ABSTRACT

Title of Document: NITROGEN SATURATION IN STREAMS AND FORESTS OF THE MARYLAND PIEDMONT

Laura Shawn Craig, Doctor of Philosophy, 2009

Directed By: Dr. Margaret Palmer
Professor, Department of Entomology and
Director, Chesapeake Biological Laboratory

Human activities have dramatically increased nitrogen (N) inputs to the landscape. Consequently, delivery of N to coastal waters, largely as nitrate (NO_3-N), has increased, resulting in widespread eutrophication and harmful hypoxic conditions. The ability to mitigate the downstream effects of elevated N inputs requires a clear understanding of the transport and transformation of N in stream ecosystems. Here, I examine N processing in urban and forested watersheds of the Maryland Piedmont.

I provide extensive evidence that three high-N streams draining urban and forested watersheds of the Maryland Piedmont are unable to remove NO_3-N as a result of both N saturation and phosphorus limitation. My findings illustrate that when elevated NO_3-N concentrations occur in the absence of other stressors that stimulate autotrophic activity (e.g. reduced canopy cover, increased nutrients) uptake cannot compensate for increased N loads. A review of the literature indicates that systems

that are similarly unable to remove NO_3-N vary widely in terms of land use and background N concentrations, highlighting the limitations of our understanding of N saturation in stream ecosystems.

I also provide the first documentation of N saturation in both the aquatic and terrestrial components of an un-manipulated forested watershed. Detailed examination of N dynamics within the forested watershed reveals that the forest is severely N-saturated despite receiving atmospheric N inputs that are small relative to other parts of the Northeast and Mid-Atlantic. Because groundwater delivers a disproportionate fraction of the N load to the channel, in-stream N concentrations are elevated when deep groundwater flowpaths dominate, and the watershed is a source of N during dry periods, I hypothesize that hydrogeologic factors that control groundwater susceptibility to NO_3-N contamination and promote delivery of NO_3-N via subsurface flowpaths may exacerbate N-saturation response.

My results suggest that we cannot rely on in-stream processing to reduce N loads even in minimally impacted watersheds. As a result, it is critical that management efforts reduce N loading to streams and take advantage of opportunities for increasing N removal in impaired systems only after other options have been exhausted.

NITROGEN SATURATION IN STREAMS AND FORESTS OF THE MARYLAND PIEDMONT

By

Laura Shawn Craig

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2009

Advisory Committee:
Dr. Margaret A. Palmer, Chair
Dr. Andrew Baldwin
Dr. Keith Eshleman
Dr. J. Patrick Megonigal
Dr. Karen Prestegaard

PREFACE

This dissertation contains a single introduction section, three research chapters, and a summary. Chapters 1, 2, and 3 are presented in manuscript form with abstract, introduction, methods, results, and discussion, followed by tables, figure legends, and figures. A single reference section occurs at the end for literature cited throughout the dissertation.

DEDICATION

For Janelle Cloud
I know that wherever you are, you are proud of me.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Margaret Palmer, who challenged me to think critically, always encouraged me to do my best, and never hesitated to give me the push I needed. Thank you to my committee − Andy Baldwin, Keith Eshleman, Pat Megonigal, and Karen Prestegaard − for providing helpful feedback, making sure I was able to see the big picture, and being incredibly patient.

Thanks to Carl Zimmerman and the Nutrient Analytical Services Laboratory for processing thousands of samples and sometimes even accepting cookies as payment. Thanks to Keith VanNess for leading me to my study watersheds and cheerfully answering requests for data. Thanks to Paul Bukaveckas, Stevan Earl, Bob Hall, Pat Mulholland, and Mike Williams for sharing their thoughts on the difficulties of interpreting non-significant results and to Rod Venterea for commenting on an early draft of Chapter 3. I am also grateful to Emily Bernhardt, Solange Filoso, and Sujay Kaushal for sharing their extensive knowledge with me.

I owe a huge debt of gratitude to my fellow students in the Palmer Lab, both past and present: Evan Grant, Brooke Hassett, Jake Hosen, Brian Laub, Owen McDonough, Chris "Junior" Patrick, Bob Smith, Chris Swan, and Lie`Ann Van-Tull. Their willingness to help me with every phase of my dissertation − from brainstorming ideas over bagels or beers, to spending long days with me in the field, to providing thoughtful comments on my writing − is unparalleled. I especially want to thank Holly Menninger, a wonderful friend and enthusiastic cheerleader, for comments that greatly improved my writing. Special thanks also to Dave Richardson for his friendship and candid criticism, and for sending encouragement in very small packages.

I would like to recognize a number of interns and friends who assisted with fieldwork and provided entertainment while "waiting for plateau": Dan Baker, Maura Browning, Kat Cappillino, Lauren Culler, Lindsay Cumella, Emily Duncan, Angela Johnson, and Matt Reardon. Thanks especially to my cousin, Sarah Heath, for coming to live with me during the summer of 2007 to help with nutrient addition experiments. I am also thankful to my friends at Maryland who helped make these last few years enjoyable, commiserated with me when I was overwhelmed, and provided the occasional distraction, particularly, Peter Blank, Chris Che-Castaldo, Scott Creary, Chris Desjardins, Julie Byrd Hébert, Eric Lind, Holly Martinson, Beth Olsen, and, of course, my fellow TAs in human anatomy and physiology, Glenn Gebler, Sara Lombardi, Dot Lundberg, and Nick Caruso.

I could not have made it through this process without the encouragement of my family. Thanks to Mom, Dad, Emily, and Dawn for lending an ear when I really needed it and for sending love (and "the force"). Thanks to my stepdad Chuck for showing genuine interest in my research; our conversations sparked a number of ideas that eventually became part of this dissertation. Thanks to James for providing me with some of the best advice I received during the last several years; the fact that my dissertation is complete is a testament to the fact that I listened to my big brother.

Finally, thank you to my best friend and love, Mike Cloud, for assisting with nearly every aspect of this dissertation and, more importantly, for supporting me every step of the way.

This research was supported by EPA-NCER-STAR Collaborative Network for Sustainability grant # X3832206. The Ann G. Wylie Doctoral Dissertation Fellowship allowed me to take time away from teaching in the fall of 2008 to focus on research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Human activities have more than doubled nitrogen (N) inputs to the landscape (Vitousek et al. 1997, Galloway et al. 2004). Consequently, delivery of N, largely as nitrate (NO_3-N), from uplands to coastal waters has increased, resulting in eutrophication along with widespread hypoxic and anoxic conditions that are detrimental to the structure and function of these systems (Howarth et al. 1996, Vitousek et al. 1997, Howarth et al. 2002, Galloway et al. 2003, Howarth 2008). Understanding how streams transport and transform N is a central focus of aquatic biogeochemistry (Ensign and Doyle 2006) and it is critical that we have a clear quantitative, understanding of these processes if we are to mitigate the downstream effects of elevated N inputs (Vitousek et al. 1997, Peterson et al. 2001).

The nutrient spiraling concept, which describes the simultaneous processes of nutrient cycling and downstream transport (Webster and Patten 1979), has provided the primary methodological framework for quantifying in-stream N removal (Newbold et al. 1981, Stream Solute Workshop 1990). As a result of research conducted largely in pristine headwaters (Peterson et al. 2001, Bernhardt et al. 2003, see review in Ensign and Doyle 2006), streams are widely viewed as important N sinks. Furthermore, several physical and biological factors that influence N uptake have been identified, including: stream size (Alexander et al. 2000, Peterson et al. 2001, Wollheim et al. 2001), channel morphology and complexity (Valett et al. 1996, Mulholland et al. 1997, Sweeney et al. 2004, Grimm et al. 2005), organic matter availability (Meyer et al. 2005, Webster et al. 2000), stream metabolism (Webster et al. 2003, Hall and Tank 2003, Niyogi et al. 2004, Hoellein et al.

2007), and background nutrient concentrations (Dodds et al. 2002, Webster et al. 2003, Earl et al. 2006); all of which may be indirectly affected by land use (Hall et al. 2009b).

Despite research demonstrating that many streams are important locations of N removal, there is mounting evidence that not all streams are efficient N sinks. Streams with chronically elevated N concentrations are often unable to remove an appreciable fraction of their N load as a result of reduced efficiency of processing (O'Brien and Dodds 2007, Mulholland et al. 2008, Hall et al. 2009) and saturation of uptake may occur if N supply exceeds biotic demand (Bernot and Dodds 2005, Earl et al. 2006). As streams approach biotic saturation, the first-order properties of N uptake should break down causing N to be transported increasingly greater distances prior to removal until, eventually, uptake lengths are immeasurable (Stream Solute Workshop 1990, Davis and Minshall 1999, Hall and Tank 2003, Earl et al. 2006, Alexander et al. 2007).

Provided that anthropogenic N inputs continue to rise, understanding how streams respond to elevated inputs will become an increasingly important priority of ecosystem research. In fact, there is already a rapidly growing body of literature documenting the effects of agricultural and urban land-use, both of which contribute to elevated N loads, on in-stream processing (e.g. Niyogi et al. 2004, Royer et al. 2004, Grimm et al. 2005, Meyer et al. 2005, Bernhardt and Palmer 2007, Hoellein et al. 2007, Mulholland et al. 2008, 2009, Hall et al. 2009b). In this dissertation, I examine N processing in forested and urban watersheds located in the crystalline Piedmont physiographic province of the Chesapeake Bay Watershed. All three study watersheds are located at the fringe of the

Washington, D.C. metropolitan area and receive large N inputs regardless of land use. A brief description of each chapter follows.

In Chapter 1, I present evidence that three streams within the Maryland Piedmont are both N-saturated and phosphorus (P) limited as a result of chronically elevated N concentrations. I use the solute addition method to quantify N removal in high-N streams draining both forested and urbanized watersheds and am unable to detect NO_3-N uptake in any stream. I use measures of whole-stream metabolism as well as additions of ammonium (NH_4-N) and potentially limiting nutrients to identify causes of non-significant NO_3-N uptake. Though I am able to detect uptake of NH_4-N and PO_4-P in all three streams, uptake velocities are low compared to most published values; possibly as a result of low absolute and relative rates of gross primary production (GPP < 1.1 g O_2 m^{-2} d^{-1}; GPP/ER < 0.22). I observe that demand for P increases as total N (TN) to total P (TP) ratios increase, suggesting P-limitation; severely elevated TN:TP ratios corroborate these findings. Combined evidence indicates that non-significant NO_3-N uptake is caused primarily by P limitation and low autotrophic demand; with longitudinal variation and masking of removal of by groundwater inputs secondarily contributing to my inability to detect NO_3N removal. Because elevated N concentrations are not accompanied by increases in the availability of light and limiting nutrients commonly observed in other high-N systems, uptake processes were unable to effectively compensate for N loading.

In Chapter 2, my goal was to determine the frequency with which non-significant results from NO_3-N addition experiments are equated with limited uptake and attempt to identify patterns among sites with limited capacity for NO_3-N removal. In a survey of the literature, I find 14 studies reporting NO_3-N uptake rate coefficients that are not statistically different from zero. I determine that negligible removal accounts for lack of detection in more than half of the studied streams. However, I observe few similarities between streams with a limited capacity for nitrate uptake; streams tend to be heterotrophic, but drain a variety of land uses and encompass a broad range of background nitrate concentrations (1.7 to 4650 μg N L^{-1}). In reviewing the literature, I discover a context-dependent bias regarding the language used to describe results of N additions -- high-N streams are more likely to be labeled as "saturated" when rates of uptake are too low to measurably reduce NO_3-N concentrations. My findings illustrate that our understanding of N saturation in lotic systems may benefit from increased knowledge of those streams where uptake cannot be detected and that our perception of the prevalence of N saturation in stream ecosystems may be distorted by publication and presentation bias.

In Chapter 3, I shift my focus from aquatic to terrestrial N processing to gain a better understanding of the biological and hydrological processes that lead to dramatically elevated NO_3-N concentrations, and ultimately in-stream N-saturation (Chapter 1), in my forested watershed. I measure several indicator metrics and observe symptoms of forest N-saturation including dramatically elevated groundwater and surface water NO_3-N concentrations and soil C:N ratios indicative of increased rates of nitrification. This is

the first un-manipulated forested watershed for which N-saturation is documented in both the terrestrial and aquatic components of the ecosystem. I model exports associated with total and baseflow discharge and find that groundwater delivers a disproportionate fraction of the N load to the channel. During dry periods, I observe dramatically elevated in-stream N concentrations and determine that the watershed is a net source of N. I hypothesize that hydrogeologic factors that control groundwater susceptibility to NO_3-N contamination and promote delivery of NO_3-N via subsurface flowpaths may exacerbate the N-saturation response in this watershed.

I conclude from this body of research that we cannot rely on in-stream processing to reduce N loads even in minimally impacted watersheds and N saturation may be occurring in more streams than we previously thought. As a result, it is critical that management efforts reduce N loading to streams (Driscoll et al. 2003, Galloway et al. 2003, Mulholland et al. 2008) and take advantage of opportunities for increasing N removal in impaired systems (Craig et al. 2008).

CHAPTER 1

Evidence for nitrogen saturation and phosphorus limitation in urban and forested streams of the Maryland Piedmont

ABSTRACT

High nitrogen (N) loads are often part of a suite of disturbances caused by human impacts. As a result, opportunities to examine the effects of elevated N concentrations independent of other stressors are rare. I used nutrient additions to quantify NO_3-N removal in high-N streams (905 to 3828 μg NO_3-N L^{-1}) draining one forested and two urbanized watersheds in Montgomery County, Maryland. Several physical, chemical, and biological variables were measured to identify factors influencing NO_3-N removal. NH_4-N additions were also conducted to quantify uptake and determine nitrification rates; and PO_4-P and DOC additions were used to determine the nutrient limitation status of each stream. NO_3-N uptake was not detected in any stream; however, significant (α = 0.1) longitudinal increases in NO_3-N were observed during three additions (one at each site). While longitudinal variation contributed to my inability to detect NO_3-N removal, variation in uptake metrics, along with evidence for limited nitrification (< 6% of NH_4-N uptake), indicated that removal at all three sites was either too low to produce a measurable reduction in NO_3-N concentrations or masked by lateral inputs. Observed NH_4-N and PO_4-P uptake velocities were also low compared to published values (1.0 to 2.5 and 0.3 to 0.9 mm min^{-1}, respectively). A negative relationship between NH_4-N demand and DIN concentrations (r = -0.9790), indicated that overall demand for N may have been reduced by dramatically elevated N concentrations. Relative demand for P ($v_{f\text{-}PO4}/v_{f\text{-}NH4}$) increased as TN:TP ratios increased (r = 0.999, p = 0.02), suggesting that P

was limiting in these systems. Molar ratios of TN:TP (368 to 875) further demonstrated the existence of P-limitation. At all three sites DOC:DIN ratios (≤ 1.0) were suggestive of possible C-limitation; however, DOC uptake was observed in only two streams. GPP and GPP/ER were consistently low (GPP: 0.05 to 1.09 g O_2 m^{-2} d^{-1}; GPP/ER: 0.01 to 0.22), possibly as a result of limited light availability in densely shaded reaches. GPP was also positively correlated to SRP (*r*: 0.999), suggesting possible P-limitation of autotrophic metabolism. Combined evidence suggests that that non-significant NO_3-N uptake was caused primarily by P limitation and low autotrophic demand; with longitudinal variation and masking of removal of by groundwater inputs secondarily contributing to my inability to detect NO_3N removal. While high-N concentrations are often observed in combination with other stressors, elevated N concentrations in my streams were not accompanied by dramatic increases in limiting nutrients or light availability commonly observed in other high-N systems. As a result, autotrophic demand could not compensate for increased N. As with other systems that have a limited capacity for N removal, the resultant increase in the delivery of N to downstream waterways is likely to have dramatic effects.

INTRODUCTION

Streams play a critical role in determining nitrogen (N) export (Howarth et al. 1996, Galloway et al. 2004) because they are capable of transporting N to downstream waterways yet may also be important sites of N removal (e.g. Peterson et al. 2001, Bernhardt et al. 2003; see review in Ensign and Doyle 2006). N uptake, and thus the potential for reducing the amount of N delivered to subsequent reaches, is influenced by a

number of physical and biological factors including stream size (Alexander et al. 2000, Peterson et al. 2001, Wollheim et al. 2001), channel morphology and complexity (Valett et al. 1996, Mulholland et al. 1997, Sweeney et al. 2004, Grimm et al. 2005), organic matter availability (Meyer et al. 2005, Webster et al. 2000), stream metabolism (Webster et al. 2003, Hall and Tank 2003, Niyogi et al. 2004, Hoellein et al. 2007), and background nutrient concentrations (Dodds et al. 2002, Webster et al. 2003, Earl et al. 2006). Streams with chronically elevated N concentrations are often unable to remove an appreciable fraction of their N load as a result of reduced efficiency of processing (O'Brien et al. 2007, Mulholland et al. 2008, Hall et al. 2009b) and saturation of uptake may occur if N supply exceeds biotic demand (Bernot and Dodds 2005, Earl et al. 2006) or if other factors becoming limiting (Earl et al. 2006, Simon et al. *in press*).

Though high N loads are known to directly alter rates of N cycling (Davis and Minshall 1999, Dodds et al. 2002, Webster et al. 2003, Hall et al. 2009b), they are often part of a suite of disturbances resulting from human impacts that could potentially influence N removal (Paul and Meyer 2001, Niyogi et al. 2004, Grimm et al. 2005, Meyer et al. 2005, Walsh et al. 2005, Bernot et al. 2006, Young et al. 2008, Wenger et al. 2009, Simon et al. *in press*). As a result, opportunities to independently examine the effects of elevated N concentrations on ecosystem function are rare. A number of studies have examined N uptake in high-N streams draining watersheds dominated by urban (e.g. Meyer et al. 2005) or agricultural (e.g. Niyogi et al. 2004, Bernot et al. 2006) land uses. Studies have also examined N removal across gradients that vary widely both in land use and N concentrations (e.g. O'Brien et al. 2007, Mulholland et al. 2008, 2009, Hall et al. 2009b).

In this study, I examined N removal in high-N streams draining both forested and urbanized watersheds in an attempt to evaluate the influence of elevated N concentrations on N uptake independent of other factors frequently associated with impacted systems. The stream draining the forested watershed provided a unique opportunity to evaluate uptake in an N-rich stream draining an otherwise unimpacted catchment.

METHODS

Site description - Study sites included three streams at the fringe of the Washington D.C. metropolitan region in Montgomery County, Maryland (Figure 1.1; Table 1.1). All streams are 2nd order tributaries of the Potomac River located in the crystalline Piedmont physiographic province of the Chesapeake Bay Watershed. Crystal Rock (CR: N39°11'56", W77°16'31") drains a 2.45 km^2 watershed that is completely (100%) urbanized and approximately 51% impervious (Maryland Office of Planning 2002 Land Use, GISHydro2000; Moglen 2007). Narrow, forested riparian buffers (< 20 m) are present at this site, but cannot be quantified at the resolution of the land use model (30 m; Moglen 2007). Sycamore Farm (SF: N39°13'53", W77°15'22") drains a rapidly urbanizing 1.10 km^2 watershed previously dominated by agriculture (46.8%) and forest (36.9%; Maryland Office of Planning 2002 Land Use, GISHydro2000; Moglen 2007). Construction began in this watershed in 2002 and was ongoing throughout the course of this study, which began in October 2004. Clearing of vegetation and grading of soil for the second phase of residential development began just prior to the onset of this study. As a result, open land associated with active construction (Figure 1.2) comprised approximately 32.3% of the total watershed area in 2004 and 22.2 % in 2006. The

remainder of the watershed was divided between residential, forested, and agricultural land uses (Table 1.1). Total impervious cover within the Sycamore Farm watershed is expected to near 40% after build-out, but could not be accurately estimated during construction (K Van Ness, Montgomery County Department of Environmental Protection, *personal communication*). In contrast to the other streams, Sopers Branch (SB: N39°16'31.1", W77°18'13.2") drains a 3.03 km^2 watershed that is primarily forested (86.6%) and less than 2% impervious (Maryland Office of Planning 2002 Land Use, GISHydro2000; Moglen 2007).

Studies in all three streams were conducted in shaded reaches with typical riffle-pool-run morphology and few (SB) or no (CR and SF) channel-spanning debris dams. Substrate was dominated by sand and gravel at all three sites; however, large cobbles armored the streambed along much of the study reach at Crystal Rock and finer particles (i.e. silts and clays), likely generated by construction activities, were frequently observed at Sycamore Farm. Physiochemical variables were measured approximately every month between October 2004 and September 2006 to characterize stream water quality. Dissolved oxygen (DO), specific conductivity, and pH were recorded at three randomly selected locations on each sampling date using a multi-parameter probe (YSI Model 556, YSI Inc., Yellow Springs, OH). Water samples collected at each location were analyzed for NO_2+NO_3-N, NH_4-N, and soluble reactive phosphorus (SRP) using an automated photometric analyzer (Aquakem 250, Thermo Fisher Scientific, Waltham, MA). Samples were analyzed for dissolved organic carbon (DOC) using high temperature catalytic oxidation (Shimadzu TOC-5000, Shimadzu Scientific Instruments, Columbia, MD).

Additional samples were collected on 12 June 2007 and analyzed for DOC, NO_2+NO3-N, NH_4-N, and SRP, as described above, as well as total nitrogen (TN) and total phosphorus (TP). These data were used to determine the relative abundances of inorganic and organic forms of N present at each site, and to establish relationships between TN and dissolved inorganic N (DIN) and TP and SRP to determine the validity of using ratios of DIN:SRP and DOC:DIN from monthly data to assess nutrient limitation status. These data also describe chemical conditions associated with nutrient additions conducted in the summer of 2007 (details below).

Nitrate additions - Short-term nitrate (NO_3-N) additions were conducted at each site in July 2005, November 2005, and June 2007, in order to calculate uptake metrics that describe nutrient spiraling (Newbold et al. 1981). I chose to measure the capacity of each stream to remove NO_3-N since it is the predominant form of N in many polluted systems (e.g. Davis and Minshall 1999, Grimm et al. 2005, Stanley and Maxted 2008) and is removed from the water column only through biotic, as opposed to abiotic processes (i.e. sorption; Figure 1.3).

I conducted multiple additions at each site, incrementally increasing the amount of NO_3-N added with each subsequent release to allow for estimation of ambient uptake lengths using the extrapolation method described by Payn et al. (2005). In July 2005, I conducted three different levels of NO_3-N additions at each site on separate dates; in November 2005 and June 2007, I conducted multiple additions, one right after the other, on the same date (Table 1.3). Study reaches were 110 m in length in 2005, with transects

20 m below the addition point and every 10 m thereafter (n = 10). In 2007, study reaches were extended to 210 m with transects located at 30, 50, 90, 130, 170, and 210 m (n = 6). Nutrient releases were conducted using standard protocols (Stream Solute Workshop 1990, Webster and Valett 2006), briefly described below.

For all additions, triplicate water samples were collected at each transect prior to each addition to establish ambient concentrations ($C_{amb\text{-}NO3}$). A concentrated solution of $NaNO_3$ and NaBr, a conservative tracer, was added at a known rate (Unispense peristaltic pump, Wheaton Industries Inc., Milville, NJ) to increase instream NO_3-N concentrations by a predetermined amount ($C_{add\text{-}NO3}$), such that the proportional increase in NO_3-N ($C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$) ranged from 0.03 to 0.74. In practice, $C_{add\text{-}NO3}$ differed slightly from predetermined goals and $C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$ ranged from 0.04 to 1.29 (Table 1.3). After solute concentrations reached a steady state (i.e. plateau), determined by monitoring conductivity at the downstream end of the reach using a multi-parameter probe (YSI Model 556, YSI Inc., Yellow Springs, OH), I collected triplicate samples at each transect. Individual additions lasted less than two hours, with series of additions lasting less than five and a half hours, and $C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$ was kept relatively low in order to minimize the possibility of inadvertently saturating biotic uptake capacity (Stream Solute Workshop 1990).

All background and plateau water samples were filtered (Whatman GF/F, 0.7µm nominal pore size) in the field into acid-washed HDPE bottles and frozen prior to analysis. Upon thawing, samples were analyzed for NO_3^- and Br^- using ion chromatography

(Dionex ICS-100 with AS18 analytical column, Dionex Corporation, Sunnyvale, CA). Data outliers, defined as any triplicate samples whose concentrations were greater than 10% from each other, were discarded (as in Williams et al. 2004).

Empirical data from each release was used to assess NO_3-N uptake capacity. Linear regression of the natural logarithm of the added NO_3-N concentration (plateau concentration corrected for background concentration and dilution) against distance downstream of the addition site was used to determine the first-order uptake rate coefficient, k_{NO3} (i.e. the slope of the linear regression). Regression analyses were conducted using SAS v9.1 (2007, SAS Institute Inc., Cary, NC; PROC REG). Similar to others who have conducted solute additions in high-N systems (e.g. Grimm et al. 2005, Meyer et al. 2005, Bernot et al. 2006), I used $\alpha = 0.1$ to identify regressions where k_{NO3} was significantly different from zero. I calculated the 90%, two-tailed, *t*-based confidence interval around the estimated value of k_{NO3} to assess strength of inference (Hall and Tank 2003, Hall et al. 2009a). Furthermore, because groundwater delivery of NO_3-N may reduce the ability to detect a significant decrease in NO_3-N along the length of the study reach when using the solute addition method, I also used linear regression to identify those additions during which plateau concentrations of the conservative tracer decreased significantly ($\alpha = 0.10$) with downstream distance. Any significant dilution of bromide was assumed to result from groundwater inputs since there were no tributary inputs to the study reaches.

Ancillary metrics – Concurrent with each uptake measurement, I measured several other variables in order to identify factors influencing NO_3-N removal at each site, including: average background NO_3-N concentrations ($C_{amb\text{-}NO3}$; from samples collected prior to additions), discharge (*Q*; from USGS gauging stations located at the downstream end of the reach), average water velocity (*v*; reach length divided by the time to half-plateau concentration of the conservative tracer), average wetted width (*w*; measured at each transect), and average reach depth (*d*; calculated as $Q/[w*v]$).

Whole-stream metabolism was measured at each site using the single-station diel oxygen method (Bott 1996). DO concentrations were recorded every hour with a Hydrolab MiniSonde 4a (Hach, Loveland, CO) at each site for eight days in the summer (12 July to 19 July) and fall (CR: 20 November to 27 November; SF: 29 November to 06 December; SB: 24 November to 01 December) of 2005, and summer of 2007 (CR: 26 June to 03 July; SF: 23 June to 30 June; SB: 25 June to 02 July). The net rate of oxygen change due to metabolism was calculated for each 1-hour interval; accounting for air-water exchange of oxygen using the surface renewal model, which estimates a reaeration coefficient based on stream velocity and depth (Owens et al. 1964). The daily rate of ecosystem respiration (ER; n=8) was calculated by scaling the average hourly rate of oxygen consumption during darkness to 24 hours. Daily gross primary productivity (GPP; n=8) was calculated as the sum of the hourly rate of change in oxygen during daylight hours minus ER.

Standing stocks of fine (FBOM; <1mm) and coarse (CBOM; >1mm) benthic organic matter were quantified using methods described in Wallace and Grubaugh (1996). Samples were collected at a randomly selected location at each of ten transects immediately following the final NO_3-N addition in July 2005, November 2005, and June 2007. At each sampling location, I inserted a corer (0.076 m^2) into the streambed, removed leaves and sticks by hand, disrupted the top 10cm of substrate, and pumped (Waterbuster Portable Pump, Attwood Marine Products, Lowell, MI) the resulting slurry through a 1mm sieve (U.S. Standard #18) into a bucket. CBOM included all of the material retained on the sieve plus material collected by hand; FBOM included all of the organic material passing through the sieve, a subsample (~125mL) of which was collected after ensuring that particles were evenly suspended. FBOM samples were filtered (pre-ashed Whatman GF/F, 0.7µm nominal pore size) upon returning to the lab, and FBOM and CBOM samples were dried at 60°C for at least 48 hours. Dried samples were weighed, ashed at 550°C, and re-weighed to calculate ash-free dry mass (AFDM, g). AFDM was normalized for sample area (CBOM and FBOM) and the volume of stream water passing through the sieve (FBOM only).

Additional measures to establish causes of undetected nitrate uptake - Lack of detectable NO_3-N uptake during 2005 led to several hypotheses regarding underlying causal mechanisms, including masking due to lateral or regenerative inputs (i.e. nitrification; (Figure 1.3) and nutrient limitation. NH_4-N additions were conducted in the summer of 2007, using methods described above, to quantify NH_4-N uptake and determine whether NO_3-N removal was masked by the microbially-mediated conversion of NH_4^+ to NO_3^-.

Additions of NH_4Cl were short in duration (< 2 h) and the proportional increase in NH_4-N over background was relatively low ($C_{add\text{-}NH4}$:$C_{amb\text{-}NH4}$: 1.9 to 3.3). For estimated values of k_{NH4} that were significantly different from zero at $\alpha = 0.10$, I calculated three uptake metrics described by Newbold et al. (1981) and the Stream Solute Workshop (1990): uptake length (S_w, m; [$^-1/k$]), uptake velocity (v_f, mm min^{-1}; [$(Q/w)/S_w$]), and areal uptake rate (U, µg m^{-2} s^{-1}; [v_f * C_{amb}]). The proportion of NH_4-N uptake attributed to nitrification was determined by fitting a two-compartment mixing model of NH_4-N and NO_3-N fluxes to the background-corrected longitudinal profile of NO_3-N measured during NH_4-N addition experiments (Mulholland et al. 2000, Bernhardt et al. 2002). This model allows for the simultaneous modeling of longitudinal decreases in NH_4-N, due to biotic uptake or nitrification, and longitudinal increases in NO_3-N during NH_4-N additions (Figure 1.3). Because NO_3-N uptake rate coefficients were essentially zero, nitrification is only assumed to occur when NO_3-N increases along the length of the reach at the same time as NH_4-N decreases.

PO_4-P (as KH_2PO_4) and DOC (as *d*-glucose) additions were also carried out at each site during the summer of 2007 to determine if NO_3-N removal was limited by P or C availability. PO_4-P and DOC concentrations were increased dramatically with proportional increases over background ranging from 65 to 197 ($C_{add\text{-}PO4}$:$C_{amb\text{-}PO4}$) for PO_4-P additions and 8 to 17 ($C_{add\text{-}DOC}$:$C_{amb\text{-}DOC}$) for DOC additions (Table 1.6). Despite the fact that additions were conducted in the same reaches used previously, P and C additions lasted 5 to 6 h because reduced streamflow led to increased solute travel times. Uptake rate coefficients (k_{PO4} and k_{DOC}) were used to calculate uptake metrics described

above. The magnitude and direction of N response to P and C amendment was determined by comparing both NO_2+NO_3-N and NH_4-N concentrations in samples collected at each transect before and during solute releases (PROC TTEST).

RESULTS

Physiochemical characteristics of each stream are summarized in Table 1.2. NO_2+NO_3-N concentrations were elevated in all three study streams and accounted for approximately 99% of DIN on all dates. NO_2+NO_3-N comprised greater than 91% of TN on the only date for which TN was analyzed. NH_4-N and dissolved organic N (DON) comprised approximately 1 and 8 % of TN, respectively, at all three sites (12 June 2007; data not shown). NO_2+NO_3-N concentrations were unrelated to land use, but NH_4-N, SRP, and DOC all increased with increasing urbanization (*r*: 0.7715, 0.9942, and 0.9544, respectively).

Molar ratios of TN:TP, measured on 12 June 2007, were extremely high at all three sites (368 to 875; Table 1.3). Molar DIN:SRP ratios were even greater, with average values ranging from 975 to 2202 between October 2004 and September 2006 (Table 1.2) and from 489 to 1144 in June 2007 (Table 1.3). Differences between TN:TP and DIN:SRP ratios result from measures of SRP capturing only 70% of TP at the two urban sites (CR and SF; data not shown). Regardless, DIN:SRP was assumed to be a reasonable surrogate for TN:TP with ratios of this magnitude (Dodds 2003). TN was also high relative to DOC, with molar DOC:TN ratios during June 2007 ranging from 0.3 at Sycamore Farm to 0.9 at Crystal Rock (Table 1.3).

Nitrate uptake was not detected at any site during the short-term nutrient addition experiments (Table 1.4). For 19 of the 22 additions, k_{NO3} was not significantly different from zero ($\alpha = 0.1$). For three of the solute injections, the slope of the regression between the natural logarithm of the corrected plateau NO_3-N concentrations and distance downstream of the addition site was both significant ($\alpha = 0.1$) and positive, indicating that NO_3-N increased along the length of the study reach. This downstream increase in NO_3-N was observed at Sycamore Farm on 11 July 2005 ($C_{amb\text{-}NO3}$: 3320 µg N L^{-1}, $C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$: 0.06), during the highest level of addition at Sopers Branch on 23 November 2005 ($C_{amb\text{-}NO3}$: 747 µg N L^{-1}, $C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$: 0.99), and at Crystal Rock on 25 June 2007 ($C_{amb\text{-}NO3}$: 2082 µg N L^{-1}, $C_{add\text{-}NO3}$:$C_{amb\text{-}NO3}$: 0.15). For the addition at Sycamore Farm on 07 July 2005, only two transects remained following the removal of outliers. Average plateau NO_3-N concentration at one of these transects was less than $C_{amb\text{-}NO3}$, which prevented further analysis since the natural logarithm of a negative number is undefined. Since nutrient spiraling metrics are ultimately based on the ability to detect a decrease in NO_3-N along the length of the study reach (i.e. a significant, negative value of k_{NO3}), I was unable to calculate NO_3-N uptake metrics at any site.

