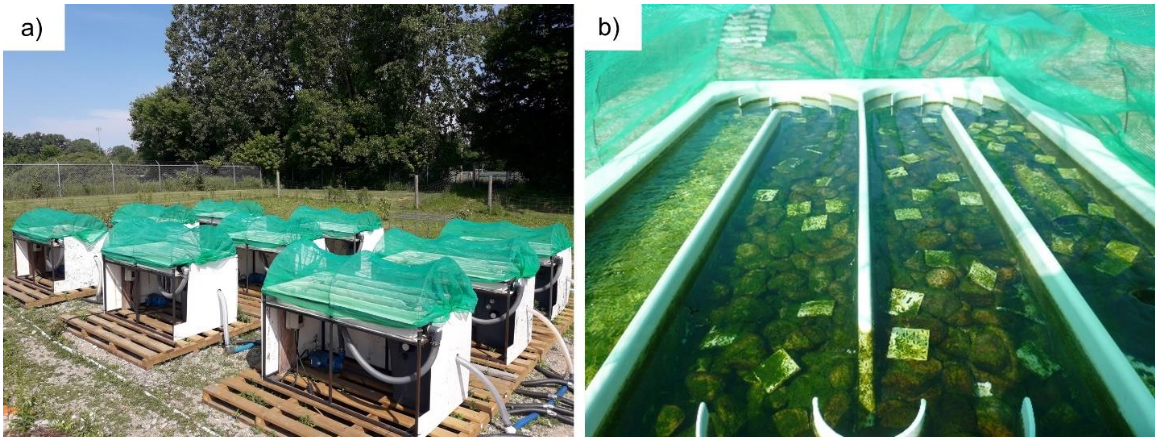

Figure 5-2. (a) Nine artificial streams located at the Thames River Experimental Stream Sciences Center in London Ontario, Canada. (b) Close up image of the artificial stream flume and substrate part way through the experiment.

Prior to the experiment, cobble substrate was placed in plastic mesh (2 x 2 cm) bags and anchored in a local stream (43.013 N, -81.281 W) for approximately 4 weeks to colonize artificial stream substrate with local biota. Colonized substrate was divided equally among each artificial stream at the beginning of the experiment to achieve a water depth of ~ 8 cm. Additional algae inoculum was collected from 5 regional streams that occupied a gradient of P enrichment (19.6 µg L^{-1} TP to 229.5 µg L^{-1} TP) by removing the biofilm from 5 to 10 cobbles randomly selected within each reach. Collected biofilms were combined in 2.7 L of water and used to seed each artificial stream (300 mL) with biota representative of the regional P conditions. Unglazed ceramic tiles (4.7 x 4.7 cm) were placed on top of the pre-colonized substrate to provide a clean surface for biofilm growth. Tiles and substrate were redistributed randomly within each treatment group 5 days into the experiment to further establish homogeneity among artificial streams. Large grazers (e.g., snails and crayfish) were removed from artificial streams at the beginning of the experiment and because substrate was inoculated in the same stream and randomly distributed among artificial streams grazing by small invertebrates was presumed to be consistent among streams.

5.2.2 Sample collection and analysis

Standardized substrata (unglazed ceramic tiles; 22.09 cm^2) were placed in each artificial stream at the start of the experiment to provide a consistent surface for benthic algal colonization and accrual (*sensu* Steinman et al., 2007). Algal samples were collected on seven occasions during the experiment: prior to the first simulated P pulse (days 7 and 10), between simulated P pulses (days 15 and 18), and after the second simulated P pulse (days 22, 25, and 29). Samples were collected for chlorophyll *a*, ash-free dry-mass (AFDM), and taxonomic identification (algal phylum and diatom species). For each measurement, a composite sample was collected by scraping a defined surface area of biofilm (5.31 cm^2) from four different tiles (21.24 cm^2). Sampled tiles were selected at random with at least one tile being selected from each of the three flume channels (Figure 5-2). Each selected tile was randomly split into two diagonal sections and a tubular sampler (Steinman et al., 2007) was used to collect one scraping of biofilm (5.31 cm^2) from each section for biomass (either chlorophyll *a* or AFDM) and taxonomy. Composite samples were collected to account for potential spatial heterogeneity in algal accumulation within individual artificial streams and a tubular sampler was used to increase the consistency in and number of samples collected. Sampled tiles were removed from the experiment.

Chlorophyll *a* samples were stored frozen and thawed prior to fluorometric analysis. Thawed samples were filtered through Whatman GF/C filters and placed in 50 mL centrifuge tubes with 10 mL of 90% ethanol. A hot ethanol non-acidification extraction was completed by partially submerging centrifuge tubes in an 80 ^{o}C hot water bath for 7 min. Liquid extract was diluted if necessary and chlorophyll *a* concentration was determined with a Turner Designs Trilogy Fluorometer (Model: 7200-000).

Ash-free dry-mass samples were stored frozen before analysis. Thawed samples were filtered onto pre-ashed Whatman GF/C filter papers for organic mass determination. Filtered samples were dried at 105 ^{o}C for a minimum of 12 hours and weighed. Samples were then ashed in a muffle furnace at 550 ^{o}C for 1 hour and subsequently weighed to determine the mass loss on ignition.

Taxonomy samples were preserved in dark bottles with Lugols iodine (~ 1% v/v) and subsampled for taxonomic identification. Coarse algal taxonomy was determined by sub-sampling 0.35 mL of well-mixed sample into a 4.25 mL Utermöhl chamber and diluting with 3.9 mL of distilled water. Algal cells were allowed to settle for 3 to 4 hours and were then enumerated (minimum 400 cells) by coarse taxonomic level (i.e., phylum) with the use of an inverted microscope at 400x magnification.

Diatom taxonomy was determined by digesting biofilm subsamples in 800 µL of 100% (v/v) nitric acid for 48 hours and 200 µL of hydrogen peroxide 30% (v/v) for an additional 48 hours to remove organic matter and clean diatom frustules. Digested samples were rinsed several times to remove any remnant nitric acid before being mounted with Naphrax® on microscope slides (refractive index: 1.74; Brunel microscopes Ltd.,Wiltshire, UK). Diatom assemblages were enumerated with use of a Reichert-Jung Polyvar microscope equipped with differential interference contrast (magnification 1250x). A minimum of 400 diatom valves were enumerated for each sample and each valve was identified to lowest possible taxonomic level, usually species, following Lavoie et al., (2008b).

5.2.3 Data analysis

Biomass data from the end of the experiment were used to evaluate differences between P treatments because of the temporal discontinuities in the cumulative experimental P load. Algal biomass measures (chlorophyll *a* and AFDM) were compared with a one-way analysis of variance and a Tukey's post hoc test was used to evaluate pairwise differences among P treatments (α = 0.05). Statistical analyses were performed in R version 3.5 (R Core Team, 2019).

Biomass measures were also used in generalized additive models (GAM) to produce nonlinear algal accrual curves and infer absolute growth rates over the experiment by estimating the rate of change in biomass across the seven sampling events (*sensu* Paine et al., 2012). GAMs were developed with the *mgcv* package (Wood 2019) for each P treatment and described algal accrual as the relationship between biomass and time (α = 0.05). Thin plate regression splines were used to smooth the data with a conservative estimated number of degrees of freedom (edf < 7). For all P treatments, model weight was added to samples collected before the first P pulse (days 7 and 10) in the GAM to prevent underfitting during algal colonization. Absolute growth rates were estimated as the first derivatives of each GAM of algal accrual with the *gratia* package in R (Simpson, 2019).

Composition of diatom assemblages before (day 10) and after (day 25) simulated P pulses were compared among P treatments using a non-metric multidimensional scaling (nMDS) ordination. Assemblages were ordered based on a Bray-Curtis dissimilarity matrix of square root transformed species relative abundance data (Legendre & Gallagher, 2001). A permutational analysis of variance was performed to evaluate the factors of P treatment, time (before and after), and their interaction (α = 0.05). Confidence intervals (95%) based on standard deviations of each P-time treatment level were computed to evaluate pairwise differences, and a similarity percentages (SIMPER) analysis was used to determine the diatom species responsible for dissimilarity among treatments. Analyses were completed with the *vegan* package in R (Oksanen et al., 2019).

5.3 Results

5.3.1 Biomass

Algal biomass measures differed among P treatments at the end of the experiment for chlorophyll *a* and AFDM (Figure 5-3). P enriched treatments were 2-fold greater in chlorophyll *a* and AFDM compared to the unenriched treatment, but no differences were observed between continuously and episodically enriched treatments that received the same cumulative P load (chlorophyll *a*: $F_{(2, 6)} = 16.35$, $p = 0.004$; AFDM: $F_{(2, 6)} = 11.31$, $p = 0.009$).

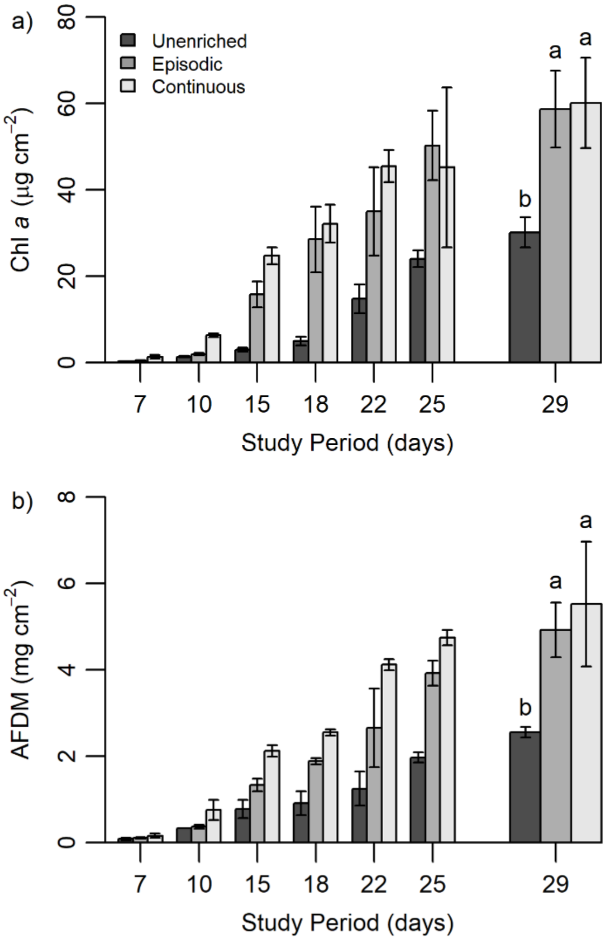

Figure 5-3. Algal biomass (mean ± 95% confidence interval) for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) measured as (a) chlorophyll *a* (Chl *a*) and (b) ash-free dry-mass (AFDM) from seven sampling events over the 29-day experiment. Letters (a, b, and c) indicate significant ($p < 0.05$) pairwise differences based on individual Tukey's post hoc test for the final sampling event.

5.3.2 Algal accrual

GAMs of algal accrual indicated that chlorophyll *a* and AFDM from each P treatment were associated with time (Figure 5-4). GAMs explained 99.0%, 97.2%, and 96.0% of the deviance in chlorophyll *a* accrual curves for unenriched (edf = 5.64, $p < 0.001$), episodically enriched (edf = 3.72, $p < 0.001$), and continuously enriched (edf = 3.56, $p < 0.001$) treatments, respectively. Likewise, deviance explained by GAMs of AFDM were 98.3% for the unenriched (edf = 5.28, $p < 0.001$), 98.0% for the episodically enriched (edf = 3.14, $p < 0.001$), and 97.0% for the continuously enriched (edf = 3.44, $p < 0.001$) treatments.

Absolute growth rates inferred from the first derivative of chlorophyll *a* accrual curves were similar between unenriched (0.29 µg cm^{-2} day^{-1}) and episodically enriched (0.32 µg cm^{-2} day^{-1}) treatments before simulated P pulses (day 7), whereas the continuously enriched treatment had a greater initial absolute growth rate of chlorophyll *a* (1.22 µg cm^{-2} day^{-1}) (Figure 5-4). Following the first P pulse (day 13), the absolute growth rate in the episodically enriched treatment (2.79 µg cm^{-2} day^{-1}) diverged from the unenriched treatment (0.27 µg cm^{-2} day^{-1}) and experienced more rapid rates of accrual. Absolute growth rates plateaued at a comparable rate of change for both continuously (day 15: 3.32 µg cm^{-2} day^{-1}) and episodically (day 17: 3.35 µg cm^{-2} day^{-1}) enriched treatments, but the episodically enriched treatment required an additional 2 days to reach a maximum. Absolute growth rates in the episodically enriched treatment remained above 3.0 µg cm^{-2} day^{-1} after the second P pulse (day 25), whereas the rate of change in chlorophyll *a* declined in the continuously enriched treatment to below 2.5 µg cm^{-2} day^{-1}. In contrast, the unenriched treatment exhibited a longer phase of lower absolute growth rates during the first half of the experiment and experienced a peak absolute growth rate (3.09 µg cm^{-2} day^{-1}) approximately 7 days later than the P enriched treatments.

Absolute growth rates inferred from AFDM accrual curves differed from chlorophyll *a* in that more time was required to reach maximum rates (Figure 5-4). Initially (day 7), the continuously enriched treatment (0.14 mg cm^{-2} day^{-1}) had a greater absolute growth rate than both episodically enriched (0.07 mg cm^{-2} day^{-1}) and unenriched (0.06 mg cm^{-2} day^{-1}) treatments. After the first P pulse (day 13) absolute growth rates in the episodically enriched treatment (0.17 mg cm^{-2} day^{-1}) increased and were 2-fold greater than the unenriched treatment (0.09 mg cm^{-2} day^{-1}), yet accrual remained lower in comparison to the continuously enriched treatment (0.25 mg cm^{-2} day^{-1}). Following the second P pulse (day 25) absolute growth rates in the episodically enriched treatment remained above

0.29 mg cm^{-2} day^{-1} whereas absolute growth rates in the continuously enriched treatment declined to less than 0.25 mg cm^{-2} day^{-1}. However, maximum absolute growth rates were comparable between continuously (0.28 mg cm^{-2} day^{-1}) and episodically (0.29 mg cm^{-2} day^{-1}) enriched treatments, and greater than the unenriched treatment (0.19 mg cm^{-2} day^{-1}). Maximum absolute growth rates occurred on days 19, 24, and 29 for continuously enriched, unenriched, and episodically enriched treatments, respectively.

