THE EFFECTS OF SEASONALITY AND RESTORATION ON STREAM NUTRIENT CYCLING AT MULTIPLE SPATIAL SCALES

A Dissertation

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by

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Human activities have increased concentrations of nutrients including nitrogen (N) and phosphorus (P) in freshwater ecosystems worldwide. Understanding how excess nutrients are processed is critical for public and ecological health. Headwater streams are important sites of nutrient transformation, but little is known about how temporal variation (e.g., seasonal change) and stream restoration strategies influence rates of nutrient cycling. My dissertation focuses on how seasonality and restoration, alone and in combination, control nutrient uptake rates at the whole-stream and substratum-specific scales in 3 headwater streams in the Upper Peninsula of Michigan.

By measuring whole-stream and substratum-specific rates of nutrient uptake across seasons, I found that although heterotrophic processes typically dominate in forested headwater streams, variation in nutrient uptake was also explained by autotrophic activity. My results suggested changes in streambed substrata composition strongly influences seasonal patterns of nutrient processing at the whole-stream scale. These conclusions are significant in the context of restoration, which often results in changes to the streambed “landscape.”
I also used seasonal measurements of nutrient uptake to document the influence of two contrasting restoration strategies on stream ecosystem function. For a trout habitat enhancement, I found that physical changes in stream habitat translated into few biological effects on uptake rates and fish communities, and concluded it may not represent a sustainable method for increasing trout abundance. For a wood addition study, I found increased nutrient uptake following intermediate disturbance (e.g. storms), contrasting with significant decreases in uptake following large storms. Therefore, the effect of wood addition on ecosystem function was variable across a disturbance gradient, and should be considered in future restoration.

My dissertation research demonstrates that nutrient uptake in headwater streams is seasonally dynamic, with significant stream-specific variability, all of which is linked to differences in streambed substrata composition. Changes to stream ecosystems such as those resulting from restoration can influence seasonal patterns of nutrient processing, altering the timing of nutrient delivery to downstream ecosystems. Overall, nutrient uptake rates are sensitive metrics for integrating changes in stream biological activity, and represent a powerful tool for characterizing the influence of seasonal change and restoration on stream ecosystem function.
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CHAPTER 1
INTRODUCTION

Ποταμοίς τοῖς αὐτοῖς ἐμβαίνομεν τε καὶ οὐκ ἐμβαίνομεν, ἐμέν τε καὶ οὐκ ἐμέν
We both step and do not step in the same rivers. We are and are not.

Ἡράκλειτος
Heraclites

1.1. Human influences on freshwater ecosystems

For millennia, human cultures have depended upon healthy freshwater ecosystems for potable water and food. As a result, freshwater ecosystems are the most sensitive of all ecosystem types to the effects of global population growth (Sala et al. 2000). Meyer (1990) suggested that in the past, rivers functioned as arteries of civilization, transporting goods and connecting cultures, but presently they frequently function as the kidneys, receiving and processing our waste products. In addition to nutrient enrichment from waste water, manure, and fertilizer effluents, modern human cultures have modified freshwater ecosystems by altering temperatures through impoundments and riparian vegetation removal, and decreasing habitat complexity via channelization and removal of structural components such as large wood (Schlessinger 1997, Harding et al. 1998, Paul and Meyer 2001).
In a classic study, Likens et al. (1970) deforested a small watershed to quantify the importance of nutrient retention by forest vegetation, which they measured as changes in stream solute concentrations. The study was successful in its objective to document the nutrient retention capabilities of the forest, but it also clearly demonstrated the sensitivity of stream ecosystems to changes in the surrounding terrestrial environments. In effect, the stream “concentrated” the influence of landscape-scale changes. As pressures on freshwater resources and biota increase from human population growth, it will be critical for researchers to document and communicate how stream ecosystems respond to further degradation (Sala et al. 2000) as well as our attempts at restoration (Poff et al. 2003), both directly in streams and throughout their watersheds.

In the Midwestern United States, an important source of inorganic nitrogen (N) and phosphorus (P) to streams is runoff from agricultural fields (Vitousek 1997). River networks transport the nutrient pollution downstream, contributing to the formation of a hypoxic zone in the Gulf of Mexico at the mouth of the Mississippi River (Rabalais et al. 2002). The Mississippi River delta is not an isolated case and similar “dead zones” have been recorded at other estuaries globally (Kennish 2002). Because nutrient availability is often limiting to primary and secondary production (Schindler 1977, Newbold et al. 1982), understanding the factors which control the ability of stream organisms to cycle nutrients is of major interest for both ecological and public health (Alexander et al. 2000, Meyer and Wallace 2001).
In the context of terrestrial biogeochemistry, streams and rivers have been viewed as “pipes” which transport material from the landscape to the ocean simply reflecting biological activity on the terrestrial landscape. More recently, studies have shown that lotic ecosystems are biologically reactive in their own right (Meyer 1990, Alexander et al. 2000, Alexander et al. 2007). In particular, small streams are thought to be especially effective at nutrient retention because of their high benthic surface area to water volume ratio (Peterson et al. 2001). In addition, headwater streams are more directly connected to the riparian landscape than larger rivers (Meyer and Wallace 2001) representing a critical point of interaction between the catchment and the broader aquatic environment, like threads stitching the fabric of terrestrial and marine ecosystems together.

My dissertation research focused on rates of nutrient processing in headwater streams, and how they are affected by seasonal changes and stream restoration strategies. In this introduction, I summarize the literature that initiated my research focus, and introduce a conceptual model that links my dissertation chapters together.

1.2. Nutrient uptake rates and microbial biofilms

Stream ecologists have used the paradigm of nutrient spiraling to quantify the movement of reactive elements such as N and P within streams and their interaction with the surrounding landscape (Webster and Patten 1979, Newbold et al. 1983). Nutrient spiraling theory incorporates the spatial orientation of elements as they move downstream in dissolved form, are removed from the water column
via uptake, and then returned in to the water column and transported downstream again (Figure 1.1). This conceptual framework has provided a powerful group of related metrics for understanding nutrient transformations through stream ecosystems (Newbold et al. 1983, Hall et al. 2000) and how natural and human influences affect these processes (Tank et al. 2000, Bernhardt et al. 2003).

Figure 1.1. The nutrient spiraling concept.

Nutrient assimilation from the water column into stream food webs is mediated by the action of microbial biofilms, which are complex assemblages of organisms including algae, fungi, and bacteria, inhabiting a mucilaginous extracellular matrix (Lock 1984). Biofilm constituents are broadly separated into two categories based on their carbon sources; autotrophs, which fix carbon dioxide (CO₂), and heterotrophs, which decompose organic carbon. Growth of biofilm organisms can be limited by the availability of inorganic nutrients such as N and P (Pringle 1990, Tank and Dodds 2003), as well as other environmental
factors such as light availability, carbon quality, and temperature. When stream conditions change, biofilms growth rates and composition will change concurrently, which can be demonstrated as changes in nutrient uptake rates as well as metabolism (i.e., rates of gross primary production, GPP, and community respiration, CR).

Many factors which control stream biofilm growth, and consequently nutrient uptake, have been well-studied including geomorphology, habitat heterogeneity, primary production, decomposition, and trophic dynamics (Mulholland et al. 1985, Rosemond et al. 1993, Hall et al. 2002, Hall and Tank 2003, McClain et al. 2003), but less attention has been focused on but the influence of seasonal variation in biofilm nutrient uptake (Martí and Sabater 1996, Simon et al. 2005, Roberts and Mulholland 2007). In addition, stream restoration projects designed to increase habitat heterogeneity for sport fisheries are rarely assessed for success using metrics of ecosystem function such as nutrient cycling (Bernhardt 2005, Lake 2007). Below I describe how stream nutrient uptake can be influenced by seasonal variation and stream restoration in particular, and why these measurements will contribute to an increased understanding of how stream ecosystems function in general.

1.3. Nutrient uptake rates and seasonal change

Ward (1989) emphasized the importance of considering the 4-dimensional nature of lotic ecosystems by integrating spatial (i.e., down into the sediments, laterally into the riparian zone, and upstream-downstream) and temporal elements
into research hypotheses. For headwater streams in temperate biomes, seasonality represents an important source of temporal variation (Mulholland 2004). In particular, seasonal variation in light and organic matter inputs influence biofilm constituents responsible for both primary production and decomposition, respectively (Mulholland et al. 1985, Webster and Benfield 1986, Roberts and Mulholland 2007). Light available to benthic organisms tracks forest canopy changes in temperate deciduous forests; light is limited by the riparian canopy in late spring due to leafout, and continues through summer and autumn until leaffall. In addition, in more northern latitudes, winter ice-cover can also limit light penetration to the benthos. During early spring prior to leafout and in late fall after leaf abscission, stream autotrophs can be released from light limitation (Hill et al. 2001), and these changes are reflected in increases in whole-stream nutrient demand and declines in ambient nutrient concentrations (Mulholland 2004, Roberts et al. 2007). In contrast, the growth of heterotrophic biofilms is limited by organic carbon quantity and quality (Tank and Webster 1998). For example, pulsed leaf inputs in autumn provide a critical carbon substrate for bacterial and fungal colonization, and this carbon source is processed and decomposed throughout autumn, winter, and spring (Webster and Benfield 1986, Webster et al. 1999). As light and carbon limitation are alleviated at different points in the year, the relative abundance of autotrophs and heterotrophs in biofilms may shift, thereby indirectly influencing whole-stream nutrient uptake (Mulholland et al. 2000, Webster et al. 2003). Despite the role of seasonal variation as a major controlling factor in temperate stream ecosystems, few
studies have analyzed how seasonal changes influence stream ecosystem processes (but see Mulholland et al. 1985, Martí and Sabater 1996, Simon et al. 2005). In addition, understanding seasonal variation in whole-stream nutrient uptake and metabolism is especially important for documenting the \textit{intra-annual} effects of stream restoration efforts.

1.4. Nutrient uptake rates and stream restoration

The increase in the number of stream restoration projects occurring globally reflects a general public awareness of the damaged ecological status of freshwater ecosystems and confirms the social and economic importance of freshwaters to our society (Society for Ecological Restoration International Science & Policy Working Group 2004). The number of stream restoration projects in the United States increased exponentially from 1970-2005, representing $14-15 billion investment since 1990 (Bernhardt et al. 2005). Unfortunately, fewer than 10% of restoration projects are monitored, and even fewer are scientifically evaluated for their effectiveness. Furthermore, of the projects that include post-restoration monitoring, most are solely short-term (Moerke and Lamberti 2004) or monitoring is directed at a single taxon such as a sport fish (Minns et al. 1996). After interviewing leaders from 317 restoration projects nationwide, Berhardt et al. (2007) found that the strongest indicators of restoration success were not metrics related to biological processes, but rather reflected post-project appearance and public perception. In summary, substantial restoration efforts continue, yet the ecosystem-scale and long-term effects of
current restoration strategies remain unclear. An increased emphasis on evaluation is critical to ensure that resources are directed towards the implementation of effective restoration that promote ongoing benefits for stream ecosystem health (Lake 2001, Poff et al. 2003).

Several recent publications have outlined pathways to increase the empirical rigor of stream restoration evaluation. These include: 1) the marriage of restoration analyses with basic tenets of ecological theory (Lake 2007), 2) the simultaneous collection of multiple types of ecosystem measurements (Minns et al. 1996), and 3) the adoption of a long-term perspective that will judge the performance of restoration structures in the context of environmental change (Palmer et al. 2005). In addition, Poff et al. (2003) suggested that if researchers and land managers cooperate to establish stream restorations as experimental manipulations, using reference conditions, pre-treatment data collection, and replication where possible, the subsequent data collection could address basic research questions and move the science of restoration ecology forward.

The use of nutrient spiraling metrics as an integral part of stream restoration evaluation represents an overlooked, but potentially powerful tool to address each of the 3 pathways for increasing the strength of stream restoration analyses described above. Nutrient uptake rates can be useful in restoration evaluation because they are integrative on two important scales: 1) they synthesize the activity of multiple organisms and/or trophic levels, and 2) they indicate the biological activity occurring at the time of measurement, which makes them sensitive to environmental change (Webster and Patten 1979,
Mulholland et al. 1985, Lake 2007). In contrast to structural measurements (e.g., chlorophyll *a* standing crop, organic matter standing stocks, or organism biomass/abundance), which represent snapshots of environmental status, functional metrics (e.g., nutrient uptake, metabolism, decomposition, and secondary production) are expressed as a rate of change over time (Gessner and Chauvet 2002). Ecosystem ecologists, including many stream ecologists, are well positioned to unite functional and structural measurements to provide more comprehensive analysis of restoration success and advance projects with improved and longer-lasting environmental benefits (Palmer 1997, Gessner and Chauvet 2002). In doing so, we follow the direction of Rachel Carson, often credited with sparking our culture’s modern environmental consciousness, who stated “like the resource it seeks to protect, wildlife conservation must be dynamic, changing as conditions change, seeking always to become more effective.”

1.5. Stream restoration in northern Michigan

From the late 1800s through the early 1900s, Michigan was the leading timber producer in the US, during which time the region was largely deforested and the rivers and streams were actively managed to effectively transport lumber (USFS 1993, Cordova et al. 2007). Ecological effects of intensive logging can influence stream and river ecosystems for decades to centuries afterwards, and include low in-stream wood abundance, channelization, and a severing of the linkage between terrestrial and stream ecosystems (Harding et al. 1998, Nilsson et
al. 2005). Currently, Michigan law prohibits logging within a 30 m riparian buffer in an effort to protect stream biota (VanDusen et al. 2005). In addition, managers have also added in-stream restoration structures to further promote stream ecosystem health; these include the addition of large wood (Roni and Quinn 2001), channel shading structures (Rosi-Marshall et al. 2006), boulders (Lepori et al. 2005), gravel for spawning habitat (Nakamura 1999, Palm et al. 2007), and settling basins to collect sand and sediment (Hansen et al. 1982, Avery 1996). The primary goal of most restoration projects is to enhance the growth and survivorship of sport fish, and effects of restoration on other ecosystem properties such as nutrient uptake and metabolism rates are not well studied (Minns et al. 1996). To produce sustained effects on target species or general ecosystem characteristics (e.g., organic matter retention and habitat heterogeneity), it will be critical to evaluate the effects of restoration structures on multiple ecosystem processes simultaneously (Palmer 1997).

The overarching objective of my dissertation is to understand how controls on nutrient spiraling parameters in forested headwater streams change with 1) seasonal variability, and 2) in response to stream channel restoration. I approached my research by quantifying ecosystem response metrics at two distinct but inter-connected spatial scales, the reach-scale (100 m) and at the substratum scale (cm) across seasons. Below I briefly describe each of my dissertation chapters and present a diagram to illustrate the conceptual relationships among the multiple research projects (Figure 1.2)
1.6. Research goals and dissertation outline

I begin **Chapter 2** by measuring whole-stream nutrient uptake and metabolism rates seasonally for 1 year in two reaches of three headwater streams in the Upper Peninsula of Michigan. This was a critical starting point for my research, because the influence of seasonality on whole-stream metabolism and nutrient uptake had not been previously quantified in this geographic region. In addition, it was necessary to document the influence of seasonal variation on ecosystem processes in reference conditions (i.e., in an un-restored state) prior to initiating analyses of reach-scale manipulations in the study streams.

Results from Chapter 2 suggested that stream substratum composition was strongly related to patterns of nutrient uptake among study streams. The whole-stream measurements I used in Chapter 2, however, simultaneously integrated the activity of biofilms colonizing all the different substrata in the stream. Because we anticipate restoration will affect the abundance and metabolic activity of different substrata in unique ways, in **Chapter 3** I measured substratum-specific nutrient uptake rates seasonally on dominant stream substrata: large wood, small wood, coarse benthic organic matter, fine benthic organic matter, sand, and rocks.
Individual chapters examined nutrient uptake at both whole-stream scale (Chapters 2, 5, 6), and substratum-specific (Chapters 3 and 4) scales. All chapters incorporated seasonal variation.
After documenting spatial and temporal variability in biofilm community functional metrics in Chapter 3, I was interested in exploring how biofilm activity (i.e., respiration rates) would respond to nutrient enrichment and how enrichment may alter the microbial community composition of stream biofilms. In Chapter 4, I measured nutrient limitation status of biofilms colonizing inorganic and organic surfaces, and explored the relationship between microbial community structure and function on organic substrata. I used a well-established assay, nutrient diffusing substrata, which exposes biofilms to different environmental conditions, and deployed them seasonally in each of our study streams. In addition, I used novel molecular techniques to describe microbial community structure on selected substrata in response to nutrient amendment.

For my final two chapters, I synthesized results generated from studying the controls on spatial and temporal variation of nutrient uptake and metabolism rates at the whole-stream scale (Chapter 2) with conclusions regarding the functional roles of biofilms on different substrata (Chapters 3 and 4), into a larger analysis of the effect of 2 common stream restoration methods on stream ecosystem function.

The restoration strategy I examined in Chapter 5 was a reach-scale manipulation completed by the US Forest Service to increase the spawning habitat and overall production of native brook trout, a method that has been in use in Michigan since the early 1980s. A ~10m sediment trap was created to collect sand and fine organic matter at the upstream end of the restoration reach, and downstream, the stream banks were stabilized by adding logs and boulders
oriented parallel to the stream channel. Finally, pea gravel was added to the channel to improve trout spawning habitat. The goal was to retain all organic matter and sand in the upstream “trap” area, which would help with continued exposure of the added gravel in the downstream spawning channel. I measured the influence of the manipulation on whole-stream nutrient uptake and metabolism, geomorphology, and fish communities in my 3 study streams to determine the physical and biological effects of this restoration strategy.

Finally, in Chapter 6, I documented the interacting effects of a reach-scale restoration via the addition of large wood with the influence of floods on rates of whole-stream nutrient uptake and metabolism. As a critical structural component of forested headwater streams, large wood retains organic matter and establishes habitat for stream biota including microbial biofilms, macroinvertebrates, and fish (Bilby 1981, Gurnell et al. 2002). I predicted that the addition of large wood would increase retention of particulate organic matter during floods, which I measured as changes in nutrient uptake rates before and after storms in control and wood-addition reaches of the 3 replicate study streams. The study was unique because ecosystem disturbance concepts have rarely been applied to evaluations of restoration efforts, and quantifying the response of restoration projects to natural disturbance will assist in the refinement of existing management strategies.
1.7. Conclusion

I began my dissertation introduction with a quote by Heraclites, which Plato interpreted into the well-known phrase that “no man steps in the same river twice,” suggesting that both the river and the person are different entities on the second visit. The “we are and are not” in the original quote implies constant change. I found this to be especially relevant for my dissertation, where I attempted to quantify changes in river ecosystems, and in doing so, developed my own scientific skills in research, instruction, and communication. Overall, it was my goal that my combined chapters followed Ward’s (1989) suggestion that “a holistic approach that employs a spatio-temporal framework, and that perceives disturbances as forces disrupting major interactive pathways, should lead to a more complete understanding of the dynamic and hierarchical structure of natural and altered ecosystems.” By doing so, I hope I have contributed to our understanding of seasonality in stream ecosystems and its interaction with the projected effects of stream restoration, thereby directly informing the design and implementation of future restoration initiatives.

1.8. Literature Cited


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2.1. Abstract

We measured whole-stream uptake of ammonium (NH$_4^+$), nitrate (NO$_3^-$), and phosphate (as soluble reactive phosphorus; SRP) in two reaches of three forested headwater streams eight times from May 2003-April 2004 (n=46 measurements per nutrient type). We also measured factors that could affect uptake including ambient nutrient concentrations, whole-stream metabolism, and organic matter standing stocks. In all three streams, we measured the highest rates of NH$_4^+$ and NO$_3^-$ uptake velocity (V$_f$) during the spring. Low ambient NH$_4^+$ concentrations limited NH$_4^+$ uptake (U) in two streams. In one stream, when ambient NO$_3^-$ concentrations increased during summer, NO$_3^-$ V$_f$ decreased. Temporal patterns of SRP V$_f$ varied among streams, but were unrelated to variability in ambient SRP concentration. However, in all three streams, seasonal variation in SRP V$_f$ was strongly influenced by heterotrophic metabolism (as measured by community respiration; State Creek $r=0.81$, $p=0.03$; Shane Creek

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Although heterotrophic processes typically dominate in forested headwater streams, we found that variability in nutrient uptake among streams was also explained by variables related to autotrophic activity (i.e., proportion coverage of large inorganic substrata and gross primary production). We suggest that the unexpected influence of autotrophy in this study was a result of stream sampling frequency, which included winter and spring, seasons not typically sampled. Our study demonstrates that examining nutrient uptake across streams and during different seasons can provide insight into factors controlling nutrient uptake parameters.

2.2. Introduction

Nutrient supply may limit rates of primary and microbial production in stream ecosystems (Elwood et al. 1981, Suberkropp and Chauvet 1995) and nutrient availability can have bottom-up effects on stream food webs and downstream ecosystems (Rosemond et al. 1993, Vitousek et al. 1997). Rates of in-stream nutrient processing are influenced by local factors such as riparian vegetation (Sabater et al. 2000), point-source nutrient input (e.g., treated wastewater; Martí et al. 2004), larger-scale variation in climate (Mulholland 1992), and cultural eutrophication (Alexander et al. 2000). Previous studies of nutrient uptake in streams have focused largely on controlling factors of nutrient uptake rates that vary geographically (i.e., across biomes, land-use types, or along a stream gradients; (Munn and Meyer 1990, Hall and Tank 2003, Webster et al. 2003, Bernot et al. 2006). In forested headwater streams, seasonal changes in
light, organic matter inputs, and temperature may also affect biotic demand for inorganic nutrients from the water column (Mulholland et al. 1985, Mulholland et al. 2000, Hill et al. 2001). Seasonal variability in nutrient uptake has been measured in grassland streams in New Zealand (Simon et al. 2005) and forested streams in the mild Mediterranean climate of Spain (Martí and Sabater 1996), but to our knowledge, no studies have conducted a similar analysis of seasonal variability in nutrient uptake rates in temperate forests where the climate is highly seasonal.

Nutrient uptake rates in streams are affected by autotrophic (i.e., algae, bryophytes, and macrophytes) and heterotrophic (i.e., fungi and bacteria) demand (Minshall 1978, Webster and Benfield 1986, Grimm 1987). Growth and activity of these organisms can be limited by inorganic nutrient availability. In addition, autotrophic activity can be limited by light availability and substratum stability (Gregory 1980, Allan 1995) and heterotrophic activity can be limited by organic carbon quantity and quality (Tank and Webster 1998). In headwater streams that drain temperate forests, changes that occur with season can influence autotrophic and heterotrophic activity; forest canopy in summer and ice cover in winter limits light and allochthonous organic matter is highest after autumn leaf-fall (Minshall et al. 1983, Hill et al. 2001). We predicted that these seasonal changes in forested headwater streams would result in concomitant variability in nutrient uptake parameters.

Our objective was to explore seasonal variability in ammonium (NH₄⁺), nitrate (NO₃⁻), and phosphate (PO₄³⁻) uptake parameters in three adjacent forested
headwater streams. We predicted that streams with a high degree of seasonal variability would provide us with a unique opportunity to explore the factors which influence inorganic nutrient uptake. The three streams that we studied are adjacent watersheds, so we hypothesized that there would little spatial variability in nutrient uptake parameter among streams. We predicted that the highest rates of nutrient uptake would occur in spring due to minimal canopy shading and increased primary production. In addition, we predicted high rates of nutrient uptake in autumn due to high rates of community respiration associated with organic matter entering streams during leaf-fall. We measured nutrient uptake in two reaches in three streams every month and predicted that any spatial and seasonal variability observed would help us identify factors that control nutrient uptake in these streams. Our use of spatial and temporal replication allowed statistical power unique in the study of whole-stream nutrient uptake rates. Furthermore, we conducted this study in the Upper Midwestern US, where there has been little research on nutrient uptake dynamics in streams. This study provides data on nutrient uptake in relatively unmodified Midwestern streams and can serve as a reference for other Midwestern streams which have been widely affected by agricultural N and P eutrophication (Alexander et al. 2000).
2.3. Methods

2.3.1. Site description

We conducted this study in three forested, first-order streams (State, Shane, and Walton Creeks) in the Ontonagon River basin of Lake Superior, in the Upper Peninsula of Michigan, USA (Figure 2.1). These streams are tributaries of the Jumbo River and are located within approximately 4.5 km of each other. These streams were chosen as replicates for this study because they have similar orientation, geology, climate, watershed area, discharge, riparian vegetation, and logging history (Table 2.1).

The climate in this region has mild summers (mean July air temperature is 18.1°C) and cold winters (mean January temperature is -10°C; (Sommers 1984). Stream water temperatures ranged from 0.0 to 18.1°C (Table 2.1). Mean annual precipitation is approximately 76.2 cm, but snowfall in portions of the Ottawa National Forest can exceed 500 cm annually (http://www.fs.fed.us/r9/ottawa/index.shtml). Bedrock consists of the slate-containing Michigamme formation, but is not exposed in any of the stream beds. Surficial geology is composed of glacial moraine, although the texture varies by stream (Table 2.1). Riparian vegetation includes white pine (Pinus strobes L.), eastern hemlock (Tsuga canadensis L.), sugar maple (Acer saccharum Marsh.), red maple (Acer rubrum L.), alder (Alnus spp.), and paper birch (Betula papyrifera Marsh.), with an understory of mixed forbes and ferns.
2.3.2. Nutrient uptake

We conducted short-term additions of ammonium (NH$_4^+$), nitrate (NO$_3^-$), and phosphate (PO$_4^{3-}$), in two 100 m reaches (separated by 20-50 m) of each stream monthly from May to October 2003, December 2003, and April 2004. Extreme winter conditions limited access from January to March 2004 at all sites and in December 2003 at Walton Creek.

We added nutrients in two separate short-term additions using standard methods (Stream Solute Workshop 1990, Webster and Ehrman 1996). One addition contained ammonium as NH$_4$Cl and NaCl as a conservative tracer measured as conductivity and the other nitrate as NaNO$_3$, phosphate as KH$_2$PO$_4$, and NaBr as a conservative tracer measured as bromide. We added NH$_4^+$ and NO$_3^-$ separately to avoid potential influence of nitrification on NO$_3^-$ uptake, but
NO$_3^-$ samples taken during previous NH$_4^+$ releases showed no detectable downstream accumulation of water column NO$_3^-$ resulting from nitrification of added NH$_4^+$ (J. Tank, unpubl. data). Because of limited daylight hours during field sampling, we added two solutes in unison (NO$_3^-$ and PO$_4^{3-}$) to maximize the number of reaches sampled in one day, but we may have potentially released limitation of one nutrient, altering uptake of the other. Although we did not test the effect of added NO$_3^-$ on SRP uptake or vice versa by comparing individual and combined releases in these study streams, we have tested it previously in low-nutrient systems and uptake of N or P was never influenced by the presence of the other during a short-term release (see Hall and Tank 2003). This is likely because the biology of these systems cannot respond quickly enough to take advantage of the short-term increase (<60min) in nutrient supply.

Prior to solute additions, we collected water samples every 20 m downstream of the release site to measure ambient solute concentrations and conductivity. We added solutes at a rate of 200 mL min$^{-1}$ (Fluid Metering, Inc. Lab pump Model RHB, Syosset, NY) to raise nutrient concentrations slightly above ambient concentrations (+5-20 μg NH$_4^+$-N or PO$_4^{3-}$- P L$^{-1}$ and + 6-130 μg NO$_3^-$-N L$^{-1}$), conductivity by 5-35 μS, cm$^{-1}$, and Br$^-$ concentration by 25-130 μg L$^{-1}$. When conservative tracer concentrations were uniform throughout the 100 m reach (plateau stage), we collected three replicate water samples at each of 5 sites within each study reach. We filtered samples in the field through glass fiber filters (GFF) with pore size of 0.45 μm (Type A/E GFF, Pall Corporation, Ann
Arbor, MI), and samples were frozen until solutes were analyzed in the
laboratory.

To measure NO$_3^-$ and Br$^-$ concentrations, we used ion chromatography
(Dionex Model DX600, Sunnyvale, CA) with AS14A analytical and guard
columns and a 500 μL injection loop. We measured ammonium concentrations
using the phenylhypochlorite technique (Solorzano 1969), and PO$_4^{3-}$
concentrations as soluble reactive phosphorus (SRP) using the molybdate-
antimony method (Murphy and Riley 1962). SRP is generally an overestimate of
PO$_4^{3-}$ (Hudson et al. 2000), so our values for ambient SRP and SRP U may
overestimate actual values. We used a YSI conductivity meter (Model 30, Yellow
Springs, OH) or Hydrolab Minisonde (Model 4A, Loveland, CO) to measure
specific conductivity. We used a Shimadzu TOC-V/TNM total organic carbon
analyzer with a total nitrogen module to measure total nitrogen (TN), and
calculated dissolved organic N (DON) concentrations as the difference between
TN and NO$_3^-$ + NH$_4^+$ concentrations.

We calculated nutrient uptake lengths (S$_w$) using background-corrected
nutrient concentrations (enriched minus ambient concentration) divided by
background-corrected tracer concentrations and plotted the natural log of this
fraction against distance downstream, taking the absolute value of the inverse of
the slope to calculate S$_w$ (Stream Solute Workshop 1990). From S$_w$ we calculated
uptake velocity ($V_t$) as (discharge/width)/S$_w$, and then calculated areal nutrient
uptake rate (U) as: $V_t$ * ambient nutrient concentration (Stream Solute Workshop
1990). Because S$_w$ is highly influenced by discharge, uptake velocity and U are
the most useful parameters for comparing nutrient uptake across spatial or temporal scales that have varying discharge (Davis and Minshall 1999, Hall et al. 2002). Although, in comparison to isotopic tracer measurements, short-term nutrient additions have been shown to overestimate $S_w$ due to saturation of benthic nutrient demand (Mulholland 1992, Hall et al. 1998); we found no relationship between enrichment concentration (proportion enriched above ambient concentration) and $S_w$ for $\text{NH}_4^+$ ($R^2=0.09$, $p=0.57$), $\text{NO}_3^-$ ($R^2=0.03$, $p=0.26$), or $\text{PO}_4^{3-}$ ($R^2=0.01$, $p=0.55$) suggesting saturation did not occur. In addition, we assume that potential overestimates of uptake parameters in this study are equal across streams and dates.

2.3.3. Whole-stream metabolism

We calculated whole-stream metabolism by measuring changes in $O_2$ concentrations and temperature in 10 min increments for 32 h immediately prior to or following the short-term nutrient additions on all dates, excluding December and April, using a field-calibrated Hydrolab minisonde (Marzolf et al. 1994, Young and Huryn 1998). Reaeration was estimated by releasing a conservative gas (propane or sulfur hexafluoride) on the same day as the nutrient additions and regressing the decline in $\text{Br}^-$-corrected gas concentrations at each downstream collection point (Wanninkhof et al. 1990). Propane and sulfur hexafluoride concentrations were measured on a gas chromatograph (Varian Model STAR 600, Varian Analytical Instruments, Walnut Creek, CA) with electron capture detector (sulfur hexafluoride) or flame ionization detector (propane).
We calculated community respiration (CR) as average reaeration-corrected O₂ flux during the dark and gross primary production (GPP) as the sum of the instantaneous change in O₂ concentration (reaeration corrected) during daylight hours minus CR. This method does not include anaerobic respiration and assumes respiration in the light is equal to that in the dark (Uehlinger 2000). There was no significant dilution within our study reaches, as measured by conservative tracers, and therefore we did not correct for groundwater O₂ inputs (Hall and Tank 2005).

2.3.4. Organic matter standing stock and substratum distribution

At five randomly selected locations along each 100 m reach, we inserted a 804 cm² core (19 L plastic bucket with the bottom removed) 10 cm into the stream benthos, vigorously stirred the substrata, removed all coarse benthic organic matter (CBOM) with a 1 mm sieve, and subsampled the remaining sediment slurry to estimate fine benthic organic matter (FBOM). In the laboratory, we separated CBOM into wood, non-wood, and bryophytes. We filtered FBOM onto a pre-ashed and weighed GFF filter, dried the filter for 3-7 days at 60°C, measured dry mass, and then determined ash-free dry mass (AFDM) following combustion (3 hours at 550°C). We extracted chlorophyll a (Chl a) from FBOM filtered onto a GFF using the non-acidification, hot ethanol method and measured Chl a concentrations on a Turner Designs Model TD-700 Fluorometer (Sartory and Grobbelaar 1984). All measurements of CBOM,
FBOM, and Chl $a$ were taken concurrently with all nutrient uptake measurements, with an additional sampling point in March 2003.

We estimated benthic coverage of various substratum types (FBOM, CBOM + wood, sand, gravel, cobble + boulder, and bryophytes) using 21 transects 5 m apart along each 100 m reach, and recorded substratum type every 10 cm across each transect in May and August 2003. We quantified percent coverage for each substratum. Large woody debris volume and density in these streams were measured in the summer of 2003 (Cordova et al. 2007).

2.3.5. Statistical analyses

We used one-way ANOVA to compare physiochemical, organic matter and Chl $a$ standing stocks, substratum percent coverage, and whole-stream metabolism measurements among the three study streams. We used repeated measures (rm) ANOVA to analyze temporal and spatial variation of nutrient uptake metrics, using the two reaches in each stream as replicates ($n=16$ for State and Shane, $n=14$ for Walton for each nutrient type). If a significant interaction between stream and date resulted, we used 1-way ANOVA across streams for each month using Bonferroni adjusted $p$-value of 0.05/8 months = 0.00625 to determine significant differences. In addition, we used rmANOVA followed by Tukey’s multiple comparison test for each individual stream to compare uptake rates among dates. For all rmANOVAs, we used a first-order autoregressive covariance structure (after Simon et al. 2005).
We used linear regression to examine the relationship between nutrient uptake and factors which directly control uptake including temperature, whole-stream metabolism, and ambient nutrient concentration (as NH$_4^+$, NO$_3^-$, and SRP independently, and as DIN:SRP ratio). Pearson’s product-moment correlation was used to determine the association between nutrient uptake and metabolism and factors which indirectly control uptake including organic matter and Chl $a$ standing stocks, large woody debris (LWD) density, and substrata percent coverage. We used all measurements (both reaches in each stream) as independent observations (V$_f$ and $U_n$=16 State and Shane, n=14 Walton; CR and GPP $n$=7 State, $n$=9 Shane and Walton). We used the coefficient of variation to compare variability in V$_f$ between reaches, among streams, and among dates. Statistical analyses were done using SAS 9.1 (SAS Institute) or SYSTAT 10.2 (SYSTAT Software).

2.4. Results

2.4.1. Stream water physiochemical parameters

In all three streams, discharge was highest in spring and lowest in summer and fall. There were no significant differences in annual discharge among streams (one-way ANOVA, $p=0.124$; Table 2.1). In general, ambient water concentrations of NH$_4^+$-N and SRP were low, ranging from 2-15 and 2-10 μg L$^{-1}$, respectively; however, NH$_4^+$-N concentrations were higher in July and December and SRP concentrations were higher in mid-summer (Table 2.1). Across all
streams, NO$_3^-$ concentrations were much higher than NH$_4^+$ (from 21-75 times higher) and SRP (from 30-108 times higher). We did not observe differences in NH$_4^+$ concentrations among streams. SRP concentrations were significantly different among streams (highest in Shane Creek and lowest in Walton Creek). Nitrate concentrations were consistently higher in Walton Creek compared to the other two streams.

2.4.2. Nutrient uptake rates ($S_w$, $V_f$, and U)

To examine temporal patterns in nutrient uptake we used $V_f$ and $U$ rather than $S_w$. Ammonium $V_f$ differed among months (rmANOVA, $p<0.0001$) and streams (rmANOVA, $p=0.0001$), but there was no significant interaction between time and stream ($p=0.18$). We measured the highest NH$_4^+$ $V_f$ in April and in State Creek (Figure 2.2A). Because concentrations were consistently low, patterns in NH$_4^+$ areal uptake ($U$) were similar to $V_f$. 
## TABLE 2.1

<table>
<thead>
<tr>
<th></th>
<th>State</th>
<th>Shane</th>
<th>Walton</th>
<th>ANOVA</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
<td>Mean</td>
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<tr>
<td>Watershed area (km²)</td>
<td>24.0</td>
<td></td>
<td></td>
<td>45.5</td>
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<tr>
<td>Stream gradient (%)</td>
<td>1.8</td>
<td>0.9</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Year last logged</td>
<td>1967</td>
<td>1967</td>
<td></td>
<td>1915</td>
</tr>
<tr>
<td>Surficial geology</td>
<td>Terminal moraine, coarse texture</td>
<td>River valley/lake plain, coarse to fine texture</td>
<td>Recessional moraine, coarse texture</td>
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<tr>
<td>Temperature (°C)</td>
<td>6.2</td>
<td>0.0-18.1</td>
<td>6.7</td>
<td>0.0-17.9</td>
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<tr>
<td>Discharge (L s⁻¹)</td>
<td>67.7</td>
<td>0.5</td>
<td>1.0-104.1</td>
<td>39.8</td>
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<tr>
<td>Conductivity (μS cm⁻¹)</td>
<td>141.5</td>
<td>9.8</td>
<td>83.2-164.3</td>
<td>83.7</td>
</tr>
<tr>
<td>Wetted width (m)</td>
<td>2.2</td>
<td>0.1</td>
<td>2.04-2.31</td>
<td>2.4</td>
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Nutrient concentrations

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>State</th>
<th>Shane</th>
<th>Walton</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺ (μg N L⁻¹)</td>
<td>5</td>
<td>1.1</td>
<td>2.0-10.8</td>
<td>8.3</td>
</tr>
<tr>
<td>NO₃⁻ (μg N L⁻¹)</td>
<td>149.4</td>
<td>5.9</td>
<td>127.3-170.6</td>
<td>169.8</td>
</tr>
<tr>
<td>SRP (μg P L⁻¹)</td>
<td>4.8</td>
<td>0.7</td>
<td>2.2-8.2</td>
<td>7.1</td>
</tr>
</tbody>
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TABLE 2.1 (Continued)

<table>
<thead>
<tr>
<th></th>
<th>State</th>
<th>Shane</th>
<th>Walton</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>DIN:SRP†</td>
<td>57.9b</td>
<td>10.8</td>
<td>28.6-122.4</td>
<td>40.3b</td>
</tr>
<tr>
<td>DON (mg N L⁻¹)</td>
<td>1.1b</td>
<td>0.1</td>
<td>0.77-1.31</td>
<td>1.0b</td>
</tr>
<tr>
<td>Organic matter standing stock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBOM (g AFDM m⁻²)</td>
<td>125.4</td>
<td>23.1</td>
<td>33.5-222.0</td>
<td>177.7</td>
</tr>
<tr>
<td>CBOM (g AFDM m⁻²)</td>
<td>8.72</td>
<td>5.96</td>
<td>0.01-48.5</td>
<td>17.8</td>
</tr>
<tr>
<td>Wood (g AFDM m⁻²)</td>
<td>145.6</td>
<td>81.4</td>
<td>0.4-673.4</td>
<td>99.08</td>
</tr>
<tr>
<td>Chlorophyll a (μg cm⁻²)</td>
<td>1.65</td>
<td>0.64</td>
<td>0.16-4.38</td>
<td>1.12</td>
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<tr>
<td>LWD volume (m³ m⁻²)</td>
<td>0.005</td>
<td>0</td>
<td>0.004-0.007</td>
<td>0.019</td>
</tr>
<tr>
<td>LWD density (no. m⁻²)</td>
<td>0.116b</td>
<td>0.01</td>
<td>0.109-0.123</td>
<td>0.183a</td>
</tr>
<tr>
<td>Substrata coverage (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBOM</td>
<td>4.4c</td>
<td>0.72</td>
<td>3.7-5.3</td>
<td>14.1b</td>
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<tr>
<td>CBOM + wood</td>
<td>17</td>
<td>2.2</td>
<td>14.8-19.2</td>
<td>25.7</td>
</tr>
<tr>
<td>Sand</td>
<td>15</td>
<td>3.3</td>
<td>11.7-18.3</td>
<td>30.1</td>
</tr>
<tr>
<td>Gravel</td>
<td>30.4</td>
<td>1.1</td>
<td>19.2-21.5</td>
<td>24.2</td>
</tr>
<tr>
<td>Cobble + boulder</td>
<td>22.5a</td>
<td>1.6</td>
<td>20.9-24.0</td>
<td>5.9b</td>
</tr>
<tr>
<td>Bryophyte</td>
<td>11.3a</td>
<td>4.9</td>
<td>6.3-16.2</td>
<td>0.0b</td>
</tr>
</tbody>
</table>
TABLE 2.1 (Continued)

<table>
<thead>
<tr>
<th>State</th>
<th>Shane</th>
<th>Walton</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Whole-stream metabolism (g O₂ m⁻² d⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPP</td>
<td>0.9</td>
<td>0.4</td>
<td>0.17-2.42</td>
</tr>
<tr>
<td>CR</td>
<td>7.6</td>
<td>1.8</td>
<td>3.44-13.31</td>
</tr>
<tr>
<td>P:R</td>
<td>0.2</td>
<td>0.1</td>
<td>0.02-0.57</td>
</tr>
</tbody>
</table>

*Groupings from Tukey's multiple comparison test after 1-way ANOVA comparing among streams*

† DIN:SRP is the molar ratio of N in DIN to P in SRP

‡ LWD volume and number of LWD pieces per stream area, from Cordova et al. *(in press)*.