Longitudinal variability contributed to the inability to detect NO_3-N uptake at all three sites (9 of 22 additions; Appendix I and II). Even when variability was minimal, uptake rates were too low to detect a decrease in NO_3-N within addition reaches. Minimum $S_{w\text{-}NO3}$ values, estimated from lower 90% confidence limits, exceeded the length of the study reach during nearly half (9 of 22) of the additions or were negative (3 of 22), confirming

longitudinal increases in NO_3-N observed at Sycamore Farm (11 July 2005), Sopers Branch (23 November 2005), and Crystal Rock (25 June 2007).

Lateral groundwater inflows were detected during seven of the 22 addition experiments as evidenced by longitudinal changes in the concentration of the conservative tracer during NO_3-N additions (Table 1.4). Bromide concentrations decreased significantly during additions at Crystal Rock on 11 July 2005, Sycamore Farm on 22 June 2007, and five of seven additions at Sopers Branch (07 July 2005, both injections on 23 November 2005, both injections on 24 June 2007). During one addition at Sycamore Farm, I observed a significant longitudinal increase in bromide, possibly resulting from poor mixing at the upstream transects. Notably, groundwater inputs were not evident during additions at Crystal Rock and Sycamore Farm during which NO_3-N increased significantly with downstream distance.

Chemical and physical parameters, measured in conjunction with each NO_3-N release, are summarized in Table 1.4. Background NO_3-N concentrations ($C_{amb\text{-}NO3}$) differed significantly across sites ($F_{(df=2)}$:63.39, $p < 0.0001$) on the dates of the solute injections. Average $C_{amb\text{-}NO3}$ was the lowest at Sopers Branch (905 µg N L^{-1}) and the highest at Sycamore Farm (3828 µg N L^{-1}). Average Q ($F_{(df=2)}$: 5.71, $p = 0.018$), depth (d; $F_{(df=2)}$: 4.70, $p = 0.031$) and width (w; $F_{(df=2)}$: 48.90, $p < 0.0001$) also differed significantly across sites. Sycamore Farm had the lowest average Q (11.5 L s^{-1}) and was shallower than the other sites (d: 0.07 m); Crystal Rock had the widest channel (w: 3.68 m). Average velocity (v) did not differ between sites ($F_{(df=2)}$: 0.31, $p = 0.74$). Biological variables

related to injections conducted in each season are summarized in Table 1.5. All three streams were heterotrophic (GPP/ER < 1) with average daily GPP/ER ranging from 0.01 to 0.22. Average daily GPP ranged from 0.05 to 1.09 g O_2 m^{-2} d^{-1} and ER ranged from 3.06 to 6.05 g O_2 m^{-2} d^{-1}. Reach-average CBOM and FBOM availability was highly variable and ranged from 2.8 to 312.6 and 19.2 to 104.7 g AFDM m^{-2}, respectively, on all sampling dates.

NH_4-N uptake occurred at all three sites (Table 1.6). $S_{w\text{-}NH4}$ ranged from 146 to 200 m; $v_{f\text{-}NH4}$ ranged from 1.0 to 2.5 mm min^{-1}. Demand for NH_4-N ($v_{f\text{-}NH4}$) decreased with increasing DIN concentrations (*r*: -0.9790) and increasing TN:TP ratios (*r*: -0.8425). Results from the two-compartment nitrification model indicated that none of the removed NH_4-N was nitrified at Crystal Rock or Sopers Branch. Less than 6% of NH_4-N uptake was attributed to nitrification at Sycamore Farm. PO_4-P uptake was also detected at all three sites (Table 1.6). $S_{w\text{-}PO4}$ ranged from 296 to 769 m and $v_{f\text{-}PO4}$ ranged from 0.3 to 0.9 mm min^{-1}. NO_2+NO_3-N and NH_4-N concentrations did not change significantly during PO_4-P additions (CR: p = 0.55, SF: p = 0.13, SB: p = 0.12). Demand for PO_4-P relative to demand for NH_4-N ($v_{f\text{-}PO4}/v_{f\text{-}NH4}$) increased significantly with increasing TN:TP ratios (p = 0.015; Figure 1.4).

DOC uptake was detected at Crystal Rock and Sopers Branch, but not Sycamore Farm (Table 1.6). Estimated uptake lengths ($S_{w\text{-}DOC}$) were 385 and 279 m at Crystal Rock and Sopers Branch, respectively; uptake velocities ($v_{f\text{-}DOC}$) were 0.9 and 1.5 mm min^{-1}, respectively. Again, NO_2+NO_3-N and NH_4-N concentrations did not respond to the

increased availability of this limiting nutrient (CR: $p = 0.77$; SB: $p = 0.24$). At Sycamore Farm, DOC uptake was not detected and $S_{w\text{-}DOC}$ was greater than 350 m on the basis of variation in the estimate of k_{DOC} at that site (Appendix I).

DISCUSSION

All streams, including the stream draining the primarily forested watershed (SB), had NO_3-N concentrations similar to those observed in high-N streams impacted by urbanization or agriculture (Grimm et al. 2005, Bernot et al. 2006, Gücker and Pusch 2006, O'Brien et al. 2007). High NO_3-N concentrations in Sopers Branch are most likely the result of elevated atmospheric N inputs to the watershed (NADP 2009; Chapter 3). While atmospheric deposition contributes to elevated N in all three streams, comparatively higher NO_3-N concentrations at Crystal Rock likely result from urban land use, which is often associated with increased NO_3-N concentrations (Paul and Meyer 2001, Walsh et al. 2005, Stanley and Maxted 2008). Concentrations observed at Sycamore Farm (> 4000 μg NO_3-N L^{-1}; Table 1.2) were similar to other suburban streams in this region (Kaushal et al. 2008) and may reflect the agricultural legacy of the watershed (Lewis et al. 2006, Bernhardt et al. 2008, Maloney et al. 2008). Reduced vegetative cover within the Sycamore Farm watershed, resulting from construction activities (Table 1.1), may have played a role in increasing the delivery of NO_3-N, both from atmospheric deposition and legacy pools, to the channel.

A variety of explanations, including longitudinal variation in NO_3-N concentrations (Earl et al. 2006), masking by groundwater inputs or nitrification (Hamilton et al. 2001,

Williams et al. 2004), and limited removal due to low biological demand or saturated conditions (Hall and Tank 2003, Earl et al. 2006, Arp and Baker 2007, Simon et al. *in press*), have been invoked to account for a lack of measurable NO_3-N uptake. Evidence for and against various causal mechanisms that may have contributed to my inability to detect NO_3-N uptake are described below. While variation and groundwater inputs may have interfered with my ability to measure NO_3-N uptake, there is considerable evidence that all three sites are both N-saturated and P-limited.

In high-N stream ecosystems, inherent variation in N concentrations makes it difficult to measure uptake (Meyer et al. 2005, Bernot et al. 2006). Longitudinal variation was high during multiple NO_3-N additions at each site, potentially impeding my ability to detect uptake. However, uptake metrics associated with estimates of k_{NO3} when minimal variation was observed (Appendix I) indicate that uptake rates were either too low to produce a measurable reduction in NO_3-N concentrations or removal was masked by lateral or regenerative inputs ($k_{NO3} \approx 0$).

Uptake lengths based on variation in the estimate of k_{NO3} confirm that NO_3-N was generated along the length of the study reach during at least one addition at each site (Table 1.4, Appendix I). Longitudinal increases in NO_3-N may result from the production of NO_3^- via nitrification or delivery of NO_3-N to the channel through groundwater flowpaths (Figure 1.3). High nitrification rates are associated with high NO_3-N concentrations (Bernhardt et al. 2002) and low C:N ratios (Strauss and Lamberti 2000); accordingly, I expected to observe high rates of nitrification at all three sites since

background NO_3-N concentrations were high (Table 1.4) and DOC:DIN ratios were generally low (Table 1.2). Surprisingly, estimates of nitrification were low at all three sites. As a result, it is doubtful that nitrifying bacteria generated enough NO_3^- to significantly increase concentrations within addition reaches. Moreover, low rates of nitrification indicate that regenerative inputs of NO_3-N were unlikely to have masked NO_3-N uptake when k_{NO3} was not significantly different from zero. It is more conceivable that lateral inputs of NO_3-N were responsible for observed increases in NO_3-N at all three sites. Although concentrations of the conservative tracer did not decrease significantly during two of the three additions for which k_{NO3} was both significant and positive (CR: 25 June 2007; SF: 11 July 2005), even small groundwater inflows could produce the observed effect if groundwater NO_3-N concentrations were considerably higher than surface water concentrations (Hamilton et al. 2001). Because groundwater in the Piedmont physiographic region is particularly susceptible to NO_3-N contamination as a result of well-drained soils and underlying geology (Nolan 2001, Nolan and Stoner 2000), lateral inputs most likely account for occasional longitudinal increases in NO_3-N. Furthermore, inputs of N-rich groundwater may have completely or partially masked NO_3-N uptake during additions where k_{NO3} was not significantly different from zero, especially at Sopers Branch where significant dilution of the conservative tracer occurred during five of seven NO_3-N additions (Table 1.4).

Minimal N uptake is a recognized consequence of N saturation in stream ecosystems (Bernot and Dodds 2005). A complete lack of measurable uptake during NO_3-N additions may also be indicative of N-saturated conditions. When biotic uptake is

saturated, any NO_3-N added to the system is expected to behave as a conservative tracer and uptake lengths will be immeasurable (Stream Solute Workshop 1990). In Crystal Rock, Sycamore Farm, and Sopers Branch, I was unable to detect a significant longitudinal decline in NO_3-N during any addition experiment (Table 1.4); however, I was able to detect NH_4-N uptake at all three sites (Table 1.6). Biota preferentially assimilate NH_4^+ because it is less energetically expensive; so, it is not surprising that I observed NH_4-N uptake but not NO_3-N uptake. Bernot et al. (2006) were also able to measure NH_4-N uptake in agriculturally-impacted streams that were identified as NO_3-N saturated. Likewise, Gücker and Pusch (2006) observed shorter relative NH_4-N uptake lengths in two eutrophic streams in Germany. While NH_4-N removal occurred at all three sites (Table 1.6), uptake velocities were less than 75% of the values (25^{th} percentile: 2.6 mm min^{-1}) reported for 2^{nd} order streams in a review by Ensign and Doyle (2006). Furthermore, because $v_{f\text{-}NO3}$ was essentially zero and $v_{f\text{-}NH4}$ decreased with increasing DIN concentrations (r: -0.9790) it is clear that overall demand for DIN was reduced, in part, because of dramatically elevated N concentrations.

Other nutrients, particularly P and C, are expected to become limiting as the relative availability of N increases (Gress et al. 2007, Simon et al. *in press*). During the summer of 2007, demand for P relative to demand for inorganic N ($v_{f\text{-}PO4}/v_{f\text{-}NH4}$) increased significantly as TN:TP ratios increased (Figure 1.4), suggesting that P was limiting in these systems. Others have demonstrated that variation in relative rates of NH_4-N and PO_4-P uptake were related to N:P ratios in low-N (Munn and Meyer 1990) and N-limited systems (Simon et al. 2005). DIN:SRP ratios were dramatically elevated (>> 100)

during the entire study period (Table 1.2), further demonstrating the existence of P-limitation (Dodds 2003). Several other authors have documented NO_3-N saturation, and corresponding P limitation, in streams with molar DIN:SRP ratios similar to those observed in this study (e.g. Earl et al. 2006, Bernot et al. 2006, Simon et al *in press*).

Interestingly, despite evidence for P-limitation, measured PO_4-P uptake velocities (Table 1.6) were well below the 25th percentile for 2nd order streams (1.6 mm min^{-1}) reported by Ensign and Doyle (2006). Furthermore, PO_4-P additions did not produce a measurable change in NO_2+NO_3-N or NH_4-N concentrations despite reducing DIN:SRP ratios to less than 13. Either added P did not stimulate NO_3-N uptake or nitrification, or both processes were stimulated but rates of NO_3-N removal and generation were in approximate balance. It is also possible that N removal was stimulated but could not be detected with coarse, reach-scale comparisons of N concentrations before and during PO_4-P additions. Co-injection of NO_3-N and PO_4-P may have been better suited for detecting the response of N uptake to P fertilization; especially since background NO_3-N concentrations were highly variable. Simon et al. (*in press*) were able to detect NO_3-N removal in a N-saturated stream using this approach.

At all three sites DOC:DIN ratios were less than or equal to 1.0 (Table 1.3), suggesting that low availability of C may have further contributed to reduced cycling of N. DOC uptake was observed at Crystal Rock and Sopers Branch, indicating that the benthic community was C-limited. At Sycamore Farm, DOC uptake was too low to be detected within the 210 m reach despite DOC:DIN ratios that were indicative of greater C-

limitation. This may have resulted from high longitudinal variation in plateau DOC concentrations (Appendix I) or increases in DOC:DIN ratios (from 0.7 to 6.5 as compared to < 3.2 to >35 at CR and SF) that were too low to stimulate C removal. Again, NO_2+NO_3N and NH_4-N concentrations did not respond to DOC amendments, signifying that increased C availability did not stimulate N uptake. Given that both heterotrophic respiration and denitrification are controlled by the availability of C (Mulholland et al. 2009), I expected to observe increased demand for both NO_2+NO_3-N and NH_4-N during the DOC addition. Again, reach-scale comparisons may have been inadequate and co-injection of N and DOC may have allowed for better detection of the response of N concentrations to increased C availability. Both Butturini et al. (2000) and Bernhardt and Likens (2002) documented increased demand for N during DOC additions using this approach.

Measured uptake velocities indicated low demand for NH_4-N and PO_4-P relative to published studies; there is also considerable evidence that metabolic demand for NO_3-N was limited. GPP measured in conjunction with NO_3-N additions fell below the median value reported in a meta-analysis of 213 streams (Young et al. 2008). GPP was extremely low on several occasions, falling below the 25th percentile (~ 0.6 mg O_2 d^{-1}) at Crystal Rock during fall 2005, at Sycamore Farm during summer and fall 2005, and at Sopers Branch during fall 2005 and summer 2007 (Table 1.5). While high nutrient concentrations have been shown to stimulate GPP (Odum 1956, Bott et al. 1985, Mulholland et al. 2001), GPP may have been reduced in these streams as a result of limited light availability in densely shaded reaches or elevated concentrations of metals

(CR only; L Van-Tull, *personal communication*). For data collected during the summer of 2007, GPP was positively correlated with SRP concentrations (*r*: 0.999, p = 0.022), suggesting possible P-limitation of autotrophic metabolism. ER typically fell towards the upper range of values reported by Young et al. (2008). Relatively high respiration rates are likely the result of high nutrient concentrations (Table 1.2) and organic matter availability (Table 1.5).

Several authors have shown that NO_3-N removal is dominated by autotrophic uptake (Hall and Tank 2003, Mulholland et al. 2008, Hall et al. 2009b). Furthermore, the demand for NO_3-N relative to NH_4-N is lower in streams with lower GPP/ER (Hall and Tank et al. 2003, Webster et al. 2003; Figure 1.5). Both GPP and GPP/ER (< 0.22) were low at all three sites, on all dates of measurement (Table 1.5), suggesting that undetectable NO_3-N uptake in my study streams may be caused by limited uptake resulting from low autotrophic demand.

CONCLUSION

Many environmental stressors occur in combinations that are broadly recognized as resulting from either agricultural or urban land uses (Paul and Meyer 2001, Niyogi et al. 2004, Walsh et al. 2005, Bernot et al. 2006, Young et al. 2008, Wenger et al. 2009). Yet, elevated N concentrations in Crystal Rock, Sycamore Farm, and Sopers Branch were not accompanied by dramatic increases in limiting nutrients or light availability commonly observed in other high-N systems. While, several factors may have contributed to my inability to detect NO_3-N uptake; evidence presented here suggests that NO_3-N removal

rates at all three sites were essentially zero as a result of N saturation, P limitation, and low autotrophic demand.

The inability to detect uptake in all three of my study streams, along with evidence that non-significant uptake resulted from N-saturated and P-limited conditions, led to a new line of questioning to determine how often non-significant NO_3-N uptake rate coefficients have been equated with low biotic removal (discussed in detail in Chapter 2). Generally speaking, neither non-significant uptake nor N-saturation is commonly encountered in the stream literature and no other study examining uptake in multiple streams has reported non-significant uptake in all study reaches. However, several other authors have concluded that a lack of detectable NO_3-N uptake is the result of limited biological demand (Chapter 2). As with other systems that have a limited capacity for N removal, the resultant increase in the delivery of N to downstream waterways from my study streams is likely to have dramatic effects (Earl et al. 2006, Alexander et al. 2007, Howarth 2008).

Table 1.1. Summary of watershed land use. Data for Crystal Rock (CR) and Sopers Branch (SB) are from the Maryland Office of Planning 2002 data set (GISHydro2000; Moglen 2007). Data for Sycamore Farm (SF) were estimated from aerial photographs taken in 2004 and 2006 since publicly available data do not reflect changes to the landscape resulting from rapid urbanization. Open urban land includes land cleared of vegetation and graded for immediate development.

Site		Urban			Agricultural	Forested
		Residential % area	*Commercial/Industrial* % area	*Open* % area	% area	% area
CR		42.0	58.0	0.0	0.0	0.0
SF	*2004*	26.0	0.0	32.3	13.4	28.3
	2006	49.9	0.0	22.2	2.7	25.2
SB		0.7	2.1	0.0	10.6	86.6

Table 1.2. Water quality characteristics for Crystal Rock (CR), Sycamore Farm (SF), and Sopers Branch (SB). Data are compiled from samples collected approximately monthly between October 2004 and September 2006; *n* is the number of dates for which each metric was assessed.

Site		Conductivity μS^C cm^{-1}	Dissolved O_2 mg L^{-1}	Dissolved O_2 % sat.	pH	DOC µg L^{-1}	NO_2+NO_3-N µg L^{-1}	NH_4-N µg L^{-1}	SRP µg L^{-1}	DOC:DIN (molar)	DIN:SRP (molar)
CR	mean	598	9.11	84.0	6.65	2159	2255	28	3	1.2	1894
	(S.E.)	(65)	(0.30)	(2.6)	(0.15)	(274)	(128)	(10)	(<1)	(0.2)	(227)
	n	19	18	18	18	19	19	19	19	18	19
SF	mean	178	8.96	83.7	6.57	1697	4142	15	6	0.57	2202
	(S.E.)	(8)	(0.27)	(2.9)	(0.12)	(277)	(288)	(3)	(1)	(0.1)	(301)
	n	21	20	20	20	20	20	20	20	19	20
SB	mean	132	8.67	81.1	6.68	3091	1140	12	3	5.4	975
	(S.E.)	(4)	(0.21)	(2.9)	(0.13)	(1723)	(182)	(3)	(<1)	(3.7)	(168)
	n	21	20	20	20	20	20	20	20	19	20

Table 1.3. Comparison of ratios used to describe nutrient limitation status calculated from triplicate samples collected at Crystal Rock (CR), Sycamore Farm (SF), and Sopers Branch (SB) on 12 June 2007.

Site		DOC:TN (molar)	DOC:DIN (molar)	TN:TP (molar)	DIN:SRP (molar)
CR	Mean	0.9	1.0	368	489
	(S.E.)	(0.1)	(0.1)	(13)	(61)
SF	Mean	0.3	0.3	875	1144
	(S.E.)	(<0.05)	(<0.05)	(8)	(18)
SB	Mean	0.7	0.7	620	641
	(S.E.)	(0.1)	(0.1)	(98)	(200)

Table 1.4. Summary of physical and chemical data from all NO_3-N additions in 2005 and 2007. Data presented for NO_3-N uptake include p-values and first-order uptake rate coefficients (k_{NO3}); positive values of k_{NO3} indicate an increase in NO_3-N along the length of the study reach. Data presented for Br^- dilution include p-values and slopes (k_{Br}) from linear regressions of the plateau concentration of the conservative tracer against downstream distance. For both, k is only reported for regressions significant at α = 0.1.

Site	Date	Reach Length	*Q*	*v*	*w*	*d*	C_{amb}	C_{add}	C_{add}:C_{amb}	NO_3-N uptake		Br^- dilution		Summary
		m	L s^{-1}	m s^{-1}	m	m	µg NO_3-N L^{-1}			p	k_{NO3}	p	k_{Br}	
CR	07-Jul-05	110	27.8	0.03	3.8	0.23	1690	728	0.43	0.961		0.785		Uptake never detected; longitudinal increase in NO_3-N during 1 addition, possible lateral inputs during 1 addition
	11-Jul-05	110	49.1	0.12	3.9	0.10	2003	2590	1.29	0.600		**0.004**	**-0.036**	
	22-Jul-05	110	36.7	0.07	3.9	0.14	1687	1531	0.91	0.421		0.728		
	19-Nov-05	110	19.1	0.06	3.6	0.09	2506	555	0.22	0.676		0.308		
								701	0.28	0.751		0.116		
								1099	0.44	0.411		0.517		
	25-Jun-07	210	21.2	0.06	3.2	0.16	2082	170	0.08	0.327		0.163		
								314	0.15	**0.080**	**+0.002**	0.213		
SF	07-Jul-05	110	6.5	0.07	2.1	0.04	3174	134	0.04	n/a		0.293		Uptake never detected; longitudinal increase in NO_3-N during 1 addition, possible lateral inputs during 2 additions
	11-Jul-05	110	14.9	0.10	2.0	0.08	3320	189	0.06	**0.091**	**+0.002**	0.983		
	22-Jul-05	110	10.1	0.09	1.8	0.06	3824	230	0.06	0.947		0.623		
	28-Nov-05	110	9.5	0.06	2.3	0.07	4691	2535	0.54	0.176		0.481		
								3863	0.82	0.604		0.512		
								4988	1.06	0.952		0.508		
	22-Jun-07	210	6.8	0.07	2.1	0.11	4131	370	0.09	0.336		**0.002**	**+0.005**	
								610	0.15	0.755		**<0.001**	**-0.005**	
SB	07-Jul-05	110	23.6	0.09	2.0	0.13	764	35	0.05	0.919		**0.069**	**-0.006**	Uptake never detected; longitudinal increase in NO_3-N during 1 addition, possible lateral inputs during 5 additions
	11-Jul-05	110	27.3	0.09	2.4	0.12	927	40	0.04	0.370		0.128		
	22-Jul-05	110	18.2	0.06	2.3	0.14	1001	94	0.09	0.283		0.339		
	23-Nov-05	110	28.3	0.11	2.8	0.09	747	463	0.62	0.124		**<0.001**	**-0.009**	
								742	0.99	**0.068**	**+0.004**	**<0.001**	**-0.019**	
	24-Jun-07	210	16.4	0.05	2.7	0.17	1085	350	0.32	0.660		**<0.001**	**-0.006**	
								780	0.72	0.792		**0.015**	**-0.010**	

Table 1.5. Summary of biological measurements corresponding with NO_3-N additions conducted in 2005 and 2007.

Site	Date		GPP g O_2 m^{-2} d^{-1} *n* = 8	ER g O_2 m^{-2} d^{-1} *n* = 8	GPP/ER *n* = 8	CBOM g AFDM m^{-2} *n* = 10	FBOM g AFDM m^{-2} *n* = 10
CR	Jul-05	mean	0.77	6.05	0.14	2.8	31.9
		(S.E.)	(<0.01)	(<0.01)	(0.02)	(0.7)	(4.3)
	Nov-05	mean	0.35	5.20	0.07	214.4	28.3
		(S.E.)	(<0.01)	(0.01)	(0.01)	(97.5)	(7.8)
	Jun-07	mean	1.09	5.56	0.20	5.6	19.2
		(S.E.)	(0.10)	(0.18)	(0.02)	(2.1)	(6.5)
SF	Jul-05	mean	0.05	5.06	0.01	27.7	73.4
		(S.E.)	(<0.01)	(0.01)	(<0.01)	(21.7)	(18.6)
	Nov-05	mean	0.46	3.69	0.13	70.1	40.3
		(S.E.)	(<0.01)	(<0.01)	(<0.01)	(31.9)	(9.5)
	Jun-07	mean	0.9	4.19	0.22	49.4	37.9
		(S.E.)	(0.10)	(0.24)	(0.02)	(28.2)	(11.4)
SB	Jul-05	mean	0.67	3.06	0.22	67.6	104.7
		(S.E.)	(<0.01)	(<0.01)	(0.01)	(57.2)	(38.4)
	Nov-05	mean	0.19	4.80	0.05	16.0	27.8
		(S.E.)	(<0.01)	(<0.01)	(<0.01)	(8.5)	(8.6)
	Jun-07	mean	.22	4.42	0.05	312.6	42.8
		(S.E.)	(<0.01)	(0.03)	(<0.01)	(295.5)	(7.5)

Table 1.6. Summary of physical and chemical data from NH_4-N, PO_4-P, and DOC additions conducted during summer 2007. All additions were conducted in 210 m reaches; ammonium additions lasted less than 2 h; phosphate and DOC additions lasted 6 h. Uptake metrics (S_w, v_f, U) are reported for regressions significant at $\alpha = 0.1$.

Added Nutrient	Site	Date	Q L s^{-1}	v m s^{-1}	w m	d m	C_{amb} µg L^{-1}	C_{add} µg L^{-1}	C_{add}:C_{amb}	p	k	S_w m	v_f mm min^{-1}	U µg m^{-2} s^{-1}
NH_4-N	CR	7-Jul-07	22.7	0.06	3.1	0.16	52	128	2.5	< **0.001**	**-0.006**	177	2.4	127
	SF	2-Jul-07	7.4	0.08	2.2	0.11	25	48	1.9	**0.035**	**-0.005**	200	1.0	26
	SB	3-Jul-07	15.0	0.05	2.4	0.13	41	135	3.3	**0.070**	**-0.007**	146	2.5	104
PO_4-P	CR	4-Sep-07	10.2	0.04	3.0	0.14	30	2738	91.3	**0.004**	**-0.001**	769	0.3	8
	SF	30-Aug-07	7.4	0.06	2.2	0.15	9	588	65.3	< **0.001**	**-0.003**	296	0.7	6
	SB	29-Aug-07	10.5	0.04	2.2	0.13	3	581	193.7	**0.001**	**-0.003**	300	0.9	3
DOC	CR	24-Jul-07	18.1	0.03	3.2	0.14	3400	57647	17.0	**0.010**	**-0.003**	385	0.9	3041
	SF	21-Jul-07	4.2	0.04	2.2	0.10	2351	18589	7.9	0.161			0.0	0
	SB	20-Jul-07	17.8	0.02	2.5	0.14	2513	25867	10.3	**0.017**	**-0.004**	279	1.5	3889

FIGURE CAPTIONS

Figure 1.1. Map of study watersheds.

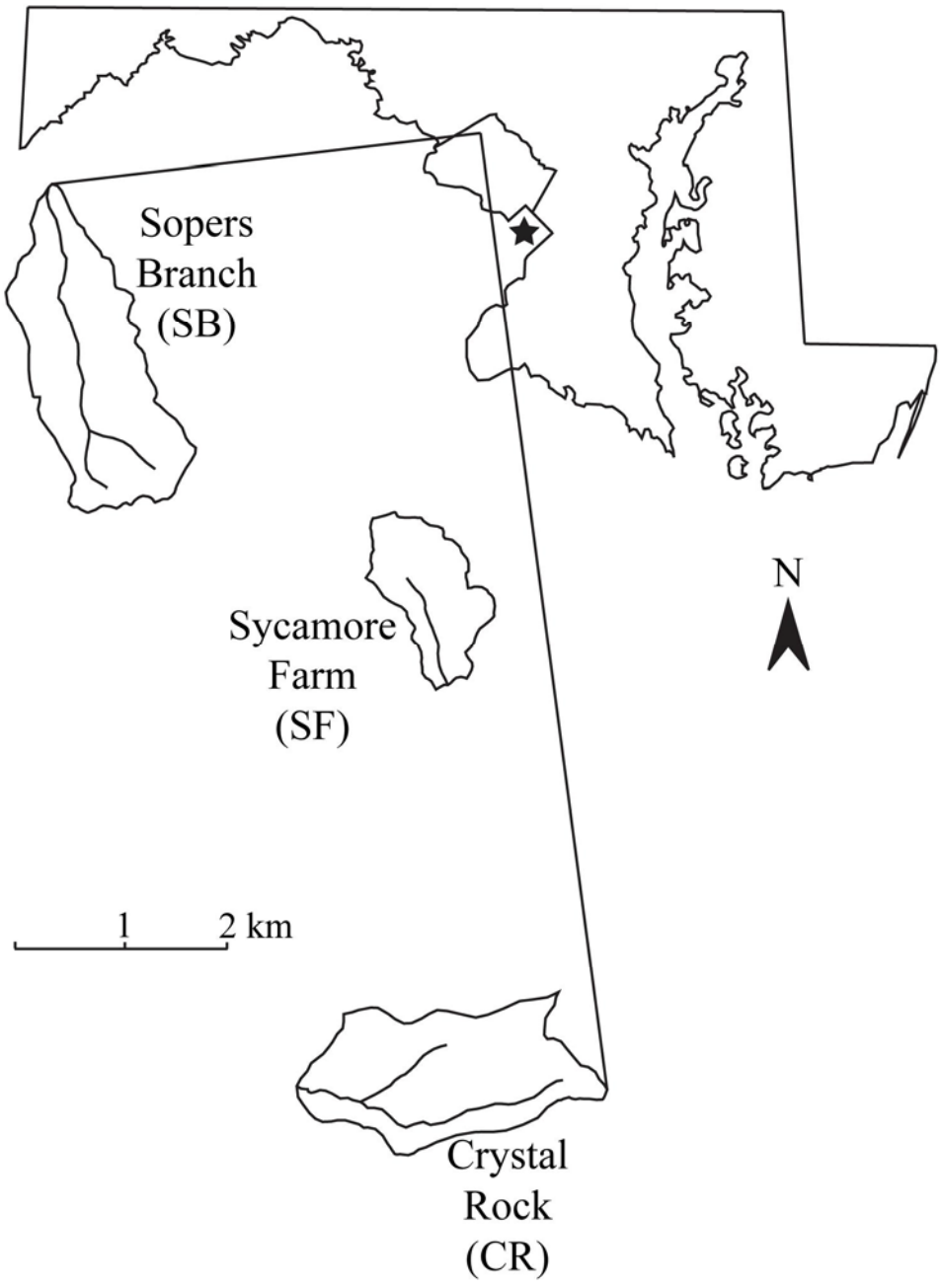
Figure 1.2. Photographs showing cleared and graded land within the rapidly urbanizing Sycamore Farm watershed; taken during May (a) and August (b) of 2005.

Figure 1.3. Illustration of the major processes that influence NO_3-N concentrations in streams.

Figure 1.4. Demand for PO_4-P ($v_{f\text{-}PO4}$) relative to demand for NH_4-N ($v_{f\text{-}NH4}$) increased with increasing TN:TP (closed circle) and DIN:SRP (open circles) ratios. Data are from summer 2007; each point represents one stream. The solid line indicates a significant ($\alpha = 0.05$) linear relationship between $v_{f\text{-}PO4}/ v_{f\text{-}NH4}$ and TN:TP.

Figure 1.5. Demand for NO_3-N ($v_{f\text{-}NO3}$) relative to demand for NH_4-N ($v_{f\text{-}NH4}$) is lower in streams with low relative autotrophic demand (GPP/ER). Data are from Webster et al. 2003 (open squares), Hall and Tank 2003 (open circles), and this study (closed triangles). The solid line indicates the significant ($\alpha = 0.05$) linear relationship between $v_{f\text{-}NO3}/ v_{f\text{-}NH4}$ and GPP/ER for published values.

