THE EFFECT OF CATCHMENT URBANIZATION ON NUTRIENT UPTAKE AND BIOFILM ENZYME ACTIVITY IN LAKE SUPERIOR (USA) TRIBUTARY STREAMS

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Dedication

This research is dedicated to my wonderful husband Aaron who has been extremely supportive and patient throughout this process, and to my amazing son Niklas who inspires me every day.

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Using an integrated approach to studying nutrient transport in stream ecosystems

The goal of the Integrated Biosciences program at the University of Minnesota is the training of researchers and practitioners emphasizing the integration of the various subdivisions of biology. Students are encouraged to conduct research involving interdisciplinary approaches to solve complex scientific problems. One important problem facing our world today is anthropogenic nutrient loading to the environment. Human activity has resulted in an estimated doubling of the amount of globally available nitrogen (Vitousek, Aber, Howarth, *et al.*, 1997). Because over 50% of the world's population lives in urban areas, the environmental impacts of increased nutrient loading are of particular importance in and around cities. As the percentage of the population living in urban centers continues to increase so too does the impact on local ecosystems. The effect of catchment urbanization on stream health has been the subject of a much research in recent years (Paul & Meyer, 2001). The term urban stream syndrome has been used to describe the attributes of flashier hydrographs, altered channel morphology, reduced biotic richness, increased sedimentation, temperatures, contaminants and nutrients associated with increased catchment urbanization (Walsh, Roy, Feminella, *et al*. 2005).

Fossil fuel burning, crop fertilization, waste disposal, and increased urbanization have saturated many terrestrial environments with excess N. Excess N is transported to streams, lakes, rivers, and oceans via runoff and groundwater (Peterson, Wollheim, Mulholland, *et al.*, 2001). Streams take up and, for a time, retain this N before it is transported downstream to recipient lakes, rivers, and oceans. Mulholland *et al.* (2008) reported that when nitrate loading to streams increases, the rates of biotic uptake and denitrification also increase, while the efficiency of the biotic uptake and denitrification significantly decreases. Excess N will flow directly downstream to receiving ecosystems and cause them to eutrophy, thereby threatening native habitats, drinking water systems, and recreational areas (Mulholland, Helton, Poole, *et al.*, 2008). The downstream effects of nitrogen and other nutrients create the need for research to better understand nutrient transport in stream ecosystems.

One way to look at nutrient transport in streams is through nutrient spiraling. Nutrient spiraling is the average downstream distance traveled while a nutrient atom completes one cycle from its dissolved inorganic form within the water column (uptake length), through a particulate phase, and a consumer phase (turnover length) before returning to the water column (Webster, 1975; Newbold *et al*,.1981). By assessing how far nutrients travel downstream before being taken up by the biota and sediment, researchers can determine how effectively streams are processing these nutrients. Uptake length, the distance traveled by a molecule before it is taken up by the biota or sediment and removed from the water column, is an aggregate measure of uptake that combines a variety of hydrologic and non-hydrologic processes that affect the fate of nutrients. Nutrient spiraling studies have greatly increased the general pool of knowledge related to nitrogen and phosphorus transport in stream ecosystems (Webster, 1975; Newbold *et al*., 1981; Fisher, Sponseller & Heffernan, 2004; Ensign & Doyle, 2006). However, measures of nutrient spiraling do not describe the biological drivers behind the regulation of uptake. Examining the biological components involved in the regulation of nutrient uptake along with spiraling indices provided a more integrated understanding of whole stream ecosystem nutrient processing. Measuring the rate of extracellular enzyme activity (EEA) in water, sediment, and biofilm is one mechanism of characterizing the biological activity of ecosystems. Previous studies have successfully correlated EEA in the biofilm to nutrient limitation and availability in streams (Hill, Elonen, Jicha, *et al.*, 2010a; Hill, McCormick, Harvey, *et al.*, 2010b).

The need to broaden our understanding of ecosystem nutrient transport is not new. In a 2000 review article in *Limnology*, Robert Wetzel outlined five key areas of current and future limnological research including, biogeochemical coupling to the environment, and a collective understanding of metabolism, nutrient cycling, bioavailability of nutrients, and growth regulators. What is new is an understanding of how the stoichiometric demands of organisms within stream ecosystems influence the overall fluctuation of nutrients. I was able to address this in my study by measuring both nutrient uptake and microbial EEA in the biofilm.

Ecological stoichiometry describes the balance of multiple chemical substances in ecological interactions and processes (Sterner & Elser, 2002). As nutrients travel through trophic levels, their relative concentrations change to meet the nutrient requirements of the consumers. If a consumer ingests food with a lower N:P ratio than itself, it is ingesting excess P. The consumer will in turn excrete that excess P in order to maintain homeostasis. Likewise, if a consumer ingests food with a lower N:P ratio than itself, it will have gained excess N and will need to excrete it to maintain homeostasis. In aquatic systems, there is a close interdependence of organic matter breakdown by microbes and the balance of multiple elements in the substrate relative to microbial demands (Sterner and Elser 2002). I examined this relationship by calculating the C:N, N:P, and C:P ratios of EEA in the biofilm (Fig. 3). From these data I was able to determine nutrient limitation and saturation within the systems. I compared the stoichiometric relationships of the biofilm EEA with the stoichiometry of nutrient uptake in the water column and found similar relationships.

Frost *et al*. (2002) specifically targeted the need for integration between nutrient cycling and trophic interactions. They set the stage for research that examines the importance of stoichiometric mechanisms in consumer-driven nutrient cycling in streams as they relate to the total biomass and N:P ratios of both the consumers and the food they ingest. Fisher *et al*. (2004) followed with a call for research related to the imbalance of nutrients between consumers and their resources with a specific focus on how this affects overall nutrient flow. In 2005, *Freshwater Biology* devoted a special issue to benthic stoichiometry following a special session on that topic at the annual meeting of the North American Benthological Society. This special issue included a study by Evans-White *et al.* (2005) on macroinvertebrate populations to provide information on elemental composition that could be used to assess the homeostasis assumptions made in ecological stoichiometry models. In the closing paper of the special issue Cross *et al*. (2005) provides a synthesis of research dealing with ecological stoichiometry in benthic systems. In a section entitled, *Ecological stoichiometry and the role of benthic communities in biogeochemistry*, Cross states, “Despite the many advances that have been accomplished

by studies of nutrient spiraling, lack of an explicit treatment of multiple elements and their relations constitutes a major shortcoming."

These "shortcomings" were addressed by Allen and Gillooly (2009) who integrated the ecological stoichiometry theory presented by Sterner and Elser (2002) with the metabolic theory of ecology (Brown, Gillooly, Allen, *et al*. 2004). They argue that by combining the two models of nutrient availability and energy we may be able to better address the factors which lead to environmental change that affects ecological communities. I have attempted to take that sort of approach in the thesis research presented here. I integrated measures of stream habitat, nutrient chemistries, spiraling metrics, extracellular enzyme activity and cellular respiration in order to understand the effects of human disturbance at the catchment scale on nutrient retention in streams. Additionally, I used the stoichiometric ratios of EEA and nutrient uptake to determine the resulting nutrient demands. By taking this integrated approach to research I was able to achieve a better understanding of the health of entire stream ecosystems.

Lake Superior is a source of drinking water, recreation and fishing for large portions of Minnesota, Wisconsin, Michigan and Canada. Its health is of great importance to the area and is the object of much scientific research. I chose nine small streams near Duluth, MN for my research because of their proximity to Lake Superior. Each either feeds directly into Lake Superior or into the St. Louis River before entering the lake. Their ability to take up nutrients and prevent excess loading to Lake Superior is essential. The streams vary in the amount of urbanization within their catchments as estimated by the percentage of impervious surface. They run through residential neighborhoods and commercial areas. Storm sewers drain into six of the nine streams and all are used for varying recreational purposes. None of the streams look particularly "disturbed" and it is likely that area residents would not characterize them as impaired or unhealthy.

Stream organisms involved in nutrient spiraling and nutrient processing have evolved in a landscape where streams are coupled to their catchments in a natural state. Urbanization replaces natural catchments with artificial catchments of impervious surfaces which alter stream chemistry, hydrology, and geomorphology (Paul & Meyer,

2001; Meyer, Paul & Taulbee 2005; Walsh *et al*., 2005; Roy, Dybas, Fritz *et al*. 2009). How organisms respond to these changes is important for understanding overall stream health and developing management strategies. I focused my thesis research on two aspects of nutrient transport in streams. The first goal of my study was to assess the effect that urbanization has on a stream's ability to effectively process nitrogen and phosphorus through the use of nutrient spiraling and microbial enzyme activity assays. By measuring habitat characteristics, stream chemistries, nutrient spiraling metrics (uptake length, uptake velocity, uptake rate), extracellular enzyme activity and respiration rates I was able to see the effect of anthropogenic disturbances at the catchment scale on nutrient retention in these streams.

The second goal of my study was to examine the relationship between nutrient uptake and microbial extracellular enzyme activity (EEA) and to use a stoichiometric approach to estimate nutrient limitation in streams. Nutrient uptake is strongly affected by nutrient availability, so examining relative nutrient limitation is important to an overall understanding of nutrient processing in streams. I measured biological activity as the rates of extracellular enzyme activity (EEA) and cellular respiration (measured as dehydrogenase activity) in stream biofilm. Microorganisms release enzymes into the biofilm for the purpose of acquiring C, N, P and other nutrients (Sinsabaugh & Foreman, 2001; Hill, *et al.*, 2010b). Production of enzymes is energetically expensive and so will theoretically only be done in response to a nutrient limitation. By measuring the EEA of groups of enzymes responsible for hydrolyzing specific nutrients; glycosidases (C acquiring), amino-peptidases (N acquiring), and acid phosphatase (P acquiring), I was able to determine the relative limitation and demands for these nutrients within a system.