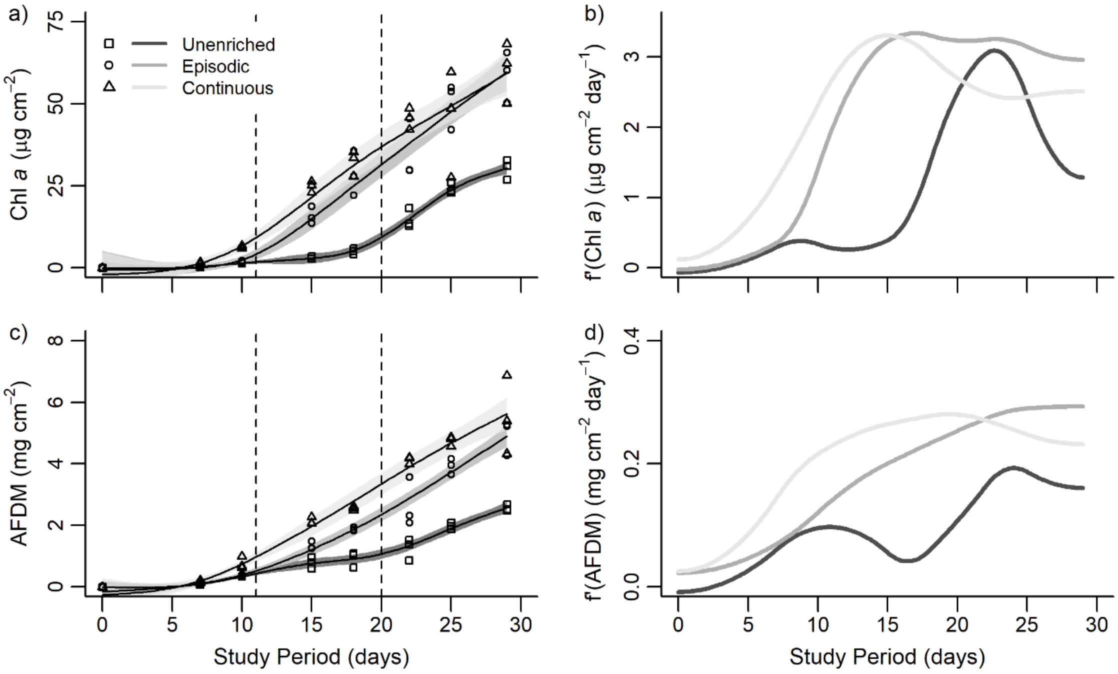

Figure 5-4. Generalized additive models (left) of algal biomass accrual over the 29-day experiment for each phosphorus treatment (dark grey: unenriched, medium grey: episodically enriched, light grey: continuously enriched) measured as (a) chlorophyll *a* (Chl *a*) and (c) ash-free dry-mass (AFDM). First derivatives of generalized additive models (right) correspond to the absolute growth rates of algal biomass measured as (b) chlorophyll *a* (Chl *a*) and (d) ash-free dry-mass (AFDM). Dashed lines represent timing of simulated phosphorus pulses.

5.3.3 Taxonomic composition

Relative abundances of algal phyla were similar between unenriched and episodically enriched treatments prior to simulated P pulses; however, benthic algal composition of both these treatments differed from that of the continuously enriched treatment (Figure 5-5). On day 10 of the experiment, communities that received continuous enrichment had

30% more chlorophytes and 25% fewer bacillariophytes compared to communities from unenriched and episodically enriched treatments. Following simulated P pulses, relative abundances of algal phyla in the episodically enriched treatment shifted and were comparable to the continuously enriched treatment with a 60% relative abundance of chlorophytes in both treatments (Figure 5-5). Proportions of chlorophytes in the unenriched treatment remained at 30% over the experiment, but the relative abundance of charophytes (e.g., *Cosmarium sp.*) increased by 20% with a 25% reduction in bacillariophytes towards the end of the experiment. Chlorophyta was the dominant phylum responsible for differences observed in P enriched and unenriched treatments after simulated P pulses.

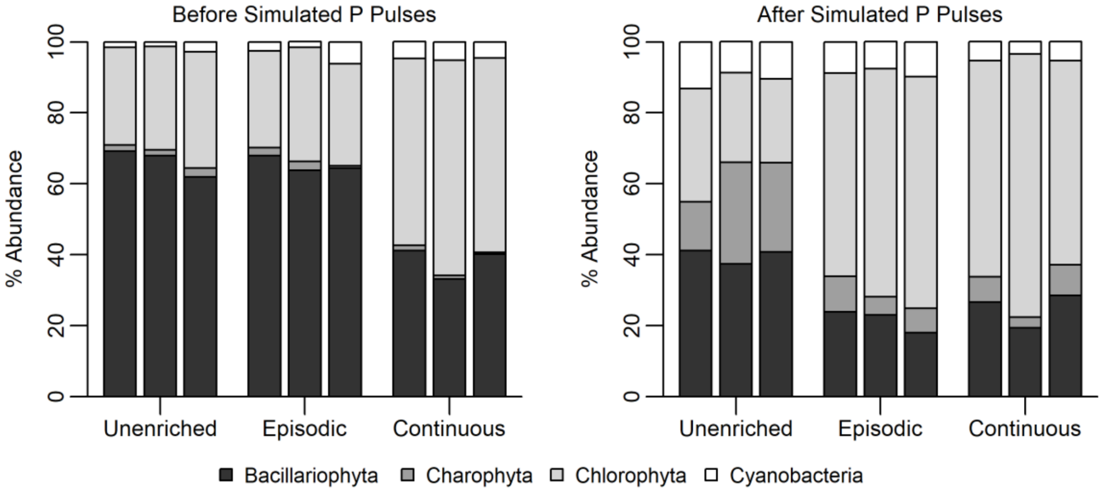

Figure 5-5. Relative abundances of algal phylum (dark grey: Bacillariophya, medium grey: Charophyta, light grey: Chlorophyta, white: Cyanobacteria) in communities of the three phosphorus treatments (three replicates per treatment) sampled before (left) and after (right) simulated phosphorus (P) pulses.

An nMDS ordination (stress = 0.06) analyzing a total of 28 diatom species observed in our artificial streams showed the separation of assemblages in two dimensions (Figure 5-6). On average, 21, 20, and 20 diatom species were recorded before and 12, 14, and 17 after simulated P pulses for the unenriched, episodically enriched, and continuously enriched assemblages, respectively. Assemblages were clustered by P treatment ($F_{(2, 17)} = 6.24$, $p < 0.001$, $R^2 = 0.21$) and time ($F_{(1, 17)} = 28.44$, $p < 0.001$, $R^2 = 0.47$), which cumulatively explained 68% of the ordination variance based on a permutational analysis of variance. A total of 81% of the variance in diatom assemblages was explained with the addition of a significant P-time interaction ($F_{(2, 17,)} = 3.83$, $p = 0.004$, $R^2 = 0.13$). For samples collected before simulated P pulses 95% confidence intervals overlapped for all P treatments. However, after simulated P pulses each treatment occupied separate ordination space. Continuously and episodically enriched diatom assemblages were positively scaled on nMDS axis 2 and were more dissimilar to the negatively scaled unenriched assemblages than to each other.

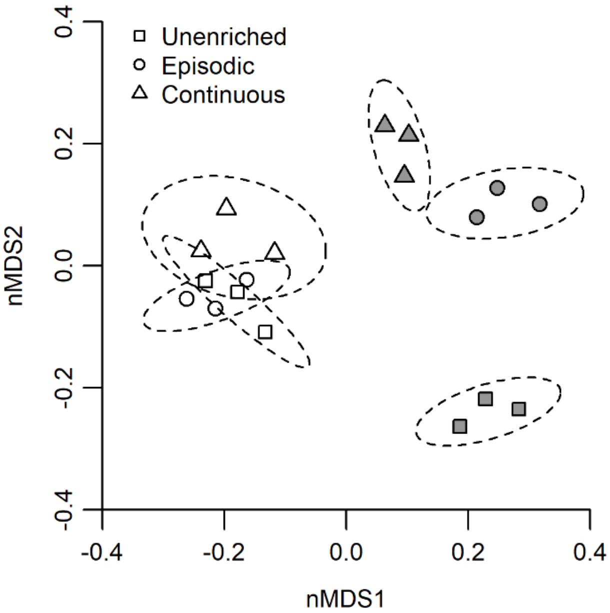

Figure 5-6. Non-metric multidimensional scaling ordination of diatom species composition of phosphorus treatments (square: unenriched, circle: episodic, triangle: continuous) collected before (open symbols) and after (closed symbols) simulated phosphorus pulses. Dashed lines represent 95% confidence intervals based on standard deviations.

SIMPER analysis identified that after simulated P pulses diatom assemblage composition of the unenriched treatment was about 40% dissimilar to continuously and episodically enriched treatments, whereas P enriched assemblages were 28% dissimilar (Table 5-1). Four influential diatom taxa were each responsible for greater than 10% of the relative dissimilarity observed among treatments. *Nitzschia palea*, *Nitzchia palea* var. *debilis,* and *Achnanthidium minutissimum* contributed most to the dissimilarity between the P enriched and unenriched diatom assemblages, whereas *Cyclotella meneghiniana* and *A.*

minutissimum contributed to the dissimilarity between episodically and continuously enriched diatom assemblages.

Table 5-1. Similarity percentages analysis of the average dissimilarly (Av. Dissim.) in diatom assemblage composition among phosphorus treatments after simulated phosphorus pulses. Influential diatom taxa were described based on the absolute contribution (Abs. Contrib.) to the total dissimilarity between treatments and identified as important based on a >10% relative contribution (Rel. Contrib.).

Treatment Comparison	Av. Dissim.	Influential Taxon	Abs. Contrib.	Rel. Contrib.
Unenriched vs. Continuous	44.8%	*Achnanthidium minutissimum*	7.9%	17.6%
		Nitzschia palea	7.8%	17.4%
		Nitzschia palea var. *debilis*	5.1%	11.4%
Unenriched vs. Episodic	36.4%	*Nitzschia palea*	6.3%	17.3%
		Achnanthidium minutissimum	4.7%	12.9%
		Nitzschia palea var. *debilis*	4.0%	11.0%
Continuous vs. Episodic	28.0%	*Cyclotella meneghiniana*	3.9%	13.9%
		Achnanthidium minutissimum	3.1%	11.1%

Relative abundances of influential diatom taxa were comparable among P treatments before simulated P pulses, but differed with the succession of algal communities (Figure 5-7). Relative abundance of *A. minutissimum* increased over the experiment in the unenriched treatment and was 2 to 3-fold more abundant than in the continuously and episodically enriched treatments after simulated P pulses. In contrast, *N. palea* and *N. palea* var. *debilis* increased in continuously and episodically enriched treatments such that after simulated P pulses both taxa had relative abundances 30 to 40% greater than the unenriched treatment. Continuously and episodically enriched treatments differed by 20%, 5%, and 10% in the relative abundances of *A. minutissimum*, *N. palea* and *N. palea* var. *debilis*, respectively, with the latter two species being more abundant in the continuously enriched treatment and the former in the episodically enriched treatment. Moreover, after simulated P pulses *C. meneghiniana* was only found to be abundant in the continuously enriched treatment (8%) in comparison to episodically enriched and unenriched treatments (< 1%).

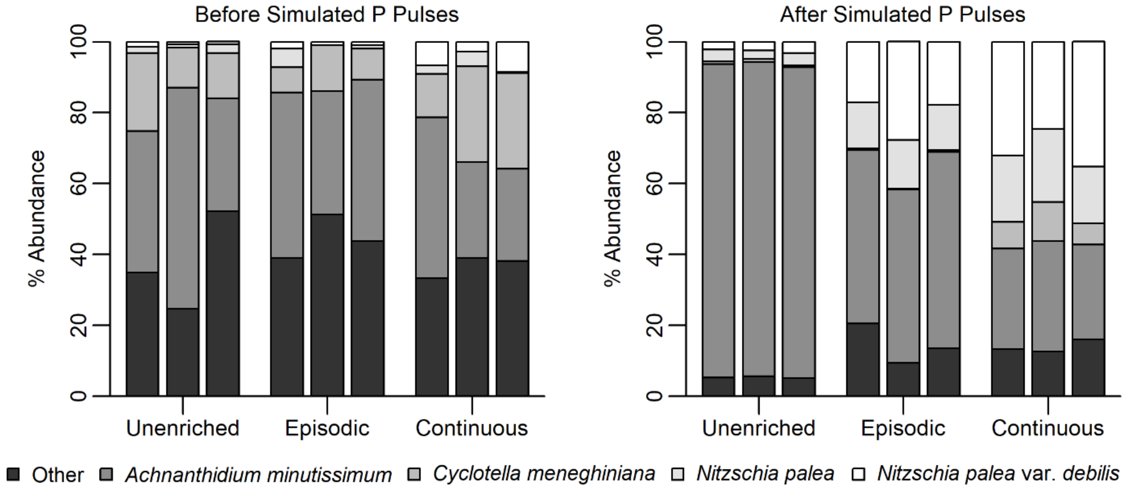

Figure 5-7. Relative abundances of influential diatom species identified through similarity percentages analysis (dark grey: Other, medium-dark grey: *Achnanthidium minutissimum*, medium grey: *Cyclotella meneghiniana*, light grey: *Nitzschia palea*, white: *Nitzschia palea* var. *debilis*) in assemblages of the three phosphorus treatments (three replicates per treatment) sampled before (left) and after (right) simulated phosphorus (P) pulses.