(Figure 1.1)

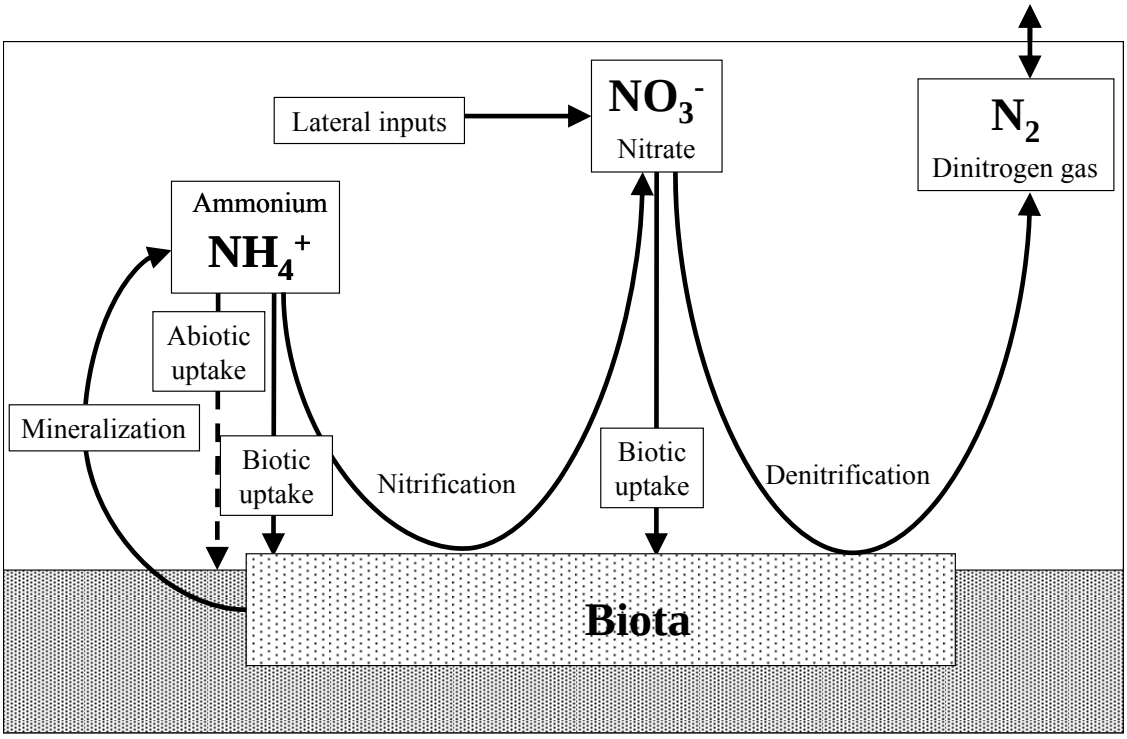


(Figure 1.2)

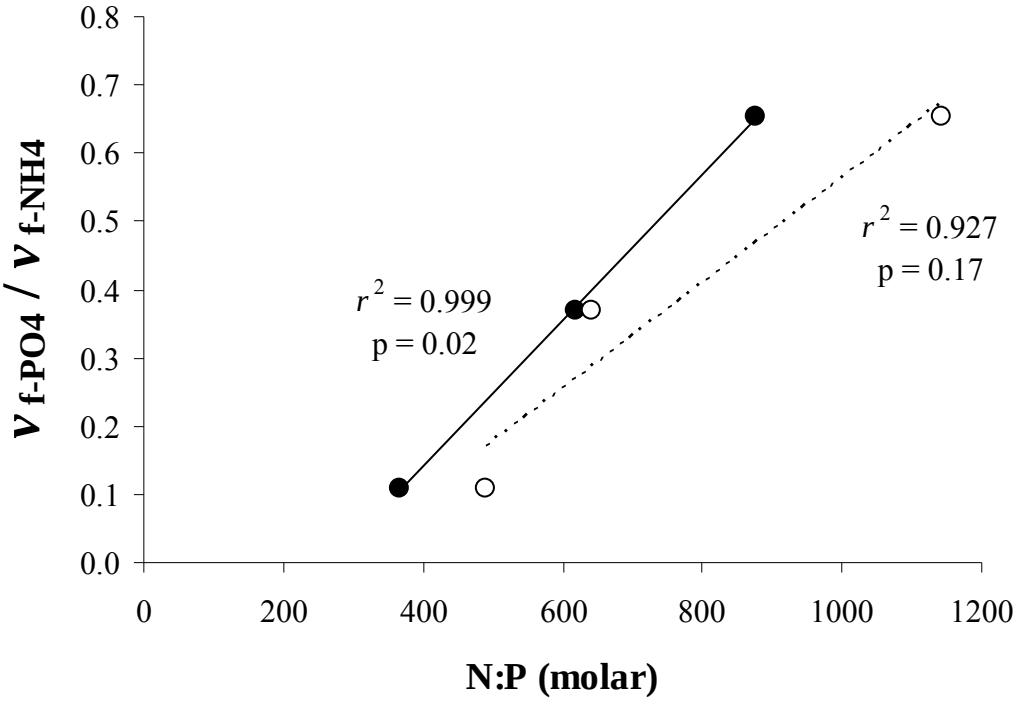
(a)

(b)

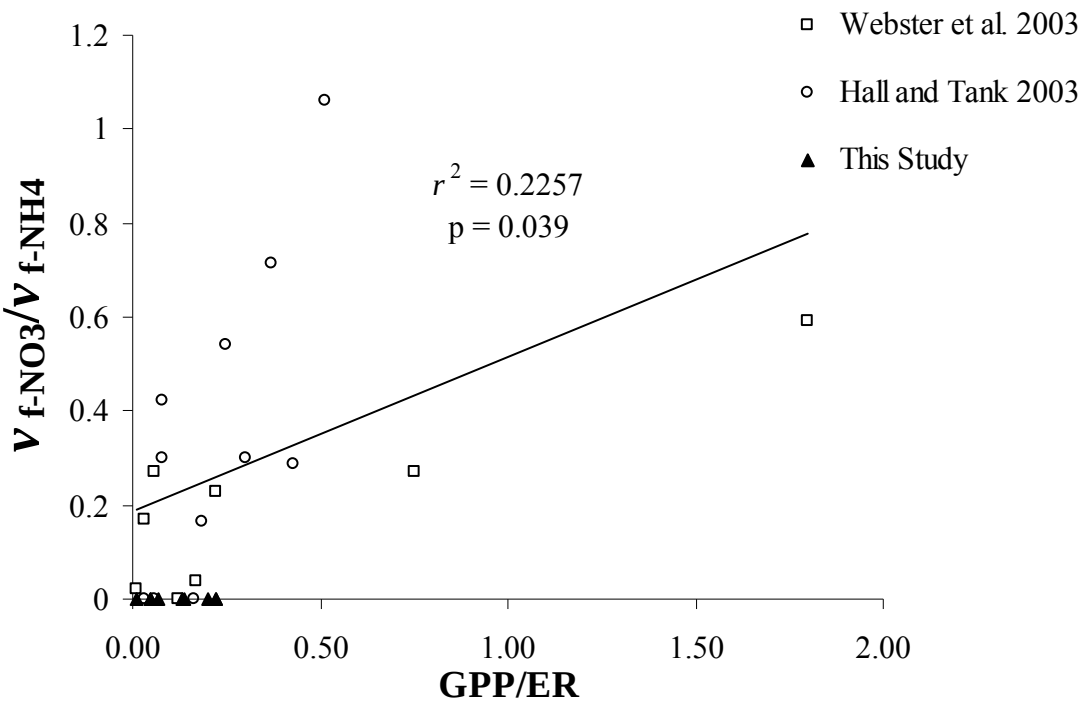
(Figure 1.3)


Lateral inputs
NO_3^-
Nitrate
N_2
Dinitrogen gas
Ammonium
NH_4^+
Abiotic uptake
Mineralization
Biotic uptake
Nitrification
Biotic uptake
Denitrification
Biota

(Figure 1.4)


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
V f-PO4 / V f-NH4
0
200
400
600
800
1000
1200
N:P (molar)
r 2 = 0.999
p = 0.02
r 2 = 0.927
p = 0.17

(Figure 1.5)


Webster et al. 2003
Hall and Tank 2003
This Study
r^2 = 0.2257
p = 0.039
V f-NO3/V f-NH4
GPP/ER
1.2
1
0.8
0.6
0.4
0.2
0
0.00
0.50
1.00
1.50
2.00

CHAPTER 2

Can lack of evidence for uptake inform our understanding of nitrate saturation in stream ecosystems?

ABSTRACT

While many streams are important locations of nitrogen (N) removal, there is mounting evidence that not all streams are able to remove an appreciable fraction of their N load as a result of reduced efficiency of processing or saturation of uptake. Unfortunately, we have limited knowledge regarding N-saturated systems partly because they are difficult to identify using commonly employed nutrient addition and isotopic tracer methods. I surveyed the literature to find all cases of nitrate uptake rate coefficients that were not statistically different from zero in order to identify patterns underlying the inability to detect removal and determine the frequency with which non-significant results could be equated with limited nitrate uptake. In more than half of the streams where nitrate uptake could not be detected there was evidence for negligible removal; background nitrate concentrations in these streams varied from 1.7 to 4650 μg N L^{-1}. Interestingly, those streams with high background concentrations and negligible uptake were described by authors as N-saturated while low-N streams were not, indicating a context-dependent bias regarding the language used to describe the outcome of N additions. Low statistical power and masking of uptake by lateral or regenerative inputs were observed less often and frequently in conjunction with negligible uptake. Non-significant uptake was the result of artificial saturation in only one study. My findings illustrate two keys points: 1) increased knowledge of those systems where nitrate uptake cannot be detected may improve our understanding of N saturation and 2) publication and presentation issues

may distort our current view of the presence and prevalence of N saturation in stream ecosystems.

INTRODUCTION

Human activities have more than doubled nitrogen (N) inputs to the landscape (Vitousek et al. 1997, Galloway et al. 2004). Consequently, delivery of N, largely as nitrate (NO_3^-), from uplands to coastal waters has increased, resulting in eutrophication along with widespread hypoxic and anoxic conditions that are detrimental to the structure and function of these systems (Howarth et al. 1996, Vitousek et al. 1997, Howarth et al. 2002, Galloway et al. 2003, Howarth 2008). Understanding how streams transport and transform N is a central focus of aquatic biogeochemistry (Ensign and Doyle 2006); more importantly, a clear quantitative, understanding of these processes is needed if we are to mitigate the downstream effects of elevated N inputs (Vitousek et al. 1997, Peterson et al. 2001).

A rich body of research has described the critical role streams play in controlling N export, influencing both the transport and fate of N in watersheds (Howarth et al. 1996, Galloway et al. 2004). As a result of research conducted largely in pristine headwater streams (Peterson et al. 2001, Bernhardt et al. 2003, see review in Ensign and Doyle 2006), streams are widely viewed as important N sinks. Recent work in large rivers (e.g. Tank et al. 2008) and human-altered stream ecosystems (e.g. Grimm et al. 2005, Bukaveckas 2007) suggests that these water bodies may also be capable of substantially reducing the amount of N delivered to downstream ecosystems.

Despite research demonstrating that many streams are important locations of N removal, there is mounting evidence that not all streams are able to remove an appreciable fraction of their N load. Efficiency of processing has been found to decrease with increasing ambient N concentrations (Dodds et al. 2002, Earl et al. 2006, O'Brien et al. 2007, Mulholland et al. 2008, 2009, Hall et al. 2009b) and saturation of uptake may occur if supply exceeds biotic demand (Wollheim et al. 2001, Bernot and Dodds 2005, Simon et al. *in press*). Unfortunately, we have limited knowledge regarding those systems that are unable to remove N due to saturation of biological demand (Bernot and Dodds 2005), in part because they are difficult to identify using methods commonly employed to assess N uptake.

The nutrient spiraling concept, which describes the simultaneous processes of nutrient cycling and downstream transport (Webster and Patten 1979), has provided the primary methodological framework for assessing N uptake in streams (Newbold et al. 1981, Stream Solute Workshop 1990). N uptake is generally quantified using short-term additions to determine the first-order uptake rate coefficient (k) for the decline in N concentration with downstream distance. This coefficient is used to calculate three metrics described by Newbold et al. (1981) and the Stream Solute Workshop (1990):

1) Uptake length (S_w, m), the average downstream distance traveled by a nutrient in its dissolved form, is the negative inverse of the first-order uptake rate coefficient ($-1/k$).

2) Uptake velocity (v_f, m s^{-1}), the speed at which a nutrient molecule moves vertically through the water column to the streambed, is equal to $[(Q/w)/S_w]$, where Q is discharge and w is the average width of the reach.

3) Uptake rate (U, μg m^{-2} s^{-1}), the nutrient load removed from the water column per unit streambed area, is equal to (v_f * C_{amb}), where C_{amb} is the ambient background nutrient concentration.

All three spiraling metrics depend on the ability to detect a decrease in the added nutrient along the length of the study reach; when k is not significantly different from zero, nutrient spiraling metrics cannot be calculated. Non-significant results are expected if N uptake is saturated under ambient conditions, since any N added to the channel will behave like a conservative tracer (Stream Solute Workshop 1990). Moreover, as streams approach biotic saturation, the first-order properties of N uptake break down and N is transported increasingly greater distances prior to removal until uptake lengths are immeasurable (Stream Solute Workshop 1990, Davis and Minshall 1999, Hall and Tank 2003, Earl et al. 2006, Alexander et al. 2007). Recent applications of stable isotope (Mulholland et al. 2008) and multiple addition approaches (Payn et al. 2005, Earl et al. 2006, 2007) have improved upon previous methods (Stream Solute Workshop 1990, Webster and Valett 2006), which are known to overestimate S_w (Mulholland et al. 2002) and ambient uptake rates (Dodds et al. 2002), but interpretation of results remains constrained when uptake cannot be detected. Regardless of the method employed, uptake rate coefficients that are not statistically different from zero are encountered and

problematic, as they prevent us from determining the true state of N uptake in these streams.

Here, I review those studies where streams were identified as N saturated or N uptake was undetectable using either nutrient addition or isotope tracer techniques. My findings illustrate two keys points: 1) knowledge of those systems where nitrate uptake cannot be detected has the potential to inform our understanding of N saturation and 2) publication and presentation issues may distort our current view of the presence and prevalence of N saturation in stream ecosystems.

METHODS

I focus specifically on nitrate uptake because nitrate is the predominant form of N in many polluted systems (Howarth et al. 1996, Davis and Minshall 1999, Royer et al. 2004, Grimm et al. 2005, Stanley and Maxted 2008). Furthermore, because nitrate uptake is a function of biotic, as opposed to abiotic processes (e.g. sorption), nitrate dynamics may more closely approximate total N dynamics. Specifically, nitrate is removed from the water column by plants, algae, and microbes for growth and metabolism ("assimilatory uptake") or it may be converted to N-containing gases via denitrification ("dissimilatory uptake"). Both processes are ultimately expected to saturate as N concentrations increase (Bernot and Dodds 2005, García-Ruiz et al. 1998), but the concentration at which saturation occurs is expected to vary both spatially and temporally (Stream Solute Workshop 1990, Bernot and Dodds 2005).

I surveyed the literature to find all reported cases of N saturation or undetected nitrate uptake resulting from solute addition experiments. References were located using Web of Science (Thompson Reuters, New York, NY; *keywords: (stream or river) and (nitrogen or nitrate) and (uptake or removal or retention or saturation*), *publication date: 1985 to 2009*), supplemental information from previous meta-analyses (Ensign and Doyle 2006, Tank et al. 2008), and review of literature cited by other studies; I also include results from my own research (Chapter 1). Explanations for non-significant results were extracted from publications and authors were contacted as necessary for additional information.

RESULTS

Though the inability to detect nitrate uptake is a relatively rare occurrence in the literature, it has been documented by several authors (Table 2.1) employing both the nutrient addition method (11 studies, 27 streams) and the isotope tracer method (3 studies, 5 streams). Three papers reporting on the Lotic Intersite Nitrogen Experiment (LINX) II dataset (e.g. Mulholland et al. 2008, 2009, Hall et al. 2009b) are regarded as a single isotope tracer study. Two publications, Earl et al. 2006 and 2007, describe a series of $^{15}NO_3^-$ injections where ambient concentrations were amended using unlabeled NO_3^-. For amended isotope additions, uptake rate coefficients were calculated using either ^{15}N tracer data (Earl et al. 2006) or bulk nitrate concentrations (Earl et al. 2007); for the purpose of accounting, studies are considered as employing the isotope tracer and nutrient addition methods, respectively. Experiments were performed in streams with discharge ranging from 1.1 to 830 L s^{-1} and background nitrate concentrations ranging

from 1.7 to 4650 μg N L^{-1}. Most studies were conducted during daylight hours (but see Fellows et al. 2006) between the spring and fall. With the exception of Hamilton et al. (2001) and Kellman (2004), studies evaluated nitrate uptake in multiple reaches or under differing conditions (e.g. Hall et al. 2009a evaluated uptake in one stream during periods of baseflow and snowmelt). Ten of 14 studies provided direct explanations for non-significant uptake rate coefficients (Table 2.1). One additional paper (Fellows et al. 2006) provided enough information to identify a probable explanation for undetected uptake. Authors were contacted for the three remaining studies (Bukaveckas 2007, Hall et al. 2009a, and LINX II) where causes of non-significant uptake were not explicitly discussed.

DISCUSSION

Quantification of nitrate uptake using solute additions is an essential part of studying N cycling in lotic ecosystems; however, interpretation of results is difficult when values of *k* are not significantly different from zero. Data from solute additions alone cannot explain undetected uptake (Stream Solute Workshop 1990), explanations are not mutually exclusive, and evidence supporting or refuting particular mechanisms may be inconclusive or limited.

Non-significant results from both nutrient and isotope tracer additions may signify that little or no assimilatory or dissimilatory uptake is occurring (Stream Solute Workshop 1990) or they may result from low statistical power owing to variability or small sample size. Interpretation of results from traditional nutrient additions is further complicated by

the potential for masking of uptake by nitrification (a dissimilatory, microbial process by which NH_4^+ is converted to NO_3^-) or lateral inputs of nitrate (e.g. via groundwater), as well as the possibility that the added nitrate will artificially saturate the system.

Methodological artifacts-Artificial saturation is a potential consequence of elevating ambient concentrations to evaluate N uptake capacity (Stream Solute Workshop 1990, Mulholland et al. 2002). Amendments that are capable of saturating uptake are system specific; dependent upon both the proportional increase over background (C_{add}:C_{amb}) and whole-stream biotic demand (Mulholland et al. 2002, Earl et al. 2007). Of the 14 studies reporting non-significant nitrate uptake rate coefficients, only Lautz and Siegel (2007) cited artificial saturation as a possible explanation, though the relative magnitude of amendments in their study (C_{add}:C_{amb} < 34) fell below the maximum value for which nitrate uptake has been quantified (C_{add}:C_{amb} = 57, Earl et al. 2006).

Masking of nitrate removal-The nutrient addition method is generally thought to quantify gross uptake (Martí et al. 1997, Hall and Tank 2003) since of rates of mineralization (i.e. release of nutrients from the benthos) are unaffected by amendments (Dodds et al. 2002). However, for nitrate additions, measurements may better reflect net uptake (Stream Solute Workshop 1990) since lateral or regenerative inputs are capable of masking removal. Evidence for or against masking is often equivocal when nitrate additions yield non-significant results, yet several studies have acknowledged that uptake was at least partially disguised by groundwater inputs (e.g. Williams et al. 2004, Chapter 1) or nitrification (e.g. Hamilton et al. 2001, Kellman 2004, Bukaveckas 2007).

Williams et al. (2004) used conservative tracer data to conclude that lateral inputs of nitrate may have contributed to the inability to detect uptake in the Ipswich River basin; I similarly concluded that groundwater inputs potentially masked nitrate uptake in three streams in the Maryland Piedmont (Chapter 1). Even when lateral inflows are minimal, groundwater inputs may obscure uptake if groundwater concentrations are considerably higher than surface-water concentrations (Hamilton et al. 2001, Chapter 1).

Bukaveckas (2007) used additions at Wilson Creek and Harts Run where nitrate increased along the length of the study reach to determine that nitrification contributed to masking; concluding that regenerative, rather than lateral, inputs masked uptake partly because groundwater inflow was small and there were no nearby sources of nitrate (P.A. Bukaveckas, *personal communication*). A groundwater-surfacewater mixing model employed by Kellman (2004) suggested that nitrification was a possible explanation for non-significant results given that in-stream nitrate concentrations exceeded estimates of surface water nitrate concentrations after accounting for groundwater dilution. Hamilton et al. (2001) identified nitrification as a possible cause of undetectable uptake in Eagle Creek by simultaneously modeling longitudinal changes of $^{15}NH_4$-N and $^{15}NO_3$-N during injections conducted immediately following the nitrate addition for which uptake was undetected.

Low statistical power-Several studies mention inadequate mixing of solutes (e.g. Kellman 2004, Earl et al. 2006, LINX II studies; P.J. Mulholland and R.O. Hall, *personal*

communication) or inherent variability in background concentrations (e.g. Chapter 1) as contributing to the inability to measure nitrate uptake. Statistical power is also reduced when sample sizes are inadequate (e.g. Hall et al 2009a; R.O. Hall, *personal communication*). Less conservative statistical tests ($\alpha = 0.1$) have been used to improve detection of N uptake in large rivers (e.g. Tank et al. 2008) and human-impacted stream ecosystems (e.g. Grimm et al. 2005, Meyer et al. 2005, Bernot et al. 2006) since they account for uncertainty associated with measuring uptake when removal capacity is low (Hanafi et al. 2007) or N concentrations are elevated (Meyer et al. 2005, Earl et al. 2007). Regardless of the selected value of α, examination of confidence limits around estimates of k may be used to assess the strength of inference when results are non-significant (Hall and Tank 2003, Hall et al. 2009a, Chapter 1).

Recognizing minimal uptake- Streams that are N-saturated are expected to exhibit zero-order uptake kinetics, where rates of nitrate uptake are independent of background concentrations (Earl et al. 2006, Alexander et al. 2007). Earl et al. (2006, 2007) used results from multiple, amended isotope tracer injections to determine that both Alta and Greenbrier Creeks were N-saturated according to this model. Interestingly, of the six streams studied by Earl et al. (2006, 2007) these were the only two identified as N-saturated; they were also the only streams where uptake could not be detected on occasion.

For several nutrient addition studies, it was possible to equate a lack of nitrate uptake with low biotic demand using information provided by supporting measurements

including direct assessment of biological activity (e.g. whole-stream metabolism), benthic resources (e.g. BOM, algal biomass), or demand for limiting nutrients (e.g. N:P ratios, P uptake). Successful additions conducted as part of the same study often provided context for interpretation of supporting measurements. For example, Hall and Tank (2003) used the relationship between relative autotrophic demand (GPP:ER) and nitrate uptake based on data from 11 streams to conclude that low autotrophic demand resulted in infinite uptake lengths in three streams with comparatively low GPP:ER (0.03 to 0.17) and background nitrate concentrations ranging from 43 to 169 μg N L^{-1}. I also concluded that nitrate removal was limited in three streams with similarly low GPP:ER (0.05 to 0.22; Chapter 1) and considerably higher ambient nitrate concentrations (747 – 4650 μg N L^{-1}). Hamilton et al. (2001) observed low relative autotrophic demand (GPP:ER = 0.1) and determined that nitrate uptake in Eagle Creek was below detection based on observed longitudinal increases in both labeled (i.e. $^{15}N\text{-}NO_3^-$) and unlabeled nitrate during a long-term addition of $^{15}NH_4Cl$. Fellows et al. (2006) also indicated that photoautotrophic metabolism was an important driver of nitrate removal based on repeated daytime and nighttime measurements of uptake, whole-stream metabolism, and benthic metabolism made in four streams. Though they did not provide a direct explanation for the inability to detect nitrate uptake during a nighttime addition at Rio Calveras, their results imply that undetected removal may have been the result of reduced photoautotrophic demand. Finally, in a study examining the effects of network position on N uptake, Arp and Baker (2007) were unable to detect nitrate uptake in eight reaches, seven of which were located below lake outlets. Based on comparisons of benthic resource availability, streambed

stability, and phosphorus (P) demand, they concluded that nitrate uptake was likely reduced because of the altered biotic demands of below-lake communities.

As N inputs increase, nitrate becomes less limiting and the demand for other nutrients, including P, increases (Vitousek et al. 1997, Gress et al. 2007). As a result, N-saturation and P-limitation are closely linked (Earl et al. 2006, Simon et al. *in press*). Simon et al. (*in press*) used simultaneous additions of N and P to determine that West Bear Brook was P-limited. Furthermore, increased availability of P enhanced nitrate uptake capacity, reversing N-saturation. I also identified P-limitation in my three study streams using P additions, but did not observe any reach-scale effects on N concentrations (Chapter 1). Molar TN:TP ratios (Dodds 2003), ranging from 368 to 875, provided additional support for the hypothesis my streams were both P-limited and N-saturated (Chapter 1).

Presentation and publication issues-Although the concentration at which uptake is expected to saturate varies both spatially and temporally (Stream Solute Workshop 1990, Bernot and Dodds 2005), there is a context-dependent bias regarding the language used to describe the response of streams to N additions. Studies conducted in impacted or high-N systems (C_{amb} > 200 μg NO_3-N L^{-1}), where non-significant results may be anticipated, often label systems as saturated when uptake is below detection and there is evidence for low biological N demand (e.g. Earl et al. 2006, 2007, Simon et al. *in press*, Chapter 1). Likewise, saturation has been used to explain low, but quantifiable nitrate removal in streams with background concentrations ranging from 50 to 5100 μg L^{-1} (e.g. Davis and Minshall 1999, Bernot et al. 2006). Conversely, unimpacted stream ecosystems are

described as having “limited” or “altered” biotic demand when uptake cannot be measured, suggesting that uptake is low, but not zero (e.g. Hamilton et al. 2001, Hall and Tank 2003, Arp and Baker 2007).

Occasionally, non-significant results are not directly addressed (e.g. Bukaveckas 2007, LINX II studies, Hall et al. 2009a) and causal mechanisms cannot be inferred. This is unfortunate because negative results do not have inherently negative connotations (Charlton 2004); assignment of positive hypotheses to non-significant results provides added value by allowing for the identification of streams where N cycling is limited. These packaging biases may influence our broader view of N saturation in stream ecosystems; it is critical that authors provide clear explanations for lack of detection and readers are aware of the use of subjective language.

Authors may also be less likely to publish results when nitrate removal cannot be detected (Csada et al. 1996), particularly when minimal uptake is inexplicable or in conflict with the widely-accepted view that streams are important N sinks (Møller and Jennions 2001). Non-significant results from nutrient additions may be especially susceptible to submission bias because, even though it is still the most common approach for measuring N removal, methodological limitations are well-documented and analysis of non-significant results is complex. Because of the potential for poor accounting, summarizing the frequency with which streams are at or nearing saturation is challenging. It is essential that non-significant results from both nutrient addition and isotope tracer studies continue to be evaluated and published to allow for recognition of

patterns underlying N saturation in stream ecosystems (Lortie and Dyer 1999, Hjältén and Price 1999).

CONCLUSION

Non-significant nitrate uptake rate coefficients are rarely encountered in the literature; however, half of the studies where nitrate uptake could not be detected provided sound evidence for negligible uptake. As a result, reduced biotic demand for nitrate accounted for non-significant results in 18 of 32 streams. An additional seven streams potentially had limited capacity for nitrate removal, but studies did not provide evidence for low uptake (Bukaveckas 2007, Kellman 2004, Williams et al. 2004; Table 2.1). Moreover, lack of detectable nitrate removal during nutrient addition experiments was more frequently attributed to minimal biotic uptake than masking effects or artificial saturation. These results imply that continued evaluation and publication of non-significant results should advance our understanding of N-saturation in stream ecosystems.

Streams with low nitrate uptake capacity were located in a variety of settings and had ambient nitrate concentrations ranging from 1.7 to 4650 μg N L^{-1}, indicating that even systems receiving relatively small N inputs may have limited capacity to retain N. Metabolic data, when available, indicated that streams were heterotrophic (GPP/ER < 1) with relatively low rates of gross primary productivity (0.05 to 0.8 mg O_2 m^{-2} d^{-1}; Young et al. 2008). This was not unexpected given the documented importance of assimilative uptake, particularly by autotrophs, to nitrate removal (Martí et al. 1997, Hall and Tank 2003, Niyogi et al. 2004, Fellows et al. 2006, Gücker and Pusch 2006, Mulholland et al.

2008, Hall et al. 2009b). Several streams were both N-saturated and P-limited, underscoring the interconnectedness of nutrient cycles. The constraints of our current understanding of N-saturation in lotic ecosystems are emphasized by the fact that no overarching patterns emerge when all streams exhibiting negligible nitrate uptake are reviewed.

Many studies highlight the potential role of in-stream processing in mitigating the downstream consequences of increased N inputs (e.g. Alexander et al. 2000, Peterson et al. 2001, Bernhardt et al. 2003, Ensign and Doyle 2006, Tank et al. 2008); yet, because not all streams are effective N sinks, we cannot rely on streams to alleviate the impacts of excess N inputs. Streams that are unable to retain substantial fractions of their N load transfer larger loads to subsequent reaches (Vitousek et al. 1997, Galloway et al. 2003, Earl et al. 2006), placing the burden of N removal on higher-order recipient systems (Alexander et al. 2007, Seitzinger 2008). Because elevated nitrate concentrations have been linked to saturation, reduced efficiency of removal, and increased rates of nitrification, delivery of larger N loads to downstream reaches may rapidly propagate through lotic networks (Strauss and Lamberti 2000, Bernhardt et al. 2002, Kemp and Dodds 2002, Bernot and Dodds 2005, Earl et al. 2006, Mulholland et al. 2008). Thus, any increase in the amount of N entering systems that are already limited in their ability to retain N will have dramatic downstream consequences (Peterson et al. 2001, Williams et al. 2004, Bernot and Dodds 2005). While our of understanding N saturation continues to develop, it is critical that management efforts reduce N loading to streams (Driscoll et

al. 2003, Galloway et al. 2003, Mulholland et al. 2008) and take advantage of opportunities for increasing in-stream N removal in impaired systems (Craig et al. 2008).

Table 2.1. Summary of published studies for which nitrate uptake could not be detected.

Citation	Method	Stream	Land use (Description)	Stream Order	Q L s^{-1}	C_{amb} ug N L^{-1}	C_{add}:C_{amb}	Explanation
Arp & Baker 2007	Nutrient addition [1]	Stanley Lake 3, ID	Unimpacted (Lake inlet)	---	670	13.3	3.0	No direct explanation for lake inlet reach; otherwise, evidence for low uptake
		Stanley Lake 4, ID	Unimpacted (Lake outlet)	---	830	1.5	7.5	
		Warm Springs, ID	Unimpacted (Lake outlet)	---	167	1.2	2.2	
		Yellow Belly 2, ID	Unimpacted (Lake outlet)	---	254	13.0	3.2	
		Yellow Belly 4, ID	Unimpacted (Lake outlet)	---	619	15.5	4.1	
		Yellow Belly 6, ID	Unimpacted (Lake outlet)	---	760	7.1	3.5	
		Stanley Lake 5, ID	Unimpacted (Below lake)	---	704	4.3	4.8	
		Warm Springs 5, ID	Unimpacted (Below lake)	---	238	1.7	3.1	
Bukaveckas 2007	Nutrient addition [1]	Harts Run, KY	Meadow (Reference)	---	62	63	---	No direct explanation in text; hypothesize nitrification or low uptake[2]
		Wilson Creek, KY	Meadow (Channelized)	---	125	375	---	
		Wilson Creek, KY	Meadow (Restored)	---	109	456	---	
Craig (Chapter 1)	Nutrient addition	Crystal Rock, MD	Urban	2^{nd}	19 – 49	1687 – 2506	0.15 – 1.29	Evidence for N saturation and P limitation; variability; possible masking by groundwater inputs
		Sycamore Farm, MD	Mixed	1^{st}	6 – 15	3174 – 4650	0.04 – 1.06	
		Sopers Branch, MD	Forested	2^{nd}	17 – 28	747 – 1066	0.04 – 0.99	
Earl et al. 2006	Amended isotope tracer[3]	Greenbrier Creek, VA	Mixed	$1^{st}/2^{nd}$	32	983	0.10	Evidence for N saturation; ^{15}N variability
Earl et al. 2007	Amended isotope tracer[4]	Alta Creek, VA	Mixed	$1^{st}/2^{nd}$	15	182	2.16	Evidence for N saturation

Table 2.1 (continued)

Citation	Method	Stream	Land use (Description)	Stream Order	Q L s^{-1}	C_{amb} ug N L^{-1}	C_{add}:C_{amb}	Explanation
Fellows et al. 2006	Nutrient addition	Rio Calaveras, NM	Meadow (Nighttime)	1st	1.1	168	2	No direct explanation; low uptake inferred from evidence in text
Hall et al. 2009a	Isotope tracer	Spring Creek, ID	Forested/Meadow	2nd	120-160	9.3	---	No direct explanation in text; low statistical power from lost samples(5)
Hall & Tank 2003	Nutrient addition	North Moran Bay Creek, WY	Unimpacted	2nd	9	43	0.28 – 0.47 (6)	Evidence for low uptake
		Moose-Wilson Road Creek, WY	Unimpacted	1st	35	89	0.13 – 0.22 (6)	
		Paintbrush Canyon Creek, WY	Unimpacted	1st	4	169	0.07 – 0.12 (6)	
Hamilton et al. 2001	Nutrient addition	Eagle Creek, MI	Forested	2nd	199	29	1.97	Evidence for low uptake and nitrification
Kellman 2004	Nutrient addition	Unnamed stream, Nova Scotia	Agricultural	1st	---	1000 - 2500	4.5 – 7.5	Evidence for nitrification, hypothesize low uptake
Lautz & Siegel 2007	Nutrient addition	Upper Red Canyon Creek, WY	---	2nd	45	2	34.0	Artificial saturation
		Cherry Creek, WY	---	1st	100	2	21.5	
LINX II(7)	Isotope tracer	Gravelly Brook, MA	Reference	1st/2nd	2	112	0.08 (8)	No direct explanation in text; variability; ^{15}N results discordant with bulk nitrate results(9)
		Hoglot Branch, NC	Agricultural	1st/2nd	52.7	154	0.08 (8)	
		Honeysuckle, MI	Reference	1st/2nd	99.4	4	0.08 (8)	
Simon et al. *in press*	Nutrient addition	West Bear Brook, ME	Forested (N saturated)	1st	2.3	443	0.51	Evidence for N saturation and P limitation
Williams et al. 2004	Nutrient addition (10)	Ipswich River Mainstem, MA	Mixed	3rd	---	210	1.02 - 1.24(11)	Possible masking; hypothesize low uptake
		Sawmill Branch, MA	Urban	1st	---	---	---	
		Lubbers Branch, MA	Mixed (Wetland reach)	2nd	---	28	1.5	

(1) PO_4^- added at the same time as NO_3^-
(2) P.A. Bukaveckas, *personal communication*
(3) Analyzed uptake using ^{15}N data
(4) Analyzed uptake using bulk nitrate data
(5) R.O. Hall, *personal communication*
(6) Hall and Tank 2003 report increases over background of 12 to 20 µg N L^{-1} for all study reaches; range of C_{add}:C_{amb} represents possible window of increase
(7) Lotic Intersite Nitrogen Experiment (LINX) II; Mulholland et al. 2008, Hall et al. 2009b, and Mulholland et al. 2009 report on same studies
(8) Mulholland et al. 2009 report an increase over background of <7.5% for all study sites
(9) P.J. Mulholland and R.O. Hall, *personal communication*
(10) NH_4^+ added at the same time as NO_3^-
(11) Williams et al. 2004 report increases over background of 214 to 260 µM NO_3^- for Ipswich mainstem; range of C_{add}:C_{amb} represents possible window of increase.