Summary

1. I used landscape, habitat, and chemistry variables, along with nutrient spiraling metrics and biofilm extracellular enzyme activity (EEA), to assess the response of streams to the level of urbanization within their catchments. For this study nine streams of similar catchment area and geomorphology were chosen along the north shore of Lake Superior near Duluth, MN. The streams were selected based on varying levels of urbanization, which I identified as the percentage of impervious surface within the catchment. A gradient of urbanization was created from the nine streams and was the basis for comparison of stream chemistry, nutrient uptake and biofilm enzymatic activity.

2. I predicted that changes in the extent of urbanization would be reflected in the N and P uptake of the study streams. I hypothesized that as the amount of urbanization in the streams' catchments increased, I would observe an elongated nutrient uptake length (S_w), a decreased uptake velocity (V_f) and an increased uptake rate (U). I was unable to support this hypothesis and found no significant correlation between uptake metrics for NH_4^+, total nitrogen (TN), total phosphorus (TP) and the urban gradient.

3. Canonical correlation analysis identified a statistically significant correlation between nutrient uptake and biofilm extracellular enzyme activity. There was a positive correlation between NH_4^+- V_f and TP- V_f, and the canonical axis of extracellular enzyme activity (total glycosidase, total peptidase, and acid phosphatase). NH_4^+- V_f and TP- V_f were also positively correlated with respiration in the biofilm as measured by dehydrogenase (DHA) activity.

4. There were significant correlations between biofilm enzyme activity and the gradient of urbanization. Total glycosidase and acid phosphatase activities decreased with the percentage of the catchment covered by impervious surfaces, storm sewer length, and water Cl^- concentration, and increased with the percentage of the catchment covered by forest. Total peptidase decreased with % impervious surface and water Cl concentration,

and increased with % forest. DHA decreased with % impervious surface, storm sewer length, % of the stream channel shaded by the riparian canopy, and water Cl, and increased with % forest, % of the catchment covered by wetland, stream width and stream depth. These findings suggest that there is a level of sensitivity to landscape disturbances measured in the EEA of stream biofilms that may not be detected with conventional measures of nutrient uptake.

5. Linear regression analyses of uptake velocity (V_f) and uptake rate (U) versus ambient NH_4^+ and TP concentrations, identified a general phosphorus limitation in the study streams. These data were further supported by linear regression analysis of biofilm total peptidase activity versus total phosphatase activity. A higher activity of phosphorus hydrolyzing phosphatases relative to nitrogen hydrolyzing peptidases indicated that the microbial community in the biofilm was producing enzymes for P acquisition in response to a P limitation. There was no statistically significant evidence of NH_4^+ saturation or limitation.

6. This study is the first to show a statistically significant correlation between nutrient uptake in streams and biofilm EEA. The significant relationship identified between biofilm EEA and the level of urbanization within stream catchments further supports the utility of EEA assays as a useful indicator of landscape disturbances in streams. Both nutrient uptake metrics and EEA stoichiometric ratios provided complimentary data identifying the study streams as P limited.

Introduction

According to the United Nations Population Division (2009), over 50% of the world's population currently lives in urban areas. As the percentage of the population living in urban centers continues to increase so too does the impact on local ecosystems. The effect of catchment urbanization on stream health has been the subject of a much research in recent years (Paul & Meyer, 2001). The term urban stream syndrome has been used to describe the attributes of flashier hydrographs, altered channel morphology, reduced biotic richness, increased sedimentation, temperatures, contaminants and nutrients associated with increased catchment urbanization (Walsh, Roy, Feminella, *et al*. 2005). Roy *et al.* (2009) reported significant effects of urbanization on the presence and hydrologic permanence of headwater streams. Such changes in hydrology can impact stream communities and lead to altered ecosystem function. Meyer *et al*. (2005) reported altered ecosystem function related to the extent of urbanization in the catchment areas of six Chattahoochee River tributary streams. They observed increased NH_4^+ and soluble reactive phosphorus (SRP) concentrations along with decreased uptake velocities related to urbanization. Groffman *et al*. (2004) measured N yields in urban and suburban watersheds to be 10 times that of a completely forested watershed.

Increasing urbanization accompanied by fossil fuel burning, fertilization, and waste disposal, has saturated many terrestrial environments with excess N (Vitousek, Aber, Howarth, *et al.*, 1997). This excess N, often in the form of nitrate, is transported to streams, lakes, rivers, and oceans via runoff and groundwater (Peterson, Wollheim, Mulholland, *et al.*, 2001). Streams take up this N before it is transported downstream to recipient lakes, rivers, and oceans. When nitrate loading to streams increases, the rates of biotic uptake and denitrification also increase, while the efficiency of the biotic uptake and denitrification significantly decrease (Mulholland, Helton, Poole, *et al.*, 2008). Excess N flowing directly downstream to receiving ecosystems causes eutrophication, and threatens native habitats, drinking water systems, and recreational areas

The harmful effects of nitrogen loading necessitate research to better understand nutrient transport in stream ecosystems. One way to study nutrient transport in stream ecosystems is through the process of nutrient spiraling. The term "nutrient spiraling" was

coined by Webster (1975) to describe the coupling of nutrient cycling and downstream transport. The concept of nutrient spiraling was further defined and quantified by Newbold (1981) who presented a mathematical model for calculating spiraling length. They defined spiraling length (S) as the average downstream distance required for a nutrient atom to complete one cycle from its dissolved inorganic form within the water column (uptake length, S_w), through a particulate phase, and a consumer phase (turnover length, S_b) before returning to the water column. The distance that nutrients are able to travel downstream before being taken up by the biota and sediment can determine how effectively streams are processing these nutrients. Uptake length describes the distance traveled by a molecule before it is taken up by the biota or sediment and removed from the water column. It is an aggregate measure of uptake that synthesizes a variety of hydrologic and non-hydrologic processes that affect the fate of nutrients; it does not, however, distinguish between main channel and storage zone processes (Runkel, 2007).

Nutrient spiraling concepts and methods were further refined by the Stream Solute Workshop (1990), whose goals were to 1) suggest a conceptual model for stream solute studies that integrates physical, chemical, and biological processes, and 2) identify advantages and limitations of various methods for studying solute transport and exchanges. The solute dynamics workshop produced a conceptual model of solute processes in streams that is useful for visualizing the pathways of nutrient spiraling. The limitations of using uptake length for comparisons between streams were also addressed. Uptake length does not distinguish between main channel and storage zone processes, or hydrologic and non-hydrologic processes, so comparing across streams that differ in these areas may be problematic. In order to isolate the effects of biogeochemical processes and provide measures of nutrient processing that may be compared across streams, equations for uptake velocity and uptake rate were developed (Stream Solute Workshop, 1990; Runkel, 1998; Davis & Minshall, 1999; Hall, Bernhardt & Likens, 2002). Nutrient spiraling studies have greatly increased the general pool of knowledge related to nitrogen and phosphorus transport in stream ecosystems (Webster, 1975; Newbold, 1981; Fisher, Sponseller & Heffernan, 2004; Ensign & Doyle, 2006)

In addition to nutrient spiraling metrics, advances in mathematical models now allow for a better understanding of the hydrologic parameters affecting solute transport in streams. The one dimensional transport with inflow and storage model (OTIS; Runkel, 1998) provides the framework to use the data from nutrient addition experiments to calculate transient storage, advection, and dispersion in streams. Transient storage occurs when solutes are held up in pockets of slow moving water or in the coarse gravel of stream beds and banks. This is important for understanding nutrient transport because the travel time for solutes carried through these areas will be significantly longer than that of solutes traveling in the main channel. Advection and dispersion are the primary transport mechanisms in the main channel. Combining measurements of uptake length (S_w), uptake velocity (V_f), and uptake rate (U), along with estimates of transient storage and dispersion provides a better overall assessment of nutrient processing within streams than could be achieved by simply looking at uptake length.

Measures of nutrient spiraling, transient storage and other physical variables, however, are affected both by the physical process of hydrology as well as the biological drivers behind the regulation of uptake. Understanding the biological components involved in the regulation of nutrient uptake along with physical variables may provide a more comprehensive understanding of nutrient and spiraling in stream ecosystems.

Measuring the rate of extracellular enzyme activity (EEA) in water, sediment, and biofilm is one mechanism of characterizing the biological activity of ecosystems. Previous studies have successfully correlated EEA in the biofilm to nutrient limitation and availability in streams (Hill, Elonen, Jicha, *et al.*, 2010a; Hill, McCormick, Harvey, *et al.*, 2010b). Microorganisms release enzymes into the biofilm for the purpose of acquiring C, N, P and other nutrients (Sinsabaugh & Foreman, 2001; Hill, *et al.*, 2010b). Production of enzymes is energetically expensive and so will theoretically only be done in response to a nutrient limitation. By measuring the EEA of groups of enzymes responsible for hydrolyzing specific nutrients; glycosidases (C acquiring), amino-peptidases (N acquiring), and acid phosphatase (P acquiring), conclusions can be drawn about the relative limitation and demands for these nutrients within a system. Respiration in the biofilm can also be measured through an enzymatic assay for dehydrogenase

activity. Dehydrogenase is a hydrolyzing enzyme and an essential component in both mitochondrial and microbial electron transport, and as such has been used to assess the response of microbial communities to landscape and chemical disturbances (Trevors, Mayfield & Inniss, 1982; Hill, Herlihy, & Kaufmann 1998)

Stream organisms involved in nutrient spiraling and nutrient processing have evolved in a landscape where streams are coupled to their catchments in a natural state. Urbanization replaces natural catchments with artificial catchments of impervious surfaces which alter stream chemistry, hydrology, and geomorphology (Paul & Meyer, 2001; Meyer *et al*., 2005; Walsh *et al*., 2005; Roy *et al*., 2009). How organisms respond to these changes is important for understanding overall stream health and developing management strategies.