Note: Abbreviations are as follows: NH₄⁺ = ammonium, NO₃⁻ = nitrate, SRP = soluble reactive phosphorus, DIN = dissolved inorganic nitrogen, DON = dissolved organic nitrogen, FBOM = fine benthic organic matter, CBOM = coarse benthic organic matter, LWD = large woody debris, GPP = gross primary production, CR = community respiration, and P:R = GPP:CR. *F* and *p*-values are the result of one-way ANOVA among streams; *p*-values ≤ 0.05 are in bold.
We measured similar trends in NO$_3^-$ V$_f$, with significant differences among months (rmANOVA, $p<0.0001$) and streams (rmANOVA, $p<0.0001$). Because there was a significant interaction between month and stream ($p<0.0001$), we analyzed the temporal pattern for each stream individually. Similar to NH$_4^+$, we observed a peak in NO$_3^-$ V$_f$ during April in State Creek and Walton Creek (Figure 2.2B). We did not observe any seasonal patterns in Shane Creek. Although NO$_3^-$ U generally mirrored V$_f$, in Walton Creek, we found no differences in NO$_3^-$ U among months (rmANOVA, $p=0.11$). We attribute this discrepancy to the fact that when NO$_3^-$ V$_f$ was low in late summer and fall there was a peak in ambient NO$_3^-$ concentration. This combination of fluctuating V$_f$ and concentrations led to consistent NO$_3^-$ U across months. Comparing among streams, we found that State Creek had significantly higher NO$_3^-$ V$_f$ than the other two streams in July and August (ANOVA, $p=0.003$ and $p=0.001$, respectively; Figure 2.2B).

We found significant differences in SRP V$_f$ among streams (rmANOVA, $p<0.0001$) and among months (rmANOVA, $p=0.001$). Because there was a significant interaction between stream and month ($p=0.0003$), we compared spatial patterns in SRP for each month separately. In Walton Creek, SRP V$_f$ was highest in May (ANOVA, $p=0.001$), State Creek SRP V$_f$ was higher than the other two streams in August (ANOVA, $p<0.001$), and in Shane Creek there was no seasonal pattern. Because SRP concentrations were consistent among streams and months, SRP U patterns were the same as V$_f$. 

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Figure 2.2. Mean (±SE) rates of (A) gross primary production (GPP) and (B) community respiration (CR) in the three study streams from May 2003 through October 2003. Data points in white indicate only one replicate, data points in black are the mean of two replicates. (*) indicates that State had higher GPP than Shane in May, but GPP in Walton was not significantly different than either (ANOVA, $p=0.05$), and that Walton had higher CR than either State or Shane (ANOVA, $p=0.03$).

2.4.3. Comparison of uptake metrics among nutrient types

We observed no differences in uptake metrics ($S_w$ or $V_f$) among solutes in State and Walton Creeks. In Shane Creek $S_w$’s were longer and $V_f$’s were lower for $\text{NO}_3^-$ than...
NH$_4^+$ or SRP (ANOVA p<0.02). NO$_3^-$ U was significantly higher than NH$_4^+$ or SRP U in all three streams, due to consistently high nitrate concentrations and $V_f$ (Table 2.2).

2.4.4. Temperature, ambient nutrient concentrations, and nutrient uptake metrics

Temperature was unrelated to nutrient uptake rates (Table 2.3) except a positive relationship to SRP $V_f$ in Walton Creek, and a negative relationship to NH$_4^+$ and SRP $V_f$ in Shane Creek. We observed similar patterns with U.

We found a positive linear relationship between ambient NH$_4^+$ concentration and NH$_4^+$ U in State Creek ($r=0.87$, $p<0.01$) and Shane Creek ($r=0.71$, $p<0.01$), but no relationship between ambient NH$_4^+$ concentration and $V_f$ (Table 2.4). There were no significant relationships between ambient NO$_3^-$ or SRP concentrations and NO$_3^-$ U or SRP U or $V_f$ (with the exception of State Creek; Table 2.4). In Walton Creek, we found a significant negative relationship between ambient NO$_3^-$ and NO$_3^-$ $V_f$.

Other studies have demonstrated that variation in inorganic N and P uptake was explained by the ratio between dissolved inorganic nitrogen and soluble reactive phosphorous (DIN:SRP; Munn and Meyer 1990, Simon et al. 2005). We did not find a consistent pattern in the relationships between DIN:SRP and $V_f$ for NH$_4^+$, NO$_3^-$, or SRP in our study streams (Table 2.3). In Walton Creek, NH$_4^+$ and SRP $V_f$ were negatively related to DIN:SRP, but in Shane Creek, SRP $V_f$ was positively related to DIN:SRP ratio. The relationships between U and DIN:SRP were identical to $V_f$, except in Shane Creek NO$_3^-$ U was positively related to DIN:SRP.
**TABLE 2.2**

ANNUAL MEAN, STANDARD ERROR (SE), AND RANGE OF NUTRIENT UPTAKE METRICS

<table>
<thead>
<tr>
<th>State</th>
<th>Shane</th>
<th>Walton</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Uptake length (Sn; m)</td>
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<tr>
<td>NH₄⁺</td>
<td>300</td>
<td>57</td>
<td>187-648</td>
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<tr>
<td>NO₃⁻</td>
<td>348</td>
<td>74</td>
<td>144-718</td>
</tr>
<tr>
<td>SRP</td>
<td>282</td>
<td>67</td>
<td>98-532</td>
</tr>
<tr>
<td>Uptake velocity (Vf; mm s⁻¹)</td>
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<td></td>
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<tr>
<td>NH₄⁺</td>
<td>0.127</td>
<td>0.021</td>
<td>0.048-0.251</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.126</td>
<td>0.03</td>
<td>0.028-0.314</td>
</tr>
<tr>
<td>SRP</td>
<td>0.179</td>
<td>0.041</td>
<td>0.039-0.323</td>
</tr>
<tr>
<td>Uptake rate (U; mg m⁻² d⁻¹)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>59</td>
<td>16</td>
<td>16-131</td>
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<td>NO₃⁻-N</td>
<td>1653</td>
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<tr>
<td>SRP</td>
<td>67</td>
<td>17</td>
<td>16-168</td>
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*ab, ab* Groupings from Tukey's multiple comparison test after 1-way ANOVA comparing among streams

Note: Abbreviations are as follows: ammonium (NH₄⁺), nitrate (NO₃⁻), and soluble reactive phosphorus (SRP). F and p-values are the result of one-way ANOVA among streams (n=23); p-values ≤ 0.05 are in bold.
**TABLE 2.3**

LINEAR REGRESSION OF NUTRIENT UPTAKE WITH DISSOLVED INORGANIC N (NH$_4^+$ + NO$_3^-$; DIN) TO SRP (DIN:SRP), TEMPERATURE, GROSS PRIMARY PRODUCTION (GPP), AND COMMUNITY RESPIRATION (CR)

<table>
<thead>
<tr>
<th>Stream</th>
<th>DIN:SRP$^*$</th>
<th>Temperature</th>
<th>GPP</th>
<th>CR</th>
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<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
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<tr>
<td>NH$_4^+$ V$_f$</td>
<td>State</td>
<td>0.020†</td>
<td>0.940</td>
<td>-0.047†</td>
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<td></td>
<td>Shane</td>
<td>0.112†</td>
<td>0.680</td>
<td>-0.662†</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>-0.809†</td>
<td>&lt;0.001</td>
<td>0.419†</td>
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<tr>
<td>NO$_3^-$ V$_f$</td>
<td>State</td>
<td>0.150†</td>
<td>0.579</td>
<td>-0.153†</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>0.478†</td>
<td>0.061</td>
<td>-0.432†</td>
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<tr>
<td></td>
<td>Walton</td>
<td>-0.431†</td>
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<td>0.244†</td>
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<td>SRP V$_f$</td>
<td>State</td>
<td>0.374†</td>
<td>0.153</td>
<td>-0.290†</td>
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<tr>
<td></td>
<td>Shane</td>
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<td>-0.489†</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>-0.637†</td>
<td>0.014</td>
<td>0.668†</td>
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</table>

$^*$DIN:SRP is the molar ratio of N in DIN to P in SRP

† Dependent variable (V$_f$) transformed (ln) because residuals strongly differed from normal distribution (KS test, $p<0.01$)

Note: Abbreviations are as follows: uptake velocity (V$_f$), ammonium (NH$_4^+$), nitrate (NO$_3^-$), soluble reactive phosphorus (SRP). For metabolism measurements $n=7$ State, $n=9$ Shane and Walton, for DIN:SRP and temperature $n=16$ for State and Shane, $n=14$ for Walton; $p$-values $\leq 0.05$ are in bold; $p$-values $\leq 0.05$ are in bold.
**TABLE 2.4**

LINEAR REGRESSION OF AMBIENT NUTRIENT CONCENTRATIONS OF AMMONIUM WITH UPTAKE VELOCITY ($V_f$) AND AREAL UPTAKE RATE (U)

<table>
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<th>Solute</th>
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<th>$V_f$ $r$</th>
<th>$p$</th>
<th>$U$ $r$</th>
<th>$p$</th>
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<tr>
<td>$\text{NH}_4^+$</td>
<td>State</td>
<td>0.329 †</td>
<td>0.214</td>
<td>0.874 †</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Shane</td>
<td>0.405 †</td>
<td>0.120</td>
<td>0.714 †</td>
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<tr>
<td></td>
<td>Walton</td>
<td>0.227 †</td>
<td>0.435</td>
<td>0.430 †</td>
<td>0.125</td>
</tr>
<tr>
<td>$\text{NO}_3^-$</td>
<td>State</td>
<td>0.428 †</td>
<td>0.099</td>
<td>0.541 †</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>0.418 †</td>
<td>0.107</td>
<td>0.395 †</td>
<td>0.130</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>-0.753 †</td>
<td>0.002</td>
<td>-0.263 †</td>
<td>0.364</td>
</tr>
<tr>
<td>SRP</td>
<td>State</td>
<td>-0.283 †</td>
<td>0.287</td>
<td>0.235 †</td>
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</tr>
</tbody>
</table>

† Dependent variables ($V_f$ or U) transformed (ln) because residuals strongly differed from normal distribution (KS test, $p<0.01$)

Note: Abbreviations are as follows: ammonium ($\text{NH}_4^+$), nitrate ($\text{NO}_3^-$), and soluble reactive phosphorus (SRP). $n=16$ for State and Shane, $n=14$ for Walton; $p$-values $\leq 0.05$ are in bold.
2.4.5. Whole-stream metabolism

We measured metabolism 25 times, which included 6 reaches and 6 different months (May through October; Figure 2.3). A complete temporal analysis of metabolism data, comparable to nutrient uptake parameters (i.e., rm-ANOVA; where \( n = 46 \)), was not possible because we lacked necessary data for all reaches and all dates. However there are sufficient measurements to statically examine spatial and temporal patterns in GPP and CR. Overall, mean GPP, CR, and P:R ratio (GPP:CR) did not differ among streams (Table 2.1). Typically, CR exceeded GPP except in Walton Creek in July and September. GPP generally declined from May through October except in Shane Creek where it was consistently low (<0.2 g O\(_2\) m\(^{-2}\) d\(^{-1}\); Figure 2.3). In May, State Creek had higher GPP than Shane Creek, but was not different in Walton Creek. CR was higher in Walton Creek in May than State and Shane Creeks (Figure 2.3).

2.4.6. Whole stream metabolism and nutrient uptake

The strongest relationship between metabolism and nutrient uptake was that CR explained 65-84% of the variation in SRP \( V_f \) in all streams (Table 2.3). For the other two solutes, the pattern was not consistent among streams. NO\(_3^-\) \( V_f \) was related to CR only in Shane and Walton Creeks, and CR and NH\(_4^+\) \( V_f \) were related only in Walton Creek. GPP was significantly related to \( V_f \) for all three nutrient types only in Walton Creek (Table 2.3). Nutrient uptake metrics were not significantly related to the ratio of GPP:CR in any case. Whole-stream uptake, \( U \), showed similar patterns as \( V_f \), except there was no
relationship between NO$_3^-$ U and CR in Walton Creek. Generally, there were no significant relationships between GPP or CR and any abiotic variables (i.e., NH$_4^+$, NO$_3^-$, or SRP concentrations, DIN:SRP, or temperature; data not shown), except in Walton Creek we observed a positive relationship between temperature and GPP ($r=0.97$, $p<0.01$).

![Graph showing GPP and CR in three streams from May 2003 to October 2003.](image)

Figure 2.3. Mean (±SE) rates of (A) gross primary production (GPP) and (B) community respiration (CR) in the three study streams from May 2003 through October 2003. Data points in white indicate only one replicate, data points in black are the mean of two replicates. (*) indicates that State had higher GPP than Shane in May, but GPP in Walton was not significantly different than either (ANOVA, $p=0.05$), and that Walton had higher CR than either State or Shane (ANOVA, $p=0.03$).
2.4.7. Organic matter standing stocks, substratum distribution, and nutrient uptake metrics

Among streams, standing stocks of FBOM ranged from 33.5 to 542.4 g AFDM m\(^{-2}\), CBOM ranged from 0.0 to 134.7 g AFDM m\(^{-2}\), and wood ranged from 0.4 to 673.4 g AFDM m\(^{-2}\) (Table 2.1). There were no significant relationships between the nutrient demand and CBOM or FBOM standing stock, Chl \(a\) concentration, or LWD density (data not shown).

In all three streams, sand and gravel accounted for approximately 45% of the substrata, while FBOM and CBOM+wood ranged from 17-24% (Table 2.1). State Creek had significantly higher gravel+cobble+boulder and bryophyte coverage than Shane or Walton Creeks (Table 2.1). We found significant positive correlations between percent coverage of gravel+cobble+boulder in the six study reaches and mean NH\(_4^+\) \(V_f\) \(r=0.86\),
\( p=0.03 \), NO\(_3^-\) \( V_f (r=0.92, p=0.01) \), and SRP \( V_f (r=0.83 p=0.04; \text{Figure 2.4}) \). There was no relationship between percent coverage of organic substrata (fine + coarse; including wood) and \( V_f \) for NH\(_4^+\) and SRP, yet there was a negative correlation with NO\(_3^-\) \( V_f (r=-0.84, p=0.04; \text{Figure 2.4}) \).

2.5. Discussion

2.5.1. Ambient nutrient concentrations and nutrient uptake metrics

Human land use (e.g., agriculture, urbanization) often changes nutrient concentrations in streams (Paul and Meyer 2001, Webster et al. 2003) and previous research has shown that ambient nutrient concentrations influence uptake metrics (Davis and Minshall 1999, Dodds et al. 2002). If nutrient availability (or concentration) is limiting nutrient uptake, then we would expect a positive relationship between concentration and \( U \) (either linear or Michaelis-Menten models) and if nutrient uptake is approaching saturation (i.e., on the curving portion of the Michaelis-Menten model), we would expect a negative relationship between concentration and \( V_f \) (Figure 2 in Davis and Minshall 1999). Ideally, testing saturation models requires multiple enrichments in the same stream over relatively short time periods, to minimize variation in biotic and abiotic factors, however, other studies have successfully used data collected in different streams and at different times to explore the relationship between ambient nutrient concentrations and uptake metrics (Davis and Minshall 1999, Simon et al. 2005, Newbold et al. 2006).
Examining the relationship between $U$ and ambient nutrient concentration can illustrate which factors (ambient concentration or $V_f$) more strongly affect $U$ (Davis and Minshall 1999) even though they are autocorrelated (because $U$ is calculated with ambient concentration). When $U$ increases with increasing concentration, thereby driving patterns in $U$, the point at which increasing concentration no longer increases $U$ is the point at which nutrient saturation should occur (Davis and Minshall 1999, Dodds et al. 2002). Because nutrient concentrations in our study streams were generally low, (except for NO$_3^-$ in Walton Creek which peaked at 762 $\mu$gNO$_3^-$-N L$^{-1}$), we did not expect to see saturation.

Ambient concentration was important in determining NH$_4^+$ $U$ in State and Shane Creeks, but not in Walton (Table 2.4). For the relationship between NH$_4^+$ concentration and NH$_4^+$ $U$, there was minimal difference between the linear (Table 2.4) and Michaelis-Menten models (State $r=0.71$; $p<0.01$, Shane $r=0.70$, $p<0.01$), which suggested that the range of NH$_4^+$ concentrations was not high enough to enter the saturation portion of the Michaelis-Menten curve. Similarly, Dodds et al. (2002) found that there was little difference in the explanatory power of linear and Michaelis-Menten models in explaining the relationship between NH$_4^+$ concentration and NH$_4^+$ $U$ in two Kansas streams. However, we hesitate to imply that there are saturation thresholds that hold across streams and biomes due to the particularly strong role that biology plays in nutrient uptake, which can vary considerably across streams of varying land use and biomes.

While we did not find any significant relationships between NO$_3^-$ concentration and $U$, the relationship between concentration and $V_f$ can also indicate that concentrations may be nearing saturation (Davis and Minshall 1999). We found that
higher NO$_3^-$ concentrations were concurrent with decreased NO$_3^-$ $V_f$ in Walton Creek, which had the greatest range of NO$_3^-$ concentrations, while State and Shane Creeks showed no significant patterns with NO$_3^-$ concentration and $V_f$. This suggests NO$_3^-$ concentration may not affect NO$_3^-$ $V_f$ below the maximum level recorded in these two streams, 240 μg NO$_3^-$-N L$^{-1}$. In agriculturally influenced streams in southwestern Michigan, saturation of NO$_3^-$ uptake was noted at the lowest NO$_3^-$ concentration recorded, ~400 μg NO$_3^-$-N L$^{-1}$ (Bernot et al. 2006). These estimated saturation values (<762 μg NO$_3^-$-N L$^{-1}$ in Walton Creek and <400 μg NO$_3^-$-N L$^{-1}$ in MI agricultural streams) are much lower than the maximum allowable daily concentration of 10,000 μg NO$_3^-$-N L$^{-1}$ for drinking water standards (Environmental Protection Agency 2002) indicating that NO$_3^-$-N concentrations may saturate biological demand in many streams in this biome prior to reaching a level that initiates mitigation.

Our study streams exhibited both high background NO$_3^-$ concentrations and relatively high NO$_3^-$ $V_f$ values, resulting in U values that are among the highest in the literature. Our highest NO$_3^-$ U occurred in spring, when peak GPP was measured as a result of a bloom of filamentous green algae prior to leaf out, and discharge was highest due to spring runoff conditions, both contributing to higher $V_f$ (because discharge is in the numerator in the $V_f$ calculation; see methods). We are not aware of other measurements of NO$_3^-$ uptake in forested headwater streams made during post-snowmelt high flows in spring which would allow direct comparison. Some previously published values have been in the same range or higher (686 mg NO$_3^-$-N m$^{-2}$ d$^{-1}$ in Davis and Minshall 1999, 7,299 mg NO$_3^-$-N m$^{-2}$ d$^{-1}$ in Webster et al. 2003, and 1,810 mg NO$_3^-$-N m$^{-2}$
$2 \text{ d}^{-1}$ in Bernot et al 2006), and these were also associated with higher background concentrations of nitrate and larger streams ($Q>50 \text{ L s}^{-1}$).

To test the plausibility of our NO$_3^-$ U values, we calculated the molar ratio of C metabolism (C respired + C fixed) to N uptake (NO$_3^-$ + NH$_4^+$-N), and found very low values (1-2.5; modified approach from Webster et al. 2003, Hall and Tank 2003). However, similar calculations for other sandy-bottomed, higher-nitrate streams in Michigan were highly variable, but included very low values as well (e.g., 0.9-40.6 for Bernot et al. 2006). Since previously published work containing both whole-stream metabolism and nitrate uptake is limited (Webster et al. 2003, Hall and Tank 2003, Bernot et al. 2006), more studies are needed to allow us to examine expanded ranges of NO$_3^-$ U values.

We found little evidence that SRP concentration influenced SRP $V_f$ in our study (Table 2.4). In contrast, SRP $V_f$ declined with increasing SRP concentration in agricultural Midwestern streams (Bernot et al. 2006) and streams in upstate New York (Newbold et al. 2006); however, ambient SRP concentrations in those streams were double the range observed in our forested, upper Midwestern streams. Mulholland et al. (1990) estimated a biological saturation level of approximately 15 $\mu$g PO$_4^{3-}$-P L$^{-1}$ in Walker Branch, TN, a forested Appalachian stream, and the maximum SRP concentration we recorded was slightly lower than this value (10 $\mu$g L$^{-1}$).

The ratio of DIN:SRP has been used to explain patterns in relative DIN and SRP uptake (Munn and Meyer 1990, Simon et al. 2005), and if relative amounts of DIN or SRP were limiting uptake, we would expect that at low DIN:SRP values, DIN $V_f$ would be high and SRP $V_f$ low. In our study, there were no consistent relationships between $V_f$
and DIN:SRP (Table 2.3). This may be because DIN values were dominated by NO₃⁻, and any variability in SRP or NH₄⁺ among streams or dates was obscured. Also, SRP is typically an overestimate of PO₄³⁻, so DIN:SRP is likely an underestimate of the DIN:PO₄³⁻ ratio (Hudson et al. 2000). Dodds et al. (2003) discouraged the use of DIN:SRP to indicate stream nutrient limitation status because the ratio gives no indication of nutrient turnover rate and disregards the effect of biologically active organic N or P.

2.5.2. Temperature and nutrient uptake metrics

Unexpectedly, temperature was not a major factor controlling variation in nutrient uptake, even though we conducted releases in all 4 seasons, spanning a large range in streamwater temperatures (0-18.1°C). There was a significant negative relationship between temperature and NH₄⁺ and SRP Vᵣ in Shane Creek, no relationships in State Creek, and a significant positive relationship between temperature and SRP Vᵣ in Walton Creek (Table 2.3). The lack of consistency may be due to the timing of resource availability (e.g., organic matter and light) which were higher during colder seasons in our forested headwater streams. Previous research has shown that temperature limitation of microbial growth rates were mitigated by the availability of high quality food (Wiebe et al. 1992), which may explain the patterns we see here – temperature limitation of biofilm growth can be overcome by the availability of limiting resources such as carbon or light, which both occur at higher amounts at colder temperatures (e.g., autumn leaf-fall or just prior to spring leafout) in forested headwater streams. Additionally, the effect of temperature on stream biofilm metabolism may vary by substratum type (Tank et al. 1993, Fuss and Smock 1996), so the effects of temperature on whole-stream rates may
change with the seasonal distribution and metabolic activity (or community composition) of biofilms on different substratum types.

Other investigators who have examined the relationship between temperature and whole-stream nutrient uptake have also seen equivocal results. A positive relationship was observed between temperature and P uptake (D’Angelo et al. 1991, Meals et al. 1999, Simon et al. 2005), and N uptake (Butturini and Sabater 1998, Simon et al. 2005). However, negative relationships were shown between temperature and P (Mulholland et al. 1985) and \( \text{NH}_4^+ \) uptake (Marti and Sabater 1996). A confounding factor in comparing among these studies is the differences in the range of temperatures recorded, which vary by biome and the number of different seasons included. A major obstacle in elucidating the relationship between nutrient uptake and temperature is that it has not been experimentally manipulated, but rather deduced from descriptive studies, in which temperature is one of multiple interacting factors. Future studies should consider hypotheses that incorporate the complexity of resource availability and temperature effects on whole-stream nutrient uptake rates.

2.5.3. Seasonal variation in nutrient uptake metrics

The peaks in \( \text{NH}_4^+\ V_f \) in winter (December) and spring (April) were likely the result of (1) increased N demand by microbial decomposers in response to the pulse of allochthonous organic matter during autumn leaf fall and (2) an increase in primary production due to higher light availability just prior to spring leafout. Although we found that benthic organic matter standing stocks and Chl \( \alpha \) densities were not good predictors of \( \text{NH}_4^+\ V_f \), we infer biotic control because the seasonal pattern of \( \text{NH}_4^+\ V_f \) was identical
in all three streams, which did not occur for the other two nutrient types (Figure 2.2). The above logic supports the results from Walton Creek, where we found significant positive relationships with metabolism and \( \text{NH}_4^+ V_f \) (Table 2.3). We might have shown this relationship in State and Shane Creeks had we measured metabolism in December and April; data collected in subsequent years demonstrate these are periods of relatively high GPP and CR across streams (T. Hoellein, unpubl. data).

Other studies have demonstrated links between \( \text{NH}_4^+ V_f \) and GPP and CR. Synthesizing data across biomes, Meyer et al. (2005) showed a positive relationship between \( \text{NH}_4^+ V_f \) and total metabolism (GPP + CR) using data from multiple studies (Hall et al. 2003, Hall and Tank 2003, Webster et al. 2003). We added our data and that of Wollheim et al. (2001), Bernot et al. (2006), and Newbold et al. (2006) to that compilation, and analyzed the regressions for GPP and CR separately (rather than as total metabolism, as in Meyer et al. 2005) to distinguish autotrophic vs. heterotrophic contributions to nutrient uptake. The relationship was significant for both GPP (\( R^2 = 0.22, p<0.01 \)) and CR (\( R^2 = 0.36, p<0.01 \); Figure 2.5A, B) and consistent across (1) streams with forested, open-canopy, urban, tundra, and agricultural riparian zones, (2) streams affected by invasive species and geothermal inputs, (3) studies employing different methods to quantify nutrient uptake (including \( ^{15}\text{NH}_4^+ \) tracer additions), and (4) studies conducted during different seasons (our data set). When U was considered the dependant variable (rather than \( V_f \)), the relationships were close to being statistically significant, explaining less variation (GPP \( R^2 = 0.06, p=0.06 \); CR \( R^2 = 0.08, p=0.05 \)), likely due to the strong influence of widely varying background concentrations across studies.
Unlike patterns for NH$_4^+$, GPP did not appear to control seasonal patterns in NO$_3^-$ $V_f$. Although a trend of higher NO$_3^-$ $V_f$ in spring was found in State and Walton Creeks, there was a significant interaction between stream and time, with higher values in State Creek in July and August, and no relationship between NO$_3^-$ $V_f$ and GPP (Figure 2.2B, Table 2.3). Despite this, biotic control of NO$_3^-$ $V_f$ can be inferred from its relationship to CR (Table 2.3), and for both GPP and CR when combined with previous studies (Hall and Tank 2003, Webster et al. 2003, Bernot et al. 2006; GPP $R^2=0.09$, $p=0.05$, CR $R^2=0.19$, $p<0.01$; Figure 2.5C,D). For U, the relationship was not significant with GPP or CR. We note that there is still substantial unexplained variation for both NO$_3^-$ and NH$_4^+$ uptake patterns in the combined data sets, indicating that reach-scale metabolism metrics may provide some insight into N processing across streams, but additional site-specific factors are clearly important in fully explaining variation in N $V_f$ (Newbold et al. 2006).
Figure 2.5. Linear regression of gross primary production (GPP; natural log) or community respiration (CR) and uptake velocity (Vf; natural log) of (A,B) ammonium (NH$_4^+$), (C,D) nitrate (NO$_3^-$), and (E,F) soluble reactive phosphorus (SRP). Data are from the present study, Mulholland et al. (1997), Wolheim et al. (2001), Webster et al. (2003), Hall and Tank (2003), Hall et al. (2003), Meyer et al. (2005), Bernot et al. (2006), and Newbold et al. (2006). Closed symbols represent whole-stream enrichment and open symbols represent isotope tracers.
Temporal patterns in SRP $V_f$ were variable among streams and more strongly related to biotic (CR; Table 2.3), than abiotic factors (ambient SRP concentration, temperature, and DIN:SRP; Tables 2.4, 2.5). The dominant pattern was an increase in SRP $V_f$ in State Creek in August, with continued high rates through December (Figure 2.2C), and a parallel pattern in CR (Figure 2.3), but the cause for this pattern is unknown. We could not account for this pattern by concurrent changes in organic matter standing stocks (leaf fall did not occur until mid-October), canopy cover, temperature, hydrology (i.e., discharge or groundwater input), or human influence. While we are unsure of the underlying mechanism, the strong relationship between CR and SRP $V_f$, which explained 65-84% of the variation in SRP $V_f$ across the study streams, suggests a biological pathway.

When our data for SRP $V_f$ were combined with other studies (Mulholland et al. 1997, Meyer et al. 2005, Bernot et al. 2006), we found evidence for heterotrophic control on SRP $V_f$ across biomes (CR $R^2=0.28$, $p<0.01$; Figure 2.5E, F). Similarly, for SRP U, there was no relationship with GPP, and a significant positive relationship with CR ($R^2=0.21$, $p<0.01$). In other forested headwater streams, researchers have observed greater P retention in December (high litter retention) relative to July (Mulholland et al. 1985) and in spring and fall relative to summer (Hall et al. 2002), indicating that seasonal variation in leaf litter retention and irradiance were responsible for increased uptake. In contrast, we did not observe a fall/winter peak in SRP uptake, but we could not measure nutrient demand in November or December 2003 in Walton Creek due to heavy snowfall.
2.5.4. Interacting effects of metabolism and ambient nutrient concentration on nutrient uptake

The influence of GPP and CR on $V_f$ may decline at higher ambient nutrient concentrations. For example, agricultural streams in Michigan with high background N (as both NH$_4^+$ and NO$_3^-$) and SRP concentrations showed evidence of saturation of uptake and no relationship between either NH$_4^+$, NO$_3^-$, or SRP $V_f$ and metabolism (Bernot et al. 2006). Similarly, urban streams in Georgia with high NH$_4^+$ and SRP concentrations also showed no relationship between $V_f$ and GPP or CR (Meyer et al. 2005). In contrast, low nutrient streams in Wyoming (Hall and Tank 2003) and northern Michigan (this data set) showed little indication of nutrient saturation and positive relationships between $V_f$ and GPP or CR. Finally, by measuring $V_f$ and metabolism across a gradient of uninhabited to urban/suburbanized watersheds, Newbold et al. (2006), showed both positive relationships between $V_f$ and metabolism, as well as indications of saturation for both NH$_4^+$ and SRP $V_f$. In these cases, explanatory power was strengthened by considering the interaction between abiotic (nutrient concentration) and biotic (GPP and CR) factors across expanded geographic and concentration gradients.

2.5.5. Variation in nutrient uptake metrics among streams

Previous research in forested headwater streams suggests that the decomposition of allochthonous organic matter, as reflected in community respiration rates, would explain the majority of variation in nutrient uptake metrics (Minshall et al. 1983, Mulholland et al. 1985, Tank and Webster 1998). Our study, however, which attempted
to capture both spatial (multiple streams) and seasonal variation, suggests that variation in GPP may also play an important role in explaining patterns in nutrient uptake among forested streams. State Creek had the highest mean $V_f$ for all three nutrient types, the highest percentage of large inorganic particles (e.g., cobble/gravel) and frequency of bryophytes, resulting in generally higher GPP compared to the other streams (Table 2.1). We found positive correlations between streambed coverage by large inorganic substrata (a key substrate for algal biofilms and bryophytes) and mean $V_f$ for all three nutrient types, and no positive relationships between streambed coverage of organic matter (fine + coarse) and NH$_4^+$ and SRP $V_f$ (Figure 2.4).

Other studies have shown that reaches with larger, more stable substrata (i.e., cobble and bedrock) have higher nutrient uptake rates than stream reaches of lower bed stability (i.e., sand and small gravel; (Munn and Meyer 1990, Marti and Sabater 1996). In these studies, reach-scale metabolism was not measured directly, but the authors inferred that moss and periphyton communities on stable substrata were responsible for higher inorganic nutrient demand. Bryophytes tend to grow in streams with stable substrata, few low flows, and higher gradient (Bowden 1999), all of which occur more in State Creek, which had bryophytes covering 6-16% of benthic surface compared to <1% in the other two streams. Bryophytes have been shown to increase particulate organic matter retention and growth of epiphytic algae, which may increase N retention (Stream Bryophyte Group 1999, Ashkenas et al. 2004) and other studies report that stream reaches containing bryophytes have higher nutrient uptake rates than reaches lacking bryophytes (Davis and Minshall 1999, Slavik et al. 2004).
TABLE 2.5

COEFFICIENT OF VARIATION (%) IN UPTAKE VELOCITY (V_f) FOR AMMONIUM (NH\textsubscript{4\textsuperscript{+}}), NITRATE (NO\textsubscript{3\textsuperscript{-}}), AND SOLUBLE REACTIVE PHOSPHORUS (SRP) ACROSS SPATIAL AND TEMPORAL SCALES FOR THIS STUDY (STATE, SHANE, AND WALTON CREEKS), AND PUBLISHED VALUES

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<th>NO\textsubscript{3\textsuperscript{-}} V_f</th>
<th>SRP V_f</th>
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</table>

\textsuperscript{a}Webster et al. (2003)
\textsuperscript{b} Simon et al. (2005)
\textsuperscript{c} Martí and Sabater (1996)
- no measurement

2.5.6. Variation in nutrient uptake metrics at multiple scales

The importance of autotrophy in explaining variation in nutrient uptake rates among our 3 forested headwater streams was unexpected, but could be due to the seasonal breadth and sample frequency in this study. The prevailing paradigm for classification of stream ecosystems is based on a gradient with endpoints being heterotrophic, forested headwater streams vs. autotrophic, open-canopy streams (Vannote
et al. 1980, Dodds 2006) based on studies that examined variation in ecosystem structure and function over large and small geographic areas (i.e., across different biomes, riparian communities, and land-use types; Minshall et al. 1983, Webster et al. 2003). In our study, seasonal variation and frequent sampling through time also provided a meaningful context to explain variation in nutrient uptake rates, which at our sites was similar to the range of variation recorded across wide geographic scales (Table 2.5; Figure 2.5). For example, in this study, the variation in NH$_4^+$ $V_f$ among our study streams was as great as variation in NH$_4^+$ $V_f$ across 10 North American streams in different biomes (49% and 46%, respectively, Webster et al. 2003), and temporal variation in $V_f$ (among dates) was generally equal to variation among streams. The use of multiple streams, replicated reaches within streams, and year-round sampling in this study incorporated gradients of controlling factors that allowed for more powerful statistical analyses of spatial and temporal variation than has been seen previously in other studies. Finally, our results demonstrate that seasonal change can provide an alternative axis to geographical variation when examining factors controlling nutrient uptake rates in streams.

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2.7. Literature cited


3.1. Abstract

Whole-stream rates of nutrient uptake and metabolism vary with season in forested headwater streams, yet there has been little research quantifying seasonal variation in rates at the substratum scale, which collectively generate whole-stream patterns. We measured gross primary production (GPP), community respiration (CR), and nitrate uptake rates (NO$_3^-$ U) on 7 substrata using in situ microcosms during 3 biologically distinct periods (spring, summer, and autumn) in three headwater streams in northern Michigan. We quantified the areal reach coverage of each substratum type, scaled NO$_3^-$ U and metabolism to the stream reach (100 m), and finally, compared scaled NO$_3^-$ U to whole-stream NO$_3^-$ U (measured using short-term additions). We found that temporal patterns of GPP, CR, and NO$_3^-$ U were distinct among substratum types, and for several substrata, their respective contribution towards scaled rates varied among sampling dates. Substratum-specific rates of NO$_3^-$ U were positively related to GPP on

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epilithic biofilms and bryophytes, and to CR on epixylic biofilms, bryophytes, and on coarse and fine benthic organic matter. Metabolism rates and NO$_3^-$ U were unrelated on sand. We found no difference between NO$_3^-$ U when calculated via scaling up the substratum-specific rates compared to short-term enrichment (repeated measures ANOVA, $p=0.24$). Our data suggest that streambed substrata composition is an important factor in controlling seasonal variability in reach-scale nutrient uptake and metabolism rates because the relative contribution of several substrata towards whole-stream rates changes through time.

3.2. Introduction
Forested headwater stream ecosystems are characterized by a heterogeneous mixture of substratum types or patches at the reach scale (Pringle et al. 1988). Stream substratum type affects the distribution of stream organisms (Reice 1980, Townsend 1989), the fate of water column nutrients (Newbold et al. 1983), and influences ecosystem processes such as metabolism (i.e., carbon fixation and respiration) and nutrient cycling rates (Fuss and Smock 1996, Kemp and Dodds 2002, Tank and Dodds 2003). The effect of substratum type on reach-scale ecosystem processes has been demonstrated previously using isotope tracers such as $^{32}$PO$_4^{3-}$, $^{15}$NH$_4^+$, and $^{13}$C-acetate (Newbold et al. 1983, Hall et al. 2000, Webster et al. 2003), and also through the experimental removal or addition of individual substratum types (Tank and Webster 1998, Cardinale et al. 2002). However, there has been little research documenting seasonal variation in metabolism and nutrient uptake on multiple stream substrata.
The relative distribution and activity of aquatic biofilms in temperate biomes is controlled by seasonally variable factors including fluctuations in light penetration to the streambed (Hill et al. 2001), water temperature (Fuss and Smock 1996), stream flow (Romaní and Sabater 2000, Ryder 2004), and the seasonality of allochthonous inputs (e.g., leaf litter; Webster and Benfield 1986). Biofilms on different substratum types may respond uniquely to seasonal changes. For example, heterotrophic microbes colonizing organic matter may be unaffected by seasonal changes in light, but strongly affected by organic matter quantity and quality (Tank and Webster 1998), whereas light may be the dominant seasonal factor influencing autotrophic biofilms (Hill et al. 2001). We predict that seasonal patterns of metabolism and nutrient uptake (as indicators of biofilm growth and activity) will differ among biofilms colonizing different substratum types. Thereby, the relative contribution of substratum types towards reach-scale rates will vary over time, ultimately controlling temporal patterns of whole-stream rates.