CHAPTER 3

Nitrogen saturation in a forested Maryland Piedmont watershed: Hydrogeological setting intensifies the effect of elevated deposition

ABSTRACT

In an increasing number of forests, atmospheric inputs of nitrogen (N) exceed the capacity of the ecosystem to store or cycle N, resulting in increased leaching of NO_3-N to groundwater and streams. It has previously been hypothesized that streams draining N-saturated forests are also likely to become saturated as surface-water NO_3-N concentrations increase; however, saturation in both the terrestrial and aquatic components of forested ecosystems has rarely been documented and no direct connection between the two is recognized. My objective was to gain a better understanding of the processes that lead to dramatically elevated NO_3-N concentrations in a forested headwater (Sopers Branch) within the Maryland Piedmont, shown previously to be N-saturated. Atmospheric inputs to the Sopers Branch watershed were between 3.6 and 5.9 kg N ha^{-1} yr^{-1}. Elevated NO_3-N concentrations in both groundwater (5.8 ± 1.3 mg L^{-1}) and surface-water (1.05 ± 0.08 and 0.67 mg L^{-1}, observed and annual flow-weighted, respectively), along with low soil C:N ratios (12) indicate that the forest is N saturated. Because groundwater in the Piedmont is susceptible to NO_3-N contamination, from both atmospheric inputs and soil nitrification, I modeled exports associated with total and baseflow discharge to evaluate the importance of groundwater delivery of NO_3-N to the channel. Average annual exports (2.5 kg ha^{-1} yr^{-1}) were typical of forests in the Northeast and Mid-Atlantic, however, baseflow accounted for a disproportionate fraction of exports (0.64 to 0.78). During dry periods, when deep groundwater flowpaths were presumed to

be the major source of NO_3-N to the channel, export frequently exceeded inputs and observed in-stream concentrations were dramatically elevated. The terrestrial and aquatic components of the Sopers Branch watershed both exhibit classic symptoms of N-saturation despite receiving smaller depositional inputs than many northeastern and mid-Atlantic watersheds. Local hydrogeologic factors may have exacerbated the N-saturation response, particularly during periods of low rainfall, ultimately leading to chronically elevated surface-water NO_3-N concentrations which exceeded the metabolic demands of stream biota. This is the first un-manipulated forested watershed for which N-saturation has been documented in both terrestrial and aquatic ecosystem components. I posit that forest streams receiving a similarly large fraction of NO_3-N through subsurface flowpaths may be more likely to exhibit symptoms of N saturation. Recognizing the frequency with which both the terrestrial and aquatic components of forested watersheds are N saturated will allow for better quantification of the contribution of undeveloped catchments to downstream pollution and improved management of impacts to coastal ecosystems.

INTRODUCTION

Human activities profoundly impact the global nitrogen (N) cycle, increasing both the availability and mobility of N (Vitousek et al. 1997). Availability has increased, in part, through activities such as energy production and transportation that rely on the combustion of fossil fuels. These activities increase the emission and subsequent deposition of trace N gases (including NO_x) to watersheds regardless of land use (Jordan and Weller 1996, Vitousek et al. 1997, Clark et al. 2000). The effects of elevated atmospheric deposition are of great interest in the Northeast and Mid-Atlantic U.S. which

receive some of the largest inputs of atmospheric N in North America (NADP 2009, Fenn et al. 1998, Nolan and Stoner 2000).

While terrestrial and aquatic ecosystems are capable of storing or denitrifying a large fraction of N inputs (Nadelhoffer et al. 1999, Lovett et al. 2002, Groffman et al. 1996, Peterson et al. 2001, Mulholland et al. 2008, 2009, Hall et al. 2009), there is substantial evidence that increased atmospheric inputs of N are having a dramatic impact on the transport of N to aquatic ecosystems (Aber et al. 1989, Stoddard 1994, Galloway et al. 2003) and that the capacity of streams to remove excess N is limited (Dodds et al. 2002, Bernot and Dodds 2005, Earl et al. 2006). As a result, the delivery of N, largely as nitrate (NO_3-N), from uplands to coastal waters has increased (Howarth et al. 1996), resulting in eutrophication along with widespread hypoxia that is detrimental to the structure and function of these systems (Vitousek et al. 1997, Howarth et al. 2002, Galloway et al. 2003, Howarth 2008). Such impacts have been observed in the Gulf of Mexico (Rabalais et al. 2002) and the Chesapeake Bay (Boesch et al. 2001), among others (Howarth 2008).

Traditionally, northern temperate forests were considered N-limited systems with sufficient biological demand during the growing season to retain nearly all of the available N from atmospheric and microbial sources (e.g. mineralization, nitrification; Aber et al. 1989, Murdoch and Stoddard 1992, Williard et al. 1997). However, in an increasing number of forests, N limitation no longer exists because inputs from atmospheric deposition exceed the capacity of the ecosystem to store or cycle N (Stoddard 1994, Aber et al. 1989, 1995, 1998). As N inputs increase beyond biological

demand in forest ecosystems, N delivery to streams, groundwater, and the atmosphere increases (Aber et al. 1989, Galloway et al. 2003). In severely N saturated forest ecosystems, losses may equal or exceed total N deposition (Vitousek et al. 1997, Peterjohn et al. 1996, Castro et al. 2007). Of particular concern are elevated amounts of N in the form of NO_3^- ("NO_3-N") because it is both a nutrient and a highly mobile anion that is readily leached from soils (Murdoch and Stoddard 1992, Ollinger et al. 1993, Vitousek et al. 1997).

The N saturation hypothesis of Aber et al. (1989, 1998) describes a series of biogeochemical responses of forests to chronic N inputs, central to which is an increase in the leaching of NO_3-N to groundwater and surface-water. Specifically, Aber et al. (1989, 1998) outlined three consecutive stages of N saturation in forest ecosystems. Stage 1 is characterized by increases in foliar N and plant production, accompanied by temporary, seasonal losses of N. In stage 2, soil microbial processes that increase available N (i.e. nitrification and mineralization) are dramatically altered and loss of N from the forest is sustained. And finally, in Stage 3 productivity decreases and the forest begins to decline. A number of studies have identified several factors that control N saturation and the associated leaching of NO_3-N, including stand age (Vitousek 1977), land use history (Aber et al. 1998), species composition (Lovett et al. 2000), phosphorus (P) availability (Gress et al. 2007), and hydrology (Creed and Band 1998, Fenn et al. 1998, Burns et al. 1998).

Stoddard (1994) further developed the model presented by Aber et al. (1989) to describe patterns in surface-water NO_3-N concentrations resulting from increased N losses to waterways during successive stages of forest saturation. Streams draining N-limited forests are typified by low, year round NO_3-N concentrations, with concentrations of NO_3-N increasing during the dormant season as forest N saturation progresses (Stoddard 1994). Ultimately, cyclical patterns in surface-water NO_3-N concentrations disappear as biotic demand in the forest is exceeded year-round and in-stream NO_3-N concentrations are continuously elevated. Eventually, the streams themselves may become N-saturated (Bernot and Dodds 2005) since the level of N saturation, and therefore the propensity to transport larger N loads to downstream reaches, has been positively correlated to ambient streamwater N concentrations (Earl et al. 2006). Yet, no direct connection between N saturation in forests (Aber et al. 1989, Stoddard 1994) and N saturation in streams (Earl et al. 2006) has been documented.

The goal of this study was to gain a better understanding of the hydrological and biological processes that lead to dramatically elevated NO_3-N concentrations in a forested headwater in the Chesapeake Bay watershed. Previous studies in this watershed indicate that the stream is N-saturated and therefore unable to remove significant quantities of N entering the channel (Chapter 1). Knowledge of the N saturation status of the forest within the watershed and the dominant factors controlling surface-water NO_3-N concentrations may provide insights to link the concepts of N saturation in terrestrial and aquatic systems that can inform efforts to manage for N delivery to coastal waters.

METHODS

Site description - Sopers Branch (SB, watershed outlet: N39°16'31.1", W77°18'13.2") is a 2nd order stream that drains 3.03 km^2 of the crystalline Piedmont Plateau province of Maryland. The crystalline Piedmont is characterized by a multilayer groundwater flow system that consists of the regolith, with its surficial soils and clay-rich decomposed bedrock (i.e. saprolite), a transition zone, and fractured bedrock of schist and gneiss along with other highly metamorphosed sedimentary and igneous rock types (Maryland Geological Survey 1981, Harned and Daniel 1989, Lindsey et al. 2006). Surface soils are dominated by Typic Hapludalfs, Ultic Hapludalfs, and Inceptic Hapludults in the uplands, with Aquic Fragiudults in the riparian zone. Approximately 40% of the watershed area is covered by shallow soils where the depth to paralithic bedrock is less than 0.5m (USDA-NRCS 2009).

The majority (86.6%) of the watershed is forested, with 57.9% of the total area as deciduous forest, 28.5% as evergreen forest, and 0.2% as mixed forest. The remainder of the watershed land use is ungrazed pasture/cropland (10.6%), including an unfertilized fallow/wheat field and open lawn, commercial and industrial (2.1%), and low-density residential (0.7%) (Figure 3.1; Maryland Office of Planning 2002 Land Use, GISHydro2000; Moglen 2007). Nearly the entire watershed is contained within a county park (Little Bennett Regional Park) which extends to the north and east of the watershed boundary; the parkland is surrounded primarily by mixed agricultural and residential land-uses and a major highway (Interstate 270) runs parallel to the stream ~0.5 to 1 km to the west of the watershed divide.

SB is well-oxygenated (% DO saturation: 81.1 ± 2.9) with circumneutral pH (6.68 ± 0.1) and specific conductivity of 132 ± 4 μS cm^{-2}. Average concentrations of NO_3-N, NH_4-N, PO_4-P, and dissolved organic carbon (DOC) from monthly samples collected between October 2004 and September 2006 were 1.14 ± 0.18 mg N L^{-1}, 0.012 ± 0.003 mg N L^{-1}, 0.0031 ± 0.0004 mg P L^{-1}, and 3.09 ± 1.72 mg C L^{-1}, respectively (mean ± S.E., Chapter 1). Dissolved organic nitrogen (DON) concentrations are relatively low, 0.12 ± 0.04 mg N L^{-1}, comprising less than 10% of total nitrogen (Chapter 1).

Precipitation - Rainfall data were obtained from the Montgomery County (Maryland) Department of Environmental Protection for a tipping-bucket gauge located less than 0.25 km from the watershed boundary. The record spans from 01 September 2004 to 30 September 2008 except for 74 d when the gauge was not operating due to maintenance or malfunction. Gaps in the precipitation record were filled using data from an identical county-operated gauge < 4 km away; leaving only two brief periods without record (7 d in May 2006 and 8 d in August 2008). For dates with a complete precipitation record for the prior 30 d, I calculated cumulative total precipitation over that period as an index of recent climatic conditions; hereafter, referred to as the 30-d precipitation index.

Nitrogen inputs - Inputs of N to the SB watershed are primarily from atmospheric deposition, since land classified as agricultural (i.e. cropland and pasture) is unfertilized and without livestock, and residences within the watershed are connected to a public sewer system (Wendy Hanley, Little Bennett Regional Park Manager, *personal*

communication). Wet deposition for water years (WY) 2005 to 2008 was calculated by multiplying the volume of precipitation during each water year by the average concentration of inorganic N in precipitation, 0.44 mg N L^{-1}, measured at a nearby (~45 km) National Atmospheric Deposition Program National Trends Network site (MD07: N39°38'25.8", W77°29'35.9") during WY2005 to WY2008 (NADP 2009). Annual estimates of total N deposition (wet + dry inputs) were based on calculated estimates of wet deposition plus estimates of dry deposition, such that dry deposition accounted for 26% of total inputs (Sheeder et al. 2002). Total and dry deposition estimates are conservative compared to those calculated using other methods (i.e. 1:1 ratio of wet to dry deposition; Fisher and Oppenheimer 1991) and may be underestimated given the proximity of the watershed to a large potential emissions source (Interstate 270; Elliott et al. 2007).

Stream hydrology - Daily mean discharge (Q) for WY2005 to WY2008 was obtained from a USGS gauge (#01643395), located at the watershed outlet. I used a recursive digital filtering method (Eckhart 2005) to estimate Q entering the stream as runoff (Q_R), baseflow discharge (Q_B, i.e. entering the stream through subsurface pathways), and baseflow index (BFI, Q_B/Q); baseflow separation was performed using the Web-based Hydrograph Analysis Tool (WHAT; Lim et al. 2005). The filter requires the input of two parameters: 1) the recession constant and 2) BFI_{max}, described below.

The recession constant is a measure of the rate at which streamflow decreases during periods without groundwater recharge. I calculated this constant, as described in Eckhart

(2008), to be 0.99 using data from the daily record for those sets of dates where *Q* decreased for at least five consecutive days. For simplicity, I used a single recession constant to perform hydrograph separation and note that *Q* decreased more rapidly during spring and summer recessions (i.e. the recession constant was less than 0.99; analysis not shown). Seasonally variable recession rates are observed in streams, such as SB, that drain watersheds with deeply rooted vegetation and shallow groundwater tables (Eckhart 2008).

The second filter parameter, BFI_{max}, represents the maximum value of the long-term ratio of Q_B to Q for the watershed. While BFI_{max} has not been directly measured for SB, there are established values for perennial streams draining porous ($BFI_{max} = 0.80$) and hard rock ($BFI_{max} = 0.25$) aquifers (Eckhart 2005). I used a weighted-average approach proposed by Eckhart (2008) to calculate BFI_{max} based on knowledge of the depth to restrictive features (i.e. bedrock or paralithic bedrock) underlying SB watershed soils. For the 40% of the watershed with soils < 0.5 m deep, I assumed $BFI_{max} = 0.25$ and for the remaining 60% of the watershed I assumed $BFI_{max} = 0.80$, yielding a parameter estimate for $BFI_{max} = 0.58$. This value is equal to the median long-term BFI for Piedmont streams in the Chesapeake Bay Watershed (Bachman et al. 1998), and is therefore a reasonable estimate of BFI_{max} for SB.

Surface-water quality - Surface-water samples were collected between 02 October 2004 and 18 November 2008. Grab samples were collected during low flow conditions approximately every month between October 2004 and September 2006, and less

regularly between September 2006 and November 2008. Stormflow samples were collected during five storm events between 20 March 2008 and 16 August 2008 using an automated sampler (ISCO 6712C with 730 Bubbler Module, Teledyne Isco, Inc., Lincoln, NE); samples represent flows ranging from 8.2 to 1253 L s^{-1}. All surface-water samples were collected within 15 m of the USGS gauging station and instantaneous *Q* was recorded at the time of collection. Triplicate grab samples were filtered (Whatman GF/F, 0.7 μm nominal pore size) into acid-washed HDPE bottles and frozen prior to analysis. Samples collected during storms were retrieved within 24 h and stored at 4°C prior to filtering; again, filtered samples were frozen prior to analysis. All surface-water samples were analyzed for NO_2+NO_3-N using an automated photometric analyzer (Aquakem 250, Thermo Fisher Scientific, Waltham, MA) following enzymatic reduction of NO_3^- to NO_2^-. The average concentration for each set of triplicate samples was calculated prior to compiling summary statistics (PROC MEANS, SAS version 9.1, 2007, SAS Institute Inc., Cary, NC) and modeling nitrate export (see methods below).

Modeling nitrate export - I used the USGS Load Estimator program (LOADEST; Runkel et al. 2004) to model NO_3-N loads and export. LOADEST employs a linear regression approach to model constituent loads using stream hydrology and chemistry; data variables entered into the model include decimal time (*dtime*), *Q*, and NO_3-N concentration (mg N L^{-1}). LOADEST calculates coefficients for a series of default models that include anywhere from one to seven explanatory variables based on these data variables. The set of explanatory variables may include the data variable *dtime*, as well as transformed data variables that improve the linearity of the model ($\ln Q$, $\ln Q^2$,

dtime^2) or model seasonal variations in loads [sin (2π *dtime)* and cos (2π *dtime*)] (Runkel et al. 2004). In order to gain a better understanding of the contribution of NO_3-N delivered via groundwater to in-stream loads, I separately modeled loads carried by Q (i.e. "total load") and Q_B (i.e. "baseflow load"). The calibration dataset used to model total load included all surface-water concentration data collected between 02 October 2004 and 18 November 2008 (n = 167). Samples collected on 09 March 2006 were excluded from the calibration dataset because of anomalously high NO_3-N concentrations observed on that date. The baseflow load calibration dataset included the subset (n = 14) of concentration data collected on dates when Q_B accounted for 90 to 100% of Q (i.e. BFI > 0.9; Schilling and Zhang 2004) according to the hydrograph separation described above; again, data from 09 March 2006 (BFI = 0.99) were excluded. Despite the small dataset used to calibrate the baseflow load model, concentration data represent all four seasons and were collected across a range of Q_B (7.6 to 42.5 L s^{-1}).

Initial model selection for both the total load and baseflow load models was carried out by LOADEST, which chooses the best fit model from a series of default models using the Akaike Information Criterion (AIC) generated for each model (Runkel et al. 2004). I assessed multicollinearity of model terms for the selected models using correlation matrices produced by LOADEST; if parameter estimates were highly correlated, I created an alternate model with fewer parameters. I calculated model fit (r^2, p-values from F-Statistics) using the extra sums of squares principle (Draper and Smith 1998), since LOADEST does not output p-values for model fit. The final model for total load included four explanatory variables: $\ln(\text{total load}) = a_0 + a_1 \ln Q + a_2 \ln Q^2 + a_3 dtime +$

$a_4 dtime^2$ (r^2: 0.9949, p < 0.0001, AIC: 0.453; Table 3.1). Model residuals were normally distributed (PPCC: 0.9826) and, thus, estimates are based on maximum likelihood estimation (MLE; Runkel et al. 2004). The final model for baseflow load included only one explanatory variable: ln(baseflow load) = $a_0 + a_1 \ln Q$ (r^2: 0.9422, p = 0.003, AIC: -0.416; Table 3.1). Again, residuals were normally distributed (PPCC: 0.9850) and loads are based on MLE. Baseflow load estimates required extrapolation for those dates (< 6% of total) on which Q_B exceeded the maximum value included in the calibration data set (42.5 L s^{-1}). All estimated loads were converted to N export per unit area to allow for comparison with published values for other watersheds.

Groundwater quality - Groundwater samples were collected on 18 November 2008 from four residential wells located within the SB watershed. While these residences rely on well water, they are connected to a public sewer system; thus, contamination of groundwater samples from residential septic systems was not an issue. At each of the four sampling locations, groundwater samples were collected in acid-washed HDPE bottles from an outdoor hose bib to ensure groundwater was not treated (i.e. passed through a filter or water softener); pipes were flushed for one minute prior to sampling. Samples were filtered within one hour of collection, frozen, and analyzed for NO_3-N using the method described above. On the same date, grab samples were collected from SB at the location of the USGS gauging station. I compared groundwater and surface-water nitrate concentrations for samples collected on 18 November 2008 using a t-test (PROC TTEST).

Soil chemistry - Mineral soil was collected from the riparian area and several upland locations within the watershed using a stainless steel soil corer (2.5 cm internal diameter) to analyze for soil carbon (C) and nitrogen. Riparian samples were collected along 11 transects perpendicular to the stream, with transects located at 300 m intervals between the watershed outlet (0 m) and the headwaters (3000 m, Figure 3.1). At each transect, I collected two cores (0 to 15 cm) at both 2 m and 15 m from the bank, on either side of the stream channel (Figure 3.1 a). Samples collected at the same distance from the channel were combined prior to analysis, resulting in two soil samples per riparian transect. Upland sampling locations consisted of six plots (0.3 km^2, radius = 100 m) located throughout the watershed (Figure 3.1). Upland plots represented each of the major land cover types (deciduous forest, evergreen forest, and cropland/pasture) and a range of elevations. For each plot, the center of the area was marked as a waypoint on a handheld GPS (eTrex Venture, Garmin International Inc., Olathe, KS) and five soil cores (0 to 15 cm) were collected randomly within each of four quadrants oriented in the cardinal directions (Figure 3.1 b). All 20 cores collected within each upland plot were pooled prior to analysis. Upon returning to the laboratory, pooled samples were weighed and then dried for a minimum of 48 hours at 60°C. Dried samples were sieved (2 mm, U.S. Standard Sieve #10) to remove roots and rocks; the remaining material was ground by hand using a mortar and pestle. I calculated bulk density for each sample and analyzed percent organic carbon (% OC) and percent total nitrogen (% TN) using a CN elemental analyzer (LECO TruSpec CN, St. Joseph, MI).

Soil characteristics (% OC, % TN, C:N) for riparian samples collected 2 m and 15 m from the stream channel were compared using paired t-tests (PROC TTEST). Because samples collected at different distances from the channel were not significantly different with respect to these characteristics ($p > 0.05$), I conducted further analysis of soils data, including comparisons between riparian and upland soils (PROC TTEST), without distinguishing between 2 m and 15 m riparian samples.

RESULTS

Precipitation - Total annual precipitation ranged from 61 cm in WY2007 to 101 cm in WY2006 (median: 95 cm; Table 3.2). WY2007 was a particularly dry year, receiving approximately half the normal annual rainfall for Montgomery County, Maryland (106cm; NOAA-NWS 2009). The highest recorded daily rainfall totals were observed during the spring and summer with the exception of a major rain event in October 2005 that was associated with the remnants of a tropical storm (Figure 3.2 a); generally, monthly precipitation was higher during the growing season ($t = -2.76$, $p < 0.01$). For all dates, the average 30-d precipitation index was 7.3 cm (range: 0 cm to 23.1 cm). The longest period without measurable rainfall was 50 d, spanning from 14 December 2006 to 01 February 2007. The percent of total precipitation leaving the watershed as *Q* ranged from 30% in WY2006 to 77% in WY2007 (median: 39%; Table 3.2).

Nitrogen inputs - Total atmospheric deposition of N ranged from 3.6 kg N ha^{-1} yr^{-1} in WY2007 to 5.9 kg N ha^{-1} yr^{-1} in WY2006 (Table 3.2). Because N inputs were estimated based on calculations of wet inorganic deposition using precipitation data, estimated total

N deposition was considerably lower in WY2007 than in years with normal precipitation; likewise, NO_3-N inputs during the dormant season months were also low (Figure 3.3). As in other watersheds in the surrounding region, atmospheric deposition was elevated relative to other parts of the country; however, wet atmospheric N inputs were lower than those recorded at two-thirds of the monitoring sites in the northeastern and mid-Atlantic U.S. (NADP 2009).

Stream hydrology - Mean daily *Q* exhibited seasonal patterns with the highest values in late-winter and spring, and the lowest values in the late-summer and fall (Figure 3.2 b). Between 01 October 2004 and 30 September 2008, mean daily *Q* ranged from 2.8 L s^{-1} (04 October 2007) to 963 L s^{-1} (12 May 2008), with a median value of 20.4 L s^{-1}. Mean daily *Q* for the middle 50% of dates was between 11.9 L s^{-1} (25^{th} percentile) and 34.0 L s^{-1} (75^{th} percentile).

Mean daily Q_B also varied seasonally, with maximum values in the winter and spring, and minimum values in the late-summer and fall (Figure 3.2b). Between 01 October 2004 and 30 September 2008, mean daily Q_B ranged from 1.9 L s^{-1} (21 October 2007) to 53.8 L s^{-1} (13 May 2008), with a median value of 14.7 L s^{-1}. For each date, BFI was calculated by dividing mean daily Q_B by mean daily *Q*; daily BFI values ranged from 0.04 to 1.0, with streamflow attributed completely to baseflow (i.e. BFI = 1.0) on 169 dates (12%). The long-term ratio of Q_B to *Q* for each water year ranged from 0.44 to 0.54 (median: 0.48; Table 3.2).

Surface-water quality - Surface-water samples collected between 02 October 2004 and 18 November 2008 coincided with instantaneous discharges ranging from 7.7 to 1253 L s^{-1} (Figure 3.2 c). For those samples collected at discharges falling within the inter-quartile range of all mean daily *Q* measurements (11.9 to 34.0 L s^{-1}), the average NO_3-N concentration was 1.05 ± 0.08 mg N L^{-1} (mean ± S.E.). The majority (95%) of samples, regardless of *Q* at the time of collection, had NO_3-N concentrations falling between 0.19 and 1.3 mg N L^{-1} (median: 1.01 mg N L^{-1}).

NO_3-N concentration was negatively correlated with *Q*, indicating a dilution effect of storms ($r = -0.50216$, $p < 0.0001$; Figure 3.4). The minimum NO_3-N concentration, observed during a storm event, was 0.14 mg N L^{-1}, on 20 April 2008. The maximum NO_3-N concentration, observed prior to leaf out in 2006 and following a relatively dry period (30-d precipitation index = 1.0 cm, BFI = 0.99), was 4.80 ± 0.04 mg N L^{-1} (mean ± S.E.) on 09 March. While surface-water NO_3-N concentrations from samples collected during low flow conditions were episodically high (e.g. 09 March 2006) they did not exhibit a clear seasonal pattern (Figure 3.2 c; $F_{(3,28)} = 0.55557$, $p = 0.065$).

Nitrate export - Total NO_3-N export from the SB watershed ranged from 1.8 kg N ha^{-1} yr^{-1} in WY2008 to 2.9 kg N ha^{-1} yr^{-1} in WY2007, with a mean value of 2.5 kg N ha^{-1} yr^{-1} (Table 3.2). Annual flow-weighted NO_3-N concentrations for WY2005, WY2006, WY2007, and WY2008 were 0.68, 0.83, 0.61, and 0.55 mg N L^{-1}, respectively. Nitrate export was the greatest in the winter and spring and the smallest in late-summer and early fall (Figure 3.5), but did not differ significantly between the growing and dormant

seasons ($t = 1.59$, $p = 0.12$; Figure 3.3). Percent retention of total inorganic N inputs (wet + dry), calculated as [(input – export)/ input]*100, ranged from 19% in WY2007 to 68% in WY2008 (median: 54%; Table 3.2). Retention of wet N inputs ranged from -10% in WY2007 to 57% in WY2008 (median: 37%). Ammonium exports were assumed to be negligible since NH_4-N accounted for < 1% of dissolved inorganic N in monthly samples.

Exports associated with Q_B (i.e. traveling along groundwater flowpaths prior to entering the stream) ranged from 1.4 kg N ha^{-1} yr^{-1} in WY2008 to 2.1 kg N ha^{-1} yr^{-1} in WY2007, with a mean value of 1.8 kg N ha^{-1} yr^{-1} (Table 3.2). Patterns in baseflow export followed patterns in Q_B, as well as patterns in total exports ($r = 0.8494$), with the largest losses in the winter and spring and the smallest losses in the late-summer and early-fall (Figure 3.5). The proportion of total annual export associated with Q_B ranged from 0.64 to 0.78 (median: 0.71) between WY2005 and WY2008 (Table 3.2).

Since baseflow export and total export were estimated using different models, it was possible to arrive at estimates that were discordant. Estimated baseflow exports exceeded estimated total exports by > 5% on approximately 13% of the dates between 01 October 2004 and 30 September 2008. The majority of these dates fell during streamflow recessions between March and July of WY2008, with the remainder falling in the spring of WY2005 and WY2007. Discordance between model estimates was largely due to the recession constant used for hydrograph separation, which overestimated the contribution of Q_B during spring and summer recessions, combined with extrapolation beyond the scope of the baseflow export model on approximately one-fourth of these dates. Even

with the occasional lack of agreement between model estimates, it is clear that Q_B contributed disproportionately to the export of NO_3-N from the SB watershed (Table 3.2).

Groundwater quality - Groundwater NO_3-N concentrations were significantly higher than surface-water concentrations ($t = -3.77$; $p = 0.0328$). The average groundwater NO_3-N concentration for residential well samples was 5.77 ± 1.34 mg N L^{-1} (mean ± S.E., n = 4) and concentrations ranged from 2.71 to 9.07 mg N L^{-1}. For surface-water samples collected on the same date, the average NO_3-N concentration was 0.71 ± 0.003 mg N L^{-1} (mean ± S.E., n = 3).

Soil chemistry - Soil characteristics did not differ between riparian and upland soils (Table 3.3). For all watershed soils, organic C and total N comprised 2.9 ± 0.01% and 0.2 ± 0.7% of mineral soil, respectively. The average soil C:N ratio was 12.1 ± 0.4 and values ranged from 9.4 to 18.3.

DISCUSSION

The response of forested watersheds to N saturation is a function of the magnitude of atmospheric inputs, hydrological flowpaths, and the cycling of N within the watershed (Murdoch and Stoddard 1992, Mitchell 2001, Aber et al 2003, Campbell et al. 2004). Several factors determine the relative importance of these controls including geology, topography, climate, soils, land use history, forest age and composition, among others. Since forests respond to chronic N inputs in a number of predictable ways (Aber et al. 1989, Stoddard 1994), there are several suitable indicators that are commonly used to

assess forest N-saturation status. These include surface-water and groundwater NO_3-N concentrations, extractable or soil solution NO_3-N, soil C:N ratios, and foliar N (Fenn and Poth 1998, Fenn et al. 1998). Other commonly reported measurements include NO_3-N export and watershed retention, which measure the transfer of N from terrestrial to aquatic systems (e.g. Lovett et al. 2002, Aber et al. 2003, Campbell et al. 2004), and rates of soil N cycling, which allow for direct quantification of the biological processes fundamental to N-saturation response (e.g. Gilliam et al. 1996, Williard et al. 1997, Fenn et al. 1998, Christ et al. 2002, Venterea et al. 2004). I used the following metrics to evaluate the response of the SB watershed to elevated atmospheric inputs: 1) magnitude of watershed export and retention, 2) surface-water NO_3-N concentrations, 3) soil C:N ratios, and 4) groundwater NO_3-N concentrations. Below, I provide rationale for assessing each metric and, through comparisons with published values, illustrate that the SB watershed is experiencing symptoms of severe N-saturation despite receiving atmospheric inputs that are low compared to other parts of the Northeast and Mid-Atlantic (NADP 2009). I close the discussion with evidence that illustrates that the local hydrogeological setting may exacerbate the N-saturation response of the SB watershed.

Watershed export and retention- Surface-waters provide a comprehensive view of the N saturation status of forests because they effectively integrate the response of the entire watershed to N deposition (Stoddard 1994, Fenn et al. 1998, Aber et al. 2003). Exports from the SB watershed (1.8 to 2.9 kg N ha^{-1} yr^{-1}) are higher than natural background values estimated from less-impacted, temperate-zone catchments and historical data (~1 kg N ha^{-1} yr^{-1}; Howarth et al. 1996) but similar to average values reported for forested

catchments in the Chesapeake Bay watershed (average: 2.1 kg N ha^{-1} yr^{-1}; DeWalle and Pionke 1995; Table 3.4) and Mid-Atlantic states (2.5 kg N ha^{-1} yr^{-1}; Clark et al. 2000). Overall, the magnitude of exports from the SB watershed were not exceptional, falling towards the middle of the range of values for forested watersheds in the Northeast and Mid-Atlantic (<0.1 to 9.4 kg N ha^{-1} yr^{-1}; DeWalle and Pionke 1995, Jordan et al. 1997, Campbell et al. 2004, Castro et al. 2007, Goodale et al. 2009; Table 3.4).