The goals of this study were 1) to assess the effect that urbanization has on a stream's ability to effectively process nitrogen and phosphorus through the measurement of the uptake parameters and biofilm enzymatic activities related to these nutrients, and 2) to examine the relationship between nutrient uptake and biofilm EEA. I measured a variety of landscape, habitat and chemistry variables along with uptake metrics (S_w, V_f, U) for NH_4^+, TN, and TP, and a suite of microbial enzyme activities. The study streams represented a gradient of urbanization that was the basis for comparison of stream chemistry, nutrient uptake and biofilm enzyme activity. I expected to see an increase in nitrogen and phosphorus concentration with increasing levels of urbanization, and hypothesized that as the amount of urbanization in a catchment increased I would observe an elongated nutrient uptake length (S_w), a decreased uptake velocity (V_f) and an increased uptake rate (U).

Previous research using biofilm enzyme activity as an indicator of nutrient limitation in streams hypothesized that if organic matter processing in the biofilm occurs in response to nutrient availability, there should be a direct linkage between enzyme activity and measures of nutrient uptake (Sinsabaugh, Hill & Follstad Shah, 2009). I measured a suite of biofilm enzyme activities along with measures of nutrient uptake to test this hypothesis. I expected to find changes in biofilm enzyme activity that correlated

with the changes in nutrient uptake, and consequently a correlation between biofilm enzyme activity and the gradient of urbanization.

Methods

Study Sites

This study was conducted on nine streams located along the North Shore of Lake Superior, near Duluth, MN. I chose first and second order streams draining catchments of similar area (16-47 km^2) and geomorphology in order to compare data across study sites. The French and Talmadge Rivers, and Amity, Chester, and Tischer Creeks flow directly into Lake Superior, while Kingsbury, Miller, Keene and Mission Creeks flow into the St. Louis River before reaching Lake Superior (Fig. 1). Streams were chosen for this study based on a gradient of urbanization. The percentage of impervious surface within each stream's catchment was used as an approximation of urban stress.

Catchment areas and land cover percentages were calculated using the ArcMap 9.3 (Environmental Systems Research Institute, Redlands, CA, USA) and Arc Hydro tools (Maidment, 2002). This suite of tools allowed catchment areas to be calculated above the sample sites, along with flow direction and flow length for each pixel in the catchment. Land use measures were summarized as the proportion of the catchment upstream of each sample point. Impervious surface cover (ISC) area was estimated using the 2001 National Land Cover Dataset (NLCD; Homer, 2004) percent impervious layer in which each 30 X 30 m pixel has a calculated percent ISC. The percent ISC was calculated by averaging all of the pixel percent ISC estimations in the catchment. The 2001 NLCD was also used to estimate the proportion of wetland and forested area in each catchment. Wetland and forest categories were aggregated from woody and emergent herbaceous wetland and deciduous, mixed and evergreen forest.

A GIS storm sewer data set was acquired from the City of Duluth, MN. Lines representing the City of Duluth's storm sewers were intersected with the study catchment boundaries and the total length of storm sewers within each catchment was summed to give a value of total storm sewer length (m) for each stream. Storm sewer length was then

used as an approximate measure of the potential for direct nutrient loading to streams from urban sources.

Habitat assessment

Cross-sectional stations were set up at 10 meter intervals along a 100m reach of each stream for a total of 11 stations per stream. Habitat metrics were measured according to US Environmental Protection Agency's Environment Monitoring and Assessment Program protocol (Kauffman, 1999). Reach slope, wood count, and median particle size were all measured once for each stream at the beginning of the sampling season, while % shading, width, depth, and velocity were measured at each sampling event throughout the season. The slope of each study reach was measured using an automatic self leveling rotary laser and a staging rod. Total wood count was measured as the sum of all large pieces of woody debris that were at least partially within the channel of each study reach. Sediment type was classified by the pooled percentages of; boulders, cobble, course gravel, fine gravel, sand, silt, and clay measured at the 11 transects of each stream. The pooled sediment type percentages were then used to calculate a median particle size for each stream (Kauffman, 1999). Vegetative canopy cover was measured at each of the 11 cross-sectional stations using a convex spherical densiometer, (Model A; Robert E Lemmon, Forest Densiometers, Bartlesville, OK, USA). Four densiometer measurements were taken (upstream, downstream, and toward each bank) from the center of the stream at each station along the stream reach. The 44 densiometer measurements collected were then used to calculate the percent of the stream channel that was shaded by riparian canopy for each stream.

Wetted width, stream depth and stream velocity measurements were also taken at each of the 11 cross-sectional stations. Depth and velocity were measured at three equally spaced points across the channel. Velocity was measured using a current probe (Flo-Mate Model 2000, Marsh-McBirney, Corporation, Frederick, MD, USA) placed at 0.6 of the total depth measured from the surface. The width, depth and velocity measurements from all cross-sections were averaged for each sampling event. Estimated discharge (Q) was calculated from the cross-sectional area (A) and averaged velocity (V) (Kauffman, 1999).

Nutrient Spiraling

I measured NH_4^+, total N (TN), and total P (TP) uptake using short-term reactive solute additions with a conservative tracer (NaCl). Background water samples were collected from each site prior to nutrient addition. A solution containing $(NH_4)_3PO_4$ and NaCl was added at a constant rate from a carboy at the first cross section point of each stream reach using a fluid metering pump (Masterflex, Cole Parmer, Vernon Hills, IL, USA). The nutrient release rate (Q_I) was determined based on the estimated discharge (Q) measured during the habitat assessment, the target nutrient concentration (C_S), and the concentration of the of the solute release solution (C_I). The target nutrient concentration for each stream was 5-10 fold higher than the ambient concentration measured during a previous sampling event. NaCl concentrations, as specific conductance, were measured continuously at the downstream end of each reach using a conductivity meter (Model HI 9828, HANNA Instruments, Woonsocket, RI, USA). When NaCl concentrations reached plateau for at least 30 minutes, indicating uniform dispersal, I took duplicate water samples at each cross-sectional site. Samples were stored at 4°C until analyzed for NH_4^+, TN, and TP using a flow-injection analyzer (Lachat Instruments, Milwaukee, WI, USA). Anion analysis for Cl was done by ion chromatography (Model AI-450, Dionex, Sunnyvale, CA, USA). The measured Cl concentrations were then used along with the concentration of the release solution, injection rate, and background concentration, to yield a more precise measure of discharge for each stream. This Cl calculated discharge was used for all further analyses unless otherwise stated.

Nutrient uptake length (S_w) was calculated as,

$$S_w = -\frac{1}{k} \qquad \text{eq. 1}$$

where k is the downstream loss rate constant (m^{-1}), which is calculated as the slope of the regression of the ln-transformed nutrient concentration with stream distance. I used the conservative tracer (Cl) concentrations to correct nutrient concentrations for dilution at each site.

Uptake lengths were normalized to water velocity, u (m s^{-1}) and stream depth, h (m) by calculating nutrient uptake velocity (V_f) as,

$$V_f = \frac{uh}{S_w} \qquad \text{eq. 2}$$

V_f is a measure of the velocity at which a nutrient molecule is taken up by the biota or sediment, and so describes biological uptake and the relative demand for nutrients compared to their concentration within the water column. V_f is corrected for flow and changes in stream size and thus allowed for comparison among study streams (Stream Solute Workshop 1990; Davis & Minshall, 1999; Valett, Crenshaw, & Wagner, 2002). .

Nutrient uptake rate (U) was calculated as,

$$U = V_f C \qquad \text{eq. 3}$$

where C is the ambient nutrient concentration (Stream Solute Workshop, 1990). Nutrient uptake rate (U) provided a measure of the areal uptake of nutrients from the water column by the benthos (Stream Solute Workshop 1990; Davis & Minshall, 1999).

Transient Storage

Transient storage area and dispersion coefficients were modeled for each stream using the OTIS model (Runkel, 1998). During each sampling event conductivity, discharge, area, and timing intervals were used to model transient storage and the dispersion coefficient.

Biofilm enzyme activity

During each sampling event, benthic biofilm samples were collected in triplicate at the most downstream cross-sectional station of each reach and at the cross-sectional station 40m from the nutrient input. Samples were frozen and stored until analyzed. Thawed samples were divided and weighed for analyses of extracellular enzyme activity, dehydrogenase activity (DHA), total N (TN), total C (TC), total P (TP), dry weight, and ash free dry weight.

TN and TC content of dried biofilm samples were determined by combustion with an elemental analyzer (Model 1112EA, Carla Erba Instruments, Milan, Italy). Dried biofilm samples for TP content were microwave digested with HNO_3 (Mars Express, Matthews, NC, USA), neutralized with NaOH, diluted with deionized water, and analyzed by the molybdate-ascorbic acid method (APHA, 1998).