Several studies have linked metabolism with nutrient uptake rates at both the whole-stream (Hall and Tank 2003, Newbold et al. 2006, Hoellein et al. 2007) and substratum scales (Fellows et al. 2006). However, previous work not addressed how links between metabolism and nutrient uptake rates may vary through time on multiple substratum types. Understanding temporal changes in the links between metabolism rates and nutrient cycles on different substrata is critical for evaluating how changes in stream substrata will affect whole-stream ecosystem processes (e.g., through stream restoration, land-use changes, or natural disturbances). This is especially relevant given the increasing desire for researchers and managers to employ measurements of ecosystem processes following restoration (Lake 2007).
We designed our research to address three objectives: 1) to compare gross primary production (GPP), community respiration (CR), and nitrate uptake rate (NO$_3^-$ U) on dominant stream substrata during 3 biologically important periods, in the spring (pre-leaf out), in the summer (during canopy cover), and in autumn (after leaf fall),  2) to quantify the relationships between metabolism and NO$_3^-$ U on different substrata through time, and 3) to measure what proportion of whole-stream NO$_3^-$ U (measured via short-term enrichment) can be accounted for by scaling up substratum-level measurements made on the dominant benthic substrata.

3.3. Methods

3.3.1. Study sites

State, Shane, and Walton Creeks are forested, first-order streams located in the Ottawa National Forest in the Ontonagon River basin of Lake Superior, in the Upper Peninsula of Michigan, USA. The streams are tributaries of the Jumbo River, and have similar orientation, geology, climate, watershed area, discharge, and logging history (Cordova et al. 2007). Riparian vegetation consists of second-growth mixed hardwood forest with the dominant species including sugar maple (*Acer saccharum* Marsh.), white pine (*Pinus strobes* L.), red maple (*Acer rubrum* L.), alder (*Alnus* spp.), eastern hemlock (*Tsuga canadensis* L.), and paper birch (*Betula papyrifera* Marsh.), with an understory consisting of mixed forbes and ferns. The climate is temperate, with annual streamwater temperatures ranging from 0-23°C (Table 3.1). In general, leaf fall occurs in mid- to late
October and the canopy re-grows in mid- to late May. This influences light availability to the streambed (lower in the summer; Table 3.1), and results in higher standing stocks of streambed coarse benthic organic matter (CBOM; i.e., leaf litter) in autumn (Entrekin et al. in press).

3.3.2. Substratum-specific microcosm incubations

We measured substratum-specific GPP, CR, and NO$_3^-$ U in field microcosms on 19-21 October 2004 (autumn), 5-7 May 2005 (spring), and 11-13 July 2005 (summer) on the dominant substrata found in each stream. We selected these dates as representative of the broader seasonal categories of spring, summer, and autumn based on temperature and canopy cover (Table 3.1). Measurements for all substrata were taken on one day per stream during each sampling period (N=9 dates; Table 3.1). The substrata we included were sand, fine benthic organic matter (FBOM; particle size <1 mm$^2$), coarse benthic organic matter (CBOM; particle size >1 mm$^2$), small wood (<10cm diameter), large wood, and bryophyte. The bryophyte category was included only in State Creek, as it represented <1% of streambed surface in the other two streams.

We collected one replicate of each substratum type from three different locations distributed throughout a 100 m reach in each of the study streams. Sand and FBOM were collected by inserting an inverted 160 mL specimen container approximately 2 cm into the substratum, sliding a spatula underneath as a seal, and then turning the container upright. We did not separate sand and FBOM in our samples, but selected habitat patches which represented as close to 100% of either type that could be obtained in situ. We collected CBOM and small wood by gathering enough material to fill approximately
75% of the container. Biofilm activity on large wood was estimated using 40-50 cm² blocks cut from a bigtooth aspen log (*Populus grandidentata* Michx.) which had been anchored to a riffle area in the benthos for biofilm colonization for approximately 5 months prior to the first collection. We collected rocks from riffle areas (and rocks with bryophytes in State Creek only), ranging from 15-100 cm² in area.

For incubations, we collected approximately 11 L of stream water during the plateau phase of a whole-stream, short-term nutrient addition (NO₃⁻ enriched water; see details below) conducted prior to gathering substrata for the microcosm incubations. The water was enriched slightly above ambient NO₃⁻ concentrations (1.1-1.45 times; Table 3.1). We filtered the water through a glass fiber filter (GFF; pore size = 1.0 μm, Type A/E, Pall Corporation, Ann Arbor, MI, USA), recorded the initial dissolved oxygen (DO) using a hand-held DO meter (YSI Model DO200, Yellow Springs, OH, USA), and gently filled each microcosm, using care to eliminate all air bubbles in the headspace.

Microcosms were 160 mL sterile specimen containers, and were air-tight when sealed (Fisher Scientific, Pittsburgh, PA, USA). We incubated the microcosms within a 0.5 m² area of the stream to maintain ambient temperature and comparable light levels among all microcosms. After two hours, we recorded final DO and then filtered microcosm water into acid-washed 60 mL bottles, which were frozen for later NO₃⁻ analysis. Preliminary analyses showed ~2 h time intervals to be the best compromise between recording minimal change in DO and NO₃⁻ in less biologically active substrata while avoiding DO and NO₃⁻ exhaustion in the most active. To measure CR, we repeated the process using fresh water and the same substrata in the dark. After approximately two hours we recorded the DO, and froze the substrata for later measurement of substratum surface area.
and ash-free dry mass (AFDM). For all light and dark incubations we used 3 replicate blanks (water only) to account for any abiotic changes in NO₃⁻ or DO.

We used still-water microcosms rather than flow-through chambers in this study. Flow-through chambers have the advantage of mimicking the effect of current velocity, which has been shown to affect biofilm metabolism (Dodds and Brock 1998). However, their expense and more time-consuming operation relative to still-water microcosms limited replication at levels we required. In addition, flow-through chambers present many identical methodological uncertainties as still-water microcosms including light attenuation by chamber material, nutrient limitation, and bubble formation (Bott et al. 1997). Solving all problems associated with chamber measurements is not possible (Dodds and Brock 1998), and still-water chambers represented the best available compromise to meet our study’s objectives.

We measured the surface area of each substratum used in the incubations in the following ways. Surface area of sand and FBOM were measured as the area of the corer used to obtain the samples (28 cm²). CBOM surface area was measured by tracing an outline of the leaves/detritus on paper, cutting out and weighing the shapes, and using a paper mass-area regression to calculate surface area. We measured dimensions of small and large wood pieces and used the geometric equation for cylinder (small wood), or cube (large wood). Rock surface area was measured by lining the surface of the rock with a single layer of aluminum foil, weighing the foil, and using a foil mass-area regression to calculate surface area. We estimated bryophyte surface area as the benthic coverage of the bryophyte, using a ruler at the base of the plant. We then added the surface area of the rock it to which was it was attached using the foil method.
For CBOM, small wood, large wood, and bryophytes we quantified substratum mass used in incubations by placing ground subsamples onto pre-ashed and weighed tins, and drying for 3-7 days at 60°C. For sand and FBOM, we did not subsample, but placed the entire sample into the tins. For epilithon, we scraped rocks with a wire brush and a squirt bottle, rinsed the slurry with a known volume of water, and filtered a subsample through a pre-ashed GFF. For all substrata, we recorded dry mass, and then determined AFDM following combustion (3 hours at 550°C). From epilithon only, we also extracted chlorophyll \( a \) from a slurry subsample using the non-acidification, hot ethanol method and measured chlorophyll \( a \) concentrations on a Turner Designs Model TD-700 Fluorometer (Sartory and Grobbelaar 1984).

Comparisons of metabolism and N uptake rates across substrata are complicated by factors associated with differences in AFDM and surface area measurements. For some substrata, (i.e., epilithon, and bryophytes), the AFDM contains mostly “active” biomass, where for CBOM, FBOM, and wood, the mass contains a larger proportion if relatively inert organic mass. In addition, we measured the actual surface area of CBOM, large wood, small wood, and rocks. For FBOM and sand, we estimated benthic surface area as the size of the container opening, and for bryophytes estimated the coverage at the base of the plant. For the latter 3 substrata, we likely underestimated the surface area for biofilm colonization. We further acknowledge the potential conflicts when comparing rates among substrata in the data interpretation.

We calculated microcosm GPP, CR, and NO\(_3^-\) U as the change in oxygen (O\(_2\)) or NO\(_3^-\) -N concentration per substratum area per time, expressed as mgO\(_2\) m\(^{-2}\) h\(^{-1}\) and \(\mu\)gNO\(_3^-\)-N m\(^{-2}\) h\(^{-1}\) respectively, as well as per unit organic matter (gAFDM) of the
substrata, mgO₂ gAFDM⁻¹ h⁻¹ and μgNO₃⁻-N gAFDM⁻¹ h⁻¹ (Kemp and Dodds 2002). For measurements expressed in terms of substratum area or substratum gAFDM, we used the abbreviations GPPsub m⁻², and GPPsub gAFDM, respectively (Table 3.2). GPP was calculated as the difference between net primary production, or change in O₂ in the light, and CR as change in O₂ in the dark (Bott 1996).

To estimate reach-scale (100 m) benthic coverage of each substratum type, we created 21 transects, 5 m apart along each reach, and recorded substratum type every 20 cm across each transect. We calculated the reach-scale areal coverage of each substratum as the total number of counts for each substratum type divided by the total number of counts for all substrata multiplied by the reach area (reach length x average width). We quantified benthic coverage within 3 days of the substrata incubations. We measured riparian canopy cover using a spherical densiometer (Model-A, Forestry Suppliers, Inc., Jackson, MS, USA), and expressed percent coverage as the mean from 5 measurements throughout each 100m reach. HOBO data-loggers (Onset Computer Corporation, Bourne, MA, USA) recorded hourly stream temperature in each stream reach.

To scale substratum-specific rates to the stream reach, we multiplied the substratum areal coverage by GPPsub m⁻², CRsub m⁻², and NO₃⁻-N Usub m⁻² for each substratum, and abbreviated them as GPPscaled, CRscaled, and NO₃⁻-N UScaled (Table 3.2). We then summed each of the three metrics GPPscaled, CRscaled, and NO₃⁻-N UScaled for all substrata to calculate a total rate for the 100m reach, (modified from Newbold et al. 1983), which we abbreviated as ΣGPPscaled, ΣCRscaled, and ΣNO₃⁻-N UScaled (Table 3.2).
3.3.3. Whole-stream nitrate uptake

On the same day as the substratum microcosm incubations, we conducted a short-term nutrient addition of NO₃⁻ in each stream to quantify whole-stream NO₃⁻ uptake rates, abbreviated NO₃⁻ U_{enrich} (Tank et al. 2006). Prior to the nutrient addition, we collected background water samples at 20, 30, 40, 60, 80, and 100 m downstream of the addition site to determine ambient solute concentrations, then we added solutes at 200 mL min⁻¹ (Fluid Metering, Inc. Lab pump Model RHB, Syosset, NY, USA) to raise background concentrations by +10 to 50 μg NO₃⁻-N L⁻¹ and +25 to 130 μg bromide L⁻¹ (Br⁻; used as a conservative tracer). At plateau, we took three replicate water samples at each collection site, filtering samples in the field through a GFF (pore size = 1.0 μm) and samples were frozen until solutes were analyzed in the laboratory. We measured NO₃⁻ and Br⁻ concentrations using ion chromatography (Dionex Model DX600, Sunnyvale, CA, USA) with AS14A analytical and guard columns and a 500 μL injection loop (Usepa 1993). We also measured ammonium (NH₄⁺) and phosphate (as soluble reactive phosphorus; SRP) concentrations in background water samples using the phenylhypochlorite technique (Solorzano 1969) and the molybdate-antimony method (Murphy and Riley 1962), respectively.

We calculated NO₃⁻ uptake lengths (S_w; or the average distance a NO₃⁻ molecule moves before being taken out of solution) using background-corrected NO₃⁻ concentrations (enriched minus ambient concentration) divided by background-corrected Br⁻ concentrations, and the natural log of this fraction was plotted against distance downstream, with the absolute value of the inverse of the slope equal to S_w (Stream Solute Workshop 1990). We converted S_w into an areal NO₃⁻ uptake rate using NO₃⁻
concentration, discharge, and width according to standard methods (Stream Solute Workshop 1990). We then compared NO$_3^-$ $U_{\text{enrich}}$ with ΣNO$_3^-$ $U_{\text{scaled}}$ from substratum microcosm incubations using identical units of mgNO$_3^-$-N m$^{-2}$ h$^{-1}$. We did not compare ΣGPP$_{\text{scaled}}$ or ΣCR$_{\text{scaled}}$ to whole-stream metabolism measurements (i.e., open-channel measurements made via data-logging sondes), because metabolism measurements are sensitive to temperature and light intensity during the incubation period, and cannot accurately be extrapolated to other times of the day. In contrast, NO$_3^-$ $U_{\text{enrich}}$ and ΣNO$_3^-$ $U_{\text{scaled}}$ are both short-term measurements (i.e., <2 h), and were made within several hours of each other on the same day (Table 3.1). We acknowledge that in some streams, hyporheic contribution towards whole-stream uptake may be significant, but is likely minimal in these low gradient, small substratum streams (Hünken and Mutz 2007). In addition, we have recorded small transient storage zones relative to water velocity in the study streams, indicating relatively low realized storage (Runkel 2002; J. Tank, unpublished data).
TABLE 3.1

PHYSIOCHEMICAL DESCRIPTORS OF STUDY STREAMS ON THE DATES OF WHOLE-STREAM (WS) AND SUBSTRATUM-SPECIFIC (SS) NUTRIENT UPTAKE MEASUREMENTS

<table>
<thead>
<tr>
<th>Date</th>
<th>Stream</th>
<th>Canopy cover (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Temp (°C)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Chl &lt;sub&gt;a&lt;/sub&gt; (µg cm&lt;sup&gt;-2&lt;/sup&gt;)</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>SRP (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>SS NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; enrich. (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>WS NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; enrich. (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>SS uptake</th>
<th>WS uptake</th>
<th>Time of day measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-Oct-04</td>
<td>State</td>
<td>-</td>
<td>5.9</td>
<td>1.29 (0.22)</td>
<td>4.1</td>
<td>4.7</td>
<td>156.6</td>
<td>367.1</td>
<td>379.1</td>
<td>8:45-9:15</td>
<td>12:19-15:57</td>
<td>8:50-9:26 12:23-16:07</td>
</tr>
<tr>
<td>20-Oct-04</td>
<td>Shane</td>
<td>81.1</td>
<td>8.6</td>
<td>1.83 (0.15)</td>
<td>5.8</td>
<td>3.8</td>
<td>107.3</td>
<td>125.1</td>
<td>122.2</td>
<td>8:50-9:26</td>
<td>12:23-16:07</td>
<td>8:50-9:26 12:23-16:07</td>
</tr>
<tr>
<td>19-Oct-04</td>
<td>Walton</td>
<td>61.5</td>
<td>4.9</td>
<td>1.40 (0.18)</td>
<td>5.0</td>
<td>7.6</td>
<td>334.7</td>
<td>379.1</td>
<td>367.1</td>
<td>9:43-10:28</td>
<td>14:32-17:29</td>
<td>9:43-10:28 14:32-17:29</td>
</tr>
<tr>
<td>7-May-05</td>
<td>State</td>
<td>69.1</td>
<td>10.0</td>
<td>0.21 (0.03)</td>
<td>2.4</td>
<td>9.8</td>
<td>173.4</td>
<td>204.1</td>
<td>196.2</td>
<td>8:24-8:50</td>
<td>14:32-17:27</td>
<td>8:24-8:50 14:32-17:27</td>
</tr>
<tr>
<td>6-May-05</td>
<td>Shane</td>
<td>87.8</td>
<td>10.5</td>
<td>0.68 (0.28)</td>
<td>2.0</td>
<td>4.6</td>
<td>149.0</td>
<td>167.2</td>
<td>170.6</td>
<td>8:58-9:21</td>
<td>13:04-17:04</td>
<td>8:58-9:21 13:04-17:04</td>
</tr>
<tr>
<td>5-May-05</td>
<td>Walton</td>
<td>82.0</td>
<td>9.7</td>
<td>0.63 (0.09)</td>
<td>14.6</td>
<td>2.9</td>
<td>287.1</td>
<td>339.7</td>
<td>325.2</td>
<td>9:46-10:24</td>
<td>14:39-17:50</td>
<td>9:46-10:24 14:39-17:50</td>
</tr>
<tr>
<td>13-Jul-05</td>
<td>State</td>
<td>91.7</td>
<td>11.6</td>
<td>0.05 (0.02)</td>
<td>1.2</td>
<td>8.3</td>
<td>174.8</td>
<td>263.9</td>
<td>227.6</td>
<td>8:35-8:59</td>
<td>12:33-15:26</td>
<td>8:35-8:59 12:33-15:26</td>
</tr>
<tr>
<td>12-Jul-05</td>
<td>Shane</td>
<td>99.6</td>
<td>17.3</td>
<td>0.12 (0.07)</td>
<td>9.2</td>
<td>9.7</td>
<td>226.2</td>
<td>250.3</td>
<td>247.7</td>
<td>9:12-9:44</td>
<td>13:19-16:31</td>
<td>9:12-9:44 13:19-16:31</td>
</tr>
<tr>
<td>11-Jul-05</td>
<td>Walton</td>
<td>97.7</td>
<td>23.2</td>
<td>0.16 (0.07)</td>
<td>48.8</td>
<td>8.0</td>
<td>153.4</td>
<td>233</td>
<td>221.7</td>
<td>10:10-11:05</td>
<td>15:38-16:41</td>
<td>10:10-11:05 15:38-16:41</td>
</tr>
</tbody>
</table>

<sup>a</sup>Canopy cover measurements were taken in 20Nov04, 15-17Apr05, and 11-13Jul05
<sup>b</sup>24 hour mean

Note: Abbreviations: Temp = temperature, Chl <sub>a</sub> = chlorophyll <sub>a</sub>, NH<sub>4</sub><sup>+</sup> = ammonium, SRP = soluble reactive phosphorus, NO<sub>3</sub><sup>-</sup> = nitrate, and enrich. = enrichment concentration.
### Table 3.2

**ABBREVIATIONS, UNITS, METHODS, AND REFERENCES FOR MEASUREMENTS OF GROSS PRIMARY PRODUCTION (GPP), COMMUNITY RESPIRATION (CR), AND NITRATE UPTAKE (NO₃⁻ U) AT SUBSTRATUM AND REACH SCALES**

<table>
<thead>
<tr>
<th>Scale</th>
<th>Metric</th>
<th>Units</th>
<th>Method</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substratum</td>
<td>GPP&lt;sub&gt;sub m²&lt;/sub&gt;</td>
<td>mgO₂ m⁻²substratum h⁻¹</td>
<td>Δ O₂ in microcosm in light minus Δ O₂ in the dark</td>
<td>Bott (1996)</td>
</tr>
<tr>
<td></td>
<td>GPP&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>mgO₂ gAFDM⁻¹substratum h⁻¹</td>
<td>Δ O₂ in microcosm in light minus Δ O₂ in the dark</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CR&lt;sub&gt;sub m²&lt;/sub&gt;</td>
<td>mgO₂ m⁻²substratum h⁻¹</td>
<td>Δ O₂ in microcosm in the dark</td>
<td>Hill et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>CR&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>mgO₂ gAFDM⁻¹substratum h⁻¹</td>
<td>Δ O₂ in microcosm in the dark</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ U&lt;sub&gt;sub m²&lt;/sub&gt;</td>
<td>μgNO₃⁻-N m⁻²substratum h⁻¹</td>
<td>Δ NO₃⁻-N in microcosm in the light</td>
<td>Kemp and Dodds (2002)</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ U&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>μgNO₃⁻-N gAFDM⁻¹substratum h⁻¹</td>
<td>Δ NO₃⁻-N in microcosm in the light</td>
<td></td>
</tr>
<tr>
<td>Scale</td>
<td>Metric</td>
<td>Units</td>
<td>Method</td>
<td>Citation</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>Reach</td>
<td>GPP\text{scaled}</td>
<td>mgO\textsubscript{2} m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>Areal benthic coverage of substratum x GPP\textsubscript{sub} m\textsuperscript{2}</td>
<td>Kemp and Dodds (2002)</td>
</tr>
<tr>
<td></td>
<td>CR\text{scaled}</td>
<td>mgO\textsubscript{2} m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>Areal benthic coverage of substratum x CR\textsubscript{sub} m\textsuperscript{2}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{3}^- U\text{scaled}</td>
<td>mgNO\textsubscript{3}^-N m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>Areal benthic coverage of substratum x NO\textsubscript{3}^- U\textsubscript{sub} m\textsuperscript{2}</td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>$\Sigma$GPP\text{scaled}</td>
<td>mgO\textsubscript{2} m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>$\Sigma$ [GPP\text{scaled}] for all substrata</td>
<td>Newbold et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>$\Sigma$CR\text{scaled}</td>
<td>mgO\textsubscript{2} m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>$\Sigma$ [CR\text{scaled}] for all substrata</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\Sigma$NO\textsubscript{3}^- U\text{scaled}</td>
<td>mgNO\textsubscript{3}^-N m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>$\Sigma$ [NO\textsubscript{3}^- U\text{scaled}] for all substrata</td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>NO\textsubscript{3}^- U\text{enrich}</td>
<td>mNO\textsubscript{3}^-N m\textsuperscript{-2} benthos h\textsuperscript{-1}</td>
<td>Short term enrichment of NO\textsubscript{3}^- with Br^- conservative tracer</td>
<td>Tank et al. (2006)</td>
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</tbody>
</table>
We used 2-way repeated measures analysis of variance (RM-ANOVA) by date and substratum to examine differences in GPP, CR, and NO₃⁻ U on substratum-specific rates. We treated streams as replicates, using the mean of the substratum rates across the three streams to analyze temporal patterns. We used this approach because our aim was not to describe differences among streams, but rather to incorporate among-stream variability within the boarder context of seasonality, thereby strengthening the applicability of our results among streams in the region. Where RM-ANOVA resulted in a significant date x substratum interaction, we analyzed each factor independently using a Bonferonni-adjusted \( p \)-value of \( \frac{0.05}{6}=0.008 \) for a RM-ANOVA to compare among dates for each substrata (\( n=6 \)), and a \( p \)-value of \( \frac{0.05}{3}=0.015 \) for an ANOVA comparing among substrata for each date (\( n=3 \)). Rates on bryophytes were considered only among dates (RM-ANOVA), because they were not replicated among streams.

We used linear regression to quantify the relationship between \( GPP_{\text{sub m}^2} \) and \( CR_{\text{sub m}^2} \) with \( NO₃⁻ U_{\text{sub m}^2} \), and performed separate regressions by date and with all data. Because all rates were measured concurrently in the same microcosm, regressions of GPP and CR with \( NO₃⁻ U \) were the same regardless of whether expressed per unit surface area or per gAFDM (i.e., uptake and metabolism rates were calculated using identical values for substratum area, gAFDM, and incubation time). In some cases, it was necessary to transform the GPP data using natural log or rank transformation (Zar 1999), due to the relatively high number of measurements with very low GPP.

We calculated a “hot-spot” index (sensu McClain et al. 2003) as the relative contribution of each substratum type (e.g., \( \frac{[GPP_{\text{scaled on rocks}}] \times 100}{\Sigma GPP_{\text{scaled}}} \)) divided by the percent coverage of that substratum type. A “hot-spot” value \( >1 \) indicates
a substratum type was a “hot-spot" of activity relative to its abundance. Finally, we used 2-way RM-ANOVA to compare $\Sigma$NO$_3^-$ $U_{scaled}$ with NO$_3^-$ $U_{enrich}$ among dates and between methods. All statistical analyses were conducted using Systat 11.0 (Systat Software Inc, Chicago, IL, USA).

3.4. Results

3.4.1. Seasonal variation in metabolism at two spatial scales

For GPP$_{sub \ m^2}$, we found a significant effect of substratum (RM-ANOVA $p<0.001$), no effect of date (RM-ANOVA, $p=0.081$), but a significant date x substratum interaction (RM-ANOVA, $p<0.001$), so we looked at each factor individually. High GPP on rock biofilms (i.e., epilithon) in May was the cause of the significant interaction. In May, GPP$_{sub \ m^2}$ was highest on epilithon (ANOVA, $p<0.001$), but there were no differences among substrata in October (ANOVA, $p=0.077$), or July (ANOVA, $p=0.349$). In addition, there were no significant differences among dates in GPP$_{sub \ m^2}$ for each individual substratum, except for epilithon, where GPP$_{sub \ m^2}$ was highest in spring, lowest in summer, and autumn was intermediate (RM-ANOVA, $p=0.008$; Table 3.3). For GPP$_{sub \ gAFDM}$, rates were highest on epilithon on every date (ANOVA, $p<0.001$) because the biofilm biomass was much lower for epilithon compared to the other substrata, where the entire organic component of the substratum was included in the AFDM measurement, be it alive (e.g., microbes), or dead (organic detritus). Among dates, GPP$_{sub \ gAFDM}$ was again only significantly different only for epilithon (Table 3.3).
TABLE 3.3

MEAN (±SE) GROSS PRIMARY PRODUCTION (GPP) COMMUNITY RESPIRATION (CR), NITRATE UPTAKE (NO$_3^-$ U), AND STREAMBED SURFACE AREA (%) AMONG STUDY STREAMS FOR EACH SUBSTRATUM AND DATE

<table>
<thead>
<tr>
<th>Substratum</th>
<th>Date</th>
<th>GPP$_{\text{sub} m^2}$</th>
<th>GPP$_{\text{sub} gAFDM}$</th>
<th>CR$_{\text{sub} m^2}$</th>
<th>CR$_{\text{sub} gAFDM}$</th>
<th>NO$<em>3^-$ U$</em>{\text{sub} m^2}$</th>
<th>NO$<em>3^-$ U$</em>{\text{sub} gAFDM}$</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large wood</td>
<td>Spring</td>
<td>10.6 (4.7)</td>
<td>0.011 (0.005)</td>
<td>19.0 (3.9)</td>
<td>0.032 (0.012)</td>
<td>444 (32)</td>
<td>5.1 (1.3)</td>
<td>9.4 (1.2)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>9.2 (4.7)</td>
<td>0.013 (0.006)</td>
<td>25.0 (1.1)</td>
<td>0.032 (0.011)</td>
<td>529 (12)</td>
<td>10.0 (2.6)</td>
<td>13.1 (2.3)</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>4.1 (2.1)</td>
<td>0.004 (0.002)</td>
<td>15.3 (1.3)</td>
<td>0.015 (0.002)</td>
<td>333 (116)</td>
<td>0.3 (0.1)</td>
<td>6.8 (1.6)</td>
</tr>
<tr>
<td>Small wood</td>
<td>Spring</td>
<td>3.9 (1.0)</td>
<td>0.013 (0.004)</td>
<td>7.8 (1.0)</td>
<td>0.033 (0.006)</td>
<td>173 (82)</td>
<td>1.9 (1.1)</td>
<td>4.9 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>4.2 (1.3)</td>
<td>0.008 (0.001)</td>
<td>12.2 (1.7)</td>
<td>0.038 (0.001)</td>
<td>-13 (71)</td>
<td>0.2 (0.5)</td>
<td>4.8 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>1.8 (0.6)</td>
<td>0.005 (0.002)</td>
<td>5.8 (0.8)</td>
<td>0.016 (0.002)</td>
<td>104 (37)</td>
<td>1.6 (0.2)</td>
<td>7.0 (2.2)</td>
</tr>
<tr>
<td>CBOM</td>
<td>Spring</td>
<td>1.8 (0.9)</td>
<td>0.039 (0.042)</td>
<td>12.7 (0.9)</td>
<td>0.264 (0.042)</td>
<td>274 (22)</td>
<td>5.2 (0.7)</td>
<td>7.3 (1.2)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3.1 (3.1)</td>
<td>0.047 (0.037)</td>
<td>6.8 (0.5)</td>
<td>0.273 (0.036)</td>
<td>34 (9)</td>
<td>0.4 (0.2)</td>
<td>3.7 (1.0)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>3.5 (1.8)</td>
<td>0.049 (0.002)</td>
<td>8.5 (0.9)</td>
<td>0.228 (0.002)</td>
<td>253 (39)</td>
<td>10.2 (2.6)</td>
<td>16.0 (1.7)</td>
</tr>
<tr>
<td>FBOM</td>
<td>Spring</td>
<td>2.7 (1.9)</td>
<td>0.021 (0.018)</td>
<td>22.1 (3.7)</td>
<td>0.244 (0.136)</td>
<td>1068 (124)</td>
<td>24.5 (7.2)</td>
<td>8.3 (3.7)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.8 (0.8)</td>
<td>0.002 (0.001)</td>
<td>49.6 (2.9)</td>
<td>0.123 (0.001)</td>
<td>1036 (495)</td>
<td>42.6 (24.9)</td>
<td>12.3 (4.8)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>2.3 (2.3)</td>
<td>0.030 (0.030)</td>
<td>22.8 (5.4)</td>
<td>0.225 (0.022)</td>
<td>82 (194)</td>
<td>2.3 (6.0)</td>
<td>11.9 (3.9)</td>
</tr>
<tr>
<td>Substratum</td>
<td>Date</td>
<td>GPP&lt;sub&gt;sub m&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;</td>
<td>GPP&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>CR&lt;sub&gt;sub m&lt;/sub&gt;</td>
<td>CR&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; U&lt;sub&gt;sub m&lt;/sub&gt;</td>
<td>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; U&lt;sub&gt;sub gAFDM&lt;/sub&gt;</td>
<td>Area (%)</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>-------------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Sand</td>
<td>Spring</td>
<td>1.6 (1.6)</td>
<td>0.032 (0.032)</td>
<td>11.2 (2.0)</td>
<td>0.154 (0.050)</td>
<td>230 (66)</td>
<td>18.0 (3.0)</td>
<td>39.9 (4.2)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>1.9 (1.9)</td>
<td>0.010 (0.010)</td>
<td>14.5 (3.0)</td>
<td>0.090 (0.011)</td>
<td>-288 (267)</td>
<td>-21.4 (19.0)</td>
<td>33.4 (7.5)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>3.7 (3.7)</td>
<td>0.047 (0.047)</td>
<td>7.26 (4.0)</td>
<td>0.093 (0.042)</td>
<td>465 (110)</td>
<td>29.6 (10.7)</td>
<td>23.8 (3.5)</td>
</tr>
<tr>
<td>Rock</td>
<td>Spring</td>
<td>37.5&lt;sup&gt;a&lt;/sup&gt; (1.6)</td>
<td>146.7&lt;sup&gt;a&lt;/sup&gt; (85.3)</td>
<td>9.0 (1.9)</td>
<td>41.9 (25.0)</td>
<td>678&lt;sup&gt;a&lt;/sup&gt; (74)</td>
<td>152.4&lt;sup&gt;a&lt;/sup&gt; (1865)</td>
<td>36.0 (5.2)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3.9&lt;sup&gt;b&lt;/sup&gt; (1.4)</td>
<td>9.9&lt;sup&gt;b&lt;/sup&gt; (3.0)</td>
<td>5.5 (2.8)</td>
<td>3.7 (1.7)</td>
<td>3&lt;sup&gt;b&lt;/sup&gt; (37)</td>
<td>9.2&lt;sup&gt;b&lt;/sup&gt; (3.5)</td>
<td>31.7 (6.3)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>16.5&lt;sup&gt;b&lt;/sup&gt; (7.3)</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt; (1.5)</td>
<td>11.3 (4.3)</td>
<td>4.6 (2.4)</td>
<td>562&lt;sup&gt;a&lt;/sup&gt; (45)</td>
<td>49.9&lt;sup&gt;b&lt;/sup&gt; (4.1)</td>
<td>33.9 (6.2)</td>
</tr>
<tr>
<td>Bryophyte&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Spring</td>
<td>9.2 (1.0)</td>
<td>-</td>
<td>50.1&lt;sup&gt;a&lt;/sup&gt; (11.7)</td>
<td>-</td>
<td>713&lt;sup&gt;a&lt;/sup&gt; (71)</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3.2&lt;sup&gt;b&lt;/sup&gt; (1.2)</td>
<td>4.47 (2.64)</td>
<td>9.2&lt;sup&gt;ab&lt;/sup&gt; (3.0)</td>
<td>1.16 (0.95)</td>
<td>36&lt;sup&gt;b&lt;/sup&gt; (56)</td>
<td>31.3 (29.0)</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>7.2 (1.2)</td>
<td>-</td>
<td>4.1&lt;sup&gt;b&lt;/sup&gt; (3.0)</td>
<td>-</td>
<td>411&lt;sup&gt;ab&lt;/sup&gt; (195)</td>
<td>-</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Significant difference among means indicated by Tukey’s multiple comparison test following significant ANOVA
<sup>c</sup>State Creek only

Note: For abbreviations and units see Table 3.2. Bold values indicate significant differences among sampling dates (repeated measures-ANOVA, p ≤ 0.008). CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter.
For CR\(_{\text{sub m}}^2\), we found also found a significant effect of substratum (RM-ANOVA, \(p<0.001\)), no effect of date (RM-ANOVA, \(p=0.089\)), but a significant date x substratum interaction (RM-ANOVA, \(p=0.002\)), so we looked at each factor individually. In this case, high CR on FBOM in July was the cause of the significant date x substratum interaction. Using Bonferroni-adjusted \(p\)-value of \(0.05/3= 0.015\), there was no difference in CR\(_{\text{m}}^2\) among substrata in October (ANOVA, \(p=0.031\)), or May (ANOVA, \(p=0.009\)), but in July, FBOM had significantly higher rates than all other substrata (ANOVA, \(p<0.001\)). Comparing among dates for each of the individual substrata, CR\(_{\text{sub m}}^2\) was highest in July for small wood (RM-ANOVA, \(p=0.006\)), and FBOM (RM-ANOVA, \(p=0.001\)), and highest in May for bryophytes (RM-ANOVA, \(p=0.003\); Table 3.3). In contrast, patterns for CR\(_{\text{sub gAFDM}}\), were similar to those for GPP\(_{\text{sub gAFDM}}\), where epilithon had much higher rates than other substrata on all dates (RM-ANOVA, \(p<0.001\)), again due to differences among substratum mass.

Results for substratum coverage mirrored the results for GPP\(_{\text{sub m}}^2\), and CR\(_{\text{sub m}}^2\), with a significant date x substratum interaction (RM-ANOVA, \(p=0.019\)), however, the cause of the interaction in this case was higher coverage of CBOM in October (RM-ANOVA, \(p=0.003\)). Because of the general lack of changes in substratum coverage for all substrata (except CBOM), the seasonal patterns of GPP\(_{\text{scaled}}\) and CR\(_{\text{scaled}}\) (i.e., GPP\(_{\text{sub m}}^2\) x relative coverage; Table 3.2) for all substratum types were identical to rates at the substratum scale (data not shown).

GPP\(_{\text{scaled}}\) for epilithon was a principal contributor to \(\Sigma\)GPP\(_{\text{scaled}}\) across dates (May=70\%, July=40\%, and Nov=84\%) because rocks were the dominant substrate for algae in the study streams (Figure 3.1A). In addition, large wood was an important site
of GPP in summer, contributing an average of 33% of $\Sigma GPP_{scaled}$. To analyze the patterns of $\Sigma CR_{scaled}$, we found it was useful to distinguish the contribution of biofilms on organic substrata (wood + CBOM + FBOM) relative to those of inorganic substrata (sand + rocks). In summer, the contribution of organic substrata to $\Sigma CR_{scaled}$ was high (66%) relative to inorganic substrata (33%), but the relative contributions of these substrata were approximately equal in spring (49%:51%) and autumn (47%:53%; Figure 3.1B).

We found that three substrata were “hot-spots” of $GPP_{scaled}$ or $CR_{scaled}$ in every date: biofilms on rocks were always “hot-spots” of GPP relative to their abundance, and large wood and FBOM were consistently “hot-spots” of CR (Table 3.4). Other substrata were “hot-spots” on a temporally variable basis (Table 3.4).
Figure 3.1. Average percent contribution of each substratum type towards the sum of substratum-specific, scaled-up rates for A) gross primary production ($\Sigma$GPP$_{\text{scaled}}$), B) community respiration ($\Sigma$CR$_{\text{scaled}}$), and C) nitrate uptake rate ($\Sigma$NO$_3^-$ $U_{\text{scaled}}$) across streams for each date. FBOM = fine benthic organic matter, CBOM = coarse benthic organic matter.
TABLE 3.4

MEAN (±SE) “HOT-SPOT” INDEX FOR GROSS PRIMARY PRODUCTION (GPP) COMMUNITY RESPIRATION (CR), AND NITRATE UPTAKE (NO$_3^-$ U) ACROSS STUDY STREAMS

<table>
<thead>
<tr>
<th>Substratum</th>
<th>Date</th>
<th>GPP</th>
<th>CR</th>
<th>NO$_3^-$ U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large wood</td>
<td>Spring</td>
<td>0.74  (0.38)</td>
<td>1.61 (0.26)</td>
<td>0.66 (0.28)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td><strong>2.19</strong> (0.72)</td>
<td><strong>1.79</strong> (0.37)</td>
<td><strong>3.29</strong> (1.97)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.42  (0.22)</td>
<td><strong>1.28</strong> (0.82)</td>
<td><strong>1.06</strong> (0.41)</td>
</tr>
<tr>
<td>Small wood</td>
<td>Spring</td>
<td>0.25  (0.13)</td>
<td>0.69 (0.10)</td>
<td>0.40 (0.16)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td><strong>1.54</strong> (0.93)</td>
<td>0.92 (0.17)</td>
<td>0.25 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.27  (0.03)</td>
<td>0.50 (0.06)</td>
<td>0.32 (0.09)</td>
</tr>
<tr>
<td>CBOM</td>
<td>Spring</td>
<td>0.12  (0.06)</td>
<td><strong>1.13</strong> (0.07)</td>
<td>0.67 (0.06)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.51  (0.51)</td>
<td>0.72 (0.62)</td>
<td>0.35 (0.25)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td><strong>1.31</strong> (1.12)</td>
<td>0.73 (0.04)</td>
<td>0.80 (0.13)</td>
</tr>
<tr>
<td>FBOM</td>
<td>Spring</td>
<td>0.16  (0.09)</td>
<td><strong>1.99</strong> (0.42)</td>
<td><strong>2.57</strong> (0.19)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.23  (0.18)</td>
<td><strong>3.77</strong> (0.70)</td>
<td><strong>3.34</strong> (1.11)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.24  (0.24)</td>
<td><strong>2.03</strong> (0.56)</td>
<td>0.56 (0.56)</td>
</tr>
<tr>
<td>Sand</td>
<td>Spring</td>
<td>0.08  (0.08)</td>
<td><strong>1.01</strong> (0.22)</td>
<td>0.56 (0.14)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>0.46  (0.46)</td>
<td>0.79 (0.21)</td>
<td>0.26 (0.26)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>0.37  (0.37)</td>
<td>0.57 (0.27)</td>
<td><strong>1.06</strong> (0.24)</td>
</tr>
<tr>
<td>Rock</td>
<td>Spring</td>
<td><strong>2.94</strong> (0.57)</td>
<td>0.77 (0.19)</td>
<td><strong>1.21</strong> (0.16)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td><strong>1.46</strong> (0.25)</td>
<td>0.27 (0.11)</td>
<td>0.17 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td><strong>1.73</strong> (0.24)</td>
<td>0.91 (0.31)</td>
<td><strong>1.40</strong> (0.16)</td>
</tr>
<tr>
<td>Bryophyte$^a$</td>
<td>Spring</td>
<td>0.44</td>
<td>0.56</td>
<td><strong>1.14</strong></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td><strong>2.3</strong></td>
<td>0.86</td>
<td><strong>1.49</strong></td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td><strong>1.9</strong></td>
<td>0.35</td>
<td>0.50</td>
</tr>
</tbody>
</table>

$^a$State Creek only

Note: Bold values are >1 (±SE). CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter.
3.4.2. Seasonal variation in nitrate uptake rates at two spatial scales

For NO$_3^-$ $U_{\text{sub} \ m^2}$ we found a significant effect of substratum (RM-ANOVA $p=0.003$), no effect of date ($p=0.209$), however, there was a significant date x substratum interaction ($p=0.001$), so we looked at each factor individually. No single substratum type or date was the cause of the significant interaction. Using Bonferonni-adjusted $p$-value of $0.05/3 = 0.015$, there was no difference in NO$_3^-$ $U_{\text{sub} \ m^2}$ among substrata in October (ANOVA, $p=0.043$), or July (ANOVA, $p=0.018$), but in May, FBOM had significantly higher rates than all other substrata (ANOVA, $p<0.001$). Comparing among dates for each of the individual substrata, NO$_3^-$ $U_{\text{sub} \ m^2}$ was lowest in summer for CBOM (RM-ANOVA, $p=0.008$) and epilithon (RM-ANOVA, $p=0.001$), and highest in spring for bryophytes (RM-ANOVA, $p=0.005$; Table 3.3). For NO$_3^-$ $U_{\text{sub} \ gAFDM}$, we also looked at date and substratum type individually, and found that in October and May epilithon had higher NO$_3^-$ $U_{\text{sub} \ gAFDM}$ than other substrata (ANOVA, $p<0.001$), but there were no difference among substrata in July (ANOVA, $p=0.079$). Comparing among dates for NO$_3^-$ $U_{\text{sub} \ gAFDM}$ showed identical results to NO$_3^-$ $U_{\text{sub} \ m^2}$, with the exception that NO$_3^-$ $U_{\text{sub} \ gAFDM}$ was not different among dates for CBOM (RM-ANOVA, $p=0.065$; Table 3.3). Finally, similar to the results for $\Sigma CR_{\text{scaled}}$ and $\Sigma GPP_{\text{scaled}}$, patterns of NO$_3^-$ $U_{\text{scaled}}$ (NO$_3^-$ $U_{\text{sub} \ m^2} \times \text{coverage}$) for all substratum types were identical to rates at the substratum scale (data not shown).