5.4 Discussion

Ecological theory suggests that resource availability regulates community composition (habitat template model; Biggs et al., 1998a). Although numerous studies have investigated how benthic algal communities are structured by instream P availability (Chételat et al., 1999; Biggs, 2000; Stevenson et al., 2008; Stevenson et al., 2012), most research has focused on ecological responses wherein the supply of P has differed spatially. Few studies have examined how algal communities respond to temporally variable P supplies associated with human activities (Humphrey & Stevenson, 1992; Rier et al., 2016). Moreover, there have been few comparisons of the eutrophication potential of P enrichment from sources differing in temporal loading pattern. By simulating continuous and episodic P enrichment patterns in artificial streams, we found that the total P load regulated algal biomass and composition of algal phyla, whereas the combination of P load and temporal loading pattern structured community growth rate and diatom species composition. Our findings suggest that episodic P pulses can have significant effects on the structure of algal communities and thus runoff driven P loading may play a largely unacknowledged role in structuring benthic algal communities in streams.

5.4.1 Algal biomass

We observed that biomass of benthic algal communities increased with P enrichment and nearly doubled with a 5-fold increase in total P load. Numerous studies have observed such increases in benthic algal biomass with increasing P supply (Chételat et al., 1999; Biggs, 2000; Stevenson et al., 2012). However, contrary to the presumption that algal P utilization is limited by the short residence time of episodic inputs we also observed that peak biomass of algal communities did not differ whether P was supplied continuously or in the form of two P pulses that, together, delivered 83% of the cumulative experimental total P load. Thus, our findings support the small number of studies that have shown structural and physiological responses of algal communities to short duration increases in P (Humphrey & Stevenson, 1992; Rier et al., 2016). Moreover, our study builds on past research by revealing that the total P load a community receives may be more important in the regulation of algal biomass than P delivery pattern. Davies and Bothwell (2012) made a similar observation in a stream mesocosm experiment that simulated microscale temporal variation in P (e.g., invertebrate grazing and excretion) whereby the hourly integrated P load controlled algal biomass opposed to the duration of sub-hourly P pulses (1–60 min h^{-1}) differing in concentration. Although we observed the same regulation of benthic algal biomass by total P load as Davies and Bothwell (2012), the simulation of P enrichment patterns associated with human land use permits our findings to be directly translatable to P enrichment in streams that drain developed watersheds.

Our finding that episodic P pulses have the potential to stimulate and sustain the development of algal mats demonstrates the importance of short-duration P subsidies on algal biomass accrual. Short-term algal accrual curves differed among P treatments such that the onset of exponential growth and maximum absolute growth rates occurred earlier in the P enriched treatments than the unenriched treatment. Observed effects of P enrichment on absolute growth rates is consistent with past studies that have demonstrated that resource availability can stimulate the rate of change in algal biomass

(Fellows et al., 2006; Rier et al., 2006) and govern temporal patterns in algal accrual (Bothwell, 1989).

We also found that although the maximum absolute growth rates attained were comparable between P enriched treatments, the onset of exponential growth and maximum absolute growth rates occurred later in the episodically enriched communities and was associated with the first P pulse. Moreover, we observed that absolute growth rates declined towards the end of the experiment in the continuously enriched treatment, but not in the episodically enriched treatment. Algal accrual often declines with community succession as peak biomass is maintained through a period of turnover and low absolute growth rates prior to biomass loss or autogenic sloughing (Biggs, 1996). Our observation of increased absolute growth rates for the episodically enriched treatment indicates the second P pulse prolonged successional transition from biomass accrual to turnover resulting in both P enriched treatments accruing the same amount of biomass at the end of the experiment. Although we did not carry out our experiment long enough to observe autogenic sloughing, biomass differed less than 1.6-fold between P enriched communities over the latter half of the experimental period, suggesting that biomass-mediated effects on resource stress in the basal layers of algal mats (e.g., light attenuation and nutrient diffusion) would have been similar (Hill & Boston, 1991). However, future studies are needed to investigate the long-term temporal patterns in benthic algal succession associated with differences in continuous and episodic P enrichment.

5.4.2 Algal community composition

Relative abundances of algal phyla responded to total P load, but not P enrichment pattern. Diatoms were most abundant at the beginning of our experiment, but P enrichment shifted the composition of algal communities towards a chlorophyte dominated community. Chlorophyte accrual is expected in P-rich environments with ample light and minimal disturbance (Biggs et al., 1998a) and algal communities amended with P in other mesocosm experiments have shown similar shifts in chlorophyte relative abundance (Bondar-Kunze et al., 2016; Bækkelie et al., 2017). Moreover, filamentous chlorophyte abundance is often associated with the increased trophic status of streams (Chételat et al., 1999; Stevenson et al., 2012). Although the largest fraction of chlorophyte taxa enumerated in our study were non-filamentous (*Scenedesmus* sp. and *Pediastrum* sp.), our study also observed an increase in filamentous chlorophytes and as a group chlorophytes appeared to be the cause of eutrophic or nuisance levels (*sensu* Welch et al., 1988; Suplee et al., 2009) of algal biomass in the P enriched communities. Thus, P enrichment from continuous and episodic sources may result in a comparable aesthetic of benthic algal communities and similar loss of valued ecological conditions.

We observed that diatom assemblage composition was initially comparable among P treatments, but diverged with enriched P loads. Our finding that P enriched diatom assemblages were dissimilar to unenriched assemblages is consistent with past studies that have investigated the response of diatoms to stream P gradients (Black et al., 2011; Taylor et al., 2018) and human land use patterns (Walsh & Wepener, 2006). However, we also found differences in diatom assemblage composition associated with the temporal pattern of P loading as continuously and episodically enriched assemblages were significantly dissimilar. The differences we observed between P enriched diatom assemblages supports past research linking temporal variability in diatom assemblage composition with changes in P availability in individual streams (e.g., Lavoie et al., 2008a; Snell et al., 2014). Thus, distinct diatom assemblages observed among P treatments indicates that both amount and pattern of P enrichment may regulate species performance and manifest in assemblage-level differences in diatom composition.

Four diatom species contributed most to the observed dissimilarity in diatom assemblage composition among P treatments. *Nitzchia palea* and *Nitzchia palea* var. *debilis* had greater relative abundances in P enriched assemblages, whereas *Achanthidium minutissimum* had a greater relative abundance in unenriched assemblages. *N. palea* has an optimal total P concentration 2-fold greater than *A. minutissimum* (Ponader et al., 2007), which coincides with the aforementioned differences observed in relative abundance. However, *N. palea* did not contribute to the dissimilarity between continuously and episodically enriched assemblages. Comparable relative abundances of *N. palea* in P enriched assemblages indicates that *N. palea* can effectively capture resources from episodic increases in P while also benefiting from continuously enriched conditions. In contrast, *Cyclotella meneghiniana* was largely responsible for the dissimilarity between continuously and episodically enriched assemblages. Yet, although *C. meneghiniana* has a total P optimum comparable to *N. palea* (Ponader et al., 2007), *C. meneghiniana* may have limited success in P limited environments through a passive cellular morphology (Grover, 1989) and low potential for P storage (Kilham et al., 1977; Tilman & Kilham, 1976). Indeed, greater relative abundances of *C. meneghiniana* observed in continuously enriched assemblages further suggests that this species may be unable to effectively capture resources in streams with high temporal variation in P concentrations. P optima of benthic diatoms may therefore not fully represent the most favourable conditions for success as the temporal availability of P appears to have a detectable effect on at least one species observed in our study.

Streams that served as the species pool for our experiment frequently receive increased loads of P from point and nonpoint sources within their catchments. Algae communities in artificial streams at the start of our experiment were thus representative of taxa commonly found in mesotrophic to eutrophic streams located in a temperate region where P enrichment is an important management concern. Therefore, the compositional differences we observed should be representative of the differential effects that continuous and episodic P enrichment have on instream benthic algae communities. As such our findings are relevant to the management of streams that drain anthropogenic landscapes with P conditions similar to the streams from which our inocula were collected. However, it is unlikely that our starting communities would fully represent those found in oligotrophic streams with catchments exposed to more limited human influence. Consequently, our findings may be less informative for the management of oligotrophic streams as the effects of P loading pattern on algal community composition in our study may have been constrained by the initial species pool used to inoculate artificial streams. For example, Taylor et al., (2018) found that benthic algae communities transferred into stream mesocosms from nutrient poor streams exhibited a larger change in diatom assemblage composition in response to differences in ambient P concentrations compared to communities from more nutrient enriched streams. Future studies are therefore needed to evaluate the compositional response of benthic algal communities found in oligotrophic streams to episodic P enrichment in order to provide further insight for the management of streams where P loadings from human activities are more limited.

5.4.3 Ecological potential of phosphorus pulses

Episodic P loading from nonpoint sources are often accompanied by additional physicochemical changes in stream conditions. Our findings demonstrate the potential of algal communities to accrue biomass from P pulses, but the observed response may not be attainable with extraneous (e.g., season and baseflow P) and co-occurring (e.g., discharge and turbidity) controls on algal development in streams. Specifically, hydrodynamic effects from runoff events can scour benthic environments and lessen the structural response of algae to P inputs (Biggs & Close, 1989). However, disturbance to algal biomass can vary spatially within a reach and temporally with the successional stage of algal communities (Peterson & Stevenson, 1992; Katz et al., 2018). Likewise, algae are resistant to small and moderate flow events (Biggs & Close, 1989; Biggs et al., 1998b) that can contribute a substantial proportion (37–52%) of the total P load in headwater streams (Macrae et al., 2007). Moderate increases in discharge from sub-scouring events can also promote the diffusion of P into algal communities, thereby increasing P uptake and production (Horner & Welch, 1981; Townsend et al., 2012). P loading from sub-scouring runoff events may therefore have a considerable effect on structuring benthic algal communities in streams (Stevenson, 1990). Moreover, a community-level response to episodic P loadings may be further regulated by background P concentrations (Cook et al., 2018). For example, many experimental studies in more nutrient enriched systems have shown no change in algal accrual or diatom assemblage composition associated with increased P concentrations (Bowes et al., 2012; McCall et al., 2017; Taylor et al., 2018). Algal communities in streams that have enriched background P concentrations may be nutrient saturated and unable to effectively utilize episodic loadings (Weigelhofer et al., 2018). Future studies are therefore needed to incorporate additional realism in order to fully understand the eutrophication potential and ecosystem-level implications of episodic P loadings in streams.

5.5 Conclusions

5.5.1 Applications to river management

The potential for algal communities to respond to episodic P inputs has important implications for watershed management. First, P based monitoring criteria to control algal biomass may be ineffective in streams that meet baseflow P guidelines, but have high temporal variability in P loading. P concentration guidelines often rely on stressor-response curves where spatial patterns of algal biomass are used to establish change points in stream trophic state and level of impairment (Jarvie et al., 2013). However, temporal variation in P may decouple spatial P concentration-biomass relationships and result in the inadequate evaluation of stream ecosystem conditions. For example, prevailing P concentrations of episodically enriched communities in our study (10 $\mu g\ L^{-1}$ SRP) would meet P management guidelines (Evans-White et al., 2013), but measures of algal biomass would be about 4-fold greater than nuisance growth (Welch et al., 1988; Suplee et al., 2009). Moreover, routine water quality monitoring programs often fail to capture short-duration inputs of P and thus may provide incomplete information needed to control eutrophication in episodically enriched streams (Cassidy & Jordan, 2011). We thus recommend that monitoring programs should consider the incorporation of event-flow P monitoring and the adoption of additional ecological indicators that can respond to temporal variation in P (e.g., diatom assemblage composition) to provide managers with a more comprehensive assessment of stream ecosystem conditions.

Second, varied success of diatom species in the acquisition of P from episodic inputs may have applications in the refinement of ecological indicators used for biological monitoring. Indeed, the results from our study suggest that many diatom species can take advantage of short-duration pulses of phosphorus. However, our study identified one species that was more successful in conditions associated with continuous P enrichment. This information could have applications for the refinement of diatom-based indices and selection of sentinel taxa based on autecological characteristics corresponding to optimal conditions of P acquisition (episodic versus continuous). Although sentinel taxa disproportionally successful under episodic P enrichment were not identified, additional information on P acquisition in diatom-based indices could be valuable in diagnosing the presence or latency of nonpoint source phosphorus pollution. Future experimental and field studies are thus required to determine the ecological success of other diatom species and the potential for sentinel diatom taxa to serve as indicators of the primary source of P to streams.

Lastly, our observation that total P load outweighed delivery pattern in the control of algal biomass can be used to inform P reduction targets. P management in developed watersheds has often differed based on the surface water type under consideration. For streams, P concentrations during summer baseflow are expected to pose the greatest eutrophication risk, whereas the total P load is most relevant to eutrophication in lakes (Stamm et al., 2014). However, our finding of no difference in peak algal biomass with P loading pattern suggests that episodic inputs of P have an equal potential to augment stream eutrophication as enriched baseflow P concentrations. As such, management interventions that reduce the total P load exported from a watershed would provide dual benefits in the reduction of eutrophication risk in downstream lakes and in the control of nuisance algal production in streams. Thus, future management strategies should consider remedial actions that provide the greatest return on investment in P load reduction.

5.6 References

Bækkelie, K. A. E., Schneider, S. C., Hagman, C. H. C., & Petrin, Z. (2017). Effects of flow events and nutrient addition on stream periphyton and macroinvertebrates: An experimental study using flumes. *Knowledge and Management of Aquatic Ecosystems, 418*, 47–58. doi: 10.1051/kmae/2017041

Biggs, B. J. F. (1996). Patterns in benthic algae of streams. In: Stevenson, R. J., Bothwell, M. L., & Lowe, R. L. (Ed.) *Algal Ecology Freshwater Benthic Ecosystems* (pp. 31-56). San Diego, CA: Academic Press.