Dehydrogenase activity was measured by mixing duplicate aliquots of biofilm sample with 2mL of sterile water and 1mL of 0.75% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) standard. Samples were sealed, agitated for 1min. and incubated at 37C in the dark for 3h (Broberg, 1985; Songster-Alpin & Klotz, 1995). The incubations were terminated with 8mL of methanol and samples were centrifuged. The supernatant was analyzed for absorbance using an ultraviolet spectrophotometer (Lambda 20, Perkin Elmer, Wellesley, MA, USA), compared to INT standard, and normalized for dry weight to yield a value of biofilm respiration (nmol INT gDW^{-1} h^{-1}). Biofilm extracellular enzyme activity (EEA) was measured using the microplate protocol developed by Sinsabaugh and colleagues (Sinsabaugh, Findlay, Franchini, *et al.*, 1997; Foreman, Franchini & Sinsabaugh, 1998; Sinsabaugh & Foreman, 2001). The potential activities of β-1,4-glucosidase (BG), α-1,4-glucosidase (AG), β-1,4-xylosidase (BX), β-1,4-galactosidase (BGAL), α-1,4-galactosidase (AGAL), β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), alanine aminopeptidase (AAP), and acid (alkaline) phosphatase (AP) were assayed fluorimetrically using 4-methylumbelliferyl (MUB)-β-D-glucoside, (MUB)- α-D-glucoside , 4-MUB-β-D-xyloside, 4-MUB-β-D-galactoside, 4-MUB-α-D-galactoside, 4-MUB-N-acetyl-β- glucosaminide, L-Leucine 7-amido-4-methylcoumarin, L-Alanine-7-amido-4-methylcoumarin, and 4-MUB-phosphate, respectively. Plates were incubated in the dark for varying lengths of time depending on the substrate. Fluorescence was measured (Model FLX800T, BioTek Instruments, Wonooski, VT, USA) using an excitation wavelength of 350nm and an emission wavelength of 450nm. Biofilm samples were run in quadruplicate for each enzyme and each reference standard with solutions prepared in deionized water. Controls for both substrate and samples, prepared in buffer, were run on the same plate. Quenching was estimated by comparing the florescence of the supernatant of the biofilm

mixed with the standard solution to the standard solution mixed with buffer. EEA is reported as substrate accumulated over time adjusted for emission coefficients calculated from the standards, corrected for quenching, and normalized to sample weight. Activities are presented in units of nmol h^{-1} gDW^{-1}. In some cases, as noted in the text, EEA is corrected for the C content of the sample and presented as nmol h^{-1} gC^{-1}.

Statistical methods

Landscape and chemistry data were compiled for all nine study streams and presented as either one time measurements or averages ±SE. Water nutrient data (NH_4^+, TN, TP) for all sampling events were analyzed using repeated measures analysis of variance (ANOVA) to test for seasonal or monthly variability. Nutrient data were also examined with ANOVA to test for variability between streams. Prism 5 (2008, version 5.02, GraphPad Software, Inc., La Jolla, CA, USA) was used to calculate linear regressions of ambient nutrient concentrations versus V_f and U to determine nutrient saturation or limitation. Linear regressions of glycosidase, peptidase and phosphatase activities were also performed to determine nutrient limitation in the biofilm. The relationships between landscape, chemistry, nutrient uptake, and EEA variables were determined using Spearman rank correlation (r). Variables found to have significant relationship by Spearman analysis were then examined by canonical correlation analysis using SAS (2008, version 9.2, SAS Institute Inc., Cary, NC, USA). Canonical correlation analysis was used to examine the co-varying responses of independent and dependant variables in order to determine which factor might explain a particular interaction.

Results

Stream chemistry and the urban gradient

All streams were sampled on a monthly basis from July through October. Water nutrient data (NH_4^+, TN, TP) for all sampling events were analyzed using repeated measures analysis of variance (ANOVA) to test for significant seasonal or monthly differences. There were no statistically significant differences in NH_4^+ ($F=1.030$,

p=0.4027), or TN (F=0.8535, p= 0.4863) background nutrient concentrations with respect to monthly sampling events. TP concentrations measured during September were significantly different than those of other months (F=6.232, p=0.0043), there were no differences amongst July, August and October. No significant difference were measured in nutrient concentrations between streams as measured by ANOVA (NH_4^+, F= 1.761, p= 0.1562; TN, F= 2.375, p= 0.0890; TP, F= 1.387, p= 0.2728). From this point forward, all of the data from each stream for all sampling dates are presented as a pooled sample set to increase statistical power (Table 1). There were measurably higher flows during the sampling events of September and October resulting in correlations between discharge and NH_4^+ (r= -0.3871, p<0.05) and TP (r= 0.4304, p< 0.05). Because of these flow differences, comparisons between streams were based on V_f, which normalized nutrient uptake for depth and velocity (Davis and Minshall, 1999).

Catchment impervious surface cover was compared with all other measured habitat variables by Spearman correlation analysis to determine if other variables might also be indicators of urbanization in the landscape. Storm sewer length, % shading and water Cl increased with impervious surface cover, while % forest and median particle size declined with increases in impervious surface cover.

Using the pooled sample set (n=34), and Spearman correlation analysis, I found water Cl concentration was significantly correlated to most of the measured habitat variables including; % impervious surface cover, % shading, % forest, % wetland, particle size, storm sewer length, wood count, stream width, depth and velocity. There were no significant relationships between impervious surface cover and ambient concentrations of NH_4^+, TN, or TP. Ambient water NH_4^+ and TN increased with % shading, while TN decreased with increases in forest cover (Tables 1, 2).

Nutrient uptake and the urban gradient

With the exception of NH_4^+-S_w with % forest, there were no significant correlations between nutrient uptake metrics and metrics related to urbanization of the catchments (% ISC, storm sewer length, Cl, % forested, and particle size). There were significant positive correlations between NH_4^+-V_f, U and TP-V_f, U, and transient storage

and a negative correlation between NH_4^+- S_w and transient storage. The dispersion coefficient increased with V_f and U for NH_4^+, TN, and TP (Tables 3, 4).

Nutrient uptake and biofilm enzyme activity

I found statistically significant correlations between nutrient uptake and biofilm EEA using Spearman correlation analysis. NH_4^+ and TP- S_w, negatively correlated with total glycosidases, total peptidases, phosphatase and dehydrogenase activities. TP- V_f and U positively correlated with total glycosidase, total peptidase, phosphatase and dehydrogenase activities, while NH_4^+- V_f,and U positively correlated with total glycosidase activity. There was no correlation between TN uptake and EEA. (Table 4).

Canonical correlation analysis of variables found to be significant by Spearman analysis showed a positive correlation between NH_4^+- V_f and TP- V_f and canonical axis 1, which was comprised of EEA, DHA, transient storage and TN and TP (Table 5). Transient storage and TP are positively correlated with nutrient uptake velocity, while water TN is negatively correlated. The correlation of V_f to enzyme activity is similar amongst glycosidases, peptidases and phosphatase, with a stronger relationship to DHA (Table 5).

Biofilm enzyme activity and the urban gradient

For comparisons of biofilm enzyme activity to habitat and stream chemistry variables all biofilm enzyme data were corrected per g C in each sample (Table 6). Carbon is the main driver of biofilm EEA so corrections for C content were made to mitigate its influence and allow for across stream comparisons to be made. EEA was negatively correlated with % ISC and several other measures of urbanization within the landscape (Table 7). Total glycosidase and acid phosphatase activities correlated negatively with % ISC, storm sewer length, and water Cl, and positively to % forest. Total peptidase activity was negatively correlated with % ISC, and water Cl, and positively correlated with % forest. DHA was correlated negatively with % ISC, storm sewer length, % shading, and water Cl, and positively to % forest, % wetland, stream width and depth (Table 7).

Canonical correlation analysis was also done for biofilm EEA, with chemistries and measures of urbanization. I found a significant correlation of all biofilm EEA to canonical axis 1 (Table 8). The main drivers of the correlation are chemical. Biofilm C and N are significantly correlated to EEA while TP is not. The urban influences of % ISC, forest and Cl are similar in the strength of their correlation.

Nutrient limitation

I estimated nutrient limitation in these study streams based on stream chemistry and biofilm EEA data. I found NH_4^+- V_f, NH_4^+-U, TP- V_f, and TP-U significantly correlated with increasing ambient water TP concentrations (Table 4). These data are further supported by linear regression analysis of V_f and U with ambient water NH_4^+ and TP concentrations. A significant positive relationship between concentration and U indicated nutrient limitation and a negative relationship between concentration and V_f indicated saturation (Hoellein, Arango, & Zak, 2007). Ambient TP plotted against TP-U yielded a significant positive slope with an r^2= 0.3676, indicating phosphorus limitation in these streams (Fig. 2-D). We found no evidence of NH_4^+ limitation or saturation (Fig. 2 A, C).

Biofilm EEA was analyzed as a function of the nutrient each hydrolyzes in response to local nutrient availability, total glycosidases (C), total peptidases (N), and acid phosphatase (P). All biofilm EEA data were pooled (n=64) and analyzed by linear regression to determine the mean stoichiometric ratios. The total glycosidase activity regressed against total peptidase enzyme activity (slope = 2.278± 0.2554, r^2= 0.5622, p< 0.0001) indicated that the microbial community in the biofilm was hydrolyzing enzymes to acquire C at a faster rate than N due to a limitation (Fig. 3-A). The total glycosidase activity regressed against acid phosphatase activity (slope= 1.025± 0.06351, r^2 =0.8077, p<0.0001) indicated similar rates of C and P acquisition (Fig. 3-B). Total peptidase activity regressed against total phosphatase activity (slope of 0.3379± 0.02072, r^2 =0.8110, p<0.0001) indicated a significant P limitation in these study streams (Fig. 3-C), which is consistent with the stream chemistry data.

Discussion

The observed measures of NH_4^+- S_w are comparable to those reported in recent studies of small streams (Davis & Minshall, 1999; Hall, Bernhardt & Likens, 2002; Hoellein, Tank, Rosi-Marshall, *et al.*, 2007; Hill, *et al.*, 2010b). My estimates of NH_4^+ -V_f and NH_4^+- U are also consistent with those of recent studies (Dodds, López, Bowden, *et al.*, 2002; Hall & Tank, 2003; Hoellein, *et al.*, 2007; Hill, *et al.*, 2010b). Increased impervious surface cover with storm sewer length and water Cl concentration is expected given that all three are directly related to human development. The increased impervious surface cover with % shaded area within the catchment was not initially expected, but makes sense given that the most urban streams were located within city parks or other public areas with grass and tree cover. ISC, storm sewer length and % shading can then all be considered indicators of urban development for this study. Consequently, % forest, % wetland, median particle size, wood count, stream depth, stream width and stream velocity are also indicators of the level of urbanization within the catchment given their negative correlation to water Cl concentration.