As for we did for $\Sigma CR_{\text{scaled}}$, we compared the contribution of biofilms on organic substrata (wood + CBOM + FBOM) relative to those of inorganic substrata (sand + rocks) towards $\Sigma NO_3^\text{-} U_{\text{scaled}}$. In summer, large wood and FBOM accounted for 86% of $\Sigma NO_3^\text{-} U_{\text{scaled}}$, and epilithon accounted for only 1.4% (Figure 3.1C). The relative
contribution of organic to inorganic substrata towards $\Sigma$NO$_3^-$ U$_{scaled}$ was approximately equal in spring (45%: 55%; Figure 3.2). Unexpectedly, in autumn, organic substrata contributed less to $\Sigma$NO$_3^-$ U$_{scaled}$ compared to inorganic substrata (26% vs. 74%, respectively), even though CBOM was most abundant at this time (Table 3.3).

In contrast to patterns for GPP and CR, the extent of “hot-spots” among substrata for NO$_3^-$ U$_{scaled}$ varied by date (Table 3.4); the “hot-spots” were FBOM and epilithon in the spring, large wood and FBOM in summer, and large wood in autumn.

3.4.3. Relationships between metabolism and NO$_3^-$ U

We found that NO$_3^-$ U was related to GPP on epilithon, to CR on organic substrata (i.e., large wood, CBOM, and FBOM), and related to both GPP and CR on bryophytes (Table 3.5). When analyzed by date, the relationship between NO$_3^-$ U and epilithon was only significant in spring; however, the pattern remained strong when all data were pooled ($r^2=0.64$, $p<0.01$). There were few other significant relationships between GPP and NO$_3^-$ U, with two exceptions: small wood in autumn ($r^2=0.44$, $p=0.05$), and CBOM in autumn ($r^2=0.67$, $p=0.01$; Table 3.5).

3.4.4. Comparison between whole-reach and reach-scaled NO$_3^-$ U

There were no significant differences between $\Sigma$NO$_3^-$ U$_{scaled}$ and NO$_3^-$ U$_{enrich}$ by date (RM-ANOVA, $p=0.422$), by method (RM-ANOVA, $p=0.240$), and no date x method interaction (RM-ANOVA, $p=0.408$), however, $\Sigma$NO$_3^-$ U$_{scaled}$ generally resulted in higher uptake rates higher than NO$_3^-$ U$_{enrich}$ (Figure 3.2).
TABLE 3.5

REGRESSION RESULTS OF NITRATE UPTAKE WITH GROSS PRIMARY PRODUCTION (GPP\textsubscript{sub.m$^2$}) AND COMMUNITY RESPIRATION (CR\textsubscript{sub.m$^2$}) ON STREAM SUBSTRATA, WITH DATA GROUPED BY SEASON AND WITH ALL DATA POOLED

<table>
<thead>
<tr>
<th>Substrata</th>
<th>GPP\textsubscript{sub.m$^2$}</th>
<th>CR\textsubscript{sub.m$^2$}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>Large wood</td>
<td>$r^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.48)$^b$</td>
<td>(0.84)</td>
</tr>
<tr>
<td>Small wood</td>
<td>$r^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>(0.17)</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.14)$^a$</td>
<td>(0.26)$^a$</td>
</tr>
<tr>
<td>CBOM</td>
<td>$r^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FBOM</td>
<td>$r^2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sand</td>
<td>$r^2$</td>
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</tr>
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<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rocks</td>
<td>$r^2$</td>
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</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
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<td>(0.80)</td>
</tr>
<tr>
<td>Bryophyte$^d$</td>
<td>$r^2$</td>
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</tr>
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</tr>
<tr>
<td></td>
<td>$p$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ natural log transformation
$^b$ rank transformation
$^c$ 2 outliers removed from Walton autumn GPP, with data included: Aut. $r^2=0.03$ $p=0.68$, All $r^2=0.16$ $p=0.05$
$^d$ State Creek only

Note: $p$-values ≤0.05 are in bold. CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter, and – indicates no regression because > half of the values were equal to zero.
Figure 3.2. Mean (±SE) nitrate uptake rates (NO$_3^-$ U) as measured using whole-stream enrichment (NO$_3^-$ U$_{enrich}$) and as the sum of scaled-up substratum-specific rates ($\Sigma$NO$_3^-$ U$_{scaled}$). Repeated-measures ANOVA indicated no difference between methods ($p=0.240$) or among dates ($p=0.422$).

3.5. Discussion

3.5.1. Variation in substratum-specific rates among substrata

Our data concur with previous studies that have shown variation in nutrient uptake and metabolism among biofilm substratum types (Munn and Meyer 1990, Tank and Webster 1998, Kemp and Dodds 2002, Webster et al. 2003). In fact, we demonstrated variability in substratum-specific rates both within/among 3 streams and 3 dates. Like other studies, we showed that epilithon and large wood were important sites of GPP compared to other substrata, that FBOM had higher rates of CR (Kemp and
Dodds 2002), and that each of these substrata were significant sites of N uptake compared to other substrata (Ashkenas et al. 2004). We contribute to the literature by showing biofilms colonizing each of those substrata (i.e., large wood, rocks, and FBOM), display distinct temporal variation in metabolism and/or N uptake rates (Table 3.3).

Comparisons of metabolism and N uptake rates across substrata are complicated by factors associated with differences in surface area and AFDM measurements. This is evident when comparing surface area and gAFDM rates of CBOM vs. FBOM. In May, CR sub m^2 for CBOM was lower than for FBOM (12.7 and 22.1 mgO_2 m^-2 h^-1, respectively; Table 3.3); in contrast, CR sub gAFDM on each substrata were about the same (0.264 and 0.244 mgO_2 gAFDM^-1 h^-1, respectively). Tank et al. (1993) recorded similar, apparently contradictory, results when comparing respiration rates on small wood and leaf litter. The influence of surface area and AFDM estimates of different substrata are important caveats for interpreting comparisons of rates among substrata and among seasons on a particular substratum.

3.5.2. Temporal variation in substratum-specific rates: large and small wood

Wood has been most commonly studied in relation to its structural role in aquatic ecosystems (i.e., organic matter reteition and pool creation; Bilby 1981, Gurnell et al. 2002), but epixylic biofilms are increasingly recognized as an important site of metabolic activity in aquatic systems (Tank and Webster 1998, Vadeboncoeur and Lodge 2000). Biofilms on large wood were shown to be important sites of nutrient uptake in streams in Oregon and North Carolina in summer using ^15NH_4^+ (Hall et al. 2000, Ashkenas et al. 2004), NO_3^- and PO_4^{3-} (Tank and Webster 1998), as well as being an important
colonization site for primary producers in a sandy bottomed stream in southeast Australia (Bond et al. 2006).

Our data show similar results, but sampling among dates indicated that epixylic biofilms played an especially large role in NO$_3^-$ uptake, GPP and CR in summer relative to autumn and spring. This temporal pattern could be related to 1) increased water temperatures (Bott et al. 1985, Tank et al. 1993, Fuss and Smock 1996) and/or 2) decreased relative abundance of CBOM (e.g., leaves: Tank and Webster 1998). Our results support both mechanisms, as we found that higher CR on wood in the summer was significantly related to higher temperatures (linear regression, $r^2=0.45$, $p=0.05$), and that NO$_3^-$ $\text{U}_{\text{sub m}^2}$ on large wood was negatively related to CBOM percent coverage (linear regression, $r^2=0.25$, $p=0.01$). Tank and Webster (1998) showed that metabolic activity of epixylic biofilms increased with experimental exclusion of leaf litter, which they attributed to alleviation of competition for inorganic nutrients from litter decomposing microbes. Our data suggest that this pattern could occur with natural seasonal fluctuations in CBOM abundance. Additionally, the data suggest common anthropogenic effects on stream ecosystems could influence wood biofilm activity. For example, decreases in leaf litter inputs and changes in temperature are common impacts in urban streams (Paul and Meyer 2001), which may have indirect effects on the role of large wood biofilms in the nutrient dynamics and metabolism in impacted streams.

Unlike large wood, biofilms on small wood did not contribute significantly to metabolism and NO$_3^-$ $\text{U}$ in our study streams (Figure 3.1). Our results agree with previously published research showing that the physical and biological role of wood in stream ecosystems differs between large and small size classes (Webster et al. 1999,
Wallace et al. 2000). We add to that literature by documenting differences in the seasonal patterns of metabolism and NO$_3^-$ uptake between large and small wood biofilms.

3.5.3. Seasonal variation in substratum-specific rates: CBOM, FBOM, and sand

Similar to seasonal patterns of leaf litter inputs to forested headwater streams, we found CBOM showed highest percent cover, CR and NO$_3^-$ U in autumn. Lower contributions to whole-stream rates in summer were likely due to a combination of decreased abundance and decreased substratum quality because biofilm growth was likely inhibited by litter softening and fragmentation at later stages of decomposition (Gessner and Chauvet 1994). Unexpectedly, we found that biofilms colonizing CBOM exhibited a relatively minor role in their contribution to $\Sigma$NO$_3^-$ U$_{scaled}$ even at the highest rates recorded ($\leq$16% of total; Figure 3.1). Our results contrast those from Tank et al. (2000), who found that uptake by leaves was responsible for most (76%) of the retained $^{15}$N in a 6-week $^{15}$NH$_4^+$ addition in a forested North Carolina stream. The discrepancy between the two studies could be due to solute specific differences in meeting biofilm inorganic N demand. Previous research has confirmed that NH$_4^+$ is the preferred source of inorganic N for biofilms (Rice and Tiedje 1989, Dortch 1990). In Upper Ball Creek, NC, NH$_4^+$ and NO$_3^-$ are both very low, <5 ug N L$^{-1}$ (Tank et al. 2000), thus demand for inorganic N, especially NH$_4^+$, was high. In contrast, in our study streams, inorganic N availability was much higher, and inorganic N demand could have been met primarily by NH$_4^+$ availability.
Biofilms on FBOM show distinct and more variable patterns in CR and NO$_3^-$ U both among seasons and among replicates (i.e., high in spring and summer), and FBOM was the only substrata for which there was a significant relationship between temperature and NO$_3^-$ U (linear regression, $r^2=0.63$, $p=0.01$). Other studies have shown that FBOM is an active site of N uptake relative to other stream substrata, likely because of the C-rich environment for microbial metabolism, and because of its relatively high substratum surface area per unit benthic surface area (Kemp and Dodds 2002, Arango et al. 2007).

Surface area estimations may also explain some of our results including lower than expected rates of metabolism and NO$_3^-$ U on CBOM and high rates on FBOM. We measured surface area of each CBOM piece in the microcosm, however, if we had considered streambed area (i.e., the stream surface area covered by an accumulation of CBOM), rather than substratum area, we would have likely documented higher rates than are shown. Conversely, FBOM surface area was set as the size of the container opening, or the amount streambed surface area that the FBOM was covering. The actual surface area of fine organic particles is much greater, and including that complexity would have likely lowered our measurements of FBOM rates.

Overall, there was little seasonal variation in rates of metabolism or NO$_3^-$ U on sand; it was not a biological “hot-spot” despite being one of the dominant substratum types in the study streams in terms of benthic coverage. Sand itself may not provide an ideal colonization site for stream biofilms due to lack of organic C availability (for heterotrophs) and low substratum stability for algal colonization (Romani and Sabater 2001). Sand may have a more important structural role in whole-stream metabolism and NO$_3^-$ U rates via its influence in scouring or burial of more biologically active substrata
(Metzler and Smock 1990, Tillman et al. 2003). Our measurements showed sand represented 24-40% of total stream surface area, however, it also occurs in combination with all other substratum types including FBOM deposits, CBOM accumulations, and interstitial spaces between rocks. This illustrates that while some simplification is required for categorization of stream substrata and measurements of their respective biological activity, more complex biotic and abiotic relationships occur among substrata in situ.

3.5.4. Temporal variation in substratum-specific rates: bryophytes and epilithon

Seasonal patterns of GPP and NO$_3$\textsuperscript{-} U for epilithon and bryophytes were most likely related to patterns of streambed light availability due changes in deciduous canopy cover (Hill et al. 2001, Roberts and Mulholland 2007). Our results also indicate that light, as it influences GPP, simultaneously influences NO$_3$\textsuperscript{-} U on epilithon and bryophytes. Furthermore, our data suggest that the activity of autotrophic biofilms (i.e., primary producers and associated microbes) has the potential to affect whole-stream NO$_3$\textsuperscript{-} U, because epilithon accounted for an average of 27% and 49% of $\Sigma$NO$_3$\textsuperscript{-} U$_{scaled}$ in spring and autumn, respectively. These findings are similar to other studies that demonstrate a coupling of NO$_3$\textsuperscript{-} uptake with autotrophic activity in high light systems (Hall and Tank 2003, Fellows et al. 2006). In addition, our data complement recent findings indicating temporal variability in light regime can also control the strength of the relationship between GPP and NO$_3$\textsuperscript{-} U at the stream-reach scale in forested, headwater streams (Mulholland et al. 2006, Roberts and Mulholland 2007).
3.5.5. Comparing $\Sigma$NO$_3^-$ $U_{\text{scaled}}$ with NO$_3^-$ $U_{\text{enrich}}$

Our data showed several merits for estimating nutrient uptake via scaled-up, substratum-specific measurements. We found no statistical differences between the methods, and each showed similar seasonal patterns, both within this study and compared to a more temporally intensive analysis of nutrient uptake in the same streams from the previous year (i.e., a trend of lower uptake rates in the summer; Hoellein et al. 2007). Additionally, the microcosm-derived $\Sigma$NO$_3^-$ $U_{\text{scaled}}$ method has several advantages for measuring NO$_3^-$ uptake under certain conditions. Unlike whole-stream nutrient enrichments, this method can elucidate which substrata or biofilms are responsible for whole-stream rates. Although similar substratum-specific rates can be obtained using an isotope labeled nutrient release (Tank et al. 2006), the $\Sigma$NO$_3^-$ $U_{\text{scaled}}$ method has two advantages over isotope releases: 1) it is much less expensive; and 2) allows for frequent repetition of substratum-specific rates within relatively short time periods, which is limited after isotope releases due to longer term “contamination” of the biofilms by the tracer.

Substratum-specific NO$_3^-$ uptake rates ( $\Sigma$NO$_3^-$ $U_{\text{scaled}}$) were, in general, higher than whole-stream values (NO$_3^-$ $U_{\text{enrich}}$; Figure 3.2), which could be attributed to several methodological artifacts. Microcosms had higher substratum surface area to water volume ratios than were present in the whole-stream enrichment approach, and microcosm water was not re-circulating, which may have increased the contact time of water with the substratum. In addition, the substratum-specific measurements were made later in the day than whole-stream measurements, when temperatures and light levels were higher, which can increase uptake rates (Mulholland et al. 2006). Future studies
comparing substratum-specific and whole-stream rates would benefit from reducing these sources of variability where possible.

3.5.6. Temporal variation in substratum-specific rates: applied considerations

The relative abundance of stream substrata can change naturally with season, but can also change as a result of stream degradation (e.g., sedimentation and channelization via changes in land-use; Wood and Armitage 1997, Bond and Lake 2003), natural disturbances such as floods, or via restoration projects such as wood or boulder additions (Lepori et al. 2005, Rosi-Marshall 2006). These influences often change the abundance of one or more substrata (e.g., FBOM, rocks, wood, and leaf litter), each of which can exhibit temporally variable strength on whole-stream rates of ecosystem processes (i.e., are “hot-spots” of biofilm activity at different times). To our knowledge, few studies have incorporated how these types of substratum changes may influence metabolism or nutrient uptake on a seasonal basis. The approach presented here may prove useful in measuring the effects of changes in stream substrata composition via natural or anthropogenic processes on whole-steam, reach scale rates of nutrient uptake and metabolism over relatively short time scales.

3.5.7. Conclusions

Our empirical measurements showed that seasonality has discrete affects on biofilm \( \text{NO}_3^\text{−} \) uptake and metabolism depending on substratum type. We also showed the relative strength of the relationship between nutrient uptake rates with metabolism rates varies through time on different substrata. Therefore, changes in substrata composition
may also change temporal patterns of metabolism and nutrient uptake rates and alter the
collection between the two processes at the whole-stream scale. Overall, our results
suggest seasonality could be a critical factor influencing how anthropogenic or natural
changes in substratum distribution will impact stream ecosystem processes.

3.6. Acknowledgements

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Graduate School at the University of Notre Dame.

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CHAPTER 4

LINKING SEASONAL VARIATION IN NUTRIENT LIMITATION OF STREAM BIOFILMS COLONIZING ORGANIC AND INORGANIC SUBSTRATA WITH MICROBIAL COMMUNITY ANALYSIS

4.1. Abstract

Human activity has increased the bioavailability of nutrients such as nitrogen (N) and phosphorus (P) in ecosystems worldwide, and therefore understanding the ability of stream biofilms to process nutrients is of interest for both public and ecological health. We examined patterns of nutrient limitation of stream biofilms colonizing inorganic and organic substrata in spring, summer, and autumn in three streams in northern Michigan. We tested for limitation of nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), ammonium (NH$_4^+$), NO$_3^-$ + PO$_4^{3-}$, and NH$_4^+$ + PO$_4^{3-}$. Additionally, in one stream, we characterized the bacterial and fungal communities on organic substrata in the autumn using denaturing gradient gel electrophoresis (DGGE) and DNA sequencing, to see if differences in community structure were linked to nutrient limitation status.

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3 I gratefully acknowledge my co-authors Jennifer L. Tank, John J. Kelly, and Emma J. Rosi-Marshall for their assistance in preparing this chapter.
Despite low background inorganic nutrient concentrations, we found that autotrophs on both inorganic and organic substrata were infrequently nutrient limited, but when limitation did occur, biofilms were limited by NH$_4^+$ and/or PO$_4^{3-}$. While community respiration on inorganic substrata was only limited in 1 out of 9 cases, community respiration on organic substrata was more frequently limited, usually by the combination of N+P. We found no evidence of NH$_4^+$ versus NO$_3^-$ preference on either substratum, although the relative nutrient response to N addition (i.e., +N treatment relative to control) was related to water column PO$_4^{3-}$ concentrations and temperature, suggesting that environmental factors were mediating response to nutrient enrichment.

Molecular analysis of DNA extracted from organic substrata showed lower fungal ribotype richness compared to bacteria, and fungal community DGGE profiles were unrelated to nutrient limitation status. Cloning and sequencing of bacterial ribotypes from each nutrient treatment, however, showed that 3 treatments, NO$_3^-$, PO$_4^{3-}$, and NO$_3^-$ + PO$_4^{3-}$, had a distinct microbial community compared to the control, NH$_4^+$, and NH$_4^+$+PO$_4^{3-}$ treatments. We also found significant nutrient limitation (i.e., higher respiration relative to the control) on the NO$_3^-$, PO$_4^{3-}$, and NO$_3^-$ + PO$_4^{3-}$ treatments. This pattern suggested that changes in respiration rates might be linked to shifts in dominant bacterial community members. Overall, our results demonstrate the potential for discrete patterns of nutrient limitation dependent upon substratum type and season, and suggest a potential link between changes in microbial community function and microbial community structure following enrichment with N + P.
4.2. Introduction

The bioavailability of nutrients is increasing globally (Vitousek 1997), resulting in eutrophication in aquatic ecosystems including streams, rivers, and estuaries (Carpenter et al. 1998, Paul and Meyer 2001, Rabalais et al. 2002). For example, in the agricultural Midwest, high inorganic nitrogen (N) and phosphorus (P) levels are of concern in most flowing waters (Alexander et al. 2000). Because N and/or P commonly limit growth of stream biota at the base of the food web (e.g. algae, bacteria, and fungi), nutrient enrichment can have significant effects on community structure and function (Tank and Dodds 2003, Cross et al. 2006). The ability of stream microorganisms to process nutrients is of major interest for maintaining high water quality and mitigating negative effects of eutrophication on ecological and public health.

Biofilms are the major mechanism for assimilation of inorganic nutrients into stream food webs. Aquatic biofilms typically have autotrophic (i.e., algae and bacteria) and heterotrophic (i.e., bacteria and fungi) constituents that are able to take up inorganic nutrients from the surrounding environment. In stream ecosystems, both autotrophic and heterotrophic assimilation have bottom-up effects on food webs (Rosemond et al. 1993, Hall and Meyer 1998). The relative importance of autotrophic and heterotrophic biofilm components is geographically and temporally variable (Webster et al. 2003). However, nutrient limitation of heterotrophic biofilms is rarely assessed (Tank and Dodds 2003, Dodds 2006), because most studies use inorganic substrata in nutrient limitation assays, which selects for a largely autotrophic community (Johnson et al. in review). As a result, there is a lack of empirical data comparing nutrient limitation of heterotrophic vs. autotrophic biofilms, which is critical for our general understanding of stream ecosystem
function, and for generating predictions regarding the effects of eutrophication in different streams, biomes, and seasons.

In temperate biomes, seasonality may affect the response of biofilms to changes in nutrient availability (Roberts et al. 2007). For example, seasonal changes in light availability have been shown to more strongly limit the growth of autotrophic biofilms than nutrient availability (Mosisch et al. 2001). In forested headwater streams, light availability to the streambed varies by season, potentially altering the relative importance of nutrient availability to biofilm growth and metabolism (Roberts et al. 2007). Similarly, carbon (C) available for heterotrophic microbial colonization (e.g., leaves) peaks in autumn, which can stimulate demand for inorganic nutrients as heterotrophs decompose the leaf litter (Mulholland et al. 1985, Roberts and Mulholland 2007). Seasonal variation in nutrient limitation status on biofilms colonizing different substrata in forested headwater streams has not been previously measured, and represents a gap in our understanding of the interaction between seasonality and nutrient processing by autotrophic and heterotrophic constituents of stream biofilms.

In addition to functional metrics such as rates of nutrient cycling and metabolism, nutrient enrichment can affect biofilm community composition (Pringle 1990, Wilcox et al. 2005, Cross et al. 2006). Understanding how microbial diversity relates to ecosystem function in the context of shifting resources (i.e., changes in nutrient or carbon availability) is a major goal of current microbial ecology research, yet addressing these questions using molecular approaches is relatively novel in stream ecology. A majority of the research using molecular analyses of lotic microbial communities has focused on biogeography (Glockner et al. 2000, Brummer et al. 2003, de Figueiredo et al. 2007,
Rubin and Leff 2007), and has less often addressed changes in community composition following experimental manipulations of biofilm resources (Olapade and Leff 2005, Das et al. 2007). In streams, understanding the relationship between diversity and function is crucial because microbial communities compose the base of the food web and are critical for the biogeochemical cycling of carbon and nutrients (Hall and Meyer 1998). A goal of this research was to couple a molecular tool for describing microbial communities, denaturing gradient gel electrophoresis (DGGE), with a functional metric, microbial community respiration, following nutrient enrichment of an organic substratum. This approach is unique because molecular tools have rarely been applied to elucidate response of heterotrophic biofilms to experimental eutrophication in stream ecosystems.

We used nutrient diffusing substrata (NDS) to address three objectives in this study: 1) to compare nutrient limitation patterns of stream biofilms in 3 forested streams in northern Michigan in spring, summer, and autumn and on two substrata types, organic (cellulose sponge) and inorganic (fritted glass disks), 2) to quantify the effect of external drivers such as temperature, light, discharge, and water column nutrients on seasonal variability in nutrient limitation status, and 3) to document whether changes in functional response (i.e., community respiration) correspond to changes in the microbial community composition of biofilms on cellulose after nutrient enrichment.
4.3. Methods

4.3.1. Site description

State, Shane, and Walton Creeks are forested, first-order streams in the Ontonagon River basin of Lake Superior, in the Upper Peninsula of Michigan, USA. All three streams are located in the Ottawa National Forest, and land-use within the stream watersheds is primarily forested (83-95%), with low human and wetland influence (Entrekin et al. 2007). We chose these streams to represent “reference” conditions for impacted agricultural streams in the upper Midwest US that have undergone eutrophication due to agricultural influences, because these streams are oligotrophic with regard to inorganic nutrient concentrations (Table 4.1). Riparian vegetation consists of second-growth mixed hardwood forest with the dominant species including white pine (Pinus strobes L.), eastern hemlock (Tsuga canadensis L.), sugar maple (Acer saccharum Marsh.), red maple (Acer rubrum L.), alder (Alnus spp.), and paper birch (Betula papyrifera Marsh.), with an understory consisting of mixed ferns and forbes. The climate is characterized by mild summers (mean July temperature is 18.1°C) and cold winters (mean January temperature is -10°C) with heavy snowfall due to the influence of nearby Lake Superior (Sommers 1984, Hoellein et al. 2007).
TABLE 4.1

MEAN (±SE) TEMPERATURE, DISCHARGE (Q), DEPTH, CANOPY COVER, AND STANDING STOCKS OF FINE AND COARSE BENTHIC ORGANIC MATTER (FBOM AND CBOM, RESPECTIVELY)

<table>
<thead>
<tr>
<th>Season</th>
<th>Stream</th>
<th>Temp. (°C)</th>
<th>Mean Q (L s⁻¹)</th>
<th>Depth (cm)</th>
<th>Can. cov. (%)</th>
<th>NH₄⁺ (μgN L⁻¹)</th>
<th>SRP (μgP L⁻¹)</th>
<th>NO₃⁻ (μgN L⁻¹)</th>
<th>FBOM (gAFDM m⁻²)</th>
<th>CBOM (gAFDM m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>State</td>
<td>6.5 (0.2)</td>
<td>83 (4)</td>
<td>15.5 (1.0)</td>
<td>42.0 (2.6)</td>
<td>3.8 (0.2)</td>
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<td>126 (111)</td>
<td>0.9 (0.6)</td>
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<td>Shane</td>
<td>6.9 (0.2)</td>
<td>98 (12)</td>
<td>5.8 (1.0)</td>
<td>46.6 (2.5)</td>
<td>6.2 (0.6)</td>
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<td>27 (7)</td>
<td>12.7 (11.5)</td>
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<td>Walton</td>
<td>9.6 (0.4)</td>
<td>56 (3)</td>
<td>12.3 (0.1)</td>
<td>33.1 (1.9)</td>
<td>3.6 (0.2)</td>
<td>5.6 (0.7)</td>
<td>263.3 (34.3)</td>
<td>49 (25)</td>
<td>38.1 (17.7)</td>
</tr>
<tr>
<td>Summer</td>
<td>State</td>
<td>11.7 (0.1)</td>
<td>47 (1)</td>
<td>5.8 (0.6)</td>
<td>87.0 (2.5)</td>
<td>5.1 (0.7)</td>
<td>6.7 (0.4)</td>
<td>131.4 (0.6)</td>
<td>34 (10)</td>
<td>&lt;0.1 (&lt;0.1)</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>15.0 (&lt;0.1)</td>
<td>27 (1)</td>
<td>8.4 (1.2)</td>
<td>86.3 (2.3)</td>
<td>6.3 (1.0)</td>
<td>9.8 (1.2)</td>
<td>186.4 (1.3)</td>
<td>109 (59)</td>
<td>0.8 (0.8)</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>11.7 (0.1)</td>
<td>27 (0.4)</td>
<td>5.7 (1.0)</td>
<td>85.3 (0.7)</td>
<td>5.8 (0.3)</td>
<td>6.6 (0.2)</td>
<td>627.9 (13.4)</td>
<td>263 (224)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>Autumn</td>
<td>State</td>
<td>2.5 (0.1)</td>
<td>52 (1)</td>
<td>6.4 (0.3)</td>
<td>54.5 (3.9)</td>
<td>8.1 (1.7)</td>
<td>2.2 (0.1)</td>
<td>170.6 (0.2)</td>
<td>90 (36)</td>
<td>15.2 (0.6)</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>1.0 (&lt;0.1)</td>
<td>26 (2)</td>
<td>22.7 (0.5)</td>
<td>60.3 (3.7)</td>
<td>14.6 (0.4)</td>
<td>6.2 (0.6)</td>
<td>239.4 (7.1)</td>
<td>119 (83)</td>
<td>95.7 (67.4)</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>3.1 (0.1)</td>
<td>30 (1)</td>
<td>14.8 (0.5)</td>
<td>36.8 (2.7)</td>
<td>4.3 (0.1)</td>
<td>3.5 (0.1)</td>
<td>308.2 (33.8)</td>
<td>100 (31)</td>
<td>105.7 (20.0)</td>
</tr>
</tbody>
</table>

Note: Temperature and discharge are the average values for the duration of the incubation period, measured facing the 4 cardinal directions at the incubation sites, and depth is the mean of 5 measurements from the NDS surface to the water surface. Water chemistry and organic matter are the mean of 10 sampling points evenly distributed 100m upstream and 100 m downstream of the substrata incubation site at the collection date (28 days following the start date). FBOM and CBOM are in units of g ash-fry dry mass (AFDM) m⁻². Walton chemistry in autumn in Walton Creek was collected in October 2003 (1.5 months prior to the data from State and Shane Creeks).
4.3.2. Nutrient diffusing substrata (NDS)

We constructed NDS from 30 ml plastic cups filled with 2% agar amended with five treatments: nitrate (0.5M NaNO₃⁻), phosphate (0.5M KH₂PO₄⁻), ammonium (0.5M NH₄⁺Cl), NO₃⁻+ PO₄³⁻, and NH₄⁺+ PO₄⁻; combined treatments contained 0.5M of both N and P (Tank et al. 2006). Cups were capped with either fritted glass disks (Leco Corporation, St. Joseph, MI, USA), or cellulose sponge (The Coburn Company, Whitewater, WI, USA). We deployed 5 replicates of each treatment type plus a control group (e.g. no nutrients added to agar) in each of the three study streams (N=30 NDS per stream per substratum type) during three seasons: summer (Jul. 24-Aug. 15, 2003), autumn after leaf fall (Nov. 22-Dec. 13, 2003), and spring prior to leaf-out (Apr. 18-May 4, 2004). We attached the NDS to a plastic L-bar (in random order), and then nailed the L-bars to the stream benthos in a riffle area. After 28 d incubation, we placed each substratum in a 50 mL centrifuge tube with stream water, which were then placed on ice for transport back to the laboratory.

Community respiration (CR) was measured within 24 h of substrata retrieval from the study sites. In the laboratory, we filled each centrifuge tube containing one NDS substratum with unfiltered stream water of known dissolved oxygen (DO; YSI Model DO200, Yellow Springs OH, USA), using care to eliminate any air bubbles. For each stream we included 3 “blank” tubes, which contained only water to correct for any background changes in DO. We incubated tubes on a shaker table in the dark for 2 h and then recorded the final DO calculating CR as the change in O₂ per substratum area per time (Hill et al. 2002). We then extracted the substrata for chlorophyll \(a\) (chl \(a\)) using the
non-acidification, hot ethanol method (Sartory and Grobbelaar 1984) and a Turner Designs Model TD-700 Fluorometer (Sunnyvale, CA, USA).

4.3.3. External drivers

We quantified a number of additional stream physiochemical and biological variables as potential predictors of NDS response to nutrient enrichment. We measured water depth of the NDS as the mean distance from the water surface to the substratum surface at the 4 corners and the center of the group of L-bars. Forest canopy cover was estimated as percent coverage using a spherical densitometer (Model-A, Forestry Suppliers, Inc., Jackson, MS, USA). We calculated mean discharge during the incubation period from a regression between the discharge of each study stream and a downstream USGS gauging station on the Ontonagon River. Finally, HOBO data-loggers (Onset Computer Corporation, Bourne, MA, USA) recorded stream temperature every hour during NDS incubation periods.

Solute concentrations were analyzed on 10 water samples collected in 60 mL acid-washed nalgene bottles from multiple stations ~100 m upstream and ~100 m downstream of the NDS on the final day of each incubation period. We filtered samples in the field through 1.0 μm glass fiber filters (GFF; Type A/E GFF, Pall Corporation, Ann Arbor, MI, USA), and froze samples for later solute analysis. In the laboratory, we used ion chromatography (Dionex Model DX600, Sunnyvale) with AS14A analytical and guard columns and a 500 μL injection loop to measure NO₃⁻ concentrations (USEPA 1993). Ammonium (NH₄⁺) was measured using the phenylhypochlorite technique (Solorzano 1969), and PO₄³⁻ was measured as soluble reactive phosphorus (SRP) with the
molybdate-antimony method (Murphy and Riley 1962). We also quantified fine and coarse benthic organic matter (FBOM and CBOM, respectively) standing stocks using a stovepipe sampler at 5 locations upstream and downstream of the NDS sites on the collection date (Entrekin et al. 2007).

4.3.4. Molecular analysis of cellulose biofilms

We incubated cellulose NDS substrata after autumn leaf-fall for 28 d in State Creek (from November 7- December 5, 2006). We used the same nutrient treatments and measured community respiration as described above. Immediately after quantifying respiration rates, one cellulose sponge from each treatment (N=6) was cut into small pieces using sterile forceps and surgical scissors, and pieces were placed in a centrifuge tube and frozen at -20°C until DNA extraction. Thirty-six hours later, DNA was extracted from the substrata using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc, Carlsbad, CA, USA).

Bacterial 16S rRNA genes were amplified from extracted DNA using primers EUB341F-GC, which contains a GC-rich clamp and is specific to most bacteria, and universal primer EUB534R (Muyzer et al. 1993). Primers used to amplify fungal ribosomal intergenic transcribed spacer region 2 (ITS2) were ITS3-GC, which contains a GC-rich clamp (Nikolcheva et al. 2005), and ITS4 (White et al. 1990). All primers were obtained from Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). Each 50 μL PCR reaction contained 0.4 μM forward primer, 0.4 μM reverse primer, 2.5 mM MgCl₂ (Promega, Madison, WI, USA), 160 μM dNTPs (Fisher Scientific), 1X GoTaq buffer (Promega), 3 units GoTaq DNA polymerase (Promega), and 3 μl environmental DNA
template, diluted 1:10 from the original extraction. PCR was run on a PTC-100 thermocycler (MJ Research, Waltham, MA, USA) with a temperature regime that included an initial denaturing temperature of 94°C for 2 minutes, followed by 40 cycles of 1) denaturing at 94°C for 1 minute, 2) annealing at 56.4°C for 1 minute, and 3) extension at 72°C for 1 minute. The final extension period was 5 minutes at 72°C.

DGGE was conducted using Biorad D-Code Universal Mutation Detection System (Biorad Laboratories, Hercules, CA, USA). We made the gels with 6% (for fungal amplicons) or 8% (for bacterial amplicons) polyacrylimide (weight/volume), with an acrylimide: bisacrylimide ratio of 36.4:1. Gels had a urea-formamide gradient of 30%-60% (for bacterial amplicons) or 20%-60% (for fungal amplicons). We cast the DGGE gel using a gradient maker and pump (GM-40 Gradient Maker and MP-100 Mini Peristaltic Pump, CBS Scientific Instruments, Del Mar, CA, USA). Electrophoresis was conducted at 60V for 14-16 h, or at 45V for 16-18 h at 60°C. Following electrophoresis, we stained the gels with GelStar nucleic acid stain (Lonza, Inc. Allendale, NJ, USA) and imaged them with Gel Doc 2000 digital gel imaging system (Bio-Rad).

Bacterial and fungal PCR amplicons were cloned with the TOPO-TA cloning kit (Invitrogen, Carlsbad, CA) using PCR-2.1 vector and were transformed into chemically competent *Escherichia coli*. Transformed *E. coli* were grown overnight on LB agar plates containing 50 μg mL⁻¹ kanamycin. Randomly selected colonies were transferred to LB broth containing 50 μg mL⁻¹ kanamycin, grown overnight at 37°C, and PCR-screened for the presence of inserts using M13F and M13R primers. Plasmids containing the insert were isolated using the Mini Plasmid Prep Kit (MoBio Laboratories). We then used DGGE to select the clones that represented the major component of the environmental
communities (Muyzer and Smalla 1998) Specifically, we used DGGE primers to amplify the clones and ran the PCR product from each clone on a DGGE gel with the original environmental sample. If the band lined up with one of the bands in the environmental sample, we considered it to be a major part of the environmental community, and we sequenced that clone (for example see Appendix A). If a clone band migrated to a position on the DGGE gel that was not in line with any band in the environmental sample, we considered this to be a minor member of the environmental community, and did not sequence it. We repeated this approach for each experimental nutrient amendment. All DNA sequencing was performed at the University of Chicago Cancer Research Center’s DNA Sequencing Facility (Chicago, IL, USA). Bacterial clone sequences were deposited to Genbank under accession numbers EU709496 to EY709512 and fungal clone sequences were deposited under accession numbers EU709513 to EU709516.