Percent retention, which is normalized for N inputs, is useful for assessing the intensity of N saturation relative to other watersheds where inputs have been similarly quantified. Measures of retention, however, do not account for the fate of N, which may be stored in the terrestrial landscape, lost to the atmosphere, potentially in the form of greenhouse gases (Fenn et al. 1998), or to deep groundwater, and thus reported values may not fully capture the detrimental impacts of chronic N deposition. In the SB watershed, inputs not accounted for by stream exports ranged from 19 to 68% of total N deposition (wet + dry, average inputs: 5.2 kg N ha^{-1} yr^{-1}; Table 3.2) between WY2005 and WY2008. In a study of 24 forested watersheds in the Northeast and Mid-Atlantic, Campbell et al. (2004) reported retention as a function of wet inputs only, citing the uncertainty associated with estimates of dry deposition at many sites. Theses sites received between 2.7 and 8.1 kg N ha^{-1} yr^{-1} (median: 6.4 kg N ha^{-1} yr^{-}) in wet deposition and retained between 24 and 99% of these inputs (median: 71%); watersheds receiving levels of wet deposition similar to the SB watershed retained a similarly broad range of inputs (24 to 96%, median: 84%). When percent retention for the SB watershed was comparably calculated, retention during WY2005, WY2006, and WY2008 (32, 42, and 57%, respectively) fell well below

the median value for watersheds receiving similar inputs. During the driest year, WY2007, SB was a net source of N, exporting approximately 110% of depositional inputs. Others have noted that percent N retention cannot be explained by the magnitude of depositional inputs alone (Williard et al. 1997) since the response of an individual watershed is determined by the complex interaction of a number of physical and biological factors (Campbell et al. 2004, Fenn et al. 1998); this suggests that comparably low retention is indicative of a more intense N-saturation response.

Surface-water nitrate concentrations - Truly pristine streams are lacking in the Northeast and Mid-Atlantic as a result of high depositional inputs (Smith et al. 2003, Dodds and Oakes 2004, Clark et al. 2000), and thus, concentrations tend to be considerably higher than predicted in the absence of chronic N deposition (Clark et al. 2000, Smith et al. 2003). In the forest saturation literature, surface-water NO_3-N concentrations are reported as either observed or flow-weighted mean concentrations; I report both here to allow for comparisons to both ranges of values. In SB, the average observed NO_3-N concentration for the mid-range of discharges was 1.05 ± 0.08 mg N L^{-1} (mean ± S.E.), more than twice the concentration of inorganic N in precipitation (~0.44 mg N L^{-1}; NADP 2009). This value falls at the upper end of the range of typical values for other predominantly forested watersheds of the Chesapeake Bay and surrounding region (<0.1 to 1.07 mg N L^{-1}; Kaufmann et al. 1991, Correll et al. 1995; Table 3.5). Moreover, it approaches the maximum, annual average concentration reported for Watershed 4 at the Fernow Experimental Forest in West Virginia (1981: 1.13 mg N L^{-1}; Adams et al. 1994), which has been cited as the "best example" of a N saturated forest in the U.S. (Peterjohn

et al. 1996, 1999). Annual flow-weighted concentrations (0.55 to 0.83 mg N L^{-1}) were also elevated relative to many other Northeastern and Mid-Atlantic forests (<0.1 to 1.37 mg N L^{-1}; Jordan et al. 1997, Clark et al. 2000, Campbell et al. 2004, Castro et al. 2007; Table 3.6), with the average annual flow-weighted concentration (0.67 mg N L^{-1}) exceeding 85% of published values.

Chronic N deposition not only affects the amount of NO_3-N present in forested streams, but also the seasonal variability of observed concentrations (Stoddard 1994). As N saturation progresses, surface-water NO_3-N concentrations are expected to vary seasonally, with lower concentrations during the growing season and higher concentrations during the dormant season, according to a model that emphasizes the importance of biological controls on N response (i.e. uptake, and immobilization; Stoddard 1994, Lovett et al. 2000). Patterns that are reversed, with maximum observed concentrations during the growing season, have also been reported in forested watersheds receiving elevated atmospheric inputs (e.g. Tague and Band 2004, Goodale et al. 2009) and are thought to reflect increases in soil nitrification during the summer months. In later stages of N saturation, the biological demands of the forest are exceeded year-round and cyclical patterns in surface-water NO_3-N concentrations are dampened (Stoddard 1994). This pattern has been documented in forested watersheds of the mid-Atlantic U.S. (Peterjohn et al. 1996), Europe (Stoddard 1994), and Japan (Ohrui and Mitchell 1997). Observed NO_3-N concentrations were similarly constant in SB, though seasonal patterns may have been obscured by infrequent sampling, especially during winter months (Stoddard 1994). Continually elevated NO_3-N concentrations, which are markedly higher

than those observed in many other forested watersheds in the Northeast and Mid-Atlantic, and high annual flow-weighted concentrations are indicative of N saturation in the SB watershed.

Soil carbon to nitrogen ratios - Soil C:N ratios convey information about the relative rates of N cycling in soils, making them suitable indicators of N saturation. Microbial processes within watershed soils, including immobilization, mineralization, nitrification, and denitrification, are critical to determining the amount of NO_3^-N entering both groundwater and surface-water (Murdoch and Stoddard 1992, Groffman et al. 1996, Williard et al. 1997, Galloway et al. 2003, Venterea at al. 2004). Several studies have shown that the majority of NO_3-N in streams is cycled within the soil prior to entering the channel; identifying microbial nitrification as the primary source of NO_3-N to streams (Mayer et al. 2002, Burns and Kendall 2002, Barnes et al. 2008, Burns et al. 2009, Goodale et al. 2009).

The balance between immobilization, the assimilation of ammonium (NH_4^+) into biomass, and mineralization, the conversion of organic N to NH_4^+, is controlled in part by the availability of C and N. Specifically, low C:N ratios are often associated with rapid cycling of N, including nitrification (e.g. Christ et al. 2002, Venterea et al. 2004). In soils with C:N < 20, net mineralization occurs and more NH_4^+ is available to be converted to NO_3^- via nitrification (Myrold 2005). Aber et al. (2003) illustrated that net nitrification increases dramatically in both organic and mineral soils below a similar threshold C:N ratio of 20 to 25. Recent work by Gress et al. (2007) showed that as soil N availability

increases with increasing deposition, vegetative uptake becomes P-limited and more NH_4^+ is available to nitrifiers. Consequently, forests that are prone to P limitation may exhibit lower soil C:N ratios than those systems where P is more readily available. Soils that are likely to become P-limited with increasing atmospheric N deposition include highly weathered and acidic soils, as well as soils derived from non-granitic bedrock, all of which are found in the SB watershed. Overall, N-enriched soils (i.e. low C:N) are susceptible to NO_3-N leaching as a result of increased rates of mineralization and subsequent nitrification (Fenn et al. 1998, Christ et al. 2002).

The average mineral soil C:N ratio in the uplands and riparian zone of the SB watershed (12) falls at the extreme low end of the range of values reported for Northeastern and Mid-Atlantic forests (10-39; Aber et al. 2003) and slightly below typical values reported for the Mid-Atlantic region (13-21; Williard et al. 1997, Castro et al. 2007; Table 3.7). Soil C:N ratios in the SB watershed are indicative of severe N saturation and soil N cycling is likely to be dominated by mineralization and nitrification. Because low C:N soils are prone to NO_3-N leaching, groundwater and surface-water concentrations are strongly impacted by the increased production of NO_3-N in soils.

Groundwater nitrate concentrations - In the Piedmont Plateau physiographic province, groundwater is particularly susceptible to NO_3-N contamination as a result of well-drained soils and underlying geology. Accordingly, groundwater concentrations in the SB watershed are high relative to published values for forests throughout the U.S. (< 2 mg N L^{-1}), including those in regions with the highest atmospheric deposition (Mueller

and Helsel 1996). While only a small number of groundwater samples were collected on one date, concentrations from all four wells exceeded the median groundwater NO_3-N concentration reported for other forested watersheds of the crystalline Piedmont region of the Chesapeake Bay watershed that receive similar inputs of atmospheric N (1.4 mg L^{-1}; Lindsey et al. 2006), providing additional support for an internal source of NO_3-N in the SB watershed (i.e. nitrification) resulting from N saturated conditions in forest soils.

Factors controlling N-saturation response - Although atmospheric N deposition was low compared to many other watersheds in the Northeast and Mid-Atlantic (NADP 2009), values of surface-water, groundwater, and soil indicators suggest late-stage N saturation. It is not immediately clear why soils within the watershed are so severely N-saturated, though, as mentioned above, acidic, weathered soils are often associated with P limitation (Gress et al. 2007), which allows nitrifying bacteria to compete for available NH_4^+. As I elaborate on below, there is evidence that the physiographic setting partially accounts for the severe N-saturation response of the SB watershed since hydrogeological controls dictate groundwater and surface-water responses to N deposition.

Groundwater in the crystalline Piedmont physiographic region is susceptible to NO_3-N contamination (Nolan 2001, Nolan and Stoner 2000) and concentrations in the SB watershed were extremely high as a result of inputs from the atmosphere and nitrification in N-enriched soils. Elevated groundwater NO_3-N concentrations translated to surface-water concentrations that increased as greater proportions of water entered the channel through subsurface pathways ($r = 0.5941$, $p < 0.0001$; Figure 3.6). In the SB watershed,

groundwater flowpaths were the predominant mode of delivery of NO_3-N to the channel with baseflow exports accounting for a disproportionately large fraction of total annual exports (median: 0.71, Table 3.2). Similarly high fractions of NO_3-N export in Q_B (median: 0.78) were reported by Bachman et al. (1998) for 58 streams draining varying land uses in Piedmont of the Chesapeake Bay watershed.

Shallow flowpaths are believed to be the major contributor of NO_3-N to the channel during much of the year, especially given the presence of shallow restrictive features in the SB watershed. However, during periods of limited rainfall, the contribution of groundwater stored in the highly permeable transition zone between the fractured bedrock and the overlying saprolite should increase (Harned and Daniel 1989, Lowrance et al. 1997). The potential for greater delivery of NO_3-N during periods when deep flowpaths dominate is high, especially if opportunities for removal in the near-stream environment are limited, since water moving through the transition zone is often oxic (Lowrance et al. 1997).

During periods of low precipitation, atmospheric N inputs were low, yet exports remained unchanged, presumably as a result of the increased contribution of N-rich groundwater from the transition zone. As such, the SB watershed retained less of the deposited N during periods of low rainfall and was a net source of NO_3-N during especially dry months (Figure 3.7). Likewise, in the driest year, WY2007, the watershed retained only a small fraction of the total atmospheric N inputs (Table 3.2). The unusually high surface-water NO_3-N concentration observed on 09 March 2006 also

corresponded with an extended dry period (i.e. low 30-d precipitation index), providing further support for the important role of subsurface flowpaths in determining the response of the SB watershed to elevated deposition.

While watershed response to N inputs may be dominated by either physical or biological controls, multiple interacting factors are likely to be responsible for observed effects (Lovett et al. 2002, Aber et al. 2003). For this reason, the biogeochemical environment encountered along subsurface flowpaths is expected to play a role in determining the response of the watershed to elevated N deposition (Cirmo and McDonnell 1997, Mitchell 2001, McClain et al. 2003, Fisher et al. 2004). Retention was significantly lower during the dormant season ($t = -3.28$, $p = 0.002$; Figure 3.3), suggesting that vegetative uptake may have reduced N losses to the channel during the growing season; however, because precipitation was also lower during the dormant season, the relative importance of biological controls to N delivery in the SB watershed cannot be determined.

CONCLUSION

The forest within the SB watershed shows symptoms of N saturation including: 1) surface-water NO_3-N concentrations that are elevated year-round, 2) groundwater NO_3-N concentrations that reflect major leaching losses, and 3) soil characteristics that suggest altered N cycling, specifically increased rates of NO_3-N production via nitrification. Rates of atmospheric deposition are not particularly high in the SB watershed relative to other watersheds in the eastern U.S. (NADP 2009), yet the forest shows several classic

symptoms of late-stage N saturation, supporting the hypothesis that these symptoms may be induced at lower levels of N deposition in some systems (Fenn et al. 1998). Previous work in this watershed (Chapter 1) documented symptoms of N saturation in the aquatic component of the ecosystem, including limited biotic uptake of dissolved inorganic N and TN:TP ratios indicative of P-limitation.

While it would be desirable to further investigate the N-saturation status of the SB watershed using other metrics (i.e. foliar N:P ratios, rates of soil N cycling) to better understand the biological mechanisms underlying watershed response, it is clear that hydrogeologic factors play a critical role is determining the central response of the SB watershed to elevated N deposition. Specifically, elevated in-stream NO_3-N concentrations are driven by a combination of well-drained soils and underlying geologic features that allow NO_3-N from atmospheric inputs and nitrification to be leached to groundwater and transported to surface-water along subsurface flowpaths. Because the effects of current N deposition to the landscape should be more readily observed in streams receiving recently recharged groundwater (Harned and Daniel 1989, Nolan and Stoner 2000), and groundwater in the crystalline Piedmont of the Chesapeake Bay watershed is relatively young ("modern" to 34 years; Focazio et al. 1998, Lindsey et al. 2003), I argue that characteristics of the Piedmont that promote leaching and the movement of NO_3-N along subsurface flowpaths may not only control but also exacerbate the impacts of N saturation on surface-water NO_3-N concentrations and export.

This is, to my knowledge, the first un-manipulated watershed for which N saturation has been recognized in both the aquatic (Chapter 1) and terrestrial components of the ecosystem (see Simon et al. *in press*). While this does not reconcile the ideas presented by Aber et al. (1989, 1998) and Earl et al. (2006) regarding N saturation in forests and streams, it raises questions regarding the N-saturation status of the many other streams draining N-saturated forests in the Northeast and Mid-Atlantic; especially those within the crystalline Piedmont of the Chesapeake Bay watershed. While the SB watershed is unique in that N saturation has been identified in both the aquatic and terrestrial components of the ecosystem, it shares commonalities with forested Piedmont watersheds studied by others. Jordan et al. (1997) observed NO_3-N concentrations exceeding 0.5 mg L^{-1} in Watershed 401 located north of Baltimore, Maryland and Correll et al. (1995) observed similarly elevated concentrations in several forested tributaries within the Gunpowder River watershed in the same region. These, and other forested streams that have dramatically elevated NO_3-N concentrations, are of particular concern given that in-stream N saturation is a function of ambient surface-water concentration (Earl et al. 2006). While it is likely that in-stream processing of N plays a role in reducing the downstream delivery of N in many of these systems (Peterson et al. 2001, Mulholland et al 2008, 2009, Hall et al. 2009), we have yet to determine if a threshold ambient concentration exists at which N removal capacity declines significantly.

As evidence presented here suggests, we cannot rely on N processing in less-impacted ecosystems to protect downstream waterways from the effects of elevated atmospheric deposition. Since the capacity of streams to remove NO_3-N declines with increasing

ambient surface-water concentrations (Earl et al. 2006), and the response of surface-water concentrations to N saturation may be exacerbated in watersheds where hydrogeologic factors promote subsurface delivery of NO_3-N, I posit that streams draining N-saturated forests in settings with hydrogeologic characteristics similar to the Piedmont may provide the best research opportunity for linking N saturation in terrestrial and aquatic systems. Recognizing the frequency at which both the terrestrial and aquatic components of forested watersheds are N saturated will also allow us to better quantify the contribution of undeveloped ecosystems to downstream N pollution and manage for detrimental impacts to coastal water bodies.

Table 3.1. Coefficients and associated p-values for total and baseflow load models, generated by LOADEST using maximum likelihood estimation (MLE; Runkel et al. 2004). See text for detailed explanation of modeling methods.

Model	Parameter	Coefficient	Value	p-value
ln(total load)	Intercept	a_0	1.2083	1.2×10^{-38}
	ln$Q$	a_1	0.6115	6.8×10^{-106}
	lnQ^2	a_2	0.0376	7.3×10^{-3}
	dtime	a_3	-0.1222	3.4×10^{-6}
	dtime^2	a_4	-0.0657	4.7×10^{-2}
ln (baseflow load)	Intercept	a_0	0.3862	5.3×10^{-7}
	ln$Q$	a_1	0.8683	1.5×10^{-7}

Table 3.2. Summary of streamflow and NO_3-N export associated with total discharge (Q) and baseflow discharge (Q_B) for all four water years. Percent loss/retention for discharge and exports were calculated using inputs from precipitation and total atmospheric N deposition (wet + dry), respectively.

Water Year	Precip.	Discharge[1]			Fraction of Q in Q_B	Atmospheric Input	Nitrate Export[2]			Fraction of Nitrate Export in Q_B
		Q_B	Q				Q_B	Q		
	cm	L ha^{-1} yr^{-1}	L ha^{-1} yr^{-1}	% loss (% retention)		kg N ha^{-1} yr^{-1}	kg N ha^{-1} yr^{-1}	kg N ha^{-1} yr^{-1}	% loss (% retention)	
2005	92	1.9 X 10^6	4.0 x 10^6	43 (57)	0.48	5.4	1.8	*2.7*	50 (50)	0.68
2006	101	1.6 x 10^6	3.1 x 10^6	30 (70)	0.54	5.9	1.6	2.5	43 (57)	0.64
2007	61	2.3 x 10^6	4.8 x 10^6	77 (23)	0.48	3.6	2.1	2.9	81 (19)	0.73
2008	98	3.3 x 10^6	3.3 x 10^6	34 (66)	0.44	5.8	1.4	1.8	32 (68)	0.78

[1] Hydrograph separation was performed using a two-parameter recursive digital filter described by Eckhart (2005); see text for details.
[2] Export calculations based on loads modeled using the USGS Load Estimator (LOADEST; Runkel et al. 2004); see text for details.

Table 3.3. Characteristics of riparian and upland mineral soils (0 to 15 cm depth). Values are mean ± S.E.; p-values are shown for t-tests comparing riparian and upland soils.

Location	Bulk Density ($g\ mL^{-1}$)	% organic carbon	% total nitrogen	C:N
Riparian	1.5 ± 0.1	2.8 ± 0.2	0.2 ± 0.02	12.1 ± 0.3
Upland	1.3 ± 0.1	3.1 ± 0.3	0.3 ± 0.02	12.4 ± 1.3
p-value	0.1465	0.4281	0.5152	0.7907

Table 3.4. NO_3-N export for Sopers Branch and other forested watersheds in the Northeast and Mid-Atlantic, U.S.. Published values are listed in descending order.

Stream/Watershed ID	State	NO_3-N Export (kg ha-1 yr-1)
Sopers Branch (WY2005)	MD	2.7
Sopers Branch (WY2006)	MD	2.5
Sopers Branch (WY2007)	MD	2.9
Sopers Branch (WY2008)	MD	1.8
Average	MD	2.5
East Branch of Neff Run (2000-2004)[1]	MD	9.4
Fernow Experimental Forest , Watershed 4[2]	WV	5.7
Monroe Run[3]	MD	5.0
Peapatch Ridge[3]	MD	4.7
Lower Big Run[3]	MD	4.3
Whiskey Hollow[3]	MD	4.3
Fernow Experimental Forest , Watershed 13[2]	WV	4.2
Fernow Experimental Forest , Watershed 9[3]	WV	4.1
Biscuit Brook[2]	NY	4.0
Miller Run[3]	MD	3.5
Fernow Experimental Forest , Watershed 3[3]	WV	3.3
Mt. Success[2]	NH	3.2
The Bowl, Lower Branch[2]	NH	2.9
Upper Big Run[3]	MD	2.8
The Bowl, East Branch[2]	NH	2.8
Huntington Forest, Archer Creek[2]	NY	2.7
The Bowl, Upper Branch[2]	NH	2.7
Watershed 401[4]	MD	2.6
West Virginia University, Watershed 1[3]	WV	2.5
Lye Brook, Watershed 6[2]	VT	2.5
The Bowl, West Branch[2]	NH	2.5
West Virginia University, Watershed 2[3]	WV	2.2
UnnamedTributary to Herrington Creek[2]	MD	2.2
Hauver Branch[3]	MD	2.2
Baldwin Creek[3]	PA	2.1
Linn Run[3]	PA	2.0
Sleepers River, Watershed 9[2]	VT	1.6
Young Woman's Creek[3]	PA	1.4

Stream/Watershed ID	State	NO_3-N Export (kg ha-1 yr-1)
Hubbard Brook Experimental Forest, Watershed 6[2]	NH	1.2
Acadia National Park, Hadlock Brook[2]	ME	1.2
Fernow Experimental Forest , Watershed 10[2]	WV	1.1
Lye Brook, Watershed 4[2]	VT	1.0
Benner Run[3]	PA	0.7
Lye Brook, Watershed 8[2]	VT	0.7
Roberts Run[3]	PA	0.6
Cornell Natural Area[5]	NY	0.6
Eastman Hill[5]	NY	0.6
East Bear Brook[2]	ME	0.6
Cayutaville Rd[5]	NY	0.5
Bessie Miller Spring[3]	PA	0.4
Stoney Creek[3]	PA	0.4
Bald Mountain[5]	NY	0.4
Honeypot Road[5]	NY	0.4
Upper Carter Creek[5]	NY	0.4
West Carter Creek[5]	NY	0.4
Hubbard Brook Experimental Forest, Watershed 9[2]	NH	0.4
East Overlook Trail[5]	NY	0.3
Pine Creek[5]	NY	0.3
Swan Road[5]	NY	0.3
White Oak Run[3]	VA	0.2
Stone Run[3]	PA	0.2
East Carter Creek[5]	NY	0.2
Michigan Hollow[5]	NY	0.2
Weymouth Point[2]	ME	0.2
Rhode River No. 110[3]	MD	0.1
West Overlook Trail[5]	NY	0.1
Acadia National Park, Cadillac Brook[2]	ME	0.1
Deep Run[3]	VA	<0.1
Leading Ridge, Watershed 1[3]	PA	<0.1
Leading Ridge, Watershed 3[3]	PA	<0.1
Cockaponset[2]	CT	<0.1
Cone Pond Inlet[2]	NH	<0.1

[1]Castro et al. 2007, [2]Campbell et al. 2004, [3]Dewalle and Pionke 1995, [4]Jordan et al. 1997, [5]Goodale et al. 2009

Table 3.5. Average observed NO_3-N concentrations for streams in the Chesapeake Bay watershed and surrounding region.

Physiographic province	Sub-region/sampling Area	NO_3-N (mg L^{-1})
Appalachian Plateau	Appalachian Plateau (NY)[1]	0.18
	Poconos-Catskills (NY, NJ, PA)[2]	0.084
	Northern Appalachians (PA, MD, WV)[2]	0.42
Ridge and Valley	Valley and Ridge (PA, NJ, MD, WV, VA)[2]	0.14
	Ridge and Valley(PA)[1]	0.40
	Great Valley (PA)[1]	1.07
Piedmont	Piedmont (MD)[1]	0.66
Coastal Plain	Coastal Plain (MD)[1]	0.12

[1]Correll et al. 1995, [2]Kaufmann et al. 1991

Table 3.6. Flow-weighted NO_3-N concentrations for Sopers Branch and other forested watersheds in the Northeast and Mid-Atlantic states. Note that published values are listed in descending order.

Stream/Watershed ID	State	NO_3-N (mg L^{-1})
Sopers Branch (WY2005)	MD	0.68
Sopers Branch (WY2006)	MD	0.83
Sopers Branch (WY2007)	MD	0.61
Sopers Branch (WY2008)	MD	0.55
Average		0.67
East Branch of Neff Run[1]	MD	1.37
Fernow Experimental Forest, Watershed 4[2]	WV	0.80
South Fork of Potomac River[3]	WV	0.77
Mt. Success, Unnamed[2]	NH	0.68
Watershed 401, Unnamed[4]	MD	0.61
Fernow Experimental Forest, Watershed 13[2]	WV	0.47
Biscuit Brook[2]	NY	0.41
Lye Brook, Watershed 6[2]	VT	0.35
Huntington Forest, Archer Creek[2]	NY	0.33
Tributary to Herrington Creek[2]	MD	0.23
The Bowl, Lower Branch[2]	NH	0.22
Sleepers River, Watershed 9[2]	VT	0.22
The Bowl, Upper Branch[2]	NH	0.21
The Bowl, East Branch[2]	NH	0.20
The Bowl, West Branch[2]	NH	0.18
Fernow Experimental Forest, Watershed 10[2]	WV	0.16
Hubbard Brook Experimental Forest, Watershed 6[2]	NH	0.13
Acadia, Hadlock Brook[2]	ME	0.11
Lye Brook, Watershed 8[2]	VT	0.09
Weymouth Point, Unnamed[2]	ME	0.07
East Bear Brook[2]	ME	0.07
Hubbard Brook Experimental Forest, Watershed 9[2]	NH	0.04
Leading Ridge, Watershed 1[2]	PA	0.02
Lye Brook, Watershed 4[2]	VT	0.02
Cone Pond, Inlet[2]	NH	0.01
Cockaponset, Unnamed[2]	CT	0.01
Acadia, Cadillac Brook[2]	ME	0.01

[1]Castro et al. 1997, [2]Campbell et al. 2004, [3]Clark et al. 2000, [4]Jordan et al. 1997

Table 3.7. Mineral soil C:N values for Sopers Branch and other watersheds in the mid-Atlantic U.S.. Note that literature values are shown in ascending order; N-enriched soils (low C:N) are susceptible to NO_3-N leaching as a result of increased rates of nitrification.

Site	State	C:N
Sopers Branch	MD	12.1
Fernow Experimental Forest, Watershed 4[1]	WV	13.2
Whiskey Hollow[1]	MD	13.3
East Branch of Neff Run[2]	MD	14.0
Stone Run[1]	PA	14.2
Fernow Experimental Forest, Watershed 10[1]	WV	14.8
Peapatch Ridge[1]	MD	15.3
Leading Ridge, Watershed 1[1]	PA	16.5
Leading Ridge, Watershed 3[1]	PA	17.0
Linn Run[1]	PA	19.0
Baldwin Creek[1]	PA	21.7

[1]Williard et al. 1997, [2]Castro et al. 2007

FIGURE CAPTIONS:

Figure 3.1. Map of Sopers Branch watershed with sampling locations for surface-water (USGS gauge), groundwater, and soil. Watershed land-use includes forest (white), cropland/pasture (light gray), low density residential (dark gray), and commercial/industrial (black). Insets illustrate soil sampling locations for riparian transects (a) and upland plots (b) with each point representing one soil core.

Figure 3.2. Precipitation, stream hydrology, and surface-water chemistry for the Sopers Branch watershed: (a) daily precipitation (cm) with shaded area indicating gaps in the data record, (b) average daily discharge (Q, L s^{-1}, solid line) and baseflow discharge (Q_B, L s^{-1}, broken line) for water years 2005 to 2008, and (c) NO_3-N concentrations (mg L^{-1}) for surface-water samples collected during low flows (n = 32, open circles) and storm events (n = 135, crosses); each symbol represents one sampling event. Note use of log scale in panels (b) and (c).

Figure 3.3. Average monthly N inputs, exports, and retention (kg ha^{-1}) during the dormant and growing seasons; asterisks (*) indicate significant differences ($\alpha = 0.05$).

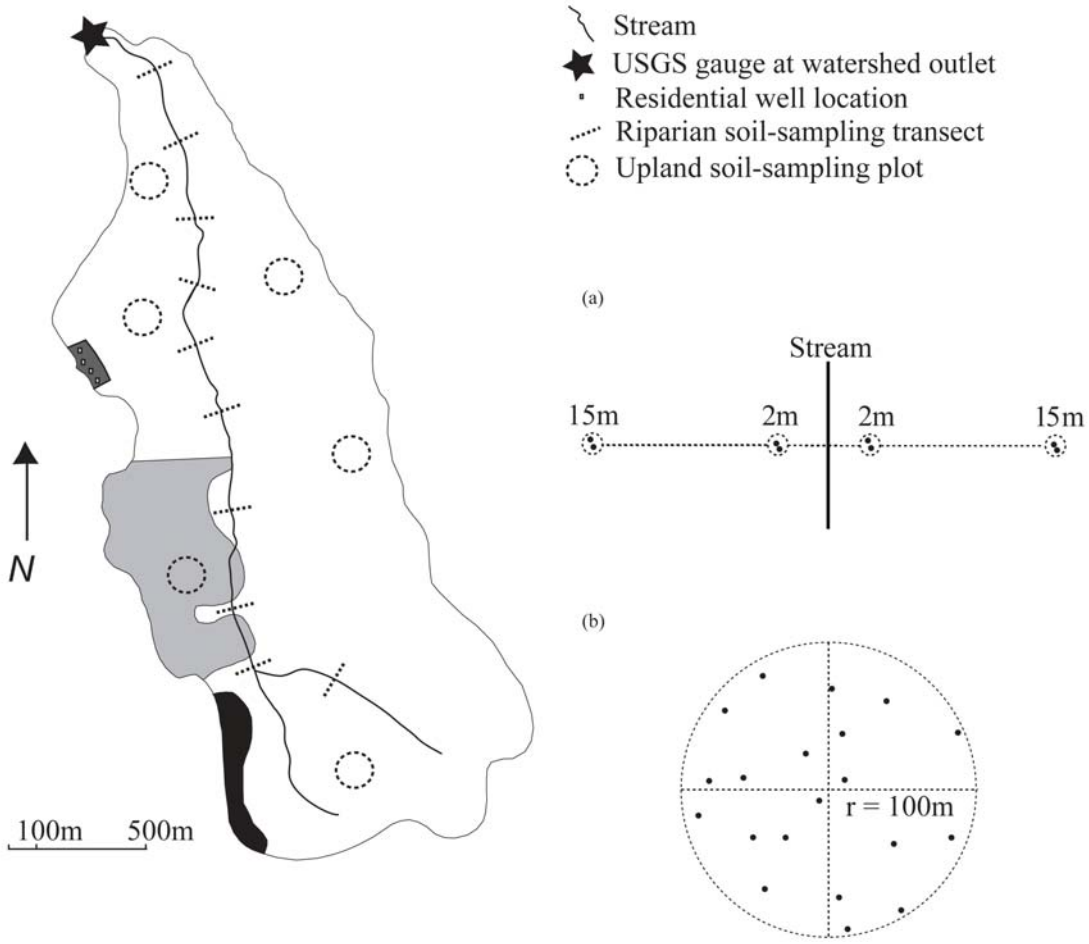
Figure 3.4. Relationship between discharge (Q, L s^{-1}) and NO_3-N concentration (mg L^{-1}) for surface-water data collected between 02 October 2004 and 18 November 2008 (n = 167; r = -0.8617); each circle represents one sampling event.

Figure 3.5. Monthly total export (kg N ha^{-1}, mean ± S.E., solid line) and baseflow export (broken line) for WY2005 to WY2008.

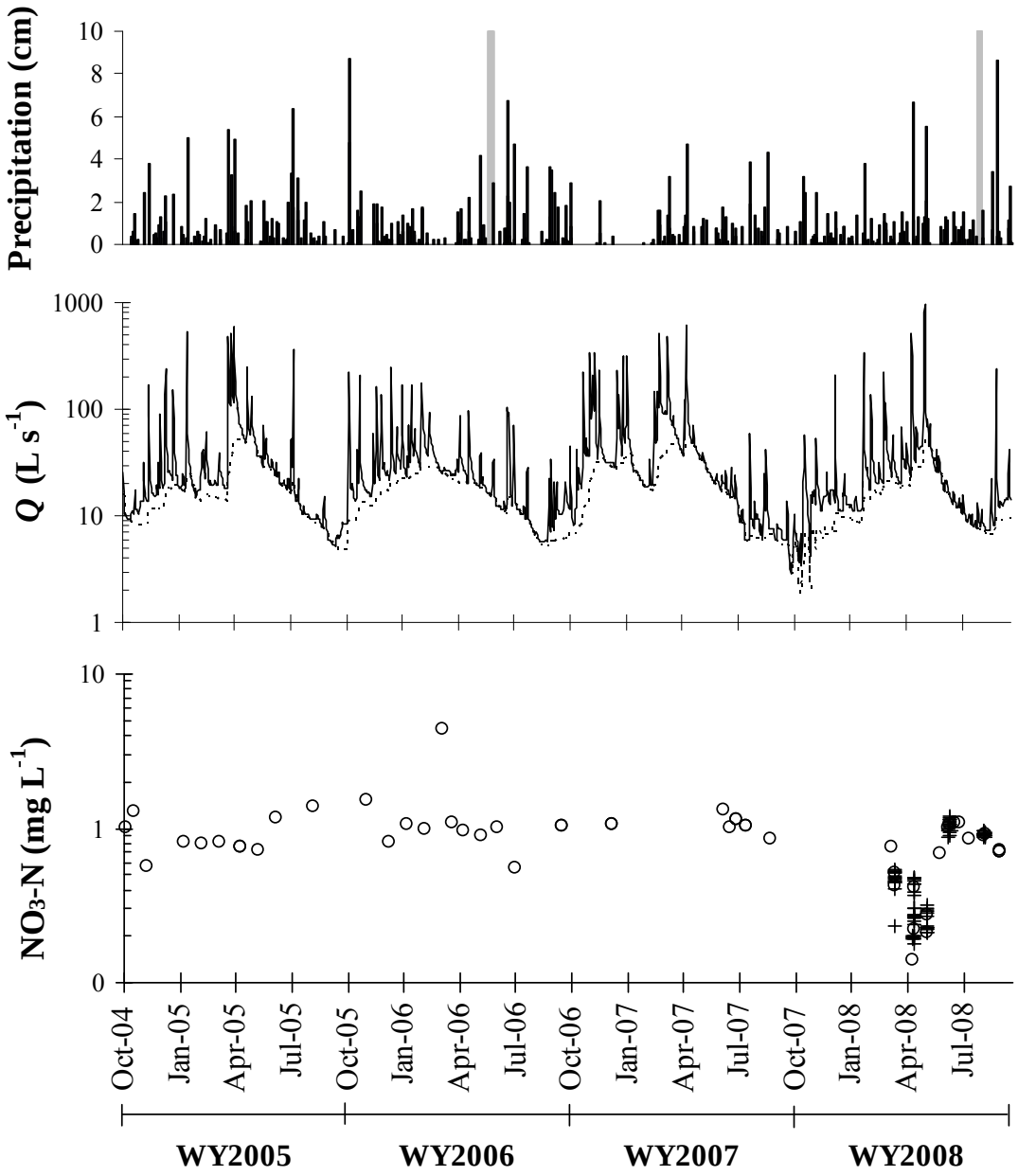
Figure 3.6. Monthly baseflow index plotted against flow-weighted NO_3-N concentration for WY2005 to WY2008.

