ANTHROPOGENIC AND LANDSCAPE FACTORS CONTROL STREAM
NITROGEN TRANSFORMATIONS AT MULTIPLE SPATIAL SCALES

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Abstract

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Anthropogenic alterations to the global nitrogen (N) cycle have doubled reactive
N flux into the biosphere and altered aquatic ecosystem function. Streams modify N
loads carried to coastal ecosystems by converting N to organic forms or removing it as
gaseous N. Understanding how streams transform N can offer insight to ecologists and
land managers about how stream ecosystems function under elevated N loads. I
researched how anthropogenic and landscape factors affect N transformations by
studying streams in two distinct biomes and at multiple spatial scales.

At the landscape scale, I studied N concentrations in streams draining the Teton
Range (Wyoming, USA), a sub-alpine and alpine ecosystem with variable lithology.
Streams draining crystalline geology had higher N compared to streams draining
carbonate geology, which had more vegetation, suggesting that lithology mediated
patterns in vegetation and terrestrial N retention. At the reach scale, I studied how land
use influenced N uptake and transformation in Midwestern streams (Michigan, USA) and
found that dissimilatory N transformation rates (i.e., nitrification and denitrification)
within streams were not affected by riparian zones, which are commonly used to mitigate water quality degradation. Dissimilatory N transformation rates were always < 10% of whole-stream N uptake and nitrification rates balanced denitrification rates, implying that denitrification did not represent net N loss from the water column. At the substratum scale, sediment organic carbon content correlated with denitrification, but only when nitrate concentration exceeded a threshold. Finally, I returned to the Tetons and found that grazing activity by invasive snails can increase periphyton N fixation rates in a stream with low N concentrations.

A synthesis of my findings from high N streams in the Midwest suggests that land-use practices have increased temporary N removal at the expense of permanent N removal. In the low N streams of the Tetons, observations from different spatial scales suggest that landscape factors that lower stream N concentrations and high rates of grazing together can influence the importance of N fixation in streams. My dissertation highlights multiple constraints on N processing in streams and emphasizes that basic ecological research can yield important results for management agencies.
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CHAPTER 1

DISSERTATION INTRODUCTION

1.1 Human Activity and Global Change

Human activities have caused global changes in ecosystem structure and function (Vitousek et al. 1997a). As a consequence, modern rates of species extinction exceed background levels by 100-1000 times (Pimm et al. 1995), and the rapidity of these changes has initiated a mass extinction on the order of that which terminated the era of dinosaurs (Hughes et al. 1997; Nee and May 1997). The drivers of this mass extinction include human-induced land cover change and fragmentation of habitats (Vitousek et al. 1997a), over-fishing and destructive harvest techniques in the ocean (Worm et al. 2006), introduction and subsequent spread of species into novel habitats (Hooper et al. 2005), and anthropogenic changes in the global carbon (C) (IPCC 2001) and nitrogen (N) cycles (Vitousek et al. 1997b).

Altered nutrient cycles can initiate widespread, and often unanticipated, changes in ecological systems. For example, excessive use of phosphates and incomplete sewage treatment has rapidly shifted many lakes from oligotrophic to eutrophic status since the mid-1900s. Knowledge of the phosphorus (P) cycle led to improvements in sewage treatment and restrictions in detergent phosphate use that helped stem blooms of toxic algae that threatened fisheries and human health (Edmondson 1970). In another example, oxides of sulfur (S) emitted by coal-burning power plants caused the acidification of
lakes and forests throughout the northeastern United States and northern Europe in the mid-1900s, initiating fish kills and degrading forest health. Research into the perturbed S cycle initiated legislative changes that reduced S emissions and decreased acid rain (Likens and Bormann 1974). By studying biologically-mediated nutrient cycles, ecosystem ecologists are uniquely situated to contribute insight into how anthropogenic changes to global nutrient cycles affect ecosystem dynamics (Schlesinger 2004). Today’s challenges in ecosystem ecology include understanding anthropogenic alterations to the C and N cycles caused by increased atmospheric carbon dioxide (CO₂) concentrations and increased availability of reactive N. Like higher concentrations of CO₂, increased availability of N has global ecological consequences (Vitousek et al. 1997b) because, among the many elements essential for ecosystems, N frequently limits primary production in terrestrial and aquatic ecosystems (Vitousek and Howarth 1991; Francoeur 2001). My dissertation research focuses on how anthropogenic activity affects N cycling in streams.

In this introduction, I begin by summarizing the scientific literature that motivated my research, and I introduce a conceptual model on page 26 linking the multiple spatial scales at which I targeted my research. Beginning on page 28, I provide a brief synopsis of the specific research gaps that my individual research chapters address.

1.2 Nitrogen Biogeochemistry: Assimilatory and Dissimilatory Processes

The largest global pool of N, dinitrogen gas (N₂), composes 78% of the atmosphere, but only specialized microbes and cyanobacteria with the enzyme nitrogenase, some of which form symbioses with terrestrial plants or aquatic algae, can directly incorporate N₂ into organic N via N fixation. Prior to anthropogenic alteration of
the N cycle, N fixation represented the most significant input of new N in an ecosystem (lightning can oxidize N₂ into a bioavailable form, but it adds a trivial amount of N), so N fixation can be considered a starting point in a variety of biologically mediated N transformations (Figure 1.1).

After N fixers incorporate atmospheric N into biomass, heterotrophs may acquire N by consuming biomass in dissolved form in the case of microbes and algae, or in particulate form in the case of higher organisms. As a metabolic waste product, organic N is mineralized to ammonium (NH₄⁺), which has a valence state of -3 and is the most reduced form of inorganic N in the environment. In contrast, nitrate (NO₃⁻), another common form of inorganic N, is the most oxidized with a valence state of +5. Many N compounds exist at valence states between NH₄⁺ and NO₃⁻, and a variety of microbes

![Biologically-mediated nitrogen transformations](image_url)

**Figure 1.1.** Biologically-mediated nitrogen transformations.
drives the global N cycle as they convert one N compound to another in redox-dependent dissimilatory N transformations. For example, in nitrification, a consortium of microbes use the energy released by oxidizing NH$_4^+$ with O$_2$ to fix CO$_2$ into organic matter, producing NO$_3^-$ as an end product (Figure 1.1). Denitrification, another dissimilatory transformation, closes the N cycle as microbes couple the oxidation of reduced organic matter to the reduction of NO$_3^-$, producing N$_2$ and lesser amounts of nitrous oxide (N$_2$O) (Figure 1.1). Because only the specialized N fixers can directly use N$_2$, denitrification effectively removes N from the ecosystem permanently, contributing to the N limitation so common in many ecosystems (Vitousek and Howarth 1991). Recent research has discovered that other, lesser-studied pathways of dissimilatory N transformation (e.g., dissimilatory NO$_3^-$ reduction to NH$_4^+$ and NO$_3^-$ reduction coupled to S or iron oxidation) may be more prevalent than once thought (Burgin and Hamilton 2007), but in the context of this dissertation, I refer to nitrification and denitrification collectively as dissimilatory N transformations.

Whereas dissimilatory processes drive the N cycle, ultimately returning N to the atmosphere, assimilatory processes retain N in the ecosystem when bacteria, fungi, algae, and plants directly incorporate NH$_4^+$ or NO$_3^-$ into biomass (Figure 1.1). A major difference between assimilatory uptake of NH$_4^+$ and NO$_3^-$ is that the latter must be reduced prior to uptake, a process that requires energy investment. Thus, thermodynamic considerations suggest that NH$_4^+$ is a more favorable N source than NO$_3^-$ though some organisms, especially plants, preferentially use NO$_3^-$ to satisfy assimilatory demand (reviewed in Schlesinger 1997). When N limits productivity in ecosystems unaffected by
anthropogenic N inputs, inorganic N is relatively rare in the environment because organisms retain it immediately after it becomes available (Schlesinger 1997).

1.3 Anthropogenic Alterations to the N Cycle

Anthropogenic activities have fundamentally altered the global N cycle by doubling N availability (Vitousek et al. 1997b). In order of largest contribution, anthropogenic N inputs include fertilizer application, fossil fuel combustion, and N-fixing crops that increase N inputs above background levels by replacing native vegetation that did not fix N (Galloway et al. 1995). Increased N availability is generally a by-product of agricultural or urban land-use activity that has improved human quality of life; fertilizer and N-fixing crops improve agricultural output and food availability, and fossil fuel combustion drives economic activity that can increase goods and services. Although human societies have reaped benefits by increasing N availability, mainly in the developed world, altering the global N cycle has had far-reaching consequences, especially in coastal ecosystems.

Most N that ultimately enters estuaries originates as anthropogenic non-point source pollution from the landscape (Boyer et al. 2006), and the biggest sources are direct application of nitrogenous fertilizer and atmospheric deposition of nitrogen oxides (Goolsby 2001). Rainfall and snowmelt export N that exceeds demand to downstream ecosystems (Howarth et al. 1996), and long-term monitoring in the Gulf of Mexico illustrates the negative consequences of increased N availability that stimulates algae blooms and causes the collapse of fisheries after spatially extensive and long-lasting zones of surface-water hypoxia ensue (Rabalais et al. 2002). Seasonal hypoxia occurs in nearly all estuaries fed by rivers that drain anthropogenically-modified landscapes in
North American and Europe because N, rather than P, limits productivity in estuaries (Howarth and Marino 2006). Because streams carry N loads from their source area in terrestrial ecosystems to receiving estuaries, stream ecosystems are also affected by increased N availability. Therefore, understanding how streams modify N loads is a critical component to understanding the ecological effects of increased N availability in aquatic ecosystems.

1.4 Nitrogen Cycle in Streams

In 1964 Luna Leopold famously said, “Streams are the gutters down which flow the ruins of continents.” In the decades since, stream ecologists have reversed this simplistic view by illuminating streams as important ecosystems in their own right, including closely examining biological controls on nutrient uptake and transformation. Streams do not simply convey excess N from the landscape to estuaries as if in a sterile pipe—biological activity processes stream-water N, transforming it via dissimilatory metabolism or retaining it in biomass via assimilatory metabolism. For example, a model predicted that 37-76% of riverine N inputs were removed during transport through a river network, with the majority removed in small streams (Seitzinger et al. 2002). Because small streams are interfaces between terrestrial and aquatic habitat, they are “hotspots” of N transformation (McClain et al. 2003), and they contribute significantly to the biological integrity of the entire river network (Meyer et al. 2007).

Despite their small size, headwater streams can exert strong control over N flux in river networks (Peterson et al. 2001; Alexander et al. 2007). First, reach-specific N removal decreases from nearly 25% of N inputs removed in low-order streams to only 5% of N inputs removed in high-order streams (Seitzinger et al. 2002). Therefore, small
streams (< 4th order), which constitute up to 90% of total length in a river network, can remove more N per unit length than a large river (Wollheim et al. 2006). Second, high width to depth ratios, more common in small streams than in large rivers, increase interaction between nutrient concentrations in the water column and the benthos, which is the focus of biological activity in streams (Peterson et al. 2001). Thus, the number of headwater streams in a river basin and the geometry of their channels can increase the ability of biological activity to modify the quantity and quality of nutrient loads transported to downstream ecosystems.

The development of the nutrient spiraling model, which couples nutrient uptake with downstream transport, facilitated the mechanistic study of N uptake and retention in streams (Newbold et al. 1981). Additionally, the use of stable isotopes in stream uptake studies has allowed researchers to partition whole-stream N uptake into assimilatory and dissimilatory processes to identify the specific fate of N removed from the water column. An interbiome \( ^{15}N \) tracer study of \( \text{NH}_4^+ \) uptake in small streams identified assimilatory N demand as the most important uptake mechanism, with autotrophic demand more important in open-canopied desert and prairie streams and heterotrophic demand more important in shaded, forested streams (Webster et al. 2003). In the same inter-biome study, nitrification generally accounted for 20-30% of \( \text{NH}_4^+ \) demand (Peterson et al. 2001). More recent research has investigated \( \text{NO}_3^- \) uptake in small streams, also using stable isotope tracers, because \( \text{NO}_3^- \) is the dominant form of N exported from human-modified landscapes to streams. In a shaded, forested stream, assimilatory demand dominated \( \text{NO}_3^- \) uptake, with only 16% of \( \text{NO}_3^- \) uptake due to denitrification (Mulholland et al. 2004); but in 9 open-canopied prairie streams, denitrification usually accounted for
about 1% of NO₃⁻ uptake (O’Brien et al. in press). Therefore dissimilatory N transformations appear to account for a smaller proportion of whole-stream N demand than assimilatory uptake.

1.5 Anthropogenic Alterations to Stream Ecosystems

In the Baldi lecture delivered to the Societas Internationalis Limnologiae in 1975, H. B. N. Hynes declared, “In every respect, the valley rules the stream.” Early nutrient uptake studies skewed our knowledge toward streams with pristine “valleys” because they were performed in streams that drain relatively unmodified reference catchments. However, most streams in the United States drain catchments heavily modified by agricultural and/or urban land-use activities that radically transform the landscape (i.e., “the valley”) (Meyer and Turner 1994). Only recently have researchers begun investigating N uptake and transformation in urban (Grimm et al. 2005; Meyer et al. 2005) and agricultural (Bernot et al. 2006; Niyogi et al. 2004) streams. Land-use activity usually increases NO₃⁻ concentrations in streams (Omernick 1976), and new research has begun to focus on how agricultural and urban land use alter NO₃⁻ delivery (Petry et al. 2002; Groffman et al. 2004; Wollheim et al. 2005) and subsequent NO₃⁻ uptake and transformation in streams (e.g., Mulholland et al. in review).

Land use alters stream ecosystems in many ways that can affect N uptake and transformation. Because nutrient concentrations frequently determine assimilatory and dissimilatory uptake rates, agricultural and urban activities that increase nutrient concentration may saturate stream uptake rates (Dodds et al. 2002; O’Brien et al. in press). Land-use activity also frequently removes riparian vegetation from stream banks, increasing light but decreasing leaf litter input to the stream, both of which should
increase the relative role of autotrophic uptake processes (Abell and Allan 2002). An increase in autotrophy could also affect relative rates of dissimilatory N transformations by altering stream redox conditions because nitrification requires oxygen (Kemp and Dodds 2001; Strauss et al. 2004) whereas denitrification requires anoxia (Seitzinger 1988). Finally, tile drainage from agricultural fields, channelizing streams into ditches, and increasing impervious surface cover in a basin collectively increase the frequency and magnitude of peak discharges in downstream ecosystems (Petry et al. 2002; Paul and Meyer 2001). The increased erosive power incises stream channels, isolating them from their floodplains (Magner et al. 2004), and lowering channel width to depth ratios, which decreases the capacity for biological uptake processes to interact with water column N concentrations (Kemp and Dodds 2002a). Returning to the quote by Luna Leopold, as we have increased our knowledge of stream ecosystems, we now see the possibility that human land use has converted streams from retentive ecosystems into the “gutters” that he envisioned, down which flow the ruins of a modified landscape—particularly excess N.

1.6 A Conceptual Model of N Dynamics Incorporating Multiple Spatial Scales

My dissertation research will help to fill our knowledge gap addressing how anthropogenic landscape change affects stream N transformations. Streams are hierarchical ecosystems where large-scale controls restrict small-scale responses (Frissell et al. 1986). For the purposes of my dissertation research, I studied N dynamics in streams at three, nested spatial scales: at the basin (broadest), the reach (intermediate), and the substratum scale (finest). Because different factors control N availability and processing in stream ecosystems at different spatial scales, the stream ecosystem response
to anthropogenic changes can also be measured at different scales, each of which provides insight into how streams transport, process, and transform N (Buck et al. 2004). I depict relationships among these spatial scales, and how my dissertation chapters relate to them, in Figure 1.2. In the following paragraphs I illustrate the importance of using multiple spatial perspectives to address my general research question: How does anthropogenic activity influence N transformations in streams?

Because of the difficulty in sampling at broad spatial scales, many researchers use spatially-explicit models to predict N dynamics in a large river network. These studies have generally found that N concentrations in large river networks are a relatively simple function of N inputs to the landscape (Peierls et al. 1991; Alexander et al. 2000; Caraco et al. 2003), which demonstrates the overriding importance of N inputs in broad-scale

![Figure 1.2. Relationships among spatial scales. Research chapters correspond to spatial scales identified on the left.](image-url)
assessments of stream N dynamics. The same models also predicted higher N concentrations than observed in small streams, suggesting the potential importance of dissimilatory N transformations and associated gaseous N losses for reducing downstream N flux (Alexander et al. 2000; Caraco et al. 2003). Despite these important findings, studies focused at a broad scale cannot easily identify the factors that control gaseous N losses from small streams, which are best investigated using reach-scale studies.

Concentrations of NH$_4^+$ and NO$_3^-$ frequently control nitrification (Kemp and Dodds 2002a) and denitrification (Inwood et al. 2005) rates respectively, so broad-scale landscape controls on stream-water nutrient concentrations also control these dissimilatory N transformations. However, as a reach-scale factor, riparian buffers can further control nutrient concentrations by intercepting N from the landscape before it enters the stream (Karr and Schlosser 1978; Peterjohn and Correll 1984). Therefore, at a given N loading level from the landscape, riparian vegetation could attenuate N concentrations, lowering N transformation rates compared to stream reaches without riparian buffers. Consequently, at the stream-reach scale, riparian buffers may moderate the effect of broad-scale determinants on N processing and transformation.

Within a stream ecosystem, the benthos is a heterogeneous assemblage of inorganic and organic substrata such as sand, cobble, coarse or fine benthic organic matter (CBOM and FBOM respectively), epilithon, or algae. These different benthic substrata have different features, such as their redox gradient or C:N ratio, that can control N transformation rates (Kemp and Dodds 2002b; Strauss et al. 2002). Substrata that sustain particularly higher N transformation rates than others are “hot-spots”
(McClain et al. 2003), and their abundance and distribution may ultimately influence whole-stream N transformation rates. In the case of epilithon, grazing by herbivores can further modify the capacity of the substratum to transform N, especially assimilatory N transformations via water-column N uptake or N fixation, by increasing nutrient diffusion rates (Lamberti and Resh 1983; Williams and Carpenter 1997). Therefore, whole-stream rates of N transformation also depend on factors that vary at the substratum scale in addition to the landscape and reach-scale factors that control N concentrations.

1.7 Dissertation Outline

In the following paragraphs, I briefly describe the motivation and approach of the individual studies that compose my dissertation. The spatial scale addressed by each research question is highlighted in Figure 1.2.

Chapter Two. Steep slopes, high elevations (with correspondingly short growing seasons), and exposed bedrock decrease N retention in alpine ecosystems, making them particularly sensitive to N deposition (Fenn et al. 1998). Lithology may influence N retention by mediating soil properties, which can also control N loss from alpine catchments (Sickman et al. 2003). I studied streams draining the Teton Range of Wyoming, where atmospheric models predict higher levels of anthropogenic N deposition than suggested by dispersed National Atmospheric Deposition Program monitoring sites. A gradient of crystalline-carbonate lithology in the Teton Range provides an optimal framework to test how lithology mediates other land cover variables that control N concentrations in alpine streams. I performed a synoptic survey of stream water among basins and within two contrasting basins to identify what landscape factors
were related to N concentrations, and I predicted that catchments with carbonate
lithology would have higher N retention.

Chapter 3. Basin-scale models indicate that anthropogenic N inputs control N
concentrations in stream basins heavily modified by land use (Alexander et al. 2000;
Caraco et al. 2003), and previous research has found a link between land use and
denitrification rates (Inwood et al. 2005). Riparian zones can decrease N concentrations
(Peterjohn and Correll 1984; Dodds and Oakes 2006), thereby mediating rates of
nitrification and denitrification. To understand how basin-wide variation in land use and
reach-scale variation in riparian zones controls dissimilatory N transformations, I studied
streams draining forested, agricultural, and urban basins with and without riparian zones.
I made monthly measurements of nitrification, denitrification, and sediment
characteristics, and I related these measurements to the configuration of the landscape to
identify how riparian zones influenced dissimilatory N transformations. I predicted that
lower nutrient concentrations in forested streams and in land-use modified streams with
riparian zones would cause lower nitrification and denitrification rates.

Chapter 4. Recent studies have indicated that cleared riparian zones make
autotrophy an important controlling factor in the biogeochemistry of agricultural (Bernot
et al. 2006) and urban streams (Grimm et al. 2005). Similarly, other studies have found
that autotrophy can be important in forested streams in the spring prior to leaf-out
(Fellows et al. 2006; Mulholland et al. 2006). Riparian zones can also contribute large
pulses of leaf litter in the fall that stimulate N uptake during decomposition. I
investigated how seasonal and land-use patterns affect whole-stream NH$_4^+$ and NO$_3^-$
uptake using short-term enrichments of NH$_4^+$ and NO$_3^-$ to measure whole-stream N
uptake, which I compared to nitrification and denitrification rates measured in the laboratory. Whereas Chapter 3 examined land-use controls on dissimilatory N transformations, Chapter 4 puts them in the context of areal N uptake rates measured at the reach scale, also identifying how land use mediates saturation of nutrient uptake. I predicted high relative demand for N in the spring in all streams due to higher autotrophic activity. I also predicted that saturation of N uptake would occur in land-use modified streams and that dissimilatory N transformation would be a small component of overall N demand.

Chapter 5. A recent meta-analysis indicated that NO$_3^-$ is the most important predictor of denitrification among aquatic habitats (Piña-Ochoa and Álvarez-Cobelas 2006), but in streams with very high NO$_3^-$ concentrations, denitrification is probably limited by organic C (Inwood et al. 2007). I compared denitrification rates in agricultural streams with seasonally stable benthic organic matter but where NO$_3^-$ concentrations varied seasonally. I also compared denitrification rates among substrata with variable organic matter and uniformly high NO$_3^-$ concentrations. I reasoned that substrata with higher C content would have higher denitrification rates, but only when NO$_3^-$ concentrations were high and unlikely to limit denitrification.

Chapter 6. The previous research chapters focus on how landscape changes and land-use activity influence stream N cycling, but humans can also influence stream N cycling by introducing invasive species. In Polecat Creek, Wyoming, an invasive snail that reaches extremely high densities (25,000-500,000 individuals m$^{-2}$; Hall et al. 2003) has been shown to dominate the N cycle through high excretion rates of NH$_4^+$. Because grazing by dominant herbivores can control epilithon quantity and quality (Feminella and
Hawkins 1995), I investigated the possibility that this invasive snail could also influence N fixation rates, thereby modifying stream N cycling in another way, not examined previously in any stream studies to date. To do this, I experimentally reduced these high ambient snail densities and measured N fixation 1 and 2 weeks after the manipulation. I predicted that treatments with higher snail biomass would have higher N fixation rates because grazing would increase resource availability for N-fixing algae.
2.1 Abstract

Steep slopes, short growing seasons, and abundant exposed rock reduce the capacity for alpine environments to retain nitrogen (N), but lithology has rarely been considered an important factor. Carbonate lithology weathers rapidly, which could increase N retention through a cascade of interactions including soil development and vegetation. We sampled streams draining a crystalline to carbonate lithology gradient in the Teton Range in Wyoming. Nitrate concentrations were higher in steep, high elevation, unvegetated catchments, but autocorrelations obscured the driving factor. Among Teton catchments, NO$_3^-$ concentrations decreased with increasing specific conductivity, which was higher in forested catchments, suggesting that vegetation was an important determinant of stream solute composition. Although we did not find a relationship between carbonate lithology and NO$_3^-$ among basins, we sampled within two basins of contrasting lithology that had equally steep slopes and high elevations. The carbonate basin had more vegetation and lower N concentrations than the crystalline basin, confirming a link between carbonate lithology and vegetation, and identifying

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$^1$ This chapter (with co-authors Jennifer L. Tank and R. O Hall, Jr.) is in review for publication.
vegetation as a more important driver of N retention than steep slopes and high elevations. High NO₃⁻ concentrations and low DOC:DON in crystalline streams suggested NO₃⁻ leaching, a symptom of N saturation. Furthermore, stream-water DIN:DON, which signals terrestrial N status in alpine ecosystems, indicated that crystalline catchments were more sensitive to N deposition, probably because of less vegetation. Collectively, these results suggest that vegetation is a primary control of N retention in alpine basins, and that lithology may indirectly mediate vegetation, and thus N retention, through soil properties.

2.2 Introduction

Watershed-ecosystems vary widely with respect to how much nitrogen (N) is retained versus exported (Fenn et al. 1998). Biological controls influencing ecosystem N retention include assimilation by vegetation (Vitousek 1977), soil bacterial biomass (Brooks et al. 1996), and sequestration of organic matter in soil pools via decomposition (Lajtha et al. 1995). Ecosystem successional status also controls N retention, with middle-aged ecosystems generally retaining more N (Vitousek and Reiners 1975). Alpine ecosystems are characterized by a host of abiotic factors that reduce their capacity to retain N including short growing season (Fenn et al. 1998), high relief topography, abundant exposed rock that decreases ecosystem water residence time (Clow and Sueker 2000), and flushing of soil nutrients during snowmelt (Sickman et al. 2003). This suite of factors makes alpine ecosystems innately sensitive to anthropogenic N deposition, and the mountain environments in Colorado show early signs of N saturation despite relatively low deposition rates (4-5 kg N ha⁻¹ y⁻¹) from nearby urban centers and
downwind agricultural activity (Baron et al. 1994; Williams et al. 1996; Baron et al. 2000).

Most N retention studies from alpine environments have been performed in areas entirely underlain by crystalline lithology, like the Sierra Nevada of California (Sickman et al. 2003) and the Front Range of Colorado (Williams et al. 1996). Lithology may indirectly increase ecosystem N retention through a cascade of interactions involving weathering rates, soil development, and vegetation, but the specific type of bedrock lithology has rarely been examined as an important factor in alpine ecosystem N retention. Because chemical weathering rates vary by rock type, and carbonate rocks weather at least twice as fast as crystalline rocks (Hembree and Rainwater 1961), rapid weathering of carbonate bedrock could cause thicker soils to develop, and soil thickness is a major control of N export from alpine environments (Sickman et al. 2002). Thicker soils should also support greater vegetation growth, if growing season permits, and nutrient demand by vegetation could cause greater ecosystem N retention (Vitousek 1977). The Teton Range, Wyoming, underlain by a mixture of crystalline and carbonate geology, is an ideal location to investigate the possibility that bedrock lithology could increase N retention in alpine ecosystems. Because lithology can regulate weathering rates and indirectly affect soil development and vegetation, we predict that lithology will explain some variability in the N retention capacity of alpine ecosystems.

Although the alpine environments of the Teton Range in northwestern Wyoming have different geology than those studied extensively in Colorado (Williams et al. 1996; Clow and Sueker 2000), they share many physical attributes, such as steep slopes and exposed rock, that would make them similarly susceptible to N deposition. National
Atmospheric Deposition Program (NADP) monitoring sites suggest relatively low N deposition (1-2 kg N ha\(^{-1}\) y\(^{-1}\)) in the rural lowlands surrounding the Tetons (2006 data are available at http://nadp.sws.uiuc.edu), but recent studies indicate that increased precipitation and cloud-water deposition in mountains may cause higher N deposition relative to lower elevations (Weathers et al. 2006). Furthermore, evergreen forests, more common in mountains than lowlands in the American West, have a high leaf-area index and can increase N deposition by scavenging airborne particles with year-round foliage (Weathers et al. 2000). Finally, ammonia volatilization and particulates from the agricultural Snake River Valley, which is immediately upwind of the Tetons, could further augment N deposition (Fenn et al. 2003a). Therefore, the Tetons likely receive higher N deposition than interpolated by low-elevation NADP monitoring sites.

As an alpine ecosystem, the Teton Range should have relatively low N retention, but we found no published data comparing solute concentrations among streams draining the Tetons. To address this gap in our knowledge, we measured element concentrations in 30 Teton Range streams to identify relationships between stream-water nutrients and landscape parameters, and to investigate the possibility that lithology regulates stream-water nutrient concentrations. In addition, we contrasted relationships between nutrient concentrations and landscape parameters at a finer spatial scale by sampling two basins underlain by contrasting lithologies, Granite (carbonate) and Paintbrush (crystalline) Canyons. We predicted that higher weathering rates, thicker soil development, and greater vegetation cover associated with carbonate parent material would cause catchments draining carbonate lithology to have lower N export compared to those draining crystalline lithology. Additionally, because even moderate increases in N
deposition could initiate N saturation in the alpine ecosystems of the Tetons, we investigated the vulnerability of these ecosystems to N deposition using indexes of sensitivity identified in lowland forests (Campbell et al. 2000a) and alpine/subalpine ecotones (Hood et al. 2003a).

2.3 Methods

2.3.1 Study Site

Nearly all of the study catchments were located in Grand Teton National Park, part of the Greater Yellowstone Ecosystem in northwestern Wyoming, USA. The Teton Range has a complex geology, with carbonate (i.e., limestone) and clastic (i.e., shale and mudstone) sedimentary formations exposed on the southern end of the range, crystalline (i.e., granite and gneiss) formations in the center of the range (Figure 2.1), and carbonate formations on the northern end of the range (Johnson and Raines 1995). Only three basins had catchments with large proportions of exposed clastic lithology, so most individual catchments were a mix of crystalline and carbonate bedrock with varying proportions of surficial debris (i.e., deposits of colluvium and alluvium).

2.3.2 Sample Collection

We sampled all major streams draining the east slope of the Teton Range (n=30) in late July 2004 to compare patterns in solute concentration across basins. Additionally, we sampled independent, non-nested sub-basins within Granite Canyon (n=13), a largely carbonate catchment, in late July 2003 and in late July 2004, we sampled Paintbrush
Figure 2.1. Synoptic sampling basins in the Teton Range, Wyoming.
compare within-basin patterns of stream-water chemistry between catchments of contrasting lithology. We sampled streams in late July when the snow-melt hydrograph was nearing base flow and solute concentrations are generally at background levels (Williams et al. 1995; Hood et al. 2003b).

We sampled most streams where they entered the Snake River valley floor (Figure 2.1), above any terminal lakes. Although individual catchments varied in their average elevation, sampling points were all at low elevation. At each sampling point, we obtained UTM coordinates with a hand-held Global Positioning System, we measured temperature and specific conductivity (YSI conductivity probe; Model EC-300), and we measured stream depth, width, and velocity (Marsh-McBirney flow meter, Model 200; Frederick, Maryland, USA) at multiple points along a channel cross-section to calculate stream discharge as the sum of partial discharges. For laboratory water chemistry analyses, we filtered stream water through ashed (550°C) Pall A/E filters (1.0 µm pore size), stored samples in 60 mL bottles triple-rinsed with filtered stream water, and we froze water samples for future analyses upon return to the laboratory.

2.3.3 Water Chemistry Analyses

We measured stream-water ammonium (NH$_4^+$) concentrations with the phenol-hypochlorite method [Solorzano 1969; detection limit as lowest standard (dl)=1.5 µg NH$_4^+$-N L$^{-1}$] using a Shimadzu UV-1601 spectrophotometer (Columbia, Maryland, USA) with a 10-cm path length. We measured nitrate (NO$_3^-$), sulfate (SO$_4^{2-}$), and chloride (Cl$^-$) with a Dionex Model 600 ion chromatograph (Sunnyvale, California, USA) equipped with ED50 electrochemical detector and AS14A guard and analytical columns (USEPA
1993; dl=2.5 µg NO$_3^-$ N L$^{-1}$, 10 µg SO$_4^{2-}$ S L$^{-1}$, and 10 µg Cl$^-$ L$^{-1}$). We measured cations [potassium (K$^+$), sodium (Na$^+$), calcium (Ca$^{2+}$), and magnesium (Mg$^{2+}$)] with a Perkin-Elmer 3100 atomic absorption spectrometer (Wellesley, Maryland, USA) using air-acetylene for the flame source (Slavin 1978; dl=0.1 mg K$^+$ L$^{-1}$, 0.1 mg Na$^+$ L$^{-1}$, 0.5 mg Ca$^{2+}$ L$^{-1}$, and 0.5 mg Mg$^{2+}$ L$^{-1}$). We simultaneously measured dissolved organic carbon (DOC), using the combustion infrared method (APHA 1995; dl=25 µg C L$^{-1}$), and total dissolved nitrogen (TDN), using high-temperature catalytic oxidation and chemiluminescence (Merriam et al. 1997; dl=25 µg N L$^{-1}$), on a Shimadzu TOC-500 analyzer equipped with a Total Nitrogen Module (Columbia, Maryland, USA). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and dissolved inorganic nitrogen (DIN), and acid neutralizing capacity (ANC) was quantified using a charge balance equation from Likens et al. (1996) using two modifications; strong acid anions that were below detection limits (i.e., fluoride and bromide) were not included in the charge balance, and we did not include Al$^{3+}$ because it only reaches high concentrations in acidic streams (Driscoll et al. 2003), and snowpack analyses indicate a meltwater pH of 6.14 (Ingersoll et al. 2005).

2.3.4 GIS Analyses

We imported GPS coordinates of the sampling locations into an ArcGIS 8.2 (ESRI, Redlands, California, USA) point coverage. We estimated the drainage area upstream of our sampling locations by digitizing polygon coverages using contour lines from USGS 1:24,000 digital raster graphics to determine drainage divides. We used 10-m digital elevation models freely available from USGS to calculate average elevation,
average slope, and following Clow and Sueker (2000), percent of each catchment as steep
(>30°) slope. We estimated surficial geology by reclassifying meso-scale lithology data
into four categories (Johnson and Raines 1995): crystalline (granite and gneiss),
carbonate (limestone and mixed carbonate), clastic (mudstone, shale, and sandstone), and
debris (landslide, glacial, and alluvial deposits). We used a Wyoming land cover dataset
(Wyoming GAP data are available at http://www.sdvc.uwyo.edu/wbn/gap.html) to
calculate the proportion of shrub, forest, meadow, tundra, and unvegetated area (i.e.,
exposed rock and soil) in each catchment. To characterize hydrologic features that might
potentially explain variability in stream N concentrations, we measured percent of each
basin occupied by wetlands and lakes using National Wetland Inventory coverages
(wetland data are available at http://wetlandsfws.er.usgs.gov) and USGS 1:24,000
hydrography digital line graphs, respectively. We could not find freely available spatial
data that provided soil depth, which has been identified as an important variable
controlling N flux from alpine catchments (Sickman et al. 2002). However, we did
analyze broad-scale soil coverages (Munn and Arneson 1998) and found that soil types
were highly correlated with the land cover data; therefore we did not include soils in our
analyses because they did not add explanatory power. In general, most soils in the
Tetons are thin inceptisols and entisols whereas surficial debris deposits are thicker.

