

# **Nitrogen spiraling in stream ecosystems spanning a gradient of chronic nitrogen loading**

**Stevan R. Earl**

Dissertation submitted to the faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy  
in  
Biology

H.M. Valett, Chair  
E. F. Benfield  
T. A. Dillaha  
C. G. Peterson  
J. R. Webster

11 October 2004  
Blacksburg, Virginia

Keywords: stream biogeochemistry, stream structure and function, nitrogen, spiraling

# **Nitrogen spiraling in stream ecosystems spanning a gradient of chronic nitrogen loading**

**Stevan R. Earl**

## **Abstract**

This dissertation is a study of the relationships between nitrogen (N) availability and spiraling (the paired processes of nutrient cycling and advective transport) in stream ecosystems. Anthropogenic activities have greatly increased rates of N loading to aquatic ecosystems. However, streams may be important sites for retention, removal, and transformation of N. In order to identify controls on NO<sub>3</sub>-N spiraling in anthropogenically impacted streams, I examined relationships among NO<sub>3</sub>-N spiraling and a suite of chemical, physical, and biological variables in streams spanning a gradient of N concentration. Across all streams, gross primary production (GPP) accounted for most NO<sub>3</sub>-N demand. Uptake of NO<sub>3</sub>-N was also related to GPP but was limited by N availability when N concentrations were low. A combination of GPP and NO<sub>3</sub>-N explained 80% of the variance in uptake. In chapter 3, I conducted a series of short-term nutrient releases in which streamwater NO<sub>3</sub>-N concentration was incrementally elevated to identify conditions leading to saturation of uptake capacity. Four of six study streams showed signs of N limitation whereas there was no significant change in uptake with increasing NO<sub>3</sub>-N amendment in two streams, suggesting N saturation. Proximity to saturation was generally correlated to N concentration but was also predicted by the ratio of N:P. My results suggest complex relationships between N spiraling and availability that depend on resident biota and other limiting factors.

In chapter 4, I examined nutrient spiraling methodology by comparing differences between ambient and amendment-derived NO<sub>3</sub>-N spiraling metrics. I quantified spiraling metrics during a short-term NO<sub>3</sub>-N amendment and under ambient conditions using a stable isotope (<sup>15</sup>NO<sub>3</sub>-N) tracer. Uptake lengths measured during amendments were

consistently longer than ambient uptake lengths. Amendment-derived  $\text{NO}_3\text{-N}$  uptake velocity and uptake were underestimated relative to ambient conditions. Using a technique to estimate ambient uptake length extrapolated from the relationship between uptake length and nutrient amendment concentration for a series of amendments at different concentrations, I found that extrapolated uptake lengths were generally better predictors of ambient uptake lengths than amendment-derived uptake lengths but the technique was less effective in high N streams that showed signs of weak N limitation.

## Acknowledgments

I am grateful for the support of my advisors, Maury Valett and Chris Peterson, and members of my doctoral committee. Maury's enthusiasm for life and ecology has been inspirational. I cannot thank Maury enough for his patience, guidance, and friendship. Chris and I did not get a chance to work together as closely as either of us had anticipated. Nonetheless, Chris has provided valuable input and I am grateful for his role in helping to make all of this possible in the first place. Fred Benfield's knowledge of Appalachian streams is unparalleled and Fred helped immensely with site selection and many other aspects of the study. Jack Webster has been an inspirational teacher and advisor. It has been an honor and a pleasure working with both Fred and Jack. Theo Dillaha provided much appreciated advice throughout the program. Although he was not a member of my committee, Steve Thomas served as a mentor and I am grateful for his guidance.

Many people helped to make this project possible. Sue Rasmussen helped navigate the bureaucracy of graduate school. The Biology Department office staff assisted with logistics. Rob Hunter provided help with IT issues. Younan Chen of the Statistical Consulting Center provided statistical advice. I am grateful to numerous landowners and to the USFS for providing access to streams.

Thanks to members of the Virginia Tech Stream Team for their support and friendship. I would especially like to thank Matt McTammany, Bobbie Niederlehner, Rob Payn, and Jack Brookshire. Matt McTammany went through the Ph.D. program a step ahead of me and shared his wisdom and insights along the way. Bobbie Niederlehner trained me to be a better analytical scientist and provided advice on nearly every aspect of my research. Rob Payn helped with many quantitative aspects of my research. I have enjoyed many stimulating conversations about life and ecology with Jack Brookshire; I look forward to many more.

Finally, I would like to thank my family. I would especially like to thank my wife, Erica Maria, for her love and support. Thanks to my parents, Ross and Kathy, my brother, Brian, and our dog, Wookie, for their support.

Funding for this project was provided by the Graduate Student Assembly (GSA) at Virginia Tech, the Virginia Tech Biology Department, the Proctor and Gamble Company, the Virginia Academy of Science, and a NSF doctoral dissertation improvement grant (DDIG).

## Table of contents

Abstract.....	ii
Acknowledgments.....	iv
Table of contents.....	vi
List of tables.....	vii
List of figures.....	viii
Chapter 1: General introduction.....	1
Literature Cited.....	5
Chapter 2: Relationships among ecosystem structure and function, and nitrogen spiraling in streams spanning a gradient of nitrogen concentration .....	8
Abstract.....	8
Introduction.....	8
Methods.....	11
Results.....	17
Discussion.....	21
Literature Cited.....	26
Chapter 3: An analysis of nitrogen spiraling and saturation in stream ecosystems using a multiple enrichment approach.....	42
Abstract.....	42
Introduction.....	43
Methods.....	47
Results.....	51
Discussion.....	55
Literature Cited.....	61
Chapter 4: Comparison of nitrogen spiraling in stream ecosystems quantified using stable isotope tracers and nutrient addition experiments .....	77
Abstract.....	77
Introduction.....	78
Methods.....	80
Results.....	84
Discussion.....	87
Conclusions.....	91
Literature Cited.....	92
Curriculum vitae .....	100

## List of tables

Chapter 2: Relationships among ecosystem structure and function, and nitrogen spiraling in streams spanning a gradient of nitrogen concentration

Table 1. Characteristics of study streams .....	33
Table 2. Stream hydraulic characteristics .....	34
Table 3. Organic matter standing stocks.....	35
Table 4. Whole-stream metabolism .....	37
Table 5. Spiraling metrics .....	38

Chapter 3: An analysis of nitrogen spiraling and saturation in stream ecosystems using a multiple enrichment approach

Table 1. Characteristics of study streams .....	65
Table 2. Results of individual releases .....	66
Table 3. Uptake kinetics .....	67

Chapter 4: Comparison of nitrogen spiraling in stream ecosystems quantified using stable isotope tracers and nutrient addition experiments

Table 1. Characteristics of study streams .....	95
Table 2. Ambient and amendment-derived spiraling metrics .....	96
Table 3. Extrapolated uptake lengths and Michaelis-Menten kinetics .....	97

## List of figures

Chapter 2: Relationships among ecosystem structure and function, and nitrogen spiraling in streams spanning a gradient of nitrogen concentration

Figure 1. Relationships between $S_w$ and select stream characteristics.....	39
Figure 2. Relationships between $v_f$ and select stream characteristics.....	40
Figure 3. Relationships between $U$ and select stream characteristics.....	41

Chapter 3: An analysis of nitrogen spiraling and saturation in stream ecosystems using a multiple enrichment approach

Figure 1. Michaelis-Menten kinetics and spiraling.....	68
Figure 2. Saturation response types (SRTs).....	69
Figure 3. Response of $S_w$ ( $\pm$ SE) to nitrogen amendments .....	70
Figure 4. Response of $v_f$ to nitrogen amendments .....	71
Figure 5. Response of $U$ to nitrogen amendments .....	72
Figure 6. Response of $S_w$ ( $\pm$ SE) to nitrogen amendments for all releases.....	73
Figure 7. Ambient $S_w$ ( $\pm$ SE), $v_f$ , and $U$ across streams .....	74
Figure 8. Truncation model.....	75
Figure 9. Relationships among streamwater $\text{NO}_3\text{-N}$ , and tracer-derived $v_f$ and $U$ .....	76

Chapter 4: Comparison of nitrogen spiraling in stream ecosystems quantified using stable isotope tracers and nutrient addition experiments

Figure 1. Extrapolations to ambient uptake lengths.....	98
Figure 2. $\text{NO}_3\text{-N}$ amendments ( $C_{ADD}$ ) and $S_w'$ for all releases.....	99

## Chapter 1: General introduction

In 1912, Fritz Haber and Carl Bosch developed a commercially viable process by which atmospheric  $N_2$  is converted to ammonia (Fisher and Fisher 2001). In the years since that discovery, the supply of anthropogenically-derived fixed nitrogen (N) has steadily increased. Supplies of fixed N to agricultural systems now exceed  $120 \text{ Tg y}^{-1}$  while an additional  $20 \text{ Tg y}^{-1}$  of fixed N is released into the atmosphere via the combustion of fossil fuels (Vitousek et al. 1997). The combination of these, and other, activities has more than doubled the annual transfer of  $N_2$  to biologically available forms compared to pre-industrial times (Vitousek et al. 1997).

The flood of anthropogenically-derived fixed N has dramatically influenced the global N cycle. Nitrogen loading to terrestrial landscapes has received considerable attention as atmospheric deposition of N compounds is leading to forest decline in many areas of North America, Europe, and Asia (Aber et al. 1998). However, it is in freshwater and marine environments where the effects of N loading may be most pronounced. Annual rates of N loading into freshwater ecosystems has increased 6 to 50-fold, causing global fertilization in continental, estuarine, and near-coastal marine environments (Carpenter et al. 1998). In an analysis of over 300 surface water sites, Smith et al. (1987) reported that an increase in the concentration of  $NO_3-N$  was the most pronounced observed change compared to more than 20 other water quality variables. The load of total dissolved N transported in rivers has increased up to 20-fold since pre-industrial times (Vitousek et al. 1997). This added flux of N is of particular concern in estuaries and coastal waters where N is typically the limiting nutrient (Nixon et al. 1996). Increased N loading has already resulted in the eutrophication of many estuaries (Officer et al. 1984, Nixon et al. 1996) and has been linked to large-scale degradation of water quality in the Gulf of Mexico (Rabalais et al. 1994, Alexander et al. 2000).

The lotic ecosystems that carry this N load have historically been viewed as relatively inert conduits. This view of streams is epitomized in classic studies of nutrient budgets in small watersheds (e.g., Likens et al. 1967, Swank 1988). In these studies, nutrient processing in the catchment is estimated as the difference between inputs and outputs where outputs are typically measured at a weir constructed at the base of the catchment. Elemental processing in the stream is thus assumed minimal (Hall 2003).

However, recent studies and a growing body of evidence suggest that streams are important sites for N processing, including N retention and removal (e.g., Peterson et al. 2001, Bernhardt et al. 2003). In fact, rates of N processing may be disproportionately higher in streams relative to other settings in the catchment due to a wide range of redox conditions at the soil-stream interface (Hedin et al. 1998). In a study of lotic ecosystems in the Mississippi River basin, Alexander et al. (2000) noted loss rates of up to 50% in headwater streams that were less than 50-cm deep. Nitrogen processing is maximal in headwater streams as they represent the most extensive interface between aquatic-terrestrial systems and are characterized by high benthic to surface-water volume ratios.

Uptake and transformations of N in lotic ecosystems are predominately functions of autotrophic and heterotrophic biological processes. As in terrestrial systems, N is a macronutrient that is frequently limiting to growth in aquatic ecosystems. Increased N concentration in streams has been shown to stimulate algal biomass and primary production (e.g., Guasch et al. 1995), heterotrophic activity (e.g., Gulis and Suberkropp 2003), nitrification (e.g., DeLaune et al. 1991), and denitrification (e.g., Kemp and Dodds 2002). However, the magnitude of N loading and the response to that disturbance is variable. In a study of epilithic and epixylic biofilms in streams throughout North America, Tank and Dodds (2003) reported that one-third of studied streams were not N limited despite low streamwater dissolved inorganic N (DIN) concentrations. These studies suggest that N uptake capacities in streams range from N-limited to N-saturated.

Nitrogen saturation occurs when the availability of N meets or exceeds metabolic demand (Aber et al. 1998) and, in turn, a different factor critical to growth becomes limiting. Aber et al. (1989, 1998) proposed the N-saturation hypothesis whereby enhanced N loading saturates forest uptake processes. In the model proposed by Aber et al. (1989, 1998), late stages of forest saturation are characterized by enhanced leaching and subsequent loading to aquatic systems. Stoddard (1994) adapted the model presented by Aber et al. (1989, 1998) and described stages of N saturation for lotic ecosystems that are delineated by the seasonal concentration of streamwater  $\text{NO}_3\text{-N}$ . Initial stages of saturation are characterized by periodic increases in streamwater  $\text{NO}_3\text{-N}$  concentration whereas late stages of saturation are characterized by elevated baseline concentrations of streamwater  $\text{NO}_3\text{-N}$ . However, nutrient concentrations in streams are, wholly or in part,

biotically mediated and reflect a balance between uptake and mineralization (Dodds 1993). Thus, the absolute nutrient concentration may not necessarily reflect nutrient-limitation or -saturation. Traditional approaches to quantify nutrient uptake kinetics, and thus identify saturating concentrations, involve measuring uptake at different nutrient concentrations at batch (e.g., Carpenter and Guillard 1971) or mesocosm (e.g., Bothwell 1989) scales. However, a similar approach of coupling nutrient uptake and concentration is possible in lotic ecosystems due to the advective properties of streams (sensu Dodds et al. 2002). This unique aspect of streams facilitates accurate estimates of nutrient availability, uptake, and, potentially, saturating concentrations.

Our knowledge of how N processing in streams responds to chronic N loading, particularly with respect to N saturation, is limited. Generalizing about relationships between N availability and uptake in stream ecosystems is particularly difficult due to the multitude of factors, both biotic and abiotic, that influence N uptake. Nevertheless, a better understanding of these relationships is needed to manage lotic ecosystems in the face of anthropogenic disturbances and to maximize their role in mediating downstream N fluxes.

My guiding objective was to investigate the relationships between N availability and spiraling in stream ecosystems, with particular emphasis on the conditions that lead to saturation of N uptake capacity. The idea for this work stemmed from the observation that while many studies have investigated the influence of specific stream attributes (e.g., hydraulic characteristics, Valett et al. 1996; riparian vegetation and light availability, Sabater et al. 2000; and metabolic activity, Mulholland et al. 2001) on N processing in streams, few studies have considered how N processing responds to N availability itself. Studies by Davis and Minshall (1999) and Dodds et al. (2002) were among the first to address relationships between streamwater N availability and whole-stream uptake, and they both contributed greatly to the ideas presented here. The lack of research addressing these issues during the first part of the 21<sup>st</sup> century must have been apparent to other researchers as these questions and ideas have been the subject of several recent papers and current projects.

In this dissertation, I present the results of field experiments conducted over two years in streams spanning a gradient of N concentration. I used a combination of NO<sub>3</sub>-N

amendments and stable-isotope tracers to quantify  $\text{NO}_3\text{-N}$  uptake and uptake efficiency employing the nutrient spiraling framework. I used a regression approach to characterize relationships among streamwater N concentration, N uptake and uptake efficiency, and a suite of chemical, biotic, and physical attributes of the study streams (Chapter 2). I then conducted a series of N amendments in which the streamwater  $\text{NO}_3\text{-N}$  concentration was incrementally elevated to characterize stream response to increased N availability. The proximity of each stream to a point of N saturation was assessed according to its response to the N amendments (Chapter 3). Finally, I take advantage of data collected during the stable isotope and multiple amendment experiments to address two methodological aspects of the spiraling framework (Chapter 4). I compared amendment- and tracer-derived estimates of  $\text{NO}_3\text{-N}$  spiraling in stream ecosystems. I then assessed the accuracy of a novel technique proposed by Rob Payn and others to quantify ambient (i.e., non-amended) spiraling metrics in streams using non-isotopic nutrient tracers. Each research product is written as an independent entity intended for publication in different journals necessitating a degree of redundancy in certain areas.

## Literature Cited

- Aber, J. D., K. J. Nadelhoffer, P. Steudler, and J. M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience* 39:378-386.
- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Bernston, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems. *BioScience* 48:921-934.
- Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effects of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758-761.
- Bernhardt, E. S., G. E. Likens, D. C. Buso, and C. T. Driscoll. 2003. In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. *Proceedings of the National Academy of Science* 100:10304-10308.
- Bothwell, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1293-1301.
- Carpenter, E. J., and R. R. L. Guillard. 1971. Intraspecific differences in nitrate half saturation constants for three species of marine phytoplankton. *Ecology* 52:183-185.
- Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559-568.
- DeLaune, R. D., L. M. Salinas, R. S. Knox, M. N. Sarafyan, and C. J. Smith. 1991. Water quality of a coastal river receiving nutrient inputs: ammonium nitrogen transformations. *Journal of Environmental Science and Health A26*:1287-1302.
- Dodds, W. K. 1993. What controls levels of dissolved phosphate and ammonium in surface waters? *Aquatic Sciences* 55:132-142.
- 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. Morall, 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.
- Fisher, D. E., and M. J. Fisher. 2001. The nitrogen bomb. *Discover* 22:50-57.
- Guasch, H., E. Martí, and S. Sabater. 1995. Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology* 33:373-383.

- Gulis, V., and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48:123-134.
- Hall, R. O., Jr. 2003. A stream's role in watershed nutrient export. *Proceedings of the National Academy of Science* 100:10137-10138.
- Hedin, L. O., J. C. von Fischer, N. E. Ostrom, B. P. Kennedy, M. G. Brown, and G. P. Robertson. 1998. Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. *Ecology* 79:684-703.
- Kemp, M. J., 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.
- Likens, G. E., F. H. Bormann, N. M. Johnson, and R. S. Pierce. 1967. The Ca, Mg, K, and Na budgets for a small forested ecosystem. *Ecology* 48:772-785.
- Mulholland, P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Martí, L. Ashkensas, 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.
- Nixon, S. W., J. W. Ammerman, L. P. Atkinson, V. M. Berounsky, G. Bilen, W. C. Boicourt, W. R. Boynton, T. M. Church, D. M. DiToro, R. Elmgren, J. Garber, A. E. Giblin, R. A. Jahnke, N. J. P. Owens, M. E. Q. Pilson, and S. P. Seitzinger. 1996. The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic Ocean. *Biogeochemistry* 35:141-180.
- 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., W. J. Wiseman, and R. E. Turner. 1994. Comparison of continuous records of near-bottom dissolved oxygen from the hypoxia zone along the Louisiana coast. *Estuaries* 17:850-861.
- Sabater, F., A. Butturini, E. Martí, I. Muñoz, A. Román, J. Wray, and S. Sabater. 2000. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. *Journal of the North American Benthological Society* 19:609-620.
- Smith, R. A., R. B. Alexander, and M. G. Wolman. 1987. Water quality trends in the nation's rivers. *Science* 235:1607-1615.

- Stoddard, J. L. 1994. Long term changes in watershed retention of nitrogen. Its causes and aquatic consequences. Pages 223-284 *in* L. A. Baker, editor. Environmental chemistry of lakes and reservoirs. Advances in Chemistry Series no. 237. American Chemical Society, Washington D. C.
- Swank, W. T. 1988. Stream chemistry responses to disturbance. Pages 339-358 *in* W. T. Swank and D. A. Crossley, Jr., editors. Forest Hydrology and Ecology at Coweeta. Springer-Verlag, New York, USA.
- Tank, J. L., and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology* 48:1031-1049.
- Valett, H. M., J. A. Morrice, C. N. Dahm, M. E. Campana. 1996. Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography* 41:333-345.
- Vitousek, P. M., J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alterations of the global nitrogen cycle: causes and consequences. *Issues in Ecology* 1: 2-15.

## **Chapter 2: Relationships among ecosystem structure and function, and nitrogen spiraling in streams spanning a gradient of nitrogen concentration**

### **Abstract**

In order to identify controls on NO<sub>3</sub>-N spiraling in anthropogenically impacted stream ecosystems, I examined relationships among NO<sub>3</sub>-N spiraling and a suite of chemical, physical, and biological variables in streams spanning a gradient of nitrogen concentration. Hydraulic characteristics, organic matter standing stocks, and rates of gross primary production (GPP) and ecosystem respiration (R) varied considerably among studied streams. Standing stocks of autotrophs and GPP were generally correlated to each other and to discharge and photosynthetically active radiation (PAR). Uptake velocity was correlated to the standing stock of autotrophs and to GPP. Rates of GPP were relatively low but varied 124-fold and explained 58% of the variance in uptake velocity. Uptake was significantly correlated to both streamwater NO<sub>3</sub>-N concentration and to GPP but was best explained (80% of variance) by a linear combination of both variables. These results suggest that demand for NO<sub>3</sub>-N is primarily driven by primary production but that N availability may limit uptake when demand is high and N concentration is low.

### **Introduction**

By the mid 1990s, the annual transformation and transfer of atmospheric N<sub>2</sub> to biologically available forms had more than doubled compared to pre-industrial times (Vitousek et al. 1997, Naiman and Turner 2000). Nitrogen (N) that is applied to landscapes through agriculture, atmospheric deposition, or in urban settings may eventually reach aquatic systems (Smith et al. 1999). Annual rates of N loading into freshwater ecosystems has increased 6 to 50-fold, causing global fertilization in continental, estuarine, and near-coastal marine environments (Carpenter et al. 1998). However, streams have the potential to retain, transform, and remove organic and inorganic materials through in-stream, biological processes that may mediate downstream fluxes of N (Peterson et al. 2001, Bernhardt et al. 2003, Hall 2003).

The nutrient spiraling concept (Webster and Patten 1979) incorporates both downstream transport and the cycling of nutrients within streams (Newbold et al. 1981), and provides the appropriate framework and tools to investigate N retention in lotic ecosystems. A nutrient spiral consists of two components; (1) uptake length ( $S_w$ ), the average distance traveled in the water column as an inorganic solute before biotic assimilation, and (2) turnover length ( $S_o$ ), the distance the atom travels in organic form before it is mineralized and introduced back to the water column (Newbold et al. 1981). In headwater streams, the uptake length is typically the longest component of a nutrient spiral and is often used to describe nutrient retention in streams. While  $S_w$  does not directly measure nutrient losses, it is sensitive to mineralization and acts as a good indicator of nutrient retention (Stream Solute Workshop 1990, Valett et al. 2002). A shorter uptake length suggests more efficient uptake and retention of nutrients (Newbold et al. 1981). However,  $S_w$  is strongly influenced by stream hydraulic features. Uptake velocity ( $v_f$ ) represents the theoretical speed at which a nutrient moves toward a sink and standardizes for stream depth and velocity, providing a more biologically meaningful descriptor to compare across streams (Davis and Minshall 1999, Valett et al. 2002). Uptake velocity reflects both nutrient uptake and supply (i.e., concentration), and thus serves as a descriptor of nutrient uptake efficiency. Areal specific uptake ( $U$ ) is the product of  $v_f$  and nutrient concentration and quantifies the mass of nutrient immobilized per area of streambed per unit time (Stream Solute Workshop 1990).

Nitrogen uptake is a function of in-stream metabolic activity, hence rates of biotic processes influence the ability of a stream ecosystem to retain N (Mulholland et al. 2000, Fellows et al. 2001, Hall and Tank 2003). Biotic processes in streams are often limited by nutrient availability and increased N availability may stimulate uptake (Dodds et al. 2002, Bernhardt et al. 2003). However, chronic N loading may influence substrate affinities by altering biotic standing stocks (Lohman et al. 1991) and composition (Peterson and Grimm 1992). Further, land use associated with N loading (e.g., agriculture, urbanization) may alter stream biota and processing rates through changes to the stream environment. For example, Sabater et al. (2000) reported greater N retention efficiency associated with more algal biomass in a Mediterranean stream following riparian deforestation. Conversely, removing vegetation may reduce the input of leaves

and wood (DeLong and Brusven 1994), substrates that contribute to heterotrophic N demand in headwater streams (Tank et al. 2000). How N uptake and uptake efficiency respond to increased N loading and to changes in the physical environment due to anthropogenic disturbance remains poorly understood.