Figure 3.7. Total monthly NO_3-N exports (kg ha^{-1}) plotted against monthly precipitation (cm). Each circle represents one month in the dormant (open) or growing (closed) seasons. The solid line represents total atmospheric N inputs (wet + dry; kg ha^{-1}) associated with varying levels of precipitation; months where the SB watershed was a net source of N fall above this line.

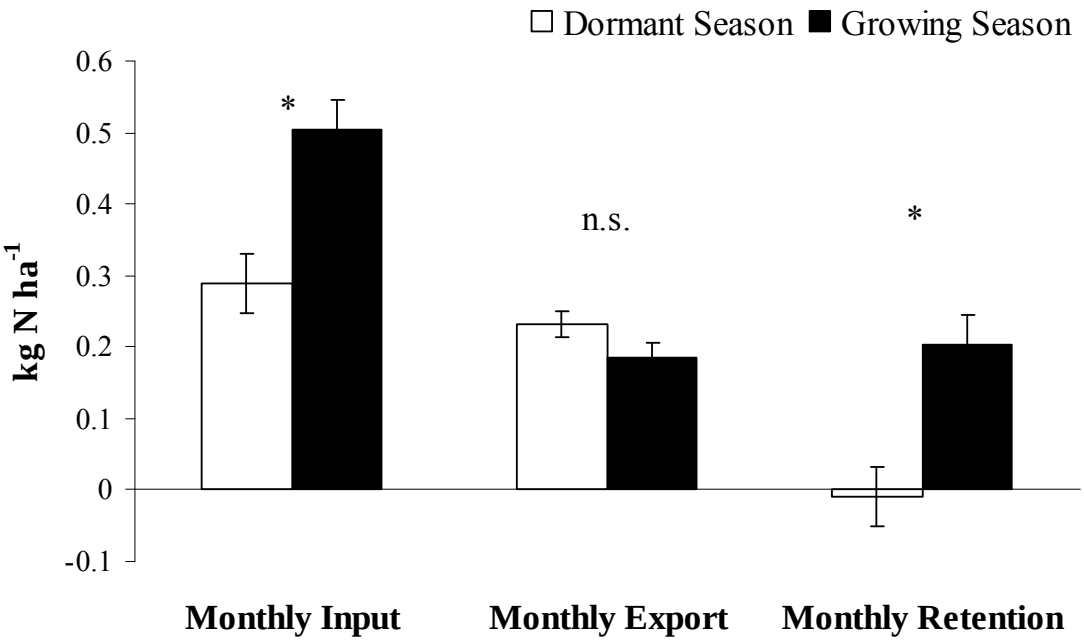
(Figure 3.1)


Stream
USGS gauge at watershed outlet
Residential well location
Riparian soil-sampling transect
Upland soil-sampling plot
N
100m
500m
(a)
Stream
15m
2m
2m
15m
(b)
r = 100m

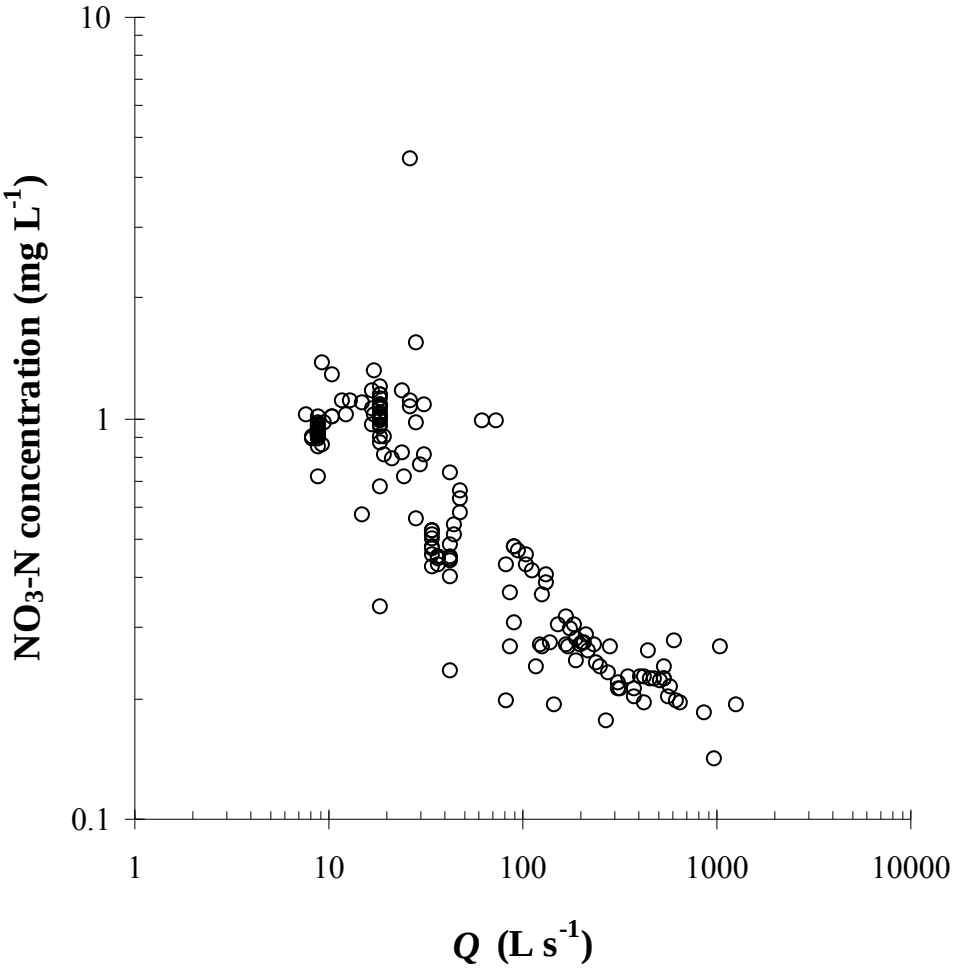
(Figure 3.2)


Precipitation (cm)
10
8
6
4
2
0
Q (L s-1)
1000
100
10
1
NO3-N (mg L-1)
10
1
0
Oct-04
Jan-05
Apr-05
Jul-05
Oct-05
Jan-06
Apr-06
Jul-06
Oct-06
Jan-07
Apr-07
Jul-07
Oct-07
Jan-08
Apr-08
Jul-08
WY2005
WY2006
WY2007
WY2008

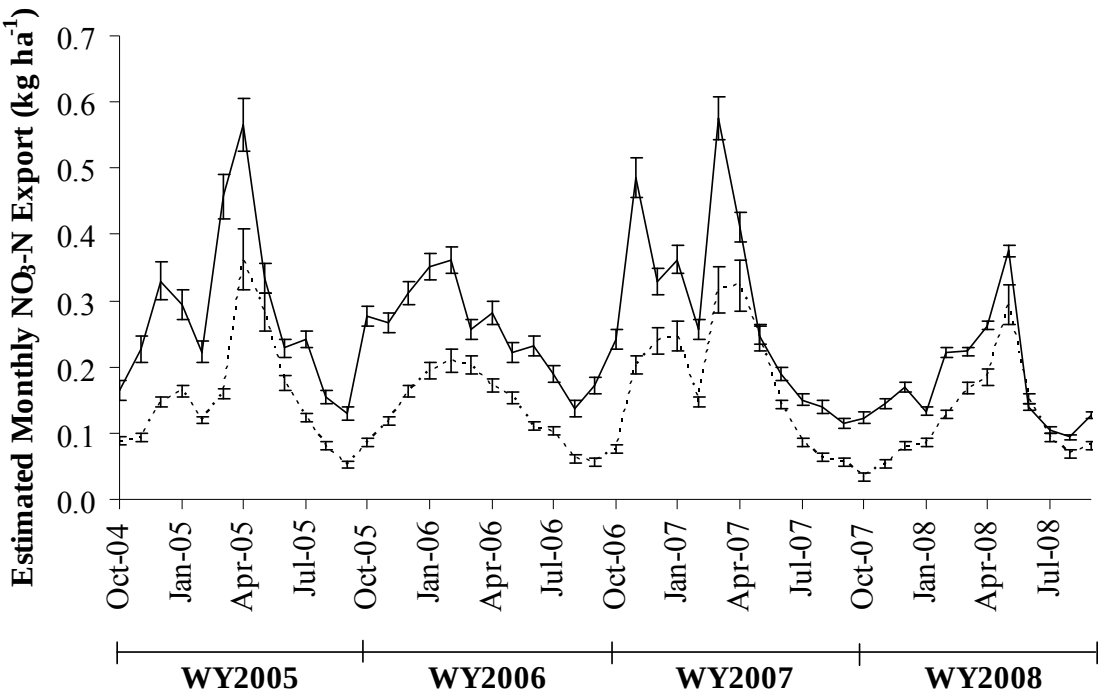
(Figure 3.3)


Dormant Season
Growing Season
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
kg N ha-1
*
n.s.
*
Monthly Input
Monthly Export
Monthly Retention

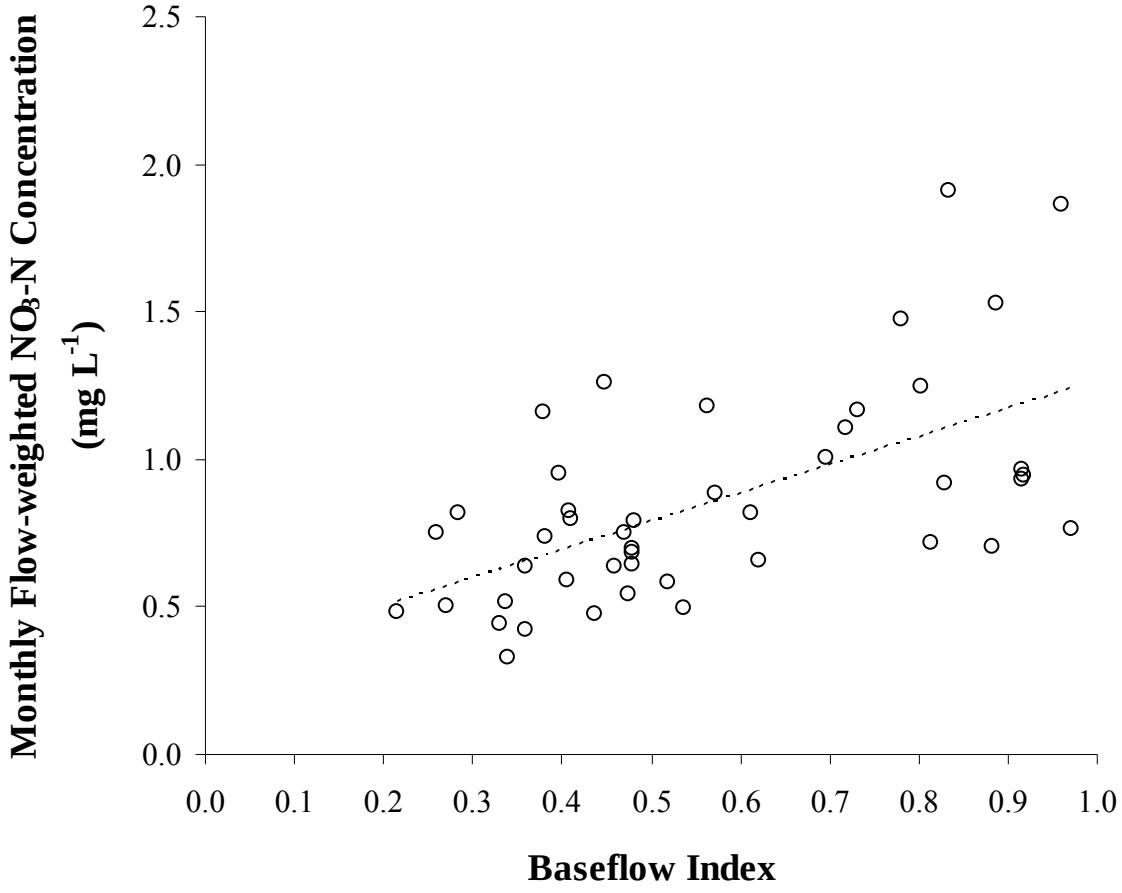
(Figure 3.4)



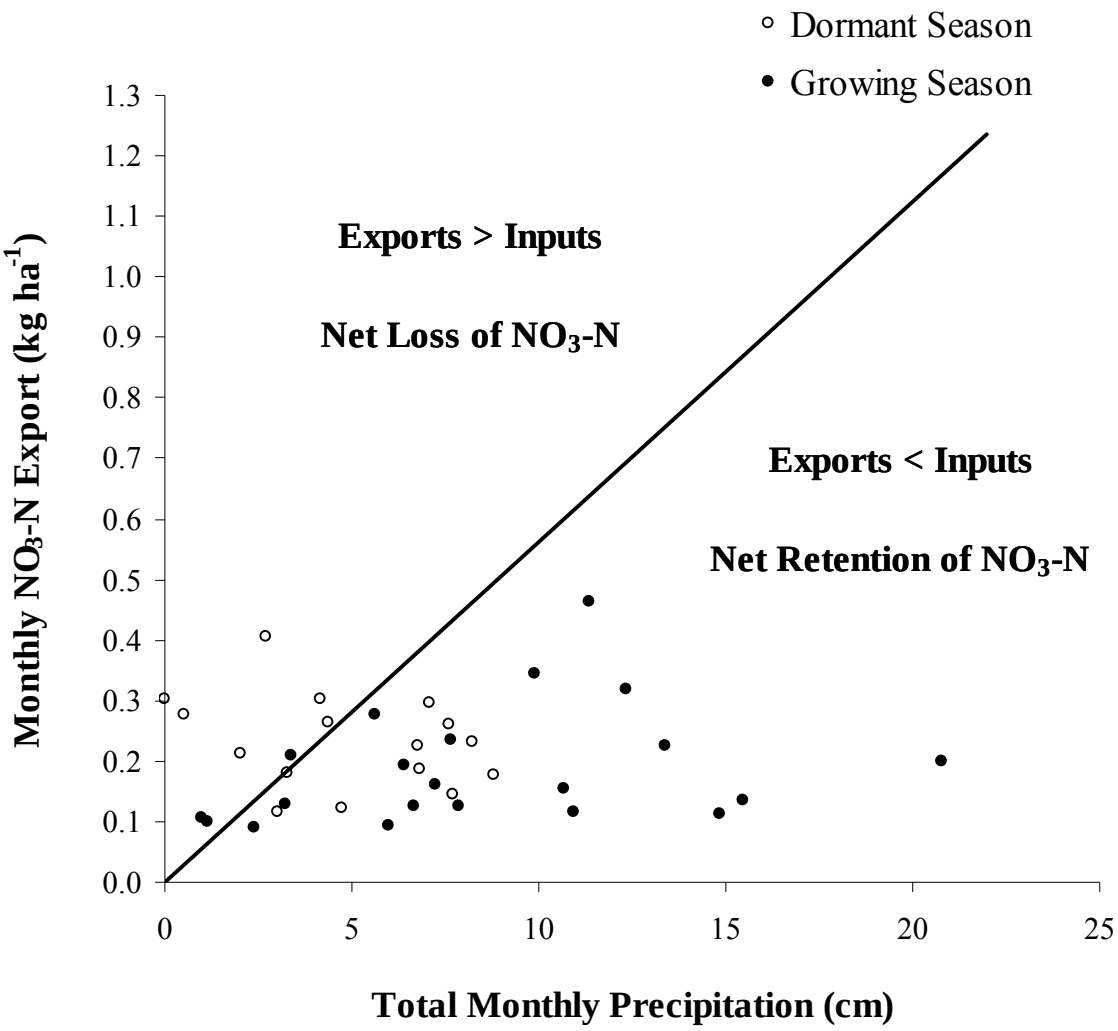
(Figure 3.5)


Estimated Monthly NO3-N Export (kg ha-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Oct-04
Jan-05
Apr-05
Jul-05
Oct-05
Jan-06
Apr-06
Jul-06
Oct-06
Jan-07
Apr-07
Jul-07
Oct-07
Jan-08
Apr-08
Jul-08
WY2005
WY2006
WY2007
WY2008

(Figure 3.6)


Monthly Flow-weighted NO3-N Concentration (mg L-1)
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Baseflow Index

(Figure 3.7)


Dormant Season
Growing Season
Exports > Inputs
Net Loss of NO3-N
Exports < Inputs
Net Retention of NO3-N
Monthly NO3-N Export (kg ha-1)
Total Monthly Precipitation (cm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
0
5
10
15
20
25

CONCLUSION

In the last several years, our view of N processing in human-impacted streams has changed dramatically. Urban streams were previously viewed as "gutters" that effectively conveyed nutrients to downstream waterways (Bernhardt and Palmer 2007). In a paper from the 1st Symposium on Urbanization and Stream Ecology (SUSE) summarizing the symptoms of the urban stream syndrome, Walsh et al. (2005) noted that urban streams were consistently less able to remove N than their unimpacted counterparts based on the findings of Grimm et al. (2005) and Meyer et al. (2005). Since that time, new data have shown that N dynamics in urban streams are much more complex. Aquatic ecologists are now moving away from the idea that all urban streams are poorly functioning ecosystems. In fact, at the 2nd SUSE, held in May 2008, presenters made it clear that not all urban streams are equal – different environmental stressors (or combinations of stressors) exist in different settings (Wenger et al. 2009). The ability of urban streams to process N reflects this variability and some urban streams have been shown to be as effective as their unimpacted counterparts at removing N (e.g. LINX II studies: Mulholland et al. 2008, 2009, Hall et al. 2009b). I provide evidence that the reverse is also true – some forested streams are as *in*effective as their urban counterparts at removing excess N. Specifically, based on extensive data from three watersheds, I conclude that high-N streams draining both urban and forested watersheds of the Maryland Piedmont may be unable to remove N as a result of both N saturation and P limitation (Chapter 1). My results and the findings of the 2nd SUSE suggest that we need

to move away from making generalizations about stream ecosystem function based on watershed land use.

Recent work has also challenged our perception of how stream ecosystems respond to chronically elevated N loads. Saturation is expected to occur as N availability exceeds biotic demand (Bernot and Dodds 2005); yet, research conducted in urban and agricultural settings indicates that saturation of uptake does not always occur in streams with chronically elevated N concentrations (O'Brien et al. 2007, Mulholland et al. 2008, Hall et al. 2009b). Instead, rates of N uptake increase with increasing N concentrations while the efficiency of processing decreases (O'Brien et al. 2007). This suggests that communities of benthic microorganisms may be able to adapt to elevated N concentrations. Here, I show that saturation, rather than efficiency loss, explains N dynamics in several high-N systems where autotrophic demand is low and other factors (e.g. light, P, hydrologic regimes) may limit the ability of the benthic community to adapt to chronically elevated N concentrations. My findings illustrate that efficiency loss may only be appropriate for describing N uptake in systems that are not otherwise limited in their ability to adapt to elevated N loads.

Many studies highlight the potential role of in-stream processing in mitigating the downstream consequences of increased N inputs (e.g. Alexander et al. 2000, Peterson et al. 2001, Bernhardt et al. 2003, Ensign and Doyle 2006, Tank et al. 2008); however, because not all streams are effective N sinks, we cannot rely on streams – even those that have been restored – to alleviate the impacts of excess N inputs (Filoso and Palmer *in*

preparation). Streams that are unable to retain substantial fractions of their N load transfer larger loads to subsequent reaches (Vitousek et al. 1997, Galloway et al. 2003, Earl et al. 2006), placing the burden of N removal on higher-order recipient systems (Alexander et al. 2007, Seitzinger 2008). Because elevated NO_3-N concentrations have previously been linked to saturation, reduced efficiency of removal, and increased rates of nitrification, delivery of larger N loads to downstream reaches may rapidly propagate through lotic networks (Strauss and Lamberti 2000, Bernhardt et al. 2002, Kemp and Dodds 2002, Bernot and Dodds 2005, Earl et al. 2006, Mulholland et al. 2008). Thus, any increase in the amount of N entering systems that are already limited in their ability to retain N will have dramatic downstream consequences (Peterson et al. 2001, Williams et al. 2004, Bernot and Dodds 2005).

In May 2009, President Barack Obama declared the Chesapeake Bay a National Treasure and within six months Senator Ben Cardin, along with three other senators, proposed the Chesapeake Clean Water and Ecosystem Restoration Act of 2009 (S. 1816). These actions signal a new commitment to Bay restoration and set the stage for establishing a credit-based nutrient trading system that requires both monitoring and accountability. Nutrient trading systems rely on our ability to define baseline conditions and determine the effectiveness of various controls, including upland best management practices (BMPs) and channel restoration. Unfortunately, given results such as those from my research in Chesapeake Bay sub-watersheds, we may lack the necessary scientific knowledge to properly support a credit-based trading system aimed at reducing aquatic N pollution (Palmer and Filoso 2009). Specifically, it may be difficult to identify

appropriate baselines given that the entire Chesapeake Bay Watershed receives elevated atmospheric N inputs (NADP 2009) which may influence N dynamics differently depending on local watershed characteristics and historical land use. As I noted in Chapter 3, our estimates of the contribution of undeveloped catchments to Bay pollution may improve once we recognize the frequency with which both the terrestrial and aquatic components of forested watersheds are N-saturated. Furthermore, our understanding of BMP efficiencies may be inadequate for assigning nutrient reduction credits. For example, my findings illustrate that when elevated NO_3-N concentrations occur in the absence of other stressors that stimulate autotrophic uptake (e.g. reduced canopy cover, increased nutrients; Young et al. 2008), streams may be unable to compensate for increased N loads (Chapter 1). This suggests that efforts commonly implemented to protect waterways, such as preservation of riparian forests and reduction of P inputs by structural BMPs, may interact with elevated N loading to aggravate water quality problems – this is not something that can easily be accounted for by a nutrient trading system.

Finally, my results suggest that we cannot assume that in-stream processing will reduce N loads even in minimally impacted watersheds (Chapter 1). Systems incapable of removing measurable amounts of N vary widely in terms of land use and background N concentrations (Chapter 2). This suggests that stream restoration may be less able to reduce the delivery of N to downstream water bodies than previously believed (Craig et al. 2008) – or at least less able to reliably reduce N loads (Filoso and Palmer *in preparation*). As such, it is critical that management efforts reduce N loading to streams

(Driscoll et al. 2003, Galloway et al. 2003) and take advantage of opportunities for increasing N removal in impaired systems only after other options have been exhausted (Craig et al. 2008).

APPENDIX I

Range of possible uptake metrics from 90%, two-tailed, *t*-based confidence intervals around estimated uptake rate coefficients for individual NO_3-N, NH_4-N, PO_4-P, and DOC additions.

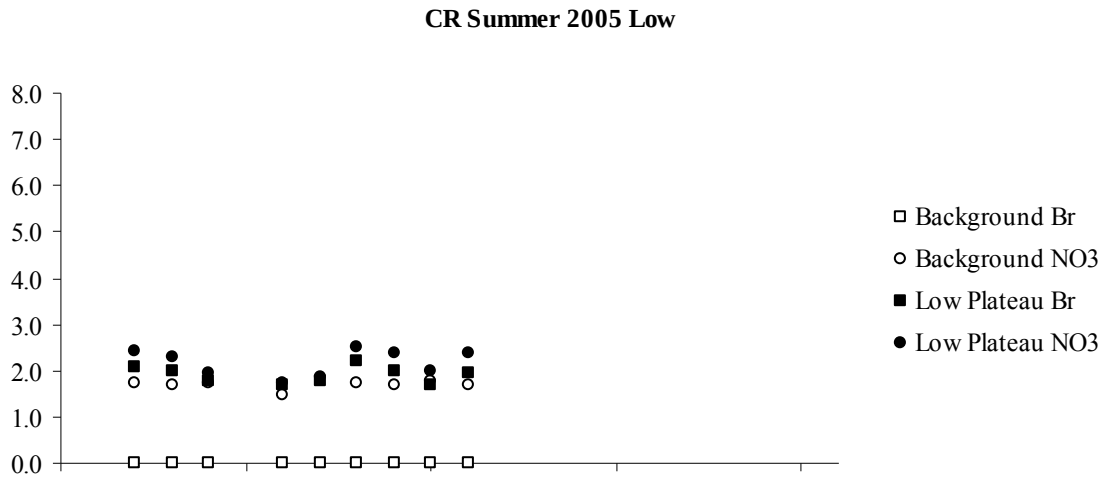
Addition Information				Regression Information						90% Confidence Limits	
Added nutrient	Date	Site	Level	n (# of transects remaining)	p-value	*k* (of added nutrient)	s.e. (*k*)	Sig. longitudinal change in nutrient?	Sig. longitudinal change in tracer?	Lower 90% CL (*k*)	Upper 90% CL (*k*)
NO_3-N	07Jul05	CR	L	8	0.96	0.0031	0.0060	no	no	-0.0086	0.0148
	11Jul05	CR	M	9	0.60	0.0045	0.0082	no	dec.	-0.0110	0.0201
	22Jul05	CR	H	4	0.42	0.0021	0.0021	no	no	-0.0040	0.0081
	19Nov05	CR	L	9	0.68	-0.0044	0.0100	no	no	-0.0233	0.0146
	19Nov05	CR	M	8	0.75	0.0013	0.0052	no	no	-0.0088	0.0113
	19Nov05	CR	H	5	0.41	-0.0058	0.0061	no	no	-0.0201	0.0085
	25Jun07	CR	L	3	0.33	0.0110	0.0621	no	no	-0.3811	0.4031
	25Jun07	CR	H	5	0.08	0.0024	0.0009	inc.	no	0.0002	0.0046
	07Jul05	SF	L	2	n/a						
	11Jul05	SF	M	4	0.09	0.0021	0.0007	inc.	no	0.0001	0.0040
	22Jul05	SF	H	3	0.95	-0.0215	0.2584	no	no	-1.6528	1.6098
	28Nov05	SF	L	6	0.18	0.0042	0.0026	no	no	-0.0013	0.0097
	28Nov05	SF	M	9	0.60	0.0007	0.0013	no	no	-0.0017	0.0031
	28Nov05	SF	H	8	0.95	-0.0001	0.0010	no	no	-0.0019	0.0018
	22Jun07	SF	L	5	0.34	-0.0040	0.0035	no	inc.	-0.0122	0.0042
	22Jun07	SF	H	6	0.76	0.0002	0.0005	no	dec.	-0.0009	0.0012
	07Jul05	SB	L	4	0.92	-0.0036	0.0312	no	dec.	-0.0946	0.0874
	11Jul05	SB	M	4	0.37	-0.0161	0.0141	no	no	-0.0571	0.0249
	22Jul05	SB	H	5	0.28	-0.0323	0.0248	no	no	-0.0905	0.0259
	23Nov05	SB	L	9	0.12	-0.0456	0.0261	no	dec.	-0.0950	0.0038
	23Nov05	SB	H	8	0.07	0.0036	0.0016	inc.	dec.	0.0005	0.0068
	24Jun07	SB	L	6	0.66	-0.0009	0.0018	no	dec.	-0.0047	0.0030
	24Jun07	SB	H	5	0.79	-0.0003	0.0012	no	dec.	-0.0031	0.0024
NH_4-N	07Jul07	CR	n/a	6	<0.01	-0.0056	0.0008	dec.	dec.	-0.0078	-0.0034
	02Jul07	SF	n/a	6	0.04	-0.0050	0.0018	dec.	no	-0.0101	0.0001
	03Jul07	SB	n/a	5	0.07	-0.0069	0.0030	dec.	dec.	-0.0164	0.0027
PO_4-P	04Sep07	CR	n/a	5	<0.01	-0.0013	0.0002	dec.	no	-0.0018	-0.0008
	30Aug07	SF	n/a	6	<0.01	-0.0034	0.0002	dec.	dec.	-0.0037	-0.0030
	29Aug07	SB	n/a	6	<0.01	-0.0033	0.0006	dec.	no	-0.0045	-0.0021
DOC	24Jul07	CR	n/a	5	0.01	-0.0026	0.0006	dec.	no	-0.0041	-0.0011
	21Jul07	SF	n/a	6	0.16	-0.0013	0.0007	no	dec.	-0.0028	0.0003
	20Jul07	SB	n/a	5	0.02	-0.0036	0.0007	dec.	dec.	-0.0053	-0.0018

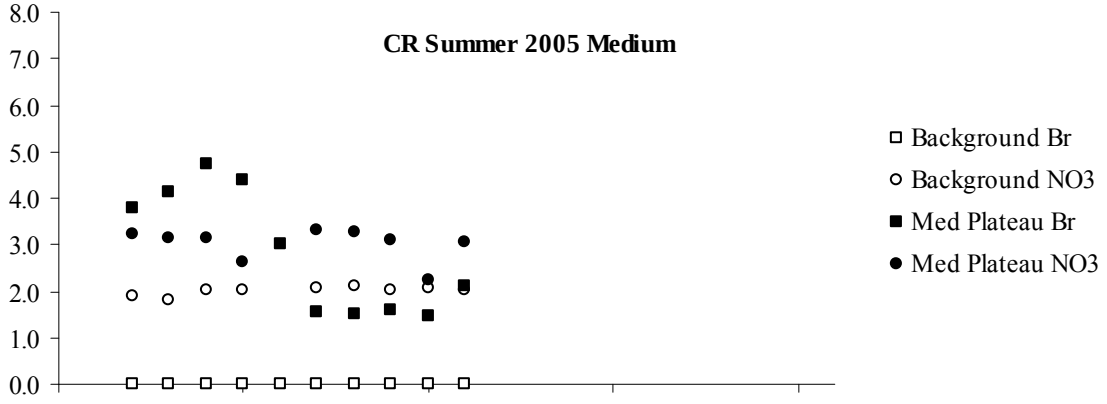
Appendix I. (continued)

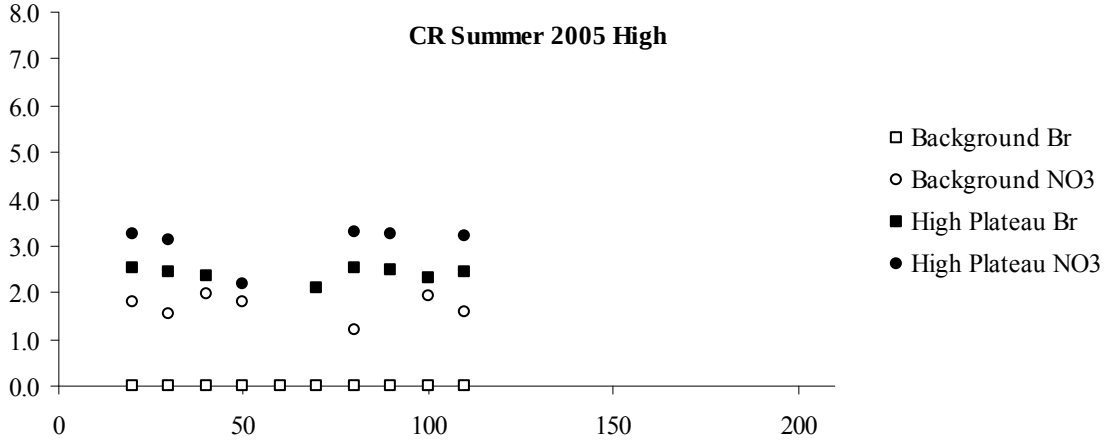
Addition Information				S_w (m)			v_f (mm min^{-1})			U (μg m^{-2} s^{-1})		
Added nutrient	Date	Site	Level	Based on Lower 90% CL	Based on Slope	Based on Upper 90% CL	Based on Lower 90% CL	Based on Slope	Based on Upper 90% CL	Based on Lower 90% CL	Based on Slope	Based on Upper 90% CL
NO_3-N	07Jul05	CR	L	116	-323	-67	3.8	-1.4	-6.5	6392	-2300	-10991
	11Jul05	CR	M	91	-222	-50	8.3	-3.4	-15.1	16687	-6824	-30335
	22Jul05	CR	H	253	-483	-124	2.2	-1.2	-4.6	3758	-1972	-7701
	19Nov05	CR	L	43	-787	-68	7.4	-0.4	-4.6	18587	-1013	-11647
	19Nov05	CR	M	114	230	-88	2.8	1.4	-3.6	7016	3470	-9042
	19Nov05	CR	H	50	173	-118	6.4	1.8	-2.7	16013	4619	-6775
	25Jun07	CR	L	3	-417	-2	151.5	-1.0	-160.2	315388	-1986	-333611
	25Jun07	CR	H	-4251	-91	-219	-0.1	-4.4	-1.8	-195	-9112	-3778
	07Jul05	SF	L									
	11Jul05	SF	M	-8803	-483	-248	-0.1	-0.9	-1.8	-169	-3072	-5975
	22Jul05	SF	H	1	47	-1	556.4	7.2	-542.0	2127817	27679	-2072459
	28Nov05	SF	L	789	-239	-104	0.3	-1.0	-2.4	1474	-4871	-11216
	28Nov05	SF	M	589	-1449	-325	0.4	-0.2	-0.8	1974	-802	-3578
	28Nov05	SF	H	525	16667	-560	0.5	0.0	-0.4	2216	70	-2076
	22Jun07	SF	L	82	251	-237	2.4	0.8	-0.8	9793	3202	-3389
	22Jun07	SF	H	1158	-6250	-845	0.2	0.0	-0.2	693	-128	-950
	07Jul05	SB	L	11	277	-11	67.0	2.6	-61.9	51185	1953	-47279
	11Jul05	SB	M	18	62	-40	39.0	11.0	-17.0	36124	10186	-15752
	22Jul05	SB	H	11	31	-39	43.0	15.3	-12.3	43028	15351	-12327
	23Nov05	SB	L	11	22	-266	57.6	27.7	-2.3	43037	20666	-1705
	23Nov05	SB	H	-2205	-275	-146	-0.3	-2.2	-4.1	-205	-1649	-3092
	24Jun07	SB	L	213	1176	-335	1.7	0.3	-1.1	1854	336	-1181
	24Jun07	SB	H	323	2941	-414	1.1	0.1	-0.9	1223	134	-954
NH_4-N	07Jul07	CR	n/a	128	178	292	3.4	2.4	1.5	177	127	77
	02Jul07	SF	n/a	99	200	-9273	2.1	1.0	0.0	52	26	-1
	03Jul07	SB	n/a	61	146	-377	6.0	2.5	-1.0	248	104	-40
PO_4-P	04Sep07	CR	n/a	559	769	1231	0.4	0.3	0.2	11	8	5
	30Aug07	SF	n/a	268	296	331	0.7	0.7	0.6	7	6	5
	29Aug07	SB	n/a	221	300	470	1.3	0.9	0.6	4	3	2
DOC	24Jul07	CR	n/a	243	385	918	1.4	0.9	0.4	4808	3041	1274
	21Jul07	SF	n/a	354	794	-3303	0.3	0.1	0.0	754	337	-81
	20Jul07	SB	n/a	188	279	547	2.3	1.5	0.8	5791	3889	1987

APPENDIX II

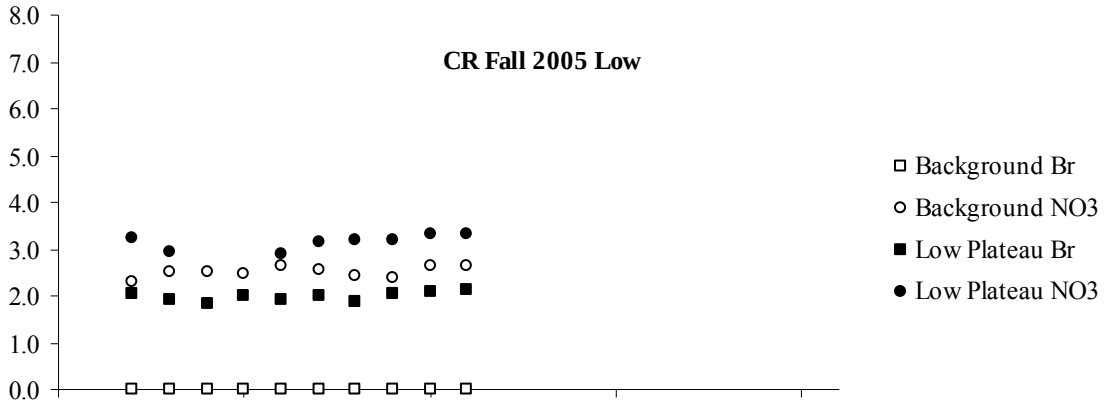
Nitrate Injection Data for Crystal Rock (CR), Sycamore Farm (SF), and Sopers Branch (SB). Data are average transect values.