2.3.5 Statistical Analyses

Because of the landscape heterogeneity in the Teton Range, the spatial variables
we calculated had many values of zero, which made transforming these important
variables for parametric statistics impossible (Zar 1999). Instead we used Spearman rank
correlation to identify significant associations among physical characteristics of the
catchments and among solute concentrations in the streams. We also used Spearman rank correlation to analyze how stream solute patterns were related to landscape variables among all Teton catchments, and within the Granite Canyon and Paintbrush Canyon datasets. We used \( t \)-tests to identify significant differences between key landscape variables in Granite Canyon and Paintbrush Canyon, and we used linear regression to identify how specific conductivity was related to \( \text{NO}_3^- \) concentration, how crystalline bedrock was related to stream-water \( \text{DIN:DON} \) ratio, and how stream-water \( \text{DOC:DON} \) ratio was related to \( \text{NO}_3^- \) concentration. All statistical analyses were performed using SYSTAT 11 (San Jose, California, USA).

2.4 Results

2.4.1 Landscape Factors

Physical characteristics varied substantially among Teton Range catchments (Table 2.1), reflecting the heterogeneity of mountain environments in general and the Teton Range in particular. For example, percent of basin as steep slope, forested and unvegetated land cover, and crystalline and debris surficial geology all varied from nearly 0-100%. Catchments with high proportions of crystalline surficial geology were located at higher elevations and had a high proportion of very steep slopes (Table 2.2). Consequently, crystalline catchments had low forest cover and a high proportion of unvegetated area relative to catchments dominated by other lithologies. In contrast, catchments with high proportions of carbonate surficial geology were large and had high meadow cover (Table 2.2). Only three catchments had extensive exposures of clastic sedimentary rock; these basins were also large but they had lower elevation and
TABLE 2.1.
PHYSICAL ATTRIBUTES (% OF BASIN) OF CATCHMENTS DRAINING THE TETONS (N=30)

<table>
<thead>
<tr>
<th>Physical attributes</th>
<th>Land cover characteristics</th>
<th>Surficial geology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elev.¹ (m)</td>
<td>Lake ¹</td>
<td>Wet.¹</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2635 (295)</td>
<td>25.8 (12.0)</td>
</tr>
<tr>
<td>Median</td>
<td>2700</td>
<td>24.7</td>
</tr>
<tr>
<td>Min-max</td>
<td>2051-3141</td>
<td>6.6-62.7</td>
</tr>
</tbody>
</table>

¹Elev.=elevation, Wet.=wetland, For.=forested, Mead.=meadow, Unveg.=unvegetated, Cry.=crystalline, Carb.=carbonate
²Steep slopes are >30°
TABLE 2.2.

SPEARMAN RANK CORRELATION COEFFICIENTS (Rₛ) FOR LANDSCAPE ATTRIBUTES (% OF BASIN) IN TETON RANGE CATCHMENTS (N=30).

<table>
<thead>
<tr>
<th>Physical attributes</th>
<th>Land cover characteristics</th>
<th>Surficial geology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basin area (ha)</td>
<td>Elev.¹</td>
<td>Slope ²</td>
</tr>
<tr>
<td>Elev.¹</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.13</td>
<td>0.92†</td>
</tr>
<tr>
<td>Steep</td>
<td>-0.16</td>
<td>0.91†</td>
</tr>
<tr>
<td>Lakes</td>
<td>0.41*</td>
<td>0.70†</td>
</tr>
<tr>
<td>Wet.¹</td>
<td>0.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Mead.¹</td>
<td>0.59†</td>
<td>-0.01</td>
</tr>
<tr>
<td>Unveg.¹</td>
<td>-0.07</td>
<td>0.76†</td>
</tr>
<tr>
<td>For.¹</td>
<td>0.32</td>
<td>-0.79†</td>
</tr>
<tr>
<td>Shrub</td>
<td>-0.30</td>
<td>-0.16</td>
</tr>
<tr>
<td>Tundra</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td>Cry.¹</td>
<td>-0.33</td>
<td>0.72†</td>
</tr>
<tr>
<td>Carb.¹</td>
<td>0.65†</td>
<td>0.09</td>
</tr>
<tr>
<td>Clast.¹</td>
<td>0.51†</td>
<td>-0.41*</td>
</tr>
<tr>
<td>Debris</td>
<td>-0.05</td>
<td>-0.59†</td>
</tr>
</tbody>
</table>

¹Elev.=elevation, Wet.=wetland, Mead.=meadow, Unveg.=unvegetated, For.=forested, Cry.=crystalline, Carb.=carbonate, Clast.=clastic
²Steep slopes are >30°
*significance at P<0.05; †significance at P<0.005
shallower slopes compared to the other basins. There was surficial debris in all basins and it positively correlated with forest cover.

2.4.2 Solute Concentrations

Among the study streams, NH$_4^+$ concentrations were uniformly low, averaging 4.5 µg N L$^{-1}$ across basins. In contrast, NO$_3^-$ concentrations were highly variable, ranging from below detection to 241 µg N L$^{-1}$ (Table 2.3). Total DIN concentrations ranged from 8-280 µg N L$^{-1}$, and NO$_3^-$ composed the majority (92%). Dissolved organic C averaged 0.8 mg C L$^{-1}$ and ranged from below detection to 3.8 mg C L$^{-1}$ whereas DON averaged 42 µg N L$^{-1}$ and ranged from 0 (i.e., TDN-DIN<0) to 193 µg N L$^{-1}$ (Table 2.3). Not surprisingly, DOC was positively correlated with DON (Table 2.4). Specific conductivity was strongly and positively correlated with most solutes we measured, including Ca$^{2+}$, Mg$^{2+}$, Na$^+$, ANC, SO$_4^{2-}$, and DOC (Table 2.4). In contrast, the most significant correlation was the negative relationship between specific conductivity and NO$_3^-$ ($r^2=0.32$, $P=0.001$), demonstrating that NO$_3^-$-rich streams were dilute with respect to other solutes whereas solute-rich streams were dilute with respect to NO$_3^-$ (Figure 2.2).
<table>
<thead>
<tr>
<th>Site</th>
<th>Basin area (ha)</th>
<th>Cry. lith. (^1) (%)</th>
<th>Carb. lith. (^1) (%)</th>
<th>Spec. cond. (^1) (µS cm(^{-1}))</th>
<th>NH(_4)^+ -N (µg L(^{-1}))</th>
<th>NO(_3)^- -N (µg L(^{-1}))</th>
<th>SRP (µg L(^{-1}))</th>
<th>DON (µg L(^{-1}))</th>
<th>DOC (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler Cr.</td>
<td>1543</td>
<td>0</td>
<td>0</td>
<td>322.0</td>
<td>1.4</td>
<td>29.8</td>
<td>38.0</td>
<td>48.7</td>
<td>1.47</td>
</tr>
<tr>
<td>Taylor Cr.</td>
<td>1613</td>
<td>0</td>
<td>0</td>
<td>264.0</td>
<td>6.6</td>
<td>1.0†</td>
<td>4.1</td>
<td>112.4</td>
<td>2.87</td>
</tr>
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<td>Mosquito Cr.</td>
<td>6319</td>
<td>1.9</td>
<td>0.4</td>
<td>279.8</td>
<td>43.6††</td>
<td>12.4</td>
<td>5.2</td>
<td>6.5</td>
<td>1.45</td>
</tr>
<tr>
<td>Trail Cr.</td>
<td>1571</td>
<td>7.7</td>
<td>37.8</td>
<td>326.4</td>
<td>0.5†</td>
<td>29.3</td>
<td>3.1</td>
<td>21.0</td>
<td>0.60</td>
</tr>
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<td>Phillips Canyon</td>
<td>2686</td>
<td>1.2</td>
<td>74.5</td>
<td>273.0</td>
<td>0.5†</td>
<td>55.4</td>
<td>6.3</td>
<td>46.1</td>
<td>0.35</td>
</tr>
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<td>Fish Cr. @ Teton Village</td>
<td>125</td>
<td>83.0</td>
<td>5.3</td>
<td>72.2</td>
<td>1.2</td>
<td>111.4</td>
<td>2.6</td>
<td>27.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Granite Canyon</td>
<td>3984</td>
<td>18.3</td>
<td>47.6</td>
<td>193.8</td>
<td>0.7</td>
<td>28.2</td>
<td>1.7</td>
<td>17.4</td>
<td>1.08</td>
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<td>Open Cr.</td>
<td>1368</td>
<td>39.5</td>
<td>17.1</td>
<td>117.3</td>
<td>1.3</td>
<td>45.6</td>
<td>1.8</td>
<td>0.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Stewart Draw</td>
<td>1101</td>
<td>36.3</td>
<td>0</td>
<td>30.3</td>
<td>1.5</td>
<td>73.6</td>
<td>2.1</td>
<td>12.8</td>
<td>0.50</td>
</tr>
<tr>
<td>Moose-Wilson Cr.</td>
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<td>21.2</td>
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<td>94.2</td>
<td>5.1</td>
<td>30.1</td>
<td>11.6</td>
<td>192.6</td>
<td>3.78</td>
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<td>Death Canyon</td>
<td>3401</td>
<td>43.8</td>
<td>34.8</td>
<td>104.6</td>
<td>0.5†</td>
<td>19.0</td>
<td>1.8</td>
<td>19.2</td>
<td>0.38</td>
</tr>
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<td>Beaver Cr.</td>
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<td>0</td>
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<td>1.0</td>
<td>87.0</td>
<td>1.8</td>
<td>10.4</td>
<td>0.47</td>
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<tr>
<td>Site</td>
<td>Basin area (ha)</td>
<td>Cry. lith. (%)</td>
<td>Carb. lith. (%)</td>
<td>Spec. cond. $^1$ (µS cm$^{-1}$)</td>
<td>NH$_4^+$-N (µg L$^{-1}$)</td>
<td>NO$_3^-$-N (µg L$^{-1}$)</td>
<td>SRP (µg L$^{-1}$)</td>
<td>DON (µg L$^{-1}$)</td>
<td>DOC (mg L$^{-1}$)</td>
</tr>
<tr>
<td>-------------------------------------</td>
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<td>----------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>--------------------------</td>
<td>------------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------------</td>
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<td>Tabbart Cr. in Avalanche Canyon</td>
<td>1412</td>
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<td>1.6</td>
<td>37.6</td>
<td>18.0</td>
<td>161.1</td>
<td>1.3</td>
<td>4.9</td>
<td>0.10</td>
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<td>751</td>
<td>98.3</td>
<td>0</td>
<td>11.3</td>
<td>38.5</td>
<td>241.1</td>
<td>3.5</td>
<td>0.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Glacier Gulch</td>
<td>498</td>
<td>86.0</td>
<td>0</td>
<td>9.2</td>
<td>0.5$^\dagger$</td>
<td>106.6</td>
<td>3.7</td>
<td>46.4</td>
<td>0.34</td>
</tr>
<tr>
<td>Broken Falls</td>
<td>182</td>
<td>74.8</td>
<td>0</td>
<td>18.4</td>
<td>0.5$^\dagger$</td>
<td>48.3</td>
<td>2.4</td>
<td>13.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Cascade Cr.</td>
<td>4255</td>
<td>73.4</td>
<td>1.6</td>
<td>33.0</td>
<td>0.5$^\dagger$</td>
<td>81.5</td>
<td>2.3</td>
<td>22.3</td>
<td>0.29</td>
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<tr>
<td>Moose Cr.</td>
<td>6450</td>
<td>36.8</td>
<td>28.0</td>
<td>115.4</td>
<td>1.3</td>
<td>63.3</td>
<td>2.6</td>
<td>53.0</td>
<td>0.47</td>
</tr>
<tr>
<td>Berry Creek</td>
<td>9532</td>
<td>28.2</td>
<td>40.9</td>
<td>187.1</td>
<td>0.5$^\dagger$</td>
<td>41.6</td>
<td>2.3</td>
<td>20.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Colter Canyon</td>
<td>924</td>
<td>50.3</td>
<td>4.6</td>
<td>64.2</td>
<td>6.2</td>
<td>109.3</td>
<td>2.4</td>
<td>21.8</td>
<td>0.22</td>
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<tr>
<td>S. Colter Canyon</td>
<td>432</td>
<td>69.9</td>
<td>0</td>
<td>34.4</td>
<td>0.5$^\dagger$</td>
<td>38.9</td>
<td>2.1</td>
<td>19.6</td>
<td>0.30</td>
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<tr>
<td>N. Waterfall Cr.</td>
<td>155</td>
<td>71.1</td>
<td>0</td>
<td>91.8</td>
<td>0.7</td>
<td>71.2</td>
<td>2.0</td>
<td>30.8</td>
<td>0.98</td>
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<tr>
<td>Waterfall Cr.</td>
<td>1147</td>
<td>55.1</td>
<td>0.8</td>
<td>47.5</td>
<td>0.5$^\dagger$</td>
<td>76.8</td>
<td>1.5</td>
<td>29.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Site</td>
<td>Basin area (ha)</td>
<td>Cry. lith.(^1) (%)</td>
<td>Carb. lith.(^1) (%)</td>
<td>Spec. cond.(^1) (µS cm(^{-1}))</td>
<td>NH(_4^+)-N (µg L(^{-1}))</td>
<td>NO(_3^−)-N (µg L(^{-1}))</td>
<td>SRP (µg L(^{-1}))</td>
<td>DON (µg L(^{-1}))</td>
<td>DOC (mg L(^{-1}))</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>-----------------------------------</td>
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<td>--------------------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>N. Moran Bay 1</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>83.4</td>
<td>2.8</td>
<td>104.4</td>
<td>2.9</td>
<td>312.9</td>
<td>3.68</td>
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<tr>
<td>N. Moran Bay 2</td>
<td>71</td>
<td>25.2</td>
<td>0</td>
<td>92.3</td>
<td>0.5†</td>
<td>66.8</td>
<td>2.7</td>
<td>76.8</td>
<td>2.18</td>
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<tr>
<td>N. Moran Cr. in Snowshoe Canyon</td>
<td>2662</td>
<td>60.9</td>
<td>0.7</td>
<td>24.9</td>
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<td>71.1</td>
<td>1.7</td>
<td>8.9</td>
<td>0.33</td>
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<tr>
<td>Moran Creek</td>
<td>4397</td>
<td>69.5</td>
<td>1.5</td>
<td>27.2</td>
<td>2.1</td>
<td>75.7</td>
<td>1.8</td>
<td>22.3</td>
<td>0.41</td>
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<td>Falling Ice Glacier</td>
<td>176</td>
<td>94.6</td>
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<td>11.3</td>
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<td>2.1</td>
<td>0.0</td>
<td>0.00</td>
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<td>Leigh Canyon</td>
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<td>22.4</td>
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<td>1.8</td>
<td>11.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Paintbrush Canyon</td>
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<td>59.0</td>
<td>0</td>
<td>32.1</td>
<td>0.5†</td>
<td>179.6</td>
<td>2.0</td>
<td>31.3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\(^1\)Cry. lith.=crystalline lithology, Carb. lith.=carbonate lithology, Spec. cond.=specific conductivity

†detection limit

††unusually high value may indicate sample contamination
### TABLE 2.4.

SPEARMAN RANK CORRELATION COEFFICIENTS ($R_S$) FOR SOLUTES IN STREAMS DRAINING TETON RANGE CATCHMENTS ($N=30$).

<table>
<thead>
<tr>
<th></th>
<th>SpCond$^1$</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>Na$^+$</th>
<th>K$^+$</th>
<th>NH$_4^+$</th>
<th>SRP</th>
<th>ANC</th>
<th>NO$_3^-$</th>
<th>Cl$^-$</th>
<th>SO$_4^{2-}$</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>0.95†</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.96†</td>
<td>0.91†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na$^+$</td>
<td>0.45*</td>
<td>0.53†</td>
<td>0.42*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K$^+$</td>
<td>0.03</td>
<td>0.09</td>
<td>0.15</td>
<td>0.38*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.19</td>
<td>0.18</td>
<td>0.17</td>
<td>0.49*</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SRP</td>
<td>0.33</td>
<td>0.36*</td>
<td>0.25</td>
<td>0.44*</td>
<td>-0.07</td>
<td>0.26</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ANC</td>
<td>0.97†</td>
<td>0.98†</td>
<td>0.96†</td>
<td>0.54†</td>
<td>0.17</td>
<td>0.20</td>
<td>0.32</td>
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</tr>
<tr>
<td>NO$_3^-$</td>
<td>-0.63†</td>
<td>-0.55†</td>
<td>-0.55†</td>
<td>-0.15</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.24</td>
<td>-0.56†</td>
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</tr>
<tr>
<td>Cl$^-$</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.72†</td>
<td>0.32</td>
<td>0.52†</td>
<td>0.26</td>
<td>0.33</td>
<td>-0.01</td>
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<tr>
<td>SO$_4^{2-}$</td>
<td>0.50†</td>
<td>0.45*</td>
<td>0.42*</td>
<td>0.18</td>
<td>0.03</td>
<td>0.19</td>
<td>-0.00</td>
<td>0.43*</td>
<td>-0.30</td>
<td>0.32</td>
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</tr>
<tr>
<td>DOC</td>
<td>0.65†</td>
<td>0.72†</td>
<td>0.59†</td>
<td>0.60†</td>
<td>0.06</td>
<td>0.26</td>
<td>0.47*</td>
<td>0.67†</td>
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<td>0.32</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>DON</td>
<td>0.28</td>
<td>0.39*</td>
<td>0.27</td>
<td>0.21</td>
<td>0.08</td>
<td>-0.06</td>
<td>0.49*</td>
<td>0.33</td>
<td>-0.05</td>
<td>0.02</td>
<td>0.25</td>
<td>0.54†</td>
</tr>
</tbody>
</table>

$^1$Specific conductivity

*significance at $P<0.05$

†significance at $P<0.005$
Figure 2.2. Relationship between nitrate concentrations and specific conductivity among Teton streams.

2.4.3 Relationship between Solute Concentrations and Topography, Land Cover, and Lithology

We correlated landscape characteristics with stream-water solutes to understand what landscape factors affected the relationship between NO$_3^-$ and specific conductivity. Streams draining crystalline catchments had generally dilute stream water, indicated by the negative relationship with specific conductivity and most other solutes, but they had high NO$_3^-$ concentration (Table 2.5). These stream-water characteristics reflect the physical attributes of crystalline basins (i.e., steep slopes, high elevations, and less vegetation; Table 2.2) that collectively reduce water residence time, decreasing the capacity for weathering to introduce solutes to meltwater and increase specific conductivity and decreasing the ability of terrestrial N retention to reduce NO$_3^-$ concentration. In contrast, streams draining clastic sedimentary catchments generally
TABLE 2.5.
SPEARMAN RANK CORRELATION COEFFICIENTS ($R_S$) BETWEEN SOLUTES AND LANDSCAPE ATTRIBUTES (% OF BASIN) IN TETON RANGE CATCHMENTS (N=30).

<table>
<thead>
<tr>
<th>Land cover characteristics</th>
<th>Physical attributes</th>
<th>Surficial geology</th>
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</thead>
<tbody>
<tr>
<td>lakes</td>
<td>Basin area (ha)</td>
<td>Cr.¹</td>
</tr>
<tr>
<td></td>
<td>Elev.¹ (m)</td>
<td>Carb.¹</td>
</tr>
<tr>
<td></td>
<td>Slope (°)</td>
<td>Clast.¹</td>
</tr>
<tr>
<td></td>
<td>Steep slope² (°)</td>
<td>Debris</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpCond¹</td>
<td>0.37*</td>
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</tr>
<tr>
<td></td>
<td>-0.70†</td>
<td>0.43*</td>
</tr>
<tr>
<td></td>
<td>-0.77†</td>
<td>0.61†</td>
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<tr>
<td></td>
<td>-0.79</td>
<td>0.43*</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.33</td>
<td>-0.77†</td>
</tr>
<tr>
<td></td>
<td>-0.72†</td>
<td>0.62†</td>
</tr>
<tr>
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<td>-0.77†</td>
<td>0.40*</td>
</tr>
<tr>
<td></td>
<td>-0.77†</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.34</td>
<td>-0.79†</td>
</tr>
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<td>-0.65†</td>
<td>0.44*</td>
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<td></td>
<td>-0.76†</td>
<td>0.52†</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Na⁺</td>
<td>-0.16</td>
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<td>-0.57†</td>
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<td>0.38*</td>
</tr>
<tr>
<td>NH₄⁺</td>
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<td>-0.24</td>
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<tr>
<td></td>
<td>-0.30</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>-0.34</td>
<td></td>
</tr>
<tr>
<td>SRP</td>
<td>0.09</td>
<td>-0.36*</td>
</tr>
<tr>
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<td>-0.22</td>
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<td>0.53†</td>
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<tr>
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<tr>
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<tr>
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<td>-0.39*</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>-0.86†</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>-0.44*</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>-0.47*</td>
<td>0.34</td>
</tr>
</tbody>
</table>

¹Elev.=elevation, Wet=wetland, Mead=meadow, Unveg=unvegetated, For=forested, Cry.=crystalline, Carb.=carbonate, Clast.=clastic, SpCond=specific conductivity

²Steep slopes are $>$30°

*significance at $P<0.05$, †significance at $P<0.005$
showed the opposite pattern in stream-water solutes, and not surprisingly, these catchments had physical attributes that were opposite those of crystalline catchments and that encouraged N retention through greater water residence time (i.e., lower elevations, shallower slopes, and greater forest cover; Table 2.2). Few catchments had significant amounts of clastic lithology, limiting its relevance in adding insight regarding the relationship between NO$_3^-$ and specific conductivity among Teton streams. Carbonate lithology and surficial debris composed more of the area studied in the Tetons than clastic geology, and although they were both positively correlated with specific conductivity, neither was correlated with NO$_3^-$ (Table 2.5). In contrast, forested land cover, which was positively related to DOC concentrations, was associated with higher specific conductivity and lower NO$_3^-$, suggesting that it was an important factor driving solute patterns. Therefore, the pattern between specific conductivity and NO$_3^-$ among Teton streams was probably driven by forests (high specific conductivity and low NO$_3^-$), which were negatively associated with crystalline lithology (high NO$_3^-$ and low specific conductivity).

2.4.4 Within-basin Solute Patterns

We compared within-basin solute patterns in two sub-basins with contrasting lithology to identify which landscape attributes contributed to the observed difference in NO$_3^-$ concentrations at the mouths of the sub-basins. Despite similarities in topography, NO$_3^-$ concentration was more than 6 times higher at the mouth of crystalline Paintbrush Canyon (177 µg N L$^{-1}$) relative to carbonate Granite Canyon (28 µg N L$^{-1}$) (Table 2.3). Several relationships in Granite Canyon agreed with the across-basin results; for example, NO$_3^-$ positively correlated with elevation and unvegetated area, and ANC
### TABLE 2.6.

**SPEARMAN RANK CORRELATION COEFFICIENTS (Rₛ) BETWEEN SOLUTES AND LANDSCAPE ATTRIBUTES (% OF BASIN) IN GRANITE CANYON SUB-CATCHMENTS (N=13).**

<table>
<thead>
<tr>
<th></th>
<th>Physical attributes</th>
<th>Land cover characteristics</th>
<th>Surficial geology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basin area (ha)</td>
<td>Elev.¹ (m)</td>
<td>Slope (°)</td>
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<tr>
<td>Ca²⁺</td>
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<tr>
<td>Mg²⁺</td>
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<td>-0.09</td>
<td>0.71*</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.01</td>
<td>0.52</td>
<td>0.04</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.09</td>
<td>0.07</td>
<td>-0.04</td>
</tr>
<tr>
<td>NH₄⁺</td>
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<td>0.48</td>
<td>0.24</td>
</tr>
<tr>
<td>SRP</td>
<td>-0.42</td>
<td>0.35</td>
<td>-0.26</td>
</tr>
<tr>
<td>ANC</td>
<td>0.12</td>
<td>0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>-0.38</td>
<td>0.65*</td>
<td>-0.27</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>-0.09</td>
<td>0.49</td>
<td>-0.30</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>-0.25</td>
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<td>-0.55</td>
</tr>
<tr>
<td>DOC</td>
<td>0.54</td>
<td>-0.42</td>
<td>0.45</td>
</tr>
<tr>
<td>DON</td>
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<td>0.01</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

Note: Shrub and tundra not represented in Granite Canyon

¹Elev.=elevation, Wet.=wetland, Mead.=meadow, Unveg.=unvegetated, For.=forested, Cry.=crystalline, Carb.=carbonate

²Steep slopes are >30°

*significance at P<0.05, †significance at P<0.005.
**TABLE 2.7.**

**SPEARMAN RANK CORRELATION COEFFICIENTS (R_s) BETWEEN SOLUTES AND LANDSCAPE ATTRIBUTES (% OF BASIN) IN PAINTBRUSH CANYON SUB-CATCHMENTS (N=8).**

<table>
<thead>
<tr>
<th></th>
<th>Physical attributes</th>
<th>Land cover characteristics</th>
<th>Surficial geology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basin area (ha)</td>
<td>Elev. (^1)</td>
<td>Slope (°)</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
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<td>-0.50</td>
<td>-0.02</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.05</td>
<td>-0.40</td>
<td>0.17</td>
</tr>
<tr>
<td>Na(^+)</td>
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<td>-0.72</td>
</tr>
<tr>
<td>K(^+)</td>
<td>-0.02</td>
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<td>0.00</td>
</tr>
<tr>
<td>NH(_4)(^+)</td>
<td>-0.17</td>
<td>-0.54</td>
<td>-0.02</td>
</tr>
<tr>
<td>SRP</td>
<td>-0.48</td>
<td>-0.02</td>
<td>-0.30</td>
</tr>
<tr>
<td>ANC</td>
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<td>-0.62</td>
<td>-0.14</td>
</tr>
<tr>
<td>NO(_3)(^-)</td>
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<td>-0.33</td>
<td>0.10</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>0.24</td>
<td>-0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>SO(_4)(^2-)</td>
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<td>-0.19</td>
<td>0.10</td>
</tr>
<tr>
<td>DOC</td>
<td>-0.60</td>
<td>-0.48</td>
<td>-0.39</td>
</tr>
<tr>
<td>DON</td>
<td>-0.10</td>
<td>0.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Note: Shrub, tundra, meadow, and carbonate not represented in Paintbrush Canyon.

\(^1\)Elev.=elevation, Wet.=wetland, Mead.=meadow, Unveg.=unvegetated, For.=forested, Cry.=crystalline, Carb.=carbonate

\(^2\)Steep slopes are >30°

*significance at P<0.05, †significance at P<0.005.
showed a positive relationship with forest cover and debris (Table 2.6). Other relationships differed from the across-basin results; for example, NO$_3^-$ and NH$_4^+$ were negatively correlated with meadow cover and carbonate surficial geology, and NO$_3^-$ was positively correlated with debris. In Paintbrush Canyon, there were few significant relationships between solute concentrations and landscape attributes, and none between land cover attributes and any N species (Table 2.7). The lack of significant relationships was probably due to the combination of relatively small sample size in Paintbrush Canyon (n=8) compared to Granite Canyon (n=13) and the landscape homogeneity in Paintbrush Canyon, which was dominated by crystalline lithology and unvegetated slopes. Proportion of unvegetated land, which was >2-fold higher in Paintbrush Canyon compared to Granite Canyon ($P=0.006$), was the only land cover characteristic that differed between basins (Figure 2.3).

![Figure 2.3](image_url)

Figure 2.3. Differences between key landscape characteristics in Granite Canyon and Paintbrush Canyon intra-basin samples. The line within the box denotes the median, the box indicates the 25th and 75th percentiles, and the error bars represent the 10th and 90th percentiles. Paintbrush Canyon had significantly more unvegetated area than Granite Canyon ($t$-test, $P=0.006$).
2.4.5 Indexes of Sensitivity to N Saturation

Stream-water DOC:DON ratios were negatively correlated to NO$_3^-$ concentration ($r^2=0.32$, $P=0.002$), and 16 out of 30 streams had DOC:DON<25 (Figure 2.4), a threshold indicating sensitivity to N deposition (Harriman et al., 1998; Gundersen et al., 1998). Although many researchers express DOC:DON as molar ratio (e.g., Hood et al., 2003a), this threshold was identified as a mass ratio (Harriman et al., 1998; Gundersen et al., 1998). Most streams that fell below this threshold had >30% of the catchment as crystalline lithology, suggesting that sensitivity to N deposition increased with crystalline lithology. We regressed crystalline lithology against stream-water DIN:DON, an alternate metric of sensitivity to N deposition that has been used in alpine ecosystems (Hood et al., 2003a). DIN:DON increased with amount of basin as crystalline lithology

![Figure 2.4](image)

Figure 2.4. Relationship between nitrate concentration and stream-water DOC:DON ratio (by mass). Shaded area represents a threshold where basins with lower DOC:DON are at higher risk for N saturation and basins with higher DOC:DON are at lower risk (Harriman et al. 1998)
Figure 2.5. Relationship between stream-water DIN:DON ratio and amount of basin as crystalline lithology. Higher DIN:DON indicates sensitivity to N saturation (Hood et al. 2003a).

\[ r^2=0.40 \]
\[ P<0.005 \]

The positive relationship between crystalline lithology and forest cover suggests that vegetation may be an important characteristic driving the sensitivity to N deposition.

2.5 Discussion

2.5.1 Landscape Factors and N Concentrations

Despite the relatively small spatial extent of the Teton Range (N-S axis is 65 km), streams had NO\textsubscript{3} concentrations that varied >100-fold (Table 2.3), and many landscape attributes correlated significantly with NO\textsubscript{3} concentration (Table 2.5). In the Teton Range, streams with high NO\textsubscript{3} concentration were associated with basins having high proportions of crystalline lithology, which was characterized by high elevation basins.
with steep, unvegetated slopes (Table 2.2). In contrast, streams with high proportions of clastic lithology, which was characterized by shallower slopes, lower elevations, and more forest cover, had low NO$_3^-$ concentrations. In alpine settings, microbial activity retains atmospherically-deposited N during early snowmelt (Brooks et al. 1996), but high nitrification rates during later snowmelt promote N export (Sickman et al. 2003). Also, N export is frequently related to catchment-scale characteristics such as steep, unvegetated slopes and thin soils that collectively reduce water residence time and decrease the potential for N immobilization (Baron et al. 1994; Clow and Sueker 2000; Sickman et al. 2002). The shallower slopes and greater forest cover of clastic basins probably contributed to lower stream concentrations of NO$_3^-$ by increasing water residence time and promoting biological N retention. The strong correlation among these landscape variables makes it difficult to determine which factor or factors played direct versus indirect roles in causing the observed pattern in NO$_3^-$ concentrations. However, the negative relationship between crystalline lithology and forested land cover and the importance of vegetation in controlling stream-water solutes in other forested landscapes (Vitousek 1977) and alpine environments (Baron et al. 1994) suggests that vegetation probably plays a direct role.

Surficial debris deposits did not correlate with stream-water NO$_3^-$ concentration across Teton catchments, but they did positively correlate with NO$_3^-$ concentration within the Granite Canyon samples. These differences in across- and within-basin samples likely reflect the differing role that distinct debris types play in the alpine N cycle. For example, glacial deposits have thicker soils that support forests which retain N (Baron et al. 1994; Campbell et al. 2000b). Among catchments, debris was positively correlated
with forest cover which, in turn, was negatively correlated with NO$_3^-$ concentration (Table 2.5), again suggesting that forest cover was an important determinant of NO$_3^-$ concentration among basins. In contrast, rockfall debris (i.e., talus) contributes NO$_3^-$ to aquatic systems because of high terrestrial nitrification rates (Campbell et al. 2002). Debris was positively correlated with NO$_3^-$ in Granite Canyon, but the spatial data we used did not allow us to distinguish between landslide and talus slope deposits, so we collectively defined them as surficial debris along with glacial and alluvial deposits. Therefore, the inconsistent relationships we found between debris and solute concentrations at different spatial scales probably reflect our aggregation of functionally distinct classes of debris, which masked their individual roles in N dynamics.

We suggested that faster weathering of calcareous bedrock would promote soil development to the benefit of vegetation, consequently increasing N retention, but we did not observe a strong relationship between specific conductivity and carbonate lithology (Table 2.5). As a direct measure of stream-water ions, specific conductivity provides an integrated metric of weathering products in a catchment hydro-system. Given the strong precedent for using stream-water solutes to infer catchment processes (e.g., Likens and Bormann 1995), the lack of an observed relationship between specific conductivity and carbonate lithology may be more a consequence of inferring weathering patterns from a map that only documents the abundance of different lithologies. The negative relationship between NO$_3^-$ concentrations and specific conductivity (Figure 2.2) supports our contention that weathering is related to N retention.