Variability in channel characteristics may also influence nutrient dynamics. Valett et al. (1996) proposed a conceptual model of stream ecosystems in which nutrient retention is represented as the product of hydrologic retention and the sum of biological and chemical processes that sequester materials. Hydrologic retention results from flow-paths that impede the downstream movement of water, thus increasing water residence times (Morrice et al. 1997). Hydrologic retention can be modeled as transient storage (sensu Bencala and Waters 1983, Stream Solute Workshop 1990), where enhanced transient storage increases the amount of time during which biological and chemical processes may influence solutes dissolved in stream water. In many headwater streams, transient storage is primarily a function of water entering subsurface flow-paths (i.e., the hyporheic zone) that move slower than the main channel (Harvey et al. 1996, Valett et al. 1996). The ratio of the cross-sectional area of the transient storage zone ( $A_S$ ;  $m^2$ ) to the cross-sectional area of the wetted stream channel ( $A$ ;  $m^2$ ) is commonly used to compare hyporheic zone size (sensu Valett et al. 1996, Fellows et al. 2001). Several studies have documented the influence of transient storage on stream metabolism (e.g., Grimm and Fisher 1984, Fellows et al. 2001) and nutrient retention (e.g., Valett et al. 1996, Mulholland et al. 1997).

Headwater streams are efficient transformers and retainers of inorganic N (Hall 2003). Peterson et al. (2001) estimated that up to 64% of the dissolved inorganic N (DIN) input to a headwater stream could be retained within a 1-km reach. Nitrogen cycling in headwater streams has received considerable recent attention due, in part, to their potential to mediate N losses from enriched landscapes. However, most studies that have addressed N cycling in the context of spiraling and retention have been conducted in relatively undisturbed stream ecosystems (sensu Webster et al. 2003). It is unclear how N processing in headwater streams is influenced by changes in streamwater N concentration due to anthropogenic disturbances. The objective of this study was to examine relationships among N spiraling and physical, chemical, and biological variables

in streams subject to varying degrees of anthropogenic disturbance. Streams were selected to span a gradient of streamwater DIN concentration yet were located within relatively close proximity to minimize large scale differences among settings (e.g., climate).

## **Methods**

I conducted N spiraling assays on a select group of first- and second-order headwater streams in the Appalachian Mountains of southwestern Virginia and western North Carolina, USA during the summer and fall of 2002 and 2003 (Table 1). Study streams in each of the two years were selected to span a gradient of background DIN concentration. Nine different streams were assayed and two of the streams (Hagar Creek and Greenbrier Creek) addressed in each of the two years. With the exception of Smith Creek, all streams had intact, forested riparian zones but land use within associated catchments was variable. Sammy Creek and Hugh White Creek were located within forested catchments relatively undisturbed by human activities over the past 80 years. Vegetation included mixed deciduous forests with thick streamside canopies of rhododendron (*Rhododendron maxium* L.). Catchments of the other streams were comprised of a mixture of forest, small farms, and residential areas. Study streams were characterized by softwater with the exception of Smith Creek and Stonecrop Creek, which drained predominately limestone terrain. A single study reach (50-388 m) was selected in each stream for assessing N spiraling, measuring whole-stream metabolism, and quantifying standing stocks of biotic compartments and hydraulic characteristics. Reach length depended on accessibility, streamwater travel time, and distance appropriate to properly measure N uptake.

### *Solute addition experiments*

Nitrogen spiraling was quantified following a short-term (3-5 h) nutrient release in which streamwater nitrate-nitrogen (NO<sub>3</sub>-N) concentration was elevated through an experimental addition to the study reach. Background samples (i.e., prior to the release) were collected from 4 to 10 transects along the study reach and analyzed for NO<sub>3</sub>-N and

bromide (Br<sup>-</sup>). A subset of the background samples was analyzed for ambient concentrations of ammonium-nitrogen (NH<sub>4</sub>-N) and orthophosphate (PO<sub>4</sub>-P).

A solution of NaNO<sub>3</sub>, NaBr, and NaCl (Br<sup>-</sup> and Cl<sup>-</sup> to act as conservative tracers, sensu Bencala et al. 1990) was released at a constant rate using a fluid-metering pump (FMI, Inc., Syosset, NY, USA). The target enrichment concentration for streamwater NO<sub>3</sub>-N was approximately 50 μg L<sup>-1</sup> but actual enrichment varied depending on hydrologic conditions and uptake. Target concentrations for Cl<sup>-</sup> and Br<sup>-</sup> were 2.00 and 0.30 mg L<sup>-1</sup>, respectively.

Three replicate samples were collected from each transect under well-mixed conditions (i.e., tracer plateau as indicated by steady-state conductivity in channel water). Samples were immediately filtered (glass-fiber filters, Gelman A/E, pore size = 1.0 μm), frozen that day, and later analyzed for NO<sub>3</sub>-N and Br<sup>-</sup>.

Uptake length was calculated by fitting an exponential decay model (SigmaPlot, SPSS, Inc., Chicago, IL) to background and dilution corrected streamwater NO<sub>3</sub>-N concentration versus distance downstream. The decay coefficient represents the longitudinal loss rate ( $k_L$ ), and its negative inverse is  $S_w$  (Newbold et al. 1981). The standard error (SE) of  $S_w$  was determined as the standard error of the uptake coefficient using a modified exponential decay model (Equation 1). In (1),  $a$  is equal to the NO<sub>3</sub>-N concentration at the upper-most transect and  $x$  is distance downstream.

$$\text{NO}_3\text{-N} = a \exp\left(\frac{x}{S_w}\right) \quad (1)$$

Uptake velocity ( $v_f$ ; mm min<sup>-1</sup>) was calculated following Stream Solute Workshop (1990):

$$v_f = \left(\frac{d}{S_w} u\right) 60000 \quad (2)$$

where  $u$  (m s<sup>-1</sup>) is the average streamwater velocity,  $d$  (m) is average stream depth, and 60000 is a conversion factor to convert units. Areal uptake ( $U$ ) was calculated as the

product of  $v_f$  and background streamwater  $\text{NO}_3\text{-N}$  concentration (Stream Solute Workshop 1990).

#### *Laboratory methods*

Anions ( $\text{NO}_3\text{-N}$  and  $\text{Br}^-$ ) were analyzed on a Dionex DX500 Ion Chromatograph (Sunnyvale, CA, USA) with an AS4A anion column. Due to low  $\text{NO}_3\text{-N}$  concentrations ( $< 10 \mu\text{g L}^{-1}$ ), samples collected at Sammy Creek and Hugh White Creek were analyzed colorimetrically following reduction by Cd (Wood et al. 1967, APHA 1998) on a Technicon Autoanalyzer (Technicon, Emeryville, CA, USA). Samples were analyzed for  $\text{NH}_4\text{-N}$  using the phenol-hypochlorite method (Solorzano 1969, USEPA 1997a) and  $\text{PO}_4\text{-P}$  as soluble reactive phosphorus using the molbydate antimony method (Murphy and Riley 1962, USEPA 1997b).

#### *Hydraulic characteristics*

Stream hydraulic characteristics were quantified by analyzing the behavior of the conservative tracer (as specific conductance) during the  $\text{NO}_3\text{-N}$  release. Specific conductance was continuously monitored at the downstream end of the reach by a data-logging Hydrolab Minisonde (Hydrolab-Hach, Inc., Loveland, CO, USA). Upon collecting plateau samples, the fluid-metering pump was turned off and specific conductance was monitored until returning to background concentrations. An advection-dispersion model and parameter estimation program (Bencala and Walters 1983, Runkel 1998) were used to compute streamwater velocity ( $u$ ,  $\text{m s}^{-1}$ ), dispersion ( $D$ ,  $\text{m}^2 \text{s}^{-1}$ ),  $A$  ( $\text{m}^2$ ),  $A_s$  ( $\text{m}^2$ ), and the storage zone exchange coefficient ( $\alpha$ ,  $\text{s}^{-1}$ ). The ratio of  $A_s$  to  $A$  was used to represent the size of the storage zone standardized to the stream cross sectional area.

Discharge ( $Q$ ) at each transect was determined by dilution gauging using the conservative tracer ( $\text{Br}^-$ ). Widths and depths of the wetted channel were measured at 5 to 10 m intervals along the study reach within one week of the release.

### *Organic matter standing stocks*

The standing stocks of epilithon, fine benthic organic matter (FBOM; particles < 1 mm), coarse benthic organic matter (CBOM; particles > 1 mm, generally small sticks and leaves), wood ( $\geq 0.5$  cm diameter), suspended particulate organic matter (SPOM, particles  $\geq 1.0$   $\mu\text{m}$ ), and bryophytes and macrophytes (if present) were measured within one week prior to or following each  $\text{NO}_3\text{-N}$  release. Epilithic biomass as ash-free dry mass (AFDM) and chlorophyll *a* was determined by sampling biofilms on 10 rocks collected randomly along the study reach. Ten sets of 3 rocks were sampled in those streams where little epilithic biomass was present (i.e., Sammy Creek, Hugh White Creek, and Stonecrop Creek). The upper surface of each rock was scrubbed and the resultant slurry was returned to the laboratory within 10 h of collection and stored frozen. Upon processing (within 1 mo.), the slurry was thawed and a homogenized subsample was filtered through a precombusted, preweighed glass-fiber filter (Gelman A/E). The sample was dried at 60 °C for  $\geq 48$  h, weighed, ashed at 500 °C for 1 h, and reweighed to determine AFDM. An additional subsample was filtered through a glass-fiber filter (Gelman A/E) for analysis of chlorophyll *a*. Filters were placed in a 90% buffered acetone solution and refrigerated for 24 h, then analyzed spectrophotometrically on a Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Pigment concentrations were calculated as outlined by Steinman and Lamberti (1996). Surface area of rocks scraped for epilithon was determined by weighing aluminum foil of known density used to cover the sampled surface. Fine benthic organic matter was collected from 8 to 10 locations in the study reach where the substratum was sand, silt, or gravel. A metal cylinder (area = 0.02 m<sup>2</sup>) was embedded into the stream bottom, the sediment was agitated to a depth of ca. 5 to 10 cm, and ca. 250 mL of the resultant slurry was collected. The height of the water in the cylinder was measured to determine the total volume of the slurry and calculate FBOM standing stock. Fine benthic organic matter samples were stored and processed using methods identical to those outlined for epilithon, except that only material passing through a 1-mm sieve was analyzed. Coarse benthic organic matter was collected from 4 to 6 locations along the study reach from areas corresponding to 100% coverage. A metal cylinder (area = 0.02 m<sup>2</sup>) was placed over the organic matter accumulation and all of the material within the cylinder was

collected. Samples were rinsed over a sieve (1 mm diameter) upon returning to the laboratory then dried at 60 °C for  $\geq 48$  h. Samples were ground, then two subsamples were weighed, ashed, and reweighed as noted above to determine standing stock as AFDM per unit area. Wood volume was estimated using the line-intercept method (Wallace and Benke 1984). The mass of wood ( $\text{g AFDM m}^{-2}$ ) was calculated using a specific gravity of  $0.356 \text{ g AFDM cm}^{-3}$  (Wallace et al. 2000). A set of three 2-L samples of streamwater was collected at the upstream end of the study reach to quantify SPOM. Each SPOM sample was filtered through a precombusted, preweighed glass-fiber filter (Gelman A/E), which was processed as above to determine the concentration of SPOM as  $\text{mg AFDM L}^{-1}$ . Bryophytes and macrophytes were collected from areas of 100% coverage and processed as outlined for CBOM.

An additional subsample from each collection, except SPOM, was analyzed for the molar ratio of carbon to nitrogen (C:N). Subsamples from a given collection were dried, homogenized, then analyzed on a VarioMax CNS Macro Elemental Analyzer (Elementar Americas, Mt Laurel, NH, USA).

Standing stocks of epilithon, FBOM, macrophytes, bryophytes, and CBOM were scaled to reflect abundance in the reach by quantifying streambed habitat associated with each compartment (cobble/boulder, sand/gravel/silt, macrophytes, bryophytes, and coarse debris, respectively). The percent streambed cover of each habitat type was determined from the relative breadth of each type along cross-sectional transects spaced at 5-10 m intervals along the study reach. Standing stocks of each organic matter compartment are expressed as habitat-weighted  $\text{g AFDM m}^{-2}$ . Standing stocks of chlorophyll *a* associated with epilithon and FBOM are expressed as habitat-weighted  $\text{mg chl } a \text{ m}^{-2}$ . The AFDM standing stock of autotrophs was determined as the sum of epilithon, bryophytes, and macrophytes. The AFDM standing stock of benthic detritus was determined as the sum of FBOM and CBOM.

#### *Whole-stream metabolism*

Whole-stream metabolism, including gross primary production (GPP) and ecosystem respiration (R), was measured in each study stream on the day of or within 1 week of the  $\text{NO}_3\text{-N}$  release. Metabolism was calculated over a  $\geq 24$  h period under clear or partly

cloudy weather conditions, similar to conditions during the NO<sub>3</sub>-N release, using the upstream-downstream diurnal dissolved oxygen (O<sub>2</sub>) change technique (Odum 1956, Marzolf et al. 1994, Young and Huryn 1999). Dissolved oxygen and streamwater temperature were monitored at the most up- and down-stream transects along the study reach of known travel time at 4- or 5-min intervals using Hydrolab data-logging Minisondes (Hydrolab-Hach, Inc., Loveland, CO, USA). Oxygen probes were calibrated in water-saturated air at the field site prior to deployment. Oxygen probes were placed at the same stream location for  $\geq 30$  min before and after each 24-h measurement period to correct for probe drift. Barometric pressure and photosynthetically active radiation (PAR) were recorded (10-min interval) at a representative location along the stream reach during the measurement period using a pressure transducer (Vaisala PTB101B, Helsinki, Finland) and quantum sensor (LI-COR Biosciences Inc., Lincoln, NE, USA), respectively, coupled to a data logger (CR10X, Campbell Scientific, Inc., Logan, UT, USA). Metabolism was measured concurrently at Purgatory Creek and Hagar Creek in 2002. Hagar Creek drains a subcatchment of the Purgatory Creek catchment and study reaches in these two streams were separated by less than ca. 500 m. Both streams share similar canopy characteristics (S. R. Earl, personal observation) and barometric pressure and PAR measured at a representative location at Hagar Creek (2002) were used to reflect conditions in both streams.

Reaeration of O<sub>2</sub> was estimated by measuring the reaeration coefficient of sulfur hexafluoride (SF<sub>6</sub>), a volatile gas-tracer. SF<sub>6</sub> was released at a constant rate through a series of submerged airstones at the same location as the solute release. In order to correct for dilution, SF<sub>6</sub> releases were conducted in concert with the NO<sub>3</sub>-N releases or with a separate conservative solute release using methods identical to those described for the nutrient releases. Replicate samples for both gas ( $n = 4$ ) and water ( $n = 3$ ) were collected from each transect upon reaching steady-state plateau in the study reach. Gas samples were obtained by drawing 6 mL of streamwater into a syringe and injecting the contents into an evacuated, gas-tight 12-mL serum vial. Serum vials were stored at room temperature for 1 to 7 d. SF<sub>6</sub> was quantified by analyzing 0.1 mL of headspace of the equilibrated samples on a SRI 8610/9300 gas chromatograph (SRI Instruments, Torrance, CA, USA) equipped with an electron-capture detector. The reaeration coefficient ( $K_2$ )

for each stream was calculated according to Wanninkhof et al. (1990) and standardized to 20°C ( $k_{2(20^{\circ}\text{C})}$ ) (Elmore and West 1961).

Gross primary production (GPP) and respiration (R) were calculated based on the reaeration-corrected change in O<sub>2</sub> concentration ( $\Delta\text{DO}$ ) between the up- and down-stream transects (Marzolf et al. 1994, Young and Huryn 1999). In some cases, equipment problems with the Minisonde located at either the up- or down-stream location limited the analysis to a single-station (Bott 1996). GPP and R were used to calculate P:R as the ratio of GPP to R and net ecosystem production (NEP) as GPP-R.

### *Statistical analyses*

Correlation analysis (Pearson product moment correlation) was used to explore relationships among measured variables. Linear regression was used in those cases where a dependent-independent relationship could be assumed. Multiple linear regression was used to determine if  $v_f$  and  $U$  could be predicted by more than one factor. All statistical analyses were performed using SigmaStat (SPSS, Inc., Chicago, IL, USA), with a significance level ( $\alpha$ ) set to 0.05 for all tests.

### **Results**

The concentration of NH<sub>4</sub>-N in the study streams was consistently low ( $\leq 5 \mu\text{g L}^{-1}$ ), whereas background concentration of NO<sub>3</sub>-N varied from 3 to 1116  $\mu\text{g NO}_3\text{-N L}^{-1}$  at the time of the releases (Table 1). The concentration of PO<sub>4</sub>-P was generally low, with the exception of Powers Branch (27  $\mu\text{g L}^{-1}$ ) and Stonecrop Creek (20  $\mu\text{g L}^{-1}$ ). Molar N:P ratios varied from 2 to 265 and reflected the substantial range in NO<sub>3</sub>-N concentration. Specific conductance was highest in each of the two hardwater streams, Smith Creek and Stonecrop Creek (specific conductance = 420 and 262  $\mu\text{S cm}^{-1}$ , respectively, versus an average of 77  $\mu\text{S cm}^{-1}$  for all other streams). Discharge of the study streams varied from 2 to 42  $\text{L s}^{-1}$  at the time of the releases (Table 1). Streamwater temperature recorded during the releases ranged slightly more than 10 °C across all dates. Photosynthetically active radiation was exceptionally high at Purgatory Creek and Hagar Creek in 2002 (337  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). In contrast, PAR varied from 2 to 90  $\mu\text{mol m}^{-2} \text{s}^{-1}$  among all other dates (Table 1). Purgatory Creek and Hagar Creek in 2002 were the only streams assayed prior

to leaf-out and lack of shading may have contributed to higher PAR values relative to all other sites.

### *Hydraulic characteristics*

All nine streams were relatively small (average depth < 12 cm, average width < 328 cm), however stream hydraulic characteristics determined from solute transport modeling of conservative tracer releases varied considerably among streams (Table 2). Stream cross sectional area varied from 0.105 to 0.310 m<sup>2</sup> and reflects the generally small size of these streams. However,  $A_s$  varied more than 30-fold from 0.013 to 0.392 m<sup>2</sup>. Cross sectional area of the transient storage zone standardized to stream size ( $A_s/A$ ) varied more than 16-fold from 0.106 to 1.717. Dispersion coefficients ( $D$ ) and  $u$  varied 4 to 6-fold, from 0.100 to 0.418 m<sup>2</sup> s<sup>-1</sup> and 0.027 to 0.173 m s<sup>-1</sup>, respectively, and  $\alpha$  varied more than 15-fold from 0.00004 to 0.00061 s<sup>-1</sup> (Table 2). Discharge was significantly correlated to both  $A$  and  $\alpha$  ( $r \geq 0.699$ ,  $P \leq 0.011$ ).

### *Organic matter standing stocks*

With the exception of macrophytes, which were only present at Greenbrier Creek in 2002 and Smith Creek (not collected), there was great variation in the standing stocks of most organic matter compartments among streams (Table 3). The standing stock of autotrophs varied from nearly undetectable ( $\leq 0.5$  g AFDM m<sup>-2</sup>) at Hugh White Creek, Stonecrop Creek, and Hagar Creek in 2003 to 14.1 g AFDM m<sup>-2</sup> at Purgatory Creek. Total benthic detritus varied from 48.5 g AFDM m<sup>-2</sup> (Hagar Creek in 2003) to 359.0 g AFDM m<sup>-2</sup> (Stonecrop Creek). The standing stock of total benthic detritus was lowest at Smith Creek (25.7 g AFDM m<sup>-2</sup>), however CBOM was not collected at that site and total benthic detritus only reflects the standing stock of FBOM. The concentration of SPOM varied from 0.3 to 12.2 mg AFDM L<sup>-1</sup>, however much of the variation was due to the particularly high concentration at Greenbrier Creek in 2003 (12.2 mg AFDM L<sup>-1</sup>) whereas the next lowest concentration was 7.2 mg AFDM L<sup>-1</sup> at Powers Branch. Although Sammy Creek was located within a catchment that was heavily forested, wood was not encountered in the study reach. In contrast, there were substantial accumulations

of wood at both Greenbrier Creek in 2003 (806 g AFDM m<sup>-2</sup>) and Hagar Creek in 2002 (1879 g AFDM m<sup>-2</sup>).

Although there was considerable variation in the standing stocks of organic matter compartments among streams, there was relatively little variation among C:N ratios within compartments (Table 3). The C:N ratio was highest for CBOM (average = 59), which also had the highest variation among all compartments (coefficient of variation [CV] = 34) due to a particularly high C:N ratio at Powers Branch (105). The C:N ratio was lower among epilithon (average = 11), bryophytes (average = 20), and FBOM (average = 18) with little variation within compartments (CV = 16, 21, and 14, respectively).

The standing stock of autotrophs generally increased with increasing discharge and light availability. The AFDM standing stock of autotrophs and chl *a* standing stocks associated with both epilithon and FBOM were significantly correlated with discharge ( $r \geq 0.647$ ,  $P \leq 0.023$ ). The AFDM standing stock of autotrophs was also significantly related to PAR ( $r \geq 0.581$ ,  $P \leq 0.048$ ) but chl *a* standing stocks were not ( $r \leq 0.517$ ,  $P \geq 0.085$ ). The relationship between the AFDM standing stock of autotrophs and PAR was influenced by the particularly high PAR values at Purgatory Creek and Hagar Creek in 2002, however the relationship was still significant and stronger when these points were omitted from the analysis ( $r = 0.803$ ,  $P = 0.005$ ,  $n = 10$ ). There were no statistically significant relationships between streamwater NO<sub>3</sub>-N concentration and organic matter standing stocks.

#### *Whole-stream metabolism*

Rates of GPP varied 124-fold from 0.01 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> at both Sammy Creek in July and Hugh White Creek to 1.24 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> at Smith Creek (Table 4). Ecosystem respiration was exceptionally low at Sammy Creek in May (0.03 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) and varied from 1.73 (Little Back Creek) to 5.83 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (Hagar Creek in 2003) among all other dates (Table 4). With the exception of Sammy Creek in May, P:R ratios were less than one and NEP was negative (Table 4). The high P:R ratio and positive NEP at Sammy Creek in May reflect the unusually low R rate while GPP at that site was within the range

of those recorded in other study streams. The reason for the uncharacteristically low R at Sammy Creek in May is unclear.

Gross primary production was significantly related to discharge ( $r = 0.869$ ,  $P \leq 0.001$ ) and most autotrophic standing stocks. Gross primary production was not significantly related to the AFDM standing stock of autotrophs ( $r = 0.543$ ,  $P = 0.048$ ) but was significantly related to epilithon (AFDM, chl *a*;  $r \geq 0.813$ ,  $P \leq 0.002$ ) and the chl *a* standing stock associated with FBOM ( $r = 0.911$ ,  $P < 0.001$ ). There was a general trend of higher GPP with larger standing stocks of autotrophs. Lack of statistical significance between AFDM standing stocks of autotrophs and GPP is due to the high rate of GPP but relatively low AFDM standing stock of autotrophs at Smith Creek (Tables 3 and 4). However, I was unable to collect macrophytes at Smith Creek, which comprised 17% of the streambed habitat (data not shown) and would have contributed to the total standing stock of autotrophs. Gross primary production was not significantly related to either streamwater NO<sub>3</sub>-N concentration ( $r^2 = 0.145$ ,  $P = 0.222$ ) or to PAR ( $r^2 = 0.106$ ,  $P = 0.301$ ). There were no general, statistically significant relationships between R and stream hydraulic characteristics, organic matter standings stocks, or streamwater chemistry.