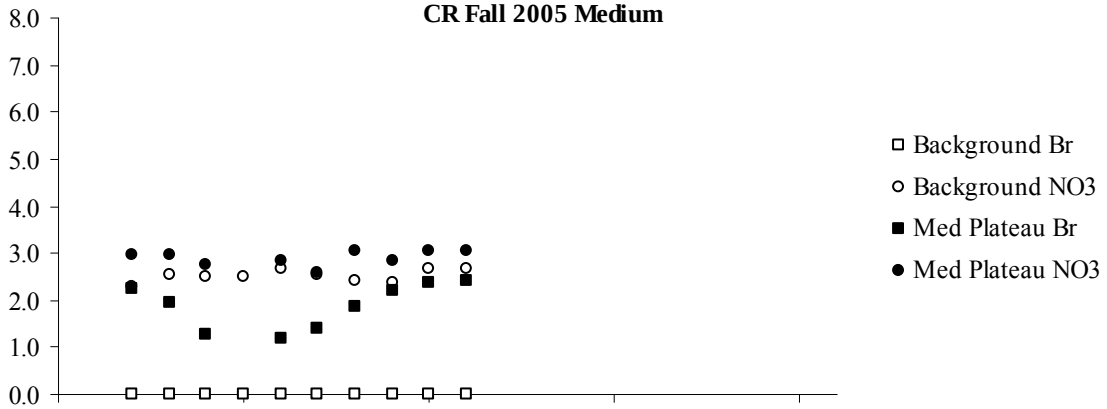


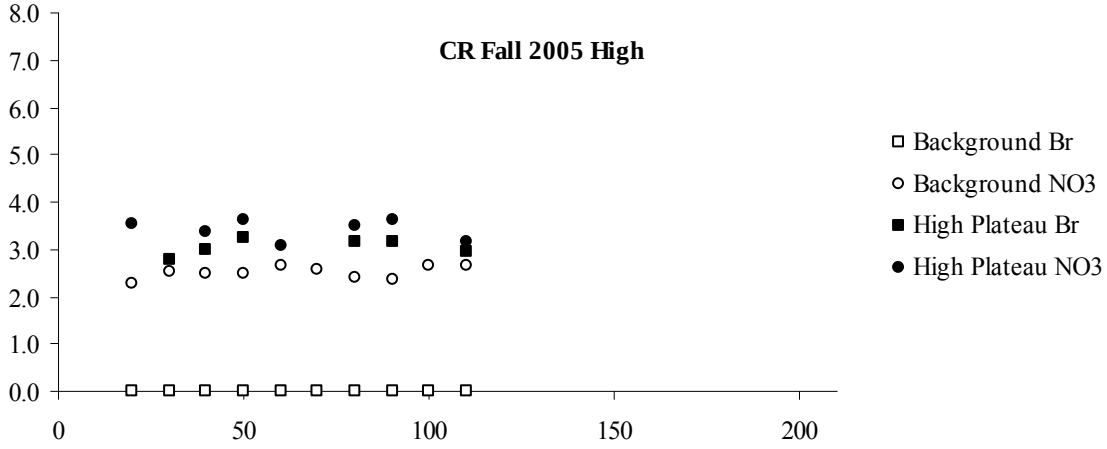




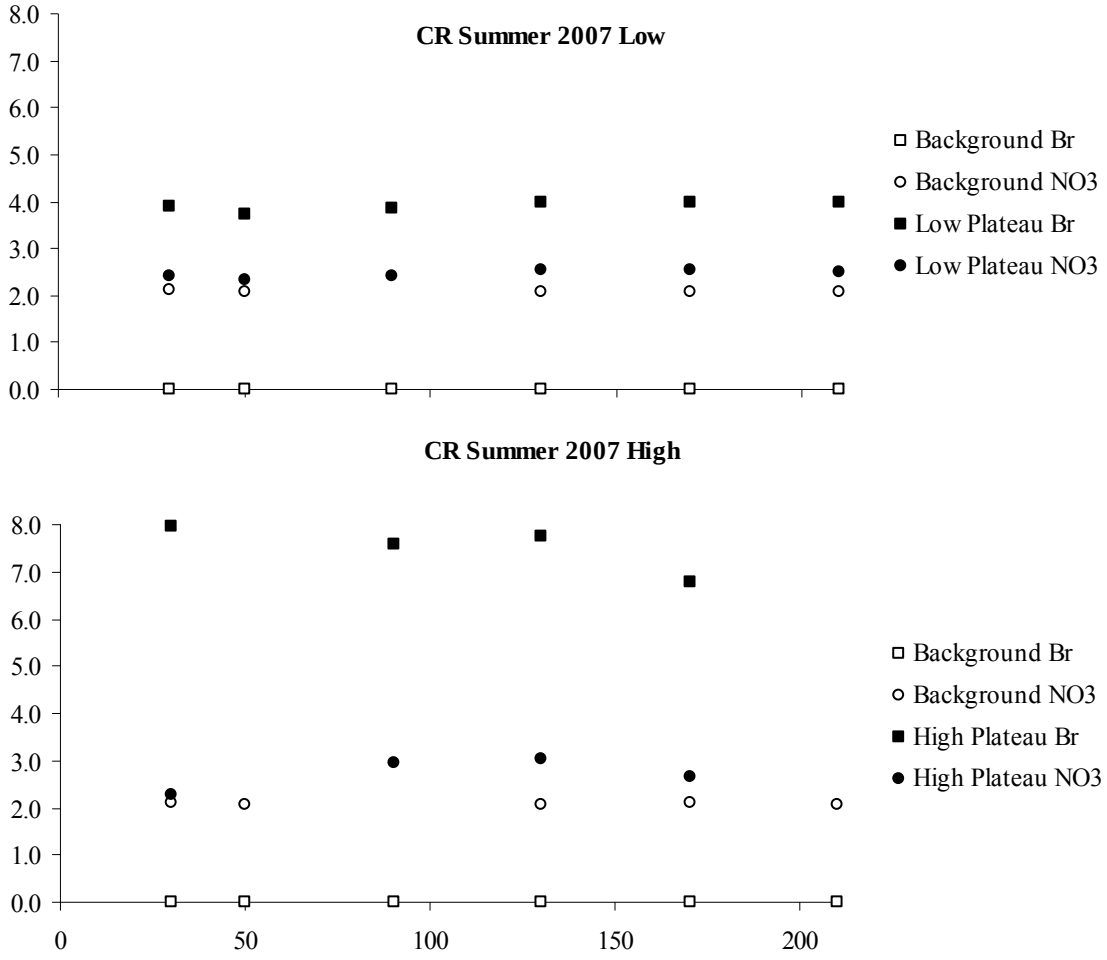
Appendix II. (continued)



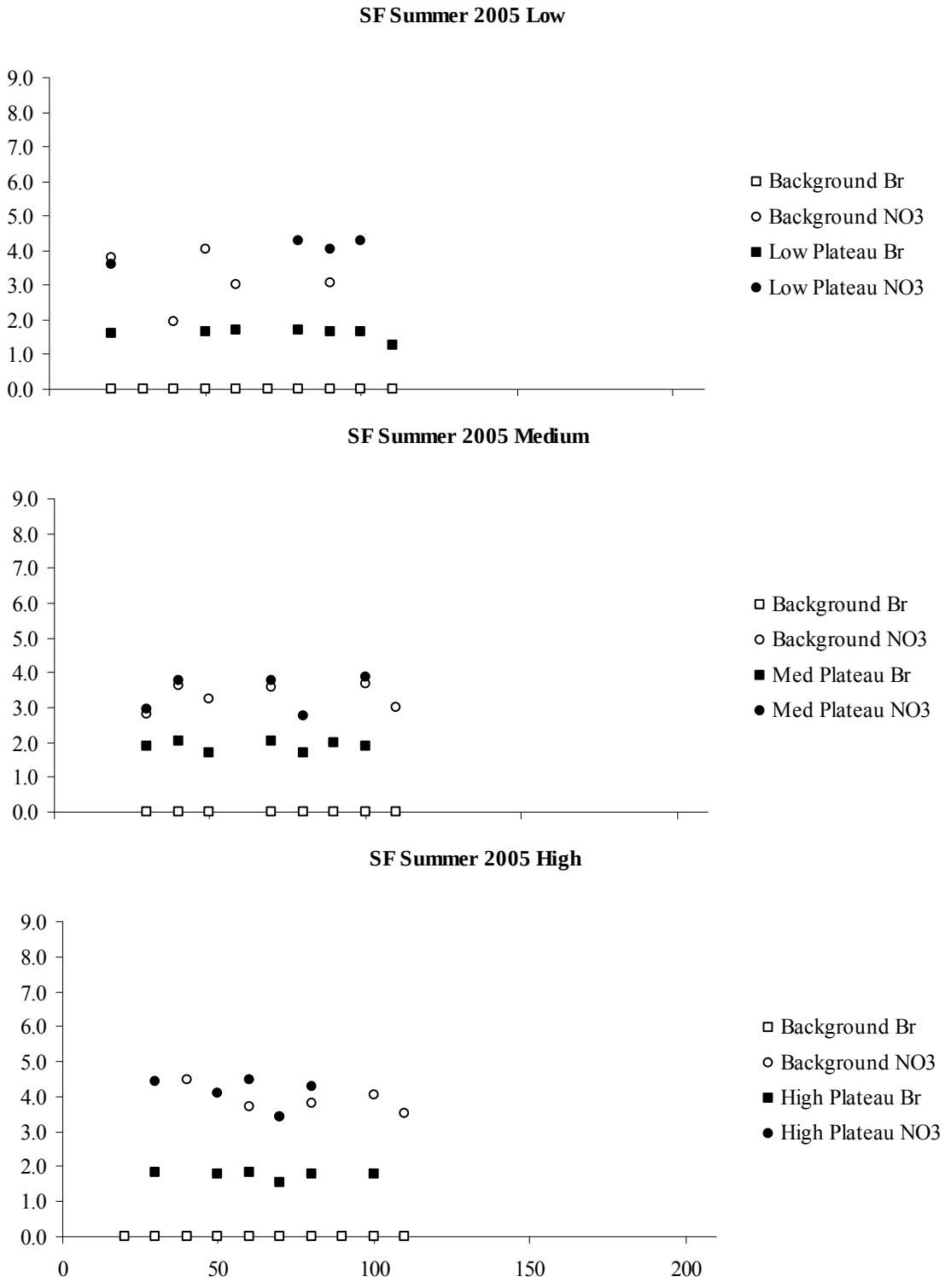




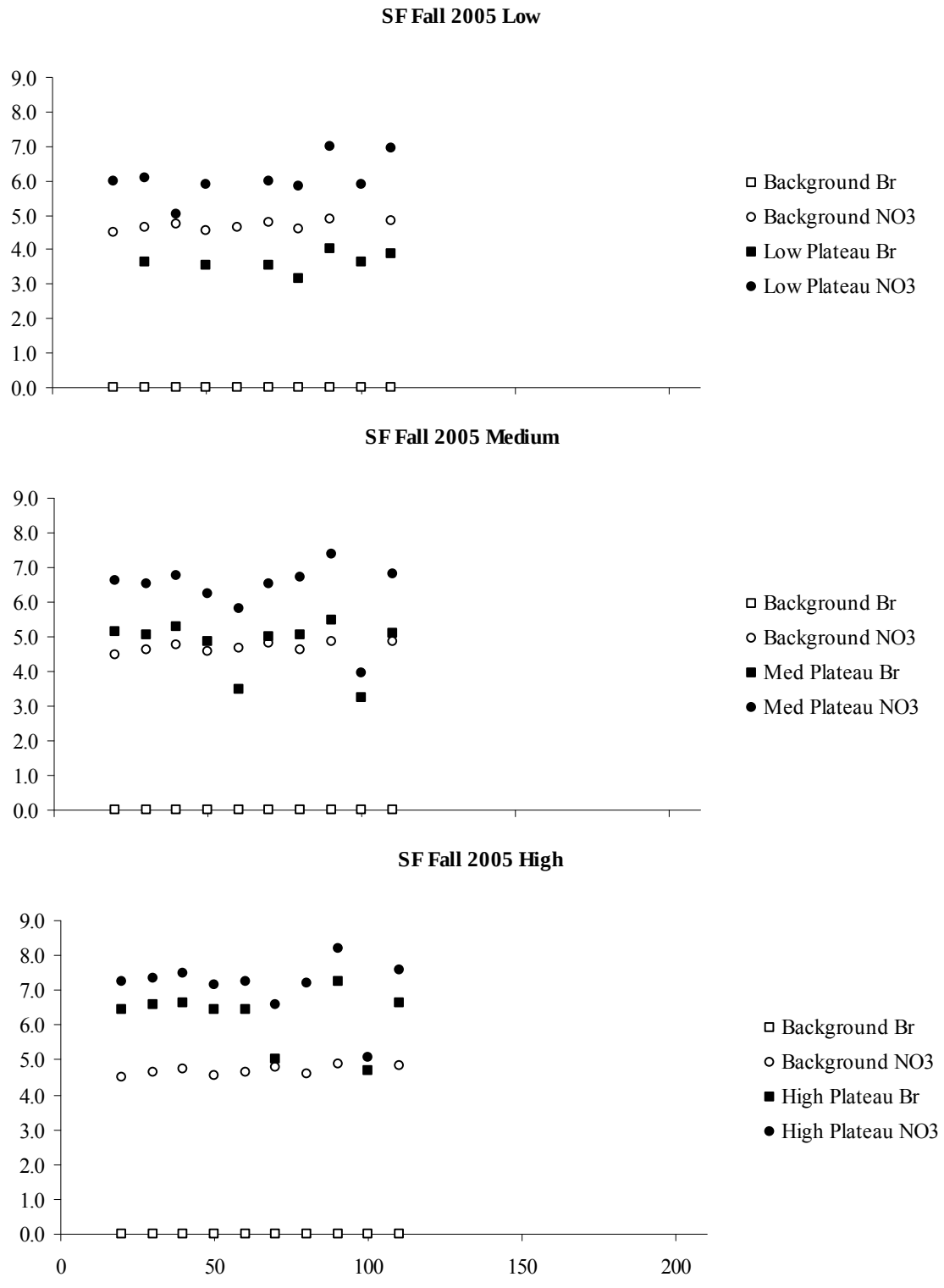
Appendix II. (continued)



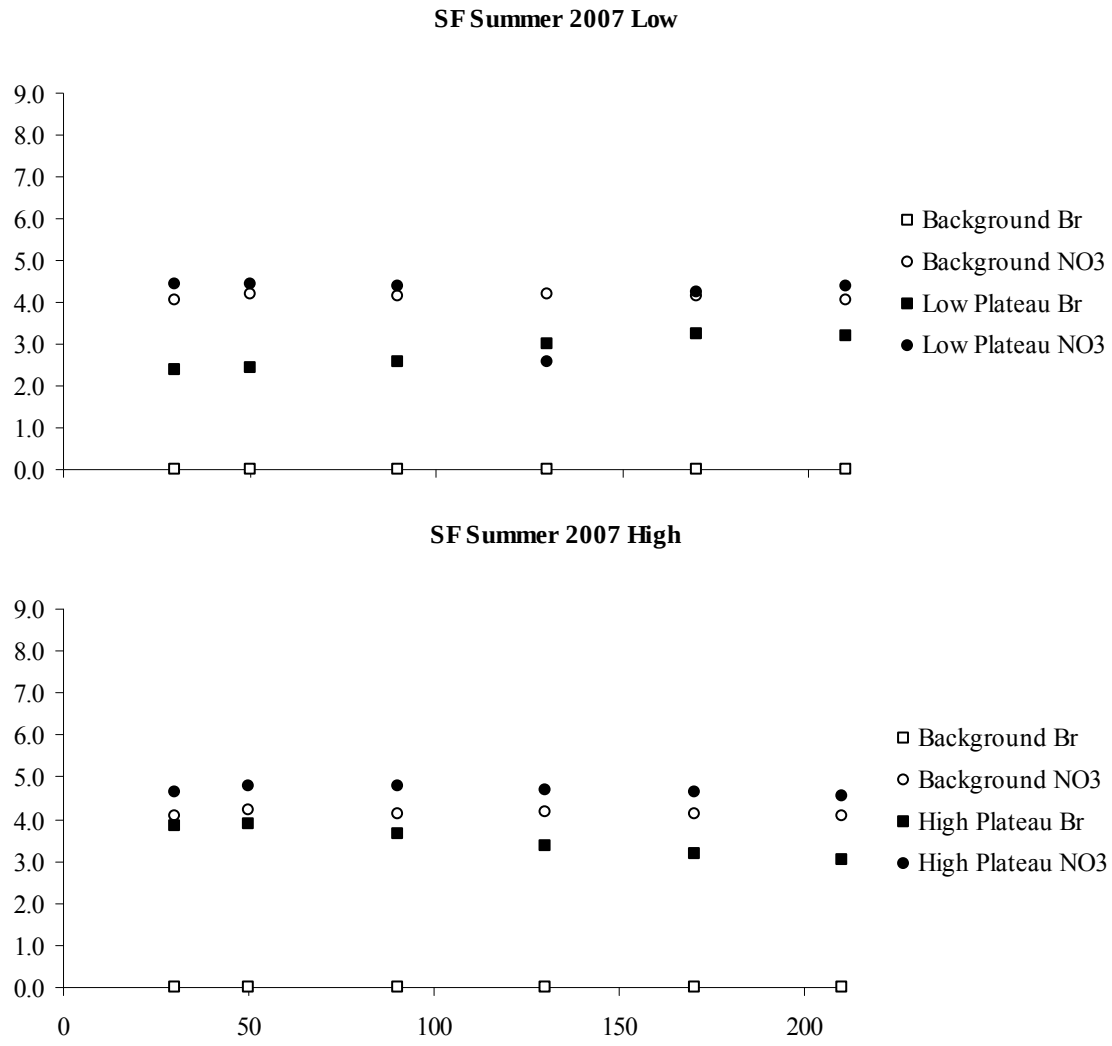
Appendix II. (continued)



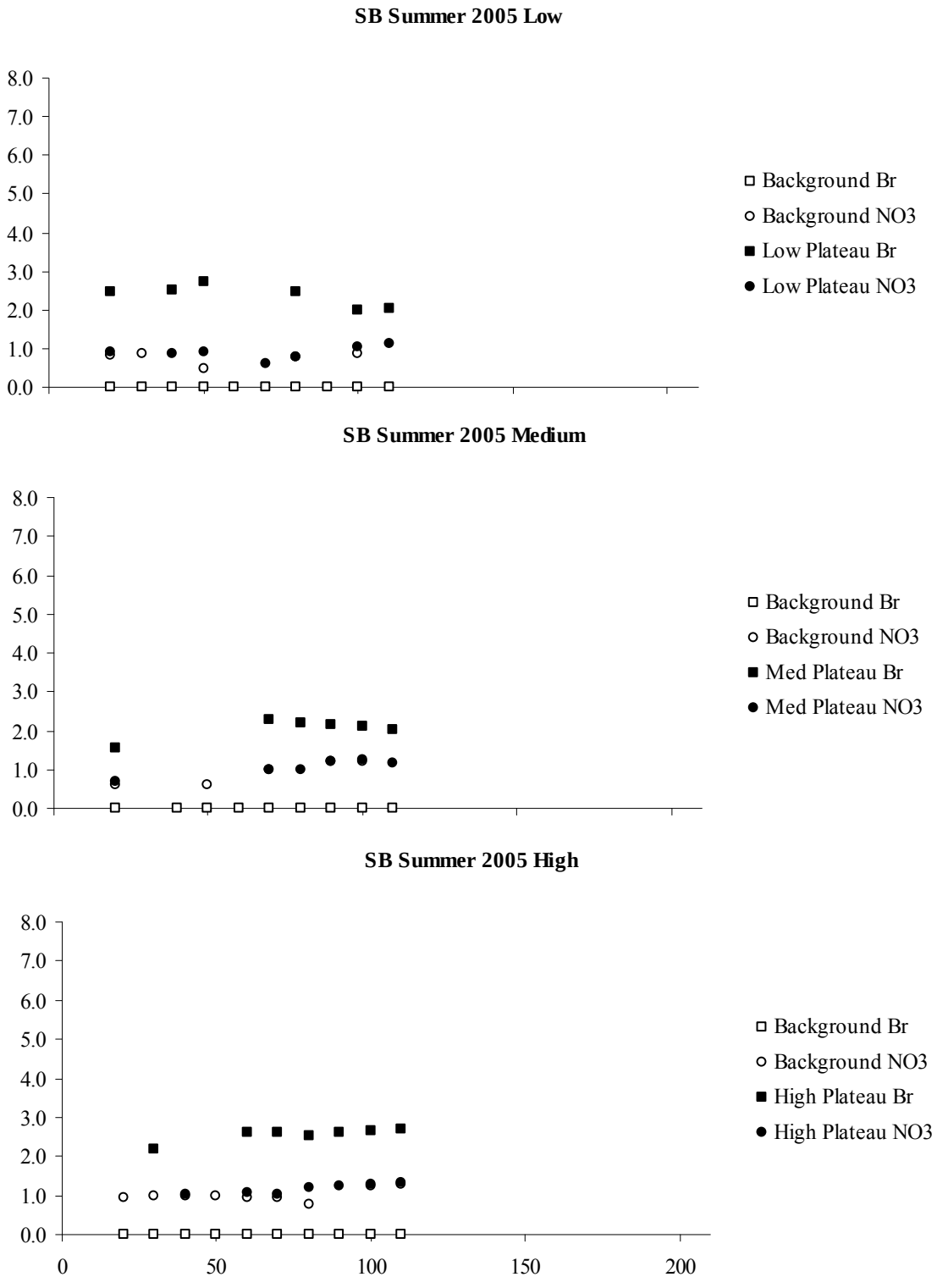
Appendix II. (continued)



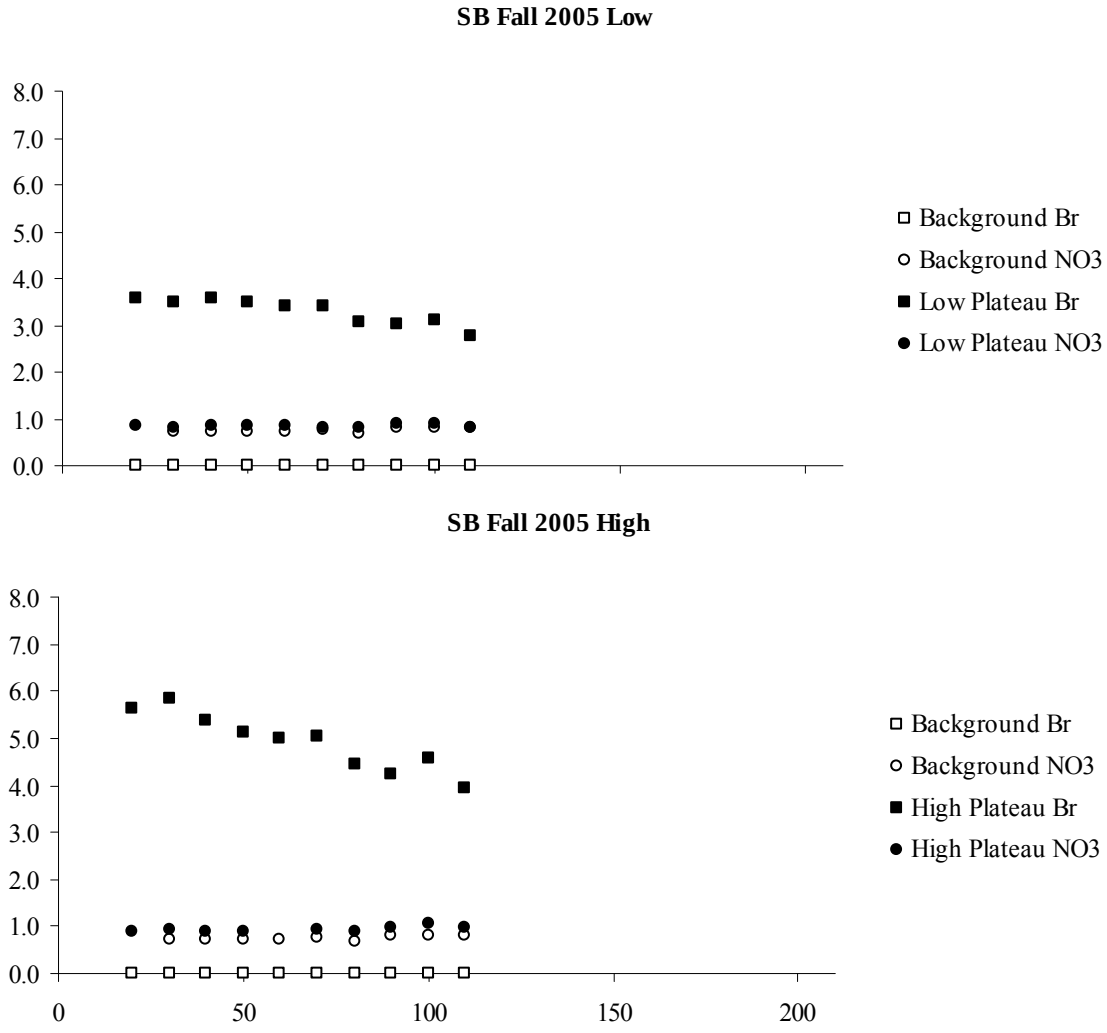
Appendix II. (continued)



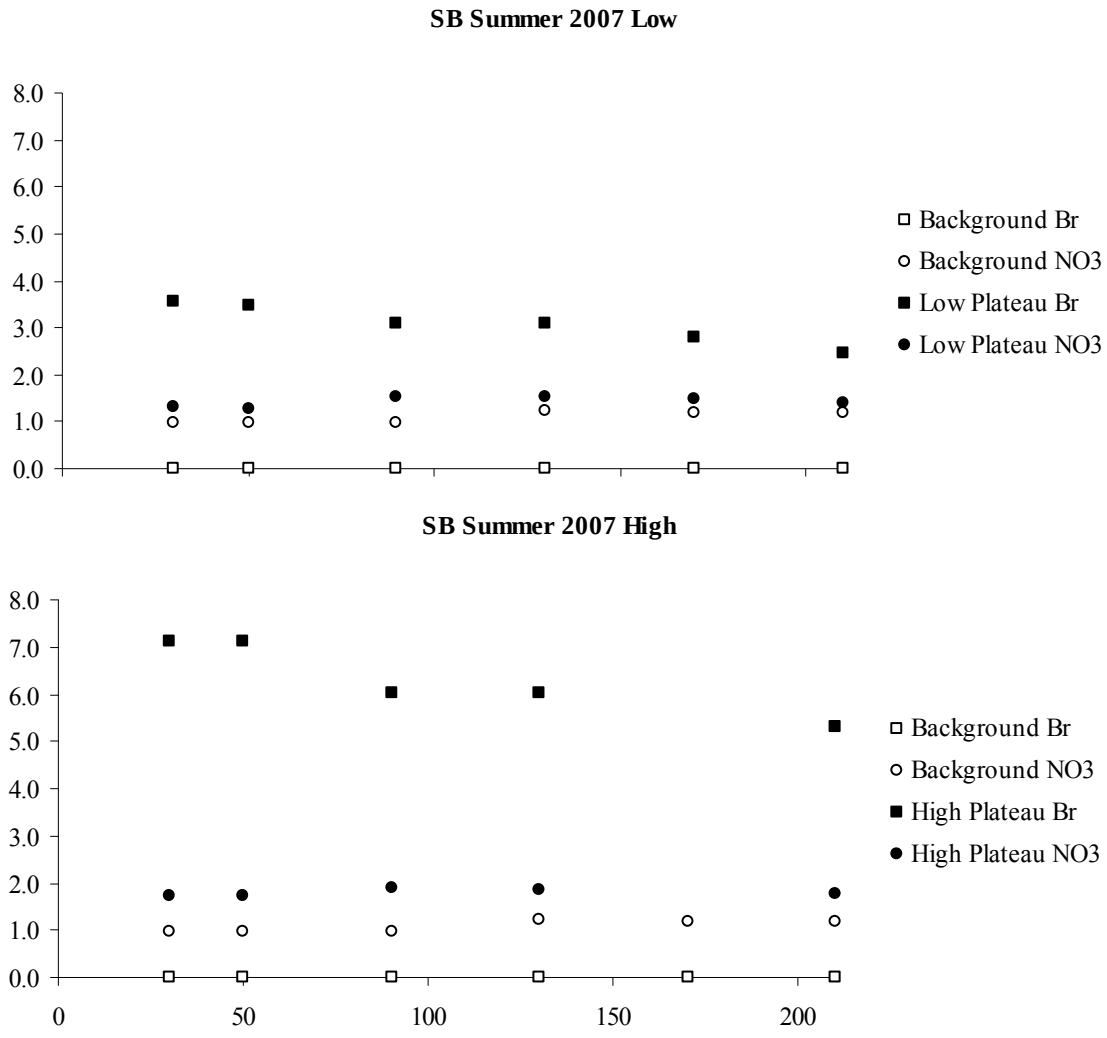
Appendix II. (continued)



Appendix II. (continued)



Appendix II (continued)



REFERENCES

Aber, J.D., C.L. Goodale, S.K. Ollinger, M.-L. Smith, A.H. Magill, M.E. Martin, R.A. Hallett, and J.L. Stoddard. 2003. Is nitrogen deposition altering the nitrogen status of Northeastern forests? **53**:375-389.

Aber, J.D., A. Magill, S.G. McNulty, R.D. Boone, K.J. Nadelhoffer, M. Downs, and R. Hallett. 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water, Air, and Soil Pollution **85**:1665-1670.