Carbonate bedrock did not correlate with NO$_3^-$ concentrations among basins but was negatively related to NO$_3^-$ concentrations in Granite Canyon (Table 2.6). Chemical
weathering of carbonate bedrock produces Ca\(^{2+}\) and Mg\(^{2+}\) ions, which in turn regulate soil fertility through cation exchange, so carbonate parent material may indirectly increase N retention by promoting more fertile soils than those derived from crystalline parent material. Additionally, rapid weathering of carbonate rock compared to crystalline rock (Hembree and Rainwater 1961; Hodson et al. 2000) would quickly break down parent material, encouraging development of thicker soils, which decrease N export from alpine environments (Sickman et al. 2002). Meadows, which grow on thick soils (Campbell et al. 2000b) and in areas with high water residence time (Fisk et al. 1998), are important sites of N retention in alpine environments; we found that meadows were negatively correlated with NO\(_3^-\) and NH\(_4^+\) (Table 2.6) in Granite Canyon but were not represented in Paintbrush Canyon (Table 2.7). Despite similarities in slope and elevation in Granite and Paintbrush Canyons, the largely carbonate Granite Canyon had >2 times the vegetative cover of the largely crystalline Paintbrush Canyon (Figure 2.3), indicating that vegetation is more important than slope or elevation for regulating N retention in alpine basins, and suggesting that carbonate lithology may indirectly regulate N retention by promoting vegetative cover. The lack of a relationship between carbonate lithology and NO\(_3^-\) concentrations across Teton catchments suggests that the indirect effect of carbonate lithology on N retention seen in within-basin samples is confounded by other landscape variables in among-basin samples.

2.5.2 Landscape Factors and Dissolved Organic Matter

Concentrations of DOC (range: below detection to 3.8 mg C L\(^{-1}\); Table 2.3) were similar to that reported in other alpine environments (e.g., Baron et al. 1991; Hood et al. 2003b), but over half these measurements were lower than snowpack DOC (0.7 mg C L\(^{-1}\);
Ingersoll et al. 2005) suggesting that many catchments retained atmospherically deposited C sometime between snowmelt and stream export. Although soils are commonly a source of DOC to aquatic ecosystems (Kaplan and Newbold 1993), dissolved organic matter can sorb to mineral surfaces in soils, making them a carbon sink (Kaiser et al. 1996). Proportion of catchment as crystalline lithology was negatively correlated with DOC (Table 4), suggesting that abundant mineral surfaces in these catchments with a high proportion of exposed rock and soil may have sorbed snowmelt DOC. Alternatively, the lack of vegetation in crystalline catchments could suggest microbial C limitation in alpine soils, especially given the relatively high snowpack N (~0.2 mg N L^{-1}; Ingersoll et al. 2005) compared to snowpack C. An alternative hypothesis is that DOC in snowmelt, which is probably very labile compared to humic terrestrial DOC, could fuel heterotrophic metabolism, resulting in N mineralization followed by nitrification, thereby contributing to the high NO₃⁻ concentrations associated with catchments having high proportions of crystalline bedrock, but this remains untested.

Alpine lakes can be important sources of DOC (Baron et al. 1991; Hood et al. 2003b), and they can retain DIN (Campbell et al. 1997; Baron and Campbell 2001), exporting it to alpine streams as DON (Wurtsbaugh et al. 2005). However, percent of basin as lakes was negatively correlated with DOC, and there was no significant relationship between DON and lakes (Table 2.5). In contrast, and as expected, DOC was positively correlated with DON (Table 2.4) across basins, suggesting a common source, and DOC was positively correlated with catchment forest cover (Table 2.5), suggesting a terrestrial origin for these organic solutes. Studies that have revealed the importance of
lakes to DOC in alpine streams have generally documented changes in DOC quality rather than quantity (e.g., Hood et al. 2003b) so simply measuring DOC concentration would not reveal the extent to which alpine lakes control DOC in the Teton streams. Our synoptic sampling design, though not ideal for addressing the role of lakes in controlling downstream DOC quality, does suggest that DOC concentration is more strongly correlated with terrestrial sources among Teton catchments. Furthermore, the lack of a relationship between lakes and DOC may be related to lakes not being a prevalent landscape feature in the Tetons (< 0.5% of the total basin area we surveyed).

2.5.3 Ecosystem N Status

Our synoptic sampling design, and the lack of direct measurements of N deposition, preclude us from determining whether Teton catchments are simply unretentive due to their alpine situation or if they are undergoing N saturation from increased atmospheric N deposition. The Tetons likely receive more N deposition than suggested by NADP monitoring sites given their higher elevations (Weathers et al. 2006), evergreen forest cover (Weathers et al. 2000), and the presence of downwind agricultural N sources (Fenn et al. 2003a). Therefore, an N deposition rate higher than background is probable, and the >4.5 kg N ha\(^{-1}\) y\(^{-1}\) deposition predicted by an atmospheric haze model (Fenn et al. 2003a) suggests that N deposition in the Tetons may approach that of the Front Range of Colorado (~5 kg N ha\(^{-1}\) y\(^{-1}\)), a similar environment that is currently undergoing N saturation (Baron et al. 1994; Nydick et al. 2004; Bowman et al. 2006).

Other evidence suggests that early N saturation may be possible. For example, comparing stream-water DOC:DON to NO\(_3^-\) concentrations showed that catchments with higher proportions of crystalline bedrock had lower DOC:DON and exported more NO\(_3^-\)
(i.e., greater NO$_3^-$ leaching; Figure 2.4), a relationship consistent with early stages of N saturation (Aber et al. 1998). Furthermore, DIN:DON, which signals sensitivity to N deposition in alpine environments (Hood et al. 2003a), increased with amount of crystalline lithology in the basin suggesting a relationship between crystalline lithology and sensitivity to N saturation (Figure 2.5). Although these relationships each explain less than half the variability among the data, probably because stream primary production may also regulate NO$_3^-$ concentration in streams (Hall and Tank 2003), they do suggest that sensitivity to N deposition varies with lithology in the Tetons. Crystalline basins have steep slopes, high elevations, and a lack of vegetation, and all these factors contribute to the innate N leakiness of alpine ecosystems. However, alpine catchments typically leak most N during the rising limb of the snowmelt hydrograph (Williams et al. 1995), and we sampled when the hydrograph was near base flow during the alpine growing season when biological demand should result in higher N retention. Therefore, high DIN:DON associated with catchments having more crystalline bedrock may indicate N in excess of demand during the growing season and could signal early stages of N saturation from atmospherically deposited N.

Watershed-ecosystems vary widely with respect to how much N may be retained based on factors such as ecosystem development (Vitousek 1977), length of growing season (Williams et al. 1996), and N deposition rate (Fenn et al. 1998). A suite of correlated landscape characteristics have been associated with low N retention in alpine environments including steep, unvegetated slopes and high elevations that have shorter growing seasons. However, our data suggest that vegetation is the most important determinant of N concentration among the high elevation Teton catchments, especially
during base flow conditions. Furthermore, carbonate lithology may play an indirect role in mediating vegetation growth in the alpine zone by promoting favorable soil conditions despite steep slopes and high elevations. However, we did not observe an effect of carbonate lithology among Teton catchments, probably because of the confounding effect of other landscape features. Although further research is needed to budget N inputs and outputs on an annual scale, the negative relationship between DOC:DON and NO$_3^-$ concentration, the high DIN:DON in streams draining crystalline catchments, and the likelihood of higher N deposition rates than indicated by NADP monitoring sites collectively suggest that the Tetons may be in early stages of N saturation. This finding is particularly important given that the Tetons lie within Grand Teton National Park, a federal Class 1 area that is legislatively mandated by the United States Congress to receive protection from the negative effects of air pollution (Williams and Tonnessen, 2000).

2.6 Acknowledgements

We thank Rachel Clavers, Natalie Griffiths, Lisa Kunza, Laura Taylor, and Elizabeth Wilson for assistance in the field. Dennis Birdsell and Jon Loftus helped with analytical equipment at Notre Dame’s Center for Environmental Science and Technology, and Steve Hamilton and David Weed helped with cation analysis at Kellogg Biological Station. Hank Harlow provided logistical support at the University of Wyoming/National Park Service Research Station. CPA received funding from the Arthur J. Schmitt Presidential Fellowship and the Bayer Predoctoral Fellowship while conducting this research. Additional funding was provided by NSF-DEB 0111410.
CHAPTER 3
LAND USE INFLUENCES THE SPATIO-TEMPORAL CONTROLS ON NITRIFICATION AND DENITRIFICATION IN HEADWATER STREAMS

3.1 Abstract

The nitrogen (N) and carbon (C) cycles in headwater streams are coupled, and land use potentially modifies these cycles by increasing N availability and removing riparian vegetation. Anthropogenically-modified streams dominate the current landscape, yet our understanding of controls on N cycling is based on a predominance of research based on forested systems. For three years, we quantified rates of two dissimilatory N transformations in a total of 18 agricultural and urban streams (with and without riparian buffers) to examine how riparian vegetation and land use influences sediment nitrification and denitrification. Nitrification rates were highest in agricultural streams in late spring and early summer, and nitrification was positively related to sediment C content and stream temperature, but negatively related to sediment C:N ratio (multiple linear regression, $R^2=0.26$, $P<<0.001$). Unexpectedly, nitrification was not related to water-column ammonium concentrations, but decomposition in the benthos may have provided more ammonium (via mineralization) to increase sediment nitrification. Denitrification rates did not differ among land-use types but were positively related to

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2 This chapter (with co-author Jennifer L. Tank) is in review for publication.
water-column nitrate, dissolved organic carbon concentrations, and sediment organic matter content, and negatively related to stream temperature (multiple linear regression, $R^2=0.47$, $P<<0.001$). Nitrate, the primary predictor of denitrification rates, was highest in agricultural streams, especially in winter, indicating that land use and seasonality were more important determinants of denitrification than coupled nitrification. Substrate availability (N and C) for N transformations did not differ between streams with buffered versus unbuffered riparian zones within a similar land use, likely due to the confounding influence of tile drainage, which effectively decoupled stream channels from their riparian zones. In this study, land use influenced the delivery of the necessary substrates for N transformations, but decreased the role of riparian zones on stream N cycling by simplifying the drainage network of headwater streams.

3.2 Introduction

Nitrogen (N) frequently limits productivity in terrestrial and aquatic ecosystems, and humans have effectively doubled N availability by fixing N on an industrial scale (Vitousek et al. 1997b). Over half of the anthropogenically-derived N is applied as nitrogenous fertilizers in agricultural or urban settings, where application frequently exceeds demand (Vitousek et al. 1997b) and excess N is exported from the landscape (Carpenter et al. 1998). Consequently, streams that drain agricultural and urban catchments often have elevated dissolved inorganic nitrogen (DIN) concentrations that modify internal N cycling and transformation rates relative to streams draining less-modified landscapes (Bernot and Dodds 2005). Biotic processing in headwater streams can control N flux to downstream ecosystems (Peterson et al. 2001), but much of our understanding of stream N cycling comes from previous studies in relatively pristine
systems, which are now somewhat rare in the landscape; most streams in the continental United States are heavily modified through anthropogenic changes in land cover (Meyer and Turner 1994). In order to improve our understanding of how land use alters N cycling in headwater streams, we quantified rates and controls on nitrification and denitrification in basins that are heavily modified by agricultural and urban land use.

Anthropogenic activities can increase both ammonium (NH$_4^+$) and nitrate (NO$_3^-$) concentrations in stream water, but the majority of the increased N load occurs as NO$_3^-$ (Peierls et al. 1991). For this reason, we quantified nitrification and denitrification rates, which can directly influence NO$_3^-$ concentrations, albeit in opposing directions. Nitrification obtains energy for the fixation of carbon dioxide by oxidizing NH$_4^+$ to NO$_3^-$, potentially increasing NO$_3^-$ concentrations, whereas denitrification releases energy by using NO$_3^-$ to oxidize organic carbon, potentially reducing stream NO$_3^-$ concentrations by producing nitrogenous gases (nitrous oxide, N$_2$O, and di-nitrogen gas, N$_2$). Because nitrification requires NH$_4^+$ as a substrate, which can be supplied from the water column or mineralized in the sediments via organic matter decomposition, and denitrification requires organic carbon (C) as an energy source, these two dissimilatory N transformations are linked through the stream C cycle (Bernhardt and Likens 2002). Therefore, agricultural or urban land-use practices can directly influence the N cycle by increasing NH$_4^+$ or NO$_3^-$ availability or indirectly influence the N cycle by altering the C cycle.

Riparian vegetation influences stream C cycling because leaf inputs provide an important energy source in many forested stream ecosystems, but agricultural and urban land use typically removes vegetation from stream banks, directly reducing allochthonous
C inputs (Golladay et al. 1989) while simultaneously increasing the importance of autochthonous C in the stream food web (Quinn et al. 1997). Removing riparian vegetation also reduces the periodic input of large wood to the stream, indirectly changing C cycling by decreasing organic matter retention (Bilby 1981). Because intact riparian vegetation can moderate daily temperature swings (Abell and Allen 2002) and improve water quality by retaining N moving from uplands to the stream (Peterjohn and Correll 1984), riparian buffer zones are often used to mitigate stream degradation associated with land-use activity (Naiman and Decamps 1997). Although riparian vegetation has the potential to reduce water-column N and alter organic matter dynamics, few studies have addressed how riparian buffers influence sediment nitrification and denitrification in streams. Therefore, we included the presence or absence of riparian vegetation when we classified basin land use in our study in order to examine riparian influence on nitrification and denitrification rates.

Riparian vegetation in temperate zones changes seasonally, thereby influencing the N and C cycles of forested streams. Although forested streams are typically considered detritally-based ecosystems, extended periods of autotrophy dominate stream metabolism before leaf-out of riparian vegetation (Mulholland et al. 2006). Prior to leaf-out, assimilatory demand for N by rapidly growing algae can dominate N uptake in streams and reduce N availability for heterotrophic microbes (Mulholland 1992). Alternatively, assimilatory demand by fungal and bacterial biofilms can dominate N uptake as they colonize leaves after abscission in the fall (Tank and Webster 1998). Additionally, in temperate streams, discharge typically varies temporally in response to seasonally shifting precipitation patterns with important consequences for N cycling. For
example, nutrient concentrations are typically controlled by hydrology in agricultural basins, especially those with subsurface tile drains that shorten terrestrial water residence time (Petry et al. 2002; Royer et al. 2004). Ecological studies performed only during the summer overlook these temporal dynamics that can have important implications for N cycling.

This study used a year-round sampling regime to investigate how nitrification and denitrification rates (hereafter collectively referred to as N transformation rates) vary among streams with specific land-use configurations, defined here as the spatial arrangement of land use with respect to the stream. We measured N transformation rates in forested streams and compared them to N transformation rates in agricultural, buffered-agricultural, urban, and buffered-urban streams to determine how N transformations varied among land-use types and in response to riparian buffers. We studied 18 streams from 6 different land-use categories, and the land-use classifications represented the range of land-use configurations found in our study area. Each year for 3 years, we selected one stream from each land-use category and sampled sediments and water monthly for 12 months. We measured nitrification and denitrification rates using laboratory assays, and we measured sediment and water parameters that were likely to explain variation in N transformation rates. We hypothesized that lower DIN concentrations in forested streams would cause lower N transformation rates compared to agricultural and urban streams, and that riparian buffers on agricultural and urban streams would lower DIN leading to N transformation rates more similar to forested streams than agricultural or urban streams, respectively. Furthermore, we hypothesized that high
assimilatory demand by algae in the spring and by decomposers in the fall would decrease dissimilatory N transformation rates by reducing overall DIN availability.

3.3 Methods

3.3.1 Land-use Classification

All 18 study streams were located within the Kalamazoo River basin of southwest Michigan (Figure 3.1), and they drained sub-basins that ranged in size from 127-3639 ha (Table 3.1). Historic native land cover in this region included mixed deciduous forest, oak woodlands, and wetlands, but currently it is a mix of row-crop agriculture and remnant forest patches. As we developed a categorical land-use classification, we

Figure 3.1. Sampling locations in the Kalamazoo River basin, southwest Michigan.
considered overall basin land use in addition to land use in the 100-m buffer immediately adjacent to the stream channel and extending upstream to the stream source (hereafter referred to as the 100-m buffer) to define land-use types as agricultural, urban, forested, buffered-agricultural, buffered-urban, or distal-agricultural. Agricultural and forested streams drained basins that were dominated by the respective land-use type (generally >70% of basin). Streams were considered urban at relatively low basin coverage (sites ranged from 15%-49% of basin as urban) because even small amounts of urban cover can have a disproportionate influence on a stream (Paul and Meyer 2001). Buffered-agricultural streams drained basins that were largely agricultural but the 100-m buffer was dominated by forest, and buffered-urban streams drained basins with a relatively large area as urban land use, but the 100-m buffer was forested or a city park. Finally, distal-agricultural streams drained basins that were largely forested but had small proportions of agriculture not immediately adjacent to the stream.

We used ArcGIS 8.2 (ESRI, Redlands, California, USA) to select candidate streams using data downloaded from the National Land Cover Database (reclassed Landsat TM imagery from 1992, Vogelmann et al. 2001). Using satellite data was problematic for three reasons: 1) the 10-m pixel size of the raw data can distort land-use proportions within a basin; 2) the imagery for the land cover data was acquired in 1992 but our study began in 2003, so 11 years of land cover change is not reflected in the basin land cover statistics; and 3) the automated procedure used to distill satellite data into land
<table>
<thead>
<tr>
<th>Stream</th>
<th>Basin area (ha)</th>
<th>Land-use category (year of study)</th>
<th>Basin</th>
<th>100-m Buffer</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Forest+Wetland (%)</td>
<td>Agricultural (%)</td>
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<td>Allegan</td>
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<td>20</td>
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<tr>
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<td>Stream</td>
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<td>Land-use category (year of study)</td>
<td>Forest+Wetland (%)</td>
<td>Agricultural (%)</td>
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<td>Tannery</td>
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<td>20</td>
</tr>
<tr>
<td>Weber</td>
<td>128</td>
<td>Distal agricultural (3)</td>
<td>48</td>
<td>52</td>
</tr>
</tbody>
</table>
cover classes can misclassify land uses. To minimize these problems, we visited each stream to confirm the land-use configuration of the candidate streams and selected 3 streams that best represented each of the 6 land-use types. We attribute apparent discrepancies between our classifications and the land-use data (Table 3.1) to problems associated with using satellite imagery (e.g., although Bullet Brook was entirely located on a military training area with no agricultural activity, the satellite imagery identifies it as 28% agriculture).

3.3.2 Sediment Nitrification Assays

In each stream, we collected monthly sediment samples for laboratory N transformation assays by taking twenty-five cores (30 cm² by 2 cm deep) from a 100-m stream reach and pooling them into 5 separate samples (~300 mL each). Sediment samples were stored on ice and returned to the laboratory where we immediately began the assays. We used the nitrapyrin-inhibition method (Hall 1984; Kemp and Dodds 2001; Strauss et al. 2004) to measure sediment nitrification rates in the study streams using 25 mL of sediment from each pooled sample (5 per stream per month) and adding 50 mL of unfiltered stream water to make a 75-mL slurry in each of two flasks. One of the flasks, designated as the production flask, received 10 µL of a 10% solution of nitrapyrin dissolved in dimethyl sulfoxide (DMSO), an organic solvent that delivered nitrapyrin across the cell membrane where it blocked the conversion of NH₄⁺ to NO₃⁻. The other flask, designated as the control, received 10 µL of DMSO, and nitrification was not blocked. During method development, we determined that an assay length between 24 and 120 hours yielded the same estimate of nitrification rate, so we incubated the flasks
for 24-48 hr on a rotary shaker at 150 rpm. At the end of the assay, we added 25 mL of 2M KCl and shook the flasks an additional 10 min to flush NH₄⁺ from cation exchange sites. We centrifuged the entire slurry and filtered the supernatant into bottles, which we froze for future NH₄⁺ analysis (method described below). We calculated nitrification rate as the difference in NH₄⁺ between the production and control flasks and scaled this by the mass of sediment and the assay length (units: µg N g⁻¹ DM h⁻¹); the nitrification rate for a given month in a given stream was the average of the 5 sets of paired flasks. We acknowledge that the nitrification rates measured using this method are probably higher than ambient rates because they were measured in oxygenated slurries (Strauss et al. 2004), but we also emphasize that they do not represent maximum potential rates because they were not incubated with amended NH₄⁺.

### 3.3.3 Sediment Denitrification Assays

We used the chloramphenicol-amended acetylene-block technique (Smith and Tiedje 1979; Royer et al. 2004; Inwood et al. 2005; Arango et al. 2007) to estimate sediment denitrification rates in the laboratory. Acetylene (C₂H₂) blocks the conversion of N₂O to N₂ by denitrifiers allowing N₂O to accumulate in the assay bottles, while the addition of chloramphenicol inhibits the de novo synthesis of denitrifying enzymes, reducing bottle effects associated with laboratory assays (Brock 1961, Smith and Tiedje 1979, Royer et al. 2004). We took 25-mL sediment sub-samples from the same pooled sediment samples as in the nitrification assays (5 pooled samples per stream) and added 50 mL of unfiltered site water with chloramphenicol at a final concentration of 0.3 mM in the 75-mL slurry. We sealed the bottles with septum caps for headspace sampling and purged them with ultra high purity helium for 5 min to induce anoxia. After purging the
bottles, we returned them to ambient atmospheric pressure and added 15 mL of pure C$_2$H$_2$, generated by reacting calcium carbide with deionized water, for a 10% atmosphere of C$_2$H$_2$ in the assay bottle. Before collecting gas samples, we shook the bottles for several seconds to equilibrate dissolved gases with the headspace, and then we removed a 5-mL headspace sub-sample from the assays, injecting the 4 mL sample into a 3-mL evacuated vial for later N$_2$O analysis. After each sampling period, we maintained constant pressure in the assay bottles by replacing the 5-mL headspace sample with 5 mL of 10% C$_2$H$_2$ in helium balance. We collected multiple gas samples during the 4.25 hr incubation with the first sample taken at elapsed time of 15 min and additional samples taken every hour thereafter.

We analyzed headspace samples for N$_2$O concentration by manually injecting 100 µL into a Varian Star 3600 gas chromatograph with a Porapak Q column and electron capture detector (injector temp = 120 °C, column temp = 40 °C, detector temp = 320 °C, with a 5% CH$_4$/95% Ar carrier gas at 30 mL min$^{-1}$), using a valve to vent C$_2$H$_2$ away from the detector. We used Bunsen coefficients to calculate total N$_2$O produced in the bottle, plotted N$_2$O production versus time, and calculated the N$_2$O production rate as the slope of the line of best fit ($r^2$>0.92). We determined denitrification rates by dividing the N$_2$O production rate by mass of sediment in the assay bottle and length of the assay (units: µg N g$^{-1}$ DM h$^{-1}$), and rates for a given month in a given stream were average denitrification rates calculated from the 5 assay bottles. Again, we recognize that the denitrification rates we report may be higher than ambient rates because we measured them in anoxic slurries (Groffman et al. 2006), but the sediments found in our study streams are naturally unconsolidated and we incubated them with chloramphenicol to
limit the response of the microbial community to the ideal anoxic conditions (Brock 1961; Smith and Tiedje 1979; Bernot et al. 2003). We also point out that the reported denitrification rates do not represent maximum potential rates because we did not amend incubations with additional NO$_3^-$ or organic C.

3.3.4 Sediment Characterization

From each pooled sediment sample we quantified sediment organic matter (%OM) by drying replicate 5-mL sub-samples to constant weight in a 60°C oven, weighing them to obtain dry mass, then ashing them at 500°C and reweighing them to obtain an ash weight. We calculated %OM as the ratio of ash-free dry mass to dry mass, expressed as a percentage. Additionally, we fumigated oven-dried samples in a dessicator with concentrated HCl to purge inorganic carbon from the sediments (Hedges and Stern 1984), and we combusted the fumigated samples in a Costech elemental analyzer (Valencia, California, USA) to measure sediment organic C (%C) and organic N (%N) content, from which we calculated molar sediment C:N. For a subset of the 3-yr dataset (91 of 216 samples), we collected an additional sediment core for exchangeable NH$_4^+$ analysis. In the lab we added 25 mL of 2M KCl to a sediment core collected as described above and shook the slurry on a rotary shaker at 150 rpm for 10 min. We centrifuged the entire slurry, filtered the supernatant (Pall A/E glass fiber filter, 1 µm nominal pore size) and froze it for future NH$_4^+$ analysis (described below). Exchangeable NH$_4^+$ was expressed as the mass of NH$_4^+$-N per volume of sediment (ng N mL$^{-1}$).
3.3.5 Physiochemical Variables and Water Chemistry

At each site, we measured stream-water velocity (Marsh-McBirney 200; Frederick, Maryland, USA), width, and depth to calculate discharge, and we used a Hydrolab Minisonde (Hach Environmental; Loveland, Colorado, USA) to measure stream-water temperature, specific conductivity, pH, and dissolved oxygen. We filtered stream water through Pall A/E glass fiber filters (1 µm nominal pore size) into HDPE bottles pre-rinsed with filtered stream water and stored them on ice, returned them to the laboratory where we froze them for future analyses. For multiple sample types (i.e., stream-water, exchangeable, and nitrification assay samples), we measured NH$_4^+$ concentrations on a Shimadzu UV-1601 spectrophotometer (Columbia, Maryland, USA) at 630 nm using the phenate method (Solorzano 1969; APHA 1995), and we measured soluble reactive phosphorus (SRP) at 885 nm using the molybdate method (Murphy and Riley 1962; APHA 1995). We measured NO$_3^-$, chloride, and sulfate simultaneously (USEPA 1993) using a Dionex 600 ion chromatograph (Sunnyvale, California, USA). Finally, we quantified water-column DOC by acidifying samples to pH < 2 and analyzing them on a Shimadzu TOC-500 carbon analyzer (Columbia, Maryland, USA) using the combustion infrared method (APHA 1995).

3.3.6 Statistical Analyses

We used either log transformation or log followed by power transformation to transform non-normal data to meet the assumptions of parametric statistics. We used repeated measures ANOVA (rmANOVA) (SAS 9.1; SAS Institute Inc., Cary, North Carolina, USA) to analyze how nitrification and denitrification rates, as well as independent variables, varied by land use and through time, and multiple linear
regression (SYSTAT 11; San Jose, California, USA) to identify which independent variables were significantly related to nitrification and denitrification rates. We used a 2-dimensional Kolmogorov-Smirnoff test to identify a threshold value in the sediment C:N versus nitrification rate relationship. We condensed the land cover data for each basin into continuous, uncorrelated ordination axes using principal components analysis (PCA) (PCord; MjM Software Design, Gleneden Beach, Oregon, USA), and we used these axes as independent variables in linear regressions to identify relationships between nitrification and denitrification rates and catchment land cover (SYSTAT 11; San Jose, California, USA).

3.4 Results

3.4.1 Land-use and Seasonal Patterns in N Transformations

Sediment nitrification rates varied over 2 orders of magnitude (Table 3.2), and they were significantly greater in agricultural streams (rmANOVA, $P=0.0090$) and in late spring and early summer (rmANOVA, $P=0.0066$) compared to forested, buffered-urban, and distal-agricultural streams and in early spring (Figure 3.2a). Temporal differences in nitrification were largely driven by the agricultural and buffered-agricultural streams, which always had the highest rates. Additionally, the pairwise comparisons indicated that buffered streams of the same general land-use category (i.e., buffered-agricultural versus agricultural) had similar nitrification rates. Denitrification rates also varied over 2 orders of magnitude (Table 3.2), and although denitrification rates did not differ among land-use types (rmANOVA, $P=0.24$), they were significantly higher in the winter
<table>
<thead>
<tr>
<th>Stream</th>
<th>Q (L s$^{-1}$)</th>
<th>NH$_4^+$-N (µg L$^{-1}$)</th>
<th>NO$_3^-$-N (mg L$^{-1}$)</th>
<th>DOC (mg L$^{-1}$)</th>
<th>Exch.$^1$ NH$_4^+$-N (ng mL$^{-1}$)</th>
<th>Organic matter (%)</th>
<th>Organic C (%)</th>
<th>Molar C:N</th>
<th>Nitrification (µg N g$^{-1}$ DM h$^{-1}$)</th>
<th>Denitrification (µg N g$^{-1}$ DM h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agricultural</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burnips</td>
<td>12 (6)</td>
<td>270 (114)</td>
<td>10.7 (0.4)</td>
<td>6.8 (2.8)</td>
<td>$X^2$</td>
<td>7.4 (0.6)</td>
<td>3.6 (0.3)</td>
<td>17.7 (0.8)</td>
<td>0.54 (0.13)</td>
<td>1.88 (0.21)</td>
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<td>3.8 (1.0)</td>
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<td>2.1 (0.3)</td>
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<td>2.5 (1.1)</td>
<td>2.2 (0.6)</td>
<td>2415 (579)</td>
<td>8.7 (0.3)</td>
<td>4.8 (0.3)</td>
<td>15.0 (0.8)</td>
<td>1.03 (0.19)</td>
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<td><strong>Land-use mean</strong></td>
<td>8 (3)</td>
<td>107 (82)</td>
<td>11.3 (5.2)</td>
<td>4.2 (1.3)</td>
<td>1279 (1136)</td>
<td>6.4 (1.7)</td>
<td>3.5 (0.8)</td>
<td>18.9 (2.6)</td>
<td>0.60 (0.23)</td>
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<td>23 (10)</td>
<td>43 (5)</td>
<td>2.1 (0.7)</td>
<td>30 (10)</td>
<td>$X^2$</td>
<td>14.2 (0.4)</td>
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<td>16.5 (0.7)</td>
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<td>977 (140)</td>
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<td>16 (4)</td>
<td>$X^2$</td>
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<tr>
<td></td>
<td>Q (L s⁻¹)</td>
<td>NH₄⁺-N (µg L⁻¹)</td>
<td>NO₃⁻-N (mg L⁻¹)</td>
<td>DOC (mg L⁻¹)</td>
<td>Exch.¹ NH₄⁺-N (ng mL⁻¹)</td>
<td>Organic matter (%)</td>
<td>Organic C (%)</td>
<td>Molar C:N</td>
<td>Nitrification (µg N g⁻¹ DM h⁻¹)</td>
<td>Denitrification (µg N g⁻¹ DM h⁻¹)</td>
</tr>
<tr>
<td>Buffered urban</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorr</td>
<td>24 (5)</td>
<td>79 (10)</td>
<td>1.5 (0.2)</td>
<td>10 (2)</td>
<td>X²</td>
<td>1.2 (0.2)</td>
<td>0.6 (0.1)</td>
<td>21.3 (0.8)</td>
<td>0.09 (0.04)</td>
<td>0.11 (0.02)</td>
</tr>
<tr>
<td>Allegan</td>
<td>20 (4)</td>
<td>23 (4)</td>
<td>1.7 (0.3)</td>
<td>17 (3)</td>
<td>38 (13)</td>
<td>0.4 (&lt;0.1)</td>
<td>0.3 (0.1)</td>
<td>30.9 (2.2)</td>
<td>0.01 (&lt;0.01)</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td>Urbandale</td>
<td>38 (5)</td>
<td>47 (8)</td>
<td>0.3 (0.1)</td>
<td>8 (1)</td>
<td>335 (40)</td>
<td>1.4 (0.1)</td>
<td>0.8 (0.1)</td>
<td>26.7 (2.0)</td>
<td>0.08 (0.02)</td>
<td>0.06 (0.01)</td>
</tr>
<tr>
<td>Land-use mean</td>
<td>28 (5)</td>
<td>49 (16)</td>
<td>1.2 (0.4)</td>
<td>12 (3)</td>
<td>186 (148)</td>
<td>1.0 (0.3)</td>
<td>0.6 (0.2)</td>
<td>26.3 (2.8)</td>
<td>0.06 (0.02)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>Distal agricultural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bellevue</td>
<td>33 (13)</td>
<td>17 (2)</td>
<td>0.2 (&lt;0.1)</td>
<td>8 (1)</td>
<td>X²</td>
<td>3.0 (0.5)</td>
<td>1.4 (0.3)</td>
<td>21.6 (2.4)</td>
<td>0.08 (0.05)</td>
<td>0.02 (&lt;0.01)</td>
</tr>
<tr>
<td>Spring Brook</td>
<td>17 (1)</td>
<td>10 (2)</td>
<td>0.7 (&lt;0.1)</td>
<td>4 (1)</td>
<td>83 (24)</td>
<td>1.1 (0.1)</td>
<td>0.6 (0.2)</td>
<td>20.1 (1.0)</td>
<td>0.04 (0.01)</td>
<td>0.03 (&lt;0.01)</td>
</tr>
<tr>
<td>Weber</td>
<td>7 (2)</td>
<td>95 (33)</td>
<td>0.03 (0.01)</td>
<td>11 (3)</td>
<td>341 (59)</td>
<td>0.8 (0.1)</td>
<td>0.3 (0.1)</td>
<td>18.0 (0.8)</td>
<td>0.06 (0.02)</td>
<td>0.02 (&lt;0.01)</td>
</tr>
<tr>
<td>Land-use mean</td>
<td>19 (8)</td>
<td>41 (27)</td>
<td>0.3 (0.2)</td>
<td>8 (2)</td>
<td>212 (129)</td>
<td>1.6 (0.7)</td>
<td>0.8 (0.3)</td>
<td>19.9 (1.0)</td>
<td>0.06 (0.01)</td>
<td>0.02 (0.01)</td>
</tr>
</tbody>
</table>

¹Exchangeable NH₄⁺ from KCl extractions
²X² indicates data not sampled
Figure 3.2. Seasonal and land-use patterns in monthly mean (±1 SE) N transformation rates (n=3 per datum). Among months, ‘a’ denotes a significantly higher value than ‘b’, and lines group land uses with similar means. (A) Nitrification rates were highest in agricultural streams and in the summer. (B) Denitrification rates did not vary among land uses but were highest in winter.

compared to the summer (rmANOVA, $P=0.0045$) (Figure 3.2b). Similar to nitrification rates, patterns in agricultural and buffered-agricultural streams drove the temporal differences in denitrification, but the high winter variability in agricultural streams
precluded detecting a significant land-use difference. Although agricultural and buffered-agricultural streams always had highest N transformation rates, the rates were temporally decoupled, with maximum nitrification in summer (i.e., May-July) compared to maximum denitrification in winter (i.e., December and February).