### *NO<sub>3</sub>-N spiraling*

Uptake length varied from 104 m (Sammy Creek in July) to over 4 km (4548 m, Greenbrier Creek in 2003) (Table 5). However, much of that variation was due to the particularly long  $S_w$  at Greenbrier Creek in 2003 and the next longest  $S_w$  was 1088 m at Hagar Creek in 2003. Streamwater NO<sub>3</sub>-N concentration did not dictate  $S_w$  ( $r^2 = 0.309$ ,  $P = 0.061$ ) nor was  $S_w$  significantly related to discharge ( $r^2 = 0.070$ ,  $P = 0.407$ ) (Figure 1).

There was a 30-fold difference in  $v_f$  among streams, varying from 0.06 (Stonecrop Creek) to 1.80 mm min<sup>-1</sup> (Purgatory Creek) (Table 5). There was no apparent relationship between  $v_f$  and NO<sub>3</sub>-N concentration ( $r^2 = 0.005$ ,  $P = 0.826$ ; Figure 2) but  $v_f$  was significantly correlated with discharge ( $r = 0.736$ ,  $P = 0.006$ ). Uptake velocity appeared to be driven, in part, by primary production because  $v_f$  was positively related to both the standing stock of autotrophs and GPP (Figure 2). Uptake velocity was significantly related to the AFDM standing stock of autotrophs ( $r^2 \geq 0.747$ ,  $P \leq 0.001$ ) and

to chl *a* standing stocks associated with both epilithon and FBOM ( $r \geq 0.750$ ,  $P \leq 0.005$ ). Uptake velocity was also significantly related to GPP ( $r^2 = 0.580$ ,  $P = 0.004$ ). However, the relationship was leveraged by high rates of GPP and high  $v_f$  at Purgatory Creek and Smith Creek (Figure 2) and was not statistically significant when these points were not included in the analysis ( $r^2 = 0.041$ ,  $P = 0.573$ ). Uptake velocity was also significantly but negatively related to R ( $P = 0.001$ ). However, the relationship was not very strong ( $r^2 = 0.424$ ). There was a trend of increasing  $v_f$  with higher PAR, however this relationship was not statistically significant ( $r = 0.408$ ,  $P = 0.108$ ).

Uptake varied considerably among streams and was exceptionally high at Smith Creek and Greenbrier Creek in 2002 ( $\geq 1000 \mu\text{g m}^{-2} \text{min}^{-1}$ ) whereas uptake varied from 1 to  $179 \mu\text{g m}^{-2} \text{min}^{-1}$  among all other streams (Table 5). There were few statistically significant relationships between  $U$  and measured stream characteristics owing to the particularly high  $U$  at Smith Creek and Greenbrier Creek in 2002. However,  $U$  was significantly related to streamwater  $\text{NO}_3\text{-N}$  concentration, N:P, and GPP ( $r^2 \geq 0.517$ ,  $P \leq 0.008$ ). Relationships between  $U$  and streamwater  $\text{NO}_3\text{-N}$  concentration and GPP were influenced by the high  $U$  at Smith Creek and Greenbrier Creek in 2002 and there was considerable scatter around the relationships (Figure 3). However, multiple regression analysis indicated that a linear combination of both streamwater  $\text{NO}_3\text{-N}$  and GPP explained more than 80% of the variance in  $U$  ( $R^2 = 0.803$ ,  $P = 0.001$ ).

## **Discussion**

### *Organic matter standing stocks and whole-stream metabolism*

I observed strong correlations between the standing stocks of autotrophs in these streams and both PAR and discharge. There was a general trend of increasing GPP with higher PAR, although this relationship was not statistically significant. However, GPP was significantly related to discharge. The strong correlations between discharge and both the standing stock of autotrophs and GPP is interesting and may indirectly reflect a gradient of light availability. With the exception of the high PAR values at Purgatory Creek and Hagar Creek in 2002, PAR was correlated with discharge. Purgatory Creek and Smith Creek had the highest discharge and rates of GPP. Purgatory Creek is located within a forested catchment with a well-developed canopy yet has a wide channel

allowing a high input of solar radiation (S. R. Earl, personal observation). The Smith Creek catchment supports both grazing and agriculture and there was little riparian cover along the study reach (S. R. Earl, personal observation). These results suggest that autotrophic biomass and, to a lesser extent, primary production in all other study streams were generally limited by light rather than nutrients. Nutrients have been shown to stimulate both algal standing stocks and primary production (Guasch et al. 1995), however only when light is not limiting (Lowe et al. 1986, Hill et al. 1992). In a comparison of 18 southern Appalachian headwater streams draining catchments of contrasting land use, McTammany (2004) reported that GPP was correlated with light but not DIN.

Although stream size and light availability accounted for much of the variance in the standing stock of autotrophs and GPP, the standing stock of all other biotic compartments and R were unrelated to each other and measured stream characteristics. Previous studies have documented relationships between R and streamwater nutrient concentrations (Mulholland et al. 2001), temperature (Bott et al. 1985, Sinsabaugh 1997), and transient storage (Fellows et al. 2001, Mulholland et al. 2001). With the exception of the unusually low R at Sammy Creek in May, R rates in these study streams were within the range of those reported for other low-order streams (e.g., Young and Huryn 1998, Fellows et al. 2001, Mulholland et al. 2001). However, excluding Sammy Creek in May, R varied < 4-fold across streams (compared to a 124-fold variation in GPP) and the lack of variation may have limited my ability to detect relationships.

#### *NO<sub>3</sub>-N spiraling*

Hydraulic characteristics determined from solute transport modeling of conservative tracer releases varied considerably but were within the range of reported values for other low-order streams (e.g., Butturini and Sabater 1999, Valett et al. 2002, Webster et al. 2003). Despite the range of values, NO<sub>3</sub>-N spiraling was generally unrelated to transient storage. Although transient storage has been shown to influence stream nutrient retention (e.g., Valett et al. 1996, Mulholland et al. 1997), these relationships are less apparent in studies involving many streams, where the influence of other variables may mask that of transient storage (Hall et al. 2002, Webster et al. 2003).

My results suggest that demand for  $\text{NO}_3\text{-N}$ , expressed as  $v_f$ , was driven primarily by primary production. These results are consistent with other studies that have documented relationships between  $\text{NO}_3\text{-N}$  demand and algal abundance (Martí et al. 1997) and primary production (Hall and Tank 2003). In contrast, the relationship between  $v_f$  and  $R$  was not as strong and was opposite in sign to that expected. Heterotrophic demand for inorganic N may have been largely satisfied by ammonium whereas demand for  $\text{NO}_3\text{-N}$  was limited except when inorganic N concentrations were very low or were fueled by relatively higher rates of GPP. Metabolic demand for inorganic N can be met by either ammonium or nitrate, however ammonium is preferentially assimilated due to lower energy requirements (Mulholland et al. 2000, Wetzel 2001). Nitrate uptake was greatest in a Tennessee mountain stream in spring when GPP was highest and ammonium availability was insufficient to meet metabolic demand (Mulholland et al. 2000). Similarly, GPP explained 75% of the variation in nitrate- $v_f$  whereas ammonium- $v_f$  was related to both GPP and  $R$  in streams of the Grand Teton National Park in Wyoming (Hall and Tank 2003). Gross primary production explained 58% percent of the variance in  $v_f$ , which is lower than the 75% reported by Hall and Tank (2003). However, GPP in these study streams was low relative to rates reported for other low-order streams (e.g., Young and Huryn 1998, Fellows et al. 2001, Hall and Tank 2003). A stronger relationship between  $v_f$  and GPP may have been evident given higher rates of GPP.

I observed a strong relationship between demand for  $\text{NO}_3\text{-N}$  (i.e.,  $v_f$ ) and GPP, however the evidence suggests more complex controls on  $U$ . Uptake was significantly and positively related to GPP and streamwater  $\text{NO}_3\text{-N}$ , however these relationships were influenced by the exceptionally high  $U$  at Smith Creek and/or Greenbrier Creek in 2002. In addition to high  $U$ , both of these streams were characterized by relatively high rates of GPP, large standing stocks of autotrophs, and high streamwater  $\text{NO}_3\text{-N}$  concentration. The combination of these features is conducive to high  $U$  but may not be evident when analyzed independently. For example, GPP was very high at Purgatory Creek and streamwater  $\text{NO}_3\text{-N}$  was very high at Greenbrier Creek in 2003 yet  $U$  measured in each of these streams was substantially lower than  $U$  measured at Smith Creek and Greenbrier Creek in 2002. The high standing stock of autotrophs and rate of GPP at Purgatory Creek likely fueled demand for inorganic N yet  $U$  was limited by low N-availability

(streamwater  $\text{NO}_3\text{-N} = 46 \mu\text{g L}^{-1}$ ). Hence, the highest  $v_f$  but low  $U$  at Purgatory Creek. Benthic algae were reported to be N limited when concentrations were below  $55 \mu\text{g NO}_3\text{-N L}^{-1}$  in a desert stream (Grimm and Fisher 1986) and below  $100 \mu\text{g NO}_3\text{-N L}^{-1}$  in an Ozark (USA) stream (Lohman et al. 1991). In contrast, high N concentration but low demand (i.e., low rate of GPP) at Greenbrier Creek in 2003 corresponded to relatively low  $U$  and, particularly low,  $v_f$ . Thus,  $U$  reflects a combination of both demand for and supply of  $\text{NO}_3\text{-N}$ . A linear combination of both streamwater  $\text{NO}_3\text{-N}$  and GPP explained 80% of the variation in  $U$  across these study streams that spanned a broad range of N concentration and stream characteristics.

Solute transport in streams is a function of hydraulic properties and previous studies have documented a positive relationship between discharge and N uptake length (Butterini and Sabater 1998, Wollheim et al. 2001, Hall et al. 2002). Uptake length was not dictated by discharge in these study streams. All of the streams included in the study were small, headwater streams and there may not have been enough variability to detect a relationship between discharge and  $S_w$ . However, rates of nutrient processing by resident biota may offset the predicted relationship between  $S_w$  and discharge based on hydraulic principles (Wollheim et al. 2001). Hall and Tank (2003) found no significant relationships between width-specific discharge and nitrate or ammonium uptake lengths even though stream discharge varied more than 57-fold. They suggested that high rates of GPP in large streams shortened N uptake lengths. The standing stock of autotrophs and rates of GPP were correlated to discharge in these study streams and  $\text{NO}_3\text{-N}$  uptake by autotrophs likely contributed to shorter uptake lengths than would have been expected given the higher discharge. These relationships further support the influence of biological processes on  $\text{NO}_3\text{-N}$  spiraling in these study streams.

### *Synthesis*

Respiration far exceeded rates of GPP yet my results suggest that  $\text{NO}_3\text{-N}$  demand was largely driven by primary production. Nitrogen enrichment has been shown to increase rates of organic matter decomposition (Meyer and Johnson 1983, Suberkropp and Chauvett 1995) and microbial respiration (Howarth and Fisher 1976). However, other studies have indicated that decomposition was not N limited (Triska and Sedell

1976, Royer and Minshall 2001). The high rates of respiration but lack of correlation to streamwater nutrient concentrations suggests that heterotrophic microbes in these study streams were not N limited. Rather, rates of decomposition may be limited by carbon quality (Melilo et al. 1983, Royer and Minshall 2001). In which case, heterotrophs would be less likely to reduce nitrate to satisfy metabolic demand for inorganic N. In contrast, autotrophs may be less energetically constrained and able to reduce nitrate to meet metabolic demand for inorganic N when light is sufficient (Gutschick 1981).

Although I did not quantify ammonium dynamics, my findings support a conceptual model of inorganic N demand in stream ecosystems proposed by Hall and Tank (2003). According to the model, ammonium is assimilated by both autotrophs and heterotrophs but  $\text{NO}_3^-$  is primarily assimilated by autotrophs. Their model was based on an analysis of net heterotrophic but high-light streams in Wyoming, USA. Respiration dominates metabolism of forested streams in the eastern USA and autotrophic demand for dissolved nutrients is generally less important relative to other factors such as allochthonous organic matter (Webster et al. 1983). Stream assays spanned April through October in each of the two years but did not include collections in autumn following leaf fall. Allochthonous input in autumn dominates the organic matter budget of streams in deciduous forest (Fisher and Likens 1973). Mulholland et al. (1985) reported that phosphorus uptake lengths were shortest in a Tennessee mountain stream in November when the standing stock of CBOM was highest and that uptake lengths increased following storms that reduced organic matter standing stocks. It is unclear if my results would have differed had the analysis included post leaf-fall assays.

In an analysis of over 300 surface water sites, Smith et al. (1987) reported that an increase in the concentration of nitrate was the most pronounced observed change compared to more than 20 other water quality variables. Results of this and other studies (Bernhardt et al. 2003, Hall and Tank 2003) suggest that in-stream photoautotrophy is an important biological process contributing to nitrate uptake and, potentially, in ameliorating downstream fluxes of nitrate.

## Literature Cited

- American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater, 20th edition. APHA, New York.
- Bencala, K. E., and R. A. Walters. 1983. Simulation of solute transport in a mountain pool-and-riffle stream: a transient storage model. *Water Resources Research* 19:718-724.
- Bencala, K. E., D. M. McKnight, and G. W. Zellweger. 1990. Characterization of transport in an acidic metal-rich mountain stream based on a lithium tracer injection and simulations of transient storage. *Water Resources Research* 26:989-1000
- Bernhardt, E. S., G. E. Likens, D. C. Buso, and C. T. Driscoll. 2003. In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. *Proceedings of the National Academy of Sciences* 100:10304-10308.
- Bott, T. L., J. T. Brock, C. S. Dunn, R. J. Naiman, R. W. Ovink, and R. C. Peterson. 1985. Benthic community metabolism in four temperate stream systems: an inter-biome comparison and evaluation of the river continuum concept. *Hydrobiologia* 123:3-45.
- 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.
- Butturini, A., and F. Sabater. 1998. Ammonium and phosphate retention in a Mediterranean stream: hydrologic versus temperature control. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1938-1945
- Butturini, A., and F. Sabater. 1999. Importance of transient storage zones for ammonium and phosphate retention in a sandy-bottom Mediterranean stream. *Freshwater Biology* 41:593-603.
- Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559-568.
- Davis, J. C., and G. W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia* 119:247-255
- Delong, M. D., and M. A. Brusven. 1994. Allochthonous input of organic matter from different habitats of an agriculturally impacted stream. *Environmental Management* 18:59-71.

- 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. Morall, 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.
- Elmore, H. L., and W. F. West. 1961. Effect of water temperature on stream reaeration. *Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers* 87:59-71.
- Fellows, C. S., H. M. Valett, and C. N. Dahm. 2001. Whole-stream metabolism in two montane streams: contribution of the hyporheic zone. *Limnology and Oceanography* 46:523-531.
- Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs* 43:421-439.
- Guasch, H., E. Martí, and S. Sabater. 1995. Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology* 33:373-383.
- Gutschick, V. P. 1981. Evolved strategies in nitrogen acquisition by plants. *American Naturalist* 118:607-637.
- Grimm, N. B., and S. G. Fisher. 1984. Exchange between interstitial and surface water: implications for stream metabolism and nutrient cycling. *Hydrobiologia* 111:219-228.
- Grimm, N. B., and S. G. Fisher. 1986. Nitrogen limitation in a Sonoran Desert stream. *Journal of the North American Benthological Society* 5:2-15.
- Hall, R. O., Jr., E. S. Bernhardt, and G. E. Likens. 2002. Relating nutrient uptake with transient storage in forested mountain streams. *Limnology and Oceanography* 47:255-265.
- Hall, R. O., Jr. 2003. A stream's role in watershed nutrient export. *Proceedings of the National Academy of Sciences* 100:10137-10138.
- Hall, R. O., Jr., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography* 48:1120-1128.
- Harvey, J. W., B. J. Wagner, and K. E. Bencala. 1996. Evaluating the reliability of the stream tracer approach to characterize stream-subsurface water exchange. *Water Resources Research* 32:2441-2451.

- Hill, W. R., H. L. Boston, and A. D. Steinman. 1992. Grazers and nutrients simultaneously limit lotic primary productivity. *Canadian Journal of Fisheries and Aquatic Sciences* 49:504-512.
- Howarth, R. W., and S. G. Fisher. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. *Freshwater Biology* 6:221-228.
- Lohman, K., Jones, J. R., and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. *Journal of the North American Benthological Society* 19:14-23.
- Lowe, R. L., S. W. Golladay, and J. R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *Journal of the North American Benthological Society* 5:221-229.
- Martí, E., N. B. Grimm, and S. G. Fisher. 1997. Pre- and post-flood retention efficiency of nitrogen in a Sonoran desert stream. *Journal of the North American Benthological Society* 16:1249-1265.
- Marzolf, E. R., P. J. Mulholland, and A. D. Steinman. 1994. Improvements to the diurnal upstream-downstream dissolved oxygen technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences* 51:1591-1599.
- McTammany, M. E. 2004. Recovery of southern Appalachian streams from historical agriculture. Doctoral dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
- Melillo, J. M., J. D. Aber, and J. F. Muratore. 1983. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63:621-626.
- Meyer, J. L., C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biology* 13:177-183.
- Morrice, J.A., H.M. Valett, C.N. Dahm, and M.E. Campana. 1997. Alluvial characteristics, groundwater-surface water exchange, and hydrologic retention in headwater streams. *Hydrological Processes* 11:253-267.
- Mulholland, P. J., J. D. Newbold, J. W. Elwood, L. A. Ferren, and J. R. Webster. 1985. Phosphorus spiralling in a woodland stream: seasonal variations. *Ecology* 66:1012-1023.
- Mulholland, P. J., E. R. Marzolf, J. R. Webster, D. R. Hart, and S. P. Hendricks. 1997. Evidence that hyporheic zones increases 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. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a  $^{15}\text{N}$  tracer addition. *Ecological Monographs* 70:471-493.
- Mulholland, P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Martí, L. 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.
- Murphy, J., and J. P. Riley. 1962. Determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.
- Naiman, R. J., and M. G. Turner. 2000. A future perspective on North America's freshwater ecosystems. *Ecological Applications* 10:958-970.
- Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 38:860-863.
- Odum, H. T. 1956. Primary production in flowing waters. *Limnology and Oceanography* 1:102-117.
- 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.
- Peterson, C. G., and N. B. Grimm. 1992. Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. *Journal of the North American Benthological Society* 11:20-36.
- Royer, T. V., and G. W. Minshall. 2001. Effects of nutrient enrichment and leaf quality on the breakdown of leaves in headwater streams. *Freshwater Biology* 46:603-610.
- Runkel, R.L. 1998. One dimensional transport with inflow and storage (OTIS): a solute transport model for streams and rivers: U.S. Geological Survey Water-Resources Investigation Report 98-4018.
- Sabater, F., A. Butturini, E. Martí, I. Muñoz, A. Romani, J. Wray, and S. Sabater. 2000. Effects of riparian vegetation removal on nutrient retention in a Mediterranean stream. *Journal of the North American Benthological Society* 19:609-620.
- Sinsabaugh, R. L. 1997. Large-scale trends for stream benthic respiration. *Journal of the North American Benthological Society* 16:119-122.

- Smith, R. A., R. B. Alexander, and M. G. Wolman. 1987. Water quality trends in the nation's rivers. *Science* 235:1607-1615.
- Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100:179-196.
- Solorzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* 14:779-801.
- Steinman, A. D., and G. A. Lamberti. 1996. Biomass and pigments of benthic algae. Pages 295-314 in F. R. Hauer and G. A. Lamberti, editors. *Methods in stream ecology*. Academic Press, San Diego.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95-119.
- Suberkropp, K., and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology* 76:1433-1445.
- Tank, J. L., J. L. Meyer, D. M. Sanzone, P. J. Mulholland, J. R. Webster, B. J. Peterson, W. M. Wollheim, and N. E. Leonard. 2000. Analysis of nitrogen cycling in a forest stream during autumn using a  $^{15}\text{N}$  tracer addition. *Limnology and Oceanography* 45:1013-1029.
- Triska, F. J., and J. R. Sedell. 1976. Decomposition of four species of leaf litter in response to nitrate manipulation. *Ecology* 57:783-792.
- U.S. Environmental Protection Agency (USEPA). 1997a. Method 349.0. Determination of ammonia in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis. Zhang, J. Z., P. B. Ortner, C. J. Fischer, and L. D. Moore in *Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition*. EPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- U.S. Environmental Protection Agency (USEPA). 1997b. Method 365.5. Determination of orthophosphate in estuarine and coastal waters by automated colorimetric analysis. Zimmerman, C. F., and C. W. Keefe in *Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition*. EPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- Valett, H. M., J. A. Morrice, C. N. Dahm, M. E. Campana. 1996. Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography* 41:333-345.

- Valett, H. M., C. L. Crenshaw, and P. F. Wagner. 2002. Stream nutrient uptake, forest succession, and biogeochemical theory. *Ecology* 83:2888-2901.
- Vitousek, P. M., J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alterations of the global nitrogen cycle: causes and consequences. *Issues in Ecology* 1:2-15.
- Wallace, J. B., and A. C. Benke. 1984. Quantification of wood habitat in subtropical coastal plain streams. *Canadian Journal of Fisheries and Aquatic Sciences* 41:1643-1652.
- Wallace, J. B., J. R. Webster, S. E. Eggert, and J. L. Meyer. 2000. Small wood dynamics in a headwater stream. *Internationale Vereinigung für Theoretische und Angewandte Limnologie, Verhandlungen* 27:1361-1365.
- Wanninkhof, R., P. J. Mulholland, and J. W. Elwood. 1990. Gas exchange rates for a first-order stream determined with deliberate and natural tracers. *Water Resources Research* 26:1621-1630.
- 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., M. E. Gurtz, J. J. Hains, J. L. Meyer, W. T. Swank, J. W. Waide, and J. B. Wallace. 1983. Stability of stream ecosystems. Pages 355-395 in J. R. Barnes and G. W. Minshall, editors. *Stream ecology*. Plenum Press, New York.
- Webster, J. R., Mulholland, P. J., J. L. Tank, H. M. Valett, W. K. Dodds, B. J. Peterson, W. B. 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, W. M. Wollheim. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology* 48:1329-1352.
- Wetzel, R. G. 2001. *Limnology lake and river ecosystems*. Third edition. Academic Press, San Diego, CA.
- Wollheim, W. M., B. J. Peterson, L. A. Deegan, J. E. Hobbie, and B. Hooker. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnology and Oceanography* 46:1-13.
- Wood, E. D., F. A. J. Armstrong, and F. A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom* 47:23-31.

Young, R. G., and A. D. Huryn. 1998. Comment: improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1784-1785.

Young, R. G., and A. D. Huryn. 1999. Effects of land use on stream metabolism and organic matter turnover. *Ecological Applications* 9:1359-1376.

Table 1. Characteristics of study streams. Nitrate-nitrogen (NO<sub>3</sub>-N), ammonium-nitrogen (NH<sub>4</sub>-N), and orthophosphate (PO<sub>4</sub>-P) are reach average concentrations prior to releases. N:P represents the atomic ratio calculated from combined concentrations of NO<sub>3</sub>-N and NH<sub>4</sub>-N to PO<sub>4</sub>-P. The lowest detectable limit (3 µg L<sup>-1</sup>) was used to calculate N:P when measured concentrations were below detectable limits (bdl). Streamwater temperature (Temp.) is the average streamwater temperature during the NO<sub>3</sub>-N release at each site. Specific conductance (Specific cond.) reflects reach-average background values. Discharge is the reach average at all transects during the NO<sub>3</sub>-N release. Photosynthetically active radiation (PAR) reflects average photon flux density from dawn to dusk during metabolism measurement.