Biggs, B. J. F., & Close M. E. (1989). Periphyton biomass dynamics in gravel bed rivers: The relative effects of flows and nutrients. *Freshwater Biology*, *22*(2), 209–231. doi: 10.1111/j.1365-2427.1989.tb01096.x

Biggs, B. J. F., Stevenson, R. J., & Lowe, R. L. (1998a). A habitat matrix conceptual model for stream periphyton. *Archiv fur Hydrobiologie, 143*(1), 21–56. doi: 10.1127/archiv-hydrobiol/143/1998/21

Biggs, B. J. F., Goring, D. G., & Nikora, V. I. (1998b). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. *Journal of Phycology, 34*(4), 598–607. doi: 10.1046/j.1529-8817.1998.340598.x

Biggs, B. F. J. (2000). Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society, 19*(1), 17–31. doi: 10.2307/1468279

Black, R. W., Moran, P. W., & Frankforter, J. D. (2011). Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams. *Environmental Monitoring and Assessment, 175*(1-4), 397–417. doi: 10.1007/s10661-010-1539-8

Bondar-Kunze, E., Maier, S., Schonauer, D., Bahl, N., & Hein, T. (2016). Antagonistic and synergistic effects on a stream periphyton community under the influence of pulsed flow velocity increase and nutrient enrichment. *Science of the Total Environment, 573,* 594–602. doi: 10.1016/j.scitotenv.2016.08.158

Bothwell, M. L. (1989). Phosphorus-limited growth dynamic of lotic periphytic diatom communities: Areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences, 46*(8), 1293–1301. doi: 10.1139/f89-166

Bowes, M. J., Ings, N. L., McCall, S. J., Warwick, A., Barrett, C., Wickham, H. D., … Singer, A. C. (2012). Nutrient and light limitation of periphyton in the River Thames: Implications for catchment management. *Science of the Total Environment, 434*, 201–212. doi: 10.1016/j.scitotenv.2011.09.082

Bowman, M. F., Chambers P. A., & Schindler, D. W. (2005). Epilithic algal abundance in relation to anthropogenic changes in phosphorus bioavailability and limitation in mountain rivers. *Canadian Journal of Fisheries of Aquatic Sciences, 62*(1), 174–184. doi: 10.1139/f04-244

Cassidy, R., & Jordan, P. (2011). Limitations of instantaneous water quality sampling in surface-water catchments: Comparison with near-continuous phosphorus time-series data. *Journal of Hydrology, 405*(1–2), 182–193. doi: 10.1016/j.jhydrol.2011.05.020y

Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A. (2012). Development of environmental thresholds for nitrogen and phosphorus in streams. *Journal of Environmental Quality, 41*(1), 7–20. doi: 10.2134/jeq2010.0273

Chételat, J., Pick, F. R., Morin, A., & Hamilton, P. B. (1999). Periphyton biomass and community composition in rivers of different nutrient status. *Canadian Journal of Fisheries and Aquatic Sciences, 56*(4), 560–569. doi: 10.1139/f98-197

Cook, S. C., Housley, L., Black, J. A., & King, R. S. (2018). Freshwater eutrophication drives sharp reductions in temporal beta diversity. *Ecology, 99*(1), 47–56. doi: 10.1002/ecy.2069

Correll, D. L. (1998). The role of phosphorus in the eutrophication of receiving waters: A review. *Journal of Environmental Quality, 27*(2), 261–266. doi: 10.2134/jeq1998.00472425002700020004x

Davies, J., & Bothwell, M. L. (2012). Responses of lotic periphyton to pulses of phosphorus: P-flux controlled growth rate. *Freshwater Biology, 57*(12), 2602–2612. doi: 10.1111/fwb.12032

Edwards, A. C., & Withers, P. J. A. (2008). Transport and delivery of suspended solids, nitrogen and phosphorus from various sources to freshwaters in the UK. *Journal of Hydrology, 350*(3–4), 144–153. doi: 10.1016/j.jhydrol.2007.10.053

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

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality, 42*(4), 1002–1014. doi: 10.2134/jeq2012.0491

Fellows, C. S., Clapcott, J. E., Udy, J. W., Bunn, S. E., Harch, B. D., Smith, M. J., & Davies, P.M. (2006). Benthic metabolism as an indicator of stream ecosystem health. *Hydrobiologia, 572*(1), 71–87. doi: 10.1007/s10750-005-9001-6

Francoeur, S. N. (2001). Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society, 20*(3), 358–368. doi: 10.2307/1468034

Grover, J. P. (1989). Influence of cell shape and size on algal competitive ability. *Journal of Phycology, 25*(2), 402–405. doi: 10.1111/j.1529-8817.1989.tb00138.x

Hill, W. R., & Boston, H. L. (1991). Community development alters photosynthesis-irradiance relations in stream periphyton. *Limnology and Oceanography, 36*(7), 1375–1389. doi: 10.4319/lo.1991.36.7.1375

Horner, R. R., & Welch, E. B. (1981). Stream periphyton development in relation to current velocity and nutrients. *Canadian Journal of Fisheries and Aquatic Sciences, 38*(4), 449–457. doi: 10.1139/f81-062

Humphrey, K. P., & Stevenson, R. J. (1992). Responses of benthic algae to pulses in current and nutrients during simulations of subscouring spates. *Journal of the North American Benthological Society, 11*(1), 37–48. doi: 10.2307/1467880

Jarvie, H. P., Neal, C., & Withers, P. J. A. (2006). Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? *Science of the Total Environment, 360*(1-3), 246–253. doi: 10.1016/j.scitotenv.2005.08.038

Jarvie, H. P., Sharpley, A. N., Withers, P. J. A., Scott, J. T., Haggard, B. E., & Neal, C. (2013). Phosphorus mitigation to control river eutrophication: Murky waters, inconvenient truths, and “postnormal” science. *Journal of Environmental Quality, 42*(2), 295–304. doi: 10.2134/jeq2012.0085

Katz, S. B., Segura, C., & Warren, D. R. (2018). The influence of channel bed disturbance on benthic Chlorophyll a: A high resolution perspective. *Geomorphology, 305*(15), 141–153. doi: 10.1016/j.geomorph.2017.11.010

Kilham, S. S., Kott, C. L., & Tilman, D. (1977). Phosphate and silicate kinetics for the Lake Michigan diatom diatoma elongatum. *Journal of Great Lakes Research, 3*(1–2), 93–99. doi: 10.1016/S0380-1330(77)72233-6

Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., & Dillon, P. J. (2008a). Are diatoms good integrators of temporal variability in stream water quality? *Freshwater Biology, 53*(4), 827–841. doi: 10.1111/j.1365-2427.2007.01935.x

Lavoie I., Hamilton, P. B., Campeau, S., Grenier, M., & Dillon, P. J. (2008b). Guide d’identification des diatomées des riviéres de l’est du Canada, ed. Presses de l’Université du Québec (PUQ).

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia, 129*(2), 271–280. doi: 10.1007/s004420100716

Macrae, M. L., English, M. C., Schiff, S. L., & Stone, M. (2007). Capturing temporal variability for estimates of annual hydrochemical export from a first-order agricultural catchment in southern Ontario, Canada. *Hydrological Processes, 21*(13), 1651–1663. doi: 10.1002/hyp.6361

Mainstone, C. P., & Parr, W. (2002). Phosphorus in rivers - Ecology and management. *Science of the Total Environment, 282–283,* 25–47. doi: 10.1016/S0048-9697(01)00937-8

McCall, S. J., Bowes, M. J., Warnaars, T. A., Hale, M. S., Smith, J. T., Warwick, A., Barrett, C. (2014). Phosphorus enrichment of the oligotrophic River Rede (Northumberland, UK) has no effect on periphyton growth rate. *Inland Waters, 4*(2), 121–132. doi: 10.5268/IW-4.2.0

Meals, D. W., Levine, S. N., Wang, D., Hoffmann, J. P., Cassell, A. E., Drake, J. C., … Brown, A. B. (1999). Retention of spike additions of soluble phosphorus in a northern eutrophic stream. *Journal of the North American Benthological Society, 18*(2), 185–198. doi: 10.2307/1468460

Menczelesz, N., Szivák, I., & Schmera, D. (2020). How do we construct and operate experimental streams? An overview of facilities, protocols, and studied questions. *Hydrobiologia, 847,* 1–10. doi: 10.1007/s10750-019-04093-0

Meyer, J. L., & Likens, G. E. (1979). Transport and transformation of phosphorus in a forest stream ecosystem. *Ecology*, *60*(6), 1255–1269. doi: 10.2307/1936971

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2019). vegan: community ecology package. Version 2.5-6.

Paine, C. E. T., Marthews, T. R., Vogt, D. R., Purves, D., Ress, M., Hector, A., & Turnbull, L. A. (2012). How to fit nonlinear plant growth models and calculate growth rates: An update for ecologists. *Methods in Ecology and Evolution, 3*(2), 245–256. doi: 10.1111/j.2041-210X.2011.00155.x

Peterson, C. G., & Stevenson, R. J. (1992). Resistance and resilience of lotic algal communities: Importance of disturbance timing and current. *Ecology, 73*(4), 1445–1461. doi: 10.2307/1940689

Ponader, K. C., Charles, D. F., & Belton, T. J. (2007). Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. *Ecological Indicators, 7*(1), 79–93. doi: 10.1016/j.ecolind.2005.10.003

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.5.0.

Rier, S. T., & Stevenson, R. J. (2006). Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia, 561*(1), 131–147. doi: 10.1007/s10750-005-1610-6

Rier, S. T., Kinek, K. C., Hay, S. E., & Francoeur S. N. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. *Freshwater Science, 35*(2), 490–502. doi: 10.1086/685859

Simpson, G. (2019). gratia: graceful ‘ggplot’-based graphics and other functions for GAMs fitted using ‘mgcv’. Version 0.2-8.

Sharpley, A. N., Mcdowell, R. W., & Kleinman, P. J. A. (2001). Phosphorus loss from land to water: Integrating agricultural and environmental management. *Plant and Soil, 237*, 287–307. doi: 10.1023/A:1013335814593

Snell, M. A., Barker, P. A., Surridge, B. W. J., Large, A. R. G., Benskin, C. McW. H., Reaney, S., … Haygarth, P. M. (2014). High frequency variability of environmental drivers determining benthic community dynamics in headwater streams. *Environmental Science: Processes and Impacts, 16*(7), 1629–1636. doi: 10.1039/c3em00680h

Solovchenko, A. E., Ismagulova, T. T., Lukyanov, A. A., Vasilieva, S. G., Konyukhova, I. V., Pogosyan, S. I., … Gorelova, O. A. (2019). Luxury phosphorus uptake in microalgae. *Journal of Applied Phycology, 31*, 2755–2770. doi: 10.1007/s10811-019-01831-8

Stamm, C., Jarvie, H. P., & Scott, T. (2014). What’s more important for managing phosphorus: Loads, concentrations or both? *Environmental Science and Technology, 48*(1), 23–24. doi: 10.1021/es405148c

Steinman, A. D., Lamberti, G. A., & Leavitt, P. R. (2007). Biomass and pigments of benthic algae. In: Hauer, F. R., & Lamberti, G. A. (Ed.) *Methods in Stream Ecology, Second Edition* (pp. 357-379). San Diego, CA: Academic Press.

Stevenson, J. J., Bennett, B. J., Jordan, D. N., & French R. D. (2012). Phosphorus regulates stream injury by filamentous green algae, DO, and pH with thresholds in responses. *Hydrobiologia, 695*, 25–42. doi: 10.1007/s10750-012-1118-9

Stevenson, R. J., Hill, B. H., Herlihy, A. T., Yuan, L. L., & Norton, S. B. (2008). Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of the North American Benthological Society, 27*(3), 783–799. doi: 10.1899/07-077.1

Stevenson, R. J. (1990). Benthic algal community dynamics in a stream during and after a spate. *Journal of the North American Benthological Society*, *9*(3), 277–288. doi: 10.2307/1467591

Suplee, M. W., Watson, V., Teply, M., & McKee, H. (2009). How green is too green? Public opinion of what constitutes undesirable algae levels in streams. *Journal of the American Water Resource Association*, *45*(1), 123–140. doi: 10.1111/j.1752-1688.2008.00265.x

Taylor, J. M., Black, J. A., Brooks, B. W., & King, R. S. (2018). Spatial, temporal and experimental: Three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems. *Journal of Applied Ecology, 55*(5), 2114–2123. doi: 10.1111/1365-2664.13150

Thomas, K. E., Lazor, R., Chambers, P. A., & Yates, A. G. (2018). Land-use practices influence nutrient concentrations of southwestern Ontario streams. *Canadian Water Resources Journal*, *43*(1), 2–17. doi: 10.1080/07011784.2017.1411211

Tilman, D., & Kilham, S. S. (1976). Phosphate and silicate growth and uptake kinetics of the diatoms asterionella formosa and cyclotella meneghiniana in batch and semicontinuous culture. *Journal of Phycology*, *12*(4), 375–383. doi: 10.1111/j.1529-8817.1976.tb02860.x

Townsend, S. A., Garcia, E. A., & Douglas, M. M. (2012). The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river. *Freshwater Science*, *31*(4), 1233–1243. doi: 10.1899/11-163.1

Walsh, G., & Wepener, V. (2009). The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. *Water SA*, *35*(5), 579–594. doi: 10.4314/wsa.v35i5.49184

Weigelhofer, G., Ramião, J. P., Puritscher, A., & Hein, T. (2018). How do chronic nutrient loading and the duration of nutrient pulses affect nutrient uptake in headwater streams? *Biogeochemistry, 141*(2), 249–263. doi: 10.1007/s10533-018-0518-y

Welch, E. B., Jacoby, J. M., Horner, R. R., & Seeley, M. R. (1988). Nuisance biomass levels of periphytic algae in streams. *Hydrobiologia, 157*, 161–168. doi: 10.1007/BF00006968

Withers, P. J. A., & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of the Total Environment, 400*(1–3), 379–395. doi: 10.1016/j.scitotenv.2008.08.002

Withers, P. J. A., Neal, C., Jarvie, H. P., & Doody, D. G. (2014). Agriculture and eutrophication: Where do we go from here? *Sustainability, 6*(9), 5853–5875. doi: 10.3390/su6095853

Wood, S. (2019). mgcv: mixed GAM computation vehicle with automatic smoothness estimation. Version 1.8-29.