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems: Hypotheses revisited. BioScience **48**:921-934.

Aber, J.D., K.J. Nadelhoffer, P. Steudler, and J.M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. BioScience **39**:378-386.

Adams, M.B., J.N. Kochenderfer, F. Wood, T.R. Angradi, and P. Edwards. 1994. Forty years of hydrometeorological data from the Fernow Experimental Forest, West Virginia. USDA Forest Service, Northeastern Forest Experimental Station, General Technical Report NE- 184, Radnor, Pennsylvania.

Alexander, R.B., R.A. Smith, and G.E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature **403**:758-761.

Alexander, R.B., E.W. Boyer, R.A. Smith, G.E. Schwarz, and R.B. Moore. 2007. The role of headwater streams in downstream water quality. Journal of the American Water Resources Association **43**:41-59.

Arp, C.D. and M.A. Baker. 2007. Discontinuities in stream nutrient uptake below lakes in mountain drainage networks. Limnology and Oceanography **52**:1978-1990.

Bachman, L.J., B. Lindsey, J. Brakebill, and D.S. Powars. 1998. Ground-water discharge and base-flow nitrate loads of nontidal streams, and their relation to a hydrogeomorphic classification of the Chesapeake Bay Watershed, Middle Atlantic coast. United States Geological Survey Water Resources Investigations Report 1998-4059.

Barnes, R.T., P.A. Raymond, and K.L Casciotti. 2008. Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested watersheds in the northeastern U.S. Biogeochemistry **90**:15–27.

Bernhardt, E.S., L.E. Band, C.J. Walsh, and P.E. Berke. 2008. Understanding, managing, and minimizing urban impacts on surface water nitrogen loading. Annals of the New York Academy of Sciences **1134**:61-96.

Bernhardt E.S., R.O. Hall, and G.E. Likens. 2002. Whole system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. Ecosystems **5**:419–430.

Bernhardt, E.S. and G.E. Likens. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology **83**:1689-1700.

Bernhardt E.S., G.E. Likens, D.C. Buso, and C.T. Driscoll. 2003. In-stream uptake dampens effect of major forest disturbance on watershed nitrogen export. Proceedings of the National Academy of Sciences **100**:10304-10308.

Bernhardt, E.S., and M.A. Palmer. Restoring streams in an urbanizing world. Freshwater Biology **52**:738-751.

Bernot, M.J., and W.K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems **8**:442–53.

Bernot, M.J., J.L. Tank, T.V. Royer, and M.B. David. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States. Freshwater Biology **51**:499-509.

Boesch, D.F., R.B. Brinsfield, and R.E. Magnien. 2001. Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. Journal of Environmental Quality **30**:303-320.

Bott, T. L. 1996. Primary productivity and community respiration. Pages 533-556 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology. Academic Press, San Diego.

Bott, T.L., J.T. Brock, C.S. Dunn, R.J. Naiman, R.W. Ovink, and R.C. Petersen. 1985. Benthic community metabolism in four temperate stream systems: An interbiome comparison and evaluation of the river continuum concept. Hydrobiologia **123**:3-45.

Bukaveckas, P.A. 2007. Effects of channel restoration on water velocity, transient storage, and nutrient uptake in a channelized stream. Environmental Science and Technology **41**:1570–1576.

Burns, D.A., E.W. Boyer, E.M. Elliott, and C. Kendall. 2009. Sources and transformations of nitrate from streams draining varying land uses: Evidence from dual isotope analysis. Journal of Environmental Quality **38**:1149-1159.

Burns, D.A., and C. Kendall. 2002. Analysis of $\delta^{15}N$ and $\delta^{18}O$ to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. Water Resources Research **38**:1051-1061.

Burns, D.A., P.S. Murdoch, and G.B. Lawrence. 1998. Effect of groundwater springs on NO_3^- concentrations during summer in Catskill Mountain streams. Water Resources Research **34**:1987-1996.

Butturini, A., T.J. Battin, and F. Sabater. 2000. Nitrification in stream sediment biofilms: The role of ammonium concentration and DOC quality. Water Research **34**:629-639.

Campbell, J.L., J.W. Hornbeck, M.J. Mitchell, M.B. Adams, M.S. Castro, C.T. Driscoll, J.S. Kahl, J.N. Kochenderfer, G.E. Likens, J.A. Lynch, P.S. Murdoch, S.J. Nelson, and J.B. Shanley. 2004. Input-output budgets of inorganic nitrogen from 24 forest watersheds in the Northeastern United States. Water, Air, and Soil Pollution **151**:373-396.

Castro, M.S., K.N. Eshleman, L.F. Pitelka, G. Frech, M. Ramsey, W.S. Currie, K. Kuers, J.A. Simmons, B.R. Pohlad, C.L. Thomas, and D.M. Johnson. 2007. Symptoms of nitrogen saturation in an aggrading forested watershed in western Maryland. Biogeochemistry **84**:333-348.

Charlton, B.G. 2004. Why a journal of negative results? Journal of Negative Results-Ecology and Evolutionary Biology **1**:6-7.

Christ, M.J., W.T. Peterjohn, J.R. Cumming, and M.A. Adams. 2002. Nitrification potentials and landscape, soil and vegetation characteristics in two central Appalachian watersheds differing in NO_3^- export. Forest Ecology and Management **159**:145-158.

Cirmo, C.P., and J.J. McDonnell. 1997. Linking the hydrologic and biogeochemical controls of nitrogen transport in near-stream zones of temperate-forested catchments: A review. Journal of Hydrology **199**:88-120.

Clark, G.M., D.K. Mueller, and M.A. Mast. 2000. Nutrient concentrations and yields in undeveloped stream basins of the United States. Journal of the American Water Resources Association **36**:849-860.

Correll, D.L., T.E. Jordan, and D.E. Weller. 1995. The Chesapeake Bay watershed: Effects of land use and geology on dissolved nitrogen concentrations. Pages 638-648 i*n* Proceedings of the 1994 Chesapeake Research Conference, Toward a Sustainable Coastal Watershed: The Chesapeake Experiment, P. Hill and S. Nelson (editors), Chesapeake Research Consortium, Inc., Edgewater , Maryland.

Craig, L.S., M.A. Palmer, D.C. Richardson, S. Filoso, E.S. Bernhardt, B.P. Bledsoe, M.W. Doyle, P.M. Groffman, B.A. Hassett, S.S. Kaushal, P.M. Mayer, S.M. Smith, and P.R. Wilcock. 2008. Stream restoration strategies for reducing river nitrogen loads. Frontiers in Ecology and the Environment **6**:529-538.

Creed, I.F., and L.E. Band. 1998. Export of nitrogen from catchments within a temperate forest: Evidence for a unifying mechanism regulated by variable source area dynamics. Water Resources Research **34**:3105-3120.

Csada, R.D., P.C. James, and R.H.M. Espie. 1996. The "file drawer problem" of non-significant results: Does it apply to biological research? Oikos **76**:591–593.

Davis, J.C. and G.W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. Oecologia **119**:247–255.

DeWalle, D.R., and H.B. Pionke. 1995. Nitrogen export from forest land in the Chesapeake Bay region. Pages 649–655 i*n* Proceedings of the 1994 Chesapeake Research Conference, Toward a Sustainable Coastal Watershed: The Chesapeake Experiment, P. Hill and S. Nelson (editors), Chesapeake Research Consortium, Inc., Edgewater , Maryland.

Dodds, W.K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. Journal of the North American Benthological Society **22**:171-181.

Dodds, W.K., A.J. Lopez, W.B. Bowden, S. Gregory, N.B. Grimm, S.K. Hamilton, A.E. Hershey, E. Martí, W.H. McDowell, J.L. Meyer, D. Morrall, P.J. Mulholland, B.J. Peterson, J.L. Tank, H.M. Valett, J.R. Webster, and W. Wollheim. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society **21**:206-220.

Dodds, W.K., and R.M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnology and Oceanography: Methods **2**:333-341.

Draper, N.R,. and H. Smith. 1998. Applied regression analysis. J.W. Wiley, Hoboken, New Jersey.

Driscoll, C.T., D. Whitall, J. Aber, E.W. Boyer, M. Castro, C. Cronan, C.L. Goodale, P. Groffman, C. Hopkinson, K. Lambert, G. Lawrence, and S. Ollinger. 2003. Nitrogen pollution in the Northeastern United States: Sources, effects, and management options. Bioscience **53**:357-374.

Earl, S.R., H.M. Valett, and J.R. Webster. 2006. Nitrogen saturation in stream ecosystems. Ecology **87**:3140-3151.

Earl, S.R., H.M. Valett, and J.R. Webster. 2007 Nitrogen spiraling in streams: Comparisons between stable isotope tracer and nutrient addition experiments. Limnology and Oceanography **52**:718-723.

Eckhardt, K. 2005. How to construct recursive digital filters for baseflow separation. Hydrological Processes **19**:507-515.

Eckhardt, K. 2008. A comparison of baseflow indices, which were calculated with seven different baseflow separation methods. Journal of Hydrology **352**:168-173.

Elliott, E.M., C. Kendall, S.D. Wankel, D.A. Burns, E.W. Boyer, K. Harlin, D.J. Bain, and T.J. Butler. 2007. Nitrogen isotopes as indicators of NO_x source contributions to atmospheric nitrate deposition cross the Midwestern and Northeastern United States. Environmental Science and Technology **41**:7661-7667.

Ensign, S.H. and M.W. Doyle. 2006. Nutrient spiraling in streams and river networks. Journal of Geophysical Research **111**: G04009, doi:10.1029/2005JG000114.

Fellows, C.S., H.M. Valett, C.N. Dahm, P.J. Mulholland, and S.A. Thomas. 2006. Coupling nutrient uptake and energy flow in headwater streams. Ecosystems **9**:788-804.

Fenn, M.E., and M.A. Poth. 1998. Indicators of Nitrogen Status in California Forests. Pages 123-13 *in* Proceedings of the International Symposium: Air Pollution and Climate Change Effects on Forest Ecosystems, A. Bytnerowicz, M.J. Arbaugh, and S.L. Shilling, technical coordinators. United States Department of Agriculture Forest Service General Technical Report PSW-GTR-166.

Fenn, M.E., M.A. Poth, J.D. Aber, J.S. Baron, B.T. Bormann, D.W. Johnson, A.D. Lemly, S.G. McNulty, D.F. Ryan, and R. Stottlemyer. 1998. Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications **8**:706-733.

Filoso, S., and M.A. Palmer. Assessing the effectiveness of stream restoration at reducing nitrogen export to downstream waters. *In preparation*.

Fisher, D.C., and M. Oppenheimer. 1991. Atmospheric nitrogen deposition and the Chesapeake Bay estuary. Ambio **20**:102-108.

Fisher, S.G., R.A. Sponseller, and J.B. Heffernan. 2004. Horizons in stream biogeochemistry: Flowpaths to progress. Ecology **85**:2369-2379.

Focazio, M.J., L.N. Plummer, J.K. Bohlke, E. Busenberg, L.J. Bachman, and D.S. Powars. 1998. Preliminary estimates of residence times and apparent ages of ground water in the Chesapeake Bay watershed, and water-quality data from a survey of springs. United States Geological Survey Water Resources Investigations Report 1997-4225.

Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Cosby. 2003. The nitrogen cascade. BioScience **53**:341-356.

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C. Cleveland, P. Green, E. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A. Townsend, and C. Vörösmarty. 2004. Nitrogen cycles: Past, present and future. Biogeochemistry **70**:153-226.

García-Ruiz, R., S.N. Pattinson, and B.A. Whitton. 1998. Denitrification in river sediments: Relationship between process rate and properties of water and sediment. Freshwater Biology **39**:467-476.

Gilliam, F.S., M.A. Adams, and B.M. Yurish. 1996. Ecosystem nutrient responses to chronic inputs at Fernow Experimental Forest, West Virginia. Canadian Journal of Forestry Research **26**:196-205.

Goodale, C.L., S.A. Thomas, G. Fredriksen, E.M. Elliott, K.M. Flinn, T.J. Butler, and M.T. Walter. 2009. Unusual seasonal patterns and inferred processes of nitrogen retention in forested headwaters of the Upper Susquehanna River. Biogeochemistry **93**:197-218.

Gress, S.E., T.D. Nichols, C.C. Northcraft, and W.T. Peterjohn. 2007. Nutrient limitation in soils exhibiting differing nitrogen availabilities: What lies beyond nitrogen saturation? Ecology **88**:119-130.

Grimm N.B., R.W. Sheibley, C.L. Crenshaw, C.N. Dahm, W.J. Roach, and L.H. Zeglin. 2005. N retention and transformation in urban streams. Journal of the North American Benthological Society **24**:626–642.

Groffman, P.M., G. Howard, A.J. Gold, and W.M. Nelson. 1996. Microbial nitrate processing in shallow groundwater in a riparian forest. Journal of Environmental Quality **25**:1309-1316.

Gücker, B. and M.T. Pusch. 2006. Regulation of nutrient uptake in eutrophic lowland streams. Limnology and Oceanography **51**:1443-1453.

Hall, R.O., M.A. Baker, C.D. Arp, and B.J. Koch. 2009a. Hydrologic control of nitrogen removal, storage, and export in a mountain stream. Limnology and Oceanography **54**:2128-2142.

Hall, R.O. and J.L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography **48**:1120-28.

Hall, R.O., Jr., J.L. Tank, D.J. Sobota, P.J. Mulholland, J.M. O'Brien, W.K. Dodds, J.R. Webster, H.M. Valett, G.C. Poole, BJ. Peterson, J.L. Meyer, W.H. McDowell, S.L. Johnson, S.K. Hamilton, N.B. Grimm, S.V. Gregory, C.N. Dahm, L.W. Cooper, L.R. Ashkenas, S.M. Thomas, R.W. Sheibley, JD. Potter, B.R.

Niederlehner, L.T. Johnson, A.M. Helton, C.M. Crenshaw, A.J. Burgin, M.J. Bernot, J.J. Beaulieu, and C.P. Arango. 2009b. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: Total uptake. Limnology and Oceanography **54**:653-665.

Hamilton S.K., J.L. Tank, D.F. Raikow, W.M. Wollheim, B.J. Peterson, and J.R. Webster. 2001. Nitrogen uptake and transformation in a Midwestern US stream: A stable isotope enrichment study. Biogeochemistry **54**:297–340.

Hanafi, S., M. Grace, J.A. Webb, and B. Hart. 2007. Uncertainty in nutrient spiraling: Sensitivity of spiraling indices to small errors in measured nutrient concentration. Ecosystems **10**: 477-487.

Harned, D.A., and C.C. Daniel III. 1989. The transition zone between bedrock and regolith: Conduit for contamination? Pages 336-348 *in* Ground Water in the Piedmont, Proceedings of a Conference on Ground Water in the Piedmont of the Eastern United States, C.C. Daniel III, R.K. White, and P.A. Stone, editors. Clemson University, Clemson, South Carolina.

Hjältén, J. and P.W. Price. 1999. To publish or not to publish? A response to Lortie and Dyer. Oikos **90**:182-184.

Hoellein, T.J., J.L. Tank, E.J. Rosi-Marshall, S.A. Entrekin, and G.A. Lamberti. 2007. Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams. Limnology and Oceanography **52**:1964-1977.

Howarth, R.W. 2008. Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae **8**:14-20.

Howarth, R.W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J.A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, Z-L Zhu. 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochemistry **35**:75-139.

Howarth, R.W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries **25**:656–76.

Jordan, T.E., D.L. Correll, and D.E. Weller. 1997. Nonpoint source discharges of nutrients from Piedmont watersheds of the Chesapeake Bay. Journal of the American Water Resources Association **33**:631-645.

Jordan, T.E., and D.E. Weller. 1996. Human contribution to terrestrial nitrogen flux: Assessing the sources and fates of anthropogenic fixed nitrogen. BioScience **46**:655-664.

Kaufmann, P.R, A..T. Herlihy, M.E. Mitch, and J.J. Messer. 1991.Stream chemistry in the eastern United States: Synoptic survey design, acid-base s tatus, and regional patterns. Water Resources Research **27**:611-627.

Kaushal, S.S., P.M. Groffman, L.E.Band, C.A. Shields, R.P. Morgan, M.A. Palmer, K.T. Belt, C.M. Swan, S.E.G. Findlay, and G.T. Fisher. 2008. Interaction between urbanization and climate variability amplifies watershed nitrate export in Maryland. Environmental Science and Technology **42**: 5872-5878.

Kellman, L. 2004. Nitrate removal in a first-order stream: Reconciling laboratory and field measurements. Biogeochemistry **71**:89-105.

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

Lautz, L.K. and D.I. Siegel. 2007. The effect of transient storage on nitrate uptake lengths in streams: An inter-site comparison. Hydrological Processes **21**:3533-3548.

Lewis, D.B., J.P. Kaye, C. Gries, A.P. Kinzig, and C.L. Redman. 2006. Agrarian legacy in soil nutrient pools of urbanizing arid lands. Global Change Biology **4**:703-709.

Lim, K.J., B.A. Engel, Z. Tang, J. Choi, K-S. Kim, S. Muthukrishnan, and D. Tripathy. 2005. Automated web GIS based hydrograph analysis tool, WHAT. Journal of the American Water Resources Association **41**:1407-1416.

Lindsey, B.D., W.F. Falls, M.J. Ferrari, T.M. Zimmerman, D.A. Harned, E.M. Sadorf, and M.J. Chapman. 2006. Factors affecting occurrence and distribution of selected contaminants in ground water from selected areas in the Piedmont aquifer system, eastern United States, 1993-2003. United States Geological Survey Scientific Investigations Report 2006-5104.

Lindsey, B.D., S.W. Phillips, C.A. Donnelly, G.K. Speiran, L.N. Plummer, J.K. Bohlke, M.J. Focazio, and W.C. Burton. 2003. Residence times and nitrate transport in ground water discharging to streams in the Chesapeake Bay watershed. United States Geological Survey Water Resources Investigations Report 2003-4035.

Lortie, C. J. and A.R. Dyer. 1999. Over-interpretation: avoiding the stigma of non-significant results. Oikos **87**:183–184.

Lovett, G.M., K.C. Weathers, and M.A. Arthur. 2002. Control of nitrogen loss from forested watersheds by soil carbon:nitrogen ratio and tree species composition. Ecosystems **5**:712-718.

Lovett, G.M., K.C. Weathers, and W.V. Sobczak. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. Ecological Applications **10**:73-84.

Lowrance, R., L.S. Altier, J.D. Newbold, R.R. Schnabel, P.M. Groffman, J.M. Denver, D.L. Correll, J.W. Gilliam, J.L. Robinson, R.B. Brinsfield, K.W. Staver, W. Lucas, and A.H. Todd. 1997. Water quality functions of riparian forest buffers in Chesapeake Bay watersheds. Environmental Management **21**:687-712.

Maloney, K.O., J.W. Feminella, R.M. Mitchell, S.A. Miller, P.J. Mulholland, and J.N. Houser. 2008. Laduse legacies and small streams: Identifying relationships between historical land use and contemporary stream conditions. Journal of the North American Benthological Society **27**:280-294.

Martí, E., N.B. Grimm, and S.G. Fisher.1997. Pre- and postflood retention efficiency of nitrogen in a Sonoran Desert stream. Journal of the North American Benthological Society **16**:805–819.

Maryland Geological Survey. 1981. A brief description of the geology of Maryland [pamphlet]. Baltimore, MD: J. Edwards Jr.

Mayer, B., E.W. Boyer, C. Goodale, N.A. Jaworski, N. Van Breemen, R.W. Howarth, S. Seitzinger, G. Billen, K. Lajtha, K. Nadelhoffer, D. Van Dam, L.J. Hetling, M. Nosal, and K. Paustian. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. Biogeochemistry **57/58**:171-197.

McClain, M.E., E.W. Boyer, C.L. Dent, S.E. Gergel, N.B. Grimm, P.M. Groffman, S.C. Hart, J.W. Harvey, C.A. Johnston, E. Mayorga, W.H. McDowell, and G. Pinay. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems **6**:301-312.

Meyer J.L., M.J. Paul, and W.K. Taulbee. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society **24**:602–612.

Mitchell, M.J. 2001. Linkages of nitrate losses in watersheds to hydrological processes. Hydrological Processes **15**:3305-3307.

Moglen, G.E. 2007. Introduction to GISHydro2000: GIS based hydrologic analysis in Maryland. Available on-line at: www.gishydro.umd.edu.

Møller, A.P. and M.D. Jennions. 2001. Testing and adjusting for publication bias. Trends in Ecology and Evolution **16**:580-586.

Mueller, D.K. and D.R. Helsel. 1996. Nutrients in the nation's groundwaters: Too much of a good thing? United States Geological Survey National Water-Quality Assessment (NAWQA) Program Circular 1136.

Mulholland, P.J., C. S. Fellows, J.L. Tank, N.B. Grimm, J.R. Webster, S.K. Hamilton, E. Martí, L.R. Ashkenas, W.B. Bowden, W.K. Dodds, W.H. McDowell, M.J. Paul, and B.J. Peterson. 2001. Inter-biome comparison of factors controlling stream metabolism. Freshwater Biology **46**:1503-1517.

Mulholland, P.J., R.O. Hall, D.J. Sobota, W.K. Dodds, S.E.G. Findlay, N.B. Grimm, S.K. Hamilton, W.H. McDowell, J.M. O'Brien, J.L. Tank, L.R. Ashkenas, L.W. Cooper, C.N. Dahm, S.V. Gregory, S.L. Johnson, J.L. Meyer, B.J. Peterson, G.C. Poole, H.M. Valett, J.R. Webster, C.P. Arango, J.J. Beaulieu, M.J. Bernot, A.J. Burgin, C.L. Crenshaw, A.M. Helton, L.T. Johnson, B.R. Niederlehner, J.D. Potter, R.W. Sheibley, and S.M. Thomas. 2009. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: Denitrification. Limnology and Oceanography **54**:666-680.

Mulholland, P.J., A.M. Helton, G.C. Poole, R.O. Hall, S.K. Hamilton, B.J. Peterson, J.L. Tank, L.R. Ashkenas, L.W. Cooper, C.N. Dahm, W.K. Dodds, S.E.G. Findlay, S.V. Gregory, N.B. Grimm, S.L. Johnson, W.H. McDowell, J.L. Meyer, H.M. Valett, J.R. Webster, C.P. Arango, J.J. Beaulieu, M.J. Bernot, A.J. Burgin, C.L. Crenshaw, L.T. Johnson, B.R. Niederlehner, J.M. O'Brien, J.D. Potter, R.W. Sheibley, D.J. Sobota, and S.M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature **452**:202-205.

Mulholland, P.M., E.R. Marzolf, J.R. Webster, D.R. Hart, and S.P. Hendricks. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. Limnology and Oceanography **42**:443-451.

Mulholland, P.J., J.L. Tank, D.M. Sanzone, W.M. Wolheim, B.J. Peterson, J.R. Webster, and J.L. Meyer. 2000. Nitrogen processing in a deciduous forest stream: Results from a tracer ^{15}N addition experiment in Walker Branch, Tennessee. Ecological Monographs **70**:471–493.

Mulholland, P.J., J.L. Tank, J.R. Webster, W.B. Bowden, W.K. Dodds, S.V. Gregory, N.B. Grimm, S.K. Hamilton, S.L. Johnson, E. Martí, W.H. McDowell, J.L. Merriam, J.L. Meyer, B.J. Peterson, H.M. Valett, and W.M. Wollheim. 2002. Can uptake length in streams be determined by nutrient addition experiments? Results from an inter-biome comparison study. Journal of the North American Benthological Society **21**: 544-560.

Munn, N.L., and J.L. Meyer. 1990. Habitat-specific solute retention in 2 small streams: An intersite comparison. Ecology **71**:2069-2082.

Murdoch, P.S., and J.L. Stoddard. 1992. The role of nitrate in the acidification of streams in the Catskill Mountains of New York. Water Resources Research **28**:2707-2720.

Myrold, D.D. 2005. Transformations of nitrogen. Chapter 14 *in* Principles and Applications of Soil Microbiology, 2nd edition, D.M. Sylvia, J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer (editors), Prentice-Hall Inc., Upper Saddle River, New Jersey, pp 333-372.

Nadelhoffer, K.J., B.A. Emmett, P. Gundersen, O.J. Kjonaas, C.J. Koopmans, P. Schleppi, A. Tietema, and R.F. Wright. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature **398**:145-148.

National Atmospheric Deposition Program (NRSP-3). 2009. NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.

National Oceanic and Atmospheric Administration-National Weather Service. 2009. Mid-Atlantic River Forecast Center, 328 Innovation Boulevard, Suite 330 State College, PA 16803.

Newbold, J.D., J.W. Elwood, R.V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiraling in streams. Canadian Journal of Fisheries and Aquatic Sciences **38**:860-863.

Niyogi, D.K., K.S. Simon, and C.R. Townsend. 2004. Land use and stream ecosystem functioning: Nutrient uptake in streams that contrast in agricultural development. Archiv für Hydrobiologie **160**:471-486.

Nolan, B.T. 2001. Relating nitrogen sources in aquifer susceptibility to nitrate in shallow ground water of the United States. Ground Water **39**:290-299.

Nolan, B.T., and J.D. Stoner. 2000. Nutrients in groundwaters of the conterminous United States, 1992-1995. Environmental Science and Technology **34**:1156-1165.

O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N.Murdoch, and J. Eichmiller. 2007. The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentrations. Biogeochemistry **84**:31–49.

Odum, H.T. 1956. Primary production in flowing waters. Limnology and Oceanography **1**:102-117.

Ohrui, K., and M.J. Mitchell. 1997. Nitrogen saturation in Japanese forested watersheds. Ecological Applications **7**:391-401.

Ollinger, S.V., J.D. Aber, G.M. Lovett, S.E. Millham, R.G. Lathrop, and J.M. Ellis. 1993. A spatial model of atmospheric deposition for the Northeastern U.S.. Ecological Applications **3**:459-472.

Owens, M., R. W. Edwards, and J. W. Gibbs. 1964. Some reaeration studies in streams. Air and Water Pollution **8**:469-486.

Palmer, M.A., and S. Filoso. 2009. Restoration of ecosystem services for environmental markets. Science **325**:575-576.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics **32**:333-365.

Payn, R.A., J.R. Webster, P.J. Mulholland, H.M. Valett, and W.K. Dodds. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. Limnology and Oceanography Methods **3**:174-182.

Peterjohn, W.T., M.B. Adams, and F.S. Gilliam. 1996. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry **35**:507-522.

Peterjohn, W.T., C.J. Foster, M.J. Christ, and M.B. Adams. 1999. Patterns of nitrogen availability within a forested watershed exhibiting symptoms of nitrogen saturation. Forest Ecology and Management **119**:247-257.

Peterson, B.J., W.M. Wollheim, P.J. Mulholland, J.R. Webster, J.L. Meyer, J.L. Tank, E. Martí, W.B. Bowden, H.M. Valett, A.E. Hershey, W.H. McDowell, W.K. Dodds, S.K. Hamilton, S. Gregory, and D.D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science **292**:86–90.

Rabalais, N.N., R.E. Turner, and W.J. Wiseman. 2002. Gulf of Mexico hypoxia, a.k.a. 'The Dead Zone'. Annual Review of Ecology and Systematics **33**:235-263.

Royer, T. V., J. L. Tank, and M. B. David. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. Journal of Environmental Quality **33**:1296–1304.

Runkel, R.L., C.G. Crawford, and T.A. Cohn. 2004. Load estimator (LOADEST): A Fortran program for estimating constituent loads in streams and rivers. United States Geological Survey Techniques and Methods Book 4, Chapter A5.

S. 1816 – 111th Congress: Chesapeake Clean Water and Ecosystem Restoration Act of 2009. *In* GovTrack.us (database of federal legislation). Available online at http://www.govtrack.us/congress/bill.xpd?bill=s111-1816.

Schilling, K., and Y-K. Zhang. 2004. Baseflow contribution to nitrate-nitrogen export from a large, agricultural watershed, U.S.A. Journal of Hydrology **295**:305-316.

Seitzinger, S. 2008. Out of reach. Nature **452**: 162-163.

Sheeder, S.A, J.A. Lynch, J. Grimm. 2002. Modeling atmospheric atmospheric nitrogen deposition and transport in the Chesapeake Bay watershed. Journal of Environmental Quality **31**:1194-1206.

Simon, K.S., M.A. Chadwick, A.D. Huryn, and H.M. Valett. Response of stream ecosystem function to chronic deposition of nitrogen and acidification at the Bear Brook Watershed in Maine. Environmental Monitoring and Assessment *in press*.

Simon, K.S., C.R. Townsend, B.J.F. Biggs, and W.B. Bowden. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. Journal of the North American Benthological Society **24**:1-18.

Smith, R.A., R.B. Alexander, and G.E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environmental Science and Technology **37**:3039-3046.

Stanley, E.H. and J.T. Maxted. 2008. Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin streams. Ecological Applications **18**:1579-1590.

Stoddard, J.L. 1994. Long-term changes in watershed retention of nitrogen: Its cause and aquatic consequences. Chapter 8 *in* L.A. Baker (ed) Environmental chemistry of lakes and reservoirs, advances in chemistry series, vol. 237. American Chemical Society, Washington D.C., pp 221-284.

Strauss, E.A. and G.A. Lamberti. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography **45**:1854-1859.

Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. Journal of the North American Benthological Society **9**:95-119.

Sweeney, B. W., T. L. Bott, J. K. Jackson, L. A. Kaplan, J. D. Newbold, L. J. Standley, W. C. Hession, and R. J. Horwitz. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Science **101**:14132-14137.

Tague, C.L., and L.E. Band. 2004. RHESSys: regional hydro-ecologic simulation system: An object-oriented approach to spatially distributed modeling of carbon, water, and nutrient cycling. Earth Interactions **8**:1-19.

Tank, J.L., E.J. Rosi-Marshall, M.A. Baker, and R.O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. Ecology **89**:2935-2945.

United States Department of Agriculture-Natural Resources Conservation Service. 2009. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/

Valett, H.M., J.A. Morrice, C.N. Dahm, and M.E. Campana. 1996. Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. Limnology and Oceanography **41**:333-345.

Venterea, R.T., P.M. Groffman, L.V. Verchot, A.H. Magill, and J.D. Aber. 2004. Gross nitrogen process rates in temperate forest soils exhibiting symptoms of nitrogen saturations. Forest Ecology and Management **196**:129-142.

Vitousek, P.M. 1977. The regulation of element concentrations in mountain streams in the northeastern United States. Ecological Monographs **47**:65-87.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737-750.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R.P. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Webster, J.R., P.J. Mulholnad, J.L. Tank, H.M. Valett, W.K. Dodds, B.J. Peterson, W.K. Bowden, C.N. Dahm, S. Findlay, S.V. Gregory, N.B. Grimm, S.K. Hamilton, S.L. Johnson, E. Martí, W.H. McDowell, J.L. Meyer, D.D. Morrall, S.A. Thomas, and W.M. Wollheim. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. Freshwater Biology **48**:1329-1352.

Webster J.R., J.L. Tank, J.B. Wallace, J.L. Meyer, S.L. Eggert,T.P. Ehrman, B.R. Ward, B.L. Bennett, P.F. Wagner, and M.E. McTammany. 2000. Effects of litter exclusion and wood removal on phosphorus and nitrogen retention in a forest stream. Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie **27**:1337-1340.

Webster, J. R., and B. C. Patten. 1979. Effects of watershed perturbation on stream potassium and calcium dynamics. Ecological Monographs **49**:51–72.

Webster, J.R. and H.M. Valett. 2006. Solute Dynamics. Chapter 8 *in* .R. Hauer and G.A. Lamberti (eds.) Methods in Stream Ecology 2nd edition. Academic Press, New York, pp 169-186.

Wenger, S.J., A.H. Roy, C.R. Jackson, E.S. Bernhardt, T.L. Carter, S. Filoso, C.A. Gibson, W.C. Hession, S.S. Kaushal, E. Martí, J.L. Meyer, M.A. Palmer, M.J. Paul, A.H. Purcell, A. Ramírez, A.D. Rosemond, K. Schofield, E.B. Sudduth, and C.J. Walsh. 2009. Twenty-six key research questions in urban stream ecology: An

assessment of the state of the science. Journal of the North American Benthological Society **28**:1080-1098.

Williams, M., C. Hopkinson, E. Rastetter, and J. Vallino. 2004. N budgets and aquatic uptake in the Ipswich River basin northeastern Massachusetts. Water Resources Research **40**:W11201 doi:10.1029/2004WR003172.

Williard, K.W.J., D.R. DeWalle, P.J. Edwards, and R.R. Schnabel. 1997. Indicators of nitrate export from forested watersheds of the mid-Appalachians, United States of America. Global Biogeochemical Cycles **11**:649-656.

Wollheim, W.M., B.J. Peterson, L.A. Deegan, J.E. Hobbie, B. Hooker, W.B. Bowden, K.J. Edwardson, D.B. Arscott, A.E. Hershey, and J. Finlay. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. Limnology and Oceanography **46**:1–13.

Young, R.G., C.D. Matthaei, and C.R. Townsend. 2008. Organic matter breakdown and ecosystem metabolism: Functional indicators for assessing river ecosystem health. Journal of the North American Benthological Society **27**:605-625.