Stream	Location	Release date	NO <sub>3</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P	N:P	Temp. °C	Specific cond.	Discharge L s <sup>-1</sup>	PAR µmol m <sup>-2</sup> s <sup>-1</sup>
			µg L <sup>-1</sup>					µS cm <sup>-1</sup>		
<b>2002</b>										
Purgatory Creek	Montgomery County, VA	22 April	46	bdl	9	5	15.4	85	42	337
Hagar Creek	Montgomery County, VA	29 April	98	bdl	12	8	13.1	82	7	337
Little Back Creek	Roanoke County, VA	22 June	179	5	11	17	20.3	128	13	15
Powers Branch	Montgomery County, VA	22 July	187	bdl	27	7	20.3	60	7	26
Smith Creek	Montgomery County, VA	11 Sept.	796	na	3	265	15.7	420	38	76
Greenbrier Creek	Roanoke County, VA	7 May	1116	bdl	5	224	15.9	152	16	90
<b>2003</b>										
Sammy Creek (May)	Giles County, VA	1 May	5	5	bdl	3	10.4	21	7	59
Sammy Creek (July)	Giles County, VA	16 July	3	bdl	bdl	2	14.9	19	6	10
Hugh White Creek	Macon County, NC	9 Oct.	3	3	bdl	2	13.1	12	7	2
Hagar Creek	Montgomery County, VA	26 Aug.	179	bdl	7	25	18.3	62	15	4
Stonecrop Creek	Giles County, VA	11 Sept.	294	5	20	16	14.2	262	2	36
Greenbrier Creek	Roanoke County, VA	14 Aug.	984	bdl	5	195	20.7	146	32	34

Table 2. Stream hydraulic characteristics. Values were determined from solute transport modeling of conservative tracer releases. Data are single values for each release derived following parameter optimization.

<b>Stream</b>	<b>Velocity</b> $u, \text{m s}^{-1}$	<b>Dispersion coefficient</b> $D, \text{m}^2 \text{s}^{-1}$	<b>Storage exchange coefficient</b> $\alpha, \text{s}^{-1}$	<b>Storage zone cross section area</b> $A_s, \text{m}^2$	<b>Stream cross section area</b> $A, \text{m}^2$	$A_s/A$
<b>2002</b>						
Purgatory Creek	0.135	0.316	0.00061	0.070	0.310	0.225
Hagar Creek	0.045	0.147	0.00015	0.097	0.155	0.623
Little Back Creek	0.096	0.281	0.00004	0.015	0.140	0.110
Powers Branch	0.087	0.140	0.00013	0.013	0.105	0.128
Smith Creek	0.151	0.343	0.00082	0.049	0.240	0.204
Greenbrier Creek	0.110	0.370	0.00004	0.038	0.155	0.243
<b>2003</b>						
Sammy Creek (May)	0.047	0.115	0.00036	0.197	0.142	1.389
Sammy Creek (July)	0.027	0.100	0.00027	0.392	0.228	1.717
Hugh White Creek	0.048	0.418	0.00020	0.158	0.145	1.091
Hagar Creek	0.057	0.393	0.00010	0.201	0.266	0.757
Stonecrop Creek	0.031	0.114	0.00017	0.053	0.065	0.821
Greenbrier Creek	0.173	0.100	0.00017	0.020	0.185	0.106

Table 3. Organic matter standing stocks. Standing stocks as ash-free dry mass (AFDM) and chlorophyll *a* are habitat-weighted reach averages. Molar C:N ratios are presented for epilithon, bryophytes, FBOM, and CBOM. Total autotrophs represents the combined mass (AFDM) of epilithon, bryophytes, and macrophytes (data not shown). Total benthic detritus represents the combined mass (AFDM) of FBOM and CBOM.

	<b>Epilithon</b>		<b>Bryophytes</b>	<b>Total autotrophs</b>
	g AFDM m <sup>-2</sup> (C:N)	mg chl <i>a</i> m <sup>-2</sup>	g AFDM m <sup>-2</sup> (C:N)	g AFDM m <sup>-2</sup>
<b>2002</b>				
Purgatory Creek	8.1 (11)	13.40	6.1 (19)	14.1
Hagar Creek	0.7 (na)	2.46	1.9 (17)	2.6
Little Back Creek	3.4 (11)	6.92	2.1 (19)	5.4
Powers Branch	1.1 (9)	1.72	0.0	1.1
Smith Creek	5.7 (na)	10.25	0.0	5.7†
Greenbrier Creek	1.7 (10)	5.14	0.0	8.6
<b>2003</b>				
Sammy Creek (May)	0.5 (9)	0.22	6.7 (19)	7.2
Sammy Creek (July)	na (11)	1.04	< 0.1 (na)	0.0†
Hugh White Creek	0.3 (12)	0.15	0.1 (28)	0.4
Hagar Creek	0.6 (11)	0.23	0.0	0.5
Stonecrop Creek	0.4 (13)	0.52	0.0	0.4
Greenbrier Creek	1.8 (15)	3.70	0.0	1.8

na = sample not available

† = missing compartment in the calculation of total autotrophs or total benthic detritus

Table 3. continued.

<b>FBOM</b>		<b>CBOM</b>	<b>Total benthic detritus</b>	<b>SPOM</b>	<b>Wood</b>
g AFDM m <sup>-2</sup> (C:N)	mg chl <i>a</i> m <sup>-2</sup>	g AFDM m <sup>-2</sup> (C:N)	g AFDM m <sup>-2</sup>	mg AFDM L <sup>-1</sup>	g AFDM m <sup>-2</sup>
23.3 (16)	5.64	35.5 (50)	58.8	0.6	113
46.0 (18)	2.05	25.7 (47)	71.8	1.2	1879
46.4 (19)	2.14	45.4 (77)	91.8	3.4	148
49.5 (17)	0.88	103.4 (105)	152.9	7.2	55
25.7 (na)	6.61	na	25.7†	0.8	310
19.0 (15)	2.46	54.9 (40)	73.9	5.1	45
0.5 (17)	0.01	315.7 (58)	316.2	na	0
0.0	0.00	243.8 (62)	243.8	0.3	79
30.4 (20)	0.06	264.6 (76)	295.0	2.3	380
15.8 (16)	0.43	32.7 (44)	48.5	2.4	61
150.9 (23)	2.34	208.2 (37)	359.0	2.4	137
30.5 (15)	1.08	48.9 (51)	79.4	12.8	806

Table 4. Whole-stream metabolism. Rates of gross primary production (GPP) and respiration (R) are based on 24-h measures of open-system oxygen balance.

Stream	GPP	R	NEP	P:R
	g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup>			
<b>2002</b>				
Purgatory Creek	0.73	1.87	-1.14	0.389
Hagar Creek	0.12	5.25	-5.13	0.023
Little Back Creek	0.13	1.73	-1.60	0.077
Powers Branch	0.03	2.10	-2.08	0.012
Smith Creek	1.24	2.72	-1.48	0.455
Greenbrier Creek	0.29	2.81	-2.53	0.101
<b>2003</b>				
Sammy Creek (May)	0.06	0.03	0.03	1.900
Sammy Creek (July)	0.01	2.15	-2.14	0.005
Hugh White Creek	0.01	5.53	-5.52	0.002
Hagar Creek	0.21	5.83	-5.63	0.035
Stonecrop Creek	0.06	5.25	-5.19	0.011
Greenbrier Creek	0.18	5.02	-4.85	0.036

Table 5. Spiraling metrics. Standard errors (SE) are presented for  $S_w$  at all sites except Greenbrier Creek in 2003.

<b>Stream</b>	<b><math>S_w</math> (<math>\pm</math>SE)</b> m	<b><math>v_f</math></b> mm min <sup>-1</sup>	<b><math>U</math></b> $\mu\text{g m}^{-2} \text{min}^{-1}$
<b>2002</b>			
Purgatory Creek	373 (72)	1.80	82
Hagar Creek	419 (26)	0.32	32
Little Back Creek	410 (60)	0.98	176
Powers Branch	815 (270)	0.32	61
Smith Creek	665 (146)	1.52	1212
Greenbrier Creek	467 (62)	0.90	1000
<b>2003</b>			
Sammy Creek (May)	168 (37)	0.89	4
Sammy Creek (July)	104 (8)	0.62	2
Hugh White Creek	223 (5)	0.29	1
Hagar Creek	1088 (217)	0.24	42
Stonecrop Creek	1047 (158)	0.06	16
Greenbrier Creek	4548 (na)	0.18	179

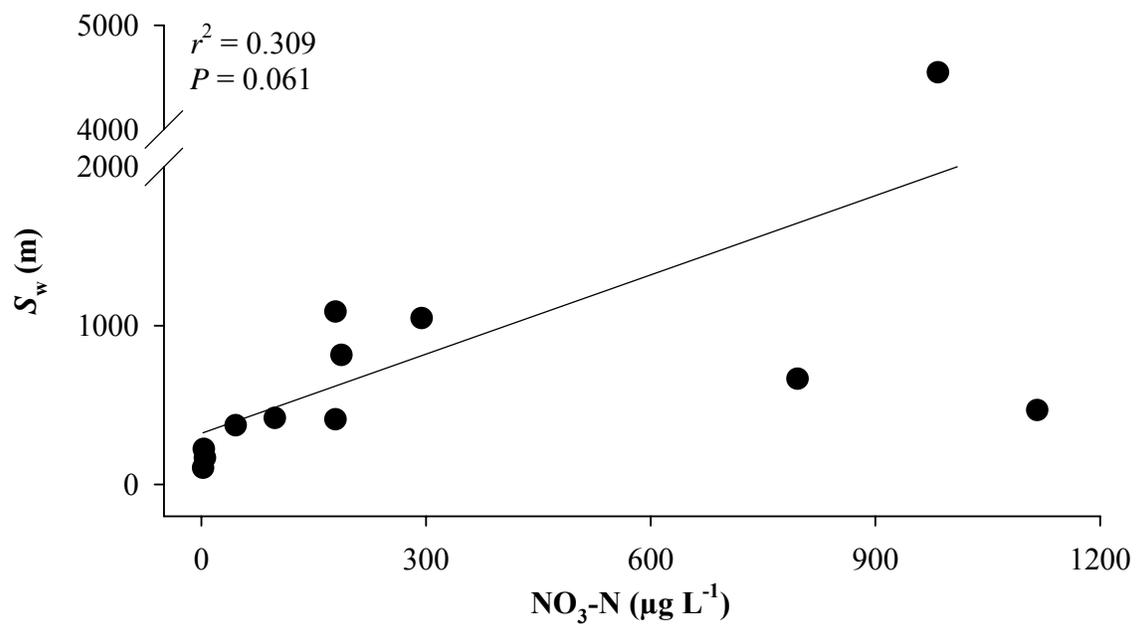
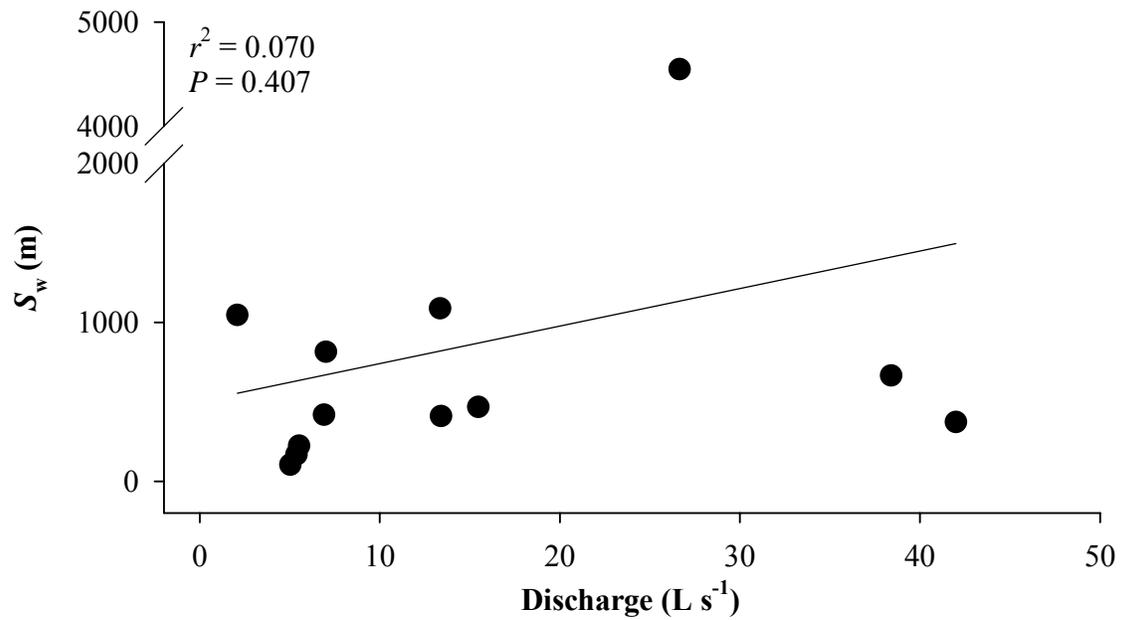


Figure 1. Relationships between  $S_w$  and select stream characteristics. Discharge is the reach average at all transects during the  $NO_3-N$  release. Nitrate-nitrogen ( $NO_3-N$ ) is the reach average background concentration.

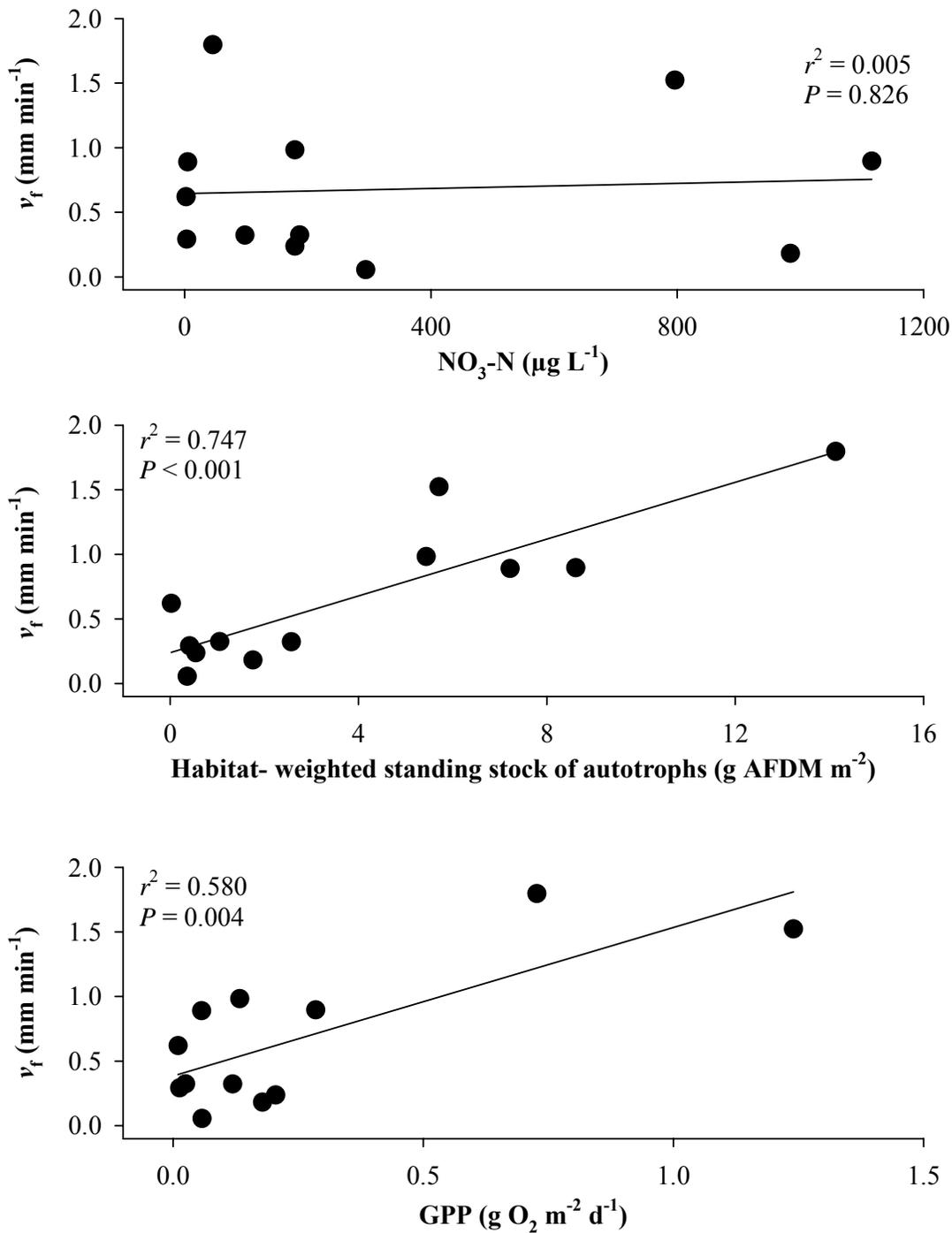


Figure 2. Relationships between  $v_f$  and select stream characteristics. Nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) is the reach average background concentration. Habitat-weighted standing stock of autotrophs reflects the combined mass (g AFDM m<sup>-2</sup>) of epilithon, bryophytes, and macrophytes. Gross primary production (GPP) is based on 24-h measures of open-system oxygen balance.

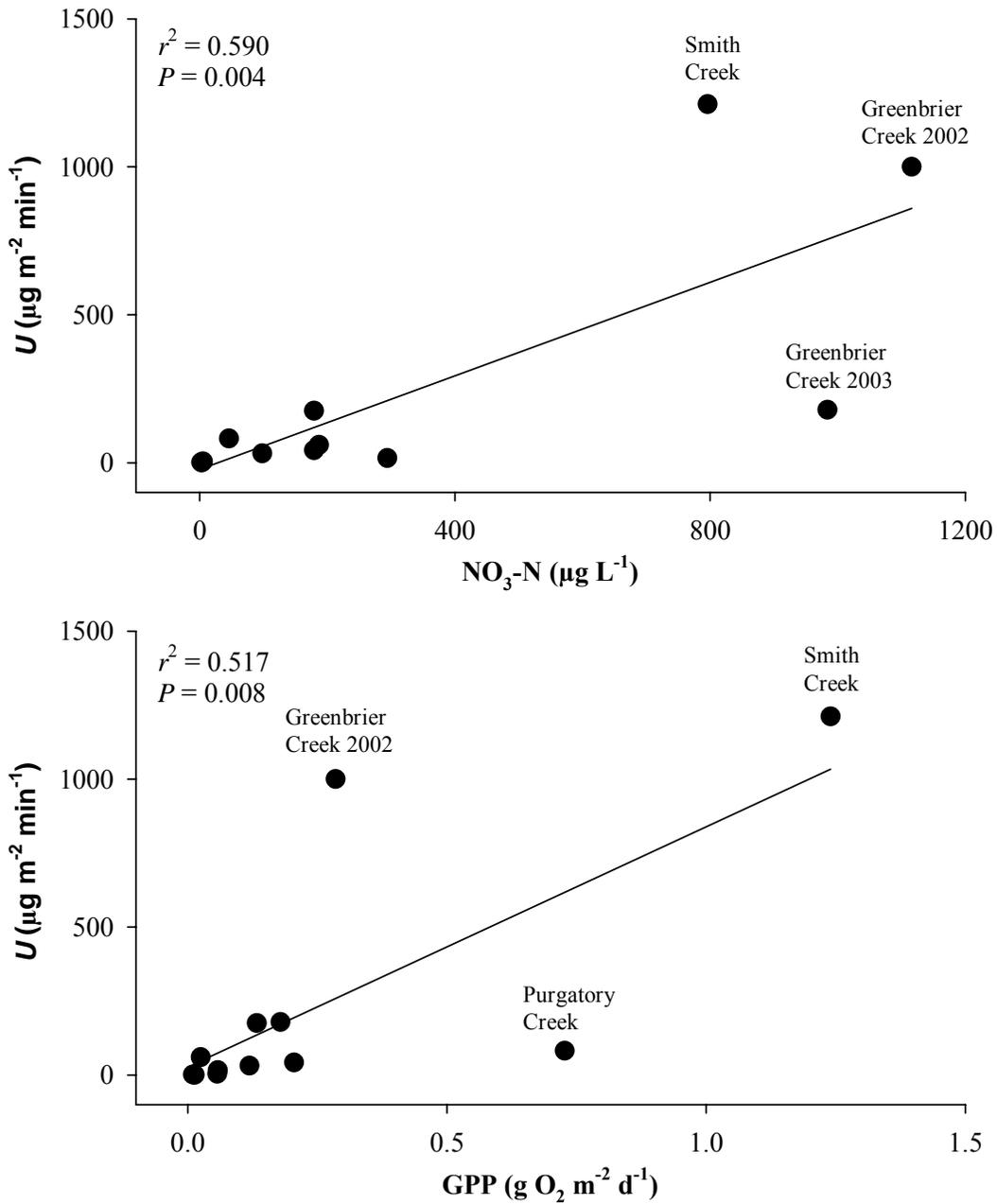


Figure 3. Relationships between  $U$  and select stream characteristics. Nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) is the reach average background concentration. Gross primary production (GPP) is based on 24-h measures of open-system oxygen balance. Site names corresponding to outlying points are identified in each panel.

### **Chapter 3: An analysis of nitrogen spiraling and saturation in stream ecosystems using a multiple enrichment approach**

#### **Abstract**

Nitrogen (N) cycling in headwater streams has received considerable attention due, in part, to their potential to serve as control points in the export of N from terrestrial landscapes. However, N uptake and N uptake efficiency may change in response to increased N loading. Identifying conditions that lead to saturation of N uptake capacity in stream ecosystems is critical to our understanding of how streams process inputs of anthropogenic N. I proposed a series of saturation response types based on Michaelis-Menten uptake kinetics, which were used to characterize the proximity of a stream to a point of N saturation. I conducted a series of short-term nutrient releases using a stable isotope tracer ( $^{15}\text{NO}_3\text{-N}$ ) to measure nitrate uptake while streamwater nitrate concentration was incrementally elevated. Experiments were conducted in streams spanning a gradient of background N concentration (3 to 983  $\mu\text{g NO}_3\text{-N L}^{-1}$ ). In response to experimental N amendments, uptake length increased and uptake velocity decreased in all streams, generally corresponding to predicted responses based on Michaelis-Menten kinetics. Uptake increased with increasing N amendments in four of six experiments, indicating that these streams were not N saturated. There was no statistically significant change in uptake with increasing N amendment in two of the study streams, suggesting that these streams were N saturated. Proximity to saturation was generally correlated to N concentration but was also related to the ratio of N:P. Ambient uptake velocity varied from 0.09 to 2.80  $\text{mm min}^{-1}$  across streams and was highest in the unsaturated, low-N streams. Ambient uptake varied from 5 to 218  $\mu\text{g m}^{-2} \text{min}^{-1}$  and was generally correlated to streamwater DIN concentration. My results suggest that N saturation in southern Appalachian headwater streams may occur at relatively high N concentrations but that the efficiency of N uptake is inversely related to N concentration. Data from this and other studies suggests that uptake velocity is consistently low in streams with  $\text{NO}_3\text{-N}$  concentrations greater than ca. 100  $\mu\text{g L}^{-1}$ . These patterns suggest that the efficiency of N removal in streams is impaired with even moderate increases in streamwater  $\text{NO}_3\text{-N}$  concentration.