Chapter 6

6 General discussion and conclusions

Human activities have resulted in the nutrient enrichment of surface waters around the globe (Bennett et al., 2001; Smith et al., 2003; Elser et at., 2007). The over-enrichment of surface waters with nitrogen and phosphorus can cause the excessive growth of algae, a process defined as eutrophication. Eutrophication can affect water quality and ecological conditions (Chislock et al., 2013) and has become a widespread problem in streams and rivers that drain human altered watersheds (Dodds & Smith, 2016). Algal biomass in streams that experience eutrophication is often high and composed of filamentous species that can cause periods of hypoxia and anoxia through autotrophic respiration (Dodds, 2006). Moreover, eutrophication-mediated changes to benthic algae communities can affect the assemblage structure of higher trophic levels (e.g., macroinvertebrates and fishes) and the rates of stream processes (e.g., decomposition) (Dodds, 2006). Thus, it is important to understand the effects of nutrient enrichment on autotrophic communities to prevent the degradation of stream ecosystems by eutrophication.

Streams in the Great Lakes-St. Lawrence basin face increasing pressure from nutrient enrichment associated with growing urban populations, agricultural intensification, and future climate changes (Wolter et al., 2006; d’Orgeville et al., 2014; DeBues et al., 2019). Moreover, research guiding the management of stream eutrophication remains broadly understudied relative to lakes (Dodds & Smith, 2016; Wurtsbaugh et al., 2019) and locally overlooked in the Great Lakes-St. Lawrence basin. This thesis provides an assessment of stream nutrient status in the lower Great Lakes-St. Lawrence basin of Ontario, Canada and investigates the influence of local management practices and human activities on stream ecosystem conditions. Furthermore, artificial streams were used to provide information applicable to managers throughout temperate regions on the ecological effects of phosphorus loading patterns associated with point and nonpoint sources.

6.1 Research findings and management implications

Chapter 2 provided evidence of widespread nutrient enrichment in streams draining the lower Great Lakes-St. Lawrence basin of Ontario, Canada. Similar to past studies (Giri et al., 2018; Staponites et al., 2019), land cover located in hydrologically connected areas of stream catchments appears to influence stream nutrient concentrations. However, increased nutrient concentrations were only found to be associated with agricultural land cover representative of nonpoint source inputs from crop production and livestock husbandry. Unlike some past studies (Shi et al., 2017; Qiu et al., 2019), urban land cover located in hydrologically connected areas was not associated with nutrient concentrations. Although impervious urban land cover may contribute to nonpoint source nutrient loading (Carey et al., 2013), nutrients from urban areas are often delivered to streams though pathways independent of water runoff (e.g., storm sewers, sewage treatment outflow) which may not be effectively represented by land cover metrics (Walsh et al., 2005).

My assessment of nutrient concentrations indicated that both nitrogen and phosphorus were equally enriched in streams draining agricultural and built-up catchments of southern Ontario. Moreover, I found that macronutrient stoichiometry of less disturbed streams in the region consistently indicated the potential for nitrogen and phosphorus co-limitation of autotrophic production. Nutrient co-limitation has been commonly observed past meta-analyses of stream nutrient limitation experiments (Francoeur, 2001; Elser et al., 2007; Beck et al., 2017). However, these findings contradict the phosphorus limitation paradigm that suggests that the autotrophic demand for nitrogen can be met through nitrogen fixation and thus phosphorus alone limits autotrophic production in freshwater ecosystems (Schindler et al., 2008; 2016). Yet, in contrast to lakes where N fixation can contribute 5-82% of total nitrogen inputs (Howarth et al., 1988), nitrogen fixation rarely contributes more than 5% of the total nitrogen load to streams (Marcarelli et al., 2008). Anthropogenic nitrogen loading may therefore have a larger ecological relevance in the eutrophication of stream ecosystems. Thus, to minimize the risk of stream degradation and bring about stream improvement in the Great Lake-St. Lawrence basin a shift in management focus from single nutrient (i.e., phosphorus) to dual nutrient (i.e., nitrogen and phosphorus) management may be required.

Findings from Chapter 2 suggested that the conversion of agriculture located in hydrologically connected areas of stream catchments to natural land covers associated with nutrient retention (e.g., forest and wetland) could provide water quality benefits in the reduction of nitrogen and phosphorus. Chapter 3 provided further evidence that increased natural vegetation located adjacent to streams (i.e., riparian buffer areas) was associated with a reduction in stream nitrogen and phosphorus concentrations. The location of riparian buffers in areas that accumulated larger amounts of water runoff were only found to be beneficial in the reduction of nitrogen during the spring. However, agricultural management practices that improve land drainage (i.e., tile drainage systems) countered the water quality benefits of natural vegetation and other nutrient management practices (i.e., manure storage structures), likely by increasing the transport of nonpoint source water runoff to streams. Tile drainage has been shown to be a significant pathway of nutrients to streams and may exacerbate the episodic delivery of nutrients from agricultural catchments (King et al., 2015). For example, Van Esbroeck et al., (2016) found that tile drains contributed 78-90% of total discharge and 40-77% of the total phosphorus load to three agricultural streams in southern Ontario. Water runoff transported through tile drains can bypass BMPs designed to retain nutrients from surface water and limit remediation benefits of BMPs on stream water quality (Lemke et al., 2011). Thus, in agricultural catchments with extensive tile drainage systems, conservation planning should be focused on BMPs that retain water runoff at tile outflows (i.e., tile plugs and retention ponds) to increase the return on investment in nutrient reduction.

Low frequency routine monitoring programs (i.e., triweekly to monthly) implemented in Chapters 2-4 are sufficient to understand spatial patterns in ambient nutrient concentrations but result in the significant loss of information on temporal patterns of nutrient loading (Bieroza et al., 2014). For example, nonpoint sources runoff events can transport nutrients to streams where high concentrations may span from hours to days (Bowes et al., 2015; Lloyd et al., 2016; Blaen et al., 2017). In addition, point sources such as sewage lagoons discharge nutrient rich effluent to streams during scheduled periods which can last days to weeks (Carlson et al., 2013). Passive monitoring programs may fail to capture these events and make it difficult to fully evaluate the effects of management practices and the potential ecological implications that temporal variation in nutrient exposure has on stream ecosystems. In particular, nonpoint source nutrient loading from agricultural watersheds has been identified as a major issue in the eutrophication of downstream waterbodies (Carpenter et al., 1998). However, few studies have evaluated the influence of episodic nutrient enrichments on the eutrophication of stream ecosystems because of the difficulty in obtaining high resolution datasets.

Chapter 4 examined autotrophic production and ecosystem respiration (i.e., stream metabolism) at a daily resolution in three streams that differed in nutrient exposure to point and nonpoint sources of nutrients over the growing season. Streams enriched with nutrients from the discharge of sewage effluent resulted in a 1.4-fold increase in autotrophic production (*aka* gross primary production) and 2.2-fold increase in ecosystem (i.e., autotrophic + heterotrophic) respiration compared to the agricultural stream. However, temporal patterns of autotrophic production and ecosystem respiration in sewage receiving streams were correlated with the stream exposed to agricultural activities. Moreover, differences in the continuity of effluent discharge between the passive and active sewage treatment facilities did not manifest in observable differences in the temporal patterns of stream ecosystem metabolism. However, all three study streams had enriched baseflow nutrient concentrations that were above regional nutrient thresholds (Chambers et al., 2008; 2012), suggesting autotrophic production may be nutrient saturated. Indeed, despite findings that that total phosphorus concentrations were nine-fold greater downstream of the passive sewage treatment facility than the active sewage treatment facility, no differences were observed in autotrophic production. Chronic nutrient enrichment may therefore support the demand for nutrients from increased autotrophic production and result in the subdued response of stream communities to temporal variation in nutrient exposure (Weigelhofer et al., 2018). Thus, managers will need to balance the nutrient load delivered to streams with the autotrophic demand for nutrients associated with desired stream ecosystem conditions in an effort to mitigate stream eutrophication.

Although study streams from Chapter 4 differed in ambient nutrient concentrations and exposure to human activities, my ability to draw conclusions on potential temporal associations between stream metabolism and nutrient concentrations was limited by the temporal resolution of nutrient data. However, temporal patterns in stream metabolism were strongly associated with continuous measurements of the dynamic physical environment of streams. Past studies have also found that light, temperature, and discharge regimes were associated with seasonal and daily variation in stream metabolism (Roberts et al., 2007; Bernhardt et al., 2018; Savoy et al., 2019). Given these observations, temporal variation in nutrient availability therefore has the potential to influence stream metabolism, but nuanced temporal effects of nutrients on autotrophic production may be hard to detect without high resolution nutrient datasets.

Stream mesocosm facilities can help ameliorate experimental and logistical limitations in study design and field data collection, similar to those I encountered in Chapter 4. Mesocosm experiments provide researchers with the ability to control extraneous variables and manipulate only variables of specific interest to examine cause and effect relationships (Menczelesz et al., 2020). Stream mesocosms have been used to investigate the effects of environmental stressors on ecological processes, benthic algae, macroinvertebrates, and fish (Menczelesz et al., 2020). Although mesocosm experiments may have limited realism, experimental research has provided invaluable information on the ecological effects of environmental pollution (Odum, 1984; Belanger, 1997; Boyle and Fairchild, 1997; Culp et al., 2000; Fordham, 2015; Menczelesz et al., 2020).

Chapter 5 investigated the response of benthic algal communities to temporal patterns of phosphorus enrichment in experimental stream mesocosms. Despite findings from Chapter 2 that nitrogen and phosphorus have the potential to limit autotrophic production in streams of the Great Lakes-St. Lawrence basin, past studies have shown that many algae species can store excess phosphorus and that biofilms can accumulate biomass from short-duration phosphorus subsidies (Humphrey & Stevenson, 1992; Davies & Bothwell, 2012; Rier et al., 2016). Chapter 5 addressed the limitation of high-resolution nutrient data in Chapter 4 and allowed for the comparison of ecological effects associated with simulated temporal patterns of point and nonpoint source phosphorus loading. Although nonpoint source phosphorus loading often co-occurs with changes in stream discharge and other physicochemical conditions (e.g., sediment, turbidity, contaminants, and light) not simulated in the mesocosm experiment described in Chapter 5, my findings provided evidence for the potential of short-duration high-concentration inputs of phosphorus to augment stream eutrophication. Indeed, episodic phosphorus enrichment stimulated and maintained growth rates of benthic algae communities. Moreover, there was no difference in algae biomass in treatments that received the same phosphorus load delivered episodically in two 48-hour enrichments or continuously throughout the experimental period. Thus, episodic phosphorus enrichment may have a largely unanticipated influence on benthic algae communities in streams. Managers should therefore consider strategies that provide the greatest return on investment in phosphorus load reduction to lower the risk of stream degradation by eutrophication.

Results from Chapter 5 build on existing research that suggest nutrient inputs from sub-scouring runoff events can stimulate algal growth and structure the composition of benthic algae communities in streams (Horner & Welch, 1981; Stevenson, 1990; Humphrey & Stevenson, 1992; Townsend et al., 2012; Rier et al., 2016). In the Great Lakes-St. Lawrence basin, small to moderate runoff events have been shown to occur frequently throughout the growing season and can contribute a substantial amount of phosphorus to streams (Macrae et al., 2007; Irvine et al., 2019a). Management strategies identified in Chapters 2 and 3 including the establishment and maintenance of riparian vegetation, wetlands, and tile drainage controls have been shown to reduce episodic nutrient loading to streams (Zedler, 2003; Polyakov et al., 2005; Sunohara et al., 2016) and may therefore benefit eutrophication management. However, Chapter 4 found that nutrient enrichment from common human activities that differed in the continuity of nutrient loading did not result in generate ecological differences in the metabolic regime of streams.

Enriched baseflow nutrient concentrations provide a potential explanation for a diminished response of autotrophic production to temporal variation in nutrient exposure. Increased nutrient loading in excess of the autotrophic demand for a stream may not be assimilated and resultantly become transported downstream without temporary retention and transformation in biological nutrient cycles. For example, past studies have reported that nutrient enrichment can saturate nutrient uptake and that spiraling length (i.e., length of downstream transport) can increase from hundreds of meters to several kilometers with increased baseflow nutrient concentrations (Haggard et al., 2001; Marti et al., 2004; Benot et al., 2006; Gücker & Pusch, 2006). Streams and downstream waterbodies in the Great Lakes-St. Lawrence basin are expected to become more susceptible to temporal variation in nutrient exposure from episodic nutrient loading (Kaushal et al., 2014; 2018; Sinha et al., 2017), thus implications from this thesis can likely provide insights on the potential effects of future climate change. Although enriched baseflow nutrient concentrations can augment stream eutrophication (Stamm et al., 2014), chronic nutrient enrichment also has the potential to reduce stream ecosystem services and increase the eutrophication risk of episodic nutrient loading in downstream waterbodies. Therefore, management actions that reduce baseflow nutrient concentrations may provide direct benefits in improving stream ecosystem conditions and wider benefits through the increased retention of episodic nutrient loads transported throughout stream networks.