4.3.5. Data analysis

We used a 2-way repeated measures analysis of variation (RM-ANOVA) to compare differences in physiochemical factors among dates and streams. We also used principal component analysis (PCA) for all physiochemical data to illustrate spatial and temporal variation of physiochemical factors among streams and seasons. For control substrata (no nutrients added), we used a 3-way RM-ANOVA to compare chl a and respiration rates on fritted glass by substratum type, date, and stream. Where we found significant interactions, we tested for the influence of each factor separately using a
Bonferonni-corrected p-value for substratum type (t-test, p=0.05/9=0.006), date (RM-ANOVA, p=0.05/3=0.017), and stream (ANOVA, p=0.05/3=0.017; Zar 1999).

To test for nutrient limitation, we used a two-factor ANOVA with the 2 factors being the presence of N or P (Tank and Dodds 2003). We performed one 2-way ANOVA for comparison among control, NO$_3^-$, PO$_4^{3-}$, and NO$_3^- +$ PO$_4^{3-}$ treatments, and a second 2-way ANOVA for comparison among control, NH$_4^+$, PO$_4^{3-}$, and NH$_4^+ +$ PO$_4^{3-}$ treatments. If the response variables did not meet assumptions of ANOVA, we log-transformed the data prior to analysis. Using Tank and Dodds (2003) protocol for analyzing NDS results, single nutrient limitation was indicated when NO$_3^-$, PO$_4^{3-}$, or NH$_4^+$ alone resulted in a positive response without a significant interaction term. Co-limitation was indicated when two treatments independently affected the response variable, or when the combination treatment (NO$_3^- +$ PO$_4^{3-}$ or NH$_4^+ +$ PO$_4^{3-}$) significantly increased the response. We recorded secondary nutrient limitation when a single treatment affected the response variable, and then the combination treatment caused a greater increase in the response variable.

We used linear regression of physiochemical parameters with the nutrient response ratio (i.e., log [treatment/control]; NRR) for chl $a$ and respiration on each substratum type across all streams and dates (N=9; Tank and Dodds 2003). Using the NRR allowed us to compare the degree of nutrient limitation across all NDS deployments as a continuous rather than categorical variable (i.e., N or P limited).

We used SeqMan and MegAlign software (DNA Star, Madison, WI, USA) to edit DNA sequences of PCR products, conduct BLAST searches for matching sequences in the National Center for Biotechnology Information (NCBI) database, and to align our
sequences to the most closely related published sequences. The phylogenetic trees were created from aligned sequences using Mega 3.1 (The Biodesign Institute, Tempe, AZ), with Jukes-Cantor neighbor joining and bootstrapping. All other statistics were done using Systat 11.0 (Systat Software, Inc, San Jose, CA, USA) or SPSS 11 (SPSS, Inc., Chicago, IL, USA). PCA was executed using PC-ORD (MjM Software Design, Gleneden Beach, Oregon, USA).

4.4. Results

4.4.1. Physiochemical parameters during NDS incubation periods

We found strong seasonal differences in the physical, chemical, and biological characteristics of the stream environments during the NDS incubation periods, which were consistent with our expectations for the temperate climate of the region. However, we also found several differences among streams, despite their similarity in size and close physical proximity (Table 4.1). In all 3 streams, we found the highest values in the summer for several factors including canopy cover (RM-ANOVA, p<0.01), temperature (p<0.01), and SRP concentrations (p<0.01). CBOM standing stock was highest in the autumn (p=0.01), and mean discharge was highest in the spring (p<0.01). In contrast, NO$_3^-$ and NH$_4^+$ concentrations and FBOM standing stocks were variable among streams and dates.

A PCA helped illustrate spatial and temporal variation of physiochemical factors among streams and seasons (Figure 4.1). PC axis 1 explained 34% of the variation among environmental variables with an eigenvalue of 3.04 (broken-stick value of 2.82).
PC axis 1 was positively correlated with water column NH$_4^+$ concentration, CBOM standing stock, and NDS depth, which were higher in autumn, and negatively correlated with mean discharge and temperature (Table 4.2). PC axis 2 explained 30% of the variation among environmental variables and had an eigenvalue of 2.70 (broken-stick value of 1.84). PC axis 2 was positively correlated to temperature, SRP concentration, and canopy cover, which were higher in the summer (Table 4.2; Figure 4.1). Together, PCA axes 1 and 2 separated the potential environmental drivers of nutrient limitation into three clusters representing, spring, summer, and autumn, which illustrated seasonal variability of controlling factors was greater than spatial variability (Figure 4.1).
Figure 4.1. Principal components analysis (PCA) illustrating spatial and temporal variation in seasonal factors during nutrient diffusing substrata incubations or on the collection date. Temp = temperature (N=28), Canopy = Canopy cover (N=4), Q = discharge (N=28), NH$_4^+$ = ammonium concentration (N=10), SRP = soluble reactive phosphorus concentration (N=10), NO$_3^-$ = nitrate concentration (N=10), and FBOM and CBOM = standing stocks of fine and coarse benthic organic matter, respectively (N=10). N represents the number of replicates used to calculate the mean of each value for each seasonal factor.
### TABLE 4.2

CORRELATION COEFFICIENTS BETWEEN PRINCIPAL COMPONENTS (PC) 1 AND 2 AND EXTERNAL DRIVERS

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>0.45</td>
<td>-0.08</td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.44</td>
<td>0.35</td>
</tr>
<tr>
<td>Canopy cover</td>
<td>-0.09</td>
<td>0.49</td>
</tr>
<tr>
<td>Mean Q</td>
<td>-0.28</td>
<td>-0.44</td>
</tr>
<tr>
<td>SRP</td>
<td>-0.22</td>
<td>0.40</td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td>0.38</td>
<td>0.13</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td>0.21</td>
<td>0.29</td>
</tr>
<tr>
<td>FBOM</td>
<td>0.14</td>
<td>0.41</td>
</tr>
<tr>
<td>CBOM</td>
<td>0.50</td>
<td>-0.11</td>
</tr>
</tbody>
</table>

Note: Q = discharge, SRP = soluble reactive phosphorus concentration, NH\(_4^+\) = ammonium concentration NO\(_3^-\) = nitrate concentration, and FBOM and CBOM = standing stocks of fine and coarse benthic organic matter, respectively. Eigenvalue for PC1 = 3.04 and for PC2= 2.70. Variance explained for PC1=34% and for PC2=30%.
4.4.2. Biofilm growth on control substrata (no nutrients added)

On control substrata, we used a 3-way RM-ANOVA with chl $a$ or respiration as dependent variables, and substratum type, date, and stream as factors. We found significant interactions among the 3 factors, so we analyzed each factor separately using Bonferonni-corrected p-values (see Methods). Overall, we found autotrophic preference for fritted glass relative to cellulose sponge, indicated by higher chl $a$ density on fritted glass (t-test, $p=0.005$; Figure 4.2), but no difference in mean respiration rates between substrata ($p=0.35$). Looking at each date separately, we found chl $a$ was always higher on fritted glass relative to cellulose sponge in summer (ANOVA, $p<0.001$), while respiration was always higher on cellulose sponge relative to fritted glass in the spring (ANOVA, $p<0.001$; data not shown). Finally, by stream, we found Shane Creek had the lowest chl $a$ in autumn relative to other dates (RM-ANOVA, $p<0.001$), and Walton Creek had the highest chl $a$ in spring (RM-ANOVA, $p<0.001$). For respiration rates, Walton Creek had the highest rates in autumn (RM-ANOVA, $p<0.001$), and Shane Creek the highest rates in summer (RM-ANOVA, $p<0.001$).
Figure 4.2. Chlorophyll $a$ (chl $a$) and microbial respiration on fritted glass and cellulose sponge for control substrata (no nutrients added). Chl $a$ was significantly different between substrata (t-test, $p=0.005$), but respiration was not (t-test, $p=0.351$). Significant difference between substrata marked with *.

Chl $a$ and respiration on control substrata were linked to environmental conditions including canopy cover and mean discharge on both substrata (Figure 4.3). Chl $a$ was negatively related to canopy cover ($r^2=0.37$, $p=0.01$), while respiration was negatively related to mean discharge ($r^2=0.24$, $p=0.04$). Other potentially important environmental drivers of biofilm growth including temperature, depth, nutrient concentrations, and organic matter standing stocks were unrelated to chl $a$ or respiration on control substrata.
Figure 4.3. Linear regression between A) canopy cover and chlorophyll a (chl a) and B) mean discharge (Q) and respiration on control substrata (no nutrients added).
4.4.3. Patterns of nutrient limitation on fritted glass and cellulose sponge

Overall, we found few instances of significant nutrient limitation on fritted glass, and where nutrient limitation was found, the patterns were different among streams and seasons (Table 4.3, Figure 4.4). There were no cases where same nutrient was limiting chl $a$ in all 3 replicate streams, and there was no season where one of the streams was not nutrient limited. For the 9 potential occurrences of nutrient limitation of chl $a$ on glass (i.e., 3 streams and 3 dates), significant nutrient limitation occurred only 3 times. All 3 cases were distinct, including limitation by $\text{PO}_4^{3-}$ alone (Walton, spring), $\text{NH}_4^+$ alone (State Creek, summer), or $\text{NH}_4^+ + \text{PO}_4^{3-}$ (State Creek, autumn). Significant limitation of respiration on fritted glass occurred only 1 out of 9 potential cases, in State Creek in spring.
## TABLE 4.3

**NUTRIENT LIMITATION AS INDICATED BY NUTRIENT DIFFUSING SUBSTRATA FOR CHLOROPHYLL A (CHL A) AND RESPIRATION ON TWO SUBSTRATUM TYPES, FRITTED GLASS AND CELLULOSE SPONGE, IN STATE, SHANE, AND WALTON CREEKS IN SPRING, SUMMER, AND AUTUMN**

<table>
<thead>
<tr>
<th>Season</th>
<th>Stream</th>
<th>Chl a</th>
<th>Respiration</th>
<th>Chl a</th>
<th>Respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>State</td>
<td>--</td>
<td>NO$_3^-$</td>
<td>--</td>
<td>PO$_4^{3-}$</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>NO$_3^-$ + PO$_4^{3-}$</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>PO$_4^{3-}$</td>
<td>--</td>
<td>--</td>
<td>NO$_3^-$ + PO$_4^{3-}$</td>
</tr>
<tr>
<td>Summer</td>
<td>State</td>
<td>NH$_4^+$</td>
<td>--</td>
<td>--</td>
<td>NO$_3^-$ + PO$_4^{3-}$</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>--</td>
<td>--</td>
<td>NH$_4^+$</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>--</td>
<td>--</td>
<td>PO$_4^{3-}$</td>
<td>--</td>
</tr>
<tr>
<td>Autumn</td>
<td>State</td>
<td>NH$_4^+$ + PO$_4^{3-}$</td>
<td>--</td>
<td>--</td>
<td>NH$_4^+$ + PO$_4^{3-}$</td>
</tr>
<tr>
<td></td>
<td>Shane</td>
<td>--</td>
<td>--</td>
<td>NH$_4^+$ + PO$_4^{3-}$</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Walton</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: NO$_3^-$ = nitrate alone, PO$_4^{3-}$ = phosphate alone, NH$_4^+$ = ammonium alone, NO$_3^-$ + PO$_4^{3-}$ = nitrate and phosphate together, NH$_4^+$ + PO$_4^{3-}$ = ammonium and phosphate together, and -- indicates no nutrient limitation.
Figure 4.4. Chlorophyll a (chl a) and respiration on fritted glass amended with no nutrients (control), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), ammonium (NH$_4^+$), NO$_3^- +$ PO$_4^{3-}$, and NH$_4^+ +$ PO$_4^{3-}$ in State, Shane, and Walton Creeks in spring, summer, and autumn. * indicates significant nutrient limitation for chl a, and + indicates significant nutrient limitation among treatments for respiration. Significant limitation calculated after Tank and Dodds (2003).
Results for nutrient limitation on cellulose sponge were similar in frequency to fritted glass for chl $a$, however, nutrient limitation of respiration was more frequent on cellulose sponge relative to the fritted glass (Table 4.3, Figure 4.5). Like for fritted glass, we found no cases where the same nutrient was limiting chl $a$. For the 9 potential occurrences of nutrient limitation of chl $a$ on cellulose, significant limitation occurred 3 times. Each of the results were distinct, including nutrient limitation of chl $a$ by PO$_4^{3-}$ alone (Walton Creek, summer), NH$_4^+$ alone (Shane Creek, summer), or NH$_4^+$+ PO$_4^{3-}$ (Shane Creek, autumn). For the 9 potential occurrences of nutrient limitation of respiration rates on cellulose sponge, significant limitation occurred 6 times, and in several cases, the same treatment was limiting across streams (Table 4.3, Figure 4.5). PO$_4^{3-}$, either alone or in combination with N, was limiting to respiration on cellulose in all 3 streams in the spring. We found NO$_3^-$ + PO$_4^{3-}$ limitation 2 times in the spring (Shane and Walton Creeks), and 1 time in the summer (Walton Creek). NH$_4^+$+ PO$_4^{3-}$ was also limiting to respiration on cellulose in 2 cases, once in spring (Walton Creek) and once in the autumn (State Creek).
Figure 4.5. Chlorophyll $a$ (chl $a$) and respiration on cellulose sponge amended with no nutrients (control), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), ammonium (NH$_4^+$), NO$_3^- +$ PO$_4^{3-}$, and NH$_4^+ +$ PO$_4^{3-}$ in State, Shane, and Walton Creeks in spring, summer, and autumn. * indicates significant nutrient limitation for chl $a$, and + indicates significant nutrient limitation among treatments for respiration. Significant limitation calculated after Tank and Dodds (2003).
We found nutrient response ratio (NRR) was related to several environmental factors for biofilms on the fritted glass, but not for cellulose sponge (Figure 4.6). Nutrient response ratio of chl \( a \) on fritted glass was positively related to temperature for \( \text{NO}_3^- \) (\( r^2=0.46 \ p=0.05 \)), \( \text{PO}_4^{3-} \) (\( r^2=0.60, \ p=0.02 \)), \( \text{NH}_4^+ \) (\( r^2=0.62 \ p=0.03 \)), \( \text{NO}_3^- + \text{PO}_4^{3-} \) (\( r^2=0.53 \ p=0.02 \)), and \( \text{NH}_4^+ + \text{PO}_4^{3-} \) (\( r^2=0.50 \ p=0.02 \)). Chl \( a \) on fritted glass was also positively related to water column SRP concentration for \( \text{NO}_3^- \) (\( r^2=0.46 \ p=0.05 \)) and \( \text{NH}_4^+ \) (\( r^2=0.60 \ p=0.02 \)). Finally, mean discharge was positively related to respiration on fritted glass for \( \text{NO}_3^- \) (\( r^2=0.52 \ p=0.03 \)), \( \text{NO}_3^- + \text{PO}_4^{3-} \) (\( r^2=0.545 \ p=0.02 \)), and \( \text{NH}_4^+ + \text{PO}_4^{3-} \) (\( r^2=0.50 \ p=0.03 \)). For the cellulose sponge, there were no significant relationships between NRR and chl \( a \) or respiration and environmental drivers.
Figure 4.6. Linear regressions between A) temperature and chlorophyll a (chl \( a \)) nutrient response ratio (NRR; treatment/control) for NO\(_3^-\) \( (r^2=0.46 \ p=0.05) \), PO\(_4^{3-}\) \( (r^2=0.60, \ p=0.02) \), NH\(_4^+\) \( (r^2=0.62 \ p=0.03) \), NO\(_3^-\) + PO\(_4^{3-}\) \( (r^2=0.53 \ p=0.02) \), and NH\(_4^+\) + PO\(_4^{3-}\) \( (r^2=0.50 \ p=0.02) \), B) water column soluble reactive phosphorus (SRP) and chl \( a \) nutrient response ratio for NO\(_3^-\) \( (r^2=0.46 \ p=0.05) \) and NH\(_4^+\) \( (r^2=0.60 \ p=0.02) \), and C) mean discharge (Q) and nutrient response ratio for NO\(_3^-\) \( (r^2=0.52 \ p=0.03) \), NO\(_3^-\) + PO\(_4^{3-}\) \( (r^2=0.545 \ p=0.02) \), and NH\(_4^+\) + PO\(_4^{3-}\) \( (r^2=0.50 \ p=0.03) \). Regressions line shown in A) indicates the regression between independent variable all significant nutrient response ratios combined.
4.4.4. Microbial community analysis on cellulose

To determine if there was a link between nutrient limitation patterns (i.e., higher respiration) and microbial community composition, we incubated cellulose substrata in State Creek (the stream which was most frequently nutrient limited) in autumn 2006. Similar to results from the NDS experiment in autumn 2003, community respiration on cellulose sponge in State Creek in 2006 indicated N and P co-limitation, but in 2006 the result indicated \( \text{NO}_3^- \) and \( \text{PO}_4^{3-} \) co-limitation (data not shown) rather than \( \text{NH}_4^+ + \text{PO}_4^{3-} \) as in 2003 (Table 4.3).

A phylogenetic analysis of bacterial 16S rRNA genes from each treatment showed differences in the dominant taxa based on which treatments were applied to the biofilms (Figure 4.7). Control, \( \text{NH}_4^+ \) and \( \text{NH}_4^+ + \text{PO}_4^{3-} \) had sequences closely matching *Flectobacillus* sp., members of the Comomonadaceae family of Beta-Proteobacteria (*Variovax* sp., *Rhodoferax* sp., and *Hylemonella* sp.), Cyanobacteria, and a picoplankton chloroplast. In the \( \text{NO}_3^- \), \( \text{PO}_4^{3-} \), and \( \text{NO}_3^- + \text{PO}_4^{3-} \) treatments, we isolated taxa with sequences most closely matching the genus *Flavobacterium* from the Cytophaga-Flavobacteria, organisms closely matching sequences from the Gamma-Proteobacteria genera *Legionella* sp., and *Pseudomonas* sp., and 2 organisms matching sequences for genera in the Beta-Proteobacteria cluster for which the family grouping is unresolved (including *Proteinimicrobium* sp.). This was consistent with the results of the community respiration rates on the nutrient treatments. \( \text{NO}_3^- \), \( \text{PO}_4^{3-} \), and \( \text{NO}_3^- + \text{PO}_4^{3-} \) treatments had higher respiration rates and a similar microbial community relative to the other treatments and the control.
Figure 4.7. Phylogenetic tree of isolated PCR products amplified with bacterial primers representing the dominant taxa in each treatment. PCR was from bacteria extracted from cellulose amended with no nutrients (Control), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), ammonium (NH$_4^+$), NO$_3^-$ + PO$_4^{3-}$, and NH$_4^+$ + PO$_4^{3-}$ incubated for 28 days in State Creek starting on November 7, 2006. Scale bar represents an estimated 5% divergence.
We found lower overall diversity of fungal relative to bacterial ribotypes. A phylogenetic analysis of fungal sequences showed a high degree of similarity between two bands, one found in the Control, NO$_3^-$, PO$_4^{3-}$, NH$_4^+$, and NO$_3^- +$ PO$_4^{3-}$ treatments (Band 2), and the other in the PO$_4^{3-}$ and NH$_4^+ +$ PO$_4^{3-}$ treatments (Band 1; Figure 4.8). A BLAST search of the Band 1 and 2 sequences revealed no matches in the NCBI database. Bands 3 and 4 in the NO$_3^-$ treatment were unique from the other treatments, and were similar to fungal sequences obtained from cellulose buried in fertilizer-amended soil samples in China (NCBI Accession No. AY704760; Zhao et al. 2005) and finished compost made from woodchips and yard debris in North Carolina, USA (NCBI Accession No. DQ900996; Figure 4.8). We had two contaminant organisms isolated from our negative control, which were shown to most closely match the cosmopolitan Ascomycota fungi *Aureobasidium pullululans* and *Cladosporium* sp.
Figure 4.8. Phylogenetic tree of isolated PCR products amplified with fungal primers representing the dominant taxa in each treatment. PCR was from bacteria extracted from cellulose amended with no nutrients (control), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), ammonium (NH$_4^+$), NO$_3^-$ + PO$_4^{3-}$, and NH$_4^+$ + PO$_4^{3-}$ incubated for 28 days in State Creek starting November 7, 2006. Scale bar represents an estimated 5% divergence. *Auerobasidium pullulans* and *Cladosporium* sp. represent contaminants in fungal primers.
4.5. Discussion

4.5.1. Autotrophic and heterotrophic colonization of two substrata types

The contrasting patterns of chl \( a \) and respiration on fritted glass and cellulose sponge substrata suggests fundamental differences in biofilm composition and function between inorganic and organic substrata. Higher chl \( a \) density on fritted glass relative to cellulose indicates that there were more autotrophs on inorganic substrata (Figure 4.1), similar to what has been found in previous NDS studies comparing substratum types (Tank and Dodds 2003, Johnson et al. in review). In contrast, community respiration rates were not different between organic and inorganic substrata (Figure 4.1) which differs with the results from Johnson et al. (in review). Using the identical substratum types, they recorded higher respiration on cellulose sponge relative to fritted glass from NDS incubated in 72 streams throughout North America. While the mean respiration rates on cellulose sponge were approximately the same between Johnson et al. (in review) and our study (~5.25 mgO\(_2\) cm\(^{-2}\) h\(^{-1}\)), mean respiration rate on fritted glass in our study was about 2 times higher (4.8 vs. 2.5 mgO\(_2\) m\(^{-2}\) h\(^{-1}\)). Despite the differing results for respiration rates on control substrata, both studies showed nutrient limitation was more infrequent on fritted glass relative to cellulose sponge (Johnson et al. in review; Table 4.3). Overall, substratum type had a strong influence on both biofilm constituents (i.e., more autotrophs on fritted glass than cellulose sponge), as well as on patterns of nutrient limitation.
Other studies have demonstrated differences in functional properties of biofilms on organic and inorganic substrata, which has implications for managing stream ecosystems in regards to nutrient enrichment. For example, epilithic (i.e., inorganic substrate) and epixylic (i.e., organic substrate) biofilm communities have been shown to have different metabolism rates, nutrient uptake rates, and nutrient limitation patterns (Sabater et al. 1998, Tank and Dodds 2003). In these study streams we have also documented significant differences in metabolism and NO$_3^-$ uptake among multiple in situ substrata (e.g., wood, sand, and rocks; Hoellein unpublished data). While most previous NDS studies have used inorganic substrata to assign the nutrient limitation status of the entire stream ecosystem, most streams are a heterogeneous mixture of substratum types or patches (Townsend 1989), which our results indicate display discrete responses to nutrient enrichment (Tank and Dodds 2003, Johnson et al. in review).

4.5.2. Nutrient limitation on fritted glass

Our results for nutrient limitation on fritted glass showed unexpectedly few cases of nutrient limitation (i.e., 3 out of 9 cases for chl $a$, 1/9 cases for respiration) even though background nutrients are very low in our study streams. High variability in nutrient limitation patterns for chl $a$ on inorganic substrata was also reported by Wold and Hershey (1999) in 6 headwater tributaries of Lake Superior. They found a mixture of N, P, and co-limitation, but also found no nutrient limitation in 19 out of 72 cases (26%), by measuring chl $a$ accrual on an inorganic substratum over a similar seasonal range of sampling dates (i.e., early
June through late November). Our results concur, demonstrating that streams in close proximity, in the same geographic region, may not necessarily show similar nutrient limitation status, even where there are minimal human impacts on watershed conditions (i.e., “pristine” conditions). In addition, our data indicated that under oligotrophic conditions, one cannot assume that periphyton are nutrient limited, but rather the response of periphyton to nutrient enrichment is often dependent on additional environmental factors besides nutrient availability.

We found the strongest relationship between physiochemical factors and response to nutrient enrichment on fritted glass was an increased response in the summer, which was a period of higher water temperature and SRP water column concentrations (Figure 4.6). While we cannot distinguish the individual influences of water column SRP vs. temperature in our study (i.e., they were both changing with the seasons), both factors could potentially influence the response of periphyton to nutrient enrichment. Water column SRP concentrations were generally very low in all 3 streams (i.e., <10 μg L⁻¹), so biofilm organisms enriched by N would be sensitive to changes in water column P. More simply, given enough N, biofilms could then respond to small changes in P availability. Periphyton has been shown to assimilate water column nutrients (as ¹⁵N) even when growing on NDS which are actively supplying nutrients from underneath the biofilm (von Schiller et al. 2007). In addition, water temperatures across all NDS deployments spanned a broader range than has previously been considered in previous NDS analyses, 1-15°C (but see Wold and Hershey 1999), which suggests temperature plays a important role in regulating biofilm response to
nutrient enrichment when considered across a large gradient. Thus, while we found significant nutrient limitation of chl \(a\) on fritted glass biofilms in only 3 out of 9 potential cases (Table 4.2), by utilizing the gradient of treatment responses across all 9 deployments, we demonstrated the unexpected influence of water column SRP concentration and temperature on the response of biofilms to nutrient enrichment in the study streams.

Among the numerous influences affecting the growth of autotrophs in lotic ecosystems, light has often been shown to be a primary controlling factor, (Mosisch et al. 2001), so we expected to record the greatest response to nutrients on fritted glass when light availability was highest, in the spring (before leaf-out) and/or autumn (after leaf-fall) (Table 4.1). Light availability was an important control on chl \(a\) growth on control substrata (Figure 4.3); however, the greatest response to nutrient enrichment was in the summer, when light availability was lowest (Table 4.1). This is contradictory to other studies, which have shown nutrient enrichment has a greater effect on autotrophic biofilms in streams with high light relative to low light availability (Tank and Dodds 2003, Johnson et al. in review). However, these previous studies included variation in light availability that occurs for streams across multiple biomes, and not among seasons within similar forested, headwater streams. Its possible that even in spring and autumn, light availability at our study sites was still low relative to other sites in the literature considered to be “open canopy” (i.e., streams in grasslands, deserts, or with anthropogenic influence on riparian zones) and this was why other factors such as temperature and water column SRP were more important than light in
determining response of chl $a$ with nutrient enrichment on fritted glass in the study streams.

4.5.3. Nutrient limitation on cellulose sponge

In contrast to patterns of nutrient limitation on fritted glass, we found consistent patterns of nutrient limitation for respiration on cellulose sponge both among seasons and among streams, which were consistent with previously published seasonal patterns of whole-stream nutrient uptake measured in the same streams. Our previous measurements of whole-stream nutrient uptake rates (via short-term enrichments conducted May 2003-April 2004), demonstrated that spring was the period of highest overall nutrient demand and in one particular, State Creek, we found the overall highest uptake rates (Hoellein et al. 2007). Patterns of nutrient limitation on cellulose sponge matched these results, as all 3 streams were nutrient limited in spring, and State Creek was always nutrient limited (Table 4.2). Because the nutrient limitation status of biofilms on organic substrata corresponded to the spatial and temporal patterns of whole-stream uptake rates, which integrates the biologically activity of all stream biofilms, results suggest that biofilms on organic substrata were a significant portion of whole-stream nutrient demand across dates. Whole-stream nutrient uptake rates are rarely paired with NDS studies (but see von Schiller et al. 2007), but our results suggest the two can be combined to provide complementary information regarding the activity of stream biofilms under both ambient (i.e., whole-stream uptake rates) and nutrient enriched (i.e., NDS) conditions.
4.5.4. Comparison of nutrient limitation by NO₃⁻ and NH₄⁺

Previous NDS studies in streams have rarely employed a direct comparison of NO₃⁻ and NH₄⁺ limitation (but see von Schiller et al. 2007). Theoretically, NH₄⁺ is the preferred inorganic N species relative to NO₃⁻ because it is the form required within the cell (Dortch 1990) and requires no additional energy to bring across the cell wall. Among the relatively few studies to experimentally address inorganic N preference, NH₄⁺ relative to NO₃⁻ preference has been shown for soil microbes (Rice and Tiedje 1989) marine phytoplankton (Dortch 1990), and more recently in stream periphyton (von Schiller et al. 2007). Our results for both inorganic and organic substrata do not support a biofilm preference for NH₄⁺ vs. NO₃⁻ as an inorganic N source. Of the 4 cases of nutrient limitation on fritted glass (3 for chl a and 1 for respiration), 2 cases responded to NH₄⁺ addition and we found 1 response to each NO₃⁻ and PO₄³⁻ addition individually (Table 4.2). Of the 6 cases of nutrient limitation on cellulose, 3 showed a response to added NO₃⁻+ PO₄³⁻ while 2 responded to the addition of NH₄⁺+ PO₄³⁻ (Table 4.2). In only one case did we find limitation of both NO₃⁻+ PO₄³⁻ and NH₄⁺+ PO₄³⁻ during the same NDS deployment (Walton Creek, spring). Thus, our NDS results did not support the hypothesis that NH₄⁺ should be the preferred inorganic N source for stream biofilm constituents on either fritted glass or cellulose sponge substrata. However, our results indicate that future NDS deployments would benefit from incorporating both inorganic N species when possible, because either can be limiting to biota (even in the same stream at the same time). Further experimentation is needed to more fully develop our
understanding of inorganic N preference for stream biofilms colonizing multiple substratum types.

4.5.5. Microbial communities on NDS

We found bacteria taxa colonizing NDS that represent common members of aquatic microbial communities, which are able to decompose high molecular weight carbon compounds such as cellulose, inhabit eutrophic environments, and are adapted to cold conditions. Cytophaga-Flavobacteria are non-spore forming, chemo-organotrophic bacteria capable of degrading polymers such as chitin and cellulose (Kirchman 2002). Members of this group are low-temperature adapted (i.e., psychrophilic) in many freshwater habitats (Battin et al. 2001). Beta-Proteobacteria have been found to be prevalent early in biofilm formation (Manz et al. 1999), with preference for the same or lower molecular weight C substrates as Cytophaga-Flavobacteria (Cottrell and Kirchman 2000) and a similar cosmopolitan distribution (Eiler et al. 2003). Gamma-Proteobacteria, in contrast, have generally been found to be less abundant in streams than either of the 2 former groups (Olapade and Leff 2005), but have been shown to respond to nutrient enrichment (Pinhassi and Berman 2003). Cyanobacteria have previously been described via DGGE and cloning, typically in water bodies with low N/P ratios (de Figueiredo et al. 2007), and they have been found concurrent with Beta-Proteobacteria and Cytophaga-Flavobateria (Roeselers et al. 2007). Collectively, these organisms are adapted to the environment provided by the NDS, which were
nutrient-enriched cellulose and deployed in November, when water temperature was ~ 2.5°C.

In autumn 2006, NDS deployed in State Creek showed significantly higher respiration rates when exposed to the NO₃⁻, PO₄³⁻, and NO₃⁻ + PO₄³⁻ treatments, and resulted in phylogenetically distinct bacterial communities among treatments (Figure 4.7). Cytophaga-Flavobacteria (as Flavobacterium sp.) colonized cellulose with PO₄³⁻ and NO₃⁻ + PO₄³⁻ treatments, but were not found on the control substratum. Olapade and Leff (2005) and Rubin and Leff (2007) each linked Cytophaga-Flavobacteria abundance with seasonal peaks in N and P concentrations in the Mahoning River, Ohio, and speculated this group may require greater nutrient availability due to their use of high molecular weight C compounds for heterotrophic metabolism. In our study, Cytophaga-Flavobacteria may also have been stimulated following nutrient enrichment + colonization of a high molecular weight polysaccharide like cellulose. In contrast, we found the organisms from the control, NH₄⁺ and NH₄⁺+PO₄³⁻ treatments were from the Cyanobacteria, the Comamonadaceae family of the Beta-Proteobacteria, and one similar to a chloroplast from picoplankton. We found no Gamma-Proteobacteria or Cytophaga-Flavobacteria (except 1, Flectobacillus sp. with NH₄⁺ enrichment) in these treatments. Overall, the communities from the control, NH₄⁺ and NH₄⁺+PO₄³⁻ treatments were more similar to one another than the communities found on the NO₃⁻, PO₄³⁻, and NO₃⁻ + PO₄³⁻ treatments. We also found respiration rates on the NO₃⁻ and PO₄³⁻ treatments (both alone and in combination) were significantly higher than on the control substrata, while the NH₄⁺ or NH₄⁺+PO₄³⁻ treatments
did not increase respiration above the control. Overall, the data suggest that significant changes in community functional properties (i.e., respiration rates) could be linked to shifts in the dominant members of the bacterial community.

The number of published studies using DGGE and sequencing to analyze fungal communities on stream substrata other than leaf litter is limited (Nikolcheva and Bärlocher 2005, Das et al. 2007). Our analysis of the fungal community on cellulose sponge NDS in State Creek showed a lower diversity of ribotypes than has been found on leaf litter in streams (Das et al. 2007). Our relatively low fungal ribotype diversity could be due to structural homogeneity of the artificial cellulose sponge substrata (i.e., relative to the chemically heterogeneous carbon polymers present in leaf litter) or the influence of nutrient enrichment, both of which could decrease diversity by selecting for taxa with narrow metabolic criteria. Similarly, Bärlocher (2006) used DGGE and conidia counts to show lower number of aquatic fungal taxa on wood compared to leaf litter, indicating wood supported a less diverse fungal community relative to leaves.

We found only 2 sequences in the NCBI database that were similar to our sequences, isolated from cellulose paper buried in bio-fertilizer amended soils in China (Zhao et al. 2005) and from woodchip-derived compost in North Carolina. These two closest matches were from terrestrial sources, supporting our assertion that this study represents a relatively early entry of fungal ITS sequences into the NCBI database from cellulolytic stream fungi. Overall, nutrient enrichment did not appear to affect fungal community composition as indicated by DGGE and
gene sequencing, however, more research is needed to 1) supply additional sequences to the database, and 2) resolve the relationship between nutrient enrichment and fungal diversity on non-leaf litter organic substrata in streams.

4.6. Conclusions

Previous work has demonstrated variability in nutrient limitation status on organic and inorganic substrata on streams in different biomes (Tank and Dodds 2003) and streams draining different land-use types (von Schiller et al. 2007). To our knowledge, ours is the first study to demonstrate that nutrient limitation on both inorganic and organic substrata types is variable by season. We add to Dodds (2006) assertion that managers need to consider both autotrophic and heterotrophic pathways in streams, by including the importance of considering the seasonal differences in the response of each biofilm type to nutrient enrichment. Further, our study represents a rare direct comparison of NH$_4^+$ vs. NO$_3^-$ limitation on both inorganic and organic substrata. The lack of NH$_4^+$ preference over NO$_3^-$ in our study is significant in the context of eutrophication, as enrichment by both inorganic N types can significantly affect the biological activity (i.e., respiration) and biomass (i.e., chl $a$) of stream biofilms. Finally, our results indicated that significant changes in biofilm function following nutrient enrichment were linked with changes in predominant members of the bacterial community, but not the fungal community. An increased understanding of the link between microbial community structure and function in stream ecosystems is critical because microbes are responsible for numerous ecosystem processes in streams such as
decomposition and nutrient cycling, which have large impacts on higher trophic levels and overall stream health.

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APPENDIX A:

DENATURING GRADIENT GEL ELECTROPHORESIS OF PCR PRODUCTS FROM ENVIRONMENTAL SAMPLES (ES) AND CLONED SEQUENCES FOR A) BACTERIAL COMMUNITY ON THE CONTROL (NO NUTRIENT) CELLULOSE, AND B) FUNGAL COMMUNITY ON THE AMMONIUM PLUS PHOSPHATE ($\text{NH}_4^+ \text{PO}_4^{3-}$) AMENDED CELLULOSE

4.8. Literature Cited


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CHAPTER 5
THE EFFECTS OF A TROUT SPAWNING HABITAT ENHANCEMENT ON GEOMORPHOLOGY, ECOSYSTEM PROCESSES, AND FISH COMMUNITIES IN THREE HEADWATER STREAMS

5.1. Abstract

Stream restorations designed to influence a single taxon (e.g., trout), can have concurrent effects on non-target ecosystem dynamics. We measured nutrient uptake and reach-scale metabolism rates in manipulated and reference reaches in 3 streams located in the Upper Peninsula of Michigan. We sampled 4 times across seasons from May 2006 to September 2007, and on the final date also measured fish community composition in each reach. The manipulated reaches in each stream included the combination of a 10m long sediment trap and a 40-60m gravel and boulder addition, designed to retain sediment, stabilize banks, and provide spawning habitat for native trout (Salvelinus fontinalis). Manipulation reaches in all streams had higher rock abundance as well as reaeration rates. In one stream, we also found associated higher nutrient uptake.

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and community respiration rates, however, we found minimal differences in fish community structure. Overall, *S. fontinalis* biomass was negatively correlated with fine sediment coverage, which was lower in the manipulated relative to reference reaches. In general, we found physical differences in habitat translated into few biological effects, and conclude that it may not represent a sustainable method for increasing *S. fontinalis* in these low-gradient, sandy-bottomed streams. Pairing measurements of nutrient uptake and metabolism rates with fish community structure for evaluation of restoration is rare, but provides a more comprehensive analysis of multiple ecosystem effects following habitat manipulations targeted at one taxon.

5.2. Introduction

The number of stream and river restoration projects in the United States has grown in recent decades, however, only a small proportion of these projects are monitored (~10%), and even fewer produce or disseminate monitoring results (Bernhardt 2005). For stream researchers, managers, and restoration professionals, an increased emphasis on evaluation is critical for the thoughtful redesign of existing strategies, and to avoid expending resources on ineffective projects (Lake 2001, Palmer et al. 2005). In addition, the lack of evaluation and monitoring represents a missed opportunity. If researchers and managers consider and design restorations as experimental manipulations (i.e., using reference conditions, pre-treatment data collection, and replication where possible),
restoration projects could address basic research questions and propel the science of restoration ecology forward (Poff et al. 2003).

Stream restorations can have both direct and indirect effects on stream biota (Figure 5.1). A manipulation can directly affect stream biota by establishing some attractive habitat type that initiates immigration and reproduction of organisms at the restored area, known as the “field of dreams” hypothesis (Palmer 1997). Additionally, a manipulation may indirectly affect biota by altering the streambed substrata composition and/or increasing the retention of autochthonous and allochthonous carbon resources available for stream biota, thereby altering rates of ecosystem processes such as organic matter decomposition, nutrient cycling, and primary and secondary production (Lepori et al. 2006, Entrekin et al. in press). To determine which mechanism is in operation following stream restoration (i.e., direct or indirect effects), measurements of biological communities and ecosystem processes should be made simultaneously; however, most monitoring projects are focused solely on abundance of target organisms (Minns et al. 1996).

In rural regions of the upper Midwestern US, stream restorations are often directed at counteracting the legacy of intensive logging that occurred from the late 1800s to early 1900s. The long-term impacts of logging on lotic ecosystems include channelization, low in-stream abundance of large wood, and increased sand and fine organic sediment accumulation (Bassett 1988, Maser and Sedell 1994). These composite influences negatively affect stream ecosystems for decades to centuries (Harding et al. 1998, Nilsson et al. 2005). On US federal
lands, streams are now protected by riparian logging regulations, and management agencies may install in-stream habitat enhancements designed to increase sport fisheries. Commonly used techniques include the addition of large wood (Roni and Quinn 2001), channel shading structures (Rosi-Marshall et al. 2006), boulders (Lepori et al. 2005b), gravel for spawning habitat (Nakamura 1999, Palm et al. 2007), and settling basins to collect sand and sediment (Hansen et al. 1982, Avery 1996). Studies evaluating the efficacy of these habitat manipulations typically record the effects on stream geomorphology and fish and macroinvertebrate communities; however, influences on critical ecosystem processes such as nutrient uptake and metabolism rates are rarely addressed (Lake et al. 2007).