3.4.2 Land-use and Seasonal Patterns of DIN and Organic C

We used rmANOVA to analyze how NH$_4^+$ and sediment %C, factors that potentially controlled nitrification rates, varied among land uses and through time. Stream-water NH$_4^+$ did not differ among land uses or through time (Figure 3.3a) though urban streams frequently had the highest stream-water NH$_4^+$ concentrations. Agricultural streams, which consistently had the highest nitrification rates, did have the highest observed NH$_4^+$ concentrations (>400 µg N L$^{-1}$), but this was driven by a rain storm that elevated NH$_4^+$ to >1600 µg N L$^{-1}$ in one agricultural stream in October (Figure 3.3a). The lack of correspondence between stream-water NH$_4^+$ concentrations and the high nitrification rates observed in spring and in agricultural streams suggests that stream-water NH$_4^+$ has little influence over benthic nitrification rates. However, sediment %C was highest in agricultural and buffered-agricultural streams (rmANOVA, $P=0.0173$), which also had highest nitrification rates, suggesting a close relationship between nitrification and sediment carbon content. Pairwise comparisons showed that buffered streams did not have significantly different sediment %C than their unbuffered counterparts. Because sediment %C did not vary through time (rmANOVA, $P=0.43$, Figure 3.4a), we explored other factors that could have influenced the seasonal pattern we observed in nitrification rates.
Figure 3.3. Seasonal and land-use patterns in monthly mean (±1 SE) DIN concentrations (n=3 per datum). Among months, ‘a’ denotes a significantly higher value than ‘b’, and lines group land uses with similar means. (A) NH$_4^+$ concentrations did not vary among land uses or by month, but (B) NO$_3^-$ concentrations were highest in agricultural streams and in the winter.
Figure 3.4. Seasonal and land-use patterns in monthly mean (±1 SE) organic C (n=3 per datum). Among months, ‘a’ denotes a significantly higher value than ‘b’, and lines group land uses with similar means. (A) Sediment organic C was highest in buffered-agricultural and agricultural streams but did not vary through time, and (B) DOC did not vary by land use but had a distinct peak among streams in October.
We also used rmANOVA to analyze how NO$_3^-$, DOC, and sediment organic matter (%OM), factors that potentially controlled denitrification rates, varied among land uses and through time. Because sediment %OM was highly correlated with sediment %C (Pearson correlation, r=0.89) and exhibited the same significant land-use pattern, we only used %OM as an independent variable. Agricultural streams had highest stream-water NO$_3^-$ concentrations (rmANOVA, $P=0.0452$), which were generally an order of magnitude higher than streams draining other land uses (Figure 3.3b), but again, pairwise comparisons indicated no significant difference between a buffered and unbuffered stream of the same land use. Furthermore, NO$_3^-$ concentrations were highest in the winter (rmANOVA, $P=0.0011$) and trended upward in the winter months in nearly all land-use types. Although dissolved organic C did not vary by land use, we observed a distinct peak corresponding to autumn leaf abscission in October that was the highest observed concentration (rmANOVA, $P=0.0005$) among streams (Figure 3.4b).

3.4.3 Factors Controlling N Transformation Rates

We used multiple linear regression (MLR) to analyze the relationship between nitrification rates and the independent variables we measured. Using our entire dataset (n=195), we found that nitrification rates were positively related to sediment %C and stream-water temperature, and negatively related to sediment C:N (MLR, $R^2=0.26$, $P<0.00001$, Table 3), but sediment %C alone explained the most variability in nitrification rates (Fig. 3.5a). About mid-way through our 3-yr study, we began sampling exchangeable NH$_4^+$ after we found no relationship between nitrification rates and water-column NH$_4^+$ concentrations during the first part of our sampling. Using linear regression to analyze this reduced dataset from the last half of the study (n=93), we found
Figure 3.5. Predictors of nitrification and denitrification rates. (A) Sediment organic C explained the most variability in nitrification in a multiple linear regression that included stream temperature and sediment C:N. (B) Stream-water NO$_3^-$ concentration explained the most variability in denitrification in a multiple linear regression that included stream temperature, sediment % organic matter, and stream-water DOC.
TABLE 3.3.

SIGNIFICANT PREDICTORS OF NITRIFICATION AND DENITRIFICATION RATES.

<table>
<thead>
<tr>
<th>N Transformation</th>
<th>Independent variable</th>
<th>Correlation</th>
<th>Partial R²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrification</td>
<td>Sediment %C</td>
<td>+</td>
<td>0.23</td>
<td>&lt;&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Stream-water temperature</td>
<td>+</td>
<td>0.02</td>
<td>0.0150</td>
</tr>
<tr>
<td></td>
<td>Sediment C:N</td>
<td>-</td>
<td>0.01</td>
<td>0.0402</td>
</tr>
<tr>
<td>Denitrification</td>
<td>Stream-water NO₃⁻</td>
<td>+</td>
<td>0.36</td>
<td>&lt;&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sediment organic matter</td>
<td>+</td>
<td>0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Stream-water temperature</td>
<td>-</td>
<td>0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Stream-water DOC</td>
<td>+</td>
<td>0.02</td>
<td>0.0201</td>
</tr>
</tbody>
</table>

Note: All variables log normalized. Multiple linear regression statistics for nitrification: R²=0.26, P<<0.0001, n=196. Multiple linear regression statistics for denitrification: R²=0.47, P<<0.0001, n=190.

A positive relationship between nitrification rates and sediment exchangeable NH₄⁺ (r²=0.38, P<0.00001, data not shown) while there continued to be no significant relationship with stream-water NH₄⁺. Taken together, the results from the entire dataset and the reduced dataset suggested that the decomposition of organic C in the sediment provided a benthic NH₄⁺ source for nitrification. We examined this hypothesis using the reduced dataset and found a positive relationship between exchangeable NH₄⁺ and sediment %C (r²=0.45, P<0.00001, data not shown), which supports the link between NH₄⁺ production and decomposition of organic C.
Although sediment C:N explained a very small amount of the variability in nitrification rates (Table 3; $r^2=0.01$), we used a 2-dimensional Kolmogorov-Smirnoff threshold test (K-S test) to identify a significant threshold of C:N=18 (K-S test, $P=0.0012$). Below this threshold, nitrification rate decreases with increasing C:N ratio, and above this threshold, nitrification rates are generally low and have no relationship with sediment C:N (Figure 3.6).

We also used multiple linear regression to analyze the relationship between denitrification rates and the independent variables we measured. Denitrification was positively related to stream-water $\text{NO}_3^-$ concentration, sediment %OM, and stream-water DOC concentration, but it was negatively related to temperature (MLR, $n=190$, $R^2=0.47$.

![Figure 3.6. Relationship between sediment nitrification rates and sediment C:N. Dotted line denotes a threshold (C:N=18) identified by a 2-dimensional Kolmogorov-Smirnoff test, below which nitrification rates were high and above which nitrification rates were low.](image-url)
Of these factors, stream-water NO$_3^-$ concentration had the most explanatory power based on coefficient of determination ($r^2=0.35$, Figure 3.5b). Higher denitrification rates associated with lower temperatures is likely a result of the increased NO$_3^-$ concentrations we observed in winter (Figure 3.3b) rather than a direct effect of temperature.

### 3.4.4 Relationship between Continuous Land-use Metrics and N Transformations

We used PCA to condense the land-use metrics we used to define each basin (i.e., basin size, % of basin as agriculture, urban, forest, and wetland, and % of 100-m buffer as agriculture, urban, forest, and wetland) into ordination axes that we used in regression analysis to explain variation in N transformation rates. This PCA identified three gradients that explained 83.7% of the variability in the basins we studied: Axis 1 was an agriculture-forest gradient that explained 45.8% of the variability in the land-use data, Axis 2 was a wetland gradient that explained 23.1% of the variability, and Axis 3 was an urban gradient that explained 14.8% of the variability. The agriculture-forest gradient represented by Axis 1 scores was significantly related to mean annual nitrification ($r^2=0.32$, $P=0.015$) and denitrification ($r^2=0.41$, $P=0.004$) rates from each stream (Figure 3.7) with higher transformation rates being associated with more agricultural land use. Regression analysis of transformation rates versus the ordination axes explained roughly the same amount of variability in N transformations as the multiple linear regressions that used various independent variables (e.g., $r^2=0.32$ versus $R^2=0.26$ for nitrification rates, $r^2=0.41$ versus $R^2=0.47$ for denitrification rates) suggesting that land-use metrics may be reasonable proxies for the abiotic variables that directly control N transformations.
3.5 Discussion

3.5.1 Seasonal Patterns of Sediment Nitrification Rates

Average annual nitrification rates spanned two orders of magnitude (0.01-1.03 µg N g⁻¹ DM h⁻¹) in the 18 streams we studied (Table 3.2), and we found highest nitrification rates in the late spring and early summer (Figure 3.2a). In order to compare our rates to published values, we multiplied our biomass-scaled rates (µg N g⁻¹ DM h⁻¹) by biomass standing stocks (g DM m⁻²) to convert them to areal units, and in doing so, nitrification rates (0.4-9.0 mg N m⁻² h⁻¹) spanned roughly the same range as those measured over a year in Appalachian mountain streams (0.4-2.0 mg N m⁻² h⁻¹, Starry et al. 2005), Kansas prairie streams (2.2-4.6 mg N m⁻² h⁻¹, Kemp and Dodds 2002), and the Upper Mississippi River (4-15 mg N m⁻² h⁻¹, Strauss et al. 2004). These studies all reported high nitrification in the late spring or early summer when we observed peak nitrification, but the reasons for the high rates varied among sites. For example, the combined effect of temperature and NH₄⁺ availability (Starry et al. 2005), increased oxygen and NH₄⁺ availability (Kemp and Dodds 2002), or temperature alone (Strauss et al. 2004) all had explanatory power with regard to variation in nitrification rates. Therefore, the major factor(s) controlling nitrification in stream ecosystems varied among sites, with the commonality that substrate availability, temperature, and oxygen status were all potential drivers.

Nitrification is a metabolic process controlled by substrate availability, specifically oxygen (Kemp and Dodds 2001) and NH₄⁺ (Strauss et al. 2002). If oxygen regulated nitrification, we would expect peak nitrification associated with high primary
Figure 3.7. Relationship between an agriculture-forest land-use gradient and N transformation rates (±1 SE). Average annual nitrification (A) and denitrification (B) rates (n=12 for each datum) were negatively related an ordination axis identified by PCA.
producer biomass because primary producers can stimulate nitrification by driving diel cycles in the depth of oxygen penetration into the benthos (Rysgaard et al. 1994).

Presence of autotrophs in our study sites was high in early spring when riparian canopies were still open and light levels were increasing (see Chapter 4), but we found highest nitrification rates in the late spring and early summer (Figure 3.2a) when the canopy is closed and primary producer biomass is lower than the early spring peak. Although our nitrification measures are potential rates with respect to oxygen because sediments were incubated in oxic assays, the presence of abundant nitrifiers with low benthic oxygen production suggests that other factors, such as NH₄⁺ availability or warming spring/summer temperatures, probably also influenced nitrification rates. We did not find a seasonal pattern in stream-water NH₄⁺ that corresponded to the seasonal pattern in nitrification, but we did find evidence that benthic NH₄⁺ was linked to sediment %C, which varied by land use.

3.5.2 Land-use Influences on Nitrification Controls

Agricultural streams had the highest nitrification rates when considering land use categorically (Figure 3.2b) or continuously (Figure 3.7a), but land use cannot directly control nitrification; rather, land use indirectly controls nitrification by mediating substrate availability. For example, sediment %C was highest in agriculturally-influenced streams (Figure 3.4a), and we found a positive relationship between sediment %C and exchangeable NH₄⁺ and a positive relationship between exchangeable NH₄⁺ and nitrification rates. Exchangeable NH₄⁺ increased nitrification in other Midwestern streams (Strauss et al. 2002) as well as in large rivers (Strauss et al. 2004), indicating that benthic decomposition of organic matter is an important NH₄⁺ source for nitrifiers (Jones
et al. 1995; Starry et al. 2005). Agricultural streams likely had highest sediment %C because agricultural fields in the Midwest are frequently sited on drained wetlands that have highly organic soils (Mitsch et al. 2001), and cultivation increases soil erosion to streams (Jones et al. 2001). Agricultural streams also have higher water temperatures because riparian zone clearing allows greater light penetration in the stream channel (Osborne and Kovacic 1993), and higher temperatures increase organic matter decomposition rates (Webster and Benfield 1986). Therefore, the positive effect of sediment %C and stream-water temperature we observed on nitrification rates (Table 3.3) is consistent with decomposition as an important N source for nitrification. Site-specific controls influencing benthic NH$_4$$^+$ production via organic matter decomposition are likely important regulators of nitrification.

In addition to the direct control of NH$_4$$^+$ supply on nitrification rates, sediment C:N ratio indirectly controls nitrification because heterotrophic microbes can outcompete nitrifiers for NH$_4$$^+$ when C:N is high (>20) but not when it is low (C:N<20) (Strauss and Lamberti 2002). Many of our study streams had sediment C:N<20 (Table 3.3), but urban streams had significantly higher sediment C:N compared to stream sediments in other land-use types (rmANOVA, $P=0.0003$, data not shown). Although multiple linear regression analysis showed that nitrification rates were most strongly related to sediment %C, we also observed a weak, negative relationship with sediment C:N (Table 3.3). Furthermore, the threshold test of nitrification rates and sediment C:N (Figure 3.6) identified a significant threshold of C:N=18 that corresponds closely to thresholds observed in previous studies of small streams [Kemp and Dodds (2002), C:N=15; Strauss and Lamberti (2002), C:N=20; and Ollinger et al. (2002), C:N=22]. Therefore high
sediment C:N may have conferred a competitive advantage to heterotrophic microbes in urban streams, lowering nitrification rates despite high temperatures from riparian clearing and intermediate sediment %C.

3.5.3 Seasonal Patterns of Sediment Denitrification Rates

Denitrification rates showed significant seasonal patterns among our study streams, and ranged nearly two orders of magnitude (0.02-1.88 µg N g⁻¹ DM h⁻¹) throughout the year with the highest denitrification rates in winter and the lowest rates in summer (Figure 3.2b). This contrasts with a meta-analysis of denitrification in aquatic habitats (i.e., oceans, coastal environments, estuaries, lakes, and rivers) with a relatively broad range of NO₃⁻ concentrations (0.01-13.58 mg N L⁻¹) concentrations showing that summer, and its associated high water temperatures, had the highest denitrification rates (Piña-Ochoa and Álvarez-Cobelas 2006). However, we found highest NO₃⁻ concentrations in the winter (Figure 3.3b), which corresponded to the highest denitrification rates and suggested that NO₃⁻ availability influences denitrification more than temperature. Therefore the factors that govern the seasonality of NO₃⁻ delivery are important regulators of nitrification in our streams.

We observed a distinct NO₃⁻ pulse with the first significant runoff in late autumn and early winter, implicating hydrology as a principal factor regulating NO₃⁻ delivery. Although southwestern Michigan has a humid climate with relatively regular rainfall, each summer of our study was characterized by below average rainfall. During periods of low rainfall, NO₃⁻ produced via terrestrial nitrification accumulates in soil water, and when rainfall flushes this concentrated soil solution into streams, stream-water NO₃⁻ concentration can increase several times (Morecroft et al. 2000). Hydrologic flushing of
NO$_3^-$ was also seen in Illinois agricultural streams where spring runoff flushed soil NO$_3^-$ into streams, increasing NO$_3^-$ concentration by up to three orders of magnitude over base-flow concentrations (Royer et al. 2006). The flushing of soil NO$_3^-$ and the peak in denitrification we observed after a dry summer and autumn suggests that sediment denitrification may be subject to seasonal NO$_3^-$ limitation in regions characterized by seasonal dry periods. Moreover, it emphasizes the importance of NO$_3^-$ as a significant predictor of denitrification in our study streams (Figure 3.5b), and it highlights the importance of hydrology in controlling the delivery of the dominant substrate for denitrification. However, NO$_3^-$ concentrations also varied by land use, so denitrification rates were influenced by land-use factors in addition to seasonal factors.

3.5.4 Land-use Influences on Denitrification Controls

Although we did not observe a significant difference in denitrification among categorical land-use types, average annual denitrification rate was positively related to agricultural land use when considered as a continuous variable (Figure 3.7b). Additionally, the two highest monthly denitrification rates in our agricultural streams (1.31 and 1.88 µg N g$^{-1}$ DM h$^{-1}$; Table 3.2) were very close the highest rates seen in agricultural streams in Illinois (2.3 µg N g$^{-1}$ DM h$^{-1}$; Schaller et al. 2004), making our rates among the highest ever reported using our method. Agricultural streams frequently have high NO$_3^-$ concentrations (e.g., Johnson et al. 1997; Inwood et al. 2005; Dodds and Oakes 2006), a pattern resulting from excess fertilizer application to adjacent crops (David et al. 1997), frequent soil disturbance that increases mineralization of organic matter (Keeny and DeLucca 1993), and tile drainage that rapidly delivers terrestrial N to
Although NO₃⁻ explained 70% of the variability in a meta-analysis comparing denitrification across aquatic habitats (Piña-Ochoa and Álvarez-Cobelas 2006), it only explained 36% of the variability among our streams (Figure 3.5b). The lower predictive power of NO₃⁻ in our data may be due to the broader temporal range we sampled (monthly compared to summer only in the meta-analysis), which included both “hot and cold moments” (sensu McClain et al. 2003) of denitrification, or it may be caused by anoxia or carbon limitation of denitrification at the upper end of the broad NO₃⁻ range we sampled (0.03-20.59 mg N L⁻¹; Table 3.2). In contrast, NO₃⁻ probably limited the denitrification rates reported in the meta-analysis since most systems had relatively low NO₃⁻ (< 1.5 mg N L⁻¹) (Piña-Ochoa and Álvarez-Cobelas 2006).

Although denitrification was most strongly related to NO₃⁻ concentration, it was also positively related to two metrics of carbon availability, sediment %OM and stream-water DOC (Table 3.3). Sediment %OM was highest in agricultural and buffered-agricultural streams for the same reasons these land uses had high sediment %C—a combination of fields located on high organic matter soils and land-use practices that increase soil erosion. Organic matter may influence denitrification by providing a C source for denitrifiers, increasing anaerobic conditions by stimulating decomposition, or both (Seitzinger 1988). However, the relatively small amount of variability explained by %OM in the regression analysis suggests that any positive effect of organic matter on denitrification is secondary to the positive effect of NO₃⁻ (Arango et al. 2007).

Denitrification was also positively related to stream-water DOC concentrations, which had a pronounced peak during autumn leaf abscission in October (Fig. 3.4b). Although DOC was not related to land use, annual average DOC concentration was
positively related to the fraction of the catchment identified as wetlands ($r^2=0.33$, $P=0.01$, data not shown), suggesting that wetlands influence stream-water DOC over the annual cycle. Dissolved organic C derived from wetlands is often dominated by refractory humic substances (Qualls and Richardson 2003) whereas leachate from freshly abscised leaves ranges in lability (Strauss and Lamberti 2002). Therefore, the autumn pulse of DOC may contribute a relatively labile C source that could stimulate denitrification, but because we did not observe a peak in denitrification associated with the peak in DOC, the effect is probably very weak and restricted to a brief window of time in the autumn.

3.5.5 Effect of Riparian Buffers on Nitrification and Denitrification

In our analyses of the controls on N transformation rates in small, mixed land-use streams, there were no factors that differed significantly between buffered and unbuffered streams of the same general land use (i.e., agricultural vs. buffered-agricultural, urban vs. buffered-urban, or forested vs. distal-agricultural), suggesting that riparian buffers do not influence water or sediment chemistry or their effects on N transformations. Many studies have shown that riparian buffers can moderate stream temperatures (Abell and Allan 2002), reduce sedimentation (Hubbard et al. 1990), and decrease nutrient flux from the terrestrial to stream ecosystems (Peterjohn and Correll 1984). However, the effectiveness of riparian buffers is dictated by their interaction with the hydrosystem (e.g., Houser et al. 2005). All 18 of our study basins had tile drains or simplified drainage networks that quickly routed runoff and shallow groundwater to the streams, decreasing interaction with the riparian buffers and enhancing the delivery of substrates required for sediment N transformations. For example, the high organic matter sediments that stimulated nitrification and denitrification rates in agriculturally-influenced streams
were most likely related to agricultural land-use practices and soil erosion, which
increases with rapid runoff from the landscape (Allan et al. 1997). Additionally, the
hydrologic flushing of soil NO$_3$ that caused high winter denitrification rates was
probably augmented by the tile drains that simplified the hydrosystem (e.g., David et al.
1997; Royer et al. 2004). The extensively modified hydrosystems of our study streams
gave hydrology the primary role in delivering substrates for nitrification and
denitrification, and riparian buffers had no effect on the variables we studied because
they were decoupled from stream channels through human modifications to the
hydrosystem.

Spatio-temporal patterns of sediment %C show further evidence of the importance
of hydrology as an overriding variable. During our field sampling, we observed natural
leaf packs in all streams in the autumn, and we expected them to become incorporated
into the sandy sediments as they fragmented into smaller pieces, which would have
resulted in peak sediment %C during autumn or early winter. However, we did not find
significant differences in sediment %C among months (Figure 3.4a). The management of
these streams as drains requires channel straightening, subsurface tile drains, and periodic
dredging that removes large woody debris dams. This combination of factors increases
the number and magnitude of peak flows and decreases channel complexity, reducing the
overall capacity of these streams to retain organic matter (e.g., Bilby and Likens 1980;
Allan et al. 1997). Thus the land-use practices that simplify basin hydrology and in-
channel complexity have likely constrained N transformations by reducing organic matter
retention in these streams.
Categorical land-use type was a significant factor for predicting nitrification but not denitrification rates, and both N transformations were related to an ordination axis that was a composite of continuous land-use variables. All the important independent variables that were related to rates of nitrification (sediment %C, stream-water temperature, and sediment C:N ratio) and denitrification (NO$_3^-$ concentration, stream-water temperature, sediment %OM, and DOC) had significant associations with categorical land-use type except DOC, which was only related to fractional wetland coverage. Our results support using categorical land-use classifications as a predictive tool to generalize across anthropogenically-modified landscapes. By including buffered-agricultural, buffered-urban, and distal-agricultural land-use types, we incorporated spatial configurations of land use that are rarely seen in other land-use studies, which typically focus only on the dominant land-use type in a basin (e.g., agricultural, urban, and native). However, these different land-use configurations did not add explanatory power to our categorical classification, implying that end-member classification schemes may adequately generalize spatio-temporal patterns in stream nutrient dynamics in some landscapes with mixed land-use patterns.

As enzyme-mediated N transformations, nitrification and denitrification are ultimately governed by substrate availability, and this study highlights land use as an important mediating factor (Figure 3.8). For example, nitrification rates were related to
Figure 3.8. Summary model of land-use influences on nitrification and denitrification. Factors with strong temporal variation, such as light levels and leaf litter input, are identified in italics and dashed lines. Although nitrification produces NO$_3^-$, we did not find evidence for coupled nitrification and denitrification and did not link these processes.

sediment %C, which provided an NH$_4^+$ source for nitrification, and agricultural land-use activity enriched sediment %C via soil erosion. For denitrification rates, NO$_3^-$ explained the most variability, and it was highest in agricultural streams, due to application of nitrogenous fertilizers. Our results also show that basin hydrology can control substrate availability by routing solutes and organic matter through the drainage network.

Furthermore, if riparian buffers do not interact strongly with water as it moves through the hydrosystem, they will not function effectively as tools to mitigate catchment degradation via land-use activity. As a whole, these results confirm that streams are
complex systems that cannot be fully understood without taking into account the spatio-
temporal linkages between the stream and the landscape through which it flows (Hynes
1975; Vannote et al. 1980).

3.6 Acknowledgements

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CHAPTER 4

ASSIMILATORY UPTAKE RATHER THAN DISSIMILATORY
TRANSFORMATIONS INFLUENCE SEASONAL PATTERNS IN NITROGEN
UPTAKE IN STREAMS OF VARYING LAND USE³

4.1 Abstract

Land-use activity modifies the ability of headwater streams to regulate
downstream nutrient export because higher nutrient concentrations can decrease whole-
stream nutrient uptake efficiency and saturate dissimilatory uptake via nitrification or
denitrification. Also, the removal of riparian vegetation can increase the importance of
autotrophy to stream metabolism. Over a three-year period, we compared whole-stream
nitrogen (N) uptake and dissimilatory N transformation rates in 18 agricultural, urban,
and forested streams during spring, summer, and autumn. We measured whole-stream
ammonium (NH₄⁺) and nitrate (NO₃⁻) uptake in the field as well as nitrification (NO₃⁻
production) and denitrification (NO₃⁻ consumption) rates using laboratory assays to
quantify their relative contribution to whole-stream NO₃⁻ dynamics. Although relative
demand for NH₄⁺ (as uptake velocity, Vf) did not vary by land use, it was highest in
spring when chlorophyll-α was highest, and among seasons and land uses, we found

³This chapter is being prepared for journal submission with the help of co-authors Jennifer
L. Tank and Laura T. Johnson.
higher relative demand for NH$_4^+$ in streams with open canopies, implying a link with primary producers. In agricultural and urban streams, whole-stream uptake (as areal uptake, $U$) of NH$_4^+$ and NO$_3^-$ and dissimilatory N transformations appear to saturate at high inorganic N concentrations. Nitrification and denitrification rates are of the same magnitude, and are therefore balanced, suggesting that site-specific redox conditions determine if stream sediments are a net source or sink of NO$_3^-$. However, nitrification and denitrification rates were always more than an order of magnitude lower than whole-stream NO$_3^-$ uptake, despite assays being measured in the laboratory (i.e., under ideal redox conditions), demonstrating the limited influence of dissimilatory N uptake on whole-stream NO$_3^-$ dynamics. In these Midwestern streams, assimilatory processes, which represent only a temporary storage of inorganic N removal from the water column, dominated whole-stream N demand and controlled downstream N flux.

4.2 Introduction

Biological uptake and transformation of nutrients in headwater streams can regulate nutrient export to downstream ecosystems (Peterson et al. 2001), and this biological activity can be measured at the whole-stream level using the nutrient spiraling techniques (Newbold et al. 1981; Stream Solute Workshop 1990). Nutrient spiraling couples nutrient uptake and release with downstream transport, and most studies of stream nutrient spiraling have been performed in either forested biomes (e.g., Tank et al. 2000; Ashkenas et al. 2004) or in stream systems minimally altered by human activities (e.g., Grimm and Fisher 1986; Dodds et al. 2000). Only recently have nutrient spiraling studies investigated streams dominated by agricultural (Niyogi et al. 2004; Bernot et al. 2006) or urban (Grimm et al. 2005; Meyer et al. 2005) land use even though human-
induced changes in land use influence most running waters in the United States (Meyer and Turner 1994). Human land-use activities typically increase dissolved inorganic nitrogen (DIN) concentrations (Carpenter et al. 1998) and can also decrease the relative importance of riparian vegetation on stream ecosystem dynamics via stream-side vegetation removal, which can reduce light limitation of aquatic autotrophs (Allan 2004). Therefore, knowledge of how land use mediates nutrient uptake and transformation processes in stream ecosystems is critical for understanding how streams regulate nutrient flux to downstream water bodies.

Although land use can influence stream nutrient cycling by affecting DIN concentrations, seasonality is also an important driver of whole-stream nutrient uptake in mid-latitude regions. For example, in temperate, forested streams, nutrient demand shows both a spring peak, when autotrophic activity increases concurrently with increasing light levels and temperature prior to leaf-out and subsequent shading, and an autumn peak, when heterotrophs actively colonize and decompose allochthonous leaf litter inputs (Mulholland 1992; Mulholland 2004). These seasonal peaks in nutrient demand have been confirmed in studies that examine nutrient uptake over an annual cycle (Simon et al. 2005; Hoellein et al. in press), so spring and autumn may be considered biologically important periods for whole-stream nutrient demand in temperate streams. However, seasonality in nutrient uptake has rarely been studied in the context of streams that are modified by agricultural and urban land use (Niyogi et al. 2004), particularly in temperate regions where seasonality plays a strong role.

Studies that have used radio- or stable-isotopes of nutrients to trace the fate of water-column uptake (e.g., Mulholland et al. 1985; Tank et al. 2000) have shown that
assimilatory demand constitutes the majority of phosphate (PO$_4^{3-}$) (Mulholland et al. 1985), ammonium (NH$_4^+$) (Webster et al. 2003) and nitrate (NO$_3^-$) uptake (Mulholland et al. 2004) whereas dissimilatory processes constitute a smaller role in N uptake (Webster et al. 2003; Mulholland et al. 2004). Although assimilatory processes may slow the downstream transport of DIN and inorganic P, they do not represent permanent removal because mineralization of organic forms will ultimately return the inorganic nutrients to the water column, albeit this could occur after export from a stream reach. Despite a smaller relative role in total N uptake, dissimilatory N transformations have been shown to be coupled in more pristine, low-N systems (Kemp and Dodds 2002) whereby nitrification converts NH$_4^+$ to NO$_3^-$, and denitrification permanently removes N from the stream by converting NO$_3^-$ to gaseous N. Therefore a complete understanding of dissolved inorganic N uptake and removal requires insight into the relative rates of dissimilatory N transformations, which can be influenced by seasonal changes in oxygen abundance and sediment redox conditions (Christensen et al. 1990; Nielsen et al. 1990), in addition to patterns of whole-stream N uptake, which integrates both assimilatory and dissimilatory processes.

We studied how land use, season, and dissimilatory N transformations influence whole-stream demand for NH$_4^+$ and NO$_3^-$. We identified 3 categorical land-use types based on dominant land cover (agricultural, forested, and urban) in our study basin, the Kalamazoo River of southwest Michigan, and we incorporated seasonal dynamics by sampling during three biologically important periods over the 3-year study: 1) spring (late April), prior to leaf emergence when we predicted high autotrophic activity in our study streams, 2) summer (late August), during base flow when we expected high stream
temperatures to maximize biological activity, and 3) autumn (late November), after leaf-fall when we expected high heterotrophic activity during microbial conditioning and decomposition of leaf litter. Thus, there were 3 samplings per year. To compliment short-term nutrient additions conducted in the field, we measured nitrification and denitrification rates using laboratory assays under optimum redox conditions to identify an upper bound on the contribution by dissimilatory N transformations to whole-stream N uptake rates.

We made three general predictions based on our study design. First, we predicted highest relative nutrient demand (i.e., $V_f$, defined in methods) in the spring due to higher autotrophic activity prior to leaf-out, and we also expected seasonality to be less significant in agricultural and urban streams because of year-round open canopies and higher light levels in streams dominated by human land use. Second, we predicted that higher nutrient concentrations in agricultural and urban streams would saturate areal uptake rates (i.e., $U$, defined in methods) and decrease relative nutrient demand ($V_f$). Third, we predicted that dissimilatory N transformations would account for a relatively small fraction of areal N uptake, but that nitrification would be more important in the spring due to algal production increasing benthic oxic habitat, whereas denitrification would be more important in the autumn when microbial respiration on decomposing leaf litter would draw down oxygen levels in the sediments as well as within decaying leaf material.
4.3 Methods

4.3.1 Land-use Classification

We studied 18 headwater streams located in the Kalamazoo River Basin of southwest Michigan (Figure 4.1), a basin with heterogeneous land cover. All streams were low-gradient and dominated by fine sediments and sand, typical of Upper Midwestern streams. Using ArcGIS 8.2 (ESRI, Redlands, California, USA) to analyze land cover data downloaded from the National Land Cover Database (reclassified

![Figure 4.1. Study sites in the Kalamazoo River basin, southwest Michigan.](image)
Landsat TM imagery from 1992, Vogelmann et al. 2001), we grouped streams as agricultural, urban, or forested, depending on which land cover dominated their sub-basin. Subsequent field reconnaissance ground-truthed the results of the GIS analysis, and we selected 6 streams from each of the 3 categories (Table 4.1). Study catchments ranged in size from 79-3639 ha, and average annual NH$_4^+$ concentrations (4-115 µg N L$^{-1}$) were much lower than NO$_3^-$ (14-17,496 µg N L$^{-1}$) (Table 4.1). Because our study basin was not heavily urbanized, urban streams represented a suburban-urban gradient, and some agricultural and urban streams had more riparian vegetation than others. Thus, we sampled the land cover variability among headwater sub-basins of the Kalamazoo basin by selecting streams dominated by a particular land cover but having a range of riparian conditions.

4.3.2 Nutrient Releases

We measured whole-stream uptake of NH$_4^+$ and NO$_3^-$ using short-term (<1 h) additions of a solution containing the reactive solute and a conservative tracer (Webster and Ehrman 1996). Prior to the releases, we sampled background solute concentrations at each of 10 stations distributed along the study reach selected for each stream (50-250 m depending on travel time). To conduct the uptake measurement, we pumped a solution of NH$_4$Cl or NaNO$_3$ with a conservative tracer (rhodamine WT, Br$^-$ as NaBr, or Cl$^-$ as NaCl) at a constant rate into the stream. We minimized interactions of the DIN species by adding NH$_4^+$ and rhodamine first and allowing time for the added solutes to leave the study reach before conducting the NO$_3^-$ release simultaneously with Br$^-$. The short-term nutrient enrichment method of measuring whole-stream uptake can overestimate uptake.
TABLE 4.1.