6.2 Recommendations for future research

This thesis examined spatial and temporal patterns in nutrient enrichment to provide recommendations for the management of stream eutrophication. Nuanced findings on the potential for episodic nutrient enrichment to augment stream eutrophication raises avenues for future research on the ecological effects of temporal patterns in nutrient exposure. Future research conducted in the field and with the use of artificial stream mesocosm facilities can increase our understanding of the influence that the dynamic physicochemical environment has on the structure and function of stream ecosystems for the improved management of anthropogenic activities.

Unlike the phosphorus loading patterns simulated in Chapter 5, episodic phosphorus loading often co-occurs with changes in many physicochemical conditions in streams. For example, overland runoff events can result in increased turbidity (Mather & Johnson, 2014) and contaminant concentrations (Solomon et al., 1996), decreased light availability (Julian et al., 2008), and altered water temperatures (Herb et al., 2008). In addition, surges in stream discharge can disturb benthic habitats through increased flow velocity and sediment abrasion (Katz et al., 2018). Future studies in artificial stream mesocosms are needed to investigate episodic phosphorus loading with additional realism and to determine the effects of co-occurring factors on the response of benthic algae communities to phosphorus enrichment. Moreover, experimental field studies, such as stream phosphorus additions (see Meals et al., 1999) and controlled runoff events (see Murdock et al., 2013), may provide opportunities to field verify ecological patterns identified in stream mesocosm experiments and better understand the wider ecological effects of phosphorus loading patterns.

Another important physicochemical condition of streams is baseflow nutrient concentration. As previously alluded to in Chapters 4 and 5, enriched baseflow phosphorus concentrations may diminish the response of autotrophic communities to temporal variation in nutrient exposure. Weigelhofer et al., (2018) found that stream phosphorus uptake of episodic additions decreased with increasing baseflow nutrient concentrations. Moreover, Taylor et al., (2018) reported that benthic algae communities in eutrophic streams were resilient to short-term changes in nutrient availability when transferred to stream mesocosms differing in nutrient concentration. Nutrient enrichment may therefore lead to increased temporal homogeneity in ecological communities of stream ecosystems (Cook et al., 2018). However, future field and mesocosm studies are needed to better understand the effects of chronic nutrient enrichment on the response of benthic algae to episodic phosphorus loading. Knowledge from this research would provide important information to managers on where to target nutrient control measures (i.e. event flow or baseflow) for the protection of stream ecosystems and to maximize the services stream ecosystems provide to downstream waterbodies in watershed nutrient retention.

Research has shown that episodic phosphorus loading to streams can vary in magnitude with the intensity of precipitation events and antecedent conditions in the watershed (Sharpley et al., 2008; Bowes et al., 2015; Lloyd et al., 2016; Blaen et al., 2017). Streams may therefore be exposed to loadings that differ in the concentration and duration of phosphorus exposure, which may have implications for autotrophic production. For example, total phosphorus concentrations measured in base and high flow conditions in agricultural streams of southwestern Ontario were observed to range from 10 to 900 µg L^{-1} over a six-year study period (Ontario Ministry of the Environment, 2012). In addition, enriched phosphorus concentrations from high flow events may persist for several hours to several days. Indeed, Chapter 5 demonstrated the potential for benthic algae communities to accrue biomass from episodic loading, however, the amount of phosphorus that can be assimilated by algal communities from episodic events remains unclear. Rier et al., (2016) evaluated the influence of pulse duration on the structure of benthic algae communities, but the gradient of pulse duration evaluated was limited to events that were less than 8 hours. Information on the capacity of benthic algae to uptake and retain phosphorus from episodic loadings that differ in concentration and duration, as observed in human-influenced streams, may help managers balance loading with instream processing to lower the amount and bioavailability of phosphorus transported downstream. Thus, future studies on the identification of potential thresholds in algal phosphorus acquisition from episodic loadings are needed to provide important information for the establishment of phosphorus management targets in developed watersheds.

Lastly, the relative availability of other macronutrients may further influence the capacity of benthic algal communities to assimilate phosphorus from episodic sources. Experimental mesocosms in Chapter 5 were enriched with nitrogen to reproduce phosphorus limiting conditions. However, results from Chapter 2 indicated that many less disturbed streams had the potential for nitrogen and phosphorus co-limitation. Ecological implications of macronutrient stoichiometry are premised on the law of the minimum (Klasumeier et al., 2004), whereby algal growth is dictated by the resource in the shortest supply. Thus, algal communities may not be able to assimilate phosphorus from short-duration high-concentration events as efficiently in streams co-limited for nitrogen and phosphorus relative to communities that are limited by phosphorus alone. Macronutrient stoichiometry can have implications for instream nutrient processing, and stochiometric rebalancing has been proposed as a potential strategy to alleviate eutrophication (Stutter et al., 2018). However, further research is needed to evaluate the response of ecosystem structure and function to differences in macronutrient stoichiometry.

A limitation of many field studies is the acquisition of high temporal resolution nutrient data (Bieroza et al., 2013). Artificial mesocosm experiments eliminate the need for such data, however, further field research is needed to better understand the ecological implications of temporal patterns in nutrient loading to streams. High temporal resolution sensors (e.g., Pellerin et al., 2016) and environmental modelling (e.g., Fu et al., 2019) provide some potential avenues to advance the science of nutrient enrichment and water resource management. Improved and emerging technologies have made it possible to accurately measure many water quality parameters (e.g., dissolved oxygen, turbidity, discharge, and nitrate concentrations) at high temporal resolutions (e.g., minutes to days) and over long periods of time. This data has been used to generate detailed information on nutrient loading (Robertson et al., 2018) and model ecosystem processes, such as nutrient uptake, primary productivity, and ecosystem respiration (Rode et al., 2016; Appling et al., 2018). Moreover, continuously measured water quality parameters have been used to model temporal changes in other physicochemical conditions that cannot be measured with the use of environmental sensors (Irvine et al., 2019b). Recent research with the use of environmental sensors has produced novel information on characteristic temporal patterns in the metabolic regimes of streams (Savoy et al., 2019) and over time will have the potential to provide nuance information on global-scale environmental change (Bernhardt et al., 2018). The intersection of big data and ecology thus presents an important opportunity improve our understanding of the relationships between the dynamic physicochemical environment and the structure and function of aquatic ecosystems.

6.3 Summary and conclusions

This thesis contributes novel information to the body of research on stream eutrophication in the Great Lakes-St. Lawrence basin and more broadly throughout temperate regions experiencing extensive nutrient loadings from mixed land uses. At the regional scale, Chapter 2 provided evidence of stream nutrient enrichment and paired nutrient concentrations with macronutrient stoichiometry to make recommendations on the specific nutrients that need to be controlled to minimize the risk of stream degradation by eutrophication. Moreover, Chapters 2 and 3 evaluated the benefits of land cover management and agricultural BMPs in the reduction of stream nutrient concentrations to provide managers with potential solutions to mitigate stream nutrient enrichment. Chapter 4 investigated the influence of nutrient exposure from common human activities on stream ecosystem metabolism and found that the ecological response to nutrient enrichment may be confounded by the dynamic physicochemical environment of streams. Artificial stream mesocosms were used in Chapter 5 to isolate the effects of phosphorus loading pattern associated with point and nonpoint sources on benthic algae communities. Contrary to the presumption that the capacity for episodic nutrient loading to augment stream eutrophication is limited by the rapid renewal of water in streams (Stamm et al., 2014; Whiters et al., 2014), Chapter 5 demonstrated that benthic algae communities can accumulate the same biomass from enriched phosphorus loads delivered continuously or in two 48-hour simulated runoff events. Particularly, novel findings from Chapter 5 generate opportunities future research on the ecological effects of phosphorus loading associated with the composition and delivery attributes of human land use activities.

6.4 References

Appling, A. P., Hall, R. O., Yackulic, C. B., & Arroita, M. (2018). Overcoming equifinality: Leveraging long time series for stream metabolism estimation. *Journal of Geophysical Research: Biogeosciences, 123*(2), 624–645. doi: 10.1002/2017JG004140

Beck, W. S., Rugenski, A. T., & Poff, N. L. (2017). Influence of experimental, environmental, and geographic factors on nutrient-diffusing substrate experiments in running waters. *Freshwater Biology, 62*(10), 1667–1680. doi: 10.1111/fwb.12989

Belanger, S. E. (1997). Literature review and analysis of biological complexity in model stream ecosystems: Influence of size and experimental design. *Ecotoxicology and Environmental Safety, 36*(1), 1–16. doi: 10.1006/eesa.1996.1487

Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impact on erodable phosphorus and eutrophication: A global perspective. *BioScience, 51*(3), 227–234. doi: 10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Bernhardt, E. S., Heffernan, J. B., Grimm, N. B., Stanley, E. H., Harvey, J. W., Arroita, M., … Yackulic, C. B. (2018). The metabolic regimes of flowing waters. *Limnology and Oceanography*, *63*(S1), S99–S118. doi: 10.1002/lno.10726

Bernot, M. J., Tank, J. L., Royer, T. V., & David, M. B. (2006). Nutrient uptake in streams draining agricultural catchments of the midwestern United States. *Freshwater Biology*, *51*(3), 499–509. doi: 10.1111/j.1365-2427.2006.01508.x

Bieroza, M. Z., Heathwaite, A. L., Mullinger, N. J., & Keenan, P. O. (2014). Understanding nutrient biogeochemistry in agricultural catchments: The challenge of appropriate monitoring frequencies. *Environmental Science: Processes and Impacts*, *16*(7), 1676–1691. doi: 10.1039/c4em00100a

Blaen, P. J., Khamis, K., Lloyd, C., Comer-Warner, S., Ciocca, F., Thomas, R. M., … Krause, S. (2017). High-frequency monitoring of catchment nutrient exports reveals highly variable storm event responses and dynamic source zone activation. *Journal of Geophysical Research: Biogeosciences*, *122*(9), 2265–2281. doi: 10.1002/2017JG003904

Bowes, M. J., Jarvie, H. P., Halliday, S. J., Skeffington, R. A., Wade, A. J., Loewenthal, M., … Palmer-Felgate, E. J. (2015). Characterising phosphorus and nitrate inputs to a rural river using high-frequency concentration–flow relationships. *Science of The Total Environment*, *511*, 608–620. doi: 10.1016/j.scitotenv.2014.12.086

Boyle, T. P., & Fairchild, J. F. (1997). The role of mesocosm studies in ecological risk analysis. *Ecological Applications*, *7*(4), 1099. doi: 10.2307/2641197

Carey, R. O., Hochmuth, G. J., Martinez, C. J., Boyer, T. H., Dukes, M. D., Toor, G. S., & Cisar, J. L. (2013). Evaluating nutrient impacts in urban watersheds: Challenges and research opportunities. *Environmental Pollution*, *173*, 138–149. doi: 10.1016/j.envpol.2012.10.004

Carlson, J. C., Anderson, J. C., Low, J. E., Cardinal, P., MacKenzie, S. D., Beattie, S. A., … Hanson, M. L. (2013). Presence and hazards of nutrients and emerging organic micropollutants from sewage lagoon discharges into Dead Horse Creek, Manitoba, Canada. *Science of The Total Environment, 445–446,* 64–78. doi: 10.1016/j.scitotenv.2012.11.100

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*(3), 559–568. doi: 10.2307/2641247

Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A. (2012). Development of environmental thresholds for nitrogen and phosphorus in streams. *Journal of Environmental Quality, 41*(1), 7–20. doi: 10.2134/jeq2010.0273

Chambers, P. A., Vis, C., Brua, R. B., Guy, M., Culp, J. M., & Benoy, G. A. (2008). Eutrophication of agricultural streams: Defining nutrient concentrations to protect ecological condition. *Water Science and Technology, 58*(11), 2203–2210. doi: 10.2166/wst.2008.815

Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. (2013). Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge 4*(4), 1-10.

Cook, S. C., Housley, L., Back, J. A., & King, R. S. (2018). Freshwater eutrophication drives sharp reductions in temporal beta diversity. *Ecology, 99*(1), 47–56. doi: 10.1002/ecy.2069

Culp, J. M., Lowell, R. B., & Cash, K. J. (2000). Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. *Environmental Toxicology and Chemistry, 19*(4), 1167–1173. doi: 10.1002/etc.5620190450

Davies, J., & Bothwell, M. L. (2012). Responses of lotic periphyton to pulses of phosphorus: P-flux controlled growth rate. *Freshwater Biology, 57*(12), 2602–2612. doi: 10.1111/fwb.12032

DeBues, M. J., Eimers, M. C., Watmough, S. A., Mohamed, M. N., & Mueller, J. (2019). Stream nutrient and agricultural land-use trends from 1971 to 2010 in Lake Ontario tributaries. *Journal of Great Lakes Research, 45*(4), 752–761. doi: 10.1016/j.jglr.2019.05.002

Dodds, W. K. (2006). Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography, 51*, 671–680. doi: 10.4319/lo.2006.51.1_part_2.0671

Dodds, W. K., & Smith, V. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters, 6*(2), 155–164. doi: 10.5268/IW-6.2.909

d’Orgeville, M., Peltier, W. R., Erler, A. R., & Gula, J. (2014). Climate change impacts on Great Lakes Basin precipitation extremes. *Journal of Geophysical Research: Atmospheres, 119*(18), 10799–10812. doi: 10.1002/2014JD021855

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

Fordham, D. A. (2015). Mesocosms reveal ecological surprises from climate change. *PLOS Biology, 13*(12), 1–7. doi: 10.1371/journal.pbio.1002323

Francoeur, S. N. (2001). Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society, 20*(3), 358–368. doi: 10.2307/1468034

Fu, B., Merritt, W. S., Croke, B. F. W., Weber, T. R., & Jakeman, A. J. (2019). A review of catchment-scale water quality and erosion models and a synthesis of future prospects. *Environmental Modelling & Software, 114*, 75–97. doi: 10.1016/j.envsoft.2018.12.008