The Lotic Intersite Nitrogen Experiment (LINX II; Mulholland *et al.* 2008) reported higher ambient NO_3^- concentrations and uptake rates in urban streams as compared to reference streams. These data suggested that higher NO_3^- concentration stimulates uptake in urban streams. They also observed that while uptake velocity was not significantly related to land use, it declined exponentially with increasing NO_3^- concentration, suggesting that streams become less efficient at NO_3^- removal as concentrations increase. Earl *et al.* (2006) also reported decreased N uptake efficiency with increased ambient N concentration, and a nonlinear decrease in uptake efficiency with increasing N input. Meyer *et al*. (2005) found negative correlations between NH_4^+ and P uptake and indicators of catchment urbanization in tributaries of the Chattahoochee River. Based on the results of these studies along with others that observed increased nutrient loading as a result of catchment level urbanization (Groffman *et al*., 2004; Walsh et al. 2005), I expected that landscape changes in urbanization would be reflected in the N and P uptake in my study streams. I hypothesized that as the amount of urbanization in

the streams' catchment increased, I would observe an elongated nutrient uptake length (S_w), a decreased uptake velocity (V_f) and an increased uptake rate (U).

I was unable to support my hypothesis, with the exception of NH_4^+-S_w and % forest, and found no significant correlation between uptake metrics for NH_4^+, total nitrogen (TN), total phosphorus (TP). Correspondingly, I did not observe an increase in ambient water levels of NH_4^+, TN or TP related to increasing levels of catchment urbanization. These data are consistent with Hoellein *et al*. (2011), who observed no relationship between NH_4^+ yield and land-use. There are two reasons why I may not have observed the expected relationship between the urban gradient and stream chemistry. First, while our impervious surface extent ranged up to 26%, the headwaters of their catchments were more natural. Second, the use of fertilizers in these catchments may be less than for most urban catchments due to public awareness of the need to protect the water quality of Lake Superior. These two qualifiers of our urban gradient result in lower than expected nutrient concentrations and loads in Lake Superior tributary streams compared to urban streams for other studies.

I found a significant correlation between NH_4^+- S_w, V_f , U and TP- V_f, U and transient storage and dispersion. TP-S_w also correlated positively with dispersion. These data show that transient storage and dispersion are important factors in both N and P processing in these study streams. Because nutrient uptake length, velocity and rate are aggregate measures of uptake and do not account for measures of transient storage or dispersion they may not be sensitive enough measures to reflect catchment scale disturbances when such variables have a significant influence (Runkel, 2007; O'Connor, Hondzo & Harvey, 2010).

Canonical correlation analysis identified a statistically significant correlation between nutrient uptake and biofilm EEA. There was a significant correlation between NH_4^+- V_f and TP- V_f, and the canonical axis of EEA (total glycosidase, total peptidase, and acid phosphatase). Hill, *et al*., (2010) hypothesized that nutrient uptake in small streams would be tightly coupled to biofilm enzyme activity under the premise that the organic matter processing by biofilm microbial assemblages is controlled by nutrient availability, but were unable to support this with their data. This is the first study to show

a statistically significant relationship between in stream nutrient uptake and biofilm EEA. NH_4^+- V_f and TP- V_f were also significantly correlated with respiration in the biofilm as measured by dehydrogenase (DHA) activity. These data support previous research by Hall & Tank (2003), who observed a strong relationship between NH_4^+-V_f and whole-stream metabolism. They asserted that biological variables are more predictive of N uptake velocity than physical variables. While I did not observe a significant relationship between N and P uptake and landscape scale disturbances caused by urbanization, I did observe significant correlations between N and P uptake velocities and the biological indicators of biofilm EEA and cellular respiration.

The findings of this study also show that there is a level of sensitivity to catchment scale disturbances measured in the biofilm EEA that was not detectable in measures of in stream nutrient uptake, as I observed significant correlations between biofilm enzyme activity and the gradient of urbanization. These data support those observed by Harbott and Grace (2005), who reported a direct relationship between EEA and the level of urbanization in their study streams. They propose that this relationship suggests measures of EEA might be used as indicators of stream health across urban gradients.

EEA was also used, along with nutrient chemistries, to estimate nutrient limitation in these study streams. Previous research proposes that if nutrient availability is approaching saturation then a negative relationship between concentration and V_f is expected, and if nutrient availability is limiting uptake then a positive relationship between concentration and U is expected (Davis & Minshall, 1999; Hoellein, *et al.*, 2007; Arango, Tank, Johnson, *et al.*, 2008; Hill, *et al.*, 2010b). Linear regression analyses of uptake velocity (V_f) and uptake rate (U) plotted against ambient NH_4^+ and TP concentrations, identified a general phosphorus limitation in these study streams (Fig. 2). There was no statistically significant evidence of NH_4^+ saturation or limitation. This finding contrasts with previous research on nutrient limitation in Lake Superior tributary streams. Using nutrient diffusing substrates, Wold and Hershey (1999) found evidence for P-limitation and N and P co-limitation in streams along the north shore of Lake Superior.

Stoichiometry of biofilm EEA was also used to examine relative nutrient availability (Sinsabaugh, *et al.*, 2009; Hill, *et al.*, 2010a). Biofilm EEA was analyzed as a function of the nutrient each hydrolyzes (glycosidases, C, peptidases, N, and acid phosphatase, P) and analyzed by linear regression to determine the mean stoichiometric ratios (Fig. 3). Total glycosidase activity regressed against total peptidase enzyme activity indicated that the microbial community in the biofilm was hydrolyzing enzymes to acquire C at a faster rate than N due to a limitation. The total glycosidase activity regressed against acid phosphatase activity indicated similar rates of C and P acquisition. Total peptidase activity regressed against total phosphatase activity indicated that the microbial community in the biofilm was producing enzymes for P acquisition in response to a P limitation. The P limitation indicated by biofilm EEA is consistent with the water chemistry and nutrient uptake data which show the same P limitation.

One goal of this study was to assess the effect of urbanization in stream catchment areas on nitrogen and phosphorus processing as measured by uptake velocities and rates in the water column and EEA in the biofilm. I did not observe changes in N or P uptake velocities or rates that correlated with the amount of urbanization within stream catchments. The strong correlation observed between NH_4^+- V_f , U and TP- V_f, U, and transient storage area and dispersion coefficients supports the assertion by others that storage zone processes can play a significant role in nutrient cycling and a transport based approach should be applied to nutrient addition experiments in order to provide better estimates of overall nutrient uptake (Runkel, 2007; O'Connor, *et al.*, 2010).

EEA in the biofilm did prove to be a significant indicator of disturbance caused by urbanization. Total glycosidase, total peptidase and phosphatase activities all correlated negatively with indicators of increased urbanization, and positively with the % of forested area within the catchments. Cellular respiration rates in the biofilm measured as DHA, also showed negative correlations to indicators of increased urbanization and positive correlations to % forest and % wetland area within the catchments. This sensitivity of EEA and cellular respiration to catchment scale disturbances supports the push by others to make the measurement of biological indicators a part of more streams studies (Hill, Herlihy & Kaufmann, 2002; Hall & Tank, 2003; Harbott & Grace, 2005;

Sinsabaugh, *et al.*, 2009; Hill, *et al.*, 2010b). Not only is the sensitivity of the data from EEA and DHA assays desirable, but the laboratory assays for both are relatively cost effective and require limited resources. The biofilm and sediment on which these assays are conducted are not as susceptible as water to discharge fluctuations and intermittent disturbances in streams which provides the potential for a more accurate analysis of stream conditions.

The second goal of this study was to examine the relationship between nutrient uptake and extracellular enzyme activity in the biofilm. I observed a statistically significant correlation between nutrient uptake in streams and biofilm extracellular enzyme activity. This relationship was further supported by the stoichiometric analysis of EEA showing the study streams to be P limited which agreed with the nutrient uptake regressed against chemistries that also indicated a P limitation. These complimentary data support previous studies that propose EEA as a useful indicator of nutrient limitation in streams (Cross, Benstead, Frost, *et al.*, 2005; Sinsabaugh, *et al.*, 2009; Hill, *et al.*, 2010a). This study shows that catchment scale disturbances caused by even moderate levels of urbanization are detectable in the microbial assemblages of the biofilm. These biological indicators can be successfully correlated with measures of nutrient uptake, chemistry and habitat to provide an overall assessment of streams.

Table 1 Summary table of landscape and chemistry data for all nine study streams. Values are presented either as one time measurements or averages for all sampling dates (±SE) *n*= 4.