The objectives of our study were to evaluate stream channel manipulations conducted in three adjacent streams in the Upper Peninsula of Michigan, USA, designed to increase the abundance of native brook trout (*Salvelinus fontinalis*). In both manipulated and upstream reference reaches of each stream, we compared stream physiochemical parameters, streambed substrata composition, basal food resources (i.e., organic matter and chlorophyll $a$), and whole-stream nutrient uptake and metabolism rates. We measured all variables during three biologically distinct periods in 2006, including spring (prior to leaf-out), summer (during full canopy cover), and autumn (post leaf-fall). Then, in late September 2007, (during leaf fall and at the start of the *S. fontinalis* spawning period), we again measured all variables in addition to sampling the fish community composition.

Our predictions regarding the potential effects of the manipulations were shaped by previous research at these sites, which has demonstrated that streambed
substrata composition is strongly related to several ecosystem processes. For example, we found that the increased abundance of large inorganic particles (e.g. gravel, cobble, and boulder) was positively correlated with uptake rates of inorganic N and P (Hoellein et al. 2007) and macroinvertebrate secondary production (Entrekin et al. 2007). Therefore, we predicted we would find higher nutrient uptake and metabolism rates in the manipulated relative to reference reaches due to added gravel and boulders. We also predicted that *S. fontinalis* would be more abundant in manipulated reaches because of 1) increased recruitment and attraction to spawning habitat (i.e., direct effect), and 2) higher macroinvertebrate secondary production associated with increased streambed coverage of gravel, cobbles, and boulders, following the pattern documented previously for nutrient uptake and rates in the reference reaches of the study streams (i.e, indirect effect; Figure 5.1).

5.3. Methods

5.3.1. Study sites

State, Shane, and Walton Creeks are forested, headwater streams in the Ontonagon River basin of Lake Superior, in the Upper Peninsula of Michigan, USA (Figure 5.2). The streams are located within the Ottawa National Forest (ONF) and are tributaries of the Jumbo River. All three streams have similar orientation, surficial geology, and watershed areas (24-46 km²) and their catchments consist mostly of second-growth mixed hardwood forest (>83%),
while the remainder consists primarily of wetland (Entrekin et al. 2007, Hoellein et al. 2007). Streams in this region were affected by intensive logging ~100 years ago, resulting in a loss of in-stream large wood, stream channelization, and increases in streambed sand and fine sediment, which ONF officials believe has degraded trout spawning habitat (USFS 1993, Cordova et al. 2007).

Figure 5.1. Manipulations of the stream channel can increase stream biota directly via attraction to the habitat (i.e., “Field of Dreams” sensu Palmer 1997), or indirectly via increases the abundance of basal food resources and changes in the rates of energy and nutrient transfer through stream biofilms, allowing for a sustained increase in food resources.
Figure 5.2. State, Shane, and Walton Creeks are tributaries of Lake Superior in the Upper Peninsula of Michigan, USA. Stream watersheds are outlined in gray. Arrows indicate location of manipulation and reference reaches.

5.3.2. Manipulation

In 2000, United States Forest Service (USFS) personnel manipulated 50-70 m reaches within each stream to increase spawning habitat for native trout, *S. fontinalis*. The creation of upstream sediment traps paired with downstream bank stabilization and habitat amendment is a common technique to manage sand bedload in upper Midwestern streams, which are typically low-gradient and strongly influenced by glacial-till geology (Hansen et al. 1982). In each
manipulation reach, a 10-m long sediment trap was created at the start of the reach by widening the stream banks (~1.5 times mean channel width). For 40 to 60 m immediately downstream of the sediment trap, the banks were lined with boulders and logs parallel to the direction of flow, and the channel was filled with pea gravel (average gravel size ~1-2 cm³). Sediment traps were maintained by removing settled sand and organic material approximately once per year from 2000 until Spring 2003, after which they were left unchanged. Our un-manipulated reference reaches were located 120-300 m upstream of the manipulated reaches in each stream. Measurements were taken in all three streams during three biologically important seasons in 2006: spring (May 22-27), summer (July 20-26), and autumn (November 1-5), and during late summer/early autumn in 2007 (September 22-27).

In this study we use the term “manipulation” to refer to the USFS habitat enhancement projects described above. The terms restoration and rehabilitation are defined in many ways, but usually imply returning an ecosystem to a similar state as prior to disturbance, or that the project is taking steps towards recovery (Society for Ecological Restoration International Science & Policy Working Group 2004). We do not apply those terms here because the purpose of the manipulation was not to mimic or recreate historical conditions but to directly influence the abundance of a target species via the creation of a particular habitat type.
5.3.3. Benthic coverage of substratum types

We estimated benthic coverage of 7 substratum types: large wood (>10 cm diameter), small wood (<10 cm diameter), coarse benthic organic matter (CBOM; i.e., leaves and organic fragments >1 mm in diameter), fine benthic organic matter (FBOM; visually identified as having dominant fine texture), sand, gravel, cobble, boulder, and bryophytes. Because we found some variability in the visual categorization of gravel, cobble, and boulder among sampling dates due to different field assistants, we condensed the three categories into one group designated as “rock.” We marked transects every 10 m along each 100 m reference reach, and every 2.5-5 m along the 50-70 m manipulated reaches. We recorded the substratum type and water depth every 20 cm across each transect. We quantified percent coverage as the total number of counts for each substratum type divided by the total number of counts for all substrata times 100.

5.3.4. Organic matter and chlorophyll a standing stock

At five randomly selected locations along each reach, we inserted a PVC corer (area = 804 cm²) approximately 10 cm deep into the benthos, continuously stirred the substrata, removed all CBOM with a 1 mm sieve, and took a subsample of the remaining sediment slurry to estimate FBOM standing stocks. Back at the laboratory, we separated CBOM into wood, non-wood, and bryophyte categories. For FBOM, we filtered slurry subsamples onto pre-ashed and weighed glass fiber filters (Type A/E GFF, pore size=1 µm, Pall Corporation, Ann Arbor, MI). We quantified OM mass by placing ground subsamples of CBOM and filtered FBOM
samples into pre-ashed and weighed tins, and drying for 3-7 days at 60°C, and then determined ash-free dry mass (AFDM) following combustion (3 hours at 550°C). We collected gravel samples for chlorophyll \( a \) (chl \( a \)) standing stocks by inserting an inverted 160 ml specimen container approximately 2 cm into the substratum, slid a flat tool under the opening for a seal, and then turned the container upright (N=5 reach \(^{-1}\)). In the laboratory, we extracted chl \( a \) directly from the gravel in the container using the non-acidification, hot ethanol method using a Turner Designs Model TD-700 Fluorometer (Sartory and Grobbelaar 1984). We converted chl \( a \) to streambed coverage by weighting density (\( \mu \) g cm\(^{-2} \)) by the total proportion of gravel+cobble habitat in the reach. We did not measure chl \( a \) on cobble separately, but assume it to be approximately the same per unit surface area as the gravel.

5.3.5. Whole-stream nutrient uptake

We measured uptake rates of ammonium (NH\(_4^+\)), nitrate (NO\(_3^-\)), and phosphate (PO\(_4^{3-}\)) using whole-stream, short-term nutrient additions. We added nutrients in two separate additions using standard methods (Stream Solute Workshop 1990). The first addition contained ammonium as NH\(_4\)Cl and the conservative tracer NaCl, and the second contained nitrate as NaNO\(_3\), phosphate as KH\(_2\)PO\(_4\), and the conservative tracer bromide as NaBr. We recognize that measuring NO\(_3^-\) and PO\(_4^{3-}\) uptake simultaneously may have potentially relieved limitation of one nutrient, altering uptake of the other. We did not test the interactive effects of either additional NO\(_3^-\) on SRP uptake or the converse (i.e.,
by comparing individual and combined releases), but it has been measured previously in oligotrophic streams like ours, and neither N nor P was influenced by the presence of the other during a short-term release (see Hall and Tank 2003). This is likely because the biology of these systems cannot respond quickly enough to take advantage of the short-term increase (<45 min) in nutrient supply (Hoellein et al. 2007).

Before each short-term addition, we collected background water samples every 10-20 m downstream of the addition site to measure ambient nutrient concentrations and conductivity. We added solutes using a peristaltic pump (rate=200 mL min⁻¹), which raised solute concentrations slightly above ambient levels (+4-24 μg NH₄⁺-N, +4-30 μg PO₄³⁻-P L⁻¹ and +15-112 μg NO₃⁻-N L⁻¹), increased conductivity by 9-42 μS cm⁻¹, and Br⁻ concentration by 34-101 μg L⁻¹. Once the addition reached plateau (i.e., the conservative tracer concentrations were consistent throughout the reach), we took three replicate water samples at each of 5 downstream stations. We filtered samples in the field through a GFF (pore size=1 μm), and froze samples until laboratory analysis of solutes could be conducted.

In the laboratory, we measured NH₄⁺ concentration using the phenylhypochlorite technique (Solorzano 1969) on a Shimadzu UV1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) or Lachat QuickChem 8500 with autoanalyzer (Lachat Instruments, Loveland, CO; Method 10-106-06-1-F), and PO₄³⁻ concentrations as soluble reactive phosphorus (SRP) using the molybdate-antimony method (Murphy and Riley 1962) via
spectrophotometry or with the Lachat Method 10-115-01-1-Q. To measure NO$_3^-$ and Br$^-$ concentrations, we used ion chromatography (USEPA 1993) with AS14A analytical and guard columns and a 500 $\mu$L injection loop (Dionex Model DX600, Sunnyvale, CA) or cadmium reduction using Latchat Method 10-107-04-1-B (NO$_3^-$ only).

We calculated nutrient uptake lengths ($S_w$) by plotting the natural log of the ratio of background-corrected nutrient concentrations divided by background-corrected tracer concentrations vs. distance downstream. The absolute value of the inverse of the slope represents $S_w$, which can be considered the mean distance traveled by a nutrient before being taken up by the stream benthos (Stream Solute Workshop 1990). We converted $S_w$ into uptake velocity ($V_f$) using discharge and average width (Stream Solute Workshop 1990) because $V_f$ allows for comparison of nutrient demand across spatial or temporal scales by accounting for variation in stream discharge and size (Davis and Minshall 1999). We estimated transient storage parameters by fitting the conductivity curve generated from the conservative tracer injection (NaCl) to a one-dimensional advection and dispersion model and solving iteratively (Hart 1995). We expressed transient storage as the ratio of the exchange rates of water between the main channel and transient storage ($k_1$), and transient storage and the main channel ($k_2$). Width was calculated as the mean of 10-21 wetted widths per reach, discharge via dilution of the conservative tracer, and reach velocity as reach length divided the time to half plateau height on the conductivity curve. We calculated average depth as discharge divided by width multiplied by velocity.
5.3.6. Whole-stream metabolism

We used a field-calibrated Hydrolab Minisodes (Models 4a or 5a, Hach Corp., Loveland, CO) to record oxygen (O2) concentration, saturation, and temperature every 10 min for 24-36 h immediately prior to or following the short-term nutrient additions (Marzolf et al. 1994, Young and Huryn 1998). To estimate reaeration, we used a conservative gas sulfur hexafluoride, SF6 (Wanninkhof et al. 1990) released concurrently with the nutrient addition, and collected 5 replicate samples in 10ml gas-tight exetainers at 10-20m intervals downstream of the addition site. In the laboratory we quantified SF6 concentrations in the exetainer headspace on a gas chromatograph with an electron capture detector (Varian CP-3800, Varian, Inc. Walnut Creek, CA). We calculated community respiration (CR) as average reaeration-corrected O2 flux during the dark and gross primary production (GPP) as the sum of the instantaneous change in O2 concentration (reaeration-corrected) during daylight hours minus CR. This method assumes respiration in the light and dark are equal, and does not include the influence of anaerobic respiration (Uehlinger 2000). We found no significant dilution within our study reaches via conservative tracer releases, so we did not correct for groundwater O2 inputs (Hall and Tank 2005).

5.3.7. Fish community composition

We sampled for fish community composition in each of the reference and manipulated reaches from September 25-27, 2007 immediately following our 4th sampling period for the metrics described above. Each reach was blocked at the
top and bottom with mesh seines (diameter = 5mm). We conducted triple-pass removal (Li and Li 1996) with a backpack electrofisher (Smith-Root Model 12, Vancouver, WA). Depletion was effective and therefore total catch was compared between reaches. Captured fish were identified to species, measured for total length and mass, and returned to the stream.

5.3.8. Statistical analyses

We used a two-way repeated measures analysis of variance (RM-ANOVA) to compare between reference and manipulation reaches (N=6) and among sampling dates (i.e., seasons; N=4). A significant date x reach interaction indicated that the effect of reach (manipulation vs. reference) was dependent upon the season. We used Tukey’s multiple comparisons test to determine differences among dates. Stream was not included as a factor in the RM-ANOVA because there was only 1 measurement per stream reach on each date (i.e., df=0). Therefore, we compared nutrient uptake and metabolism between reaches for each stream individually using a paired t-test. To compare nutrient uptake and metabolism among seasons, we averaged the ratio of manipulation/reference among streams, and used a RM-ANOVA to compare among sampling dates. We also used 1-way ANOVA to compare abundance of sand and organic matter in the reference reach with the two sub-reaches of the manipulation reach (i.e., sediment trap and stabilized channel). We used simple linear regression (SLR) to quantify the relationships between nutrient uptake rates and geomorphology and metabolism rates.
To examine differences in the fish communities among reaches, we used non-metric multidimensional scaling (NMDS) with Sørenson similarity, using fish species biomass (fourth-root transformed). We added a secondary matrix to NDMS to explore relationships among stream geomorphology, ecosystem function, and fish community metrics. Finally, we used multiple linear regressions to examine the relationship between total fish biomass and abundance and *S. fontinalis* biomass and abundance with physiochemical and biological variables. If necessary, data were transformed to meet assumptions of parametric statistics. All statistics were conducted using SPSS 11 (SPSS, Inc. Chicago, IL) and the NMDS was executed using PC-ORD Version 4 (MJM Software Design, Gleneden Beach, OR).

5.4. Results

5.4.1. Physiochemical parameters and streambed substrata

We documented several differences in physiochemical parameters between reaches and among dates (Table 5.1). Between reaches, reaeration rates were always higher in the manipulation reach (RM-ANOVA, $F_{1,6}=31.44$, $p=0.03$), although there was a significant date x reach interaction (RM-ANOVA, $F_{3,6}=4.75$, $p=0.05$), because rates were approximately identical between reaches in summer. Transient storage was higher in the manipulated relative to the reference reaches (RM-ANOVA, $F_{1,5}=233.42$ $p=0.042$) but among dates, transient storage
was lowest in May 2006 and highest in September 2007 (late summer/early autumn; RM-ANOVA, $F_{3,6}=13.25$, $p=0.031$). Among dates, all reaches were shallowest in July 2006 (RM-ANOVA, $F_{3,6}=17.53$, $p=0.002$), SRP concentrations were lowest in May 2006 and highest in September 2007 (RM-ANOVA, $F_{3,6}=15.98$, $p=0.003$), and temperature was lowest in November 2006 (RM-ANOVA, $F_{3,6}=15.46$, $p=0.003$; Table 5.1).

Streambed substratum composition did not differ between reaches or among dates for most substrata including large wood, small wood, and fine benthic organic matter, but we found differences in rock and sand abundance (Table 5.1). Rock coverage was always higher in the manipulation reaches (RM-ANOVA, $F_{1,6}=52.17$, $p=0.019$), while sand was lower in the manipulated reaches (RM-ANOVA, $F_{1,6}=19.19$ $p=0.048$). Among dates, the proportion of rock substrata was lower in November 2006 and September 2007 (RM-ANOVA, $F_{3,6}=4.77$, $p=0.05$), and was likely due to higher CBOM coverage on those dates (RM-ANOVA, $F_{3,6}=12.28$, $p=0.005$).
### TABLE 5.1

MEAN (± SE) VALUES FOR PHYSIOCHEMICAL MEASUREMENTS, WATER CHEMISTRY, STREAMBED SUBSTRATA, CHLOROPHYLL A (CHL A) AND ORGANIC MATTER STANDING STOCKS, NUTRIENT UPTAKE VELOCITIES (V_F), AND METABOLISM RATES IN THE REFERENCE AND MANIPULATION REACHES OF THE STUDY STREAMS (N=4 REACH \(^{-1}\))

<table>
<thead>
<tr>
<th>Physical variables</th>
<th>State Creek</th>
<th>Shane Creek</th>
<th>Walton Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference</td>
<td>Manipulation</td>
<td>Reference</td>
</tr>
<tr>
<td>Discharge (L s(^{-1}))</td>
<td>53.0 (5.3)</td>
<td>51.9 (8.0)</td>
<td>33.9 (7.0)</td>
</tr>
<tr>
<td>Width (m)</td>
<td>2.31 (0.02)</td>
<td>2.13 (0.10)</td>
<td>2.16 (0.19)</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>12.5 (0.9)</td>
<td>15.7 (1.3)</td>
<td>9.7 (0.7)</td>
</tr>
<tr>
<td>Velocity (m min(^{-1}))</td>
<td>10.9 (1.3)</td>
<td>9.2 (0.3)</td>
<td>9.1 (0.7)</td>
</tr>
<tr>
<td>kO(_2) at 20°C (min(^{-1}))</td>
<td>0.109 (0.012)</td>
<td>0.150 (0.021)</td>
<td>0.070 (0.012)</td>
</tr>
</tbody>
</table>
TABLE 5.1 (Continued)

<table>
<thead>
<tr>
<th></th>
<th>State Creek</th>
<th>Shane Creek</th>
<th>Walton Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference</td>
<td>Manipulation</td>
<td>Reference</td>
</tr>
<tr>
<td>Trans. stor. ($k_1/k_2$)</td>
<td>0.052 (0.039)</td>
<td>0.122 (0.037)</td>
<td>0.076 (0.034)</td>
</tr>
<tr>
<td>Temperature (°C)*</td>
<td>7.2 (1.9)</td>
<td>8.6 (2.0)</td>
<td>12.4 (3.1)</td>
</tr>
<tr>
<td>Water Chemistry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$ (µg L$^{-1}$)</td>
<td>8.7 (2.4)</td>
<td>9.7 (2.0)</td>
<td>8.9 (1.2)</td>
</tr>
<tr>
<td>SRP (µg L$^{-1}$)</td>
<td>6.3 (1.7)</td>
<td>5.9 (1.6)</td>
<td>7.1 (2.0)</td>
</tr>
<tr>
<td>NO$_3^-$ (µg L$^{-1}$)</td>
<td>185.4 (10.6)</td>
<td>175.3 (12.2)</td>
<td>141.3 (21.8)</td>
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<tr>
<td>DO (% saturation)*</td>
<td>97.7 (0.6)</td>
<td>95.8 (1.1)</td>
<td>94.1 (1.1)</td>
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<td>Streambed substrata</td>
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<td></td>
</tr>
<tr>
<td>Large wood (%)</td>
<td>6.2 (0.3)</td>
<td>4.0 (1.1)</td>
<td>13.1 (2.2)</td>
</tr>
<tr>
<td>Small wood (%)</td>
<td>2.3 (0.5)</td>
<td>1.7 (0.4)</td>
<td>3.1 (0.5)</td>
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<tr>
<td>CBOM (%)</td>
<td>10.5 (3.5)</td>
<td>11.5 (4.3)</td>
<td>12.5 (3.9)</td>
</tr>
<tr>
<td>FBOM (%)</td>
<td>6.5 (2.3)</td>
<td>8.1 (2.6)</td>
<td>13.5 (2.6)</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>19.8 (3.8)</td>
<td>6.0 (1.5)</td>
<td>32.7 (2.7)</td>
</tr>
<tr>
<td>Rock (%)</td>
<td>52.2 (2.6)</td>
<td>67.3 (3.0)</td>
<td>25.1 (2.8)</td>
</tr>
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**TABLE 5.1 (Continued)**

<table>
<thead>
<tr>
<th></th>
<th>State Creek Reference</th>
<th>Shane Creek Reference</th>
<th>Walton Creek Reference</th>
<th>State Creek Manipulation</th>
<th>Shane Creek Manipulation</th>
<th>Walton Creek Manipulation</th>
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<tr>
<td>Chl a and organic matter standing stock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chl a (µg cm⁻²)</td>
<td>0.46 (0.05)</td>
<td>0.93 (0.29)</td>
<td>0.04 (0.01)</td>
<td>0.29 (0.10)</td>
<td>0.05 (0.01)</td>
<td>0.51 (0.28)</td>
</tr>
<tr>
<td>FBOM (gAFDM m⁻²)</td>
<td>66.4 (27.3)</td>
<td>86.7 (16.7)</td>
<td>82.9 (23.3)</td>
<td>138.1 (67.8)</td>
<td>154.4 (28.3)</td>
<td>77.9 (19.9)</td>
</tr>
<tr>
<td>CBOM (gAFDM m⁻²)</td>
<td>45.8 (10.2)</td>
<td>35.5 (5.5)</td>
<td>55.8 (17.9)</td>
<td>42.8 (5.1)</td>
<td>371.3 (166.0)</td>
<td>61.5 (27.8)</td>
</tr>
<tr>
<td>Nutrient uptake velocity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ Vᵣ (mm s⁻¹)</td>
<td>0.135 (0.025)</td>
<td>0.157 (0.052)</td>
<td>0.058 (0.016)</td>
<td>0.135 (0.011)</td>
<td>0.058 (0.015)</td>
<td>0.074 (0.019)</td>
</tr>
<tr>
<td>NO₃⁻ Vᵣ (mm s⁻¹)</td>
<td>0.235 (0.040)</td>
<td>0.217 (0.032)</td>
<td>0.089 (0.037)</td>
<td>0.173 (0.038)</td>
<td>0.083 (0.025)</td>
<td>0.100 (0.043)</td>
</tr>
<tr>
<td>SRP Vᵣ (mm s⁻¹)</td>
<td>0.088 (0.011)</td>
<td>0.080 (0.029)</td>
<td>0.036 (0.013)</td>
<td>0.073 (0.022)</td>
<td>0.041 (0.007)</td>
<td>0.035 (0.017)</td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPP (gO₂ m⁻² d⁻¹)</td>
<td>1.33 (0.40)</td>
<td>2.30 (0.74)</td>
<td>0.80 (0.20)</td>
<td>0.83 (0.39)</td>
<td>1.43 (0.42)</td>
<td>0.79 (0.45)</td>
</tr>
<tr>
<td>CR (gO₂ m⁻² d⁻¹)</td>
<td>4.96 (1.46)</td>
<td>9.16 (2.13)</td>
<td>6.06 (1.00)</td>
<td>14.92 (1.52)</td>
<td>14.71 (3.33)</td>
<td>9.75 (4.14)</td>
</tr>
</tbody>
</table>

*24 h mean

**Note.** Abbreviations as follows: kO₂ at 20°C=reaeration rate, Tran. Stor.=transient storage, NH₄⁺=ammonium, SRP=soluble reactive phosphorus, NO₃⁻=nitrate, DO=dissolved oxygen, CBOM=coarse benthic organic matter, and FBOM=fine benthic organic matter, GPP = gross primary production, and CR = community respiration.
Figure 5.3. Percent abundance of sand (A-C) and fine + coarse organic matter (E-F) in the reference (Ref.) and manipulated (Manip.) reaches of the three study streams. The two sub-reaches of manipulation reach are separated into the sediment trap region (trap) and downstream stabilized channel region (stablized). p-values indicate results from 1-way ANOVA among reaches, and the small letters indicate significant difference among reaches per Tukey’s multiple comparison test.
The manipulation consisted of two distinct sub-reaches, the sediment trap and the stabilized channel where gravel was added for spawning habitat. We analyzed the sub-reaches separately to compare differences in benthic coverage of sand and organic matter (CBOM+FBOM) to ensure differences between manipulation and reference reaches were not masked when the manipulation reach was considered as a single unit (Figure 5.3). In Shane Creek, we found significantly more sand in the trap portion of the manipulated reach compared to the reference reach and the stabilized channel (ANOVA, $F_{2,9} = 22.14$, $p < 0.001$, Figure 5.3A). There was a similar pattern in Walton Creek ($F_{2,9} = 5.14$, ANOVA $p = 0.032$, Figure 5.3C), although the reference reach did not differ from the sub-reaches of the manipulation. In contrast, we measured more organic matter in the trap relative to the reference reach and spawning channel in State Creek (ANOVA, $F_{2,9} = 6.98$, $p = 0.015$, Figure 5.2E).

5.4.2. Organic matter and chlorophyll a standing stock

We found that chl $a$ standing stocks were always higher in the manipulated reach (RM-ANOVA, $F_{1,6} = 20.74$, $p = 0.045$), but no differences in FBOM or CBOM standing stocks between reaches or among dates (Table 5.1). While not significant, we found trends in organic matter standing stocks among dates. FBOM standing stock tended to be higher in the manipulated reach in July 2006, while CBOM standing stocks in the manipulation reaches were slightly lower than the reference in May 2006, November 2006, and September 2007 (data not shown).
5.4.3. Reach-scale nutrient uptake and metabolism rates

Overall, we documented few significant differences in nutrient uptake and metabolism rates between manipulated and reference reaches or among dates (Table 5.1). The exception was SRP $V_f$, which was highest in May 2006 and lowest in November 2006 (RM-ANOVA $F_{3,6}=7.93$, $p=0.016$). However, when we compared the rates between reaches for each stream individually (Figure 5.4A-E), Shane Creek had higher CR (paired t-test, $t_{0.05,3}=-3.37$, $p=0.043$), SRP $V_f$ (paired t-test, $t_{0.05,3}=-5.55$, $p=0.012$), and a trend of higher NH$_4^+$ $V_f$ (paired t-test, $t_{0.05,3}=-2.67$, $p=0.075$) in the reference reach relative to the manipulated reach (Figure 5.4A,B,E respectively). The Manipulation/Reference ratio was not different among dates for either nutrient uptake or metabolism rates (RM-ANOVA $p>0.05$, Figure 5.4F-J).
Figure 5.4. A-F) Mean (±SE) values for uptake velocity ($V_f$) of ammonium ($\text{NH}_4^+$), soluble reactive phosphorus (SRP), and nitrate ($\text{NO}_3^-$), gross primary production (GPP), and community respiration (CR) for reference (Ref) and manipulation (Manip.) reaches ($N=4$ stream$^{-1}$). * indicates a significant difference between reaches for Shane CR (paired t-test, $t_3=-3.37$, $p=0.043$), SRP $V_f$ ($t_3=-5.55$, $p=0.012$). G-K) Mean (±SE) values for Manip./Ref ratio for $\text{NH}_4^+$, SRP, and $\text{NO}_3^-$ $V_f$, GPP, and CR on each sampling date ($N=3$ date$^{-1}$). There were no significant differences in Manip./Ref ratio among dates (ANOVA, $p>0.05$).
Figure 5.5. Simple linear regression between uptake velocity ($V_f$) of ammonium ($\text{NH}_4^+$), soluble reactive phosphorus (SRP), or nitrate ($\text{NO}_3^-$), with gross primary production (GPP), community respiration (CR), or % gravel+cobble+boulder for A,B) Shane Creek, C) State Creek, and D) Walton Creek.

From previous studies, we know that substrata distribution, especially % gravel+cobble+boulder, as well as GPP and CR, explained variation in nutrient $V_f$ in the reference reaches of the study streams (Hoellein et al. 2007). We used simple linear regression to see if the relationships were conserved across reference and manipulated reaches in each stream when the data were pooled. We found no significant relationships when data from all streams were pooled, so we looked at each stream individually. In Shane Creek, variation in $\text{NH}_4^+$ $V_f$ was...
significantly related to % gravel+cobble+boulder (SLR, \( r^2 = 0.67 \ p = 0.01 \); Figure 5.5A) and CR (SLR, \( r^2 = 0.64 \ p = 0.01 \); data not shown). Also in Shane Creek, SRP \( V_f \) was related to % gravel+cobble+boulder (SLR, \( r^2 = 0.54 \ p = 0.04 \); data not shown), and \( \text{NO}_3^- \ V_f \) was related to CR (SLR, \( r^2 = 0.64 \ p = 0.02 \); Figure 5.5B). In State Creek, GPP was significantly related to \( \text{NH}_4^+ \ V_f \) (\( r^2 = 0.59 \ p = 0.03 \); Figure 5.5C). Finally, in Walton Creek, SRP \( V_f \) was significantly related to GPP (\( r^2 = 0.59 \ p = 0.03 \); data not shown) and CR (\( r^2 = 0.61 \ p = 0.02 \); Figure 5.5D). Note that for Shane Creek (Figure 5.5A, 5.5B), the data points for manipulation and references reaches cluster together on the x and y axes; however, on the regressions for State and Walton Creeks (Figure 5.5C, 5.5D) the manipulation and reference reach data points are evenly distributed across the full range of the x-axis variable.

5.4.4. Fish community structure

On 25-27 September 2007, when our fish sampling was conducted, each reach contained 5-7 species of fish, all typical of cold-water streams in the region (Table 5.2). All species were native except for two sport fish taxa: brown trout (\textit{Salmo trutta}), introduced from Europe, and juvenile coho salmon (\textit{Oncorhynchus kisutch}), introduced from the Pacific coast of North America. These introduced species contributed <10% of total fish biomass and abundance. The most abundant fish were brook trout (\textit{S. fontinalis}; all three streams), blacknose dace (\textit{Rhinichthys atratulus}; State and Shane Creeks), central mudminnows (\textit{Umbra limi}; Walton Creek), and mottled sculpin (\textit{Cottus bairdii}; Walton Creek). \textit{S. fontinalis} represented >45% of total fish biomass across the 6 reaches.
Among the three study streams, there was some variability in fish community structure between manipulation and references reaches (Table 5.2). Total fish abundance and biomass in reference and manipulation reaches were about identical in Walton Creek. In State Creek, there was higher total abundance and biomass in the manipulated reach, whereas the opposite pattern was found in Shane Creek. *S. fontinalis* abundance was approximately the same between reaches in State and Shane Creeks, and in Walton Creek biomass was lower in the manipulated reach. Patterns for *S. fontinalis* biomass were similar to abundance, except in State Creek, where biomass was higher in the manipulated reach. Among streams, State Creek had the highest overall *S. fontinalis* abundance and biomass. Overall, paired t-tests showed no differences in total or *S. fontinalis* biomass and abundance between reaches (p>0.22). We found no detectable differences in the size-frequency distribution of *S. fontinalis* between reference and manipulation reaches in State and Shane Creeks (Figure 5.6A-B), but in Walton Creek, the reference reach contained ~2.5 times more age-0 fish compared to the manipulation reach (Figure 5.6C).
TABLE 5.2

ABUNDANCE AND BIOMASS OF FISH SPECIES FOUND IN THE REFERENCE (REF.) AND MANIPULATION (MANIP.) REACHES OF THE THREE STUDY STREAMS. “–“ INDICATES ABSENCE

<table>
<thead>
<tr>
<th></th>
<th>State Creek</th>
<th>Shane Creek</th>
<th>Walton Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abundance (Number m⁻²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salvelinus fontinalis</td>
<td>0.350</td>
<td>0.362</td>
<td>0.168</td>
</tr>
<tr>
<td>Salmo trutta</td>
<td>0.047</td>
<td>0.044</td>
<td>-</td>
</tr>
<tr>
<td>Oncorhynchus kisutch</td>
<td>0.006</td>
<td>-</td>
<td>0.077</td>
</tr>
<tr>
<td>Rhinichthys atratulus</td>
<td>0.148</td>
<td>0.231</td>
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</tr>
<tr>
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<td>0.011</td>
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<tr>
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<td>-</td>
<td>-</td>
</tr>
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<td><strong>0.770</strong></td>
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<th><strong>Biomass (g m⁻²)</strong></th>
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<td>0.51</td>
<td>0.64</td>
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<td>-</td>
<td>0.14</td>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
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<td><strong>4.89</strong></td>
<td><strong>2.22</strong></td>
<td><strong>2.10</strong></td>
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Figure 5.6. Size-frequency histogram of brook trout (*Salvelinus fontinalis*) in the reference and manipulation reaches of the three study streams.
NDMS analysis assisted us in clustering the reaches based on fish community biomass characteristics (Figure 5.7). We used a 2 axes solution where stress <0.00001 and final instability =0.0565 after 28 iterations. Axis 1 was negatively correlated with *U. limi* and positively correlated *O. kisutch* and *R. atratulus*. Axis 2 was positively correlated with, *S. trutta*, *S. fontinalis*, and *R. atratulus* (Table 5.3). *R. atratulus* is related to both axes because it projects at a 45 degree angle from the origin (highest in State and Shane reference reaches). Data points for each reach on the NDMS plot were not simply clustered by stream nor by treatment (reference vs. manipulation). Both reaches of Walton Creek clustered by themselves due to high biomass of *U. limi*. In contrast, State and Shane Creek reference reaches were more similar to each other, while the manipulation resulted in contrasting trajectories making them less similar to each other (Figure 5.7). We also added a second matrix that included 5 physiochemical and biological factors that were variable among reaches and we hypothesized would affect fish species: reaeration, FBOM and rock coverage, NH$_4^+$ $V_f$, and CR, but we found that none were significantly aligned with NDMS axes. The strongest relationship was that reaeration was correlated with Axis 1 (Spearman’s correlation, $\rho$=-0.640, $p$=0.162).
Figure 5.7. A) Nonmetric multidimensional scaling of fish species biomass in the study streams, with a 2-axes solution (28 iterations, stress <0.00001, final instability=0.0565). Species with significant correlations to axes (p≤0.05) are represented as vectors, with the length and direction indicating the correlation strength. All correlation coefficients and p-values are reported in Table 5.3. Species are *Oncorhynchus kisutch* (coho salmon), *Rhinichthys atratulus* (blacknose dace), *Umbra limi* (central mudminnow), *Salvelinus fontinalis* (brook trout), and *Salmo trutta* (brown trout).
TABLE 5.3

SPEARMAN’S CORRELATION COEFFICIENT (ρ) BETWEEN FISH SPECIES BIOMASS (FOURTH ROOT TRANSFORMED) AND NONMETRIC MULTIDIMENSIONAL SCALING AXES 1 AND 2. P-VALUES ≤0.05 ARE IN BOLD

<table>
<thead>
<tr>
<th>Fish</th>
<th>Axis 1 ρ</th>
<th>p</th>
<th>Axis 2 ρ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-0.99</td>
<td>&lt;0.01</td>
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<td>0.23</td>
</tr>
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<td>Oncorhynchus kisutch</td>
<td>0.84</td>
<td>0.04</td>
<td>0.03</td>
<td>0.96</td>
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<tr>
<td>Rhinichthys atratulus</td>
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<td>0.05</td>
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<tr>
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<td>0.37</td>
<td>0.47</td>
<td>0.83</td>
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<td>Salmo trutta</td>
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<td>0.39</td>
<td>0.84</td>
<td>0.05</td>
</tr>
<tr>
<td>Semotilus atromaculatus</td>
<td>0.43</td>
<td>0.40</td>
<td>0.31</td>
<td>0.54</td>
</tr>
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<td>Cottus bairdii</td>
<td>-0.76</td>
<td>0.08</td>
<td>-0.34</td>
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</tr>
<tr>
<td>Catostomus commersoni</td>
<td>-0.13</td>
<td>0.81</td>
<td>0.66</td>
<td>0.16</td>
</tr>
<tr>
<td>Notropis heterolepis</td>
<td>-0.66</td>
<td>0.16</td>
<td>-0.39</td>
<td>0.44</td>
</tr>
</tbody>
</table>

We examined all physiochemical variables, streambed substrata composition data, and rates of nutrient uptake and metabolism from September 2007 as independent variables in a multiple linear regression analysis to attempt to find predictors of total fish abundance and biomass and *S. fontinalis* abundance and biomass. In general, we found that total fish abundance and biomass were unrelated to geomorphology, organic matter standing stocks, chl *a*, or rates of nutrient uptake and metabolism. In contrast, we did find that *S. fontinalis* biomass specifically was negatively related to FBOM percent coverage ($r^2=0.673$, p=0.045; Figure 5.8). The distribution of reference vs. manipulated reaches in the
regression showed that FBOM coverage was lower in the manipulation reaches of all 3 study streams; but *S. fontinalis* biomass was higher in the manipulated reaches only in Shane and State Creeks relative to the reference reaches (Figure 5.8). In Walton Creek, *S. fontinalis* biomass was not measurably different between the two reaches. We also found a trend of decreased *S. fontinalis* abundance with increased sand, although the relationship was not statistically significant (SLR, $r^2=0.445$, $p=0.148$; data not shown).

![Figure 5.8. Simple linear regression between brook trout (*Salvelinus fontinalis*) biomass and coverage of streambed surface by fine benthic organic matter (FBOM; %).](image)

5.5. **Discussion**

We found that the manipulation reaches designed to enhance trout habitat showed significant differences in many non-target parameters in all 3 study streams, including altered physiochemical variables (e.g. higher reaeration), changes in streambed substrata (e.g. greater rock abundance), and increases in chl $a$ standing crop (Table 5.1). However, strong evidence that the manipulation
influenced stream ecosystem function was limited to one stream, Shane Creek (Figure 5.5). Finally, the target species, *S. fontinalis*, was higher in abundance in the manipulated reach in 2 of the 3 streams, which was related to reduced coverage of fine sediments (Figure 5.8). Overall, results from our study indicate a subtle effect of the manipulation on *S. fontinalis* via changes stream structure (i.e., direct effect), rather than changes in ecosystem processes (i.e., indirect effect; Figure 5.1).

Other studies have shown mixed results of habitat enhancements on salmonid fish, with examples of both neutral (Avery 1996, Kondolf et al. 1996, Moerke et al. 2004, Rosi-Marshall et al. 2006) and positive results (Nakamura 1999, Merz et al. 2004, Palm et al. 2007). However, these studies are based on measurements of geomorphology combined with abundances of periphyton, macroinvertebrates, and fishes. We know of no previous studies that have simultaneously quantified metrics of ecosystem function (e.g. metabolism, nutrient uptake rates) with fish community composition to compare directly to our results. Overall, possible explanations for the lack of a strong and consistent biological response in ecosystem function and community composition for our study include 1) the 50-100 m reaches were too short relative to operational scale of the biological response variables (sensu Lepori et al. 2005a), 2) lack of sediment trap maintenance, 3) natural variation inherent in the 3 replicate study streams, and 4) the biota were also affected by factors other than the habitat enhancement.
5.5.1. Spatial and temporal scales of response variables

Matching the scale of restoration to the scale of desired response variables remains a major challenge in restoration ecology (Lake et al. 2007). For example, increasing the abundance of rocks (i.e., gravel, cobble) in the 50-70 m manipulation reaches increased the overall abundance of chlorophyll $a$ relative to the 70-100 m upstream reference reaches (Table 5.1), most likely because algae were able to colonize the new solid substrate once they were added to the formerly sand-dominated stream reaches; in essence the algae were habitat-limited while all other conditions remained suitable (e.g. light availability, nutrients). Conversely, measurements such as metabolism (which integrates changes in DO over spatial scales larger than the experimental reaches) and fishes (which can range over larger areas than the 50-100 m study reaches during their life cycle) may be influenced by environmental drivers operating at larger scales compared to the size of the manipulation reaches. In contrast, the nutrient uptake rate measurements reflect the identical scale of the manipulation reaches, as they were measured along each reach length specifically. By combining all of these metrics together, researchers can obtain a more comprehensive understanding of the biological responses operating at multiple spatial scales following habitat manipulations.