## Introduction

Because they represent the initial and most extensive interface between terrestrial and aquatic ecosystems, low-order streams may serve as important control points (sensu Hedin et al. 1998) in the export of nitrogen (N) to more downstream aquatic environments (Alexander et al. 2000). These streams do not act merely as pipelines for the downstream transport of material. Instead, they have the potential to retain, transform, and remove organic and inorganic materials through in-stream biological processes (Peterson et al. 2001). Due to their potential as retainers and transformers of dissolved inorganic nitrogen (DIN), N cycling in headwater streams has received considerable attention. Numerous studies have investigated the influence of hydraulic (e.g., Wollheim et al. 2001), geomorphic (e.g., Munn and Meyer 1990), and biological (e.g., Hall and Tank 2003, Webster et al. 2003) controls on N uptake in headwater streams. However, N loading may alter N uptake and uptake efficiencies and little is known about the relationships between N availability and N retention capacity in streams.

Solutes in streams are simultaneously affected by processes that assimilate and transform nutrients and those physical forces that cause the downstream transport of dissolved and suspended material. The nutrient spiraling concept (Webster and Patten 1979) incorporates both the downstream transport and the cycling of nutrients within streams and provides the appropriate conceptual and technical framework for measuring nutrient dynamics in streams (Newbold et al. 1981). Three commonly measured indices are used to describe spiraling in streams. Uptake length ( $S_w$ ) is the average distance traveled as an inorganic solute in the water column before being immobilized. Uptake length is a powerful descriptor of nutrient demand, however it is strongly influenced by stream hydraulic features. Uptake velocity ( $v_f$ ) represents the theoretical speed at which a nutrient moves toward a sink and standardizes for stream depth and velocity. Thus, it provides a meaningful descriptor to compare across streams (Davis and Minshall 1999). Uptake velocity reflects both nutrient uptake and supply (as concentration), thus serves as a descriptor of nutrient uptake efficiency. Areal specific uptake ( $U$ ) quantifies the mass of nutrient immobilized per area of streambed per unit time (Stream Solute Workshop 1990).

Because nutrient retention reflects the capacity for a stream to use available nutrients, retention efficiency should vary with nutrient concentration. When a nutrient is available in short supply compared to biotic needs (i.e., limiting), biotic assimilation will maintain low concentration. Uptake may increase with increasing concentration of the limiting nutrient. Under these conditions, uptake efficiency, as uptake relative to supply, reflected in  $v_f$ , will be high. Nutrient saturation occurs when the rate of biological processes are no longer limited by the availability of the nutrient (Aber et al. 1998). As nutrient concentration increases from limiting to saturating, abrupt changes in uptake (Bothwell 1989) and, correspondingly, uptake-length and -velocity may occur (Davis and Minshall 1999). When biota are nutrient-saturated, uptake is maximal and is unaffected by increasing nutrient concentration. At the same time, uptake efficiency declines with increasing nutrient concentration.

*Coupling spiraling and uptake kinetics*

The Michaelis-Menten (M-M) model (equation 1) is an asymptotic function that was originally developed to model enzyme kinetics but has proven to be robust in describing nutrient uptake as a function of concentration at a broad range of scales.

$$U = \frac{U_{\max} C}{C + K_m} \quad (1)$$

where:

$U$  = uptake

$C$  = nutrient concentration

$K_m$  = half-saturation constant

$U_{\max}$  = maximum uptake

For example, this model has been used successfully to describe nutrient uptake by algal monocultures (Dortch et al. 1991), diatom communities (Bothwell 1989), and stream reach ecosystems (Dodds et al. 2002). According to the M-M model, uptake increases with increasing nutrient concentration until asymptotically approaching a point of maximum uptake ( $U_{\max}$ ). Maximum uptake and the half-saturation constant ( $K_m$ ); the

concentration at which uptake is one-half of  $U_{\max}$ ) vary widely among organisms and in response to environmental conditions, and together provide insightful indices of organismal or system affinity for a nutrient.

Because the spiraling metrics ( $S_w$ ,  $v_f$ , and  $U$ ) are mathematically related, applying the M-M model to  $U$  also dictates how  $S_w$  and  $v_f$  will respond to changing nutrient concentration (Figure 1). The uptake velocity ( $v_f$ ) is described by a non-linear decrease with increasing nutrient concentration according to the following equation:

$$v_f = \frac{U_{\max}}{K_m + C} \quad (2)$$

At the same time,  $S_w$  increases linearly with nutrient concentration according to the following equation:

$$S_w = \frac{ud}{U_{\max}} C + \frac{udK_m}{U_{\max}} \quad (3)$$

where  $u$  and  $d$  are streamwater velocity and depth, respectively. Both  $K_m$  and  $U_{\max}$  can be derived from relationships between nutrient concentration and any of the spiraling metrics ( $S_w$ ,  $v_f$ ,  $U$ ) as described in equations (1), (2), and (3).

Empirical estimates of the relationship between nutrient concentration and uptake are prerequisite in order to determine  $K_m$  and  $U_{\max}$ . Typically, they are determined in batch (e.g., Carpenter and Guillard 1971) or mesocosm (e.g., Bothwell 1989) experiments. Because of the technical difficulties of measuring nutrient uptake and, particularly, manipulating nutrient concentration, nutrient uptake kinetics have rarely been addressed empirically for entire ecosystems (but see Dodds et al. 2002). However, the advective characteristics of streams and the nutrient spiraling concept provide the framework to quantify instantaneous, *in situ* nutrient uptake at the whole-ecosystem level. Spiraling can be coupled to experimental nutrient additions to characterize uptake responses to changing nutrient concentration, facilitating investigation of ecosystem-level nutrient uptake kinetics.

### *Saturation, spiraling, and stream response types (SRTs)*

The M-M model predicts a continuum of uptake response from no uptake when nutrient concentration is zero to maximum uptake when the nutrient concentration meets or exceeds metabolic demand. However, the continuum can be segregated into a series of discrete, recognizable zones. At low concentrations, when nutrient concentration is well below saturating conditions (i.e.,  $C \ll K_m$ ), uptake approximates a linear relationship with increasing nutrient concentration (Kim et al. 1990). As nutrient concentration increases to near-saturating concentrations,  $U$  approaches asymptotic plateau. When nutrient concentration meets or exceeds metabolic demand, the system is saturated and the relationship between nutrient concentration and uptake has a slope approximately equal to zero. I contend that these regions of the M-M curve can be abstracted to represent three distinct stages of response to increasing nutrient concentration. These saturation response types (SRTs; Figure 2) correspond to a stream's proximity to uptake saturation. Thus, SRTs are delineated as: (1) Type I – exhibiting a constant, positive, linear slope ( $\beta > 0$ ), indicating a stream that is well below saturation, (2) Type II – uptake increasing to an asymptotic plateau, indicating a stream that is approaching saturation, and (3) Type III – systems where the slope relating nutrient concentration and uptake is not significantly different from zero ( $\beta = 0$ ), indicating a stream that is saturated.

Enrichment of terrestrial landscapes is leading to increased N loading to aquatic systems. Aber et al. (1989, 1998) proposed the N-saturation hypothesis whereby anthropogenic N saturates forest uptake processes and their phases of N saturation that are conceptually similar to the SRTs I propose. Late stages of forest saturation are characterized by enhanced loading to aquatic systems. While headwater streams have been shown to be important sites for processing and retaining nutrients (e.g., Hall 2003), it is unclear how nutrient retention efficiency is related to concentration. It should be the case that just as saturated forests result in enhanced loading to streams, saturated streams contribute to enhanced N loads to downstream reaches. Here I present an analysis of the relationship between streamwater N concentration and uptake in stream ecosystems. I used a stable isotope tracer ( $^{15}\text{N}$ ) coupled to a multiple enrichment approach to determine how N uptake among streams spanning a gradient of chronic DIN concentration responds

to artificial increases in N concentration. My goal was to use the M-M framework and proposed SRTs to identify if and when streams spanning a gradient of DIN concentration are N saturated.

## **Methods**

I examined five first- or second-order headwater streams in the southern Appalachian Mountains of southwestern Virginia and western North Carolina, USA (Table 1). Study streams were selected to span a gradient of background DIN concentration. All streams had intact, forested riparian zones but land use within associated catchments was variable. Two streams (Sammy Creek and Hugh White Creek) were located within forested catchments relatively undisturbed by human activities since the 1920's. Vegetation included mixed deciduous forests with thick streamside canopies of rhododendron (*Rhododendron maximum* L.). Catchments of the other streams were comprised of a mixture of forest, small farms, and residential areas. Study streams were characterized by softwater with the exception of Stonecrop Creek which drained a predominately limestone catchment. I selected a single study reach (50-388 m) in each stream for assessing N uptake. Reach length depended on accessibility, streamwater travel time, and distance appropriate to properly measure N uptake.

### *Solute addition experiments*

I conducted a series (2-4) of short-term (3-5 h) nutrient releases in which streamwater nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) concentration was successively elevated through experimental additions to the study reaches. At the same time,  $^{15}\text{NO}_3\text{-N}$  was added as a tracer and quantified to document uptake response to variable  $\text{NO}_3\text{-N}$  concentrations. Releases were generally conducted on consecutive days except at Greenbrier Creek where four releases were conducted over two days (Table 2). Releases were planned for periods without storms in order to maintain generally similar climatic and hydraulic conditions.

Background samples (i.e., prior to the release) were collected from 4 to 7 transects along the study reach and analyzed for  $\text{NO}_3\text{-N}$ , bromide ( $\text{Br}^-$ ), and  $^{15}\text{NO}_3\text{-N}$ . A subset of

the background samples was analyzed for ambient concentrations of ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and orthophosphate ( $\text{PO}_4\text{-P}$ ).

For the first release, a solution of  $\text{K}^{15}\text{NO}_3$  (98 atom %; Cambridge Isotope Laboratories, Andover, MA, USA),  $\text{NaBr}$ , and  $\text{NaCl}$  ( $\text{Br}^-$  and  $\text{Cl}^-$  to act as conservative tracers, sensu Bencala et al. 1990) was released at a constant rate designed to increase the streamwater  $\delta^{15}\text{NO}_3\text{-N}$  by 500‰. Tracer  $^{15}\text{NO}_3\text{-N}$  additions elevated streamwater  $\text{NO}_3\text{-N}$  concentrations by less than  $0.45 \mu\text{g L}^{-1}$ . Target concentrations for  $\text{Cl}^-$  and  $\text{Br}^-$  were  $2.00$  and  $0.30 \text{ mg L}^{-1}$ , respectively.

Three replicate plateau samples were collected from transects under well-mixed conditions (i.e., tracer plateau as indicated by steady-state conductivity in channel water). Samples for the analysis of  $^{15}\text{NO}_3\text{-N}$  were collected in acid-washed 4-L bottles, chilled until filtered (glass-fiber filters, Whatman GF/F, pore size =  $1.0 \mu\text{m}$ ), and refrigerated ( $\sim 4 \text{ }^\circ\text{C}$ ) for less than 1 week until processed. Additional samples ( $n = 3$  per transect) were collected for analysis of  $\text{NO}_3\text{-N}$  and  $\text{Br}^-$ , filtered (glass-fiber filters, Gelman A/E, pore size =  $1.0 \mu\text{m}$ ), and frozen that day.

Identical methods were employed for subsequent releases at each stream, however  $\text{NaNO}_3$  was added to the release solution in order to increase the streamwater  $\text{NO}_3\text{-N}$  concentration. Enrichment concentrations were variable and ranged from  $\sim 40$  to  $600 \mu\text{g NO}_3\text{-N L}^{-1}$  (Table 2). Target  $\delta^{15}\text{NO}_3\text{-N}$  (i.e., 500‰) was maintained by adjusting the amount of  $\text{K}^{15}\text{NO}_3$  in the release solution as necessary.

Conductivity was continuously monitored at the downstream end of the reach by a data-logging Hydrolab Minisonde (Hydrolab, Inc., Loveland, CO, USA). Streamwater velocity was measured by analyzing conservative tracer curves using an advection-dispersion model with transient storage and inflow (Bencala and Walters 1983, Runkel 1998). Discharge ( $Q$ ) at each transect was determined from the dilution of the conservative tracer ( $\text{Br}^-$ ) and assumed to be temporally constant throughout background and plateau sampling. Widths and depths of the wetted channel were measured at 5-10 m intervals along the study reach prior to or following the series of releases.

### *Laboratory methods*

Anions ( $\text{NO}_3\text{-N}$  and  $\text{Br}^-$ ) were analyzed on a Dionex DX500 Ion Chromatograph (Sunnyvale, CA, USA) with an AS4A anion column. Due to low  $\text{NO}_3\text{-N}$  concentrations ( $< 10 \mu\text{g L}^{-1}$ ), samples collected at Sammy Creek and Hugh White Creek were analyzed colorimetrically following reduction by Cd (Wood et al. 1967, APHA 1998) on a Technicon Autoanalyzer (Technicon, Emeryville, CA, USA). Samples were analyzed for  $\text{NH}_4\text{-N}$  using the phenol-hypochlorite method (Solorzano 1969, USEPA 1997a) and  $\text{PO}_4\text{-P}$  as soluble reactive phosphorus using the molbydate antimony method (Murphy and Riley 1962, USEPA 1997b). DIN was calculated as the sum of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ . Atomic N:P ratios were determined using DIN and  $\text{PO}_4\text{-P}$  concentrations.

$^{15}\text{NO}_3\text{-N}$  was measured by headspace diffusion according to Sigman et al. (1997). Ammonium was removed by boiling samples to a final volume of approximately 100 mL under basic conditions by adding MgO. Nitrate-nitrogen was converted to ammonia ( $\text{NH}_3$ ) by adding Devarda's Alloy. A precombusted, acidified ( $25 \mu\text{L } 2.5 \text{ M KHSO}_4$ ) glass-fiber filter (Whatman GF/D) encased by Teflon® tape was added to the sample immediately after adding the Devarda's alloy. Sealed samples were incubated at  $60^\circ\text{C}$  for 48 h and then gently shaken for 10 d during which time  $\text{NH}_3$  was captured by the acidified filter. The filters were then removed from the Teflon® sandwich, dried, tinned, and shipped to the UC Davis Stable Isotope Facility (Davis, CA) for analysis of  $^{15}\text{N}$  on a Europa Integra mass spectrometer (Sercon Ltd., Cheshire, UK).

In the two streams with background  $\text{NO}_3\text{-N}$  concentrations  $< 80 \mu\text{g L}^{-1}$  (Sammy Creek and Hugh White Creek; Table 1), background and plateau samples were spiked with a known amount of  $\text{NO}_3\text{-N}$  to ensure a minimum mass ( $\geq 80 \mu\text{g}$ ) of N required to measure  $^{15}\text{NO}_3\text{-N}$  in a reasonable sample volume (i.e.,  $\leq 1 \text{ L}$ ). Thus, the target  $\delta^{15}\text{NO}_3\text{-N}$  was elevated such that the sample  $\delta^{15}\text{NO}_3\text{-N}$  would reflect an enrichment of 500‰ after spike addition.

### *Data analysis*

$^{15}\text{NO}_3\text{-N}$  fluxes were calculated at each transect at both background and plateau according to the following equation:

$$^{15}\text{NO}_3\text{-N flux}_i = \text{AF}_i [\text{NO}_3\text{-N}]_i Q_i \quad (4)$$

where  $^{15}\text{NO}_3\text{-N flux}_i$  is the downstream transport ( $\mu\text{g s}^{-1}$ ) of  $^{15}\text{NO}_3\text{-N}$ ,  $\text{AF}_i$  (atomic fraction) is the ratio of  $^{15}\text{N}:^{14}\text{N}+^{15}\text{N}$ ,  $[\text{NO}_3\text{-N}]_i$  is the mean concentration ( $\mu\text{g L}^{-1}$ ) of  $\text{NO}_3\text{-N}$ , and  $Q_i$  is the discharge ( $\text{L s}^{-1}$ ) each at transect  $i$ .  $^{15}\text{NO}_3\text{-N}$  fluxes at plateau were corrected for background by subtracting background flux at each transect.

Uptake length ( $S_w$ ) was calculated by fitting an exponential decay model (SigmaPlot, SPSS, Inc., Chicago, IL, USA) to the background and dilution corrected flux of  $^{15}\text{NO}_3\text{-N}$  versus distance downstream. The decay coefficient represents the longitudinal loss rate ( $k_L$ ), and its the negative inverse is the uptake length (Newbold et al. 1981). The standard error (SE) of  $S_w$  was determined as the standard error of the uptake coefficient using a modified exponential decay model (equation 5), where  $a$  is equal to the  $^{15}\text{NO}_3\text{-N}$  flux at the upper-most transect and  $x$  is distance downstream.

$$^{15}\text{NO}_3\text{-N flux} = a \exp\left(\frac{x}{S_w}\right) \quad (5)$$

Uptake velocity ( $v_f$ ;  $\text{mm min}^{-1}$ ) was calculated following Stream Solute Workshop (1990):

$$v_f = \frac{d}{S_w} u \quad (6)$$

where  $u$  ( $\text{mm min}^{-1}$ ) is the average streamwater velocity, and  $d$  (m) is average stream depth. Areal uptake ( $U$ ) was calculated as the product of  $v_f$  and the streamwater  $\text{NO}_3\text{-N}$  concentration (Stream Solute Workshop 1990):

$$U = v_f [\text{NO}_3\text{-N}] \quad (7)$$

Streamwater  $\text{NO}_3\text{-N}$  reflects the geometric mean of  $\text{NO}_3\text{-N}$  at all transects in the study reach. The geometric mean is used in the calculation of  $U$  as it most closely approximates the availability of  $\text{NO}_3\text{-N}$  along the study reach.

Linear and nonlinear regression were used to compare spiraling metrics ( $S_w$ ,  $v_f$ , and  $U$ ) to  $\text{NO}_3\text{-N}$  concentration associated with amendments within streams and to the background streamwater concentration across streams. Linear regressions were performed using SigmaStat (SPSS, Inc., Chicago, IL, USA). The nonlinear regression function in SigmaPlot was used to compare amendment  $\text{NO}_3\text{-N}$  to  $v_f$  and  $U$  according to equations (1) and (2), respectively. Whole-stream estimates of  $K_m$  and  $U_{\max}$  were calculated from the relationship between  $\text{NO}_3\text{-N}$  amendment and  $S_w$ ,  $v_f$ , and  $U$ , according to equations (1), (2), and (3), respectively. The slopes of the relationships between  $S_w$  and  $\text{NO}_3\text{-N}$  amendment were compared by analysis of covariance (ANCOVA) using the PROC REG procedure in SAS (SAS, Inc., Cary, NC, USA). Significance level ( $\alpha$ ) was set to 0.05 for all statistical tests.

## Results

The concentration of  $\text{NH}_4\text{-N}$  in the five study streams was consistently low ( $\leq 5 \mu\text{g L}^{-1}$ ), whereas the background concentration of  $\text{NO}_3\text{-N}$  varied from 3 to  $982 \mu\text{g L}^{-1}$  at the time of the releases (Table 1). The concentration of  $\text{PO}_4\text{-P}$  was generally low, with the exception of Stonecrop Creek ( $20 \mu\text{g L}^{-1}$ ). Atomic N:P varied from 2 to 195. Discharge of the study streams varied from 2 to  $31 \text{ L s}^{-1}$  at the time of the releases (Table 1), and background streamwater  $\text{NO}_3\text{-N}$  was positively correlated ( $r = 0.862$ ,  $P = 0.027$ ) to discharge.

### *Response to multiple enrichments*

Uptake length increased with increasing  $\text{NO}_3\text{-N}$  amendment in all of the experiments (Figure 3). Significant, linear relationships were observed at Hagar Creek, Stonecrop Creek, and Hugh White Creek ( $P \leq 0.013$ ). Although  $S_w$  increased with  $\text{NO}_3\text{-N}$  amendment during the two experiments at Sammy Creek (April/May and July), the nature of the relationship could not be determined from the limited number of data points. Uptake length also increased with increasing  $\text{NO}_3\text{-N}$  amendment at Greenbrier Creek, however the relationship was not significant ( $r^2 = 0.990$ ,  $P = 0.063$ ). There was considerable error surrounding estimates of  $S_w$  at Greenbrier Creek (Table 2, Figure 3), likely due to the high  $\text{NO}_3\text{-N}$  concentration and relatively high discharge at this site. The

relationship between  $^{15}\text{NO}_3\text{-N}$  flux and distance downstream during the first amended release at Greenbrier Creek (14 August 2003,  $1081 \mu\text{g NO}_3\text{-N L}^{-1}$ , Table 2) was not significant ( $P = 0.141$ ) resulting from unusually high variability of  $\delta^{15}\text{NO}_3\text{-N}$  values during that release. This was the only occurrence of a nonsignificant estimate of  $S_w$  and was not plotted or included in the analyses.

There was a nonlinear decline in  $v_f$  with increasing  $\text{NO}_3\text{-N}$  amendment at most sites. Significant ( $P \leq 0.029$ ) relationships between  $v_f$  and  $\text{NO}_3\text{-N}$  amendment according to equation (2) were evident at Hagar Creek, Stonecrop Creek, and Hugh White Creek (Figure 4). Uptake velocity decreased with  $\text{NO}_3\text{-N}$  amendment during both collections at Sammy Creek (Figure 4). Ambient  $v_f$  was highest in each of the low-N streams (Sammy Creek and Hugh White Creek). However,  $v_f$  decreased substantially from ambient to the first  $\text{NO}_3\text{-N}$  amended release among the low-N streams (average of 66%) (Table 2). Uptake velocity decreased an average of 33% from ambient to the first  $\text{NO}_3\text{-N}$  amended release among the relatively higher-N streams (Stonecrop Creek, Hagar Creek, and Greenbrier Creek).

Relationships between  $U$  and  $\text{NO}_3\text{-N}$  amendment differed among sites (Figure 5). Uptake increased with  $\text{NO}_3\text{-N}$  amendment at both Stonecrop Creek and Hugh White Creek. The relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment at Stonecrop Creek was best described by an asymptotic model ( $r^2 = 0.989$ ). Although there was an apparent nonlinear trend in the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment at Hugh White Creek, the relationship was best described by a linear model ( $r^2 = 0.947$ ). At Sammy Creek,  $U$  increased with increasing  $\text{NO}_3\text{-N}$  amendment during both experiments (April/May and July). Uptake generally declined with  $\text{NO}_3\text{-N}$  amendment at Greenbrier Creek and Hagar Creek. However, the slope of the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment was not significant at either Greenbrier Creek ( $r^2 = 0.767$ ,  $P = 0.320$ ) or Hagar Creek ( $r^2 = 0.651$ ,  $P = 0.193$ ) when analyzed by linear regression.

Although there was some variability among uptake kinetics ( $U_{\text{max}}$  and  $K_m$ ) independently calculated from the three spiraling metrics at each site, calculated values generally corresponded to observed responses to  $\text{NO}_3\text{-N}$  amendments. However, values for  $K_m$  were negative and  $U_{\text{max}}$  values were far below observed  $U$  at both Greenbrier Creek and Hagar Creek where  $U$  was unrelated to  $\text{NO}_3\text{-N}$  amendment (Table 3). Similar

values of  $K_m$  and  $U_{max}$  were calculated from each of the spiraling metrics at those sites (Table 3).

Slopes relating  $S_w$  and  $\text{NO}_3\text{-N}$  amendment were significantly greater at both Greenbrier Creek and Hagar Creek relative to the other study streams (Figure 6). Further, negative intercepts for these two streams corresponded to negative  $K_m$  values (equation 3, Table 3). ANCOVA results indicate that the slopes of  $S_w$  versus  $\text{NO}_3\text{-N}$  amendment at both Greenbrier Creek and Hagar Creek are significantly greater ( $P \leq 0.002$ ,  $\alpha = 0.008$  after Bonferroni correction) than slopes at both Stonecrop Creek and Hugh White Creek (Figure 6). Due to the limited number of data points, experiments at Sammy Creek were not included in the statistical comparison but slopes from these experiments were similar to those at Stonecrop Creek and Hugh White Creek (Figure 6).