LAND-USE CHARACTERISTICS AND NUTRIENT CHEMISTRY (±1 SE) IN THE STUDY BASINS

<table>
<thead>
<tr>
<th>Stream</th>
<th>Basin area (ha)</th>
<th>Land-use category (year of study)</th>
<th>Basin</th>
<th>100-m Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Forest+ Wetland (%)</td>
<td>Agricultural (%)</td>
<td>Urban (%)</td>
</tr>
<tr>
<td>Allegan</td>
<td>127</td>
<td>Urban (2)</td>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>Arcadia</td>
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<td>Urban (2)</td>
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<td>40</td>
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<tr>
<td>Axtell</td>
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<td>Urban (3)</td>
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<td>10</td>
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<tr>
<td>Bellevue</td>
<td>528</td>
<td>Forested (1)</td>
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<td>55</td>
</tr>
<tr>
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<td>Forested (2)</td>
<td>64</td>
<td>28</td>
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<td>Burnips</td>
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<td>Dorr</td>
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<td>Urban (1)</td>
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</tr>
<tr>
<td>Ellis</td>
<td>366</td>
<td>Agricultural (3)</td>
<td>22</td>
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<td>Richland</td>
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<td>Agricultural (2)</td>
<td>26</td>
<td>73</td>
</tr>
<tr>
<td>Shelbyville</td>
<td>154</td>
<td>Agricultural (2)</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Stream</td>
<td>Basin area (ha)</td>
<td>Land-use category (year of study)</td>
<td>Basin area (ha)</td>
<td>Land-use category (year of study)</td>
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</tr>
<tr>
<td>Sherman</td>
<td>260</td>
<td>Agricultural (2)</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Silver Creek</td>
<td>79</td>
<td>Forested (3)</td>
<td>97</td>
<td>3</td>
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<tr>
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<td>70</td>
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<td>Spring Brook</td>
<td>358</td>
<td>Forested (2)</td>
<td>49</td>
<td>49</td>
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<tr>
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<td>Urbandale</td>
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<td>Weber</td>
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<td>Forested (3)</td>
<td>48</td>
<td>52</td>
</tr>
</tbody>
</table>
length ($S_w$), thereby underestimating uptake velocity ($V_f$) and uptake rate ($U$) (Mulholland et al. 2002), but we minimized this effect by targeting an increase of 10-20 µg NH$_4^+$-N. However, when NO$_3^-$ concentrations were high (> 1 mg L$^{-1}$), we increased NO$_3^-$ up to 200 µg N L$^{-1}$ so that we could reliably measure a downstream decline. For conservative tracers, we targeted an enrichment of 10 µg rhodamine WT L$^{-1}$, 50 µg Br$^-$/L$^{-1}$, or 20 µSeimens cm$^{-1}$ for Cl$^-$. When the conservative tracer reached a constant concentration throughout the reach (i.e., plateau concentration), we took stream-water samples at the 10 sampling locations downstream.

After measuring nutrient concentrations in the laboratory (methods described below), we calculated nutrient uptake length ($S_w$) using the linear form of the exponential model:

$$\ln N_x = \ln N_0 - ax$$  \hspace{1cm} (4.1)

where $N_0$ is nutrient concentration at the injection site, $N_x$ is nutrient concentration at $x$ m downstream, and $a$ is the m$^{-1}$ uptake rate (Newbold et al. 1981). We used the conservative tracer to correct plateau nutrient concentrations for dilution, and we estimated the parameter $a$ in eqn. (1) by plotting the dilution-corrected tracer nutrient concentrations versus distance from the injection site. We calculated $S_w$ as $-a^{-1}$ (Newbold et al. 1981), which represents the average distance (m) traveled by a nutrient molecule before uptake from the water column. However, $S_w$ is sensitive to stream depth and velocity so variations in stream size can confound inter-site or temporal comparisons of $S_w$ (Davis and Minshall 1999). Therefore, we calculated uptake velocity ($V_f$) as:

$$V_f = Qa/w$$  \hspace{1cm} (4.2)
where $Q$ is stream discharge ($m^3 \text{min}^{-1}$) and $w$ is wetted width (m) (Stream Solute Workshop 1990). We calculated $Q$ by balancing the mass of conservative tracer released (Webster and Ehrmann 1996) and measured wetted width at 10 transects in the study reach. Uptake velocity (units, $m \text{min}^{-1}$), the velocity at which a nutrient is drawn from the water column toward the benthos, represents nutrient demand relative to water-column concentration, and it can be compared among streams because it is normalized for stream size. Areal uptake rate ($U$, mg $m^{-2} \text{min}^{-1}$) is calculated as:

$$U = V_f N_b$$ (4.3)

where $N_b$ is background nutrient concentration in the study reach, and $U$ represents the areal flux of nutrients from the water column into the streambed. Hereafter, we use “relative nutrient demand” to refer to $V_f$, which we use for comparisons among streams and season, and we use “whole-stream nutrient uptake” to refer to $U$, which we use in Michaelis-Menten models to investigate the potential for nutrient uptake saturation. Because stream size varied across sites and over seasons, we do not use uptake length ($S_u$) as a comparative nutrient spiraling metric (Davis and Minshall 1999).

4.3.3 Dissimilatory N Transformations: Nitrification Assays

We measured dissimilatory N transformation rates (i.e., nitrification and denitrification) in the laboratory to compare with whole-stream NO$_3^-$ and NH$_4^+$ uptake rates, which represent a combination of assimilatory and dissimilatory processes. We estimated sediment nitrification rates using the nitrapyrin-inhibition method (Hall 1984; Kemp and Dodds 2001; Strauss et al. 2004). This procedure is described in detail in Arango and Tank (in review), but we summarize the methods briefly here. Within one
week of the whole-stream nutrient releases, we collected sediment cores and stored them on ice until we returned to the laboratory and immediately began the assays. In paired sets of flasks (5 pairs per stream), we made 75-mL slurries by adding 25 mL of sediment and 50 mL of unfiltered site water. One flask in the pair (the production flask) received 10 µL of a 10% solution of nitrapyrin dissolved in dimethyl sulfoxide (DMSO), which blocks the conversion of NH$_4^+$ to NO$_3^-$. The other flask (the control flask) received 10 µL of DMSO, and nitrification was not blocked with nitrapyrin. We incubated the assays on a rotary shaker at 150 rpm for 24-48 h, and we terminated the assay by adding 25 mL of 2M KCl and shaking for an additional 10 min to flush NH$_4^+$ from binding sites. We centrifuged the entire slurry and froze the filtered supernatant for future NH$_4^+$ analysis (methods described below). We calculated nitrification rate (average of 5 paired flasks per stream) as the difference in NH$_4^+$ between the production and control flasks, which we scaled by the mass of sediment and the assay length (units: µg N g$^{-1}$ DM h$^{-1}$) and multiplied by sediment standing stock in each reach (g DM m$^{-2}$) to convert to an areal nitrification rate (mg N m$^{-2}$ h$^{-1}$) for comparison to whole-stream areal N uptake (i.e., $U$).

Using eqn. 3, we calculated relative demand for NH$_4^+$ via nitrification ($V_f$) (Royer et al. 2004).

We recognize that nitrification rates measured using this method are likely to be higher than ambient rates because we removed redox-limitation by measuring them in oxygenated slurries (Strauss et al. 2004). However, they do not represent maximum potential rates because we did not incubate them with amended NH$_4^+$, but instead used ambient NH$_4^+$ contained in stream water and sediment at the time of sediment collection.
4.3.4 Dissimilatory N Transformations: Denitrification Assays

We estimated denitrification rates in the laboratory using the chloramphenicol-amended acetylene-block method (Smith and Tiedje 1979; Royer et al. 2004; Inwood et al. 2005; Arango et al. 2007). Acetylene (C$_2$H$_2$) blocks N$_2$ production by denitrifiers, allowing N$_2$O to accumulate, and the antibiotic chloramphenicol restricts the microbial response to ideal redox conditions by preventing new enzyme production (Brock 1961; Smith and Tiedje 1979; Royer et al. 2004). Again, this procedure is described in detail in Arango and Tank (in review), but we summarize the methods briefly here. We collected sediment and made slurries as described for nitrification assays above, except we amended slurries with chloramphenicol (final concentration 0.3 mM) before incubating them in bottles with septum caps for headspace gas sampling. We purged each headspace with ultra high purity helium (He) to induce anoxia, returned the bottles to ambient atmospheric pressure, and injected 15 mL of C$_2$H$_2$ into the bottles resulting in a 10% atmosphere of C$_2$H$_2$. We collected 5 headspace samples for N$_2$O analysis throughout the 4.25 h incubation, with the first sample taken 0.25 h after adding C$_2$H$_2$ and the remaining samples taken at 1 h intervals. Before taking each headspace sub-sample, we shook the bottles to equilibrate dissolved gases with the headspace and water, and then we removed a 5 mL sub-sample, and injected 4 mL into a pre-evacuated glass vial. We maintained a constant pressure in the assay bottles by replacing each sub-sample with 5 mL of 10% C$_2$H$_2$ in He balance.

We measured N$_2$O concentration in headspace gas samples by manually injecting 100 µL into a Varian Star 3600 gas chromatograph (Palo Alto, California, USA) with a Porapak Q column and electron capture detector (injector temp = 120 °C, column temp =
40 °C, detector temp = 320 °C, with a 5% CH₄/95% Ar carrier gas at 30 mL min⁻¹). We used Bunsen coefficients to calculate total N₂O produced in the bottle, plotted N₂O production versus time, and calculated N₂O production rate as the slope of the line of best fit (r²>0.92, indicating linear N₂O production rates). We divided the N₂O production rate by the mass of sediment in the assay bottle and length of the assay to calculate sediment denitrification rates (units, µg N g⁻¹ DM h⁻¹), which we multiplied by benthic sediment standing stock in each reach (g DM m⁻²) to convert denitrification to an areal rate to compare to \( U_{NO₃} \). Using eqn. 3, we calculated relative NO₃⁻ demand via denitrification (\( V_{D,DEN} \)) (Royer et al. 2004).

Again, we acknowledge that denitrification rates we report may be higher than ambient rates because we removed redox-limitation by inducing anoxia in the slurries (Groffman et al. 2006), but denitrification rates measured in the assays do not represent maximum potential rates because we did not amend incubations with NO₃⁻ or organic C. Instead, assays were conducted at ambient stream-water NO₃⁻ or organic C levels at the time of sediment collection (e.g., Royer et al. 2004; Inwood et al. 2005).

4.3.5 Organic Matter Standing Stocks and Substratum Distribution

We used a stratified random sampling design to quantify organic matter standing stocks of sand, fine benthic organic matter (FBOM), leaves, and seasonal macrophytes and benthic algae. Sand and FBOM were the most common habitats, making up 80% ± 3 (SE) of the benthos. From 10 locations within each reach, we selected a benthic area with 100% cover of each substratum and we sampled FBOM, leaves, and macrophytes with a 475 cm² core, and sand and benthic algae with a 30 cm² core. We dried sub-samples of organic matter to constant weight at 60°C and measured dry mass, and then
we combusted ground samples for 3 h at 550°C, rewetted and redried them to constant weight at 60°C and measured mass after ashing. We calculated organic matter content as the difference between dry mass and ash free dry mass. We scaled substratum standing stocks to the stream reach by weighting each substratum according to its proportional abundance, which we estimated by recording benthic cover at 10 cm intervals along 10 transects equally-spaced throughout each study reach (Hoellein et al. in press).

Additionally, we extracted chlorophyll-\(a\) (chl-\(a\)) from sand and FBOM using the hot ethanol method (Sartory and Grobbelaar 1984) and a Turner Designs TD-700 fluorometer (Sunnyvale, California, USA) at 436 nm excitation and 680 nm emission wavelength.

4.3.6 Water Chemistry

We collected filtered (1 \(\mu\)m nominal pore size; Pall A/E, East Hills, New York, USA) background and plateau water samples in acid-washed HDPE bottles triple-rinsed with filtered stream water. We measured \(\text{NH}_4^+\) concentrations (from short-term nutrient releases and nitrification assays) on a Shimadzu UV-1601 spectrophotometer (Columbia, Maryland, USA) at 630 nm using the phenate method (Solorzano 1969; APHA 1995), and we quantified SRP at 885 nm using the molybdate method (Murphy and Riley 1962; APHA 1995). Concentrations of \(\text{NO}_3^-\) and \(\text{Br}^-\) (USEPA 1993) were measured simultaneously using a Dionex 600 ion chromatograph with AS14A analytical and guard columns and ED50 electrochemical detector (Sunnyvale, California, USA). Rhodamine WT was quantified in the lab using a Turner Designs TD-700 fluorometer (Sunnyvale, California, USA) at 530 nm excitation and 555 nm emission wavelengths. Finally, we measured background and plateau specific conductivity (YSI EC-300 conductivity probe; Yellow Springs, OH, USA) in the field when we used NaCl as a conservative tracer.
4.3.7 Statistical Analyses

We normalized data that did not meet the assumptions of parametric statistics using log transformation or log followed by power transformation. We used a one-way analysis of variance (ANOVA) to identify significant differences in canopy closure among our study streams (SYSTAT 11; San Jose, California, USA) and repeated measures ANOVA (rmANOVA) (SAS 9.1; SAS Institute Inc., Cary, North Carolina, USA) to detect seasonal and land-use differences in chl-\(\alpha\) biomass, leaf standing stocks and \(V_f\) among the study streams. We identified significant differences between \(V_{f-NH_4}\) and \(V_{f-NO_3}\) using a paired \(t\)-test. We used simple and multiple linear regressions (SYSTAT 11; San Jose, California, USA) to identify the independent variables that were related to organic matter standing stocks and nutrient demand, and we used non-linear regressions (SigmaPlot 10.0; San Jose, California, USA) to fit Michaelis-Menten uptake models to the measured nutrient uptake rates.

4.4 Results

4.4.1 Seasonal and Land-use Controls on Stream Predictors

We predicted that the riparian zone would control seasonal variations in light and organic matter, which in turn would drive variability in solute uptake, so we analyzed how summer canopy closure and seasonal patterns of benthic chl-\(\alpha\) and leaf litter varied by land use. Forested streams had greater summer canopy closure than urban streams (one-way ANOVA, \(P=0.047\), Fig. 4.2a), with channelized, agricultural streams having intermediate values due to moderate shading from heavy grass cover and steep slopes. Despite significant differences in canopy closure among land uses, when data were
Figure 4.2. Summer shading and standing stocks of chlorophyll-\(a\) and leaf litter (±1 SE) in the study streams. (A) Forested streams had more riparian shading than urban streams. (B) Each season had significantly different chlorophyll-\(a\) standing stocks, which were highest in spring and lowest in autumn. (C) Leaf litter was highest in autumn, and lowest in summer and spring.
pooled across seasons, there were no land-use differences in chl-\(a\) or leaf litter standing stocks (Figures 4.2b and 4.2c). However, we did find higher chl-\(a\) in spring (rmANOVA, \(p<0.0001\)) and higher leaf litter standing stocks (rmANOVA, \(P=0.0003\)) in autumn among streams, indicating that seasonality had stronger control than land use over benthic cover and organic matter in these headwater streams.

4.4.2 Seasonal and Land-use Influence on Relative Nutrient Demand (\(V_f\))

We predicted higher relative N demand (\(V_f\)) in the spring due to increased autotrophic uptake associated with open canopies, and we predicted that urban and agricultural streams would have less seasonality in \(V_f\) because a year-round lack of shading associated with open riparian canopies would stimulate autotrophic activity. We observed highest relative NH\(_4^+\) demand (\(V_{f,NH4}\)) in the spring (rmANOVA, \(P=0.02\)), but we found no differences among land uses (rmANOVA, \(P=0.12\)) (Figure 4.3a). Contrary to our expectations, urban and forested streams were more similar to each other with highest \(V_{f,NH4}\) in the spring and lowest in autumn. In contrast, agricultural streams had lowest \(V_{f,NH4}\) in the summer, probably because grasses in the channel shaded the bottom and curtailed autotrophic activity. We found no seasonal (rmANOVA, \(P=0.10\)) or land use (rmANOVA, \(P=0.79\)) differences in relative NO\(_3^-\) demand (\(V_{f,NO3}\)) (Figure 4.3b). Among streams and seasons, \(V_{f,NH4}\) exceeded \(V_{f,NO3}\) (paired \(t\)-test, \(P=0.0001\), data not shown), indicating higher relative demand for NH\(_4^+\) compared to NO\(_3^-\), not surprising given that NO\(_3^-\) concentrations were consistently higher than NH\(_4^+\), sometimes by orders of magnitude (Table 4.1).
Figure 4.3. Seasonal patterns in relative nutrient demand ($V_f$) (±1 SE) among streams of different land use. (A) Relative NH$_4^+$ demand ($V_{f,NH4}$) was highest in spring and lowest in fall but did not differ by land use. (B) Relative NO$_3^-$ demand ($V_{f,NO3}$) does not differ among seasons or land uses.

4.4.3 Relationship between N Concentrations and Whole-stream $U$ and $V_f$

Land-use activity frequently increases nutrient concentrations in streams; a linear relationship between areal uptake rates ($U$) and background nutrient concentrations indicates that nutrient uptake and diffusion are proportional whereas a hyperbolic relationship (i.e., Michaelis-Menten) indicates that uptake saturates with increasing concentration (Dodds et al. 2002). For areal uptake of NH$_4^+$ ($U_{NH4}$) and NO$_3^-$ ($U_{NO3}$), both linear and Michaelis-Menten (MM) models were significant, but MM models had a
better fit, suggesting saturation of uptake among streams and seasons (Figures 4.4a and 4.4b). However, the MM models only explained 33-43% of the variability, indicating that biological factors also contributed to the spatio-temporal variation in whole-stream uptake. Higher NH$_4^+$ concentrations in urban streams (Table 4.1) and higher NO$_3^-$ concentrations in agricultural streams (Table 1) saturated $U_{\text{NH}_4}$ and $U_{\text{NO}_3}$, respectively, indicating that land use affected areal uptake by influencing inorganic N concentrations (Figures 4.4a and 4.4b).

We confirmed uptake saturation by plotting $V_f$ versus nutrient concentration, which showed negative relationships for relative demand for NH$_4^+$ ($V_{f\text{NH}_4}$) and NO$_3^-$ ($V_{f\text{NO}_3}$) and indicated decreased uptake efficiency at higher concentrations (Figures 4.4c and 4.4d). The linear relationship between relative NH$_4^+$ demand ($V_{f\text{NH}_4}$) and NH$_4^+$ concentration is insignificant ($P=0.053$), and the linear model fits better than an exponential decay function ($P=0.10$), which is expected given that abiotic sorption dynamics are likely important for NH$_4^+$ (Davis and Minshall 1999). Although the relationship between relative NO$_3^-$ demand ($V_{f\text{NO}_3}$) and NO$_3^-$ concentration appears to follow an exponential decay pattern, which we expected given that NO$_3^-$ is not strongly affected by abiotic sorption processes (Davis and Minshall 1999), we could not fit an exponential decay function to these data ($r^2=0.02, P=0.27$). Our NO$_3^-$ concentrations span a range not previously reported in the literature, and clearly $V_{f\text{NO}_3}$ declines with increasing availability.
Figure 4.4. Relationship between nutrient concentrations and areal uptake ($U$) and relative nutrient demand ($V_f$). (A) Areal NH$_4^+$ uptake ($U_{NH4}$) plateaus at 17.7 mg N m$^{-2}$ h$^{-1}$ with a half-saturation constant ($K_s$) of 63.5 µg NH$_4^+$-N L$^{-1}$. (B) Areal NO$_3^-$ uptake ($U_{NO3}$) plateaus at 475.7 mg N m$^{-2}$ h$^{-1}$ with $K_s=2155.9$ µg NO$_3^-$-N L$^{-1}$. (C) Relative NH$_4^+$ demand ($V_{fNH4}$) varies widely at low concentration, and a linear model fits better than a negative exponential model ($r^2=0.05, P=0.10$). (D) Although relative NO$_3^-$ demand ($V_{fNO3}$) appears to decline exponentially, a negative exponential model does not fit ($r^2=0.02, P=0.27$).
4.4.4 Relationship between N Concentrations and Dissimilatory N Demand

We examined the relationship between relative NH$_4^+$ demand via nitrification ($V_{fNIT}$) and NH$_4^+$ concentration, which declined exponentially ($r^2=0.23$, $P=0.003$; Figure 4.5a) and suggested saturation of $V_{fNIT}$ with increasing NH$_4^+$ concentrations, which were

![Graph A](image1.png)

![Graph B](image2.png)

Figure 4.5. Relative NH$_4^+$ demand via nitrification ($V_{fNIT}$) versus NH$_4^+$, and NO$_3^-$ concentrations versus nitrification rate ($U_{NIT}$). (A) Relative NH$_4^+$ demand via nitrification declines exponentially. (B) Stream-water NO$_3^-$ concentrations are not related to production of NO$_3^-$ via nitrification rates ($U_{NIT}$).
generally higher in urban streams. Because nitrification rates ($U_{\text{NIT}}$) can contribute NO$_3^-$ to the water column, we plotted them versus NO$_3^-$ concentration but did not find a significant positive relationship, probably because NO$_3^-$ loading related to land-use activity had stronger control over NO$_3^-$ concentrations.

High water-column NO$_3^-$ concentrations can increase denitrification rates, and we found that uptake of NO$_3^-$ via denitrification ($U_{\text{DEN}}$) saturated as NO$_3^-$ concentration increased ($r^2=0.61$, $P<0.0001$; Figure 4.6a), with agricultural streams more likely to be saturated than urban or forested streams due to their higher NO$_3^-$ concentrations. We also found that relative NO$_3^-$ demand via denitrification ($V_{f,\text{DEN}}$) declined exponentially with increasing NO$_3^-$ concentrations ($r^2=0.51$, $P<0.001$; Figure 4.6b), confirming saturation of $V_{f,\text{DEN}}$.

4.4.5 Relationship between Dissimilatory N Transformation and Inorganic N Uptake

We used multiple linear regressions (MLR) to identify relationships between whole-stream relative nutrient demand ($V_f$) and benthic organic matter standing stocks, land use in the basin and in riparian buffers, and dissimilatory uptake parameters (both $U$ and $V_f$ for nitrification and denitrification). Relative NH$_4^+$ demand ($V_{f,\text{NH4}}$) was positively related to relative NH$_4^+$ demand via nitrification ($V_{f,\text{NIT}}$) and negatively related to canopy cover (MLR, $R^2=0.33$, $P<0.0001$) (Table 2), but it was not related to total organic matter standing stocks, organic matter compartments (i.e., FBOM v. leaves), or any metric of land use. Because relative NH$_4^+$ demand ($V_{f,\text{NH4}}$) was highest in the spring (Figure 3.3a), the relationships identified in the MLR suggest that autotrophy influences nitrification rates. Relative NO$_3^-$ demand ($V_{f,\NO3}$) was negatively related only to nitrification rates ($U_{\text{NIT}}$, $r^2=0.13$, $P=0.01$) (Table 2), suggesting that NO$_3^-$ produced in the sediments via
nitrification may reduce relative NO$_3^-$ demand from the water column. The lack of a relationship between relative NO$_3^-$ demand ($V_{fNO3}$) and denitrification suggests that denitrification is not a significant component of whole-stream NO$_3^-$ dynamics.

We compared nitrification rates ($U_{NIT}$) to whole-stream areal NH$_4^+$ uptake ($U_{NH4}$) to identify the relative magnitudes of these processes. Nitrification rates
TABLE 4.2.
FACTORS CONTROLLING INORGANIC NUTRIENT DEMAND AMONG STREAMS AND SEASONS.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Independent Variable</th>
<th>Correlation</th>
<th>Partial r²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{f,NH4}$</td>
<td>$V_{f,NH4}$ via nitrification</td>
<td>+</td>
<td>0.16</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Canopy closure</td>
<td>-</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$V_{f,NO3}$</td>
<td>Nitrification rate (mg N m⁻² h⁻¹)</td>
<td>-</td>
<td>0.13</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Note: Multiple linear regression model for analysis of $V_{f,NH4}$ is $R²=0.33, P<0.0001$.

accounted for 53% ± 11 (SE) of whole-stream NH₄⁺ uptake (Figure 4.7a), but our laboratory assays for measuring nitrification rates probably overestimated ambient rates. Because nitrification converts NH₄⁺ to NO₃⁻, nitrification rates ($U_{NIT}$) are equivalent to NO₃⁻ production rates, so we compared $U_{NIT}$ to NO₃⁻ consumption via denitrification ($U_{DEN}$) to understand the potential for dissimilatory N metabolism to affect whole-stream NO₃⁻ uptake ($U_{NO3}$). Redox-optimized nitrification rates measured with assays sometimes exceeded denitrification and vice versa, but the rates were of the same magnitude and nearly always balanced (Figure 4.7b). Rates of nitrification ($U_{NIT}$) and denitrification ($U_{DEN}$) did not differ among seasons as we predicted, but whole-stream $U_{NO3}$ always exceeded $U_{NIT}$ and $U_{DEN}$ by at least an order of magnitude. Even under optimum redox conditions, nitrification produced NO₃⁻ equivalent to 3.3% ± 0.5 (SE) of whole-stream NO₃⁻ uptake whereas denitrification permanently removed NO₃⁻ equivalent to 2.5% ± (0.3) SE of whole-stream uptake. Therefore, dissimilatory N transformations
Figure 4.7. Rate of dissimilatory N transformations compared to whole-stream uptake (±1 SE). (A) Areal NH₄⁺ uptake ($U_{NH4}$) exceeds NH₄⁺ uptake via nitrification ($U_{NIT}$). (B) Areal NO₃⁻ uptake ($U_{NO3}$) exceeds NO₃⁻ uptake via denitrification ($U_{DEN}$) by nearly two orders of magnitude among streams and seasons (±1 SE). Nitrification, symbolized as the hatched bar, produces NO₃⁻, but it is included to emphasize the balance between nitrification and denitrification under optimum redox conditions.
did not contribute substantially to whole-stream NO$_3^-$ dynamics, which were dominated by assimilatory demand among streams and seasons.

4.5 Discussion

4.5.1 Land-use-mediated, In-stream Autotrophy as a Determinant of Inorganic N Demand ($V_f$)

Higher relative NH$_4^+$ demand ($V_{f,NH4}$) in the spring (Figure 4.3a) confirmed our prediction that in-stream autotrophic activity would stimulate N demand among streams in the spring. Primary producers are important determinants of NH$_4^+$ demand in streams with high light levels such as in desert (Webster et al. 2003), prairie (Dodds et al. 2000), and tundra (Peterson et al. 1997) environments, and in forested streams with logged riparian zones (Sabater et al. 2000). However, autotrophy can also be important in the spring in temperate forested streams prior to leaf emergence when high light levels cause a peak in assimilatory N demand by primary producers (Simon et al. 2005; Hoellein et al. in press). Relative NH$_4^+$ demand ($V_{f,NH4}$) was not related to indicators of heterotrophic demand as organic matter standing stocks, whether expressed at the reach scale or as individual compartments (e.g., FBOM vs. leaf standing stock). In contrast, we found higher relative NH$_4^+$ demand ($V_{f,NH4}$) with less canopy closure (Table 4.2), suggesting an autotrophic basis for NH$_4^+$ demand across all seasons, in addition to the spring peak we observed. The unstable, fine sediments (i.e., sand and FBOM) that dominated the benthos in our study streams did not provide enough stability for filamentous algae or macrophytes, which have previously been identified as important determinants of $V_{f,NH4}$ (Bernot et al. 2006). However, sand and FBOM had highest chl-$a$ content in the spring.
(Figure 4.2b), indicating that diatoms colonizing fine particles were an important component of the algal community that probably drove patterns in $V_{f,NH4}$.

Urban streams trended toward higher relative NH$_4^+$ demand ($V_{f,NH4}$) compared to other land uses we studied, but not enough to produce a significant land-use difference in $V_{f,NH4}$ (Figure 3.3a). Lower riparian canopy coverage in urban streams compared to agricultural and forested streams probably caused the trend toward higher relative NH$_4^+$ demand ($V_{f,NH4}$) due to a stronger influence of autotrophic activity, which has been shown in other urban streams (Grimm et al. 2005; Meyer et al. 2005). Despite the importance of autotrophy in urban streams, frequent scouring flows due to increased impervious surface cover export algae (Grimm et al. 2005) and FBOM (Meyer et al. 2005), resulting in lower $V_{f,NH4}$ compared to more pristine streams with unmodified hydrology. However, land-use changes can sometimes cause a subsidy-stress biological response (Allan 2004), where an initial positive response turns negative at higher levels. The urban streams we studied were much less urbanized (22.8% of basin as urban land cover; Table 4.1) than those studied by Meyer et al. (2005; 64.6-79.3%), and we may have identified a stimulating response of light urbanization that augmented NH$_4^+$ demand by increasing primary productivity through riparian clearing.

The seasonal pattern in relative NO$_3^-$ demand ($V_{f,NO3}$) in agricultural and urban streams suggested an autotrophic influence because of higher $V_{f,NO3}$ in the spring (Figure 4.3b), but we found no seasonal differences because forested streams had high variability and a seasonal pattern that diverged from the other streams. Recent studies indicate the importance of NO$_3^-$ as a whole-stream N source when autotrophic activity is high (Hall and Tank 2003; Fellows et al. 2006; Mulholland et al. 2006). However, substratum-
specific (Munn and Meyer 1990; Kemp and Dodds 2002) and whole-stream (Martí and Sabater 1996) studies frequently show that NH$_4^+$ is the first choice as an N source. In our study streams, high N demand by autotrophic production in the spring probably cannot be met solely by the low concentrations of NH$_4^+$, so NO$_3^-$ supplements autotrophic demand. The lack of a strong seasonal signature in relative NO$_3^-$ demand ($V_{f,NO3}$) among our study streams probably reflects the consistently high NO$_3^-$ concentrations (>2 mg N L$^{-1}$ averaged among streams) and the preference for using a moderate supply of NH$_4^+$ (overall average 37 $\mu$g N L$^{-1}$) as an primary N source, which would both mask patterns in NO$_3^-$ demand.

In general, we anticipated that land use would mediate seasonal patterns in the relative demand for inorganic N (as $V_f$), with agricultural and urban streams exhibiting less seasonality than forested streams due to open canopies year-round, resulting in greater autotrophic influence through the year. Although seasonal patterns were influenced by land use, it was not in the way we expected. For example, relative NH$_4^+$ demand ($V_{f,NH4}$) in forested and urban streams paralleled the seasonal pattern in chl-$\alpha$, which was highest in spring and lowest in autumn, but agricultural streams had lowest $V_{f,NH4}$ in summer despite having more open canopies than forested streams. In the upper Midwest, most low-order agricultural streams are shallow and have low water velocities at summer base flow. In combination with the high light levels in these streams, channel geometry and hydrology encourage the growth of grasses on the channel edges, which ultimately restrict light availability during summer.
4.5.2 Land-use Influence on Saturation of Whole-stream Uptake

Because land-use activity frequently increases nutrient concentrations in streams (Carpenter et al. 1998), we predicted that urban and agricultural streams would show saturation of inorganic N uptake more than forested streams, and we tested our prediction by regressing $U$ and $V_f$ against water-column N concentrations. Although uptake kinetics are more appropriately used to examine the uptake response to different nutrient concentrations in one stream (Dodds et al. 2002), comparing across streams and time periods has been used effectively to investigate saturation kinetics from a regional or seasonal perspective (Simon et al. 2005; Newbold et al. 2006; Hoellein et al. in press). Our data set is bolstered by the additional component of varying land use which expands the concentration range further than has been examined previously. Among streams and seasons, areal NH$_4^+$ uptake ($U_{NH4}$) and NO$_3^-$ uptake ($U_{NO3}$) saturated with increasing concentrations, and saturation varied by land use, with higher NH$_4^+$ concentrations in urban streams saturating $U_{NH4}$ and higher NO$_3^-$ concentrations in agricultural streams saturating $U_{NO3}$.

Relationships between $U$ and nutrient concentration were explained better by Michaelis-Menten (MM) models as opposed to linear models for areal uptake of inorganic N ($U_{NH4}$ and $U_{NO3}$), yet the variability explained by MM models was relatively low (Figure 4.4a and b). This is not surprising as the total variability in areal N uptake among streams and seasons is a combination of nutrient concentration and relative nutrient demand (i.e., $V_f$, see eqn. 3), which varies throughout the year (Newbold et al. 2006). Therefore the variability in $U_{NH4}$ and $U_{NO3}$ not explained by N concentration was
probably due to previously discussed influences of autotrophy on $V_f$ as well as additional ecosystem metrics we did not measure such as ecosystem respiration.

Saturation of biological uptake is indicated when streams exhibit MM dynamics, but decreased uptake efficiency at higher nutrient concentrations (i.e., $V_f$ declines with increasing concentration) confirms saturation of biological demand (Dodds et al. 2002). Additionally, when abiotic sorption also removes solutes from the water column (e.g., NH$_4^+$ or PO$_4^{3-}$) then $V_f$ should decline linearly with increasing nutrient concentration, particularly at low concentrations. When a solute is not influenced by abiotic sorption (e.g., NO$_3^-$), $V_f$ declines exponentially with increasing nutrient concentration (Davis and Minshall 1999). We observed a significant ($P=0.05$) linear decline in $V_f$-NH$_4^+$ (Figure 4.4c), and although we observed lower $V_f$-NO$_3^-$ at higher NO$_3^-$ concentrations (Figure 4.4d), the apparent exponential decline could not be described with a negative exponential model. Because biological demand for NH$_4^+$ was saturated across the concentration range found in our study streams, we were able to calculate the half-saturation constant ($K_s$) for NH$_4^+$ at ${\sim}64\ \mu g\ NH_4^+-N\ L^{-1}$, which is somewhat higher than that from Simon et al. in low-nutrient New Zealand streams (2005; 0.5-19.0 $\mu g\ NH_4^+-N\ L^{-1}$). The higher saturation values we report probably reflects higher NH$_4^+$ concentrations in our mixed land-use study streams compared to the pristine grassland streams studied by Simon et al. (2005).