5.5.2. Fish community composition

Fish populations in headwater streams are temporally variable, as individuals move to meet requirements for food, habitat/protective cover,
temperature, and reproduction at different stages in their life cycles. The central purpose of the USFS manipulation was to attract *S. fontinalis* during their search for suitable gravel habitat for spawning. When we sampled in late September 2007, it was during the spawning period (late summer through autumn), which was evident by the ventral gold-red coloration of the larger males we collected (Behnke 2002). Yet when pooled across the 3 study streams, our data indicated no significant difference in *S. fontinalis* biomass or abundance between reaches (Table 5.2), nor on the overall fish community composition between manipulated and reference reaches (Figures 5.6 and 5.7). However, we acknowledge that our description of the fish community represents only a snapshot of where the fish are during the sampling period, and we did not document other potentially important life history traits including egg production, egg to fry survival, or production rates (Avery 1996, Merz et al. 2004, Palm et al. 2007). In addition, we did not sample the trap and stabilized channel portions of the manipulations separately, and including the trap portion in our reach-scale sampling may have lowered our overall estimate of fish abundance in the stabilized channel portion of the manipulated reach.

Among streams, our data were consistent with previous studies that have demonstrated a negative relationship between *S. fontinalis* and the abundance of fine sediment (Saunders and Smith 1965, VanDusen et al. 2005, Hartman and Hakala 2006) and sand (Alexander and Hansen 1986). We found that the sediment trap portion of the manipulation retained sand in Shane and Walton Creeks, but retained organic matter in State Creek (Figure 5.3). Because fine
sediment, and to a lesser extent, sand, were the major controlling factors on *S. fontinalis* distribution among all 6 reaches (Figure 5.8), this may explain the slight increase in *S. fontinalis* biomass in manipulation reaches in State and Shane Creeks.

In their description of the potential use of sediment traps to enhance trout habitat in Michigan streams, Hansen et al. (1982) list several disadvantages of the method. These include the need for periodic sand removal, the difficulty in removing and re-purposing collected “spoils,” the potential physical hazard of sediment traps for anglers, and the lack of ability of basins to significantly reduce fine organic sediment. Hansen et al. (1982) recommended building small basins in trout streams, because while they are less effective at trapping fine sediment than larger basins, they will not retain water long enough for it to be warmed. 

FBOM coverage (as %) was slightly lower in the manipulated reaches, and was related to *S. fontinalis* biomass (Table 5.1, Figure 5.8), even though periodic maintenance (i.e., removal of sand and sediment) at our study sites had ceased 3 years prior to our analysis. Anecdotally, the reasons for suspending sediment trap maintenance in our study streams were due to the pursuit of a new restoration strategy in neighboring reaches and the concurrent retirement of the USFS employee advocating this restoration protocol. Other restoration projects requiring regular maintenance could encounter equivalent fates via changes in personnel, funding, or research interests of the responsible parties.
5.5.3. Metabolism and nutrient uptake rates

Similar to fish community composition metrics, the 1-station method for measuring reach-scale metabolism incorporates biological activity occurring at a scale potentially larger than that of our treatment reaches; quantifying solely the metabolism within a defined reach length requires use of the 2-station method combined with sufficient travel time to capture a significant change in DO (Bott 1996). To place our single-station data in context, the reach length over which 95% of the DO signal was integrated can be estimated as $3V/k$, where $V$ is water velocity, in m day$^{-1}$, and $k$ is reaeration rate, day$^{-1}$ (Chapra and Di Toro 1991, Beaulieu et al. 2008). This analysis gives us a mean reach length integrated by the DO signal of 396 m across all streams, which is longer than our study reach lengths. Results suggest that the metabolism of biofilms outside the study reaches was likely included in our reach scale measurements likely hindering our ability to detect a metabolism effect across reaches. However, our use of the 1 station method was necessary in order to measure metabolism in both reaches of 3 replicate streams during the relatively short sampling periods in each season using our available equipment. We also recognize that our reach lengths, constrained by the already constructed manipulation, would have also made it challenging to measure significant DO change between 2 stations because of the low GPP in our shaded study streams. Despite this, in Shane Creek we detected concurrent increases in CR and subsequent linkages to both NH$_4^+$ $V_f$ and SRP $V_f$. Because $V_f$ measurements were measured only within the study reaches, this suggests a relatively large contribution of reach-specific metabolism to overall rates. Future
studies that quantify the response of metabolism to reach-scale restoration would benefit from using the 2-station approach, ensuring that the DO signal is integrated at the same spatial scale as the restoration, which would strengthen linkages with concurrent ecosystem processes quantified at the reach scale such as nutrient uptake rates.

In contrast to the fish community and metabolism metrics, nutrient uptake measurements represent the composite activity of biofilms at the very same scale as the study reaches. While our prediction of higher overall uptake rates in our manipulated reaches was not supported by the data in State and Walton Creeks, Shane Creek showed higher SRP $V_f$ in manipulated relative to the reference reach (Figure 5.4), and a positive relationship between percent gravel, cobble, and boulder and SRP $V_f$, NH$_4^+$ $V_f$, and CR (Figure 5.5). The significant manipulation effects on ecosystem metrics in Shane Creek, relative to the lack thereof in State and Walton Creeks, could be due to the magnitude of difference in substrata composition between reaches. The manipulation reach in Shane Creek had 2 times the abundance of rocks as the reference reach, while in State and Walton Creeks the difference was 1.2 and 1.5 times, respectively. It is possible that the greater magnitude of change in Shane Creek resulted in significant differences in $V_f$ and CR.

5.5.4. Temporal variation in nutrient uptake rates

Previous research in these same study streams has shown nutrient uptake and metabolism are sensitive to seasonal change (Hoellein et al. 2007), and that
the relative contribution of epilithic biofilms towards whole stream metabolism and nutrient uptake rates is lowest in the summer (Hoellein, unpublished data). Since rocks made up a greater proportion of the streambed in the manipulation reaches, we predicted lower nutrient uptake and GPP rate in July 2006 in the manipulation relative to the reference reaches (i.e., manipulation/reference <1), but the data did not support this prediction as there were no difference in the manipulation/reference ratio among dates for nutrient uptake velocity or metabolism (Figure 5.4F-J). However there was a trend of lower NO$_3^-$ V$_f$ and GPP in Summer 2006 relative to the other dates (Figure 5.4H-I). Overall, while the manipulation reaches were different than the reference reaches for some aspects of substrata composition, this did not translate into differences in the absolute or relative rates of whole-stream ecosystem processes between reaches (in 2 out of the 3 study streams) or among dates (in all 3 streams).

Combining nutrient uptake rates with more traditional restoration metrics such as biological community composition (e.g. macroinvertebrate and fish sampling), represents an overlooked, but potentially powerful combination in the analysis of stream restoration at the reach scale, because it integrates the activity of multiple trophic levels (e.g. biofilms to higher organisms) and the scale of the measurement can be set to match the scale of the manipulation (e.g. nutrient uptake metrics). While we did not record many significant spatial or temporal differences in this study, we suggest it was not due to a lack of sensitivity of the metrics, but rather due to the lack of effect of the manipulation, combined with
the inherent but unexpected variability captured by using measurements from 3 replicate streams.

5.5.5. Natural variation among replicate streams

Previous studies on the effects of restoration efforts of fish habitat have been replicated in two ways: 1) within a stream, where replicate measurements are taken through time (Kondolf et al. 1996, Rosi-Marshall et al. 2006, Palm et al. 2007) or in replicated manipulations along a reach of a single stream (Nakamura 1999, Moerke and Lamberti 2004), or 2) restorations can be replicated in multiple streams (Pretty et al. 2003, Lepori et al. 2005a). The latter approach is more infrequent due to increased costs and added logistical consideration (e.g. sampling effort). Both replication approaches have complementary strengths and weaknesses. Replicating manipulations along a single stream may be confounded by upstream-downstream effects of manipulated reaches on one another, are not considered statistically independent, but rather pseudoreplicated (sensu Hurlbert 1984), and may have limited applicability for streams in a broader geographic region. However, differences between reaches of the same stream may be easier to detect, as sites are not confounded by among-stream variability of reference conditions; a phenomena we have demonstrated robustly in our study (Tables 5.1 and 5.2, Figures 5.4 and 5.7).

Tests of restoration replicated among streams entails incorporating the natural variability inherent to stream ecosystems, even those in very close proximity (Figure 5.1), which may confound any generalizations about
manipulation effects due to different outcomes in different replicate streams. In our study, several physiochemical and biological characteristics of the reference reaches in the 3 replicate streams were different from one another (i.e., each “started” from a different place; Table 5.1). As a result, we found it necessary to analyze streams individually for several response variables (Figures 5.3, 5.4, and 5.5). Because of this a priori inter-stream variability, we found that analyzing data using the distribution of points and a regression approach, rather than categorically, was the most insightful for describing treatment effects on ecosystem processes and S. fontinalis biomass within and among replicate streams (Figures 5.5 and 5.8).

5.5.6. Statistical challenges for post-hoc restoration analyses

Our analysis of paired manipulation and reference reaches and the 6-year lag between manipulation and evaluation presented 2 statistical challenges in the context of the study design. Our post-hoc analysis assumed that the reaches were identical prior to the manipulation, that is, we considered the reference reach to represent the status of the manipulation reach were it not for the channel modifications. Also, we violated assumption of equal independence in our linear regressions (i.e., not all data points are equally independent; Figure 5.5, Figure 5.8). While we acknowledge the statistical limitation of post-hoc evaluation and equal independence violation, we hold it should not be an impediment to appraising the effects of restoration projects in general. Post-hoc data collection is still more beneficial than collecting no data at all, and can provide some insight
into the long-term effectiveness of projects for which no pre-treatment data are available or spatial replication is not possible.

5.5.7. Other potential influences on ecosystem processes and fish communities

Besides the availability of spawning habitat, other factors likely strongly influenced ecosystem processes and *S. fontinalis* populations in our study streams. For example, logging practices, both past and present, have been shown to affect stream biota in this geographic region (USFS 1993). Although clear-cut logging ceased in the upper Midwestern US ~1960 (VanDusen et al. 2005), the influence of logging on stream biota can persist for centuries (Harding et al. 1998). In addition, clear-cutting was replaced with single-tree selection logging, which VanDusen et al. (2005) demonstrated was correlated w decreased *S. fontinalis* abundance in headwater tributaries of the neighboring Sturgeon River watershed. The range of *S. fontinalis* abundance found in VanDusen et al. (2005) encompassed the range in *S. fontinalis* abundance from our study (i.e., 0.05-0.4 and 0.16-0.35 individuals m⁻², respectively), and selective logging was occurring during our sampling period at least at 1 site, Shane Creek (T. Hoellein, personal observation). We did not attempt to quantify the effects of logging practices specifically in our study, but we recognize that the overarching influence of both past and present land-use practices on both stream ecosystem processes and *S. fontinalis* biomass may have been stronger than the reach-scale habitat enhancements.
5.5.8. Long-term success of trout enhancement

Palmer et al. (2005) suggested that one important criterion for stream restoration success is enhanced resilience to environmental change; more specifically, restoration strategies should enhance ecosystem function in the context of a dynamic environment. Unfortunately, the requirement of regular maintenance for this strategy follows the opposite philosophy: that normal environmental processes will eventually degrade the structure of the habitat enhancements. In the well-studied salmon fisheries of the Pacific Northwest, researchers have also indicated that artificial structures (e.g., gravel beds, spawning enhancements) should be considered short-term measures until natural watershed conditions are restored (Kondolf et al. 1996, Roni et al. 2002). Without re-establishing the sediment basins and gravel reaches via human maintenance, the manipulation strategy at our study sites is unlikely to increase ecosystem function in the future, but more likely will remain the same or come to physically and biologically resemble reference conditions. Managers in the region may obtain greater ecological benefits for their resource investments via strategies for habit enhancement that will produce long-lived, resilient habitat changes requiring minimal human maintenance.

5.6. Acknowledgements

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Kenton, MI, for implementing habitat enhancements, allowing access to the study sites, and the Ottawa National Forest for logistical assistance. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (Managed Ecosystems Program grant 2003-35101-12871) to JLT and GAL. TJH was supported by an Arthur J. Schmitt Presidential Fellowship from the Graduate School at the University of Notre Dame.

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CHAPTER 6
THE EFFECT OF AN EXPERIMENTAL WOOD ADDITION ON STREAM
ECOSYSTEM RESISTANCE ACROSS A DISTURBANCE INTENSITY
GRADIENT

6.1. Abstract

Stream restorations are rarely evaluated for their influence on ecosystem response to disturbance, even though ecosystem disturbance concepts (e.g., resistance, resilience) have provided insight into how biological communities are structured by environmental change. Wood addition is an increasingly common approach for restoration in streams that have been affected by wood/snag removal for flood reduction, recreation, historic transport of timber, as well as a lack of new wood recruitment due to ongoing riparian forest harvest.

In May 2004, we added twenty-five logs (2.4m L x 0.5m D) to 100-m experimental reaches of three forested headwater streams where in-stream wood was low due to intense forest harvesting ~100y ago and selective logging for the past 40y. We predicted that the addition of large wood would increase retention

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of particulate organic matter and associated microbial biofilms during storms, thereby conferring increased resistance to storms in experimental reaches with wood. To quantify resistance, we measured reach-scale nutrient uptake rates before and after multiple storms in both wood addition (i.e., treatment) and upstream control reaches in all 3 streams for 4 years following the experimental addition of wood.

We found that uptake rates of both nitrate (N) and phosphate (P) were higher following an intermediate-sized flood (i.e., return interval of 0.1-1 y), but decreased after a large flood (i.e., return interval of ≥1 y) compared to pre-flood measurements. In one stream, Shane Creek, the treatment reach had higher P uptake rates in autumn relative to the control reach following an intermediate sized flood. Changes in P uptake rates were positively related to changes in reach-scale community respiration suggesting a heterotrophic pathway for P uptake via biofilms colonizing organic matter retained by added wood. In contrast, changes in N uptake were related to reach-scale GPP indicating autotrophic control.

Overall, our data indicate that the experimental addition of wood had the strongest influence over change in nutrient uptake rates following intermediate-sized floods in autumn, however, there was no effect of wood addition following the largest storms. The ability of restoration structures such as large wood to maintain crucial ecosystem functions including nutrient uptake across a gradient of disturbances of should be considered an integral component for restoration success.
6.2. Introduction

Disturbance, recovery, and succession of ecosystems is a fundamental concept addressed by all disciplines of ecological research (Cowles 1899, Clements 1936, Likens et al. 1970, Connell 1978). Disturbances which result in disruption of community structure and loss of biomass are thought to adjust the “stage” on which biotic interactions occur, beginning a successional process leading to a return to pre-disturbance conditions at some future time (Pickett and White 1985). Regular disturbance can increase habitat heterogeneity and biodiversity by allowing for the coexistence of early and late successional species (Sousa 1984). The application of regular disturbance is employed as a management technique in many ecosystems such as forests, grasslands, and rivers (i.e., controlled fires and floods; Little and Moore 1949, Stanford et al. 1996).

Streams have been used as a model for examining ecosystem response to disturbance because stream biological communities experience regular restructuring via naturally recurring floods and droughts (Grimm 1987, Dodds et al. 2004) and previous research has supported that disturbance is a major structural factor influencing lotic ecosystems in general and species diversity in particular (Resh et al. 1988, Poff 1992, Lake 2000). Despite this, it has proven difficult to apply general theories of disturbance, such as the intermediate disturbance hypothesis and dynamic equilibrium (Connell 1978, Huston 1979) to stream ecosystems largely because there is debate as to whether stream
ecosystems are ever in equilibrium prior to disturbance (Ward and Stanford 1983, Resh et al. 1988). Nevertheless, many studies have quantified the effects of disturbance on stream organic matter dynamics, algae, macroinvertebrates, and fishes (Bilby 1981, Grimm and Fisher 1989, Power 1990). However, the effects of disturbance on ecosystem processes such as nutrient cycling have been less frequently addressed (Grimm 1987, Golladay et al. 1992, Martí et al. 1997), and represent a gap in our ability to more fully develop and apply disturbance paradigms to stream ecosystems.

The most common way to quantify community response to disturbance is to quantify organism density and biomass before and after the event (Connell and Sousa 1983, Sutherland 1990). In forested headwater streams, however, nutrient assimilation and uptake is mediated by microbial biofilms, including bacteria, fungi, and algae, whose biomass and abundance are difficult to quantify and varies naturally even in steady-state conditions (Martens 1994, Hall et al. 2000). Stream ecologists have developed the concept of nutrient spiraling to quantify the biological activity of biofilm organisms as the movement of nutrients from the water column to the benthos (Webster and Patten 1979, Newbold et al. 1983). Spiraling theory provides a powerful group of related metrics for understanding transformations of bioreactive solutes through stream food webs (Peterson et al. 2001, Bernhardt et al. 2003, Argerich et al. 2008), including nutrients such as nitrogen (N) and phosphorus (P), which are often limiting to primary and secondary production in stream ecosystems (Newbold et al. 1983, Grimm 1987). Functional measurements (e.g., nutrient uptake and metabolism rates) are
advantageous in that they synthesize the activity of multiple organisms and/or
trophic levels, and are indicators of biological activity. In contrast, structural
measurements (e.g., microbial biomass and cell counts) often do not differentiate
living and dead organisms, so may be less indicative of ecological processes
occurring at the time of measurement (Gessner and Chauvet 2002).

In forested headwater streams, allochthonous (i.e., terrestrially derived)
organic matter (OM) is an important carbon and energy source for stream biota
matter abundance has been linked to nutrient uptake rates, especially in autumn
following leaf fall (Mulholland et al. 1985, Mulholland 2004). However, OM
resources can be quickly exported during floods, with the majority of OM loss
from headwater streams occurring during just a few storms over the course of a
year (Meyer and Likens 1979). Golladay et al. (1992) used nutrient budgets to
show that forested streams with clear-cut riparian zones retained less N and P than
reference streams after storms, and speculated this was due to decreased retentive
structures (e.g., large wood) in disturbed streams, but the role of retentive
structures in buffering nutrient loss following storms was not empirically
addressed.

Experimental manipulations of large wood in headwater streams, either as
additions or removals, have shown that wood has the potential to increase
retention of OM and habitat heterogeneity (Bilby 1981, Trotter 1990, Wallace et
al. 1995). The placement of wood in streams is gaining acceptance as a restoration
strategy for fish conservation (Naiman et al. 2000, Rosi-Marshall 2006), however
it is rare that restorations include monitoring to quantify the influence of wood addition on ecosystem function (Moerke and Lamberti 2004, Bernhardt 2005). Researchers have postulated that quantifying how restoration efforts withstand periodic disturbance is one of the most the critical factors affecting restoration success (Carpenter et al. 2001, Palmer 2005), yet applications of disturbance theory are rarely invoked in the evaluation of ecosystem restoration projects (Lake 2007).

Our goal was to measure the influence of an experimental wood addition on stream ecosystem resistance to storms by measuring changes in whole-stream nutrient uptake before and after multiple storms over a 4.5 yr period (2003-2007) in both treatment (wood-added)and upstream control reaches of three forested headwater streams. We predicted that because of increased organic matter retention in reaches with added wood, nutrient uptake rates in treatment reaches would be less strongly influenced by storms relative to the upstream control reaches (i.e., increased resistance with wood addition). Additionally, we expected that the difference between control and treatment reaches would be greatest in autumn, when coarse particulate organic matter inputs and standing stocks (i.e., leaf litter) were highest in the study streams.
6.3. Methods

6.3.1. Study sites

We studied State, Shane, and Walton Creeks, which are 3 forested, headwater tributaries of the Jumbo River in the Ontonagon River basin of Lake Superior, located in the Upper Peninsula of Michigan, USA. Our streams were chosen as replicates for the wood addition experiment because they have similar geology, climate, orientation, watershed area, riparian vegetation, and logging history (Cordova et al. 2007, Entrekin et al. 2007). The stream catchments are entirely within the Ottawa National Forest, with >83% of the watershed area in second-growth mixed hardwood forest, and the remainder consisting primarily of wetland (Entrekin et al. 2007, Hoellein et al. 2007). Riparian vegetation includes white pine (Pinus strobes L.), eastern hemlock (Tsuga canadensis L.), sugar maple (Acer saccharum Marsh.), red maple (Acer rubrum L.), alder (Alnus spp.), and paper birch (Betula papyrifera Marsh.), with an understory of forbes and ferns. Streams and rivers in the Upper Peninsula of Michigan were impacted by intensive logging ~100 y ago, resulting in a paucity of in-stream large wood, stream channelization, and increases in benthic sand and fine sediment on stream bottoms (VanDusen et al. 2005, Cordova et al. 2007).

6.3.2. Wood addition

In May 2004, 25 bigtooth aspen (Populus grandidentata Michx.) logs (2.4m L x 0.5m D) were added to 100 m treatment reaches in the 3 replicate study
streams. Logs were individually transported to the stream banks via snowmobile in March 2004, and then hand-placed into the streams at haphazard angles and locations throughout each 100 m treatment reach immediately following snowmelt in May. Neither log transport nor placement caused damage to riparian vegetation. By the end of the current study, in January 2008, no logs had moved out of the treatment reaches, and we have been quantifying all metrics of ecosystem response in both the wood-addition reach (treatment) as well as within an upstream 100 m control reach (separated by 20-50 m buffer) in all three study streams.

6.3.3. Nutrient uptake rates

Over the 5 study years, we have conducted short-term nutrient additions of nitrogen (N) as nitrate (NO₃⁻), and phosphorus (P) as phosphate (PO₄³⁻) in both control and treatment reaches of the 3 study streams at intervals ranging from 1 week to bimonthly depending on storm history from May 2003 to December 2007 (N=41 dates for State and Shane Creeks, N=33 for Walton Creek). Data from May 2003-April 2004 (one year pre-wood addition) have been presented in Hoellein et al. (2007) and Entrekin et al. (2007). Extreme winter conditions limited access to Walton Creek, although we were able to collect data at State and Shane Creeks in December 2003, 2005, January 2007, and March 2006, and at all 3 sites in December 2007.

We conducted short-term nutrient additions using standard methods (Stream Solute Workshop 1990) and the addition contained nitrate as NaNO₃,
phosphate as KH$_2$PO$_4$, and NaBr and/or NaCl as conservative tracers, measured as bromide and conductivity, respectively. Prior to solute additions, we collected background water samples at 5-6 stations evenly-spaced downstream of the addition site for ambient solute concentrations and conductivity. We added solutes at a rate of 200 mL min$^{-1}$ (Fluid Metering, Inc. Lab pump Model RHB, Syosset, NY) to raise nutrient concentrations slightly above ambient concentrations ($+3-54$ μg PO$_4^{3-}$ P L$^{-1}$ and $+5-142$ μg NO$_3^{-}$-N L$^{-1}$), Br$^-$ concentration by 20-158 μg L$^{-1}$, and specific conductivity by 5-40 μS cm$^{-1}$. At the plateau stage (i.e., when conservative tracer concentrations were uniform throughout the 100 m reach), we collected three replicate water samples at each of the 5-6 downstream stations, filtering samples in the field through glass fiber filters (GFF) with pore size of 1.0 μm (Type A/E GFF, Pall Corporation, Ann Arbor, MI). Water samples were frozen until solutes concentrations were measured in the laboratory.

We quantified NO$_3^-$ and Br$^-$ concentrations using ion chromatography (Dionex Model DX600, Sunnyvale, CA) with AS14A analytical and guard columns and a 500 μL injection loop, or we measured NO$_3^-$ using cadmium reduction on a Lachat QuickChem 8500 with an autoanalyzer (Lachat Instruments, Loveland, CO; Method 10-107-04-1-B). We measured PO$_4^{3-}$ concentrations as soluble reactive phosphorus (SRP) using the molybdate-antimony method (Murphy and Riley 1962) via spectrophotometer on a Shimadzu UV1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) or on the Lachat using Method 10-115-01-1-Q. Finally, we measured ammonium
(NH₄⁺) concentration in background water samples using the phenylhypochlorite technique (Solorzano 1969) on a spectrophotometer or on the Lachat using Method 10-106-06-1-F. We used a YSI conductivity meter (Model 30, Yellow Springs, OH) or Hydrolab Minisonde (Model 4A, Loveland, CO) to measure specific conductivity.

We calculated nutrient uptake lengths (Sw; or the average distance a nutrient molecule travels before being biologically removed from the water column) using background-corrected nutrient concentrations (enriched minus ambient concentration) divided by background-corrected tracer concentrations to account for any dilution. We then plotted the natural log of this fraction vs. distance downstream, where the absolute value of the inverse of the slope equaled Sw (Stream Solute Workshop 1990). Because Sw is highly influenced by discharge, uptake velocity (Vf) and areal nutrient uptake rate (U) are the most useful parameters for comparing nutrient uptake across spatial or temporal scales that have varying discharge (Davis and Minshall 1999). From Sw, we calculated Vf, which represents demand relative to ambient concentration, as (discharge/width)/Sw, and U, representing nutrient uptake rate per stream unit surface area, as Vf times ambient nutrient concentration (Stream Solute Workshop 1990).

On the same day as each nutrient addition, we calculated stream discharge (Q) as the dilution of the conservative tracer where \( Q_s = \frac{Q_a \times C_a}{C_s} \) where \( Q_s \) = stream discharge, \( Q_a \) = rate of solute addition, \( C_a \) = solute concentration of the addition, and \( C_s \) = solute concentration at each of the downstream collection
stations. We averaged discharge across all 5-6 stations for stream Q. We calculated stream wetted-width as the mean of 11-21 measurements throughout each 100 m reach on the same day as the nutrient addition.

6.3.4. Whole-stream metabolism

Previous research in our study streams indicated nutrient uptake rates are strongly linked to primary production and/or community respiration, as measured by whole-stream metabolism (Hoellein et al. 2007), therefore we used metabolism as a predictor variable to correlate with nutrient uptake rates. We calculated whole-stream metabolism by measuring changes in O_2 concentrations and temperature every 10 min for ~32 hours immediately prior to or following the short-term nutrient additions using field-calibrated Hydrolab Minisondes placed at the bottom of each reach (Marzolf et al. 1994, Young and Huryn 1998). Reaeration was measured by releasing a conservative gas (propane or sulfur hexafluoride) simultaneous with the nutrient additions. Propane and sulfur hexafluoride (SF_6) concentrations were measured on a gas chromatograph (Varian Model STAR 600, Varian Analytical Instruments, Walnut Creek, CA) with electron capture detector (for SF_6) or flame ionization detector (for propane). To calculate reaeration, we regressed the decline in gas concentrations, corrected for dilution using Br^- or conductivity, at each sample site vs. distance downstream (Wanninkhof et al. 1990).

We calculated community respiration (CR) as mean reaeration-corrected O_2 flux during the dark and gross primary production (GPP) as the sum of the
instantaneous change in reaeration-corrected O$_2$ concentration during daylight hours minus CR. This method does not include anaerobic respiration and assumes respiration in the light is equal to that in the dark (Uehlinger 2000). There was no significant dilution within our study reaches, as measured by conservative tracers, and therefore there was no need to correct for groundwater O$_2$ inputs (Hall and Tank 2005).

6.3.5. Continuous stream discharge monitoring

Pressure transducers were installed in the fall of 2006 and recorded stream water height every 10 minutes (Water Logger Model RXWL15X, Global Water Instrumentation, Inc., Gold River, CA). We used a regression between stream discharge (measured via solute dilution, see above) and stage height to calculate stream discharge for 2007 (State $r^2=0.542$, $p=0.006$, Shane $r^2=0.692$, $p=0.003$, Walton $r^2=0.893$, $p=0.001$). We then used a regression between each stream discharge from 2007 and stage height for a downstream USGS gauge on the Ontonagon River to calculate discharge from 2003-2006 (State $r^2=0.632$, $p<0.001$, Shane $r^2=0.610$, $p<0.001$, Walton $r^2=0.841$, $p<0.001$).

6.3.6. Data analysis

To characterize changes in discharge for comparison of floods over the 5 year study period and to compare among the 3 study streams, we calculated the return interval of the mean daily discharge measurements. Return interval (T) was calculated for each stream by ranking the mean daily flow from January 1,
2003- December 31, 2007 from maximum to minimum, where \( T = (n+1)/m \), and \( m = \) flood rank and \( n = \) number of days on record (Gore 1996). We then calculated probability of exceedance (\( P; \) as \% ) as \( 1/T \times 100 \). We then grouped flood return intervals into 4 categories: <0.01 year (i.e., discharge level occurs more than 100 times per year), 0.01-0.1 year (i.e., discharge level occurs 11-100 times per year), 0.1-1 year (i.e., discharge level occurs 2-10 times per year) and \( \geq 1 \) year (i.e., discharge level occurs on a return interval of 1 or more years). We can refer to these categories as base flow, small disturbance, intermediate disturbance, and large disturbance, respectively.

We also tabulated the number of days since the last storm for each sampling (e.g. nutrient addition) date as the number of days after a flood, where a flood was defined as having a peak flow with a return interval >0.1 y (i.e., intermediate or large disturbance). We used regression to link nutrient uptake parameters (i.e., SRP and \( \text{NO}_3^- \cdot V_f \) and \( U \)) with days since flood. We analyzed all regressions in 4 distinct groupings: 1) including all streams and seasons, 2) analyzing streams separately, with seasons combined, 3) analyzing streams combined, with seasons separate, and 4) analyzing streams and seasons separately. We grouped data into seasons based on our 5 years of experience of year-round data collection at the study sites (Entrekin et al. 2007, Hoellein et al. 2007, Entrekin et al. in press). Winter was categorized as the period containing ice and snow cover (i.e., December through March), and summer as the time period when there was full riparian canopy at each stream (i.e., June through September). Spring was designated as April and May which is the short time
period after snowmelt, but before riparian leaf-out occurs, and autumn is in October and November, which is also a time period with open canopy cover post leaf-fall, and before snow and ice accumulation occurs.

Next, we compared the change in N and P uptake rates between sampling dates according to the change in stream discharge between dates. Change in uptake rate was equal to the rate on 1 date minus the rate on the previous date, where a value <0 indicates a decline and a value >0 indicates an increase in uptake. Change in discharge was categorized as the return interval of the maximum flow between dates (i.e., base flow, small disturbance, intermediate disturbance, and large disturbance). For changes in uptake rates and discharge we only considered dates when the time interval between sampling dates was < 2 months, as any period >2 months was likely to be confounded by concurrent seasonal change in addition to change associated with floods (Hoellein et al. 2007). We used a 2-way ANOVA to compare change in uptake rates by return interval category (i.e., base flow, small disturbance, intermediate disturbance, and large disturbance) and reach (i.e., control and treatment). We analyzed all data in 4 groupings based on season and stream: all data combined, all streams combined by season, all seasons combined by stream, and streams and seasons individually. In addition, we expected to find the strongest influence of wood addition on nutrient uptake rates following storms in autumn, so we also compared the absolute rates of nutrient uptake between reaches using a paired t-test within each return interval category for this season. Finally, we used simple linear regression (SLR) to quantify the relationship between change in uptake rates versus change
in GPP and CR, also using in the 4 season and stream groupings listed above. All
statistics were done using SPSS 11 (SPSS, Inc. Chicago, IL) and SigmaPlot 10
(Systat Software, Inc, San Jose, CA).

6.4. Results

6.4.1. Hydrograph

Over the 4.5 year study period (May 2003 – December 2007), the
hydrograph of the study streams was dominated by high spring flows from
snowmelt, low flows over summer, and ice-cover in the winter, but hydrologic
patterns in the fall were variable among years. In 2005 and 2007, there were high
flows in the fall (reaching ~2/3rd of the spring peak flow), but there were no high
flows in the autumn in 2003, 2004, and 2006 (Figure 6.1). Overall, we found that
the 2003-2007 patterns were typical of the long-term record in the Ontonagon
River; mean daily discharge has been recorded since 1942, and fall floods
occurred in 21 out of 66 years, or 32% of years on record (data not shown).
Figure 6.1. Mean daily discharge of A) State Creek, B) Shane Creek, and C) Walton Creek from January 1, 2003 through January 1, 2008. Sampling dates are indicated by a white triangle (N=41, State and Shane Creeks, N=33 for Walton Creeks). Large wood was added to the treatment reach May 8-9, 2004.
6.4.2. Physiochemical parameters

Physiochemical data from our 41 sampling dates reflected seasonality in hydrology patterns and indicated the streams were generally oligotrophic with regard to nutrient concentrations (Table 6.1). On individual sampling dates, Q was higher in spring, and lower in summer across all three streams; however, stream width in these square-channeled streams was relatively constant among seasons. Across sampling dates and streams, SRP was uniformly low (i.e., >10.2 µg L\(^{-1}\)), but dissolved inorganic N concentrations were variable. Ammonium showed similar low levels in State and Shane Creeks (i.e., <15 µg L\(^{-1}\)), but in Walton Creek, we recorded summer peaks of NH\(_4^+\) in 2004 - 2007, ranging from 29-49 µg L\(^{-1}\). Similarly NO\(_3^-\) remained relatively low in State and Shane Creeks, ranging from 140-251 µg L\(^{-1}\) across dates, but we recorded higher values in Walton Creek, especially in the summer of 2003 and 2004 where concentrations peaked at 776 and 442 µg L\(^{-1}\), respectively.

In general, spatial and temporal patterns of nutrient uptake and metabolism rates were similar to the patterns documented in the study streams for the pre-wood addition period covering May 2003- April 2004 and presented in Hoellein et al. (2007). Uptake velocities of SRP were generally higher than for NO\(_3^-\) (Table 6.2). In contrast, because ambient NO\(_3^-\) concentrations were ~10 times higher than SRP, and because aerial uptake is calculated using ambient concentration data (see above), we found that NO\(_3^-\) U was also generally an order of magnitude higher than SRP U throughout the sample period (Table 6.2).

Spring was the period of highest GPP across all streams, and was generally lowest
in summer. We do not probe these patterns further here because the objective of this study was not to analyze spatial and temporal trends in uptake rates and metabolism but rather to address how rates change between sampling dates in relationship to changes in hydrology in the context of wood addition.