When Greenbrier Creek and Hagar Creeks are omitted from the comparison of uptake kinetics among streams,  $K_m$  varied from 3 to 330  $\mu\text{g L}^{-1}$  and  $U_{max}$  varied from 16 to 158  $\mu\text{g m}^{-2} \text{min}^{-1}$  (Table 3). However,  $K_m$  and  $U_{max}$  calculated from the two collections at Sammy Creek should be treated with caution because they were estimated from two-point regressions. In a cross-stream comparison,  $K_m$  calculated from each spiraling metric was positively and significantly related ( $r^2 \geq 0.913$ ,  $P \leq 0.045$ ) to the background streamwater  $\text{NO}_3\text{-N}$  concentration, however this relationship was driven entirely by Stonecrop Creek which had a substantially higher  $K_m$  (254, 325, and 330  $\mu\text{g L}^{-1}$  calculated from  $S_w$ ,  $v_f$ , and  $U$ , respectively) and streamwater  $\text{NO}_3\text{-N}$  concentration (298  $\mu\text{g L}^{-1}$ ) relative to Sammy Creek and Hugh White Creek (Tables 1 and 3). There was no discernible relationship between  $U_{max}$  and the background streamwater  $\text{NO}_3\text{-N}$  concentration among streams.

The considerable error associated with estimates of  $S_w$  at Greenbrier Creek translated into a substantial range of slope and intercept combinations. By modeling  $S_w$  within the range of calculated standard error for all three data points,  $K_m$  varied from -749 to 1475  $\mu\text{g L}^{-1}$  and  $U_{max}$  varied from 81 to 374  $\mu\text{g m}^{-2} \text{min}^{-1}$ . There was less error ( $r^2 = 0.636 - 0.815$ ) in the relationships between distance downstream and  $^{15}\text{NO}_3\text{-N}$  flux at Hagar Creek, and a series of uptake lengths within the range of standard error such that the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment approximated a slope of zero could not be modeled.

### *Patterns across streams*

Ambient  $S_w$  (i.e., non-amended  $^{15}\text{NO}_3\text{-N}$  tracer) varied from 23 to 3652 m and was positively and significantly ( $r^2 = 0.979$ ,  $P < 0.001$ ,  $n = 6$ ) related to the background streamwater  $\text{NO}_3\text{-N}$  concentration (Figure 7). The longest ambient  $S_w$  was measured at Greenbrier Creek ( $S_w = 3652$  m), the site with the highest streamwater  $\text{NO}_3\text{-N}$  concentration ( $983 \mu\text{g L}^{-1}$ ). The next longest  $S_w$  was only 632 m (Stonecrop Creek). The linear relationship between  $S_w$  and ambient streamwater  $\text{NO}_3\text{-N}$  was still significant ( $r^2 = 0.926$ ,  $P = 0.009$ ,  $n = 5$ ) when the long  $S_w$  at Greenbrier Creek was not included in the statistical assessment. While this pattern suggests a strong relationship between N concentration and  $S_w$ , the pattern likely also reflects the influence of stream size (Davis and Minshall 1999, Wollheim et al. 2001) as both  $\text{NO}_3\text{-N}$  and  $S_w$  were significantly correlated with discharge ( $r \geq 0.862$ ,  $P \leq 0.027$ ,  $n = 6$ ) as well as to each other. Ambient  $v_f$  varied from 0.09 to  $2.80 \text{ mm min}^{-1}$  and decreased nonlinearly (equation 2,  $K_m = 12 \mu\text{g L}^{-1}$ ,  $U_{\max} = 34 \mu\text{g m}^{-2} \text{ min}^{-1}$ ) with background streamwater  $\text{NO}_3\text{-N}$  concentration ( $r^2 = 0.887$ ,  $P = 0.005$ ,  $n = 6$ ) (Figure 7). Ambient  $U$  varied from 5.1 to  $222.4 \mu\text{g m}^{-2} \text{ min}^{-1}$  and was generally positively related to background streamwater  $\text{NO}_3\text{-N}$  (Figure 3). However, ambient  $U$  at Stonecrop Creek ( $26.5 \mu\text{g m}^{-2} \text{ min}^{-1}$ ) was lower than at Hagar Creek ( $84.0 \mu\text{g m}^{-2} \text{ min}^{-1}$ ) despite a higher background  $\text{NO}_3\text{-N}$  concentration at Stonecrop Creek. Ambient  $U$  was significantly related to the background streamwater  $\text{NO}_3\text{-N}$  concentration using linear regression ( $r^2 = 0.767$ ,  $P = 0.022$ ,  $n = 6$ ) and an asymptotic model (equation 1;  $r^2 = 0.902$ ,  $P = 0.004$ ,  $n = 6$ ). Although regression statistics from the asymptotic model indicated a good fit to the data, the model resulted in nonsensical uptake kinetics ( $K_m > 1.9 \times 10^6 \mu\text{g L}^{-1}$ ,  $U_{\max} > 4 \times 10^5 \mu\text{g m}^{-2} \text{ min}^{-1}$ ), and a visual inspection indicated that the model resulted in a poor fit to the data. However, when Stonecrop Creek, the only hardwater stream, was omitted from the analysis, the relationship was still significant using the asymptotic model ( $r^2 = 0.998$ ,  $P < 0.001$ ,  $n = 5$ ) and estimates of  $K_m$  and  $U_{\max}$  were realistic ( $K_m = 583 \mu\text{g L}^{-1}$  and  $U_{\max} = 354 \mu\text{g m}^{-2} \text{ min}^{-1}$ ).

## Discussion

### *Michaelis-Menten kinetics*

My results generally support biological uptake of  $\text{NO}_3\text{-N}$  as a function of M-M kinetics. The relationships between  $S_w$ ,  $v_f$ ,  $U$ , and  $\text{NO}_3\text{-N}$  amendments corresponded to predicted patterns given M-M kinetics in most of the experiments. Dodds et al. (2002) reported uptake continued to increase despite very high amendment concentrations in prairie streams and that lack of biotic saturation may be due to an influence of hydrodynamic limitation across solid-water boundaries (mass transport limitation). If uptake was limited solely by mass transport, uptake would be a linear function of concentration and both  $S_w$  and  $v_f$  would remain constant (Dodds et al. 2002). Uptake is likely influenced by some combination of biotic and mass transport processes (Sanford and Crawford 2000). The fact that relationships between N amendment and both  $S_w$  and  $v_f$  followed those predicted by M-M kinetics suggests that biotic processes dominated uptake in these study streams. The diffusion of nutrients may be limited by biofilm thickness and increasing velocity has been shown to stimulate periphyton growth (Horner et al. 1990). The biomass of benthic biofilms was low in these streams (S. R. Earl unpublished data) and mass transport may be more important in streams with greater biomass standing stocks. The sorptive capacity of the study streams is unknown, however  $\text{NO}_3\text{-N}$  has limited propensity to adsorb to stream sediments and uptake is likely predominantly controlled by biotic processes (Triska et al. 1993).

The whole-stream estimates of  $K_m$  (varying from 3 to 330  $\mu\text{g L}^{-1}$ , excluding Hagar Creek and Greenbrier Creek) are similar to those reported for freshwater algae. Reported  $K_m$  values include 59  $\mu\text{g NO}_3\text{-N L}^{-1}$  for a periphyton assemblage (Kim et al. 1990), 1.4 – 130  $\mu\text{g N L}^{-1}$  for diatoms (USEPA 1985), and 102 to 213  $\mu\text{g NO}_3\text{-N L}^{-1}$  for *Cladophora glomerata* (Lohman and Priscu 1992). The general agreement between estimates of  $K_m$  and those reported in the literature for micro- and mesocosm studies where the influence of mass transport and adsorption is negligible further supports biotic regulation of uptake in these study streams.

### *Saturation Response Types*

The study sites spanned a continuum from potentially N-limited to N-saturated streams. An increase in  $U$  with increasing N amendment at Sammy Creek, Stonecrop Creek, and Hugh White Creek corresponds to SRTs I and II and suggests that N uptake capacity in these streams was not saturated. I observed evidence of either SRT I or SRT II at Hugh White Creek. Although the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment at this site was best described by a linear model, visual inspection of the relationship suggested a slight, nonlinear trend. This pattern may reflect a subtle distinction between SRTs I and II but may also reflect the substantial range of  $\text{NO}_3\text{-N}$  amendment resulting from the very low background  $\text{NO}_3\text{-N}$  concentration at this site. The ratio of  $\text{NO}_3\text{-N}$  amendment to  $\text{NO}_3\text{-N}$  background concentration varied from  $\sim 1$  to 164 across the four injections at Hugh White Creek versus  $\sim 1$  to 58 at all other sites. SRTs predict the relationship between  $U$  and added N based on a stream's proximity to N saturation, however I did not define the magnitude of enrichment above background concentration necessary to characterize these relationships. High concentrations of added N may exceed uptake capacity even in non-saturated streams (i.e., SRT I) and it is not surprising that the study streams exhibit signs of more than one SRT. Both experiments at Sammy Creek (April/May and July) indicate that this stream is well below N saturation (SRT I). Although the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment could not be fully resolved due to the limited number of data points, ambient  $U$  is well below estimated  $U_{\text{max}}$ , and modeled data based on  $K_m$  and  $U_{\text{max}}$  indicate that  $U$  measured during the releases was in the near-linear range of the curve. Together, these data suggest a SRT I designation for this stream. I observed evidence of SRT II at Stonecrop Creek, where  $U$  increased with increasing  $\text{NO}_3\text{-N}$  amendment according to an asymptotic relationship and was approaching  $U_{\text{max}}$  at the highest enrichment. The slope of the relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment at Greenbrier Creek was not significantly different from zero, corresponding to a SRT III and suggesting that uptake capacity in this stream was saturated. Similarly,  $U$  did not change significantly with increasing  $\text{NO}_3\text{-N}$  amendment when assessed with a linear model at Hagar Creek, suggesting that this system was also N saturated.

The nonsignificant relationship between  $U$  and  $\text{NO}_3\text{-N}$  amendment at both Hagar Creek and Greenbrier Creek suggests saturation, however visual inspection of the relationships indicated that  $U$  declined with increasing  $\text{NO}_3\text{-N}$  concentration at each site. The apparent decline in  $U$  with increasing  $\text{NO}_3\text{-N}$  amendment is inconsistent with the M-M model. While the linear increase in  $S_w$  and nonlinear decline in  $v_f$  with increasing  $\text{NO}_3\text{-N}$  amendment correspond to predicted patterns given M-M kinetics, each of these relationships resulted in similar, nonsensical values for  $K_m$  and  $U_{\max}$  at both Hagar Creek and Greenbrier Creek. Further, the significantly greater slope of the relationship between  $S_w$  and  $\text{NO}_3\text{-N}$  amendment at both Hagar Creek and Greenbrier Creek relative to all other sites suggests that these streams are behaving differently with respect to N uptake. Alternate models of nutrient uptake may be more applicable when streams are no longer nutrient limited (i.e., saturated). The asymptotic relationship between  $U$  and limiting-nutrient concentration given M-M kinetics may be truncated when another factor that is critical to growth becomes limiting (Dugdale et al. 1981). After truncation, the relationship between  $U$  and nutrient concentration resembles a flat-line with a slope of zero (Dugdale et al. 1981). This deviation from M-M kinetics would result in an increase in the rate of decay of  $v_f$  and an increase in the slope of the relationship between  $S_w$  and nutrient concentration (Figure 8). The significantly steeper slopes of the relationship between  $S_w$  and  $\text{NO}_3\text{-N}$  amendment at Hagar Creek and Greenbrier Creek correspond to expected patterns following truncation, providing further evidence that N uptake capacity in these streams is saturated. Standard error of  $S_w$  was highest in each of these streams and that variability may have contributed to the apparent decline in  $U$ .

The proximity of streams to N saturation, as indicated by their respective SRT, was generally correlated to streamwater nutrient availability. I classified Sammy Creek, during both experiments, and Hugh White Creek as SRT I, indicating that N in these streams is below saturating concentrations. At the opposite end of the spectrum, Greenbrier Creek was N saturated (SRT III), corresponding to the high N concentration in that stream. However, my analysis indicated that Stonecrop Creek was not N saturated, although it was approaching saturation (i.e., characteristic of SRT II), despite a high N concentration. Lack of saturation at Stonecrop Creek may reflect the high concentration of  $\text{PO}_4\text{-P}$  in that stream which resulted in a streamwater molar N:P ratio of

16. In a study of benthic algae, Shanz and Juon (1983) determined that N was limiting at N:P ratios < 10, P was limiting at N:P ratios > 20, and the limiting nutrient was ambiguous at N:P ratios between 10 and 20. Nutrient ratios are useful for identifying limiting nutrients when concentrations are near growth-limiting concentrations (Borchardt 1996). My analysis of N saturation in these study streams is consistent with the thresholds identified by Shanz and Juon (1983) and suggest that Stonecrop Creek may not be N or P limited whereas Hagar Creek and Greenbrier Creek may have been P limited.

The study streams spanned a substantial range of N concentration and included streams that showed characteristics of N limitation and N saturation. My findings that low-N streams are below saturating concentrations concur with studies that reported N limitation at relatively low N concentrations. Benthic algae were reported to be N limited when concentrations were below  $55 \mu\text{g NO}_3\text{-N L}^{-1}$  in a desert stream (Grimm and Fisher 1986) and below  $100 \mu\text{g NO}_3\text{-N L}^{-1}$  in an Ozark (USA) stream (Lohman et al. 1991). The limited number of data points makes it difficult to predict a specific concentration at which southern Appalachian headwater streams reach a point of N saturation. Data from Hagar Creek suggests that saturation of uptake capacity may occur at concentrations as low as  $180 \mu\text{g NO}_3\text{-N L}^{-1}$ . However, the lack of saturation at Stonecrop Creek suggests that saturation may occur at much higher concentrations when sufficient phosphorus is available. Saturation at generally high concentrations corresponds to observed patterns across streams. I was able to fit an asymptotic model (equation 1) to the relationship between  $U$  and background streamwater DIN concentration when Stonecrop Creek was omitted from the analysis. In addition to having the highest streamwater concentration of  $\text{PO}_4\text{-P}$ , Stonecrop Creek was also the only hardwater stream included in the study and productivity has been shown to differ in streams of contrasting ionic strength (Krueger and Waters 1983). Although saturating concentrations cannot be determined from  $K_m$  alone, the high  $K_m$  estimated from the comparison across streams suggests that saturation may occur at concentrations  $> 583 \mu\text{g NO}_3\text{-N L}^{-1}$ .

Ultimately, nutrient saturation occurs when the availability of a nutrient increases to a point at which another factor critical to growth becomes limiting. This study focused on N availability and did not address other aspects of stream structure and function that

may influence the relationship between N concentration and uptake. For example, nutrient uptake may be related to hydraulic residence time (Valett et al. 1996) and gross primary production (Hall and Tank 2003, Webster et al. 2003). The concentration at which a specific nutrient exceeds metabolic demand is likely influenced by a combination of these and other factors. Further, this study was limited to an analysis of DIN and did not consider concentrations of total N, which may show stronger relationships to biotic standing stocks (Dodds et al. 1997) and, in turn, rates of biotic processes. Although a specific concentration at which N may be saturating is elusive, the strong correlation among N concentration and the spiraling metrics is robust and illustrates the importance of N concentration as a controlling factor in N processing.

#### *Implications for N retention in headwater streams*

My results indicate that N uptake and N uptake efficiency, as indicated by  $v_f$ , was generally a function of N concentration. The low N (SRT I) streams were characterized by high N uptake efficiency but low uptake which was likely constrained by limited N availability. In contrast, uptake efficiency was low at Greenbrier Creek but uptake reflected the high background  $\text{NO}_3\text{-N}$  concentration and I observed the highest areal uptake at Greenbrier Creek. Variation in uptake efficiency was also evident within streams as  $v_f$  consistently declined with increasing N amendment. However, the low uptake efficiency at Stonecrop Creek was puzzling. I would have expected  $v_f$  to be lowest in those streams showing clear signs of saturation (i.e., Hagar Creek, Greenbrier Creek), yet I observed the lowest  $v_f$  at Stonecrop Creek. These findings suggest that uptake efficiency is not always indicative of nutrient saturation. Although the results from Stonecrop Creek were anomalous, uptake efficiency generally decreased with increasing N concentration. Thus, despite higher uptake, uptake relative to supply decreased and the downstream flux of nitrate increased with increasing nitrate concentration both within and across streams. A pattern of decreasing N uptake efficiency with increasing N concentration is not unique to this study. I compared my results of tracer-derived  $v_f$  and  $U$  across a N gradient to those reported by Webster et al. (2003) and Valett et al. (unpublished data) (Figure 9). Data included a cross-biome comparison of 10 different streams (Webster et al. 2003) and a seasonal analysis of

headwater streams in NC, TN, and NM (Valett et al. unpublished). Although  $v_f$  spans a wide range in relatively low-N streams,  $v_f$  is markedly lower in all streams with background  $\text{NO}_3\text{-N}$  concentrations  $> 100 \mu\text{g L}^{-1}$  (Figure 9). This pattern further suggests that the efficiency of N uptake in stream ecosystems is impaired with even moderate increases in N availability.

Although they were most efficient at retaining external inputs of N, uptake efficiency was most affected by  $\text{NO}_3\text{-N}$  addition in the low-N streams. Despite significantly greater slopes of  $S_w$  versus  $\text{NO}_3\text{-N}$  amendment at Hagar Creek and Greenbrier Creek, I generally observed large % decreases in  $v_f$  between ambient and  $\text{NO}_3\text{-N}$  amended releases in the low-N streams. This pattern may reflect the greater increase in streamwater  $\text{NO}_3\text{-N}$  relative to background concentrations during amendments in the low-N streams. However, even with substantial increases in N concentration associated with N amendments and the corresponding decrease in uptake efficiency, uptake efficiency in the low-N streams was still equal to or greater than in the comparatively higher N streams under both amended and non-amended conditions. Higher loss rates (i.e., export) associated with decreased uptake efficiency in high-N streams are consistent with N saturation (Stoddard 1994), suggesting availability has exceeded uptake capacity.

The disparity between ambient  $U$  and  $U_{\text{max}}$  in low-N streams suggests that these systems have the capacity to assimilate substantial inputs of N. These results support the conclusions of Peterson et al. (2001) that headwater streams may retain and transform considerable amounts of DIN. However, nutrient uptake and growth are only coupled under steady-state conditions (Borchardt 1996), thus the uptake kinetics calculated from my short-term N amendments do not necessarily reflect kinetics that might be associated with long-term N loading. Nutrient concentration reflects a balance between uptake and mineralization (Dodds 1993), and while increased uptake may mitigate downstream N fluxes associated with short-term external inputs of nutrients, mineralization may eventually reach a steady state with uptake and streamwater nutrient concentrations will reflect the additional inputs. Given that the goal of eliminating all sources of anthropogenic inputs of N to stream ecosystems is impractical, resource managers should strive to reduce inputs to the lowest feasible concentration in order to maximize the efficiency of streams as transformers and retainers of N.

## Literature Cited

- Aber, J. D., K. J. Nadelhoffer, P. Steudler, and J. M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience* 39:378-386.
- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Bernston, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems. *BioScience* 48:921-934.
- 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* 503:758-761.
- American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater, 20th ed. APHA, New York.
- Bencala, K. E., and R. A. Walters. 1983. Simulation of solute transport in a mountain pool-and-riffle stream: a transient storage model. *Water Resources Research* 19:718-724.
- Bencala, K. E., D. M. McKnight, and G. W. Zellweger. 1990. Characterization of transport in an acidic metal-rich mountain stream based on a lithium tracer injection and simulations of transient storage. *Water Resources Research* 26:989-1000
- Borchardt, M. A. 1996. Nutrients. Pages 183-227 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. *Algal Ecology*. Academic Press, Inc., San Diego, CA, USA.
- Bothwell, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1293-1301.
- Carpenter, E. J., and R. R. L. Guillard. 1971. Intraspecific differences in nitrate half saturation constants for three species of marine phytoplankton. *Ecology* 52:183-185.
- Davis, J. C., and G. W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia* 119:247-255
- Dodds, W. K. 1993. What controls levels of dissolved phosphate and ammonium in surface waters? *Aquatic Sciences* 55:132-142.
- Dodds, W. K., V. H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. *Water Res.* 31:1738-1750.

- 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. Morall, 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.
- Dortch, Q., P. A. Thompson, and P. J. Harrison. 1991. Variability in nitrate uptake in *Thalassiosira pseudonana* (Bacillariophyceae). *Journal of Phycology* 27:35-39.
- Dugdale, R. C., B. H. Jones, Jr., and J. J. MacIssac. 1981. Adaptation of nutrient assimilation. *Canadian Bulletin of Fisheries and Aquatic Sciences* 210:234-350.
- Grimm, N. B., and S. G. Fisher. 1986. Nitrogen limitation in a Sonoran Desert stream. *Journal of the North American Benthological Society* 5:2-15.
- Hall, R. O., Jr., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography* 48:1120-1128.
- Hall, R. O., Jr. 2003. A stream's role in watershed nutrient export. *Proceedings of the National Academy of Science* 100:10137-10138.
- Hedin, L. O., J. C. von Fischer, N. E. Ostrom, B. P. Kennedy, M. G. Brown, and G. P. Robertson. 1998. Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. *Ecology* 79:684-703.
- Horner, R. R., E. B. Welch, M. R. Seeley, and J. M. Jacoby. 1990. Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. *Freshwater Biology* 24:215-232.
- Kim, B. P., A. P. Jackman, and F. J. Triska. 1990. Modeling transient storage and nitrate uptake kinetics in a flume containing a natural periphyton community. *Water Resources Research* 26:505-515.
- Krueger, C. C., and T. F. Waters. 1983. Annual production of macroinvertebrates in three streams of different water quality. *Ecology* 64:840-850.
- Lohman, K., J. R. Jones, and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. *Journal of the North American Benthological Society* 10:14-23.
- Lohman, K., and J. C. Priscu. 1992. Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. *Journal of Phycology* 28:443-448.

- Munn, M. D., and J. L. Meyer. 1990. Habitat-specific solute retention in two small streams: an intersite comparison. *Ecology* 71:2069-2082.
- Murphy, J., and J. P. Riley. 1962. Determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.
- Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 38:860-863.
- 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.
- Runkel, R.L. 1998. One dimensional transport with inflow and storage (OTIS): a solute transport model for streams and rivers: U.S. Geological Survey Water-Resources Investigation Report 98-4018.
- Sanford, L. P., and S. M. Crawford. 2000. Mass transfer versus kinetic control of uptake across solid-water boundaries. *Limnology and Oceanography* 45:1180-1186.
- Shanz, F., and J. Juon. 1983. Two different methods of evaluating nutrient limitations of periphyton bioassays using water from the River Rhine and eight of its tributaries. *Hydrobiologia* 102:187-195.
- Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine chemistry* 57:227-242.
- Solorzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* 14:779-801.
- Stoddard, J. L. 1994. Long term changes in watershed retention of nitrogen. Its causes and aquatic consequences. Pages 223-284 *in* L. A. Baker, editor. *Environmental chemistry of lakes and reservoirs*. Advances in Chemistry Series no. 237. American Chemical Society, Washington D. C.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95-119.
- Triska, F. J., J. H. Duff, and R. J. Avanzino. 1993. Patterns of hydrological exchange and nutrient transformations in the hyporheic zone of a gravel-bottom stream: examining terrestrial-aquatic linkages. *Freshwater Biology* 29:259-274.