	Talmadge	French	Amity	Mission	Keene	Chester	Kingsbury	Tischer	Miller
Catchment area, *A* (km^2)	13.6	46.9	42.8	27.2	14.2	15.9	23.6	18.9	22.3
Reach slope (m/m)	0.023	0.011	0.014	0.014	0.012	0.022	0.011	0.019	0.021
Imperviousness (%)	0.13	0.18	2.18	3.41	7.70	13.3	13.6	15.7	25.7
Storm sewer (km)	0.00	0.00	18.1	0.00	9.58	41.3	2.75	76.8	23.1
Wetland (%)	14.3	7.39	0.91	2.13	0.77	0.24	4.77	0.68	1.95
Forest (%)	79.4	88.3	82.7	75.9	71.0	66.2	60.1	60.1	49.4
Particle size (mm)	1265	1308	1024	389	354	816	550	1120	474
Wood count sum	66	33	49	9	1	7	0	12	14
Shading (%)	61.1 (0.5)	44.6 (0.5)	50.6 (0.3)	34.9 (1.1)	81.5 (0.2)	67.6 (1.5)	60.2 (1.2)	74.7 (1.4)	86.6 (0.5)
Stream width, *w* (m)	3.78 (0.15)	8.08 (0.07)	6.77 (0.23)	4.53 (0.28)	2.92 (0.10)	4.37 (0.10)	4.96 (0.36)	4.64 (0.16)	4.52 (0.19)
Stream depth, *z* (m)	0.10 (0.01)	0.21 (0.01)	0.15 (0.01)	0.12 (0.02)	0.13 (0.01)	0.16 (0.01)	0.11 (0.02)	0.12 (0.01)	0.14 (0.01)
Velocity, *v* ($m\ s^{-1}$)	0.17 (0.02)	0.20 (0.02)	0.21 (0.02)	0.14 (0.04)	0.21 (0.03)	0.21 (0.02)	0.18 (0.05)	0.25 (.02)	0.18 (0.02)
Discharge, *Q* ($m^3\ s^{-1}$)	49.9 (15.0)	271 (72.7)	82.5 (17.4)	85.1 (32.1)	62.9 (15.0)	92.2 (29.2)	86.4 (27.3)	122 (29.4)	107 (20.1)
Transient Storage, A_s/A	0.27 (0.13)	0.58 (0.18)	0.23 (0.08)	0.12 (0.04)	0.18 (0.06)	0.19 (0.06)	0.17 (0.09)	0.45 (0.08)	0.49 (0.13)
Dispersion coefficient D	0.38 (0.10)	0.80 (0.24)	0.12 (0.05)	0.30 (0.17)	0.20 (0.05)	0.68 (0.16)	0.37 (0.18)	0.70 (0.15)	0.32 (0.08)
Water Cl ($mg\ L^{-1}$)	11.6 (0.6)	5.0 (0.1)	31.4 (1.2)	78.0 (2.1)	68.7 (8.0)	83.4 (9.4)	112 (24.7)	69.6 (3.2)	150 (14.5)
Water NH_4^+ ($\mu g\ L^{-1}$)	12.0 (1.8)	5.7 (0.5)	9.5 (1.4)	11.5 (2.6)	16.6 (3.2)	18.8 (2.4)	20.2 (6.0)	31.1 (5.8)	12.9 (3.5)
Water TN ($\mu g\ L^{-1}$)	605(42.1)	471 (25.8)	392 (38.5)	564 (44.3)	518 (36.3)	1283(234.6)	650(29.2)	664 (60.1)	996 (205)
Water TP ($\mu g\ L^{-1}$)	22.3 (2.7)	21.7 (3.0)	9.8 (1.9)	13.5 (4.4)	23.7 (4.9)	25.6 (2.7)	16.4 (2.6)	25.8 (4.5)	15.7 (2.0)
Biofilm TN (mg/kg)	6558 (1751)	2882 (1061)	3063 (395)	1267 (266)	3237 (855)	5892 (1963)	5633 (2520)	4973 (968)	3248 (1014)
Biofilm C (mg/kg)	97887 (20649)	29560 (10814)	59615 (11075)	15026 (3617)	66125 (17408)	87965 (32222)	93420 (29943)	86513 (24269)	50723 (15583)
Biofilm TP (mg/kg)	766 (87.6)	708 (159)	621 (31.6)	596 (68.6)	705 (38.9)	699 (49.3)	806 (137)	882 (68.9)	871 (170)

Table 2 Spearman correlations coefficients (r) of background in-stream nutrient concentrations (NH_4^+, TP, TN, Cl), with landscape and chemical variables for all water sampling events *n*=34. Not applicable (na) is used to indicate variables that are derived from other variables. (-) represent correlations with p> 0.05.

	Water Cl	Water NH_4^+	Water TN	Water TP
Imperviousness	0.82	-	-	-
Shading	0.59	0.35	0.48	-
Forest	-0.84	-	-0.42	-
Wetland	-0.45	-	-	-
Particle size	-0.68	-	-	-
Storm sewer	0.51			
Wood count	-0.60	-	-	-
Stream width	-0.47	-0.46	-	-
Stream depth	-0.43	-0.34	-	0.41
Stream velocity	-0.36	-	-	0.53
Discharge	-	-0.36	-	0.58
Reach slope	-	-	-0.35	-
Transient Storage	-	-	-	-
Dispersion	-	-	-	0.48
Water Cl	na	0.36	0.38	-
Water NH_4^+	0.36	na	0.53	-
Water TN	0.38	0.51	na	-
Water TP	-	-	-	na
Biofilm C	-	-	-	-
Biofilm N	-	-	-	-
Biofilm P	-	-	-	-

Table 3 Summary table of nutrient uptake data for all nine study streams. Values are presented as averages for all sampling dates (range) *n*= 4.

	Talmadge	**French**	**Amity**	**Mission**	**Keene**	**Chester**	**Kingsbury**	**Tischer**	**Miller**
S_w **NH_4^+** (m)	144 (122-167)	161 (66.7-435)	576 (58.8-2083)	379 (90.9-667)	418 (83.3-1000)	372 (179-625)	323 (90.9-556)	301 (256-345)	574 (28.2-297)
S_w **TN** (m)	1666 (625-3704)	289 (175-526)	646 (625-667)	2119 (238-4000)	1032 (345-2381)	1407 (333-2500)	403 (90.9-714)	742 (714-769)	482 (76.9-1695)
S_w **TP** (m)	341 (182-500)	307 (71.4-833)	1441 (55.6-5263)	1208 (139-1923)	286 (227-345)	361 (233-556)	1886 (200-3571)	534 (135-1000)	397 (132-625)
V_f **NH_4^+** (mm/min)	1.08 (1.06-1.10)	28.4 (0.52-74.4)	8.1 (0.2-21.2)	13.3 (0.18-26.3)	4.99 (0.41-13.0)	7.27 (0.93-19.3)	1.79 (0.43-3.16)	2.01 (0.38-3.36)	10.9 (0.15-29.0)
V_f **TN** (mm/min)	0.61 (0.04-1.44)	7.13 (1.28-14.5)	1.31 (0.35-2.28)	0.74 (0.60-0.89)	2.42 (0.89-5.97)	3.62 (0.13-10.3)	1.75 (0.31-3.16)	2.78 (1.30-4.26)	3.64 (1.82-4.91)
V_f **TP** (mm/min)	2.41 (0.96-3.85)	15.7 (0.27-39.3)	7.70 (0.12-27.4)	5.80 (0.08-17.2)	5.08 (0.43-9.72)	3.71 (0.94-10.7)	0.75 (0.07-1.4)	6.25 (0.13-13.6)	7.60 (1.63-17.0)
U **NH_4^+** (ug/ m2 min)	13.9 (9.2-18.5)	132 (4.5-345)	101 (2.5-355)	64.9 (1.7-128)	160 (5.7-457)	196 (10.8 -560.0)	67.2 (5.1-129)	63.7 (12.2-115)	66.2 (0.8-152)
U **TN** (ug/ m2 min)	333 (25.1-631)	2982 (719-6106)	573 (124-1023)	470 (399 -541)	1582 (187-4234)	2044 (334-5389)	995 (240-1750)	1197 (704-1691)	2309 (1658-2960)
U **TP** (ug/ m2 min)	48.3 (10.0-86.5)	338 (5.2-924)	53.1 (2.6-191.5)	164 (0.2-491)	256 (7.4-505)	85.5 (19.0-240)	9.5 (0.7-18.2)	236 (1.9-715)	141 (28.2-297)

Table 4 Spearman correlations coefficients (r) of NH_4^+, TN, and TP uptake length (S_w), uptake velocity (V_f), and uptake rate (U) for all water sampling events n=34 as compared with landscape, chemical, and enzyme activity variables. Not applicable (na) is used to indicate variables that are derived from other variables. (-) represent correlations with p> 0.05.

	NH_4^+			**TN**			**TP**		
	S_w	V_f	U	S_w	V_f	U	S_w	V_f	U
Imperviousness	-	-	-	-	-	-	-	-	-
Storm sewer	-	-	-	-	-	-	-	-	-
Wetland	-	-	-	-	-	-	-	-	-
Forest	-0.39	-	-	-	-	-	-	-	-
Shading	-	-	-	-	-	-	-	-	-
Particle size	-	-	-	-	-	-	-	-	-
Wood count sum	-	-	-	-	-	-	-	-	-
Reach slope	-			0.53	-	-	-	-	-
Stream width	-	-	-	-	-	-	-	-	-
Stream depth	-	na	na	-	na	na	-	na	na
Stream velocity	-	na	na	-	na	na	-	na	na
Discharge	-0.41	na	na	-	na	na	-0.44	na	na
Transient Storage	-0.54	0.63	0.63	-	-	-	-	0.47	0.48
Dispersion	-0.58	0.77	0.70	-	0.56	0.45	-0.61	0.71	0.73
Water NH_4^+	-	-	-	-	-	-	-	-	-
Water TP	-	0.52	0.53	-	-	-	-	0.40	0.67
Water TN	0.50	-0.40	-	-	-	-	-	-	-
Water Cl	0.41	-	-	-	-	-	-	-	-
Biofilm C	-	-	-	-	-	-	-0.35	-	-
Biofilm N	-	-	-	-	-	-	-0.36	-	-
Biofilm TP	-	-	-	-	-	-	-	-	-
Total glycosidase activity	-0.32	0.32	0.28	-	-	-	-0.59	0.42	0.42
Total peptidase activity	-0.34	-	-	-	-	-	-0.52	0.34	0.32
Phosphatase activity	-0.30	-	-	-	-	-	-0.50	0.31	0.32
Dehydrogenase activity	-0.44	0.38	-	-	-	-	-0.47	0.40	0.33

Table 5 Canonical correlations of $V_f NH_4^+$ and V_f TP for all water sampling events *n*=34 with canonical gradients W1 and W2 comprised of, biofilm enzyme activity, transient storage and water TN and TP.