6.4.3. Flood return intervals

Flow-duration curves for mean daily discharge in each study stream over the 4.5 year period showed relatively flat curves (Figure 6.2; Gore 1996); the flow-duration curve was flattest in State Creek, steepest in Walton Creek, and was intermediate in Shane Creek (note the scale difference between Walton Creek and the other two streams). Baseflow (i.e., return interval <0.01 year) made up 75% of the mean daily flows over the 4.5 year period. In all three streams, the lowest flows in this category occurred during a drought that occurred in Summer 2007, which caused the flow in Walton to decrease to the lowest discharge throughout the entire study period at ~1 L s\(^{-1}\).
TABLE 6.1

MEAN (± SE) DISCHARGE (Q), WIDTH, AND WATER COLUMN AMMONIUM (NH₄⁺), SOLUBLE REACTIVE PHOSPHORUS (SRP), AND NITRATE (NO₃⁻) CONCENTRATIONS BY SEASON IN CONTROL (C) AND WOOD-ADDED (TREATMENT; T) REACHES FROM MAY 2003-DECEMBER 2007 (C), AND MAY 2004 – DECEMBER 2007 (T). A “-“ INDICATES NO DATA

<table>
<thead>
<tr>
<th>Stream</th>
<th>Reach</th>
<th>Season</th>
<th>N</th>
<th>Q (L s⁻¹)</th>
<th>Width (m)</th>
<th>NH₄⁺ (µg L⁻¹)</th>
<th>SRP (µg L⁻¹)</th>
<th>NO₃⁻ (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>C</td>
<td>Spring</td>
<td>10</td>
<td>67.2 (5.7)</td>
<td>2.2 (&lt;0.1)</td>
<td>4.4 (0.4)</td>
<td>5.5 (1.0)</td>
<td>182.2 (9.4)</td>
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<td>Summer</td>
<td>18</td>
<td>54.7 (3.7)</td>
<td>2.2 (&lt;0.1)</td>
<td>8.2 (1.0)</td>
<td>7.9 (0.7)</td>
<td>161.2 (6.2)</td>
</tr>
<tr>
<td></td>
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<td>Fall</td>
<td>9</td>
<td>62.2 (7.0)</td>
<td>2.4 (0.1)</td>
<td>4.4 (1.3)</td>
<td>8.6 (2.1)</td>
<td>192.2 (11.2)</td>
</tr>
<tr>
<td></td>
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<td>Winter</td>
<td>5</td>
<td>63.7 (4.3)</td>
<td>2.3 (&lt;0.1)</td>
<td>6.6 (0.5)</td>
<td>5.1 (1.6)</td>
<td>233.1 (17.3)</td>
</tr>
<tr>
<td>T</td>
<td>Spring</td>
<td>8</td>
<td>67.1 (6.6)</td>
<td>2.3 (0.1)</td>
<td>4.8 (0.7)</td>
<td>6.0 (1.0)</td>
<td>185.4 (11.0)</td>
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</tr>
<tr>
<td></td>
<td>Summer</td>
<td>14</td>
<td>55.3 (5.5)</td>
<td>2.3 (&lt;0.1)</td>
<td>8.7 (1.0)</td>
<td>8.8 (1.0)</td>
<td>170.6 (7.1)</td>
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<td>64.6 (7.0)</td>
<td>2.5 (0.1)</td>
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<td>8.4 (1.4)</td>
<td>193.7 (11.8)</td>
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<tr>
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<td>2.4 (0.1)</td>
<td>6.7 (0.3)</td>
<td>6.4 (1.7)</td>
<td>251.0 (9.8)</td>
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<td>Stream</td>
<td>Reach</td>
<td>Season</td>
<td>N</td>
<td>Q (L s⁻¹)</td>
<td>Width (m)</td>
<td>NH₄⁺ (µg L⁻¹)</td>
<td>SRP (µg L⁻¹)</td>
<td>NO₃⁻ (µg L⁻¹)</td>
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<td>11.5 (1.1)</td>
<td>7.0 (1.3)</td>
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<td>T</td>
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<td>54.9 (4.2)</td>
<td>2.5 (0.1)</td>
<td>7.6 (1.1)</td>
<td>6.8 (1.0)</td>
<td>139.8 (9.1)</td>
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<td></td>
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<td>2.3 (0.1)</td>
<td>7.8 (0.6)</td>
<td>10.2 (1.0)</td>
<td>174.3 (12.3)</td>
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<td>6.0 (1.3)</td>
<td>8.2 (1.2)</td>
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<td>12.5 (1.1)</td>
<td>7.1 (1.9)</td>
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<td>9.0 (1.9)</td>
<td>4.7 (0.2)</td>
<td>203.2 (25.1)</td>
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<td>24.8 (4.4)</td>
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<td>31.3 (-)</td>
<td>1.9 (-)</td>
<td>-</td>
<td>6.9 (-)</td>
<td>247.1 (-)</td>
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<td>11.3 (2.1)</td>
<td>4.7 (0.7)</td>
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<td></td>
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<td>Summer</td>
<td>11</td>
<td>27.1 (9.0)</td>
<td>2.2 (0.1)</td>
<td>27.7 (3.8)</td>
<td>6.7 (0.7)</td>
<td>233.7 (34.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>8</td>
<td>57.4 (11.8)</td>
<td>2.6 (0.1)</td>
<td>9.1 (2.2)</td>
<td>6.4 (0.6)</td>
<td>191.7 (49.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>1</td>
<td>31.1 (-)</td>
<td>2.3 (-)</td>
<td>-</td>
<td>7.3 (-)</td>
<td>241.5 (-)</td>
</tr>
</tbody>
</table>
TABLE 6.2

MEAN (± SE) UPTAKE VELOCITY (V_f) AND AREAL UPTAKE (U) SOLUBLE REACTIVE PHOSPHORUS (SRP), AND NITRATE (NO_3\^-), AND RATES OF GROSS PRIMARY PRODUCTION (GPP) AND COMMUNITY RESPIRATION (CR) IN THE 3 STUDY STREAMS IN BOTH THE CONTROL (C) AND WOOD-ADDED (TREATMENT; T) REACHES FROM MAY 2003-DECEMBER 2007 (C), AND MAY 2004 – DECEMBER 2007 (T). A “–” INDICATES NO DATA ARE AVAILABLE

<table>
<thead>
<tr>
<th>Stream</th>
<th>Reach</th>
<th>Season</th>
<th>N</th>
<th>SRP V_f (mm s(^{-1}))</th>
<th>SRP U (mg m(^{-2}) d(^{-1}))</th>
<th>NO_3^- V_f (mm s(^{-1}))</th>
<th>NO_3^- U (mg m(^{-2}) d(^{-1}))</th>
<th>GPP (gO_2 m(^{-2}) d(^{-1}))</th>
<th>CR (gO_2 m(^{-2}) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>C</td>
<td>Spring</td>
<td>10</td>
<td>0.115 (0.023)</td>
<td>55.8 (15.4)</td>
<td>0.106 (0.025)</td>
<td>1515 (290)</td>
<td>1.8 (0.4)</td>
<td>10.1 (3.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>18</td>
<td>0.110 (0.019)</td>
<td>69.3 (9.7)</td>
<td>0.083 (0.011)</td>
<td>1174 (171)</td>
<td>0.7 (0.2)</td>
<td>5.8 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>9</td>
<td>0.143 (0.041)</td>
<td>78.9 (17.7)</td>
<td>0.075 (0.009)</td>
<td>1248 (168)</td>
<td>0.7 (0.3)</td>
<td>8.2 (1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>5</td>
<td>0.164 (0.040)</td>
<td>57.5 (8.2)</td>
<td>0.092 (0.042)</td>
<td>1957 (1027)</td>
<td>1.4 (0.2)</td>
<td>5.5 (1.3)</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>Spring</td>
<td>8</td>
<td>0.171 (0.033)</td>
<td>86.8 (22.8)</td>
<td>0.119 (0.030)</td>
<td>1739 (360)</td>
<td>1.4 (0.3)</td>
<td>13.6 (2.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>14</td>
<td>0.123 (0.013)</td>
<td>89.7 (12.3)</td>
<td>0.087 (0.011)</td>
<td>1269 (177)</td>
<td>0.7 (0.2)</td>
<td>7.0 (2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>8</td>
<td>0.147 (0.021)</td>
<td>114.4 (26.9)</td>
<td>0.110 (0.017)</td>
<td>1823 (272)</td>
<td>0.4 (0.1)</td>
<td>10.3 (3.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>4</td>
<td>0.157 (0.021)</td>
<td>87.7 (31.1)</td>
<td>0.110 (0.046)</td>
<td>2472 (1119)</td>
<td>1.0 (0.5)</td>
<td>3.9 (1.2)</td>
</tr>
<tr>
<td>Stream</td>
<td>Reach</td>
<td>Season</td>
<td>N</td>
<td>SRP (V_f) ((\text{mm s}^{-1}))</td>
<td>SRP (U) ((\text{mg m}^{-2} \text{ d}^{-1}))</td>
<td>(\text{NO}_3) (V_f) ((\text{mm s}^{-1}))</td>
<td>(\text{NO}_3) (U) ((\text{mg m}^{-2} \text{ d}^{-1}))</td>
<td>GPP ((\text{gO}_2 \text{ m}^{-2} \text{ d}^{-1}))</td>
<td>CR ((\text{gO}_2 \text{ m}^{-2} \text{ d}^{-1}))</td>
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<td>--------------------------------</td>
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<td>-------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Shane</td>
<td>C</td>
<td>Spring</td>
<td>10</td>
<td>0.104 (0.017)</td>
<td>54.5 (5.3)</td>
<td>0.052 (0.010)</td>
<td>627 (133)</td>
<td>0.7 (0.2)</td>
<td>5.5 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>18</td>
<td>0.052 (0.008)</td>
<td>41.8 (5.5)</td>
<td>0.043 (0.007)</td>
<td>680 (121)</td>
<td>0.4 (0.1)</td>
<td>4.8 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>9</td>
<td>0.045 (0.008)</td>
<td>33.3 (11.4)</td>
<td>0.037 (0.008)</td>
<td>338 (93)</td>
<td>0.4 (0.1)</td>
<td>4.3 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>5</td>
<td>0.073 (0.013)</td>
<td>45.3 (12.0)</td>
<td>0.048 (0.015)</td>
<td>936 (281)</td>
<td>0.6 (0.3)</td>
<td>10.3 (6.0)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>Spring</td>
<td>8</td>
<td>0.161 (0.026)</td>
<td>82.1 (6.7)</td>
<td>0.061 (0.008)</td>
<td>730 (107)</td>
<td>0.6 (0.1)</td>
<td>4.7 (1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>14</td>
<td>0.067 (0.007)</td>
<td>63.4 (11.6)</td>
<td>0.045 (0.005)</td>
<td>632 (79)</td>
<td>0.3 (0.1)</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>8</td>
<td>0.107 (0.015)</td>
<td>80.0 (17.3)</td>
<td>0.057 (0.013)</td>
<td>466 (140)</td>
<td>0.4 (0.1)</td>
<td>5.9 (1.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>4</td>
<td>0.109 (0.021)</td>
<td>72.1 (27.3)</td>
<td>0.044 (0.017)</td>
<td>838 (304)</td>
<td>0.0 (-)</td>
<td>2.7 (-)</td>
</tr>
<tr>
<td>Walton</td>
<td>C</td>
<td>Spring</td>
<td>8</td>
<td>0.120 (0.022)</td>
<td>47.9 (9.7)</td>
<td>0.077 (0.018)</td>
<td>1331 (361)</td>
<td>2.2 (0.3)</td>
<td>12.5 (2.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>15</td>
<td>0.056 (0.012)</td>
<td>29.1 (8.7)</td>
<td>0.049 (0.020)</td>
<td>822 (150)</td>
<td>0.6 (0.1)</td>
<td>12.6 (8.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>9</td>
<td>0.079 (0.021)</td>
<td>44.2 (14.2)</td>
<td>0.056 (0.017)</td>
<td>911 (263)</td>
<td>0.4 (0.1)</td>
<td>9.6 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>1</td>
<td>0.008 (-)</td>
<td>4.8 (-)</td>
<td>0.072 (-)</td>
<td>1533 (-)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>Spring</td>
<td>5</td>
<td>0.131 (0.028)</td>
<td>53.8 (14.6)</td>
<td>0.097 (0.032)</td>
<td>1491 (435)</td>
<td>1.5 (0.3)</td>
<td>11.5 (1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>11</td>
<td>0.067 (0.017)</td>
<td>40.2 (12.4)</td>
<td>0.058 (0.031)</td>
<td>699 (182)</td>
<td>0.5 (0.1)</td>
<td>7.0 (0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fall</td>
<td>8</td>
<td>0.089 (0.027)</td>
<td>54.1 (18.4)</td>
<td>0.056 (0.023)</td>
<td>401 (81)</td>
<td>0.9 (0.4)</td>
<td>13.9 (2.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>1</td>
<td>0.023 (-)</td>
<td>14.4 (-)</td>
<td>0.067 (-)</td>
<td>1391 (-)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 6.2. Mean daily discharge (Q) and probability of exceedance (%) from May 15, 2003 through December 31, 2007 in A) State Creek, B) Shane Creek, and C) Walton Creek. Different symbols apply to the return interval category of the discharge rate.
6.4.4. Relationship between days since flood and nutrient uptake

Given our *a priori* hypotheses, we found that nutrient uptake was related to the number of days since the last flood (i.e. intermediate or large, where T= 0.1 - >1 year) only in State Creek in the spring (Figure 6.3B). In State Creek, SRP Vf was significantly related to days since flood in both the treatment and control reaches following a curvilinear Gaussian function ($r^2=0.51$, $p=0.01$; reaches combined), where $y= a*e^{-0.5[(x-x_o)/b]^2}$, and $a=0.26$, $b=6.71$, and $x_o=16.65$. The curve peaked at 15 d post flood, and then declined through 30+ days post flood (Figure 6.3B). In contrast, there were no significant relationships between nutrient uptake rates and days since the last flood when the data were pooled across streams ($r^2=0.01$, $p=0.31$), or in Shane ($r^2=0.17$, $p=0.25$) or Walton Creeks individually ($r^2<0.01$, $p=1.00$; Figure 6.3A, 6.3C, 6.3D). In summer, autumn, and winter for SRP, and in all seasons for NO$_3^-$ uptake parameters, there was no relationship between uptake rates and days since flood when data from all streams were pooled or considered separately (data not shown) and data look very much like the non-significant data in Figure 6.3A, 6.3C, and 6.3D. However, winter discharges were uniformly low (i.e., no storms) due to ice cover, and we had the fewest data points available due to unfavorable sampling conditions. In all streams, we never found any distinct patterns between control and treatment reaches.
Figure 6.3. Days post flood (where a flood = peak return interval of >0.1 y; intermediate and large disturbance) and uptake velocity (Vf) of soluble reactive phosphorus (SRP) in spring (April and May) in the control and treatment (wood added) reaches of A) all 3 study streams combined, B) State Creek, C) Shane Creek, and D) Walton Creek.
6.4.5. Influence of return interval on change in nutrient uptake rates

We examined the influence of return interval category on changes in uptake rates between sampling dates. Pooling data from all streams and seasons, we found that when an intermediate flood (T = 0.1-1 y) occurred between sampling dates, nutrient uptake rates increased relative to a large flood (T ≥ 1 y) for SRP Vf (ANOVA, p=0.004, Figure 6.4A), SRP U (ANOVA, p=0.046, Figure 4B), NO3⁻ Vf (ANOVA, p=0.004, Figure 6.4C) and NO3⁻ U (ANOVA, p=0.046, Figure 6.4D). Small floods (T= 0.01-0.1 y) or base flows (T <0.01 y) were associated with a decline or no change in uptake rates (Figure 6.4A-D). Between control and treatment reaches, there was no differences in the change in rates between for SRP Vf (ANOVA, p=0.811, Figure 6.4A), SRP U (ANOVA, p=0.999, Figure 6.4B), NO3⁻ Vf (ANOVA, p=0.810, Figure 6.4C) and NO3⁻ U (ANOVA, p=0.919, Figure 6.4D), and there were no significant interactions between reach and interval categories for any of the nutrient uptake parameters.

In autumn, which was when we predicted there would be the largest effect of wood addition on change in nutrient uptake rates following floods, we the same pattern in SRP and NO3⁻ uptake parameters as with all data pooled (see above). However, we also looked at absolute uptake rates (i.e., the rate recorded on the date of measurement as opposed to the difference in the rate between sampling dates), and compared between control and treatment reaches using a paired t-test. We found higher uptake rates in the treatment reach relative to the control in the intermediate flood category for SRP Vf (paired t-test, p=0.03, Figure 6.5A) and
SRP U (paired t-test, p=0.07, Figure 6.5B) whereas there was no difference between reaches in the 0.1-1 y category for NO$_3^-$ $V_f$ (paired t-test p=0.119, Figure 6.5C) or NO$_3^-$ U (paired t-test p=0.283, Figure 6.5D). We analyzed the streams individually by season, and found that the pattern in SRP U was driven by SRP U in Shane Creek in autumn, which was significantly higher in the treatment reach relative to the control in the intermediate flood category (paired t-test, p=0.016). There were no other significant differences between wood-addition and control reaches.

![Graph showing mean (±SE) change in uptake velocity ($V_f$) and areal uptake rate (U) of A, B) soluble reactive phosphorus (SRP) and C, D) nitrate (NO$_3^-$) between sampling dates, grouped by the return interval of the maximum discharge between dates. Data are pooled across dates and streams, with and control and treatment (wood-added) reaches shown separately. Small letters indicate differences in the change in rates among return interval categories after Tukey’s multiple comparison test, p-values are from ANOVA comparing among return interval categories.](image-url)

Figure 6.4. Mean (±SE) change in uptake velocity ($V_f$) and areal uptake rate (U) of A, B) soluble reactive phosphorus (SRP) and C, D) nitrate (NO$_3^-$) between sampling dates, grouped by the return interval of the maximum discharge between dates. Data are pooled across dates and streams, with and control and treatment (wood-added) reaches shown separately. Small letters indicate differences in the change in rates among return interval categories after Tukey’s multiple comparison test, p-values are from ANOVA comparing among return interval categories.
Figure 6.5. Mean (±SE) uptake velocity (Vf) and areal uptake rate (U) of A) soluble reactive phosphorus (SRP) Vf, B) SRP U, C) nitrate (NO3-) Vf, and D) NO3- U between sampling dates in autumn (October-November), grouped by the return interval of the maximum discharge between dates. Data are grouped across dates, with control and treatment (wood-added) reaches separate. Small letters indicate differences in the change in rates among return interval categories after Tukey’s multiple comparison test, p-values are from ANOVA comparing among return interval categories. Uptake rates were higher in the treatment reach in the return interval category 0.1-1 y for SRP Vf (paired t-test, p=0.03) and SRP U (paired t-test, p=0.07), indicated by a *.

6.4.6. Metabolism as a predictor of changes in nutrient uptake

Our data set for metabolism was not as spatially and temporally extensive as that for nutrient uptake, so we were unable to analyze changes in metabolism at different flood intervals within each season. Instead, we used linear regression to relate changes in uptake rates with changes in metabolic rates across streams and
reaches when data were available. Changes in NO$_3^-$ $V_f$ and U were unrelated to change in CR, however change in NO$_3^-$ U was positively related to GPP in the spring (SLR, $r^2=0.611$, p=0.004, Figure 6.6A). Change in SRP U was positively related to change in CR in autumn when data from all streams was combined (SLR, $r^2=0.390$, p=0.006, Figure 6.6B), and in Shane Creek individually (SLR, $r^2=0.643$, p=0.017, data not shown).

![Figure 6.6](image)

**Figure 6.6.** A) In spring, change in NO$_3^-$ U was positively related to change in gross primary production (GPP; $r^2=0.611$, p=0.004), and B) in autumn, change in SRP U was positively related to community respiration (CR; $r^2=0.390$, p=0.006).

### 6.5. Discussion

#### 6.5.1. Flood effects on autotrophic vs. heterotrophic stream biofilm communities

In headwater stream ecosystems, biofilm organisms are broadly separated into 2 categories, autotrophic algae and heterotrophic fungi and bacteria, which all assimilate inorganic nutrients from the water column, but have unique growth limitations and disparate responses to disturbance via flooding (Uehlinger and
Naegeli 1998, Acuña et al. 2007). During flooding, autotrophs can be scoured from inorganic substrata (Grimm 1987, Roberts et al. 2007), in contrast, heterotrophic biofilms are transported, intact, along with their organic substrata (Bilby 1981, Golladay et al. 1992). Studies examining the response of autotrophic biofilms to disturbance have shown that following scour, algal communities regrow in a predictable pattern of community succession (Grimm 1987, Uehlinger and Naegeli 1998), and that during that succession, N retention increases from early to middle stages, followed by late stage declines (Grimm 1987, Martí et al. 1997). These data from streams support the early model from Vitousek and Reiners (1975) describing succession and nutrient retention proposed for forests.

In our study, we found that in spring in State Creek, whole-stream nutrient uptake rates were related to days since flood in a similar curvilinear shape found by Grimm (1987) in Sycamore Creek, AZ using a mass-balance budget approach, suggesting that our stream exhibited similar scour and re-growth patterns typical of an autotrophic community (Figure 6.3B) in a forested stream prior to summer leafout. We did not find a similar pattern in the other streams or in other seasons (Figures 6.3A, 6.3C, and 6.3D). However, in these forested, headwater streams, spring is the period of highest GPP (Hoellein et al. 2007), and State Creek was the stream with the overall highest GPP of all streams (Table 6.1), so State Creek was the mostly likely candidate where we would expect to see a disturbance response typical of an autotrophic community. While Grimm (1987) showed increasing N retention until ~30 d post flood in a Sonoran desert stream, in State Creek we found that the decline in nutrient uptake occurred after ~15 days. Acuña et al.
(2007) demonstrated a similar pattern in a headwater stream in Spain, where GPP initially increased, then declined ~20 d following a spring storm. Forest canopy emergence in spring limits algal growth in forested headwater streams (Hill et al. 2001, Roberts et al. 2007), so shading likely caused nutrient uptake rates to decline after 15 days post flood, which contrasts with previous work in the open-canopy, desert stream where the autotrophic community could develop unhindered by changes in light (Grimm 1987).

The response of heterotrophic biofilm constituents (i.e. fungi and bacteria) to flooding is not well studied, but rather the general research focus has been on the export vs. retention of organic C material that the heterotrophs colonize, including wood, leaves, and sediment (Golladay et al. 1992, Acuña et al. 2007, Argerich et al. 2008). Because heterotrophic organisms move with their C substrata, the response of heterotrophic biofilm activity following disturbance is not necessarily dependent on time since disturbance as with autotrophic communities. Heterotrophs are most likely not scoured away from their organic substrata but instead are exported along with it, although this is not well studied. Instead, the critical factor for heterotrophic metabolism is the influence of the disturbance on the quantity of OM available for colonization and subsequent decomposition (Acuña et al. 2007). Therefore, heterotrophic biofilm response to stream floods should be controlled by 1) disturbance intensity, and 2) OM retention (Uehlinger and Naegeli 1998).
6.5.2. Disturbance intensity

We predicted that nutrient uptake rates would show greater declines following floods of increased intensity, but we unexpectedly found that intermediate-level disturbance resulted in a significant increase in uptake rates between sampling dates, relative to changes in uptake rates at base flow or after low- and high-level disturbances (Figure 6.4). We suggest that changes in OM export and retention were responsible for the observed changes in nutrient uptake rates across the disturbance gradient. If stream OM standing stocks are conceptualized as the net change in import vs. export, it would follow that negligible changes in discharge would not affect net OM standing stocks while large floods would shift the OM balance towards export, and intermediate sized floods would move the OM balance towards net import. More simply, we suggest that intermediate sized storms were large enough to move new allochthonous material into the stream reach from upstream and riparian sources, but not large enough to export it all from the reach. While we did not directly create OM matter budgets before, during, and after storms (which is notoriously difficult to do, especially during large floods when streams overflow their banks), our data indicate the strong link between changes in nutrient uptake rates and community respiration (Figure 6.6), not primary production, suggesting a predominantly heterotrophic (i.e., OM-biofilm) influence on uptake rates in autumn.

Our data appear to support the well-known hypothesis developed by Connell (1978) suggesting that intermediate disturbances, in terms of frequency or intensity, will result in greater biodiversity than larger, smaller, or more
frequent disturbances. We note that our support of the intermediate disturbance hypothesis comes not from enumerating biofilm biodiversity of algae, fungi, and bacteria, but instead by using rates of biofilm-mediated processes such as nutrient uptake and metabolism at the scale of a 100 m stream reach as an alternative metric than that proposed by Connell (1978). We did not explicitly attempt to describe any relationship between biofilm biodiversity and function, but instead used functional metrics as tools to assess the status of community activity and response to disturbance where community measurements were impractical. This approach has been used previously to document stream biofilm response to floods (Uehlinger and Naegeli 1998, Acuña et al. 2007, Argerich et al. 2008). However, previous studies have not evaluated the response of stream biofilms across disturbance gradients (i.e., large, intermediate, small, or none) as we have done here.

6.5.3. Relationship between disturbance and the addition of large wood

Fisher et al. (1998) hypothesized that cross-linkages between riparian zones and stream ecosystems would enhance ecosystem stability. In forested headwater streams, riparian zone-stream linkages take the form of inputs of wood, sediment, and leaves (Wallace et al. 1995, Wallace et al. 1997). Since the addition of large wood to headwater streams is intended to strengthen the riparian-stream linkage through retention of OM (Gurnell et al. 2002), we predicted wood-conferred resistance to changes in nutrient uptake rates in response to floods would be most evident in autumn when leaf litter is most abundant.
Unexpectedly, our prediction was supported only in one of our 3 study streams (i.e., Shane Creek) but not in the other two streams. We assumed that in using 3 replicate study streams, we would see similar response to floods, but it is now evident that stream-specific factors were critical in determining response of nutrient uptake rates to changes in discharge. Potential explanations for the sensitivity of Shane Creek to wood-conferring resistance relative to the other two streams include differences in streambed substrata and pre-treatment rates of nutrient uptake.

Among streams, Shane Creek had the greatest benthic coverage of sand combined with the lowest abundance of large inorganic particles (i.e., gravel, cobble, and boulder). Previous work at these same study streams has demonstrated that increased sand combined with decreased large inorganic particle coverage are negatively correlated with nutrient uptake (Hoellein et al. 2007) and macroinvertebrate secondary production (Entrekin et al. 2007). This was consistent with the generally low nutrient uptake and metabolism rates in Shane Creek relative to the other two streams, especially State Creek (Hoellein et al. 2007). We suggest then that Shane Creek had the most “room to improve” in the context of increases in nutrient uptake rates as a result of changes in substrata composition following wood addition. Similarly, a second reach-scale manipulation further downstream in the 3 study streams (i.e., a gravel and boulder addition designed to increase trout spawning habitat) also showed that Shane Creek was the only site in which there was higher nutrient uptake and metabolism rates in the manipulated relative to the control reach (Hoellein et al in prep).
Overall, our data emphasize that using multiple streams as replicates for environmental manipulation may result in stream-specific outcomes, even when the replicates are similar in many physiochemical and biological parameters, and geographically located very close together. We advocate the collection of pre-treatment and long-term data when possible, which in our case helped explain, in part, the disparate effects of the treatment in response to disturbance among replicate streams. We predict that similar outcomes may eventually occur in State and Walton Creeks, but that these ecosystems may require longer time intervals (i.e., >5 y) to document differences in nutrient uptake rates between treatment and control reaches in response to flooding.

6.5.4. Stream resistance vs. resilience

Our data set included measurements before and after numerous high discharge events in multiple seasons, streams, and stream reaches, however, we did not follow the effect of an individual peak flow at multiple times following an event. More simply, we did not measure ecosystem resilience, or time to recovery. We acknowledge that differences in the amount of time “post flood” at which our measurements were taken could be a conflicting factor for calculating ecosystem resistance, as the nutrient uptake rates could have been changing in the days since the flood occurred (i.e., returning to pre-disturbance levels; Argerich et al. 2008). However, by compiling data across multiple years and streams, we found no relationships between days since flood and nutrient uptake rates, suggesting that
days since flood was not the most critical factor for change in uptake rates (with the exception of State Creek in spring; Figure 6.3B).

A second potential caveat we recognize is that our calculation of the change in rates included data that were not statistically independent (i.e., some dates were both a “before” and an “after”), due to the pattern and seasonal timing of storms. Peak flows in the study streams tended to be bundled over a period of several weeks, particularly during our most intense sampling period in Fall 2007 (Figure 6.1). We acknowledge the potentially important influence of resilience on our resistance calculations, and the conflict of non-independence. However, given the size of the data set, which we analyzed in multiple configurations, including among streams (3 sites), within each stream (2 reaches), and over time (4.5 yrs), we assert that the patterns we demonstrated here are robust. In addition, our results represent a unique contribution to the literature, because we included both a gradient of disturbance size and the influence of experimental wood addition in our study design.

6.5.5. Long-term effects of wood addition

Studies have projected that over long time periods (i.e., centuries), forest succession will result in highly retentive stream reaches resulting from increased channel complexity due to large wood recruitment from aging riparian forests, thereby continually increasing the influence of heterotrophic metabolism on nutrient uptake rates (Valett et al. 2002). Our data suggest this is not a smooth transition where nutrient uptake rates gradually increase over time, especially in
the shorter term, where this change might occur in a jagged pattern, in which accumulated organic matter is periodically exported by large storms. However, our data covers a relatively short time period relative to forest succession (5 years vs. centuries, respectively), and its possible that increasing complexity of debris accumulations over longer time intervals will confer resistance to increasing flood size.

6.5.6. Wood addition as a restoration tool

The success of wood addition as a stream restoration strategy will be partially dependent on local hydrology, which is ultimately controlled by climate and land-use in the stream catchments. Forested headwater streams experience some natural buffering to rising water levels during storms as rainwater is intercepted by forest vegetation, including percolation and soil absorbance (Gore 1996). In other biomes and catchement land-uses, the same volume of water can produce higher and more intense floods. In urban streams, for example, channel and catchement modifications such as increases in impervious surface cover increase the speed and intensity of floods (Paul and Meyer 2001). Similarly, in different biomes (i.e., other than the temperate deciduous forest in this study), flooding can be more severe due less interference of rainwater by soils and vegetation, and/or more extreme patterns of drought and rainfall (Busch and Fisher 1981).

The ability of restoration structures to function in light of the expected disturbance regime should be considered for the ecosystem in question.
Researchers and managers should consider if local hydrology is compatible with wood addition as a potential restoration tool, so that the intended effects (i.e., organic matter retention) are not continually lost via hydrologic export. If not, it may be more appropriate to address the broader issue of modified hydrology prior to using wood addition as a restoration technique. Quantifying the effects of disturbance on the influence of ecosystem restoration is a critical factor in advancing our understanding of stream ecosystems in general and the science and engineering of stream restoration in particular.

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6.7. Literature Cited


CHAPTER 7

CONCLUSION

7.1. Ecological restoration and seasonality

Ecological research has long been important in revealing the multitude of complex interactions between organisms and their environment, but in recent decades, it has become compulsory for ecologists to include a landscape perspective in their research encompassing human interactions and influences (Palmer et al. 2004). As a result, the division between basic and applied ecological research has blurred, such that to justify national funding requires an explicit statement regarding the broader impacts for society at large (National Science Foundation 2007).

Concurrent with a new focus on anthropogenic environmental impacts, there has been increased interest in incorporating ecological paradigms in the thoughtful construction of long lasting restoration projects (Society for Ecological Restoration International Science & Policy Working Group 2004). The sub-discipline of restoration ecology is a relatively young science so a framework for the placement of restoration ecology within broader scientific and social paradigms is still being developed (Aronson and Vallejo 2006). Regardless, there is an increasing desire to utilize ecological tools in the assessment and design of restoration projects, especially in stream ecosystems (Lake 2007).
A critical factor in the success of an ecosystem restoration will be its performance under the expected climate, including seasonal changes in temperature, weather, and hydrology (Palmer et al. 2005, Lake et al. 2007). However, there are few published studies examining how restoration strategies influence ecosystem function under varying climatic conditions. For streams draining temperate forests, seasonality may mediate the efficacy of restoration on functional attributes such as stream nutrient cycling. However, the effects of seasonality itself on nutrient cycling rates in stream ecosystems are not well known.

The goal of my dissertation research projects was to use ecosystem metrics to quantify the influence of seasonality and restoration in headwater streams. My research addressed a series of ecological questions regarding how both seasonality and restoration would affect nutrient cycling rates, and how those rates were related to other ecosystem properties such as stream metabolism. In this synthesis, I will review these questions in the context of my major findings, which will contribute to a greater understanding of stream ecology in particular, and restoration ecology in general.

7.1.1. How does seasonal change influence nutrient cycling in three neighboring headwater streams at the whole-stream scale?

Nutrient uptake rates can limit rates of ecosystem productivity (Elwood et al. 1981, Suberkropp and Chauvet 1995) and influence stream food webs (Rosemond et al. 1993, Cross et al. 2006). Many factors controlling rates of in-stream nutrient processing having been documented including the influence of riparian vegetation (Sabater et al. 2000), organic matter standing stocks (Hall et al. 2000), primary production (Hall and
Tank 2003), and pollution (Martí et al. 2004, Alexander and Allan 2007). While previous studies of nutrient uptake in streams have focused on geographic variation (Munn and Meyer 1990, Hall and Tank 2003, Webster et al. 2003, Bernot et al. 2006), the importance of temporal variation (i.e., seasonal change) has been infrequently addressed in temperate biomes (Mulholland et al. 1985, Martí and Sabater 1996, Roberts et al. 2007). In Chapter 2, I quantified seasonal patterns in nutrient uptake rates in three forested, headwater streams, employing an unprecedented degree of replication, both spatially and temporally.

Previous research suggests that the decomposition of allochthonous organic matter would explain the majority of variation in nutrient uptake metrics (Minshall et al. 1983, Mulholland et al. 1985, Tank and Webster 1998). My research, however, suggested that variation in primary production was also critical for explaining temporal patterns in nutrient uptake rates even in these forested headwater streams. My results suggested that the classification stream ecosystems as either “heterotrophic” (e.g., forested headwater streams) or “autotrophic” (e.g., open-canopy streams) may be too restrictive given the dynamic temporal nature of stream ecosystems (Vannote et al. 1980, Dodds 2006). This categorical paradigm of heterotrophic vs. autotrophic streams was developed based on studies that examined variation in ecosystem structure and function over geographic areas (i.e., across different biomes, riparian communities, and land-use types; Minshall et al. 1983, Webster et al. 2003), while in my study; I captured the same range of variability over a seasonal gradient of variation. My results were supported by concurrent research into the food sources of macroinvertebrates in the same study streams, where primary producers were shown to be a more important C source for biota.
than predicted (Entrekin et al. in preparation). Three decades ago, Minshall (1978) clearly established the under-appreciated importance of autotrophy, even in forested ecosystems, and my research in these forested streams support his insightful observations.

7.1.2. How does seasonality influence nutrient cycling and nutrient limitation at the substratum-specific scale?

As discussed above, one of the major findings from Chapter 2 was that streambed substratum composition was important in explaining overall variation in nutrient uptake rates among my study reaches. However, the whole-stream, short-term nutrient addition method cannot distinguish the relative contribution of individual substrata on overall nutrient uptake. An alternative method using isotopic tracers would identify substratum-specific contributions, but cannot be repeated within seasonal time intervals due to long-lasting labeling of food web compartments (i.e., <1 year; Tank et al. 2006). As is the case for studies examining variation in whole-stream rates of nutrient uptake, previous research into the contribution of biofilms colonizing different stream substrata towards whole-stream rates has been based on studies conducted across geographic, rather than temporal, scales of variability (Webster et al. 2003). In Chapter 3, my unique contribution to the literature has been to measure substratum-specific nutrient uptake and metabolism rates seasonally using microcosms, and then scaling-up these rates for comparison to measurements made using whole-stream methods.

Results from Chapter 3 suggested that temporal patterns for gross primary production (GPP), community respiration (CR), and nitrate uptake (NO$_3^-$ U) were distinct
among substratum types, and for several substrata, their respective contribution towards scaled rates varied among sampling dates. For example, epilithon was not a significant contributor to rates of GPP or nitrate uptake at the whole-stream scale in the summer, but was a significant location for both processes in spring and autumn, when there was open-canopy either pre-leafout or post-leaffall respectively. If I had measured rates only during a limited summer field season, as is common in many studies in temperate systems, I would have missed this critical seasonal change. Overall, research from **Chapter 3** supported earlier results from **Chapter 2** regarding the influence of autotrophic processes on whole-stream nutrient uptake rates. In addition, the results suggested that biofilms colonizing different substrata respond to seasonality in distinct ways. In summary, changes in riparian vegetation or streambed substratum composition (i.e., sedimentation) could cause significant changes in the seasonal demand for nutrients, altering the timing and form of nutrient delivery to downstream ecosystems.

Eutrophication is a common result of human impacts on stream ecosystems in the Midwestern US. After demonstrating variability in substratum-specific biofilm function in **Chapter 3**, I pursued several additional aspects of substratum-specific nutrient dynamics including 1) how different substrata types (i.e., organic vs. inorganic) would respond to nutrient enrichment, 2) whether stream biofilms exhibited preference between different inorganic N species, and 3) how nutrient enrichment would affect biofilm community composition. To address these questions I used nutrient diffusing substrata (NDS), deployed seasonally, and molecular tools (i.e., cloning and denaturing gradient gel electrophoresis) to document the influence of enrichment on bacterial and fungal communities colonizing organic substrata.
My major findings were that despite low ambient nutrient concentrations, biofilms on inorganic and organic substrata were not always nutrient limited. Results suggested that multiple factors can control the response of biofilms to enrichment including light availability and ambient phosphate (P) concentrations. In contrast to studies in the literature, I found no preference for ammonium vs. nitrate, although there are few studies comparing the two inorganic N forms, so my study represents a significant contribution to the existing data examining nutrient limitation and N species. Finally, I showed that the addition of phosphate and nitrate (alone and in combination) increased community respiration and changed the microbial community relative to control substrata where no nutrients were added. Overall, the results from Chapters 3 and 4 increase our collective understanding regarding the role of biofilms colonizing different stream substrata under distinct environmental conditions (i.e., in nutrient enriched conditions and in the face of seasonal change). Because shifts in composition and coverage of streambed substrata are common in response to human influence (Paul and Meyer 2001) and restoration (Minns et al. 1996, Lepori et al. 2005), results from Chapter 3 and 4 will add to our general understanding regarding the effects of seasonality on nutrient processing by different biofilm types.

7.1.3. How does a reach-scale substratum manipulation affect seasonal variation whole-stream nutrient uptake rates?

A reach-scale manipulation in the three study streams presented an ideal opportunity to extend the results from Chapters 2-4 towards understanding the influence of a substrata manipulation on metrics of ecosystem function. In Chapter 5, I analyzed
the addition of a paired sediment trap and gravel-amended spawning reach on numerous metrics including nutrient uptake, metabolism, organic matter dynamics, epilithon chlorophyll \(a\) standing stocks, and fish community structure. Because I found that epilithon contributed significantly towards whole stream primary production and nutrient uptake in the spring and autumn in Chapter 3, my expectation was that the manipulation would increase nutrient uptake and metabolism in these seasons, and increase native brook trout (\(S.\ fontinalis\)) abundance overall as a result of the addition of gravel, cobble, and boulders.

However, my results did not completely support the prediction, as nutrient uptake and metabolism were unaffected by the manipulation in two of the three study streams, and in one stream, increased uptake rates were related to increased community respiration rather than primary production. Despite these unexpected results, we were successful in employing a unique combination of structural and functional measurements that have not previously been used to evaluate the success of habitat manipulations in streams. By measuring both structural elements (i.e., organic matter, chlorophyll \(a\), fish biomass and abundance) and functional metrics (i.e., nutrient uptake and metabolism rates), I suggest that the subtle changes in fish community composition, were likely related to changes in ecosystem structure, but not necessarily reflected in function. Therefore, any benefits of the manipulation are likely entirely dependent on maintaining the physical structure of the manipulation, and were not related to changes in rates of energy transfer through biofilm resources.
7.1.4. How does disturbance influence the influence of wood addition on seasonal patterns in whole-stream nutrient uptake?

In addition to changes in season, it is critical for a restoration strategy to function in light of the endemic disturbance regime. While disturbance has been implemented as a management technique in many ecosystems such as forests, grasslands, and rivers (i.e., controlled fires and floods; Little and Moore 1949, Stanford et al. 1996), the interaction between restoration and disturbance is rarely addressed. In Chapter 6, I examined the influence of floods on stream response to an experimental wood addition by measuring nutrient uptake before and after storms in the three study streams. I found that nutrient uptake rates increased following intermediate-sized floods in both control and wood-addition reaches across all seasons. After the largest floods, both control and wood-addition reaches showed significantly lower nutrient uptake in all streams and seasons. These results were novel and had not been previously documented in streams, most likely because previous studies did not include a gradient of storm sizes in their analyses. In one stream, Shane Creek, I found a significant increase in phosphate uptake rates in the wood-addition relative to the control reaches following an intermediate sized storm in autumn. Shane Creek was also the stream which showed increases in nutrient uptake due to the Forest Service habitat manipulation examined in Chapter 5. The reasons why Shane Creek may have been more sensitive to reach-scale manipulations are discussed in Chapters 5 and 6, and in both cases I credit a greater proportion of benthic surface area covered by sand in Shane Creek prior to restoration. This contention is supported by results from Chapter 3 where I showed that sand was never a “hot-spot” for nutrient uptake or metabolism across seasons. The overall success of wood addition as a stream
restoration strategy will be partially dependent on local hydrology and streambed substrata composition, and both of these should be considered critical factors in determining restoration success.

7.2. Unique research approaches

My dissertation research explored questions relevant to current topics in both stream ecology and the broader ecological literature. For my research, I used three replicate study streams, and a year-round sampling regime, which resulted in an unexpected degree of both spatial and temporal variability. Unexpectedly, the 3 sites exhibited different whole-stream nutrient uptake rates, nutrient limitation patterns, and responses to reach-scale manipulations. The seasonal variation in nutrient uptake I found across my three streams in Chapter 2 was similar to the variation recorded among 10 sites in multiple biomes throughout North America (Webster et al. 2003). The documentation of this natural stream variation despite apparent external “similarity” of the 3 systems and their close proximity emphasizes that using “replicate” streams for environmental manipulation may result in stream-specific outcomes, even when the replicates are similar in many physiochemical and biological parameters, and geographically located very close together. In addition, throughout all my dissertation chapters I found that seasonality significantly influenced ecosystem processes. These results provided a useful source of variation for my analyses, but moreover, should be a critical consideration in the success of restoration techniques.

My dissertation research asked 2 topical questions of relevance for general ecological research: specifically 1) what is the role of disturbance in controlling rates of
ecosystem function, and 2) what is the relationship between ecosystem function and community structure? In Chapter 6, I found that intermediate disturbance resulted in increases in nutrient uptake relative to larger disturbances. Measurements of ecosystem response to disturbance (i.e., resistance and resilience) have not previously been invoked to evaluate the success of stream restoration via measurements of nutrient uptake rates. In Chapter 4, I showed that the microbial biofilms community was related to changes in ecosystem function following nutrient enrichment. In both cases, these questions were born simply from my own attempts to describe the influence of nutrient dynamics in the environment of the study streams, but results should prove applicable to research in other ecosystem types.

7.3. Management Implications

Recent papers have suggested that there is a need for increase use of metrics of ecosystem function for the evaluation of restoration (Lake 2007). Results from my dissertation answer that call, and provide multiple examples where measurements of nutrient uptake were useful for restoration evaluation. Stream restoration strategies often involve altering the physical template of a stream, thereby changing the abundance of different stream substrata (Minns et al. 1996). Results from Chapters 2-4 indicated that nutrient uptake rates are strongly related to streambed substrata composition, which can influence the seasonal patterns of nutrient export from headwater streams. In addition, stream restoration monitoring is relatively rare (Moerke et al. 2004, Bernhardt 2005). In Chapters 5 and 6, I showed that nutrient uptake measurements are a valuable, and potentially overlooked tool for quantifying biological differences between reference and
manipulated reaches following two different reach-scale restorations. Overall, my dissertation research provides an example of how functional measurements can be directed towards evaluating reach-scale stream manipulations, ultimately contributing to the evaluation and refinement of long-lasting and effective restoration strategies.

7.4. Literature Cited


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