- U.S. Environmental Protection Agency (USEPA). 1985. Rates, constants, and kinetics: formulations in surface water quality modeling. USEPA/600/3-85/040 - 2<sup>nd</sup> edition. Washington, D. C.
- U.S. Environmental Protection Agency (USEPA). 1997a. Method 349.0. Determination of ammonia in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis. Zhang, J. Z., P. B. Ortner, C. J. Fischer, and L. D. Moore *in* Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition. USEPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- U.S. Environmental Protection Agency (USEPA). 1997b. Method 365.5. Determination of orthophosphate in estuarine and coastal waters by automated colorimetric analysis. Zimmerman, C. F., and C. W. Keefe *in* Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition. USEPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- Valett, H. M., J. A. Morrice, C. N. Dahm, M. E. Campana. 1996. Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography* 41:333-345.
- 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., Mulholland, P. J., J. L. Tank, H. M. Valett, W. K. Dodds, B. J. Peterson, W. B. 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, W. M. Wollheim. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology* 48:1329-1352.
- Wollheim, W. M., B. J. Peterson, L. A. Deegan, J. E. Hobbie, and B. Hooker. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnology and Oceanography* 46:1-13.
- Wood, E. D., F. A. J. Armstrong, and F. A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom* 47:23-31.

Table 1. Characteristics of study streams. Nitrate-nitrogen (NO<sub>3</sub>-N), ammonium-nitrogen (NH<sub>4</sub>-N), and orthophosphate (PO<sub>4</sub>-P) are reach average means of background concentrations prior to all releases. N:P represents the atomic ratio calculated from combined concentrations of NO<sub>3</sub>-N and NH<sub>4</sub>-N to PO<sub>4</sub>-P. The lowest detectable limit (3 μg L<sup>-1</sup>) was used to calculate N:P when measured concentrations were below detectable limits (bdl).

Stream	Location	NO <sub>3</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P	N:P	Specific	Discharge
		μg L <sup>-1</sup>				conductance	
Sammy Creek (April/May)	Giles County, VA	5	5	bdl	3	21	7
Sammy Creek (July)	Giles County, VA	3	bdl	bdl	2	20	6
Hugh White Creek	Macon County, NC	3	3	bdl	2	12	7
Hagar Creek	Montgomery County, VA	182	bdl	7	25	62	15
Stonecrop Creek	Giles County, VA	298	5	20	16	262	2
Greenbrier Creek	Roanoke County, VA	983	bdl	5	195	145	31

Table 2. Results of individual releases. Standard errors (SE) are presented for uptake lengths. NO<sub>3</sub>-N represents the geometric mean of the NO<sub>3</sub>-N concentration (µg L<sup>-1</sup>) in the study reach during plateau sampling. ns = non-significant ( $P \geq 0.05$ ) regression.

Stream	Date	Discharge L s <sup>-1</sup>	NO <sub>3</sub> -N µg L <sup>-1</sup>	S <sub>w</sub> (SE) m	v <sub>f</sub> mm min <sup>-1</sup>	U µg m <sup>-2</sup> min <sup>-1</sup>
Sammy Creek	29 April 2003	9.0	5	128 (21)	1.57	7.8
	1 May 2003	6.8	47	181 (22)	0.83	39.0
Sammy Creek	15 July 2003	6.1	3	42 (3)	2.09	5.1
	16 July 2003	6.1	140	88 (9)	0.74	103.7
Hugh White Creek	8 October 2003	6.8	3	23 (2)	2.80	8.8
	9 October 2003	7.0	39	175 (2)	0.37	14.6
	10 October 2003	7.7	260	508 (14)	0.14	36.8
	11 October 2003	8.2	537	816 (15)	0.09	48.1
Hagar Creek	25 August 2003	15.5	181	587 (81)	0.46	84.0
	26 August 2003	15.3	223	1157 (135)	0.22	49.8
	27 August 2003	15.6	360	3128 (543)	0.08	30.2
	28 August 2003	13.5	577	5464 (715)	0.05	26.8
Stonecrop Creek	10 September 2003	2.2	287	632 (24)	0.09	26.5
	11 September 2003	2.0	361	684 (84)	0.09	30.7
	12 September 2003	1.9	575	1002 (38)	0.06	35.7
	13 September 2003	2.1	850	1263 (131)	0.05	41.8
Greenbrier Creek	14 August 2003	32.0	981	3652 (1037)	0.23	222.4
	14 August 2003	32.0	1081	ns		
	15 August 2003	29.0	1240	5519 (1264)	0.14	173.1
	15 August 2003	29.0	1568	7194 (1136)	0.11	167.9

Table 3. Uptake kinetics. Half-saturation constant ( $K_m$ ) and maximum uptake ( $U_{max}$ ) values were estimated from regressions among  $\text{NO}_3\text{-N}$  amendments,  $S_w$ ,  $v_f$ , and  $U$  at five study streams from April through October 2004.

Stream	$K_m$ ( $\mu\text{g L}^{-1}$ )			$U_{max}$ ( $\mu\text{g m}^{-2} \text{min}^{-1}$ )		
	$S_w$	$v_f$	$U$	$S_w$	$v_f$	$U$
Sammy Creek (April/May)*	96	42	42	138	74	74
Sammy Creek (July)*	118	73	73	228	158	158
Hugh White Creek	57	3	117	48	16	57
Hagar Creek	-126	-142	-141	21	18	19
Stonecrop Creek	254	325	330	52	57	57
Greenbrier Creek	-354	-503	-482	133	108	112

\*  $K_m$  and  $U_{max}$  values derived from two-point regressions

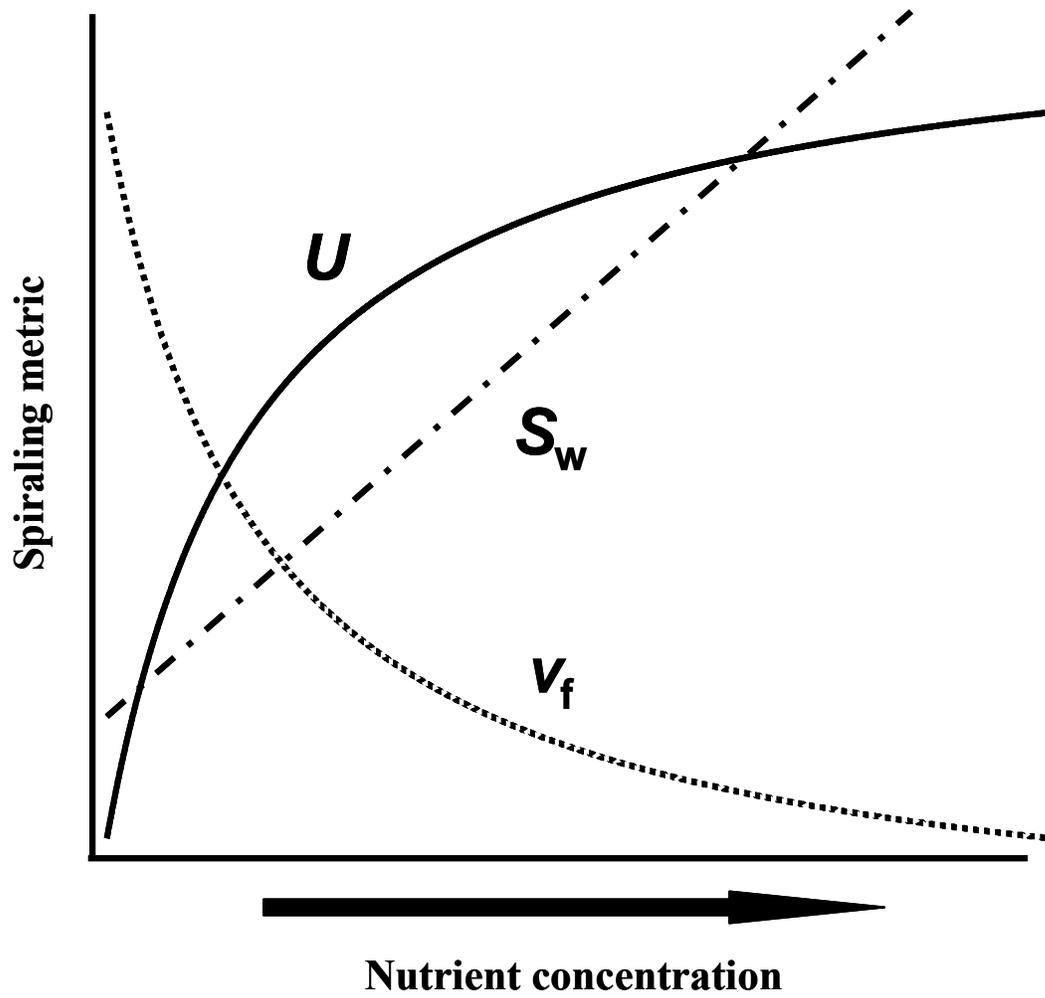


Figure 1. Michaelis-Menten kinetics and spiraling. Theoretical relationship between  $S_w$ ,  $v_f$ ,  $U$ , and streamwater nutrient concentration provided nutrient uptake ( $U$ ) follows Michaelis-Menten kinetics.

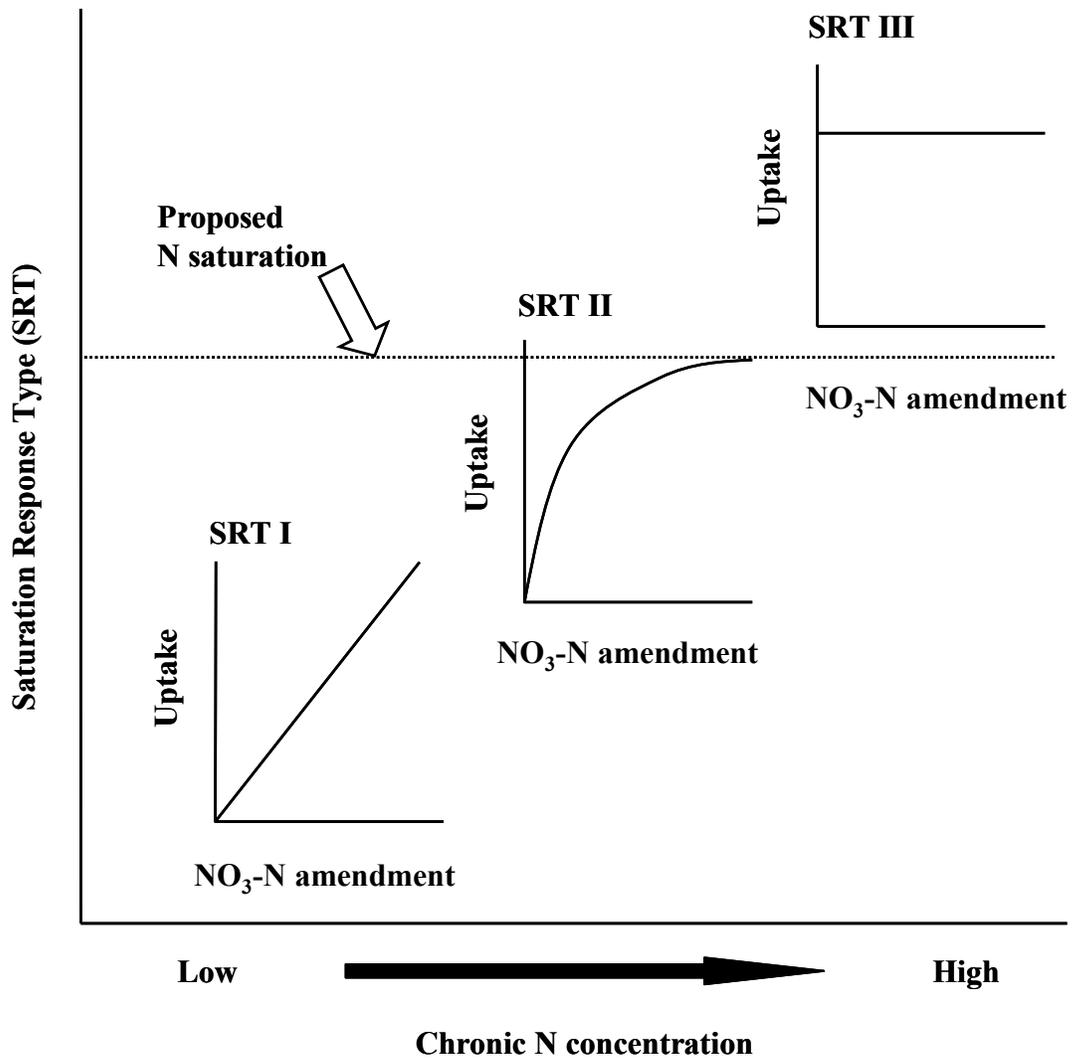


Figure 2. Saturation response types (SRTs). SRTs are theoretical relationships between streamwater nitrogen concentration and individual stream response to increased  $\text{NO}_3\text{-N}$  concentration (amendments). The response of each stream, as described by the change in uptake with increasing concentrations of streamwater  $\text{NO}_3\text{-N}$ , will vary depending on chronic N exposure (depicted on the x-axis). Further, chronic N exposure is expected to dictate the proximity of the stream to a proposed streamwater DIN concentration at which biotic uptake is nitrogen saturated.

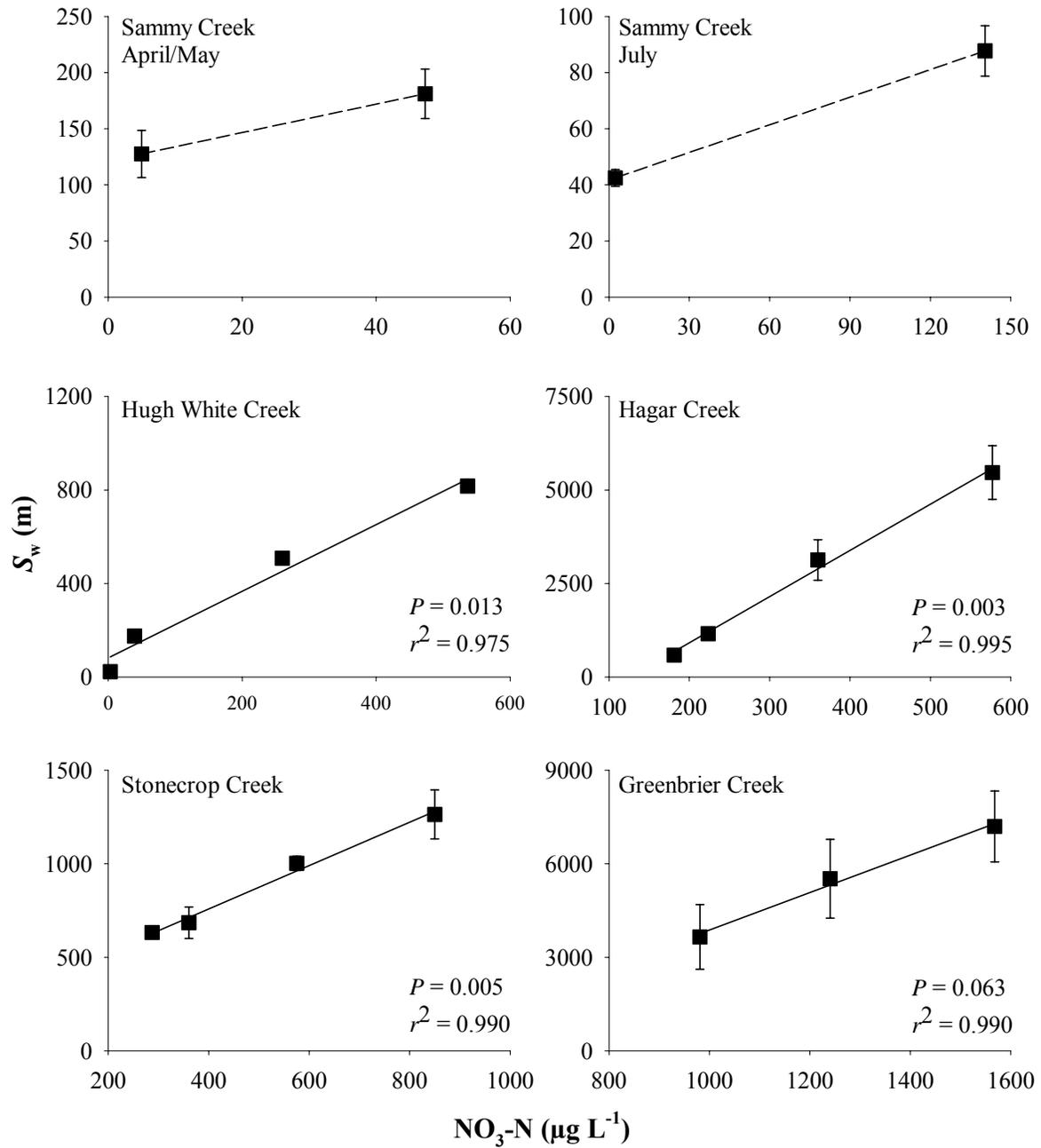


Figure 3. Response of  $S_w$  ( $\pm$ SE) to nitrogen amendments. The first point in each panel corresponds to  $S_w$  measured at the ambient streamwater  $\text{NO}_3\text{-N}$  concentration. Nitrogen amendments are presented as the geometric mean of plateau  $\text{NO}_3\text{-N}$  concentration ( $\mu\text{g L}^{-1}$ ). Results of linear regressions are presented for those streams in which more than two releases were conducted.

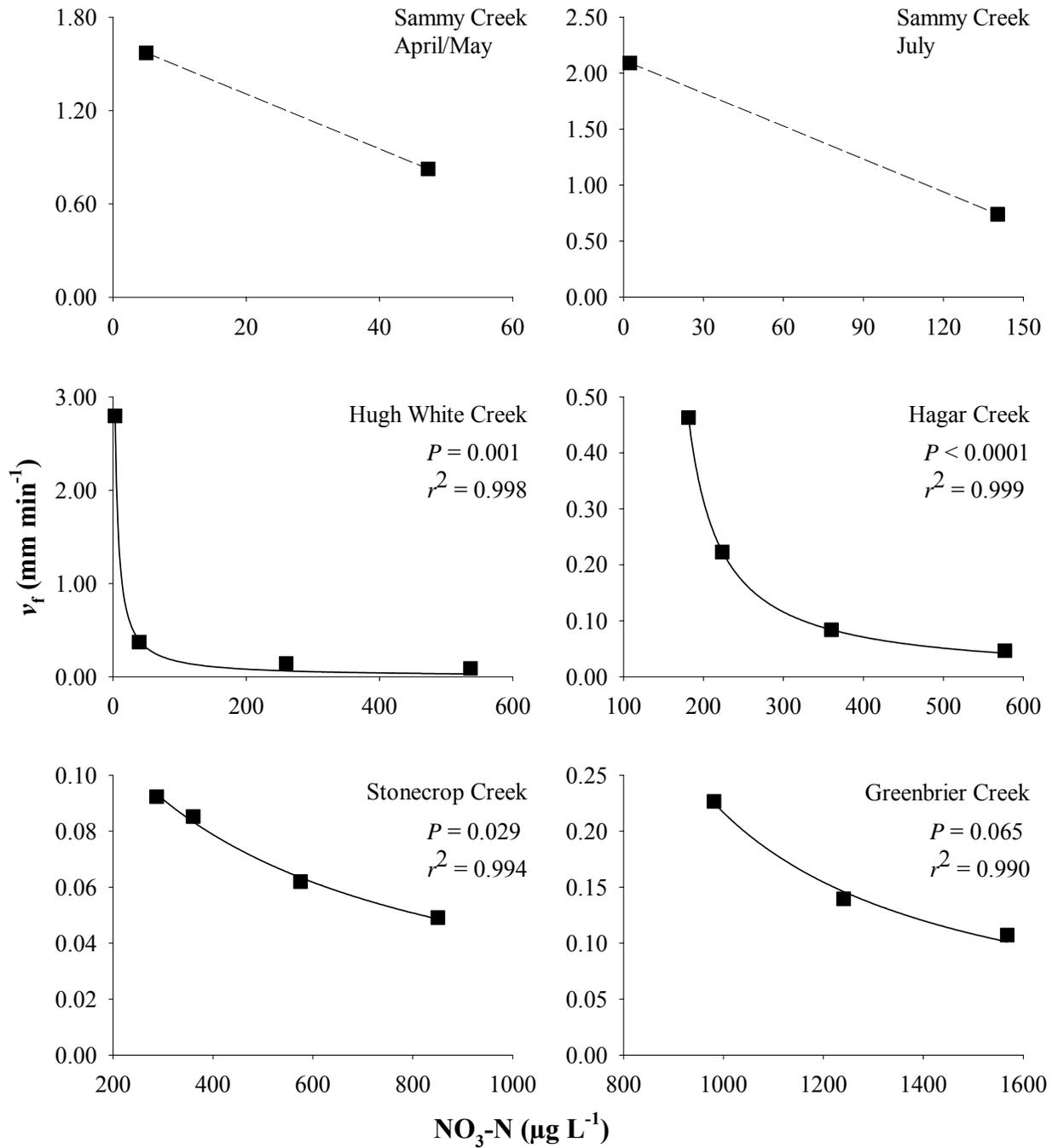


Figure 4. Response of  $v_f$  to nitrogen amendments. The first point in each panel corresponds to  $v_f$  measured at the ambient streamwater  $\text{NO}_3\text{-N}$  concentration. Nitrogen amendments are presented as the geometric mean of plateau  $\text{NO}_3\text{-N}$  concentration ( $\mu\text{g L}^{-1}$ ). Results of nonlinear regressions following equation (2, *see text*) are presented for those streams in which more than two releases were conducted.

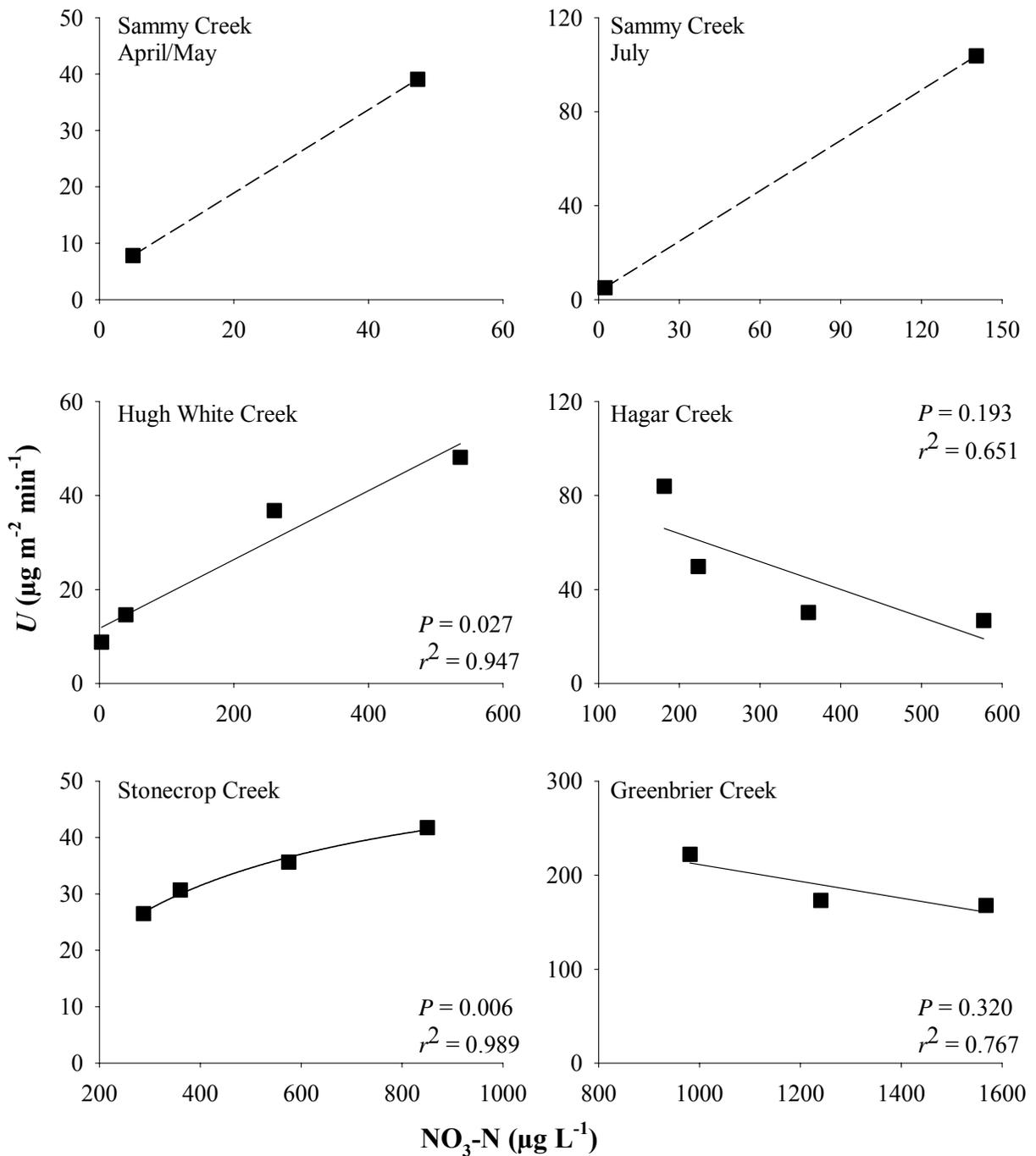


Figure 5. Response of  $U$  to nitrogen amendments. The first point in each panel corresponds to  $U$  measured at the ambient streamwater  $\text{NO}_3\text{-N}$  concentration. Nitrogen amendments are presented as the geometric mean of plateau  $\text{NO}_3\text{-N}$  concentration ( $\mu\text{g L}^{-1}$ ). Best fit regression (linear or nonlinear following equation 1, *see text*) results as indicated by  $r^2$  are presented for those streams in which more than two releases were conducted.