	W1	W2
V_f NH_4^+	0.7689	0.0432
V_f TP	0.6648	0.2525
Total glycosidase activity	0.3598	0.5112
Total peptidase activity	0.2330	0.3619
Phosphatase activity	0.3258	0.4043
Dehydrogenase activity	0.5205	0.3693
Transient Storage	0.7268	-0.1671
Water TN	-0.4603	0.2588
Water TP	0.5137	-0.1289

Table 6 Summary table of biofilm enzyme activity data for all nine study streams. Units of activity are nmol h^{-1} per gC^{-1} and values are presented as averages (±SE) of all biofilm samples collected at each stream *n*= 8. Total glycosidases are calculated as the sum of, α-D-Galactosidase, β-D-Galactosidase, 0.5* β-N-acetylglucosaminidase, β-D-Xylosidase, α-D-Glucosidase, and β-D-Glucosidase activities. Total aminopeptidases are calculated as the sum of, 0.5* β-N-acetylglucosaminidase, L-Alanine aminopeptidase, and L-Leucine aminopeptidase.

	Talmadge	**French**	**Amity**	**Mission**	**Keene**	**Chester**	**Kingsbury**	**Tischer**	**Miller**
α-D-Galactosidase	225	98.6	158	45.5	134	173	151	122	61.1
	(47.0)	(26.6)	(25.8)	(14.5)	(34.9)	(61.7)	(57.6)	(30.4)	(27.7)
β-D-Galactosidase	211	116	169	51.0	211	239	191	180	83.6
	(28.7)	(30.4)	(33.0)	(12.6)	(52.1)	(99.6)	(69.8)	(46.3)	(34.8)
β-N-acetylglucosaminidase	1561	1334	802	222	898	1511	632	774	633
	(280)	(458)	(166)	(65.1)	(181)	(621)	(176)	(256)	(209)
β-D-Xylosidase	375	289	231	71.8	245	236	172	244	121
	(48.0)	(88.6)	(47.7)	(20.8)	(51.0)	(97.3)	(62.9)	(85.4)	(45.8)
α-D-Glucosidase	158	142	161	79.5	174	242	155	160	68.8
	(33.6)	(40.1)	(28.2)	(21.8)	(35.2)	(91.1)	(40.1)	(49.8)	(20.6)
β-D-Glucosidase	2908	3236	1145	646	2487	2635	1760	2090	817
	(388)	(1635)	(120)	(183)	(663)	(965)	(584)	(686)	(164)
Total glycosidases	4658	4548	2264	1005	3699	4281	2746	3183	1469
	(685)	(2050)	(337)	(286)	(926)	(1626)	(902)	(1026)	(397)
L-Alanine aminopeptidase	503	377	240	126	264	310	124	2278	134
	(191)	(94.9)	(43.4)	(36.8)	(41.0)	(74.2)	(14.6)	(47.0)	(39.9)
L-Leucine aminopeptidase	201	291	227	120	256	376	118	197	119
	(48.5)	(96.4)	(40.3)	(41.1)	(44.8)	(136)	(23.9)	(42.2)	(28.0)
Total aminopeptidases	1484	1335	868	357	969	1442	558	812	569
	(379)	(420)	(167)	(111)	(176)	(521)	(127)	(217)	(172)
Acid phosphatase	4222	5438	2062	864	2283	3754	1629	1836	1160
	(609)	(2145)	(333)	(257)	(549)	(1052)	(418)	(590)	(321)
Dehydrogenase	39829	13462	12890	22351	26390	20616	70115	92960	27832
	(9441)	(2870)	(3505)	(7624)	(12168)	(8661)	(40826)	(59027)	(4804)

Table 7 Spearman correlations coefficients (r) of biofilm extracellular enzyme activity (total glycosidase, total peptidase, phosphatase, dehydrogenase), for all biofilm sampling events *n*=64 as compared with landscape, chemical and uptake variables. Not applicable (na) is used to indicate variables that are derived from other variables. (-) represent correlations with p> 0.05.

	Total Glycosidase activity	Total Peptidase activity	Phosphatase activity	Dehydrogenase activity
Imperviousness	-0.33	-0.31	-0.39	-0.34
Storm sewer	-0.27	-	-0.31	-0.33
Wetland	-	-	-	0.25
Forest	0.37	0.40	0.43	0.41
Shading	-	-	-	-0.39
Particle size	-	-	-	-
Wood count	-	-	-	-
Reach slope	-	-	-	-
Stream width	-	-	-	0.34
Stream depth	-	-	-	0.26
Stream velocity	-	-	-	-
Discharge	-	-	-	-
Transient Storage	-	-	-	-
Dispersion	-	-	-	-
Water Cl	-0.32	-0.33	-0.33	-0.39
Water NH4	-	-	-	-0.34
Water TN	-	-	-	-
Water TP	-	-	-	-
Biofilm C	-0.44	-0.65	-0.51	-0.55
Biofilm N	-0.36	-0.55	-0.40	-0.44
Biofilm P	-	-	-	-

Table 8 Canonical correlations of biofilm enzyme activity (total glycosidase, total peptidase, phosphatase, dehydrogenase) for all sampling events *n*=64 with canonical gradients W1 and W2 comprised of (%impervious surface, % forested, background in stream Cl, biofilm C, N and TP).

	W1	W2
Total glycosidase activity	0.5200	0.0040
Total peptidase activity	0.7691	0.0052
Phosphatase activity	0.6241	0.1725
Dehydrogenase activity	0.7295	-0.0130
Biofilm N	-0.7676	0.0720
Biofilm C	-0.8186	-0.1269
Biofilm TP	-0.1779	-0.2080
Imperviousness	-0.3905	-0.2577
Forest	0.4979	0.3001
Water Cl	-0.4088	0.3516

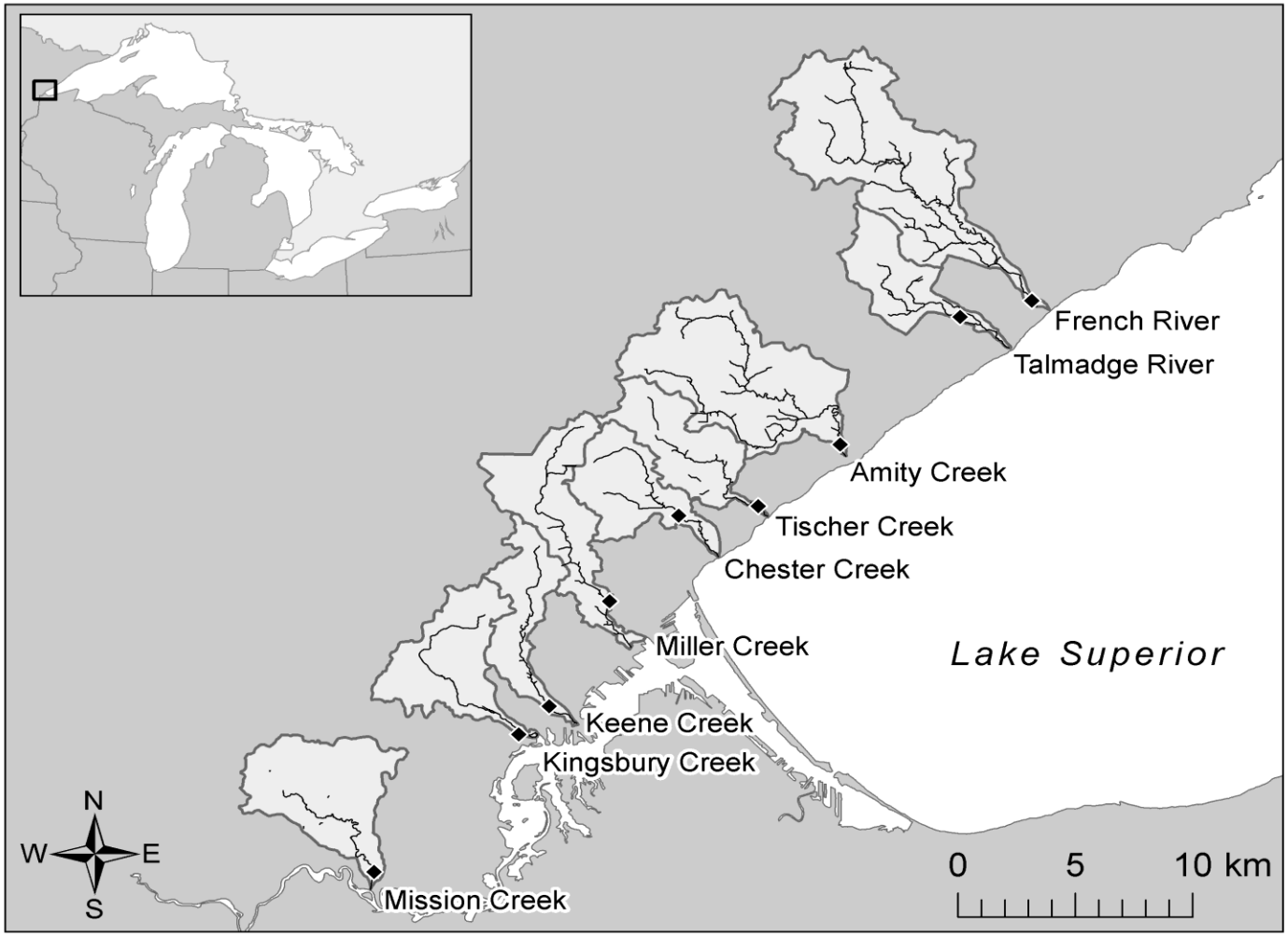
French River
Talmadge River
Amity Creek
Tischer Creek
Chester Creek
Miller Creek
Lake Superior
Keene Creek
Kingsbury Creek
Mission Creek
N
W
E
S
0
5
10 km