Areal NO$_3^-$ uptake ($U_{NO_3}$) reached a significant plateau (Figure 4.4b), but we did not see an associated statistically significant decline in relative NO$_3^-$ demand ($V_f$) (Figure 4.4d) that would allow us to attribute the plateau in $U_{NO_3}$ to lower relative nutrient demand. However, the half-saturation constant ($K_s=2,156\ \mu g\ NO_3^--N\ L^{-1}$) was orders of
magnitude above Simon et al. (2005; $K_s=1.4 \mu g N L^{-1}$) because our streams had substantially higher NO$_3^-$ concentrations. Taken together, the $K_s$ values we report are much different than those found in the literature, and they suggest that the capacity for whole-stream N uptake can increase considerably, presumably through biological changes, in response to chronically high NO$_3^-$ concentrations, despite uptake becoming inefficient at higher concentrations.

4.5.3 Land-use Influence on Saturation of Dissimilatory N Transformations

Water-column concentrations of NH$_4^+$ and NO$_3^-$ can also influence benthic nitrification and denitrification (e.g., Kemp and Dodds 2002), so we analyzed patterns in relative NH$_4^+$ demand via nitrification ($V_{FNIT}$). As NH$_4^+$ concentration increased, relative NH$_4^+$ demand via nitrification decreased exponentially (Figure 4.5a), which is consistent with saturation of biological demand. Although abiotic sorption dynamics typically cause NH$_4^+$ to decline linearly with relative demand (Davis and Minshall 1999), the exponential decay relationship may indicate saturation of sediment binding sites at high pore-water NH$_4^+$ concentrations, which would cause NH$_4^+$ to behave non-linearly with respect to relative NH$_4^+$ demand ($V_f$) because it is driven by biology.

We also examined the relationship between NO$_3^-$ produced via nitrification ($U_{NIT}$) and water-column NO$_3^-$ concentrations, which was not significant (Figure 4.5b) due to land-use practices that increased NO$_3^-$ availability (Inwood et al. 2005). Because denitrification requires NO$_3^-$, increasing concentrations typically increase denitrification rates (Bernot and Dodds 2005). We observed saturation of denitrification rates ($U_{DEN}$) at high NO$_3^-$ concentrations (Figure 4.6a), as have other studies (Seitzinger 1988; García-Ruiz et al. 1998a), and we confirmed saturation of biological demand with a significant
exponential decay model (Figure 4.6b). Agricultural streams with high NO₃⁻ concentrations were the only ones in the saturated portion of the curve, and the MM curve predicted maximum denitrification at 11.4 mg N m⁻² h⁻¹, within the range of the theoretical maximum $U_{DEN}$ reported in Bernot and Dodds (2005; 4.2-20.8 mg N m⁻² h⁻¹). Our data suggest that denitrification capacity in agricultural streams was near the maximum possible level, and was probably limited by organic C as a reductant or overall anoxic habitat (Arango et al. 2007). Additionally, the high coefficient of determination for $U_{DEN}$ ($r^2=0.61$, Figure 4.5b) indicates that NO₃⁻ concentration is a better predictor of denitrification compared to whole-stream $U_{NO3}$ ($r^2=0.43$), probably because denitrification requires NO₃⁻ whereas whole-stream N demand can be met by NH₄⁺, NO₃⁻, or DON, which would add variability to the relationship between NO₃⁻ concentration and $U_{NO3}$.

4.5.4 Contribution of Dissimilatory N Transformations to Whole-stream N Uptake

Among streams and seasons, areal NH₄⁺ uptake via nitrification ($U_{NIT}$) accounted for about half of whole-stream NH₄⁺ uptake ($U_{NH4}$) (Figure 4.7a), which was within the range identified by an interbiome comparison using $^{15}$NH₄⁺ tracer additions (3-60% of whole-stream uptake) (Peterson et al. 2001), and was also very close to the proportion identified by Hamilton et al. (2001) in a nearby Michigan stream (57%). Although we interpret our nitrification rates cautiously because we measured them in the laboratory under oxic conditions, they compare well with nitrification rates measured in situ using isotopic tracer methods. Nitrification converts N from NH₄⁺, whose sorption dynamics may promote its removal from the water column as well as biotic uptake, to NO₃⁻, a form that is more mobile in sediments and travels longer distances in streams before uptake.
Consequently, nitrification, if high enough, can add to chronically high NO$_3^-$ loads and exacerbate downstream N exports (Bernot and Dodds 2005). In the more N-rich streams, any NH$_4^+$ converted to NO$_3^-$ via nitrification in our streams may be exported far downstream prior to uptake because whole-stream NO$_3^-$ uptake ($U_{NO3}$) and NO$_3^-$ uptake via denitrification ($U_{DEN}$) approach saturation at high N concentrations (Figures 4.4b and 4.6a) as a result of increased N availability via human land use.

Areal NO$_3^-$ removal rates ($U_{NO3}$) measured using short-term, whole-stream additions reflect the balance between assimilatory uptake (i.e., temporary NO$_3^-$ storage) denitrification (i.e., permanent NO$_3^-$ removal), and nitrification (i.e., NO$_3^-$ production), but few studies have measured each of these processes. We recognize that lab assays of nitrification and denitrification were measured under optimum redox conditions, but note that inorganic N availability represented ambient conditions. Additionally, whole-stream NO$_3^-$ uptake lengths ($S_u$) are likely slightly longer because we used short-term nutrient additions, which may underestimate $U_{NO3}$ (Mulholland et al. 2002). Therefore, comparing these rates represents a best-case scenario in the potential for dissimilatory N transformations to affect whole-stream NO$_3^-$ dynamics. Under optimum redox conditions, sediment nitrification and denitrification rates approximately balanced each other (Figure 4.7b), suggesting that site-specific factors controlling sediment redox potential will determine net production or removal of NO$_3^-$ in the benthos. For example, diel oxygen changes may shift the sediment from net production of NO$_3^-$ in the day, when increasing oxygen stimulates nitrification, to net consumption at night, when increasing respiration stimulates denitrification (Risgaard-Petersen et al. 1994). Additionally, seasonal changes could influence sediment redox potentials, favoring nitrification in the
spring when autotrophic activity peaks and denitrification in the autumn when heterotrophic activity peaks (Christensen et al. 1990).

The streams we studied had high NO$_3^-$ concentrations due to land-use activity, so we asked whether the benthos is a net producer of NO$_3^-$ via nitrification or a net consumer of NO$_3^-$ via denitrification. Our data indicate that dissimilatory transformation rates were a small fraction of areal whole-stream NO$_3^-$ uptake ($U_{NO3}$) because nitrification and denitrification were always 1 to 2 orders of magnitude less than $U_{NO3}$ (Figure 4.7b), emphasizing the importance of assimilatory uptake in the removal of inorganic N from the water column which dominated $U_{NO3}$ in our study streams (Figure 4.8). A whole-stream $^{15}$NO$_3^-$ tracer experiment using much more accurate and much more expensive methods than ours confirmed that assimilatory demand controlled NO$_3^-$ uptake and that $U_{DEN}$ was a relatively small fraction (16% compared to our data showing 2.5%) of whole-stream $U_{NO3}$ (Mulholland et al. 2004), the same result found in 72 $^{15}$NO$_3^-$ tracer experiments conducted in 8 biomes of varying land use across North America (Mulholland et al. in review). Although we calculate assimilatory $U_{NO3}$ as the fraction of areal NO$_3^-$ removal not accounted for by denitrification, this fraction cannot be solely attributed to assimilatory demand. Other processes such as dissimilatory NO$_3^-$ reduction to NH$_4^+$ (DNRA) or NO$_3^-$ reduction coupled to iron oxidation, may account for an unknown fraction of $U_{NO3}$, but we know comparatively little about these processes in the context of total dissimilatory NO$_3^-$ uptake (Burgin and Hamilton 2007).
Figure 4.8. Whole-stream nitrogen (N) uptake processes. Numbers represent rates (mg N m\(^{-2}\) h\(^{-1}\)) of whole-stream areal uptake (\(U_{\text{NH4}}\) and \(U_{\text{NO3}}\)) and areal uptake via nitrification (\(U_{\text{NIT}}\)) and denitrification (\(U_{\text{DEN}}\)), averaged among land uses and seasons. Assimilatory demand dominates uptake of inorganic N, and \(U_{\text{NIT}}\) and \(U_{\text{DEN}}\) approximately balance.

Although assimilatory N demand is only temporary, it does slow the downstream flux of NO\(_3^-\) by removing it from the water column and transforming it into a particulate organic form (Mulholland 2004). Despite documenting the importance of assimilatory N demand in stream ecosystems, previous studies have rarely considered the ultimate fate of assimilated N. Scaling the total DIN uptake we measured (\(U_{\text{NH4}} + U_{\text{NO3}}=115\) mg N m\(^{-2}\) h\(^{-1}\)) to an annual rate corresponds to the production of nearly 1 kg organic N m\(^{-2}\) y\(^{-1}\). However, we did not observe significant accumulations of biomass over the year that we studied each of our streams, thus we assume little reach-scale organic matter storage.
Although organic matter produced in our study reaches could potentially be buried in particulate form *in situ*, given the apparent lack of accumulation of organic material, it is more likely exported downstream in dissolved form following mineralization, or exported downstream in particulate form to be later buried or mineralized elsewhere. Previous research has shown that episodic export of organic matter can dominate downstream material flux in streams (Meyer and Likens 1979; Royer and David 2005). Although we did not explicitly budget annual N stocks and fluxes in our streams, the lack of organic matter accumulation suggests that much of the assimilated N we measured was either exported or mineralized. Future studies that consider both the short- and long-term fate of assimilated N in streams will make valuable contributions to our overall understanding of stream N cycling.

4.5.5 Summary

In the mixed land-use streams we studied, seasonality exerted strong control over relative nutrient demand ($V_f$), which was highest in the spring when autotrophy was dominant. Although the land-use category of a stream was not a significant predictor of $V_f$, it did influence areal uptake rates of NH$_4^+$ and NO$_3^-$ because higher nutrient concentrations associated with agriculture and urbanization saturated biological demand. The half-saturation constants we found were quite different than those reported in the literature, reflecting the different ranges in nutrient concentrations that we studied and indicating that stream nutrient uptake rates can increase substantially under chronically high nutrient loads. Denitrification rates were saturated in agricultural streams and near a theoretical maximum (Bernot and Dodds 2005), implying that additional NO$_3^-$ loads could travel far downstream with minimal biological processing in agricultural streams.
with high NO$_3^-$ concentrations. Although nitrification and denitrification approximately balanced each other when measured under ideal redox conditions, both processes were small contributions to whole-stream NO$_3^-$ demand, which was dominated by assimilatory uptake. Our results suggest that assimilatory demand dominates nutrient uptake in headwater streams, ultimately controlling the capacity of streams to reduce downstream N flux, if only temporarily.

4.6 Acknowledgements

We thank the many private landowners who granted us access to their property. The Drain Commissions of Allegan, Barry, Calhoun, Eaton, and Kalamazoo Counties, the City of Kalamazoo, Allegan County Parks Department, and Fort Custer Training Center helped us coordinate site access. Jake Beaulieu, Denise Bruesewitz, Kathryn Docherty, Sally Entrekin, Natalie Griffiths, Jon Loftus, and Kris Premier provided assistance in the field and Rachel Clavers and Carrie DePalma provided assistance in the laboratory. CPA was funded by the Arthur J. Schmidt Presidential Fellowship and Bayer Predoctoral Fellowship during the execution of this study. Additional funding was provided by NSF-DEB 0111410.
CHAPTER 5
BENTHIC ORGANIC CARBON INFLUENCES DENITRIFICATION IN STREAMS
WITH HIGH NITRATE CONCENTRATION

5.1 Abstract

Anthropogenic activities have increased reactive nitrogen availability, and now many streams carry large nitrate (NO₃⁻) loads to coastal ecosystems. Denitrification is potentially an important nitrogen sink, but few studies have investigated the influence of benthic organic carbon on denitrification in NO₃⁻-rich streams. Using the acetylene-block assay, we measured denitrification rates associated with benthic substrata having different proportions of organic matter in agricultural streams in Illinois and Michigan. In Illinois, benthic organic matter varied little between seasons (5.9-7.0% of stream sediment), but NO₃⁻ concentrations were high in summer (>10 mg N L⁻¹) and low (<0.5 mg N L⁻¹) in autumn. Across all seasons and streams, the rate of denitrification ranged from 0.01-4.77 µg N g⁻¹ DM h⁻¹ and was positively related to stream-water NO₃⁻ concentration. Within each stream, denitrification was positively related to benthic organic matter only when NO₃⁻ concentration exceeded published half-saturation constants. In Michigan, streams had high NO₃⁻ concentrations and diverse benthic substrata which varied from 0.7-72.7% organic matter. Denitrification rate ranged from

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0.12-11.06 μg N g⁻¹ DM h⁻¹ and was positively related to the proportion of organic matter in each substratum. Taken together, these results indicate that benthic organic carbon may play an important role in stream nitrogen cycling by stimulating denitrification when NO₃⁻ concentrations are high.

5.2 Introduction

Reactive nitrogen (N) in the form of nitrate (NO₃⁻) and ammonium (NH₄⁺) has increased as a result of anthropogenic activities including fertilizer use and fossil fuel combustion (Galloway 1998). Anthropogenic activities contribute 140 Tg N y⁻¹ to the biosphere and have effectively doubled N availability compared to historic inputs (Vitousek et al. 1997b). Long term monitoring of the Mississippi River and the Gulf of Mexico illustrates the negative consequences of increased N availability in aquatic ecosystems, where excess anthropogenic N stimulates extensive algae blooms that senesce and decompose, causing large zones of hypoxia (Turner & Rabalais 1991; Rabalais et al. 2002).

Nonpoint sources of N, primarily contributed by agriculture, now exceed point sources as the largest contributor of N to U.S. surface waters (USEPA 1996; Goolsby et al., 2001). Precipitation transports nitrogenous fertilizer applied in excess of crop demand to aquatic ecosystems (Carpenter et al. 1998), leaching up to 80% of applied N into ground and surface waters (Howarth et al. 1996). Drainage tiles installed in agricultural fields expedite N export by rapidly draining NO₃⁻-rich subsurface runoff into streams (David et al. 1997; Petry et al. 2002). Feedlot runoff and leaky manure containment ponds associated with a high density of livestock may also increase water-
column N concentrations via nonpoint source pollution (Carpenter et al. 1998), which is difficult to regulate.

Denitrification can reduce in-stream N permanently by removing it from stream ecosystems as nitrous oxide (N\textsubscript{2}O) and dinitrogen (N\textsubscript{2}) gases (Alexander et al. 2000). A respiratory process used by facultatively anaerobic microbes, denitrification requires supplies of NO\textsubscript{3}\textsuperscript{-}, organic carbon (C), and the presence of anoxic habitat (Knowles 1982; Seitzinger 1988). In this dissimilatory metabolic pathway, organic C compounds serve as electron donors and NO\textsubscript{3}\textsuperscript{-} serves as an oxidant. Denitrifying microbes harness the energy released by the simultaneous oxidation of reduced C to CO\textsubscript{2} and the reduction of NO\textsubscript{3}\textsuperscript{-} to N\textsubscript{2}O and/or N\textsubscript{2}. Because denitrification is an anaerobic process, many studies have investigated how the concentration of dissolved oxygen mediates denitrification rates in streams (e.g., Duff et al. 1984; Christensen et al. 1990; Schaller et al. 2004), but other studies have emphasized the role of NO\textsubscript{3}\textsuperscript{-} supply in controlling denitrification rates (e.g., Holmes et al. 1996; Pattinson et al. 1998; Martin et al. 2001). The prevalence of denitrification studies in relatively low NO\textsubscript{3}\textsuperscript{-} streams (< 1 mg NO\textsubscript{3}\textsuperscript{-}-N L\textsuperscript{-}1) has emphasized how NO\textsubscript{3}\textsuperscript{-} supplies control denitrification at the expense of understanding the role of C in stream denitrification (but see Hedin et al, 1998; LeFebvre et al. 2004).

Furthermore, studies that have examined how C influences denitrification have focused on dissolved (DOC) rather than particulate organic C (POC), and POC can positively influence denitrification in two ways. First, denitrifiers are limited to using dissolved substances that can be actively or passively transported across their cell membranes, but abiotic leaching and exoenzyme activity can extract DOC from POC, providing denitrifiers with a C source for NO\textsubscript{3}\textsuperscript{-} reduction (Seitzinger 1988). Second, aerobic
decomposition of POC can influence denitrification by reducing oxygen concentrations and expanding the anaerobic habitat.

Because NO$_3^-$ is plentiful in agricultural streams in the Midwestern United States, the latter are ideal systems for studying how organic C influences denitrification. Previous research in Illinois and Michigan shows that the addition of NO$_3^-$ does not increase denitrification rate, suggesting C limitation and also that high stream-water NO$_3^-$ concentrations represent a fair proxy for NO$_3^-$ availability at the point of denitrification despite the likelihood that pore-water and stream-water concentrations differ. In the Midwestern United States, agricultural streams typically drain former wetlands, which contribute recalcitrant DOC to streams (Royer and David 2005), and the sandy sediments often entrain POC, which leaches DOC. Therefore, sediment POC (expressed as sediment organic matter; %OM) may represent a better measure of C availability, at the point of denitrification in the anoxic benthos, than stream-water DOC. Inorganic N concentration in agricultural streams in Illinois frequently exceeds 10 mg NO$_3^-$-N L$^{-1}$ in early summer and then decreases below 0.5 mg NO$_3^-$-N L$^{-1}$ in late summer and autumn, a pattern driven by interactions between agricultural practices, hydrology, and biotic demand (David et al. 1997; Royer et al. 2004), whereas sediment organic matter does not generally vary between summer and autumn. We predicted that the rate of denitrification in Illinois streams would be closely related to sediment organic matter only when NO$_3^-$ concentrations were high. Michigan streams have relatively high NO$_3^-$ concentrations (> 0.5 mg N L$^{-1}$) that do not vary seasonally, as in Illinois streams. However, Michigan streams have diverse POC sources, hereafter referred to as substrata, the organic fraction of which varies by nearly two orders of magnitude. Given these relatively high NO$_3^-$
concentrations, we predicted that denitrification rates in Michigan would be positively related to the organic fraction of the substrata across all streams.

5.3 Methods

5.3.1 Site Description

Our eight study streams were located in east-central Illinois and southwest Michigan, where row-crop agriculture dominates land use. The study streams, described in detail by Royer et al. (2004) and Inwood et al. (2005), have little riparian vegetation, are deeply channelized, and have flashy hydrographs. However, Michigan streams generally have more riparian vegetation than Illinois streams, and consequently receive more allochthonous organic matter. The study streams ranged from first to third order. As anticipated, Michigan streams had high stream-water NO$_3^-$ concentrations whereas NO$_3^-$ concentrations in Illinois streams were high during summer sampling but low during autumn (Table 5.1). Sediment organic matter in Illinois streams had low spatial variability whereas Michigan streams had patchy organic matter accumulations dominated by four substratum types that ranged in organic fraction (%OM) over two orders of magnitude: sand, fine benthic organic matter (FBOM, organic matter that passes through a 1 mm sieve but is retained by a 63 µm sieve), coarse benthic organic matter (CBOM, organic matter retained by a 1 mm sieve), and bacterial biofilms (Table 5.2). Bacterial biofilms were distinguished from FBOM by having a green/brown color.
### TABLE 5.1.

STREAM CHARACTERISTICS AT THE TIME OF SAMPLING

<table>
<thead>
<tr>
<th>Stream Name and Location</th>
<th>Stream Order</th>
<th>Width (m)</th>
<th>Stream Temperature (°C)</th>
<th>Discharge (L s⁻¹)</th>
<th>Nitrate (mg N L⁻¹)</th>
<th>DOC(mg C L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>Autumn</td>
<td>Summer</td>
<td>Autumn</td>
<td>Summer</td>
</tr>
<tr>
<td>Big Ditch (BDO), IL</td>
<td>2</td>
<td>8.6</td>
<td>6.6</td>
<td>16.8</td>
<td>19.8</td>
<td>1030</td>
</tr>
<tr>
<td>Black Slough (BLS), IL</td>
<td>1</td>
<td>3.3</td>
<td>1.9</td>
<td>17.7</td>
<td>21.1</td>
<td>324</td>
</tr>
<tr>
<td>Lake Fork, Kaskaskia R. (LFK), IL</td>
<td>3</td>
<td>14.1</td>
<td>9.6</td>
<td>26.3</td>
<td>21.3</td>
<td>1270</td>
</tr>
<tr>
<td>Sand Cr. (SNC), MI</td>
<td>1</td>
<td>X</td>
<td>0.4</td>
<td>X</td>
<td>X</td>
<td>9.9</td>
</tr>
<tr>
<td>Swan Cr. (SWC), MI</td>
<td>1</td>
<td>X</td>
<td>0.7</td>
<td>X</td>
<td>X</td>
<td>4.5</td>
</tr>
<tr>
<td>Black Cr. (BLC), MI</td>
<td>2</td>
<td>X</td>
<td>2.0</td>
<td>X</td>
<td>X</td>
<td>115</td>
</tr>
<tr>
<td>Dorr &amp; Byron Cr. (DBC), MI</td>
<td>3</td>
<td>X</td>
<td>2.7</td>
<td>X</td>
<td>X</td>
<td>279</td>
</tr>
<tr>
<td>Tributary to Little Rabbit R. (RAB), MI</td>
<td>1</td>
<td>X</td>
<td>1.5</td>
<td>X</td>
<td>X</td>
<td>16.4</td>
</tr>
</tbody>
</table>

Note: ‘X’ indicates data were not collected.
TABLE 5.2.
MEAN (±1 SE) SUBSTRATUM CHARACTERISTICS IN THE STUDY STREAMS

<table>
<thead>
<tr>
<th>State</th>
<th>Season sampled</th>
<th>Substratum type</th>
<th>Standing stock (g AFDM$^1$m$^{-2}$)</th>
<th>Benthic cover (%)</th>
<th>Organic matter (%)</th>
<th>Chl-$\alpha$$^2$ (µg cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL</td>
<td>Summer</td>
<td>Homogenous sediment</td>
<td>X</td>
<td>100</td>
<td>7.0 (0.2)</td>
<td>X</td>
</tr>
<tr>
<td>IL</td>
<td>Autumn</td>
<td>Homogenous sediment</td>
<td>X</td>
<td>100</td>
<td>5.9 (0.3)</td>
<td>X</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>Sand</td>
<td>69.5 (10.1)</td>
<td>36.2 (5.7)</td>
<td>0.7 (0.1)</td>
<td>2.3 (0.7)</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>Biofilm</td>
<td>84.8 (49.4)</td>
<td>5.1 (1.8)</td>
<td>9.9 (1.4)</td>
<td>3.3 (1.5)</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>CBOM$^3$</td>
<td>272.7 (79.1)</td>
<td>17.5 (3.6)</td>
<td>72.7 (2.3)</td>
<td>X</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>FBOM$^4$</td>
<td>343.4 (108.2)</td>
<td>40.6 (5.9)</td>
<td>18.0 (2.1)</td>
<td>7.5 (3.5)</td>
</tr>
</tbody>
</table>

Notes: $^1$ash free dry mass; $^2$chlorophyll-$\alpha$; $^3$coarse benthic organic matter; $^4$fine benthic organic matter; ‘X’ indicates data not sampled.

suggesting an autotrophic component. Previous research in these streams has demonstrated that NO$_3^-$ limits denitrification in Illinois only in autumn (Inwood et al. 2007; Wall et al. 2005). Together, these different conditions allowed us to assess the importance of benthic organic matter to in-stream denitrification in situations where NO$_3^-$ concentrations are high and unlikely to limit denitrification (Michigan streams and Illinois in summer); further, the seasonally distinct NO$_3^-$ concentrations in Illinois streams allowed us to compare the importance of benthic organic matter when NO$_3^-$ was low and high.

5.3.2 Field Sampling

We sampled Illinois streams twice in 2002, once in early summer when stream NO$_3^-$ concentrations are typically high and once in late summer/early autumn when
stream NO$_3^-$ concentrations are typically low (David et al. 1997; Royer et al. 2004). In each Illinois stream, we delineated a sampling grid of five transects spaced 12 m apart along a 50 m stream reach. At each transect we collected sediment cores at 0.25, 0.5, and 0.75 channel width by sampling the top 5 cm of the stream bottom using a 28 cm$^2$ corer. We sampled to a depth of 5 cm because previous studies found that > 90% of sediment denitrification occurs in the top 5 cm of the sediments in these streams (Inwood et al., 2007). Because Illinois stream sediments were visually uniform, we did not distinguish between sediment types and considered each core equally representative of the stream bottom. We sampled Michigan streams once in autumn 2003 but because the Michigan streams had patchy distributions of distinct benthic substrata, we used a stratified design to sample each substratum randomly from 10 locations within a 100-m stream reach. We sampled sand, FBOM, CBOM, and biofilms by selecting a known area with 100% cover of a given substratum type. We used cores to sample sand as described above, but we sampled CBOM by taking material off the stream bottom, and we sampled FBOM and biofilms using a turkey baster to suck the material off the stream bottom. Of the 10 samples for each substratum, we retained five for calculating substratum standing stock and pooled the remainder into one composite substratum sample from each stream for denitrification assays.

5.3.3 Denitrification Assays

We measured denitrification in the laboratory using the chloramphenicol-amended acetylene (C$_2$H$_2$) block method (Smith and Tiedje 1979; Royer et al. 2004; Inwood et al. 2005). Acetylene (C$_2$H$_2$) blocks the final step of the complete denitrification pathway allowing N$_2$O, which is more easily measured than N$_2$, to
accumulate in the assay bottles. Chloramphenicol is an antibiotic that inhibits de novo protein synthesis (Brock 1961) and reduces bottle effects associated with laboratory slurry incubations, allowing for more accurate estimates of in situ rates (Smith and Tiedje 1979). Sediments from Illinois streams were incubated at stream temperature, but Michigan substrata were incubated at room temperature to minimize variability not associated with substratum characteristics. We incubated four analytical replicates of each pooled sample in 125 mL media bottles. For Illinois stream sediments, and for sand, FBOM, and biofilm substrata from Michigan sites, we made slurries and added 25 mL to each bottle; CBOM samples were broken into small pieces to facilitate homogenization before adding them to each bottle (~10 g wet weight). Bottles were filled to 75 mL with chloramphenicol-amended site water for a final concentration of 5 mM chloramphenicol (Inwood et al. 2005; Schaller et al. 2004). Each bottle was sealed with a septum cap, purged with ultra high purity helium (He) to create anoxia, and vented to relieve excess pressure. We added 15 mL of C$_2$H$_2$ to three of the four assay bottles, and the fourth bottle received no C$_2$H$_2$ to control for background N$_2$O production. We incubated the bottles for 4 h and took four headspace samples throughout the incubation (0:15, 1:30, 2:45, 4:00 h). Prior to headspace sampling, we shook the bottles for 10 sec to equilibrate N$_2$O between the water and headspace. Using a 5 mL syringe, we took a 4 mL headspace sample from each bottle and immediately injected it into a 3.5 mL pre-evacuated vial. We returned the assay bottles to the original positive pressure by replacing the subsample with 4 mL of 10% C$_2$H$_2$ in He balance.

We analyzed headspace subsamples by manually injecting 100 µL into a Varian 3600 gas chromatograph (Palo Alto, CA, USA) equipped with a Porapak Q column,
electron capture detector, and a valve to vent C$_2$H$_2$ away from the detector (injector temp, 120° C; column temp, 40° C; detector temp, 320° C, ultra high purity N$_2$ carrier gas, 30 mL min$^{-1}$). Total concentration of N$_2$O at each sampling period was calculated using the appropriate Bunsen coefficient to determine the amount of gas dissolved in water at a given headspace concentration (see Inwood et al. 2005, for detailed equations). We plotted N$_2$O concentration against time and calculated denitrification rate as the slope of the line of best fit ($r^2 \geq 0.92$ for all rates). We expressed N$_2$O production rate as denitrification rate by converting N$_2$O to N and normalizing by substratum dry mass (DM) ($\mu$g N g$^{-1}$ DM h$^{-1}$). Many published denitrification rates are scaled only by stream-bottom surface area. To facilitate inter-study comparisons, we scaled our rates to area (mg N m$^{-2}$ h$^{-1}$) by multiplying DM normalized rates by substratum standing stocks (g m$^{-2}$). Because we measured denitrification in slurries, this may have somewhat over-estimated our rates compared to other studies that measured denitrification in situ or by using intact cores.

For rates measured in Michigan, we calculated nutrient spiralling metrics for denitrification using the methods of Royer et al. (2004). Briefly, we calculated the uptake velocity of NO$_3^-$ due to denitrification ($V_{f, \text{DEN}}$) as:

$$V_{f, \text{DEN}} = \frac{U}{C}$$  \hspace{1cm} (5.1)

where $U$ is the substratum denitrification rate scaled to area (mg N m$^{-2}$ s$^{-1}$) and $C$ is the stream-water NO$_3^-$ concentration (mg N m$^{-3}$). We calculated the loss rate (-$k$) of NO$_3^-$-N from the water column via denitrification as:

$$-k = \frac{V_{f, \text{DEN}}}{h}$$  \hspace{1cm} (5.2)

where $h$ is stream depth (m). We converted fraction of load s$^{-1}$ to % of load d$^{-1}$.  

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Subsamples of all substrata were dried at 60°C, weighed to obtain DM, ashed at 550°C and reweighed to obtain ash-free dry mass (AFDM). We calculated %OM of each subsample as the ratio of AFDM to DM, and this value represents mass lost upon ignition. We also combusted oven-dried samples in a Costech Elemental Analyzer (Valencia, California, USA) to measure substratum %C. To calculate total reach standing stock of each substratum, we multiplied substratum standing stock by its proportional abundance in the reach, determined by identifying benthic cover at 10 cm intervals along 10 regularly placed transects in each reach. For sand, FBOM and biofilm samples collected in Michigan, we retained a third set of subsamples for chlorophyll-a (chl-a) analysis. Chlorophyll-a was extracted using the hot ethanol method (Sartory & Grobbelaar, 1984). We measured chl-a using a Turner Designs TD-700 fluorometer (Sunnyvale, CA, USA) at 436 nm excitation wavelength and 680 emission wavelength, and we determined concentration using a calibration curve made from spectrophotometrically analyzed concentrated stock solution.

5.3.5 Water Chemistry

We used filtered water samples collected from each site to measure NO₃⁻-N and DOC. Water samples were filtered with 0.7 μm Whatman GF/F (Brentford, UK) glass fiber filters and collected in acid-washed bottles, pre-rinsed with site water, stored on ice in the field, and frozen upon return to the laboratory no later than 12 h after collection. Nitrate concentration was measured using a DIONEX 600 ion chromatograph (Sunnyvale, CA, USA) with ED50 electrochemical detector and AS14A guard and analytical columns (USEPA 1993). Dissolved organic carbon samples were acidified to
pH 2 and measured on a Shimadzu TOC-5000 analyzer (Columbia, MD, USA) using the
combustion-infrared method (APHA, 1995) or persulfate oxidation using a Dohrmann-
Xertex DC-80 analyzer (Sunnyvale, CA, USA) equipped with a Horiba infrared gas
analyzer (Model PIR-2000).

5.3.6 Statistical Analyses

Statistical analyses were performed using SYSTAT 11 (Systat Software Inc.,
Richmond, CA). To meet the assumptions of parametric statistics, we transformed non-
normal data using either a logarithmic, or logarithmic followed by power, transformation,
and statistical significance was determined at the 0.05 level. For Illinois streams sampled
in two seasons, we determined seasonal influence on stream-water NO₃⁻ and DOC
concentrations and sediment %OM using paired t-tests. We used simple linear
regressions to analyze relationships between denitrification rates and %OM within
streams, among streams and sampling periods, and between NO₃⁻ load removed via
denitrification and substratum %C. In the Michigan streams, we analyzed how
substratum type influenced denitrification rates and chl-a using one-way ANOVA. When
an ANOVA detected significant differences among means, we used Tukey’s post hoc
multiple comparison test to determine which means differed.

5.4 Results

5.4.1 Stream-water and Sediment Characteristics

We anticipated that all the Illinois sites would have NO₃⁻ concentrations below
0.5 mg N L⁻¹ during autumn sampling. Although site BLS had higher NO₃⁻ concentration
than expected, stream-water NO$_3^-$ concentrations were significantly higher in summer than in autumn in the Illinois streams ($P=0.047$) (Table 5.1). Stream-water DOC concentration did not vary between seasons, despite the high value at LFK in the autumn (Table 5.1). In Michigan streams, NO$_3^-$ concentration ranged from 0.4-6.4 mg N L$^{-1}$ and DOC concentration from 2.4-3.9 mg C L$^{-1}$ (Table 5.1).

Stream sediments in Illinois were a homogeneous mixture of sand, mud, and fine organic particles, and we did not differentiate among these sediments by constituent substratum pools. Although mean sediment %OM was relatively low in the summer and autumn, it was significantly higher in summer than in autumn ($P<0.001$) (Table 5.2). However, this seasonal difference (1.1%) was numerically small compared to the range of %OM found in Michigan streams (0.7-72.7%) (Table 5.2). Of the Michigan substrata, CBOM had the highest %OM while sand had the lowest ($P<0.001$), and FBOM and CBOM (both as g AFDM m$^{-2}$) had the highest standing stocks ($P=0.023$), whereas sand and biofilm had the lowest (Table 5.2). Sand and FBOM combined comprised 71-80% of the benthic cover (Table 5.2). Sand, FBOM, and biofilm each had an autotrophic component as measured by chl-$a$ biomass, but we detected no differences in chl-$a$ among substrata.