Giri, S., Qiu, Z., & Zhang, Z. (2018). Assessing the impacts of land use on downstream water quality using a hydrologically sensitive area concept. *Journal of Environmental Management, 213*, 309–319. doi: 10.1016/j.jenvman.2018.02.075

Gücker, B., & Pusch, M. T. (2006). Regulation of nutrient uptake in eutrophic lowland streams. *Limnology and Oceanography, 51*(3), 1443–1453. doi: 10.4319/lo.2006.51.3.1443

Haggard, B. E., Storm, D. E., & Stanley, E. H. (2001). Effect of a point source input on stream nutrient retention. *Journal of the American Water Resources Association, 37*(5), 1291–1299. doi: 10.1111/j.1752-1688.2001.tb03639.x

Herb, W. R., Janke, B., Mohseni, O., & Stefan, H. G. (2008). Thermal pollution of streams by runoff from paved surfaces. *Hydrological Processes, 22*(7), 987–999. doi: 10.1002/hyp.6986

Horner, R. R., & Welch, E. B. (1981). Stream periphyton development in relation to current velocity and nutrients. *Canadian Journal of Fisheries and Aquatic Sciences, 38*(4), 449–457. doi: 10.1139/f81-062

Howarth, R. W., Marino, R., Lane, J., & Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography, 33*(4), 669–687. doi: 10.4319/lo.1988.33.4part2.0669

Humphrey, K. P., & Stevenson, R. J. (1992). Responses of benthic algae to pulses in current and nutrients during simulations of subscouring spates. *Journal of the North American Benthological Society, 11*(1), 37–48. doi: 10.2307/1467880

Irvine, C. A., Backus, S., Cooke, S., Dove, A., & Gewurtz, S. B. (2019b). Application of continuous turbidity sensors to supplement estimates of total phosphorus concentrations in the Grand River, Ontario, Canada. *Journal of Great Lakes Research, 45*(4), 840–849. doi: 10.1016/j.jglr.2019.05.007

Irvine, C., Macrae, M., Morison, M., & Petrone, R. (2019a). Seasonal nutrient export dynamics in a mixed land use subwatershed of the Grand River, Ontario, Canada. *Journal of Great Lakes Research, 45*(6), 1171–1181. doi: 10.1016/j.jglr.2019.10.005

Julian, J. P., Doyle, M. W., & Stanley, E. H. (2008). Empirical modeling of light availability in rivers. *Journal of Geophysical Research, 113*(G3), G03022. doi: 10.1029/2007JG000601

Katz, S. B., Segura, C., & Warren, D. R. (2018). The influence of channel bed disturbance on benthic Chlorophyll a: A high resolution perspective. *Geomorphology, 305*, 141–153. doi: 10.1016/j.geomorph.2017.11.010

Kaushal, S. S., Gold, A. J., Bernal, S., & Tank, J. L. (2018). Diverse water quality responses to extreme climate events: an introduction. *Biogeochemistry, 141*(3), 273–279. doi: 10.1007/s10533-018-0527-x

Kaushal, S. S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., Newcomer, T. A., … Belt, K. T. (2014). Land use and climate variability amplify carbon, nutrient, and contaminant pulses: A review with management implications. *Journal of the American Water Resources Association, 50*(3), 585–614. doi: 10.1111/jawr.12204

King, K. W., Williams, M. R., Macrae, M. L., Fausey, N. R., Frankenberger, J., Smith, D. R., … Brown, L. C. (2015). Phosphorus transport in agricultural subsurface drainage: A review. *Journal of Environmental Quality, 44*(2), 467–485. doi: 10.2134/jeq2014.04.0163

Klausmeier, C. A., Litchman, E., Daufresne, T., & Levin, S. A. (2004). Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature, 429*(6988), 171–174. doi: 10.1038/nature02454

Lemke, A. M., Kirkham, K. G., Lindenbaum, T. T., Herbert, M. E., Tear, T. H., Perry, W. L., & Herkert, J. R. (2011). Evaluating agricultural best management practices in tile-drained subwatersheds of the Mackinaw River, Illinois. *Journal of Environmental Quality, 40*(4), 1215–1228. doi: 10.2134/jeq2010.0119

Lloyd, C. E. M., Freer, J. E., Johnes, P. J., & Collins, A. L. (2016). Using hysteresis analysis of high-resolution water quality monitoring data, including uncertainty, to infer controls on nutrient and sediment transfer in catchments. *Science of The Total Environment, 543,* 388–404. doi: 10.1016/j.scitotenv.2015.11.028

Macrae, M. L., English, M. C., Schiff, S. L., & Stone, M. (2007). Capturing temporal variability for estimates of annual hydrochemical export from a first-order agricultural catchment in southern Ontario, Canada. *Hydrological Processes, 21*(13), 1651–1663. doi: 10.1002/hyp.6361

Marcarelli, A. M., Baker, M. A., & Wurtsbaugh, W. A. (2008). Is in-stream N2 fixation an important N source for benthic communities and stream ecosystems? *Journal of the North American Benthological Society, 27*(1), 186–211. doi: 10.1899/07-027.1

Marti, E., Aumatell, J., Godé, L., Poch, M., & Sabater, F. (2004). Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. *Journal of Environmental Quality, 33*(1), 285–293. doi: 10.2134/jeq2004.2850

Mather, A. L., & Johnson, R. L. (2014). Quantitative characterization of stream turbidity-discharge behavior using event loop shape modeling and power law parameter decorrelation. *Water Resources Research, 50*(10), 7766–7779. doi: 10.1002/2014WR015417

Meals, D. W., Levine, S. N., Wang, D., Hoffmann, J. P., Cassell, E. A., Drake, J. C., … Brown, A. B. (1999). Retention of spike additions of soluble phosphorus in a northern eutrophic stream. *Journal of the North American Benthological Society, 18*(2), 185–198. doi: 10.2307/1468460

Menczelesz, N., Szivák, I., & Schmera, D. (2020). How do we construct and operate experimental streams? An overview of facilities, protocols, and studied questions. *Hydrobiologia, 847*(1), 1–10. doi: 10.1007/s10750-019-04093-0

Murdock, J. N., Shields, F. D., & Lizotte, R. E. (2013). Periphyton responses to nutrient and atrazine mixtures introduced through agricultural runoff. *Ecotoxicology, 22*(2), 215–230. doi: 10.1007/s10646-012-1018-9

Odum, E. P. (1984). The mesocosm. *BioScience, 34*(9), 558–562. doi: 10.2307/1309598

Ontario Ministry of the Environment. (2012). Water quality of 15 streams in agricultural watersheds of Southwestern Ontario 2004-2009. Ontario Ministry of the Environment Environmental Monitoring and Reporting Branch. Queens Printer for Ontario. PIBS 8613e.

Pellerin, B. A., Stauffer, B. A., Young, D. A., Sullivan, D. J., Bricker, S. B., Walbridge, M. R., … Shaw, D. M. (2016). Emerging tools for continuous nutrient monitoring networks: Sensors advancing science and water resources protection. *Journal of the American Water Resources Association*, *52*(4), 993–1008. doi: 10.1111/1752-1688.12386

Polyakov, V., Fares, A., & Ryder, M. H. (2005). Precision riparian buffers for the control of nonpoint source pollutant loading into surface water: A review. *Environmental Reviews*, *13*(3), 129–144. doi: 10.1139/a05-010

Qiu, Z., Kennen, J. G., Giri, S., Walter, T., Kang, Y., & Zhang, Z. (2019). Reassessing the relationship between landscape alteration and aquatic ecosystem degradation from a hydrologically sensitive area perspective. *Science of The Total Environment, 650*, 2850–2862. doi: 10.1016/j.scitotenv.2018.10.036

Rier, S. T., Kinek, K. C., Hay, S. E., & Francoeur, S. N. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. *Freshwater Science, 35*(2), 490–502. doi: 10.1086/685859

Roberts, B. J., Mulholland, P. J., & Hill, W. R. (2007). Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems*, *10*(4), 588–606. doi: 10.1007/s10021-007-9059-2

Robertson, D. M., Hubbard, L. E., Lorenz, D. L., & Sullivan, D. J. (2018). A surrogate regression approach for computing continuous loads for the tributary nutrient and sediment monitoring program on the Great Lakes. *Journal of Great Lakes Research, 44*(1), 26–42. doi: 10.1016/j.jglr.2017.10.003

Rode, M., Halbedel née Angelstein, S., Anis, M. R., Borchardt, D., & Weitere, M. (2016). Continuous in-stream assimilatory nitrate uptake from high-frequency sensor measurements. *Environmental Science & Technology, 50*(11), 5685–5694. doi: 10.1021/acs.est.6b00943

Savoy, P., Appling, A. P., Heffernan, J. B., Stets, E. G., Read, J. S., Harvey, J. W., & Bernhardt, E. S. (2019). Metabolic rhythms in flowing waters: An approach for classifying river productivity regimes. *Limnology and Oceanography, 64*(5), 1835–1851. doi: 10.1002/lno.11154

Schindler, D. W. (2012). The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society B: Biological Sciences, 279*(1746), 4322–4333. doi: 10.1098/rspb.2012.1032

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., … Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences, 105*(32), 11254–11258. doi: 10.1073/pnas.0805108105

Sharpley, A. N., Kleinman, P. J. A., Heathwaite, A. L., Gburek, W. J., Folmar, G. J., & Schmidt, J. P. (2008). Phosphorus Loss from an agricultural watershed as a function of storm size. *Journal of Environmental Quality, 37*(2), 362–368. doi: 10.2134/jeq2007.0366

Shi, P., Zhang, Y., Li, Z., Li, P., & Xu, G. (2017). Influence of land use and land cover patterns on seasonal water quality at multi-spatial scales. *Catena, 151*, 182–190. doi: 10.1016/j.catena.2016.12.017

Sinha, E., Michalak, A. M., & Balaji, V. (2017). Eutrophication will increase during the 21st century as a result of precipitation changes. *Science, 357*(6349), 405–408. doi: 10.1126/science.aan2409

Smith, R. A., Alexander, R. B., & Schwarz, G. E. (2003). Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology, 37*(14), 3039–3047. doi: 10.1021/es020663b

Solomon, K. R., Baker, D. B., Richards, R. P., Dixon, K. R., Klaine, S. J., La Point, T. W., … Williams, W. M. (1996). Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry, 15*(1), 31–76. doi: 10.1002/etc.5620150105

Stamm, C., Jarvie, H. P., & Scott, T. (2014). What's more important for managing phosphorus: Loads, concentrations or both? *Environmental Science & Technology, 48*(1), 23–24. doi: 10.1021/es405148c

Staponites, L. R., Barták, V., Bílý, M., & Simon, O. P. (2019). Performance of landscape composition metrics for predicting water quality in headwater catchments. *Scientific Reports, 9*(1), 14405. doi: 10.1038/s41598-019-50895-6

Stevenson, R. J. (1990). Benthic algal community dynamics in a stream during and after a spate. *Journal of the North American Benthological Society, 9*(3), 277–288. doi: 10.2307/1467591

Stutter, M. I., Graeber, D., Evans, C. D., Wade, A. J., & Withers, P. J. A. (2018). Balancing macronutrient stoichiometry to alleviate eutrophication. *Science of The Total Environment, 634*, 439–447. doi: 10.1016/j.scitotenv.2018.03.298

Sunohara, M. D., Gottschall, N., Craiovan, E., Wilkes, G., Topp, E., Frey, S. K., & Lapen, D. R. (2016). Controlling tile drainage during the growing season in Eastern Canada to reduce nitrogen, phosphorus, and bacteria loading to surface water. *Agricultural Water Management, 178*(3), 159–170. doi: 10.1016/j.agwat.2016.08.030

Taylor, J. M., Back, J. A., Brooks, B. W., & King, R. S. (2018). Spatial, temporal and experimental: Three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems. *Journal of Applied Ecology, 55*(5), 2114–2123. doi: 10.1111/1365-2664.13150

Townsend, S. A., Garcia, E. A., & Douglas, M. M. (2012). The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river. *Freshwater Science, 31*(4), 1233–1243. doi: 10.1899/11-163.1

Van Esbroeck, C. J., Macrae, M. L., Brunke, R. I., & McKague, K. (2016). Annual and seasonal phosphorus export in surface runoff and tile drainage from agricultural fields with cold temperate climates. *Journal of Great Lakes Research, 42*(6), 1271–1280. doi: 10.1016/j.jglr.2015.12.014

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & Morgan, R. P. (2005). The urban stream syndrome: Current knowledge and the search for a cure. *Journal of the North American Benthological Society, 24*(3), 706–723. doi: 10.1899/04-028.1

Weigelhofer, G., Ramião, J. P., Puritscher, A., & Hein, T. (2018). How do chronic nutrient loading and the duration of nutrient pulses affect nutrient uptake in headwater streams? *Biogeochemistry, 141*(2), 249–263. doi: 10.1007/s10533-018-0518-y

Withers, P., Neal, C., Jarvie, H., & Doody, D. (2014). Agriculture and eutrophication: Where do we go from here? *Sustainability, 6*(9), 5853–5875. doi: 10.3390/su6095853

Wolter, P. T., Johnston, C. A., & Niemi, G. (2006). Land use land cover change in the U.S. Great Lakes Basin 1992 to 2001. *Journal of Great Lakes Research, 32*(3), 607–628. doi: 10.3394/0380-1330(2006)32[607:LULCCI]2.0.CO;2

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *Wiley Interdisciplinary Reviews: Water, 6*(5), 1–27. doi: 10.1002/wat2.1373

Zedler, J. B. (2003). Wetlands at your service: Reducing impacts of agriculture at the watershed scale. *Frontiers in Ecology and the Environment, 1*(2), 65. doi: 10.2307/3868032

Appendices

Appendix A: Supplementary material for Chapter 2

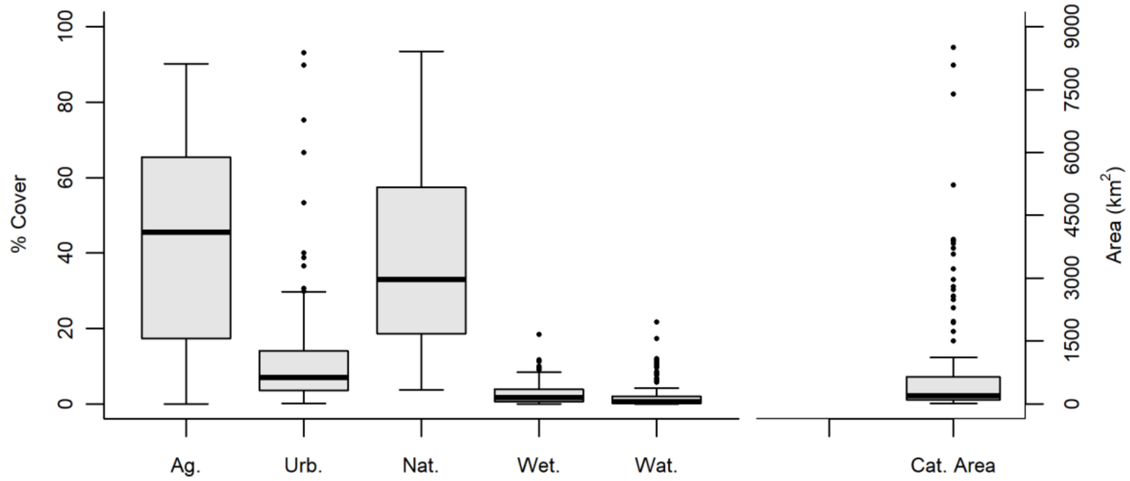

Figure A-1. Quantile plots summarizing the land cover composition and catchment area of the 127 study streams selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network.