Figure 1

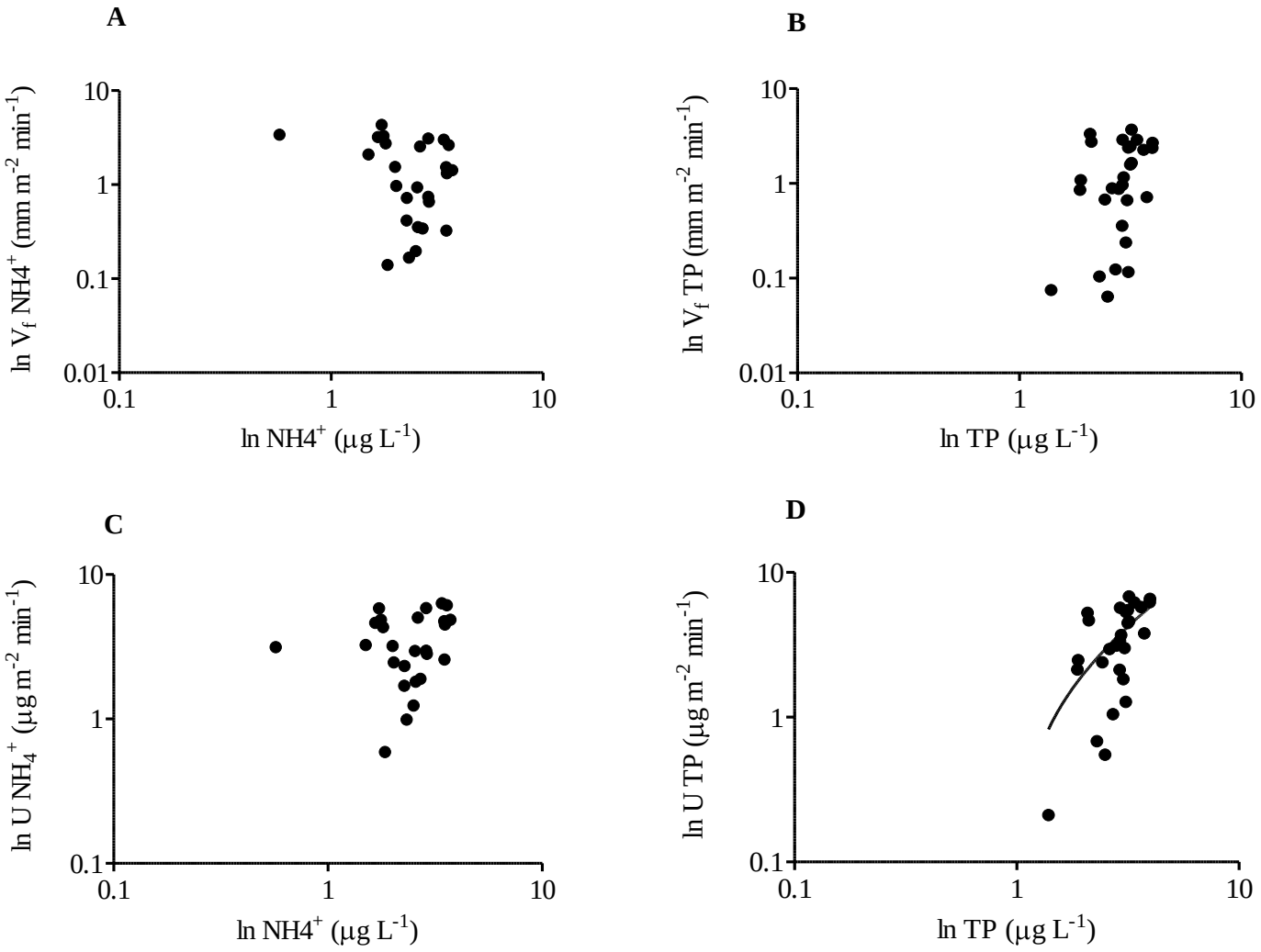
A
B
C
D
10
1
0.1
0.01
0.1
1
10
ln V_f $NH4^+$ (mm m^{-2} min^{-1})
ln $NH4^+$ (µg L^{-1})
ln V_f TP (mm m^{-2} min^{-1})
ln TP (µg L^{-1})
ln U NH_4^+ (µg m^{-2} min^{-1})
ln U TP (µg m^{-2} min^{-1})

Figure 2

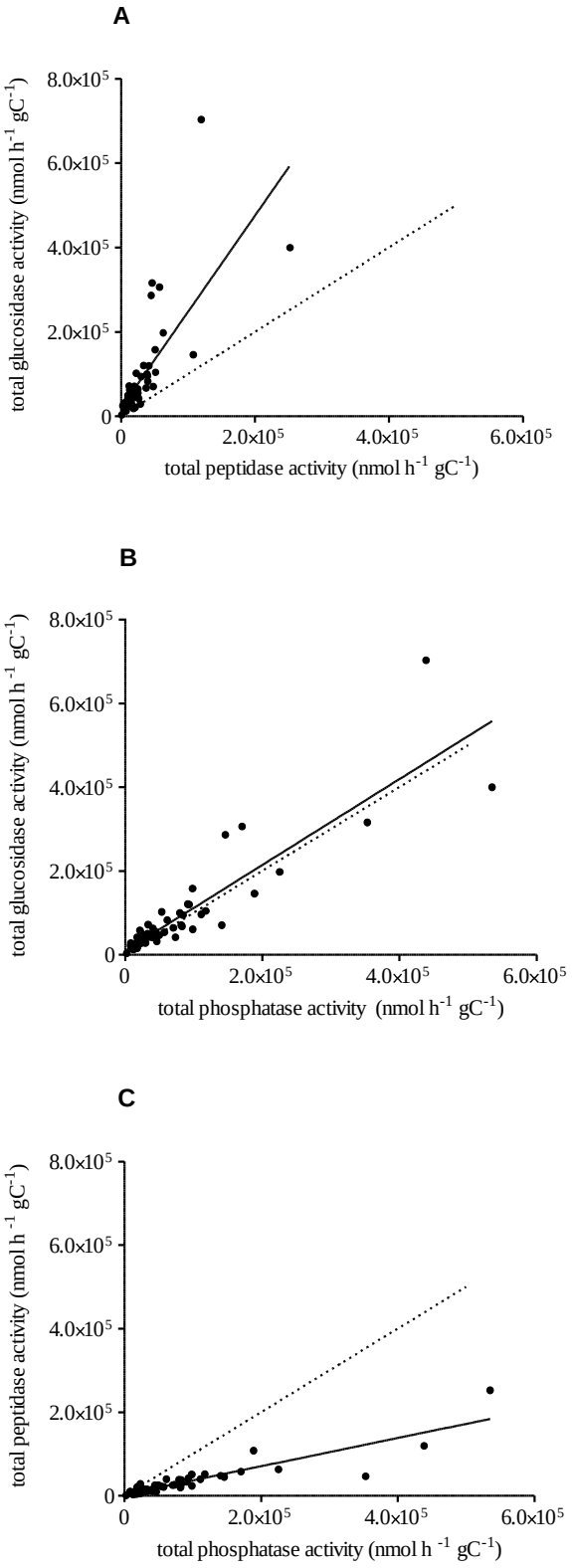
A
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0
total glucosidase activity (nmol h^-1 gC^-1)
0
2.0x10^5
4.0x10^5
6.0x10^5
total peptidase activity (nmol h^-1 gC^-1)
B
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0
total glucosidase activity (nmol h^-1 gC^-1)
0
2.0x10^5
4.0x10^5
6.0x10^5
total phosphatase activity (nmol h^-1 gC^-1)
C
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0
total peptidase activity (nmol h^-1 gC^-1)
0
2.0x10^5
4.0x10^5
6.0x10^5
total phosphatase activity (nmol h^-1 gC^-1)

Figure 3

Figure 1 Map of nine study sites and stream catchment areas located along the North Shore of Lake Superior, near Duluth, MN.

Figure 2 Regression plots of NH_4^+ and TP uptake velocity (V_f) and uptake rate (U) against background NH_4^+ and TP concentrations, for all water sampling events n=34. The significantly positive regression slope of plot D indicates an overall phosphorus limitation for these streams, r2= 0.3676, p<0.0006. The absence of a significant positive slope in plot C does not indicate NH_4^+ limitation. The absence of negative slopes for plots A and B indicate no evidence of significant nutrient saturation.

Figure 3 Linear regression plots of A) total glycosidase enzyme activity and total peptidase enzyme activity, slope=2.278 ±0.2554, r^2= 0.5622, p< 0.0001, B) total glycosidase enzyme activity and total phosphatase enzyme activity, slope = 1.025 ± 0.06351, r^2 =0.8077, p<0.0001, C) total peptidase enzyme activity and total phosphatase enzyme activity, slope= 0.3379, r^2 =0.8110, p<0.0001, for all biofilm sampling events n=64. The dotted lines represent a theoretical 1:1 relationship. Plot A indicates a limitation of and demand for C as compared to N, plot B shows a near 1:1 relationship of C:P, and plot C indicates an overall P limitation and demand for these streams.

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