### 5.4.2 Denitrification Rates

We measured denitrification in three replicates of each substratum from each site, and the fourth replicate was a control for background N$_2$O production. Standard error of our denitrification measures averaged 15% of the mean within a substratum from a site (e.g., sand denitrification in SWC) compared to 30% of the mean among substrata from all sites (e.g., sand denitrification in all Michigan streams). This indicates greater
variability in denitrification on the same substratum among sites than within a substratum from a single site. The low variability within a site also suggests that our method for measuring denitrification is reasonably precise even with relatively low replication.

Denitrification rates were generally higher in summer compared to autumn in Illinois streams (1.50-4.77 compared to 0.01-1.00 µg N g⁻¹ DM h⁻¹) (Table 5.3) despite generally lower stream-water temperatures caused by the larger proportion of stream-flow originating from cool, sub-surface tile flow to the stream in summer. However, the difference in denitrification was marginally insignificant (P=0.061), probably due to small sample sizes (n=3 for each season). Overall, average denitrification rates on sediments were positively related to NO₃⁻ concentration among streams and between seasons in Illinois (r²=0.83, P=0.012) (Figure 5.1), but there was no relationship between denitrification rates and DOC concentrations. For a season within each Illinois stream, we regressed denitrification rate against sediment %OM. At NO₃⁻ concentrations < 1 mg N L⁻¹, there was no relationship between sediment %OM and denitrification, but at NO₃⁻
TABLE 5.3.
MEAN (±1 SE) SUBSTRATUM-SPECIFIC DENITRIFICATION RATES AMONG STREAMS

<table>
<thead>
<tr>
<th>State</th>
<th>Season sampled</th>
<th>Substratum type</th>
<th>Denitrification (µg N g⁻¹ DM h⁻¹)</th>
<th>Denitrification (mg N m⁻² h⁻¹)</th>
<th>Nitrate load removed by denitrification; -k (% d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL</td>
<td>Summer</td>
<td>Homogenous sediment</td>
<td>3.0 (0.4)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IL</td>
<td>Autumn</td>
<td>Homogenous sediment</td>
<td>0.4 (0.2)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>Sand</td>
<td>0.1 (0.0)</td>
<td>2.0 (0.9)</td>
<td>10.2 (3.8)</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>Biofilm</td>
<td>2.3 (0.6)</td>
<td>1.7 (0.8)</td>
<td>15.1 (4.9)</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>CBOM</td>
<td>11.1 (2.6)</td>
<td>3.8 (1.7)</td>
<td>39.4 (18.1)</td>
</tr>
<tr>
<td>MI</td>
<td>Autumn</td>
<td>FBOM</td>
<td>4.2 (1.5)</td>
<td>8.5 (3.8)</td>
<td>118.0 (61.4)</td>
</tr>
</tbody>
</table>

Note: n=3 in IL, n=5 in MI. ‘X’ indicates data were not collected.
concentrations > 1 mg N L\(^{-1}\) there was generally a positive effect on denitrification rate from increased sediment %OM (Figure 5.2).

In the Michigan streams, denitrification rates varied significantly among substrata \( (P<0.001) \), with CBOM supporting the highest rate and sand the lowest when normalised by DM (Table 3). In general in Michigan, denitrification rate increased with substratum %OM \( (P<0.001, r^2=0.77; \text{Figure 5.3}) \). We found a positive relationship between water-column NO\(_3\)^\(-\) concentrations and denitrification on CBOM \( (\text{mg N m}^{-2} \text{ h}^{-1}; P=0.032, r^2=0.83, \text{data not shown}) \) and between water-column NO\(_3\)^\(-\) concentration and denitrification on biofilms \( (\mu\text{g N g}^{-1} \text{ AFDM h}^{-1}; P=0.023, r^2=0.86, \text{data not shown}) \), but we found no such relationships with denitrification rates on sand or FBOM. Substratum-
Figure 5.2. Relationship between denitrification rate and sediment organic fraction in Illinois streams. Plots a) - f) correspond to the datum with the same letter (a-f) in Figure 5.1: a) LFK, autumn; b) BDO, autumn; c) BLS, autumn; d) LFK, summer; e) BLS, summer; and f) BDO, summer.
Figure 5.3. Denitrification rates (± 1 SE) in Michigan streams in relation substratum organic fraction. n=5 for each datum.

Specific denitrification rates were not significantly related to water-column DOC concentration for any substratum and, although substratum-specific $V_{fDEN}$ did not vary among substrata (despite ranging widely: 10.2-118.0%; Table 5.3), it was positively related to substratum %C ($P$=0.035, $r^2$=0.22, Figure 5.4). For each stream, we calculated a whole-stream denitrification rate by multiplying the substratum-specific denitrification rate ($\mu$g N g$^{-1}$ DM h$^{-1}$) by the substratum standing stock (g m$^{-2}$) and then weighting the resulting areal rate (mg N m$^{-2}$ h$^{-1}$) by the proportional abundance of the substratum. The summation of the weighted rates yields an estimate of the whole-stream denitrification rate. We found no relationship between whole-stream denitrification and either water-column NO$_3^-$ concentration or DOC concentration.
Figure 5.4. Removal rate of stream-water nitrate via denitrification (±1 SE) in relation to substratum carbon content in Michigan streams. n=3 for each datum

5.5 Discussion

5.5.1 Comparison of Denitrification Rates

Denitrification can be measured by different methods, which can confuse cross-study comparisons. We used C$_2$H$_2$-inhibition, which can underestimate actual denitrification when NO$_3^-$ is low and denitrification is strongly coupled with nitrification (Seitzinger et al. 1993). Therefore, for better comparability, we compared our substratum-specific rates with previously published C$_2$H$_2$-inhibited rates. Furthermore, we measured denitrification with slurries, which generally give higher estimates than intact cores, so we used chloramphenicol to reduce our over-estimation of actual values (Smith and Tiedje 1979). When making comparisons, we also note when we compare our slurry-measured rates to those from intact cores.
Among Michigan streams, substratum-specific denitrification rates varied over two orders of magnitude, indicating that some substrata were “hotspots” of denitrification (sensu McClain et al. 2003). Our measures were similar to the few studies that also reported substratum-specific denitrification rates, even though none reported the entire suite of substrata that we measured. For example, denitrification on sand was identical to García-Ruíz et al. (1998b) at 2.0 mg N m\(^{-2}\) h\(^{-1}\), though they used intact cores. Denitrification on FBOM (27.4 µg N g\(^{-1}\) AFDM h\(^{-1}\)) was lower than reported by Bonin et al. (2003; 57 and 100 µg N g\(^{-1}\) AFDM h\(^{-1}\)), but they measured denitrification potential (i.e., C\(_2\)H\(_2\)-inhibited assays amended with NO\(_3^-\) and DOC), which likely increased denitrification relative to our unamended assays. Using the same method as ours, Triska and Oremland (1981) measured a denitrification rate of 0.66 µg N g\(^{-1}\) DM h\(^{-1}\) on decomposing periphyton communities; the biofilm we sampled was a similar substratum due to the autotrophic presence mixed with detrital material, but we measured somewhat higher rates (2.30 µg N g\(^{-1}\) DM h\(^{-1}\)). Each substratum had physical characteristics (e.g., particle size) and a chemical composition (e.g., C content) that likely enhanced or diminished denitrification, and the similarity of our results to other studies suggests that substrata may have intrinsically different denitrification rates among biomes.

Whereas a particular substratum may support inherently high denitrification rates, the absolute magnitude probably depends primarily on site-specific variability in the controls of denitrification. For example, denitrification in Michigan streams was highest on FBOM and CBOM (Table 3), and Kemp and Dodds (2002) reported the same using intact cores in Kansas streams. However, our denitrification rates are several orders of magnitude higher than theirs (8.5 and 3.8 mg N m\(^{-2}\) h\(^{-1}\) compared to 0.004 and 0.005 mg
N m$^{-2}$ h$^{-1}$ on FBOM and CBOM respectively). Differences between Kansas and Michigan streams may explain this disparity. Streams in Michigan had higher NO$_3^-$ (0.42-6.4 mg N L$^{-1}$) than in Kansas (0.005-0.774 mg N L$^{-1}$), which would likely cause higher denitrification rates in Michigan. Also, Michigan streams are largely heterotrophic (Hamilton et al. 2001) compared to autotrophic Kansas streams (Dodds et al. 2000), so primary producers may outcompete denitrifiers for NO$_3^-$ (Kemp and Dodds 2002) or may repress denitrification by pumping oxygen into the benthos (Rysgaard et al. 1994).

We scaled our substratum-specific denitrification rates to a whole-stream rate using habitat-weighting so we could compare our rates to many other published data. Whole-stream rates we calculated from slurries probably overestimate denitrification, but our data compare with those from many denitrification studies in streams from widely differing biomes (Figure 5.5), despite methodological differences. Because some benthic substrata supported higher denitrification rates than others, the habitat-weighting technique probably provided a reasonable whole-stream measure of \textit{in situ} denitrification rates in the patchy Michigan streams. As an indirect estimate of whole-stream denitrification, habitat-weighting incorporates more error than a direct measure. However, given sufficient replication within each substratum and thorough characterization of the habitat-weighting factors, it should provide a reasonable estimate of whole-stream denitrification.

5.5.2 Factors Controlling Denitrification Rates

Denitrification requires NO$_3^-$, organic carbon and anoxic conditions (Knowles 1982; Seitzinger 1988), and comparing among biomes suggests that denitrification rate
increases across a broad range of NO$_3^-$ concentrations (Figure 5.5). Denitrification is a metabolic process, however, and is subject to saturation with respect to reactants (i.e., NO$_3^-$ and C); we can therefore analyze denitrification rate using uptake kinetics.

Examined in this context, denitrification will approach saturation when NO$_3^-$ concentrations exceed the half-saturation constant. A study using intact cores to measure denitrification found half-saturation constants between 0.18-1.27 mg NO$_3^-$-N L$^{-1}$ along a river continuum (García-Ruiz et al. 1998a), and a study using slurries in estuarine environments found half-saturation constants between 0.38-0.74 mg NO$_3^-$-N L$^{-1}$
Others have used a two-dimensional Kolmogorov-Smirnov test (Garvey et al. 1998) to determine a threshold concentration beyond which factors other than NO₃⁻ control denitrification. These thresholds, 0.4 mg NO₃⁻-N L⁻¹ in small streams (Inwood et al. 2005) and 0.9 mg NO₃⁻-N L⁻¹ in a river and its receiving reservoir (Wall et al. 2005), were within the range of half-saturation constants measured using intact cores. Because the thresholds were measured using C₂H₂-block slurries, they are directly comparable with our data. In our study, stream-water NO₃⁻ concentrations equalled or exceeded these threshold values (but see BDO and LFK in autumn, Table 5.1), suggesting NO₃⁻ saturation of denitrification, but we found a positive relationship between denitrification and NO₃⁻ concentration among streams and sampling periods in Illinois (Figure 5.1), suggesting NO₃⁻ limitation of denitrification. Although these results appear contradicting, they depict two distinct relationships among the data. First, among streams and seasons, denitrification rates are positively related to NO₃⁻ concentration (Figure 5.1). Second, within a season at a given stream, denitrification is positively related to the sediment organic fraction only when NO₃⁻ is high (Figure 5.2).

Denitrification may also be limited by the presence of anoxic microhabitats, which are created by the aerobic decomposition of organic matter. Anoxia often occurs deep in stream sediments (e.g., Holmes et al. 1996; Morrice et al. 2000; LeFebvre et al. 2004) and is related to whether advection or diffusion drive oxygen flux into the benthos. In the sand-bottom streams we studied, Tank (unpublished) found an anoxic layer just below the first few mm of sediment. This suggests that the active zone of denitrification begins immediately below the sediment surface, and the shallow depth to anoxia may be related to fine particles that slow penetration of oxygenated stream water into the benthos.
(Vervier et al. 1992; Pretty et al. 2006). Anoxia may additionally occur in particle-associated microsites in an otherwise oxic environment (Sørensen et al. 1979; Sakita and Kusuda 2000). We measured denitrification on substrata (i.e., FBOM, CBOM, and biofilm) taken from the oxic streambed surface. Although using anoxia to measure denitrification in the laboratory exposes these substrata to radically different conditions than in the field, the high denitrification rates we measured demonstrate the potential for denitrification on anoxic microsites. These microsites may supplement the denitrification that occurs at depth in the sandy stream sediments, increasing overall denitrification capacity in these streams.

Denitrifying bacteria are heterotrophic microbes that use DOC as a C substrate for assimilatory and dissimilatory metabolism, so they may also be limited by organic C. We define two distinct forms of organic C in our study streams: dissolved and particulate organic C (DOC and POC, respectively). Dissolved organic C was < 0.7 μm, the mesh size we used to filter the water in which we measured DOC, whereas POC was >63 μm diameter and included FBOM and CBOM. Studies from soils (e.g., Burford and Bremner 1975; Jandl and Sollins 1997), riparian zones (Hill et al. 2000), and streams (Inwood et al. 2005) have shown positive relationships between DOC and denitrification. Denitrification assays done in similar streams to ours found that denitrification was C limited when NO$_3^-$ concentrations were high (Inwood et al. 2007). However, we did not observe a relationship between benthic denitrification rates and stream-water DOC in any of our streams, suggesting that sediment POC is a more important C source for denitrifiers than stream-water DOC.
Sediment POC produces DOC directly via abiotic leaching and indirectly via microbial degradation with exoenzymes (Fenchel et al. 2000) or exudates from shredding macroinvertebrates (Meyer and O’Hop 1983). We found a strong relationship between denitrification rates and substratum %OM across a range of high NO$_3^-$ concentrations in the Michigan streams (Figure 5.3), and when NO$_3^-$ was high in Illinois streams (Figure 5.2). We also found that stream-water NO$_3^-$ load removed via denitrification was positively related to substratum %C content (Figure 5.4), with FBOM and CBOM generally removing more NO$_3^-$ than sand or biofilms (Table 5.3). Sediment POC may facilitate denitrification by leaching DOC or by creating more extensive anoxic habitat via heterotrophic decomposition. Particulate organic C amendments have stimulated denitrification in NO$_3^-$-rich groundwater (Schipper and Vojvodić-Vuković 2002) and in marine sediments (Dahllof and Karle 2005) although the mechanism for increased denitrification (i.e., increased C availability as an electron source or increased anoxic habitat) was not identified. However, POC has been used as the sole C source for denitrification in wastewater treatment applications (e.g., van Oostrom and Russell 1994; Rocca et al. 2005), demonstrating the feasibility of POC as an electron source for denitrification. The relationships we found between denitrification and substratum %OM, a metric of POC, but not between denitrification and whole-stream DOC indicate that POC plays an important role in regulating stream denitrification when NO$_3^-$ concentrations are high. However, more research is required to identify when POC influences denitrification via direct or indirect mechanisms.

These results have important implications when considering streams as ecosystems that may attenuate NO$_3^-$ export to downstream water bodies (e.g., Alexander
et al. 2000; Peterson et al. 2001). To promote drainage from agricultural landscapes, management agencies commonly clear riparian vegetation and remove woody debris from streams to hasten water flow (Allan and Flecker 1993). Practices that promote C removal from agricultural streams may accentuate already high NO$_3^-$ concentrations typical of these systems by limiting the denitrification potential of the stream. Engineers manipulate C levels to optimize NO$_3^-$ removal in remediation settings (e.g., van Oostrom and Russell 1994; Schipper and Vojvodić-Vuković 2002), and the same principles could apply to optimize denitrification in agricultural streams with chronically high NO$_3^-$ concentrations. Allowing large organic matter (e.g., LWD) to accumulate in agricultural streams would create more heterogeneous flow conditions and generate settling zones in which FBOM and CBOM could accumulate. These organic matter accumulations could provide a C source for denitrifiers and may enhance the spatial extent of anoxia in stream sediments, further stimulating denitrification.

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CHAPTER 6

HERBIVORY BY AN INVASIVE SNAIL INCREASES NITROGEN FIXATION IN A NITROGEN-LIMITED STREAM

6.1 Abstract

Despite anthropogenic contributions that have doubled nitrogen (N) flux into the biosphere, biological N fixation still accounts for nearly half of new biosphere N inputs. N fixation is rarely studied in streams because low autochthonous production due to shading and/or high inorganic N concentrations may prohibit photo-autotrophic N fixation. However, many western North American streams have high light levels and low inorganic N concentrations, and N fixation can be an important source of bioavailable N. Plant-animal interactions can control algal biomass and productivity in streams with abundant algae, but it is not known how herbivory may affect N fixation. In Polecat Creek, Wyoming, the invasive New Zealand mudsnail (*Potamopyrgus antipodarum*) dominates the N cycle. Using this as our model system, we experimentally reduced *Potamopyrgus* biomass on periphyton-covered rocks and we measured N fixation *in situ* using acetylene-reduction chamber assays one and two weeks after exclusion. We found highest N fixation rates at highest (i.e., representing ambient) snail biomass when N fixation was expressed per unit chlorophyll-a (two-way ANOVA, $P=0.002$). Although

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5This chapter (with co-authors Jennifer L. Tank, Leslie A. Riley, and R. O. Hall, Jr.) is being prepared for journal submission.
snail herbivory changed the relative composition of the algal assemblage by reducing green algal taxa and increasing N-fixing diatoms, which were dominated by *Epithemia* spp., the absolute abundance of N-fixing algal cells did not vary among snail treatments. Therefore, higher snail densities increased biomass-specific N fixation by increasing nitrogenase efficiency. Incorporating our N fixation rates into a previously published whole-stream N budget for Polecat Creek, we found that N flux into the periphyton was 50% higher when we included previously unmeasured N fixation. Herbivory by an invasive snail can increase whole-stream N fixation by changing the composition of, and controlling resource availability to, the algal community in N-limited streams.

6.2 Introduction

Industrial nitrogen (N) fixation has perturbed the global N cycle by doubling N flux into the biosphere (Vitousek et al. 1997b), but biological N fixation still represents nearly half the global N flux. On a global scale, biological N fixation in terrestrial ecosystems exceeds that in marine ecosystems by nearly an order of magnitude (Schlesinger 1997), and of the aquatic ecosystems where N fixation has been well-studied (e.g., lakes, estuaries, and oceans), N fixation contributes the largest N inputs to eutrophic lakes (Howarth et al. 1988). Among aquatic ecosystems, N fixation has received little attention in streams, where conditions often inhibit N fixation. For example high N loading from atmospheric deposition and/or land-use activities puts N-fixing algae at a competitive disadvantage in many streams (Howarth et al. 1988). Also, many well-studied stream ecosystems are forested (e.g., Hubbard Brook, NH; Coweeta Hydrologic Laboratory, NC; Walker Branch, TN), and low light levels limit the growth of algae in general, including N fixers (Horne and Carmiggelt 1975; Grimm and Petrone 1997).
However in unshaded stream systems, like many in the western United States where vegetation is sparser, N fixation is a more significant source of bioavailable N, as these streams frequently have lower water-column N:P ratios (Grimm and Fisher 1986) and higher light levels (Minshall 1978).

Previous studies of N fixation in stream ecosystems have demonstrated the importance of temperature (Marcarelli and Wurtzbaugh 2006), light (Horne and Carmiggelt 1975; Grimm and Petrone 1997), and phosphorus (P) availability (Marcarelli and Wurtzbaugh 2006) as controls on N fixation, but to our knowledge, no published studies have examined how herbivory might control N fixation in streams. Grazers can regulate periphyton by reducing algal biomass (e.g., Lamberti and Resh 1983; Mulholland et al. 1991) and changing algal community composition (e.g., Steinman et al. 1987; Rosemond et al. 1993), and it follows that rates of N fixation could be influenced as well (Figure 6.1). For example, grazing can selectively remove N-fixing taxa (e.g., Tuchman and Stevenson 1991), which could decrease N fixation rates. Alternatively, grazers may avoid the N-fixing *Nostoc* spp. because heavy sheath material and microcystin-like toxins make them unpalatable (Dodds et al. 1995), in which case N fixation could increase due to this selective herbivory. Indiscriminant grazers, like snails, could increase or decrease N fixation depending on grazing intensity. Heavy grazing pressure could decrease N fixation on an area-specific basis, as it does for periphyton
primary production (Mulholland et al. 1991; Hill et al. 1992; but see Lamberti et al. 1989 for an exception). Conversely, moderate grazing could reduce self-shading and improve nutrient flow to the periphyton, increasing N fixation rates on a biomass-specific basis (i.e., increased nitrogenase efficiency), much as moderate grazing tends to enhance primary production (Lamberti and Resh 1983; Williams and Carpenter 1997). The N fixation response to moderate grazing could also depend on what algal forms are present, with filamentous taxa responding negatively due to their position in the periphyton overstory, whereas adnate, prostrate, or crustose growth forms could respond positively to increased light and nutrient availability (Stevenson 1997; Wellnitz and Ward 1998).

Plant-herbivore interactions are a cornerstone of ecological theory, and they have highlighted the role of animal consumers in regulating resource availability at the ecosystem level (Power 1990; Carpenter et al. 1987; Vanni 2002). The importance of
plant-animal interactions is exemplified by invasive species, which can have widespread impacts by re-routing nutrient fluxes through the food web, fundamentally altering ecosystem dynamics (Vitousek et al. 1987; Vitousek 1990; Taylor et al. 2006). For example, exceptionally high filtration rates of invasive zebra mussels consume the vast majority of phytoplankton in large water bodies (Strayer et al. 1999), and grazing by the golden apple snail releases enough phosphorus to initiate phytoplankton blooms in wetlands (Carlsson et al. 2004). In Polecat Creek, a stream in the Greater Yellowstone Ecosystem in northwest Wyoming, very high densities of the exotic New Zealand mudsnail (*Potamopyrgus antipodarum*) consume nearly all net primary production, and snails in this system directly control stream N cycling with exceptionally high area-specific N excretion rates (Hall et al. 2003). Consequently, herbivory by *Potamopyrgus* dominates the N cycle in Polecat Creek.

We tested the role of herbivory in controlling N fixation in Polecat Creek, an optimal system to experimentally reduce ambient densities of the invasive snail, which are routinely found at extremely high densities (20,000-500,000 snails m\(^{-2}\); Hall et al. 2003). Pilot studies indicated the presence of the prostrate diatom *Epithemia* spp., a species with N-fixing endosymbionts. Because prostrate diatoms can increase when grazers remove overstory algae, we hypothesized that a reduction in snail herbivores would increase filamentous algae, consequently decreasing *Epithemia* spp. and reducing N fixation. Because N fixing algae can modify water-column nutrient ratios by increasing N availability (Schindler 1977), we quantified changes in water chemistry in benthic chambers, anticipating an increase in dissolved N as a result of N fixation. Finally, we incorporated our measured N fixation rates into a detailed N budget reported
in a previous study of Polecat Creek (Hall et al. 2003) to quantify the importance of N fixation in the context of whole-stream N dynamics.

6.3 Methods

6.3.1 Study Site and Experimental Design

Polecat Creek is a geothermal, spring-fed stream located near Yellowstone National Park, WY, and it has optimum conditions for N fixation due to year-round warm temperatures and stable flows, low stream-water N:P ratios, and an open channel with high light levels and high primary production (Hall et al. 2003). In order to extrapolate our measured N fixation rates to a whole-stream N budget previously constructed for Polecat Creek, we performed our experiment using native rocks instead of tiles, avoiding artifacts caused by artificial substrates (Cattaneo and Amireault 1992). We covered the bottom of 40 cages (~90 cm² each) with rocks from which we carefully removed all invertebrates, leaving the epilithic community intact. We placed the cages, which had 1 mm mesh screens on each side to promote stream-water exchange, on a floating raft so the rocks were completely submerged, and we anchored the raft to the stream bank (Lamberti et al. 1987a). We randomly assigned one of four treatment levels to each cage, and we added snails of uniform length (2.5 mm) collected from the stream to achieve four different grazer biomass levels (0, 6.6, 13.3, and 33.2 g AFDM m⁻²) where the highest standing stock approximated ambient *Potamopyrgus* biomass (Hall et al. 2003). Our highest (i.e., ambient) biomass treatment corresponds to 180 g DM m⁻², much higher than the average natural standing stock of *Elimia* spp. (5 g DM m⁻²) in streams (Rosemond et al. 2000) but reflecting the extremely high snail biomass in Polecat Creek. We also
randomly assigned each cage a sampling time of 1 or 2 weeks post-manipulation. In week one, there were 5 replicate cages for each treatment, but in week two, we measured metabolism on rocks in one replicate cage, so each treatment had 4 replicates.

6.3.2 *In situ* N Fixation Assay

We measured N fixation rates *in situ* using the acetylene (C$_2$H$_2$) reduction assay (Flett et al. 1976; Grimm and Petrone 1997; Marcarelli and Wurtzbaugh 2006) conducted in chambers in which nitrogenase, the enzyme that mediates N fixation, reduces C$_2$H$_2$ to ethylene and provides a measure of N fixation activity. We saturated unfiltered stream water with C$_2$H$_2$ that we had first bubbled through a 10% HCl solution to remove impurities. After placing the rocks into 2.2 L clear plastic chambers, we added 1.5 L of unfiltered stream water and amended it with 150 mL of C$_2$H$_2$-saturated water to achieve a 10% partial pressure of acetylene in the headspace. We sealed each chamber with a septum-equipped lid for headspace sampling and incubated the chambers in the stream for 3-4 h. At the end of the assay, we gently swirled the chambers for 30 sec to equilibrate gases between the water and headspace, and we immediately took replicate 5 mL headspace gas samples and injected them into pre-evacuated 3 mL glass vials. To control for background ethylene production, we incubated rocks in chambers without C$_2$H$_2$-saturated water, and to control for potential ethylene contamination of C$_2$H$_2$, we incubated unfiltered stream water with C$_2$H$_2$-saturated water. In neither case did we find background ethylene production (data not shown).

We quantified ethylene concentrations in gas samples within 48 h of sample collection by manually injecting 1 mL of gas from each sample into a Shimadzu GC-14A gas chromatograph (Columbia, MD, U.S.A.) with Hayesep T column and flame.
ionization detector (injector temp = 100 °C, column temp = 35 °C, detector temp = 180 °C, ultra high purity helium carrier gas at 50 mL min⁻¹). We used temperature-dependent Bunsen coefficients to calculate total ethylene produced given headspace concentrations and water volumes, and we divided total ethylene produced by the length of the assay to calculate an ethylene production rate. It is understood that moles of ethylene produced rarely equals moles of N₂ fixed, and the actual stoichiometry can only be measured by incubating the specific algal community with ¹⁵N₂ as a tracer, but in the absence of this calibration procedure, we follow the common practice of assuming a conversion factor of 1 mole of N₂ fixed for every 3 moles of ethylene produced (Capone 1993; Grimm and Petrone 1997; Marcarelli and Wurtzbach 2006). After converting the ethylene production rate to a nitrogen fixation rate (as µg N h⁻¹), we converted it to an areal N fixation rate (mg N m⁻² h⁻¹) based on the rock surface area sampled, quantified by tracing the planar area of assayed rocks onto paper that we subsequently weighed and converted to area using a weight-to-area conversion. To account for differences in algal biomass per unit surface area, we also expressed N fixation as a chlorophyll-specific rate (µg N µg⁻¹ chl-a h⁻¹) based on chlorophyll-a (chl-a) standing stock (methods described below).

### 6.3.3 Metrics of the Algal Community

In week 1, we measured ecosystem metabolism after collecting gas samples from the N fixation assays, but our results appeared to have been confounded by incubating rocks with acetylene. Therefore in week 2, we placed one replicate from each treatment in sealed chambers that we incubated in the stream and recirculated with a Watson-Marlow 323 pump (Wilmington, MA, USA) at approximately 8 mL s⁻¹. We did not
additionally measure N fixation on the rocks we incubate for metabolism, and this unreplicated measure of ecosystem metabolism serves as an index of metabolism in the snail treatments. We carefully placed rocks in clear, 0.5 L PVC chambers for approximately 1 h and used a dissolved oxygen meter (YSI-85; Yellow Springs, Ohio, USA) to measure net primary production (NPP) as the increase in oxygen during the chamber incubation (Bott 1996). Then we incubated rocks in dark chambers for another hour and measured community respiration (CR) as the oxygen decline during the incubation. We calculated gross primary production (GPP) as the sum of NPP and the absolute value of CR, and scaled these metrics by the rock area we assayed (mg O₂ m⁻² h⁻¹) and by chl-a standing stock (see method below) on the rocks we assayed (mg O₂ mg chl-a⁻¹ h⁻¹).

After performing the acetylene reduction assays for N fixation and metabolism, we used brushes to remove periphyton from the assayed rocks. We filtered a known volume of periphyton slurry onto pre-ashed Pall A/E filters (1 µm pore size, East Hills, New York, USA), freezing the filters for subsequent chl-a analysis, and the remaining slurry was preserved in 1% Lugol’s solution for later algae identification. We extracted chl-a from the filters using hot ethanol technique (Sartory and Grobbelaar 1984) buffered with magnesium carbonate, and we measured the supernatant on a Turner Designs TD-700 fluorometer (Sunnyvale, CA, USA) at 436 nm excitation wavelength and 680 nm emission wavelength. We sub-sampled 0.1 L of preserved slurry into Palmer-Maloney chambers and used a Nikon Diaphot TMD inverted microscope (Melville, N.Y., USA) at 400x to identify algae according to Prescott (1978). Sampling effort curves indicated that 500 cells per sample adequately characterized the algal community. Algae were
identified to genus where possible or until we could classify them into one of 5 functional groups: green algae, diatoms, N-fixing diatoms, cyanobacteria, and N-fixing cyanobacteria.

6.3.4 Water Chemistry

To determine how the periphyton community affected chamber water chemistry during assays, we collected filtered (Pall A/E, 1 µm pore size; East Hills, New York, USA) water samples in acid-washed HDPE bottles pre-rinsed with filtered site water, stored them on ice in the field, and froze them upon return to the laboratory for future analysis. We used a Shimadzu UV-VIS spectrophotometer (Columbia, MD, U.S.A.) and 10 cm path length to measure ammonium (NH$_4^+$) with the phenate method (Solorzano 1969) at 630 nm. We quantified soluble reactive phosphorus (SRP) with the molybdate method (Murphy and Riley 1962) at 885 nm. We measured nitrate (NO$_3^-$) on a DIONEX 600 ion chromatograph (Sunnyvale, CA, USA) with ED-50 electrochemical detector (USEPA 1993). Dissolved inorganic nitrogen (DIN) is the sum of NH$_4^+$ and NO$_3^-$ concentrations.

6.3.5 Statistical Analyses

Any data that were not normally distributed were log-transformed to meet the assumptions of parametric statistical analysis (Zar 1999). We used two-way analysis of variance (ANOVA) to test for significant differences in chl-$a$ standing stocks and N fixation as a result of snail grazing (4 levels) or length of incubation after the biomass manipulation (2 levels). We analyzed total algal cells and percent composition of the algal community with multiple ANOVA (MANOVA), using 2-way ANOVA with a
Bonferroni-adjusted $P$-value of 0.05/5 algal categories = 0.01 to determine significant differences in *post-hoc* tests. We used multiple linear regression to identify which continuous independent variables (e.g., percent of algal community as N fixers, chl-$a$, snail biomass) were related to N fixation rates, and we used simple linear regressions to identify relationships between snail biomass and GPP and between GPP and N fixation. We analyzed periphyton nutrient flux (i.e., release or uptake) during the assays using 2-way ANOVA to detect significant differences among treatments and between incubation times, and we used simple linear regression to examine the relationship between DON flux from the benthos and proportion of the algal community as N-fixing diatoms. We performed all statistical analyses using SYSTAT 11.2 software (San Jose, California, USA).

6.4 Results

6.4.1 Grazing Effects on N Fixation and the Algal Community

We predicted that rocks incubated at highest (i.e., ambient) snail biomass (33.2 g AFDM m$^{-2}$) would have higher N fixation because grazing would remove green algae and maintain resource availability (i.e., light and/or nutrients) to N-fixing diatoms in the basal layers of the periphyton. We found highest chl-$a$ standing stock at lowest snail biomass and lowest chl-$a$ standing stock at highest snail biomass (2-way ANOVA, $P=0.045$; Figure 6.2a), but chl-$a$ values among treatments fell within the range of ambient chl-$a$ biomass normally found in this region (0.77-6.35 µg cm$^{-2}$; Riley, unpublished data). Standing stock of chl-$a$ did not vary between weeks 1 and 2, implying that cage effects on periphyton standing stocks were minimal.
Figure 6.2 Chlorophyll-α (chl-α) standing stocks and nitrogen (N) fixation (±1 SE) among experimental snail standing stocks. Letters group the weekly means by treatment. (A) Chl-α standing stock declined with increasing snail biomass. (B) Areal N fixation was highest in the 13.3 g AFDM m⁻² treatment. (C) N fixation scaled by chl-α was highest in the 33.2 g AFDM m⁻² treatment, and it was marginally lower in the second week.
Areal N fixation rates differed among treatments (2-way ANOVA, \( P=0.012 \); Figure 6.2b), but the N fixation response did not match our prediction of lowest N fixation rates without snails and highest rates at highest snail densities; however by week 2, N fixation rates were higher with highest snail biomass. Scaling N fixation rates by chl-\( \alpha \), which incorporates the effects of grazing on algal biomass (Figure 6.2c) shows a consistent trend by week 2 with highest N fixation rate at highest snail biomass and lowest rate in the no-snail treatment (2-way ANOVA, \( P=0.002 \)). Overall, week 2 N fixation rates, pooled across treatment, were slightly lower when scaled by chl-\( \alpha \) (2-way ANOVA, \( P=0.048 \)), but because the two highest snail densities had similar rates between weeks 1 and 2, this temporal difference was driven by the two lowest snail densities (Figure 6.2c). Lower N-fixation per unit chl-\( \alpha \) in week 2, especially at low snail densities, is consistent with our prediction that reduced grazing pressure allows algal biomass to accrue, which reduces light and nutrient penetration to the basal, N-fixing diatoms.