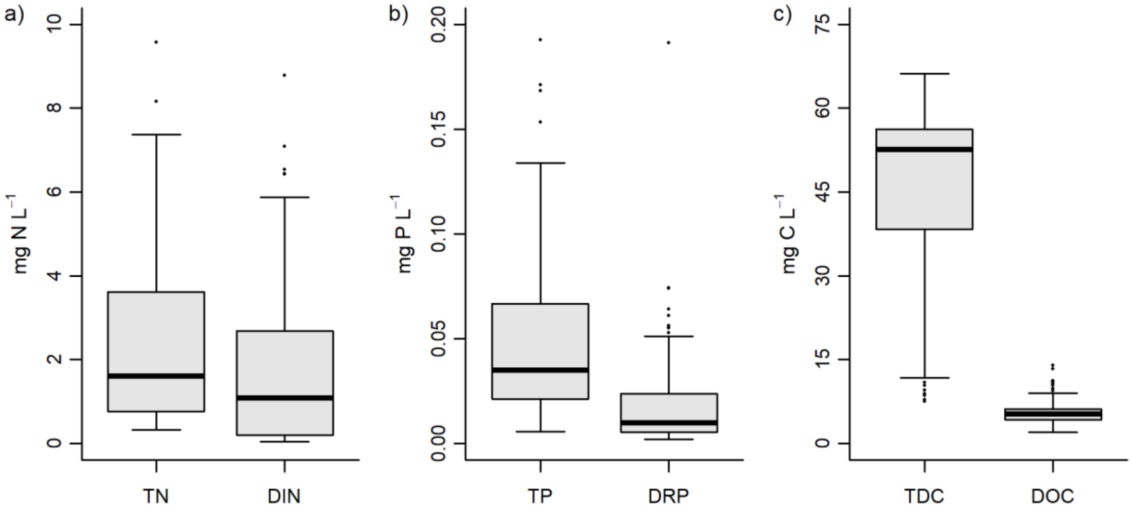

Figure A-2. Quantile plots summarizing 10-year average (a) nitrogen (TN: total nitrogen; DIN: dissolved inorganic nitrogen), (b) phosphorus (TP: total phosphorus; DRP: dissolved reactive phosphorus), and (c) carbon (TDC: total dissolved carbon; DOC: dissolved organic carbon) concentrations of the 127 selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network.

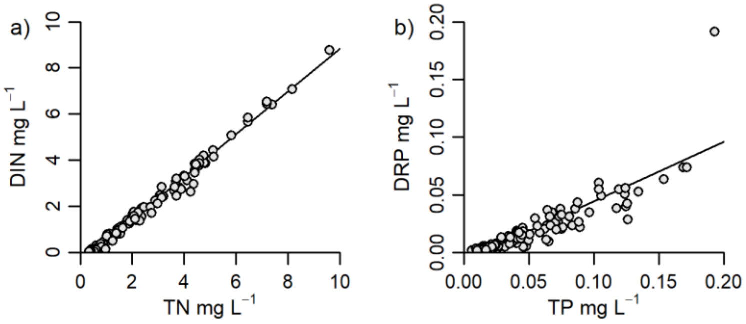

Figure A-3. Scatterplots summarizing the associations between 10-year average (a) total nitrogen (TN) and dissolved inorganic nitrogen (DIN), (b) total phosphorus (TP) and dissolved reactive phosphorus (DRP), and (c) total dissolved carbon (TDC) and dissolved organic carbon (DOC) concentrations of the 127 selected study streams monitored by Ontario's Provincial Water Quality Monitoring Network. Solid trend lines indicate linear best fit.

Table A-1. Summary of the multiple Dirichlet regression model between Redfield transformed total nitrogen (TN_R), total phosphorus (TP_R), and total dissolved carbon (TDC_R) compositions and spatially weighted land cover variables.

Land Cover Variable	Coefficient (± Standard Error)	Z-value	p-value
TN_R			
Natural	-2.028 ± 2.363	-0.858	0.391
Wetland	0.734 ± 2.616	0.281	0.779
Agriculture	-1.492 ± 2.671	-0.559	0.576
Urban	-2.323 ± 2.516	-0.923	0.356
TP_R			
Natural	-3.990 ± 2.065	-1.932	0.053
Wetland	2.793 ± 2.714	1.029	0.303
Agriculture	-2.655 ± 2.364	-1.123	0.261
Urban	-2.758 ± 2.315	-1.191	0.234
TDC_R			
Natural	-1.313 ± 2.141	-0.613	0.540
Wetland	3.490 ± 2.908	1.200	0.230
Agriculture	-2.261 ± 2.523	-0.896	0.370
Urban	-2.571 ± 2.356	-1.091	0.275

Appendix B: Supplementary material for Chapter 5

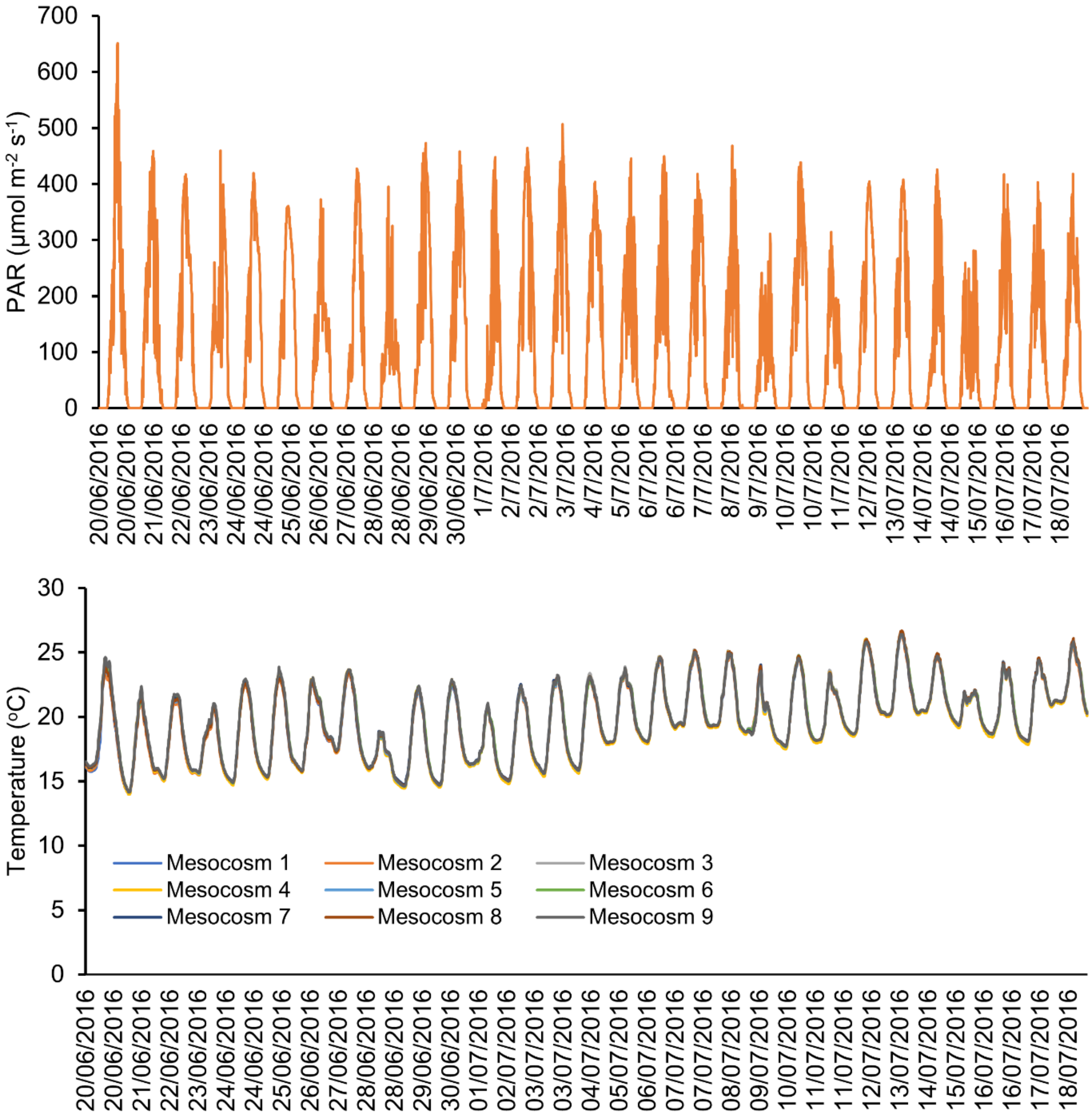

Figure B-1. Temporal patterns in photosynthetically active radiation (PAR) and temperature in artificial stream mesocosms over the 29-day experiment.

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Pearce, N.J.T., & Yates, A.G. (2020). Spatial and temporal patterns in macronutrient concentrations and stoichiometry of tributaries draining the lower Great Lakes-St. Lawrence basin. Journal of Great Lakes Research 46(4): 1-12.

Pearce, N.J.T., Thomas, K.E., Venkiteswaran J.J., Chambers, P.A., & Yates, A.G. (2020). Metabolic regimes of three mid-order streams in southern Ontario, Canada exposed to contrasting sources of nutrients. Hydrobiologia, 847: 1925-1942.

Webb, J.R., Pearce, N.J.T., Painter, K.J., & Yates, A.G. (2019). Hierarchical variation in cellulose decomposition in least disturbed reference streams: a multi-season study using the cotton strip assay. Landscape Ecology, 34(10): 2353-2369.

Pearce, N.J.T., & Yates, A.G. (2017). Intra-annual variation of the association between agricultural best management practices and stream nutrient concentrations. Science of the Total Environment, 586: 1124-1134.

Pearce, N.J.T., & Yates, A.G. (2015). Agricultural best management practice abundance and location does not influence stream ecosystem function or water quality in the summer season. Water (BMP Development, Implementation, and Performance), 7(12); 6861-6876.

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Pearce, N.J.T., & Yates, A.G. (2019). Algae-mediated phosphorus uptake and retention in stream ecosystems. Poster presentation at Ontario Phosphorus Research Workshop, Upper Thames River Conservation Authority, London, Ontario Canada.

Pearce, N.J.T., Thomas, K.E., Lavoie, I., Chambers, P.A., & Yates, A.G. (2019). Composition of algal assemblages differs with the temporal pattern of phosphorus loading. Oral presentation at the Society for Freshwater Science Annual Meeting, Salt Lake City, Utah USA.

Lucas, C.P., Pearce, N.J.T., & Yates, A.G. (2019). Stream biofilm function impacted by nutrients, but not velocity, associated with simulated storm events. Poster presentation at the Society for Freshwater Science Annual Meeting, Salt Lake City, Utah USA.

Pearce, N.J.T., Thomas, K.E., Lavoie, I., Chambers, P.A., Yates, A.G. (2019). Additive effects of sewage effluent on instream biological communities of agricultural rivers. Poster presentation at the Canadian Conference for Fisheries Research and Society of Canadian Limnologists Annual Meeting, London, Ontario Canada.

Kroeze, S.L., Boreux, M., Pearce, N.J.T., Robinson, C., Roy, J., & Yates, A.G. (2019). The relationship between decomposition rate and groundwater in streams. Poster presentation at the Canadian Conference for Fisheries Research and Society of Canadian Limnologists Annual Meeting, London, Ontario Canada.

Pearce, N.J.T., Venkiteswaran, J.J., Chambers, P.A., & Yates, A.G. (2018). Temporal patterns of stream metabolism in three streams exposed to different sources of anthropogenic nutrients. Oral presentation at the Society for Freshwater Science Annual Meeting, Detroit, Michigan USA.

Webb, J.R., Painter, K.J., Pearce, N.J.T., Bailey, R.C., Tiegs, S.D., & Yates, A.G. (2018). Hierarchical variation in cellulose decomposition across southern Ontario reference streams. Poster presentation at the Society for Freshwater Science Annual Meeting, Detroit, Michigan USA.

Pearce, N.J.T., & Yates, A.G.Y. (2016). Seasonal variation of agricultural best management practice effects on stream water quality. Oral presentation at the International Association for Great Lakes Research Annual Conference, Guelph, Ontario Canada.