In addition to the prediction that snail grazing would result in higher N fixation rates, we also predicted that grazing would alter algal community composition (Table 6.1). Total numbers of algal cells per unit area and numbers of cells in each component of the algal community did not differ between weeks 1 (Figure 6.3a) and 2 (Figure 6.3b) or among treatments. However, comparing weeks 1 and 2 reveals that the total number of diatoms tended higher in all treatments that contained snail herbivory, and the highest snail biomass (33.2 g AFDM m\(^{-2}\)) had a notable, though insignificant, increase in N-fixing diatoms between weeks 1 and 2. Although cell density (i.e., cells cm\(^{-2}\)) did not differ among treatments, the relative community composition (i.e., % abundance)
TABLE 6.1.
CHANGES IN THE RELATIVE COMPOSITION OF THE ALGAL COMMUNITY

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<thead>
<tr>
<th></th>
<th>Treatment 1 (0 g snails m⁻²)</th>
<th>Treatment 2 (6.6 g snails m⁻²)</th>
<th>Treatment 3 (13.3 g snails m⁻²)</th>
<th>Treatment 4 (33.2 g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1 (%)</td>
<td>Week 2 (%)</td>
<td>% Change</td>
<td>Week 1 (%)</td>
</tr>
<tr>
<td>N-fixing diatoms*</td>
<td>11.8</td>
<td>12.5</td>
<td>+0.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Diatoms</td>
<td>11.0</td>
<td>11.8</td>
<td>+0.8</td>
<td>15.5</td>
</tr>
<tr>
<td>N-fixing cyanobacteria</td>
<td>0.9</td>
<td>3.7</td>
<td>+2.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Non-N-fixing cyanobacteria</td>
<td>9.3</td>
<td>7.6</td>
<td>-1.7</td>
<td>14.8</td>
</tr>
<tr>
<td>Green algae*</td>
<td>67.1</td>
<td>64.4</td>
<td>-2.7</td>
<td>56.5</td>
</tr>
</tbody>
</table>

Note: The community composition was significantly different in week 1 compared to week 2 (MANOVA, Wilks’ lambda, p=0.001). Asterisks next to algal types indicate a significantly different relative abundance between weeks 1 and 2 in post-hoc tests. There were no significant differences among treatments.
Figure 6.3. Total algal cells (±1 SE) by functional group did not vary between weeks 1 (A) and week 2 (B) or treatment. Algae capable of fixing nitrogen (N) are symbolized with hatching.
changed between weeks 1 and 2 because N-fixing diatoms increased and green algae decreased significantly (Wilks’ lambda, \(P=0.001\); Table 6.1). Therefore, grazing by *Potamopyrgus* shifted the community composition toward a higher relative abundance of N-fixing diatoms.

### 6.4.2 Predicting N Fixation Rates

We used multiple linear regression (MLR) to identify the metrics that were significantly related to areal N fixation rates. Pooling the data across weeks, % of algal community as N fixers, chl-a standing stock, and snail biomass were all positively related to N fixation, explaining almost half the variation in areal N fixation rates (MLR, \(R^2=0.47\), \(p<0.001\)). For illustrative purposes, highlighting differences in predictive relationships among weeks, we plotted the relationships between areal N fixation and the predictor variables from the MLR using simple linear regressions (Figures 6.4a-c, trend lines correspond to all data). We also regressed the predictor variables by weeks individually to identify how their predictive power varied between weeks (Figure 6.4, inset table). The most significant predictor variable for N fixation in the MLR (i.e., weeks 1 and 2 together) was % of the algal community represented by N fixers, showing a positive relationship with N fixation (Figure 6.4a). This factor was also a significant predictor for weeks 1 and 2 when we considered weeks separately (Figure 6.4, inset table). The next most significant variable in the MLR (i.e., weeks 1 and 2 together), chl-a standing stock, was positively related to N fixation (Figure 6.4b). However, when we analyzed the weeks separately, chl-a was significantly related to N fixation only in week 1 (Figure 6.4, inset table). The final significant variable in the MLR, snail biomass, was
Figure 6.4. Significant predictor variables for nitrogen (N) fixation. All variables were significant in a multiple linear regression ($R^2=0.47$, $p<0.001$), and they appear in order of significance in the multiple linear regression. Individual relationships are depicted with N fixation rather than with residual variation for easier interpretation. Regression statistics in scatter-plots correspond to weeks 1 and 2 together whereas week-specific relationships are described in the inset table. “N fixers” denotes the sum of N-fixing diatoms and N-fixing cyanobacteria.
significantly related to N fixation when we analyzed weeks 1 and 2 together (Figure 6.4c), but it was only related to N fixation rates from week 2 when we considered the weeks separately (Figure 6.4, inset table). Therefore, the relative importance of predictive metrics for N fixation varied through incubation time.

Nitrogen fixation is energetically expensive, so we measured ecosystem metabolism (i.e., GPP, NPP, and CR) using oxygen change in assay chambers to relate carbon dynamics to N fixation. However, because we measured metabolism on only one of the treatment replicates, we could not include these metrics in MLR analysis. Despite limited metabolism measurements, GPP per unit chl-\(a\) increased with snail biomass \((r^2=0.94, P=0.03;\) Figure 6.5a), implying increased efficiency of photosynthesis at higher grazing pressure. Additionally, GPP was positively related to N fixation \((r^2=0.95, P=0.03;\) Figure 6.5b), but CR was not \((r^2=0.80, P=0.11,\) data not shown).

### 6.4.3 Nutrient Exchange between the Periphyton and Water Column

We measured concentrations of NH\(4^+\), NO\(_3^-\), DON, and SRP before and after the N fixation incubations to calculate net nutrient exchange between the periphyton and the water column. Because low water-column DIN:SRP molar ratios (mean = 0.99 ± 0.03 SE) and low inorganic N concentrations (NH\(4^+\), mean <1 \(\mu g\) N L\(^{-1}\); NO\(_3^-\), always below detection limit of 2.5 \(\mu g\) N L\(^{-1}\)) in Polecat Creek suggest N limitation, and N fixation imports new bioavailable N into the system, we predicted net N release from the periphyton with increasing N fixation. Rate of change in dissolved solute concentrations did not vary across treatments, and only net DON release was marginally higher in week 2 compared to week 1 (Figure 6.6; 2-way ANOVA; \(P=0.044\)). Although all forms of N
Figure 6.5. Relationships between snail density, gross primary production (GPP) and nitrogen (N) fixation. In week 2, I estimated treatment GPP by measuring metabolism on 1 of the 5 replicates. I did not measure metabolism in week 1.
increased during the assays, net release of NH$_4^+$ and NO$_3^-$ from the periphyton was very low. In contrast, DON release was 8x higher than NH$_4^+$ and NO$_3^-$, suggesting that organic N may be a significant periphyton N loss mechanism. Background concentrations of SRP are relatively high in this geothermal stream (mean = 11.9 ± 0.3 SE), and the lack of SRP change through time (Figure 6.6) suggests low SRP demand.

We regressed periphyton DON exchange versus many potential explanatory variables, including snail, chl-\( \alpha \), and organic matter standing stocks, N fixation rates, individual components of the algal community, and the aggregated N-fixing and non-N-fixing proportions of the algal community. The only significant predictor of DON release
Figure 6.7. Relationship between periphyton dissolved organic nitrogen (DON) exchange and proportion of N-fixing diatoms. Positive values represent net periphyton release whereas negative values represent net periphyton uptake.

was proportion of N-fixing diatoms in the algal community (Figure 6.7), implying a positive relationship between DON exchange and the dominant N fixer in the algal community.

6.5 Discussion

6.5.1 Comparing N Fixation among Aquatic Habitats

N fixation has been well-studied in oceans, estuaries, and eutrophic lakes, but not in streams, so we compared our N fixation rates to those reported from other aquatic habitats and to the few published rates from streams (Figure 6.8). We found that streams had among the highest areal N fixation rates reported in aquatic ecosystems. This
comparison comes with the caveat that the average stream rate we report, as well as the mean from this study, are all studies from streams in the western United States (Horne and Carmiggelt 1975; Grimm and Petrone 1997; Marcarelli and Wurtzbaugh 2006), where optimum conditions for N fixation (high light levels, low DIN, and low N:P ratios) are common. Therefore we do not know what a more representative “average” stream N fixation rate might be. In many streams the combination of factors that promote N fixation do not occur, so this process may not be widespread in streams in other regions of the United States. For example, many streams in the Midwest and eastern United States have high N concentrations from non-point source pollution (Carpenter et al. 1998), which discourages N fixation. Streams with low nutrient concentrations in the

![Bar chart showing nitrogen fixation rates in different aquatic habitats.](image)

**Figure 6.8.** Nitrogen (N) fixation rates (±1 SE) among aquatic habitats. Note log scale. Data are from Howarth et al. (1988; Table 1), except “eutrophic lakes” includes Howarth et al. (1988; Table 1), Gu and Alexander (1993), and Presing et al. (2001), and “streams” includes Horne and Carmiggelt (1975), Grimm and Petrone (1997), and control values reported in Marcarelli and Wurtzbaugh (2006). Data in “our study” include measurements from the 33.2 g AFDM m² snail treatment, which represent ambient snail biomass in Polecat Creek, Wyoming.
eastern United States are frequently forested, so N fixation may be limited to brief periods of net autotrophy in the spring before leaf out (Mulholland et al. 2006). However, even with high light and low nutrient levels that would otherwise favor N fixation during spring in forested eastern streams, high spring flows might limit N fixation by scouring algae from the benthos (Grimm and Petrone 1997). Thus, high N fixation rates in streams are spatially and temporally restricted to stream ecosystems with low N, high light, and stable flows, but when those conditions are met, N fixation rates are very high compared to other aquatic habitats (Figure 6.8).

6.5.2 Relationship between Herbivory and N Fixation

In our experiments, rocks incubated with highest biomass of an invasive snail (at 33.2 g AFDM m\(^{-2}\) (representing ambient levels in Polecat Creek) had higher N fixation rates compared to rocks with lower snail biomass (Figure 6.1), demonstrating that herbivory has the potential to increase N fixation rates in stream ecosystems. Grazing and herbivory have been shown to influence N fixation in other aquatic ecosystems, but the response by N fixers varies. For example, zooplankton grazing decreases phytoplankton N fixation by restricting filament length and N fixation efficiency in lentic ecosystems (Chan et al. 2004) and by suppressing the colony size of N fixers in estuarine ecosystems (Chan et al. 2006). Benthic grazing by snails in the littoral zone of oligotrophic arctic lakes decreased N fixation, though the mechanism was not identified (Gettel et al. in press). Examples given above show that herbivory decreases N fixation in lentic freshwater and estuarine ecosystems, but herbivory by fish (Wilkinson and Sammarco 1983) and sea urchins (Williams and Carpenter 1997) has been shown to increase N fixation in coral reefs.
Stream grazers can alter N fixation by changing the algal community structure, and although studies in streams commonly report herbivory-induced changes in the algal communities in general, associated changes in N fixation specifically have not been measured to our knowledge. Usually, the effect of snail grazing on algae depends in part on the growth forms making up the algal community, with filamentous forms typically decreasing (Tuchman and Stevenson 1991; Evans-White and Lamberti 2005) and prostrate forms increasing (Rosemond et al. 1993) in relative abundance. In our study, grazing significantly decreased the proportion of green algae (i.e., % of total cells) and increased the proportion of N-fixing diatoms, which were dominated by the prostrate diatom *Epithemia* spp. (Table 6.1), but the change in relative community structure was not accompanied by a change in absolute numbers (i.e., cells cm\(^{-2}\)) (Figure 6.2). Many other studies have observed an increase in the proportion of prostrate diatoms via grazing (Lamberti et al. 1987b; Wellnitz and Ward 1998; Hillebrand et al. 2000; Holomzuki et al. 2006) because moderate grazing pressure by snails removes erect and filamentous algal forms rather than prostrate forms, which snails only consume under experimentally induced starvation (Steinman 1991).

Three metrics accounted for nearly half the variability in N fixation rates and demonstrated the importance of algal community composition, chl-\(a\) (as a proxy for primary producer biomass), and snail grazing pressure in determining N fixation rates (Figure 6.4). Even though diatoms dominated the N-fixing community, % N-fixers was the most important predictor of N-fixation throughout the experiment (Figure 6.4a) and when we analyzed the weeks separately (Figure 6.4, inset table), suggesting that cyanobacteria also contributed to N fixation rates despite their lesser relative and absolute
abundance. Although chl-α was significant when we analyzed weeks 1 and 2 together (Figure 6.4b), this relationship was driven by week 1 (Figure 6.4, inset table) because the change we observed in the algal community structure between weeks 1 and 2 (Table 6.1) disrupted the predictive power of chl-α in week 2 (Figure 6.4, inset table). However, after changing the algal community by week 2, actual snail biomass in cages predicted N fixation rates, demonstrating that the importance of grazing pressure increased as the experiment progressed (Figure 6.4, inset table).

Although N fixation was positively related to the proportion of N fixers in the community (Table 6.1), we did not see an associated change in the total number of N-fixing algal cells at higher snail biomass (Figure 4.3). Therefore, higher N fixation rates can only be explained by higher biomass-specific efficiency of N fixers (i.e., nitrogenase activity) at higher snail densities. Although N fixation has never been examined in this context in streams, grazing has been shown to increase the efficiency of photosynthesis by increasing light penetration and nutrient diffusion from the water column (Lamberti and Resh 1983; Steinman 1996); we observed highest GPP per unit chl-α with the highest snail biomass (Figure 6.5a), demonstrating increased photosynthetic efficiency at higher grazing levels. Because N fixation requires a large amount of energy, it is frequently related to the rate of photosynthesis in terrestrial environments (Bormann and Gordon 1984; Giraud and Fleishman 2004) and to light availability in streams (Horne and Carmiggelt 1975; Grimm and Fisher 1997) and lakes (Higgins et al. 2001). *Epithemia* spp. have endosymbiotic N fixers, and we observed higher N fixation with higher GPP (Figure 6.5b), which is consistent with a link between photosynthesis and N fixation. Therefore, our data are consistent with our hypothesis that snails would increase N
fixation by cropping periphyton and increasing nutrient and light availability to N-fixing diatoms in the basal periphyton layers, consequently increasing N fixation.

6.5.3 Changes in Water Chemistry during Assays—Implications for N Cycling

The net change in NH$_4^+$, NO$_3^-$, DON, and SRP concentrations represented nutrient exchange between the periphyton and the water column, and we predicted that N fixation would increase N concentrations by added new, bioavailable N to the system. Although we observed net periphyton release of all forms of N that we measured (Figure 6.6), NH$_4^+$ and NO$_3^-$ release was very low in comparison to DON. In contrast to DON, concentration changes of inorganic N were likely neither ecologically nor biologically significant (<2 µg N L$^{-1}$). The periphyton community was composed of N-fixing and non-N-fixing constituents, and factors such as meiofaunal grazing (Møller 2007) or cell leakage during photosynthesis (Kaplan and Bott 1982) have been shown to influence DON release. In our experiments, real-time snail grazing did not directly contribute to nutrient exchange because, prior to the N fixation assay, we removed snails and gently rinsed the periphyton to minimize N leaching from snail egestate. However, the only factor related to DON release from the periphyton was the proportion of N-fixing diatoms (Figure 6.7), suggesting that this dominant component of the N-fixing community had stronger control over DON release than other potential factors.

Nitrogen fixation introduces new bioavailable N into the environment as fixed N in organic form, and $^{15}$N$_2$ tracer studies demonstrate that organic N leaks from cells during N fixation, providing a direct source of N to the ecosystem such as we see in our experimental incubations. For example, concentrations of labeled glutamate and glutamine paralleled N fixation rates over the diel cycle in marine environments, where
DON loss corresponded to approximately 25% of fixed N (Capone et al. 1994). In another study, $^{15}$N-labeled DON losses accounted for between 27-76% of N fixed by a marine cyanobacterium (Glibert and Bronk 1994). Recent research indicates that DON can be an important source of N for heterotrophs in N-limited streams (Kaushal and Lewis 2005; Brookshire et al. 2005) as well as for marine phytoplankton (reviewed by Zehr and Ward 2002). We did not use $^{15}$N$_2$ to trace the fate of fixed N in our experiments, and to our knowledge, this technique has not been performed in stream ecosystems. However, if N fixers in streams release DON in the same proportions as N fixers in the ocean (Glibert and Bronk 1994), release of DON resulting from periphyton N fixation could make newly fixed N immediately available for whole-stream uptake, bypassing retention in particulate form (as algal biomass) that delays newly fixed N from becoming immediately available to non-N fixers.

6.5.4 Incorporating N Fixation into the Whole-stream N Budget

We compared the N fixation rates we measured in the highest snail biomass (i.e., ambient densities in Polecat Creek) to whole-stream N fluxes measured by Hall et al. (2003) to understand the relative role of N fixation to the N budget in Polecat Creek. First, we compared the importance of periphyton DON flux to the whole-stream N budget. Average DON flux among treatments and incubation times was 1.0 mg N m$^{-2}$ h$^{-1}$ which, compared to an ambient N fixation rate of 1.1 mg N m$^{-2}$ h$^{-1}$, represented 90% of fixed N released from periphyton as DON. Without using $^{15}$N$_2$ to trace the fate of fixed N, it is impossible to quantify how much of the DON loss was from direct leaking during N fixation and how much was due to sloppy feeding by meiofauna or leakage during photosynthesis. However, if the range of DON loss from marine N fixers is applicable to
stream N fixing algae, then 27-76 % of the DON flux may be attributed to direct losses during N fixation (Glibert and Bronk 1994). Because exported DON would not satisfy N demand at the point of N fixation, direct DON loss that is subject to immediate downstream export might maintain periphyton N limitation despite relatively high N fixation rates.

We compared our measured N fixation rate of 1.1 mg N m\(^{-2}\) h\(^{-1}\) to whole-stream \(\text{NH}_4^+\) uptake measured by Hall et al. (2003, their Table 2), which was 2.1 mg N m\(^{-2}\) h\(^{-1}\). Incorporating N fixation into the whole-stream N budget indicates that total flux of N into the periphyton is about 50% greater than measured using short-term releases of \(\text{NH}_4^+\) alone; but it must be noted that Polecat Creek has relatively high assimilatory demand for \(\text{NH}_4^+\) due to high primary production stimulated by geothermal phosphorus inputs. In other systems, the contribution of N fixation to stream N budgets could be much higher. For example, our N fixation rate is lower than the maximum rate reported from a desert stream (51 mg N m\(^{-2}\) h\(^{-1}\); Grimm and Petrone 1997) and a mountain stream with high light levels (11 mg N m\(^{-2}\) h\(^{-1}\); Horne and Carmiggelt 1975). Nevertheless, our results combined with previous studies indicate that N budgets in streams with N-fixing taxa should include N fixation to gain a more complete understanding of whole-stream N fluxes and periphyton N demand.

Although N fixation constitutes a relatively large N flux compared to whole-stream \(\text{NH}_4^+\) uptake in Polecat Creek, excretion from *Potamopyrgus* contributes 7.8 mg N m\(^{-2}\) h\(^{-1}\) (Hall et al. 2003, their Table 1). Therefore, N fixation is only 14% of the \(\text{NH}_4^+\)-N flux driven by grazing, and an even smaller fraction in comparison to total N flux by *Potamopyrgus* (i.e., excretion + egestion = 15.7 mg N m\(^{-2}\) h\(^{-1}\)). Including N fixation in
the Polecat Creek N budget only reinforces the conclusion that invasive snails dominate the N cycle of Polecat Creek.

Animals can affect whole-system nutrient cycling via direct effects, such as increasing nutrient availability via waste excretion, or by indirect effects, such as changing the community structure and subsequently altering nutrient dynamics (Vanni 2002). Hall et al. (2003) documented that the invasive *Potamopyrgus* affected the N cycle directly because its excretion accounted for nearly two-thirds of the whole-stream NH$_4^+$ demand. Our data show that this invasive snail can also indirectly affect the N cycle by altering the composition of the algal community and increasing the rate and efficiency of N fixation. However, increased N fixation may not be a universal response to herbivory if the N-fixing component of the periphyton community is dominated by filamentous taxa that could easily be grazed. Although rarely studied in stream ecosystems, N fixation can contribute significantly to whole-stream N flux into periphyton, and not accounting for this N flux in streams with high light availability and low N:P ratios could underestimate N uptake at a whole-system level. The use of $^{15}$N-labelled N$_2$ tracers would elucidate the relative importance to the food web of the net production of DON we observed during N fixation and to the consumption of newly fixed particulate N incorporated into algal biomass. Our results also suggest the possibility that the very high densities of invasive snails in Polecat Creek may have increased N fixation rates over pre-invasion levels, ultimately increasing N flux into the ecosystem and perhaps increasing ecosystem production in this nitrogen limited stream.
6.6 Acknowledgements

We thank Gretchen Gettel for her nitrogen fixation protocols and for allowing us to use her chambers for our pilot study. Scott Tarbutton helped set up the experiment, and Lisa Kunza shared her equipment for acetylene reduction assays. Gary Lamberti provided access to a microscope, and Konrad Kulacki and Michelle Evans-White helped with algae identification. Hank Harlow provided logistical support at the University of Wyoming/National Park Service Research Station. CPA received funding from the Arthur J. Schmitt Presidential Fellowship and the Bayer Predoctoral Fellowship while conducting this research. Additional funding was provided by NSF-DEB 0111410, USGS, and Wyoming Water Development Commission.
CHAPTER 7
DISSERTATION CONCLUSION

7.1 Summary

Increased N loading has negatively affected aquatic ecosystems, particularly in coastal environments (Howarth and Marino 2006). Although many studies have identified small streams as sites of rapid biogeochemical transformations that control downstream nutrient export (e.g., Peterson et al. 2001; Wollheim et al. 2006; Alexander et al. 2007), few have mechanistically addressed how anthropogenic landscape alterations influence the controls on N retention and removal in stream ecosystems. My dissertation research contributes novel information that enhances our basic ecological understanding of how streams transform elevated N loads, and it provides insight into what management strategies might effectively reduce N loads. In the following paragraphs, I summarize the key ecological findings, placing them in the context of the current body of knowledge, and I explore the management implications from each of my research chapters. At the end of my conclusion, I pool the results from each of my research chapters into a unified conceptual model of how anthropogenic activity influences N transformations in streams, emphasizing the synergism of my individual research chapters and how they contribute to the overarching focus of the dissertation.

Chapter 2. Researchers have identified many factors that contribute to high N export from mountain basins including short growing seasons at high elevations (Fenn et
al. 1998), lower water residence time associated with steep slopes that decreases the potential for biotic retention (Clow and Sueker 2000), and soil depth (Sickman et al. 2002). My research confirms that these factors were related to N concentrations among streams in the Tetons, but it also indicates that bedrock lithology was more important than elevation or slope in predicting N export within basins, probably by controlling soil development and vegetation. This finding complements the body of basic ecological research on alpine ecosystem N retention conducted in the west, mainly in Colorado (e.g., Baron et al. 2000; Fenn et al. 2003b; Hood et al. 2003a). Also, models suggest that the Tetons receive higher N deposition than NADP sites suggest, so I used the approach of Hood et al. (2003a) to document evidence that the less retentive, crystalline catchments of the Teton Range show early signs of N saturation. Therefore, my research also has important management implications because Grand Teton National Park is congressionally mandated to receive protection from air pollution (Williams and Tonnessen 2000).

Chapter 3. Riparian zones can reduce N concentrations of inflowing waters (Peterjohn and Correll 1984) and control C dynamics in streams (Golladay et al. 1989; Quinn et al. 1997). Although the N and C cycles are strongly linked in stream ecosystems (Bernhardt and Likens 2002; Fellows et al. 2006), and despite research that confirms the importance of riparian zones in determining reach-scale nutrient concentrations (Dodds and Oakes 2006), I did not find evidence that riparian zones influenced in-stream, reach-scale nitrification and denitrification over an annual cycle in streams in the Kalamazoo River basin in southwestern Michigan. My results validate a growing body of evidence indicating that riparian zones cannot be used exclusively as
management tools to buffer catchment-level disturbances (Baker et al. 2006; Houser et al. 2005), probably because they become less effective in anthropogenic landscapes due to channel modifications (Magner et al. 2004) and impervious surface cover (Paul and Meyer 2001; Bernhardt and Palmer 2007) that quickly route water from the landscape. My monthly, year-round study design allowed for ecological insight that would have been overlooked had I just conducted summer sampling. For example, all streams had highest denitrification rates in early winter, when stream temperatures were cold and when runoff flushed elevated soil-water NO$_3^-$ from the landscape into streams. Other studies that have found stream denitrification responds more to elevated NO$_3^-$ concentrations despite lower water temperature (Inwood et al. 2005; Wall et al. 2005), and my monthly sampling approach emphasizes the importance of performing ecological studies at times other than solely summer.

**Chapter 4.** I complemented my findings from Chapter 3 with a study of how land use and seasonality affect areal N uptake and the relative contribution of dissimilatory N transformations to that uptake. My results confirmed recent research that agricultural and urban landscape changes increase the importance of autotrophy in whole-stream NH$_4^+$ and NO$_3^-$ uptake (Grimm et al. 2005; Bernot et al. 2006), especially in the spring when canopies were open (Mulholland et al. 2006; Hoellein et al. in press). I also identified that higher N concentrations associated with land use can saturate areal uptake in streams, suggesting streams would not be able to retain additional N inputs from increasing urbanization (Wollheim et al. 2005) or intensifying agriculture (Schilling and Spooner 2006). High N concentrations also saturated nitrification and denitrification uptake, and examining these N transformations at the whole-stream level rather than in
the laboratory was a novel approach. When I compared the relative magnitudes of dissimilatory N transformations and whole-stream uptake, redox-optimized nitrification and denitrification approximately balanced each other, but both were always less than 10% of whole-stream NO$_3^-$ uptake, a similar proportion seen in detailed $^{15}$N tracer studies (Peterson et al. 2001; Mulholland et al. 2004; O’Brien et al. in press). Although many modeling studies indicate that gaseous N losses from small streams are an important N loss mechanism (Alexander et al. 2007), my comparisons indicate that optimizing denitrification to reduce coastal N loading would be less effective than reducing N inputs to streams in the first place (Howarth 2005).

**Chapter 5.** A meta-analysis of denitrification in various aquatic habitats indicated that NO$_3^-$ concentration was the single-most important predictor of denitrification (reviewed by Piña-Ochoa and Álvarez-Cobelas 2006). However, the meta-analysis included few streams with high NO$_3^-$ concentrations (> 5 mg N L$^{-1}$), where denitrification would be more likely limited by organic C as an electron donor (Hedin et al. 1998). I showed that carbon becomes an important predictor for denitrification at high NO$_3^-$ concentrations, confirming recent studies that have also investigated the factors limiting denitrification when anthropogenic activities increase stream-water NO$_3^-$ (Groffman et al. 2005; Inwood et al. 2007). Agricultural streams typically have very low organic matter retention (Magner et al. 2004), so management strategies that improve C retention, even as simple as not clearing riparian vegetation, might enhance NO$_3^-$ removal via denitrification and make it a more important NO$_3^-$ uptake mechanism. At the same time, my results from Chapter 4 call into question how effective this management
approach would be in the context of a stream with very high NO$_3^-$ loads, given the limited biological capacity of streams to retain or remove N overall.

**Chapter 6.** I returned to the low N streams of the Tetons to investigate N fixation, an understudied biogeochemical transformation in streams. I experimentally reduced the ambient, albeit very high, densities of an invasive snail in a Wyoming stream (Hall et al. 2006) to investigate how its grazing activity mediates substratum N fixation rates. At the highest biomass of snails (i.e., ambient levels), grazing increased N fixation by cropping green algae and increasing light and nutrient availability for prostrate N-fixing diatoms. We also found evidence suggesting that loss of DON during N fixation might represent a substantial, direct flux of newly-fixed N to the ecosystem, similar to what occurs in marine environments (Capone et al. 1994; Glibert and Bronk 1994). Upon publication, this experiment will be the first to investigate how herbivory affects N fixation in stream ecosystems, joining fewer than 10 other studies of N fixation published in streams. Additionally, in demonstrating a link between consumer activity and ecosystem N cycling, it suggests that species that govern algal-herbivore interactions may have substantial effects on stream nutrient cycling, analogous to how a detritivorous fish dominates the C cycle of tropical streams (Taylor et al. 2006).

### 7.2 Research Synthesis, Implications, and Conclusion

A hierarchical framework for studying ecological phenomena necessarily expands the scope of a research question, yet individual studies cannot often employ this approach. In the context of a dissertation, separate studies can be synthesized into a broader context than would have been possible with only the results from a single study. I researched stream N dynamics in two distinct regions: in the Midwest, where streams
with high DIN concentrations drained anthropogenically-transformed basins, and in the Tetons, where streams had low DIN concentrations and drained a minimally modified landscape. The results from these two regions, synthesized in Figure 7.1, each provide a different perspective on how anthropogenic and landscape influences modify the stream N cycle. I discuss the implications of my research from each region in turn.

A synthesis of my findings from the Midwest suggests that land-use practices may have shifted the relative balance in streams toward increased temporary N storage via assimilatory processes and decreased permanent N removal via denitrification. For example, assimilatory processes dominated whole-stream N uptake, which was more closely related to autotrophic (i.e., chl-α) rather than heterotrophic (i.e., organic matter

Figure 7.1. Conceptual model of stream nitrogen (N) transformations linking multiple spatial perspectives. Means, standard error, and ranges for N transformations identified by number are given in Table 7.1.
TABLE 7.1.
SUMMARY OF N FLUXES MEASURED IN THIS DISSERTATION

<table>
<thead>
<tr>
<th>Number from Figure 7.1</th>
<th>N Flux</th>
<th>Overall Mean (mg N m⁻² h⁻¹)</th>
<th>Standard Error of Mean</th>
<th>Range (mg N m⁻² h⁻¹)</th>
<th>Chapter</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>N Fixation</td>
<td>0.68</td>
<td>0.07</td>
<td>0.09-1.58</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Periphyton DON flux</td>
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<td>0.15</td>
<td>-0.23-3.79¹</td>
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<td>Whole-stream NH₄⁺ uptake</td>
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<td>0.15-29.79</td>
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<td>(U) Assimilatory NH₄⁺ uptake (U)</td>
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<td>0.95</td>
<td>0.03-27.24</td>
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<td>3.17</td>
<td>0.29</td>
<td>0.10-19.72²</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Whole-stream NO₃⁻ uptake</td>
<td>110.58</td>
<td>25.52</td>
<td>0.02-999.38</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(U) Assimilatory NO₃⁻ uptake (U)</td>
<td>108.21</td>
<td>25.19</td>
<td>0.25-983.13</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Denitrification</td>
<td>2.90</td>
<td>0.53</td>
<td>0.01-50.30</td>
<td>3</td>
</tr>
</tbody>
</table>

Notes: ¹negative value indicated periphyton uptake; ²One value of 0 was not included in this range.

Standing stock) metrics (Chapter 4). Clearing riparian vegetation by converting forests to agricultural and urban land use has probably enhanced assimilatory uptake by increasing light availability (Bernot et al. 2006). Although burial of assimilated N in sediments can slow downstream transport, subsequent decomposition remineralizes NH₄⁺ which may then be converted to NO₃⁻ through nitrification, resulting in a much more mobile (i.e., less retentive) form of N (Chapter 3). Even though nitrification and denitrification rates approximately balance (Chapter 4), the use of sediment NH₄⁺ by nitrification (Chapter 3) decreases total N retention in the stream by replacing stream-water NO₃⁻ removed via denitrification or reducing the need for denitrifiers to use stream-water NO₃⁻. Finally, the inorganic, sandy sediments that dominated our study streams provided a poor substratum
for denitrification compared to C-rich CBOM and FBOM (Chapter 5). Clearing riparian vegetation has probably curtailed the efficacy of denitrification by decreasing inputs of C-rich substrata and the potential for large woody debris or other channel complexity to store those inputs within stream reaches (Allan 2004). Consequently, land-use practices have most likely decreased the relative role of permanent N removal pathways in these streams.

Streams in and surrounding the Teton Range are fundamentally different from those in Michigan due to lower anthropogenic N inputs and their placement within a relatively unmodified landscape. Synthesizing the results from my research there affords an opportunity to discuss the implications of landscape variability on stream N cycling in low N streams. Bedrock lithology controlled N concentrations in Teton streams through a cascade of interactions involving soil development and vegetation, where streams draining crystalline catchments had higher DIN than streams draining carbonate catchments (Chapter 2). Because Teton streams had low P concentrations, the N:P ratio was driven by variation in DIN, and because N:P is a fundamental control on N fixation rates (Schindler 1977), the N fixation potential of Teton streams may ultimately be controlled by variability in lithology. In streams where N fixation occurs, the presence of heavy grazing, in this case by an invasive snail, plays a role in determining the rate of N fixation because their grazing activity can improve resource availability to N fixers in the periphyton (Chapter 6). Therefore, landscape variation and biological activity together play important roles in determining N inputs to streams when overall DIN is low. This insight would not have been possible in mixed land-use streams in the Midwest, where anthropogenic N inputs overwhelm landscape variability and biological controls.
Despite fundamental differences between mixed land-use catchments in the Midwest and streams draining the Teton Range, both regions share a common factor that influences stream N cycling: *humans*. Although the magnitude of anthropogenic influence differs between the two regions, humans influence stream N cycling in the Midwest and the Tetons simply because humans dominate today’s global N cycle (Galloway et al. 2004). Anthropogenic N fixation will grow with the human population, and ecosystem ecologists will continue to have opportunities to investigate the consequences a human-dominated N cycle (Schlesinger 2004). Research in this field will continue to uncover important ecological findings that will enhance our understanding of how organisms control elemental cycling, and this understanding will be vital to society as policy-makers grapple with balancing human needs and environmental stewardship as the pace of global change quickens.
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