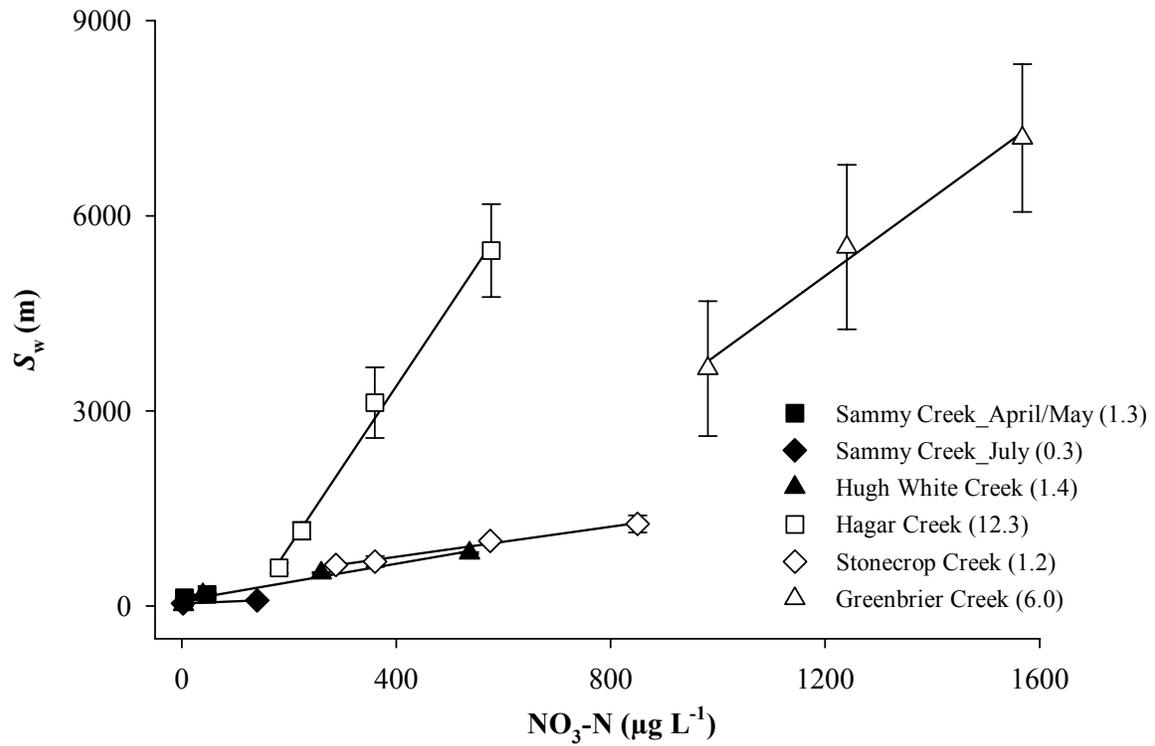


Figure 6. Response of  $S_w$  ( $\pm$ SE) to nitrogen amendments for all releases. The first point for each stream corresponds to  $S_w$  measured at the ambient streamwater  $\text{NO}_3\text{-N}$  concentration. Nitrogen amendments are presented as the geometric mean of plateau  $\text{NO}_3\text{-N}$  concentration ( $\mu\text{g L}^{-1}$ ). The slope of the relationship between  $S_w$  and  $\text{NO}_3\text{-N}$  amendment is indicated in parenthesis.

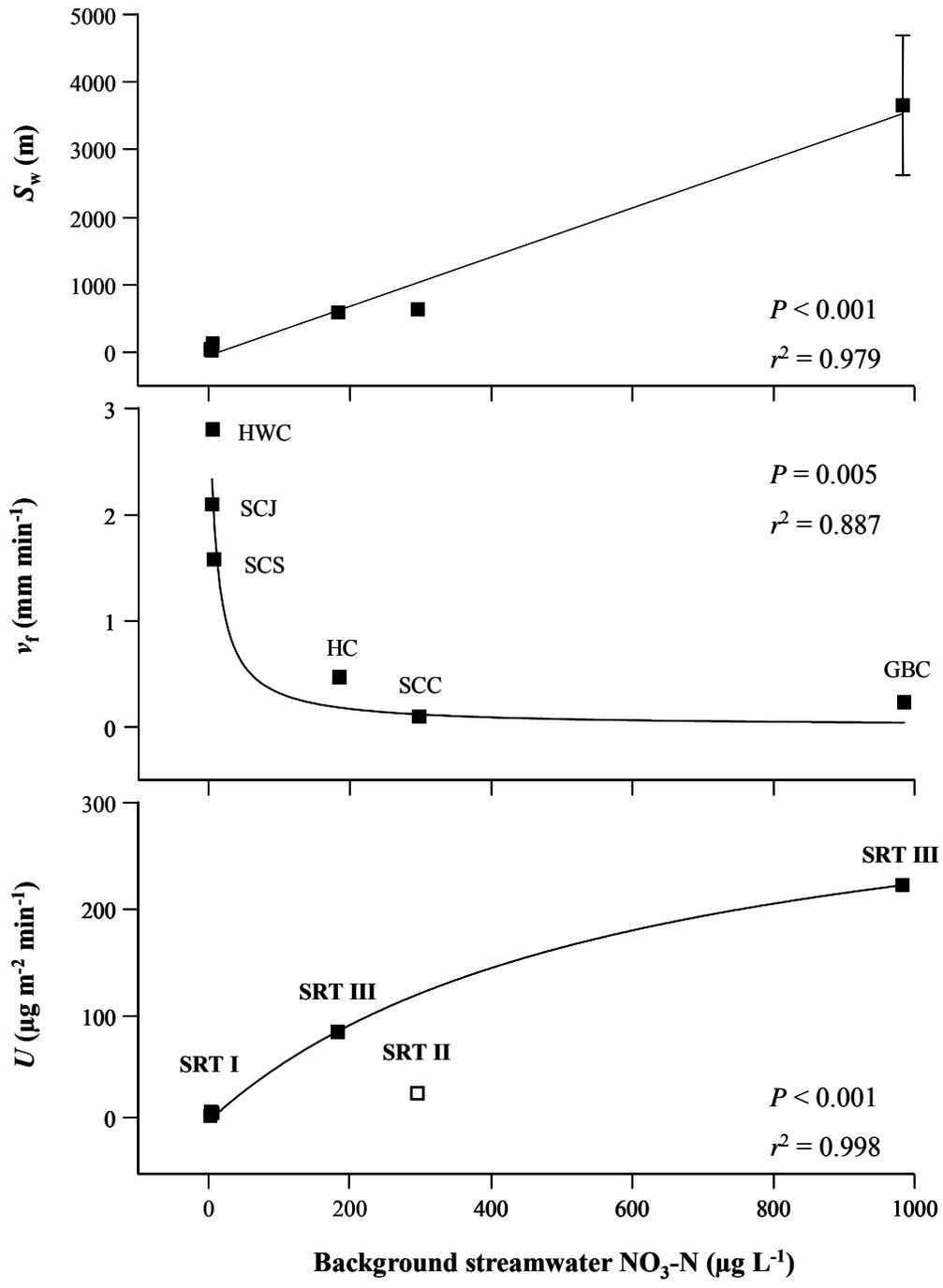


Figure 7. Ambient  $S_w$  ( $\pm$ SE),  $v_f$ , and  $U$  across streams. Site names for individual points are noted in the middle panel ( $v_f$  versus  $\text{NO}_3\text{-N}$ ): HWC = Hugh White Creek, SCJ = Sammy Creek (July), SCS = Sammy Creek Spring (April/May), HC = Hagar Creek, SCC = Stonecrop Creek, and GBC = Greenbrier Creek.  $P$  and  $r^2$  values correspond to regression results according to equations (1), (2), and (3), for  $S_w$ ,  $v_f$ , and  $U$ , respectively. Stonecrop Creek (open square in bottom panel) is not included in the regression of  $U$  versus  $\text{NO}_3\text{-N}$ . Saturation Response Types (SRTs) for individual streams are delineated in the bottom panel.

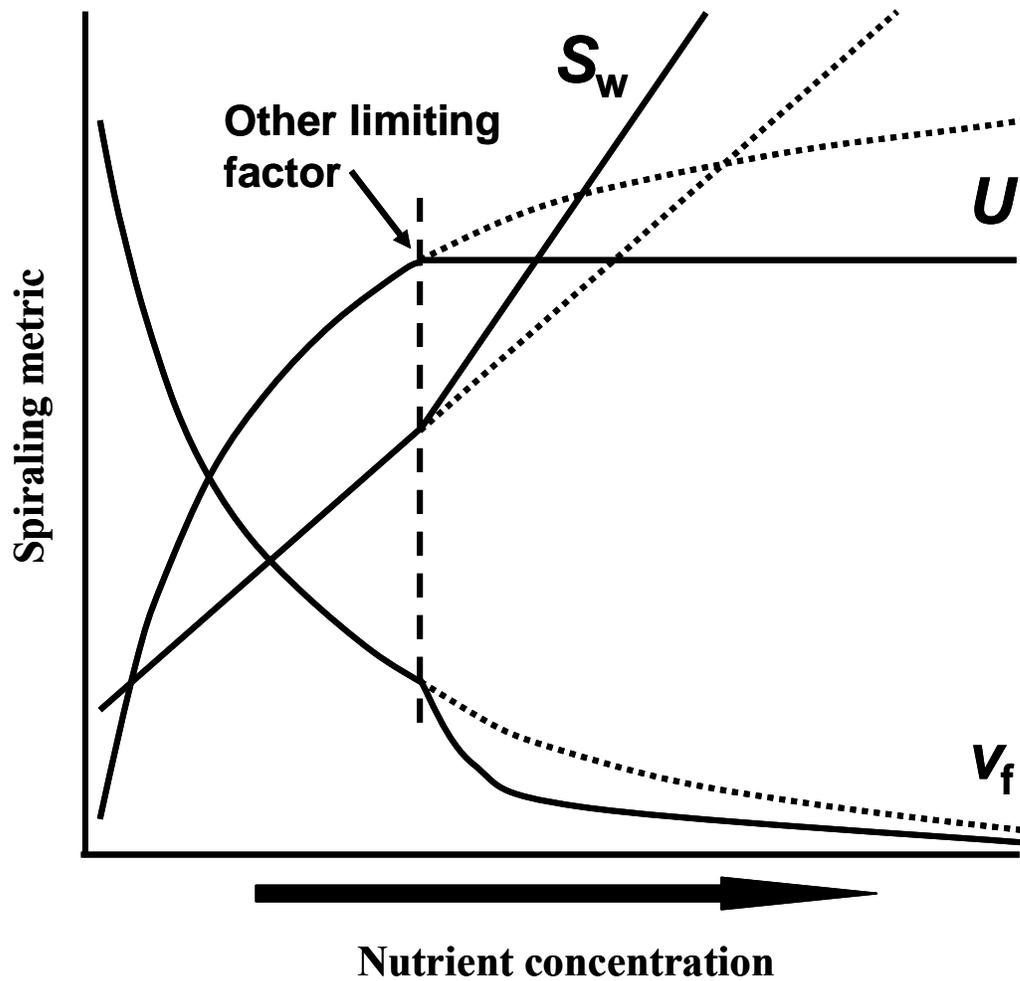


Figure 8. Truncation model. The asymptotic relationship between nutrient concentration and nutrient uptake corresponding to Michaelis-Menten kinetics is truncated when some other factor that is critical to growth becomes limiting. Following truncation, the slope of the relationship between  $U$  and nutrient concentration resembles a flat-line with a slope of zero. Deviation from Michaelis-Menten kinetics following truncation (solid lines) results in an increase in the decay rate of  $v_f$  and a steeper slope in the relationship between  $S_w$  and nutrient concentration.

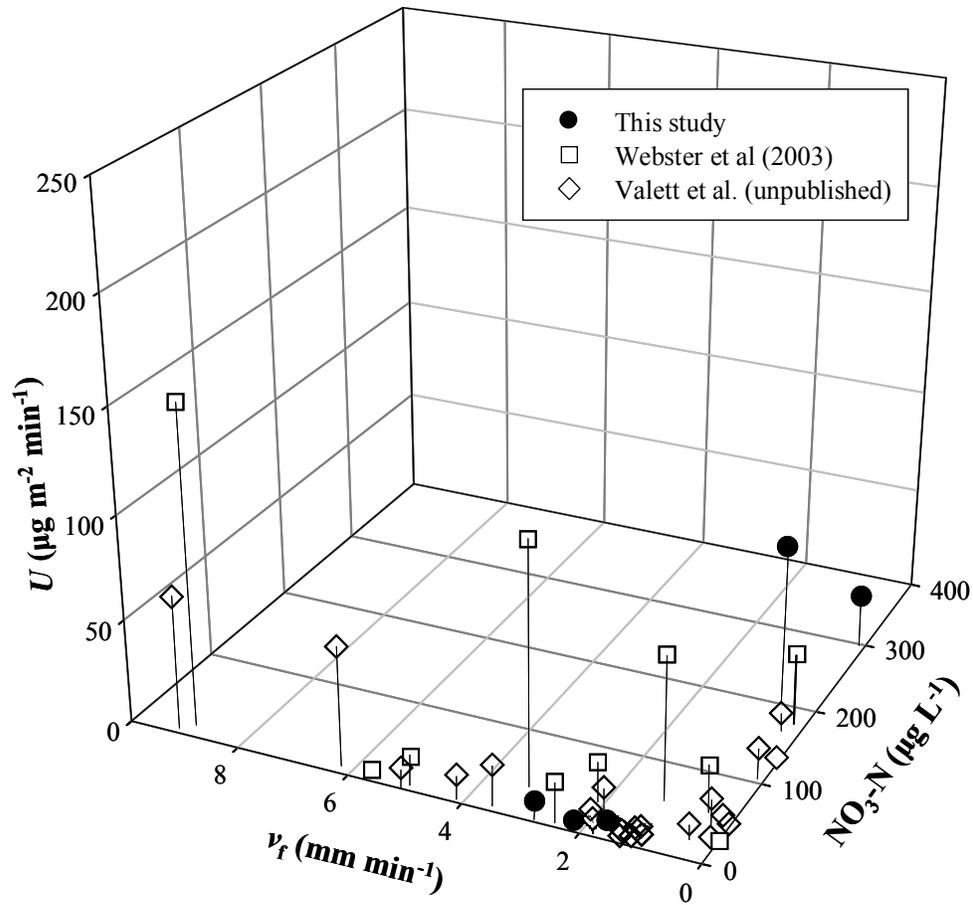


Figure 9. Relationships among streamwater  $\text{NO}_3\text{-N}$ , and tracer-derived  $v_f$  and  $U$ . Data from this study are ambient (i.e., non-amended) values from each site except Greenbrier Creek which was omitted to limit the scale of the  $\text{NO}_3\text{-N}$  axis. Data from Webster et al. (2003) include a cross-biome comparison of 10 (Little Miami River excluded) different streams. Data from Valett et al. (unpublished) include a seasonal comparison of streams in NC, TN, and NM.

## **Chapter 4: Comparison of nitrogen spiraling in stream ecosystems quantified using stable isotope tracers and nutrient addition experiments**

### **Abstract**

Nutrient spiraling provides the conceptual and technical framework for describing nutrient cycling in stream ecosystems. Spiraling is frequently quantified through short-term nutrient additions in which the streamwater nutrient concentration is elevated. However, elevating the nutrient concentration of the stream alters nutrient uptake, and spiraling metrics quantified through amendment experiments do not necessarily reflect spiraling under ambient conditions. Several studies have documented that uptake lengths quantified during nutrient amendments consistently overestimate ambient uptake lengths, and that overestimation is related to the magnitude of the nutrient increase. I examined differences between ambient and amendment-derived  $\text{NO}_3\text{-N}$  spiraling metrics in streams spanning a gradient of streamwater dissolved inorganic nitrogen concentration (DIN). Spiraling metrics were quantified during a short-term  $\text{NO}_3\text{-N}$  amendment and under ambient conditions using a stable isotope ( $^{15}\text{NO}_3\text{-N}$ ) tracer. Uptake lengths measured during amendments were consistently longer than ambient uptake lengths. Amendment-derived  $\text{NO}_3\text{-N}$  uptake velocity and uptake were underestimated relative to ambient conditions. The ratio of amendment-derived to ambient uptake lengths ranged more than 8-fold across streams but was not related to background streamwater DIN concentration or the concentration increase associated with amendments.

Investigators have proposed a method whereby the ambient uptake length can be extrapolated from the relationship between uptake length and nutrient amendment concentration for a series of amendments at different concentrations. I used a series of short-term  $\text{NO}_3\text{-N}$  amendments to compare extrapolated ambient uptake length to ambient uptake length measured using the stable isotope ( $^{15}\text{NO}_3\text{-N}$ ) tracer. Extrapolated uptake lengths were better predictors of ambient uptake lengths than amendment-derived uptake lengths in three of five experiments. However, extrapolated uptake lengths were negative in two streams. Evidence suggests that nitrogen limitation was weak in these two streams and that the back extrapolation technique may be less effective in streams with a high background streamwater nitrogen concentration.

## Introduction

The nutrient spiraling concept (Webster and Patten 1979) provides the theoretical framework to describe the paired processes of nutrient cycling and advective flow in stream ecosystems. The mathematical framework for quantifying nutrient spiraling was detailed by Newbold et al. (1981) and is commonly described by a triad of three interrelated metrics. The uptake length ( $S_w$ ) is the average distance traveled by a nutrient in dissolved form before being immobilized through abiotic or biotic processes into a particulate form. Uptake length reflects both the downstream flux of nutrients and the removal of the nutrient through uptake and is a powerful descriptor of nutrient retention. However,  $S_w$  is strongly influenced by hydraulic properties of streams and typically increases with increasing discharge (Wollheim et al. 2001). Uptake velocity ( $v_f$ ) corrects for stream size and hydraulics and reflects both nutrient uptake and supply (as concentration), thus serves as an index of nutrient uptake efficiency. Finally, uptake ( $U$ ) quantifies the areal specific uptake of a nutrient per unit time.

Uptake length is equal to the negative inverse of the longitudinal loss rate ( $k_L$ ) of a nutrient, which can be quantified by the downstream decline of a nutrient tracer (Newbold et al. 1981). The ideal method for quantifying uptake length is to add a tracer in the form a radioactive or stable isotope of a nutrient. By using an isotopic tracer, the extant nutrient concentration of the stream is maintained and measured uptake length reflects uptake under background (ambient) conditions. However, safety and regulatory concerns prohibit the use of radioactive isotopes in most stream studies, and stable isotope studies are costly and time-consuming (Mulholland et al. 1990, 2002). As an alternative, many investigators have used short-term nutrient additions (amendments) to elevate the nutrient concentration of a stream and monitor the downstream decline of the added nutrient to quantify uptake length (e.g., Munn and Meyer 1990, Davis and Minshall 1999, and many others).

Several studies have documented that uptake lengths measured by nutrient amendments ( $S_w'$ ) consistently overestimate ambient uptake lengths ( $S_w$ ) measured under background concentrations using isotopic tracers (Mulholland et al. 1990, 2000, 2002). This discrepancy occurs because uptake increases with increasing streamwater nutrient

concentration and uptake lengths measured under ambient and nutrient-amended conditions will only be equal if uptake increases proportionately to the increase in streamwater nutrient concentration. Because isotope tracers do not alter the streamwater nutrient concentration, isotope tracer studies quantify gross uptake of the available nutrient in the stream. However, gross uptake under ambient conditions cannot be segregated from uptake of the added nutrient during amendment studies and uptake quantified using short-term nutrient amendments reflects net uptake of the added nutrient. The over-prediction of amendment-derived uptake lengths relative to ambient uptake lengths can be assessed through the ratio of  $S_w':S_w$  (Mulholland et al. 2002).

#### *Extrapolating ambient $S_w$*

The relationship between nutrient concentration and uptake is commonly described using an asymptotic function analogous to the Michaelis-Menten (M-M) equation originally developed to model enzyme kinetics:

$$U = \frac{U_{\max} C}{C + K_m} \quad (1)$$

where  $U_{\max}$  is the maximum uptake capacity,  $C$  is the streamwater nutrient concentration, and  $K_m$  is the half-saturation constant (the concentration at which uptake is one-half of  $U_{\max}$ ). According to this model, uptake increases with increasing nutrient concentration until asymptotically approaching a point of maximum uptake ( $U_{\max}$ ). This relationship has been shown to be applicable at a broad range of scales, including whole-stream uptake (Dodds et al. 2002, S. R. Earl unpublished data).

Mulholland et al. (2002) demonstrated, through both modeled data based on M-M kinetics and empirical data, that the relationship between amendment concentration and  $S_w'$  is linear. This relationship can be described by the following equation (Payn et al. submitted):

$$S_w' = \frac{ud(K_m + C_{BKG})^2}{U_{MAX} K_m} + C_{ADD} \left[ \frac{ud(K_m + C_{BKG})}{U_{MAX} K_m} \right] \quad (2)$$

where  $u$  is the streamwater velocity,  $d$  is the average stream depth,  $C_{BKG}$  is the background streamwater nutrient concentration, and  $C_{ADD}$  is the nutrient amendment concentration above background. Based on this relationship, Payn et al. (submitted) have proposed a method whereby the ambient uptake length can be estimated using a multiple amendment approach to characterize the relationship between  $C_{ADD}$  and corresponding uptake lengths for a given stream. The ambient uptake length can be estimated by extrapolating this relationship to the negative ambient streamwater nutrient concentration ( $-C_{BKG}$ ). Uptake kinetics ( $K_m$  and  $U_{max}$ ) can also be determined from the relationship, and together these measures characterize nutrient affinity. The slope of the relationship between  $S_w'$  and  $C_{ADD}$  is indicative of the severity of nutrient limitation: steeper slopes correspond to less nutrient limitation (Mulholland et al. 2002).

Here I present an analysis of ambient and amendment-derived nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) spiraling in streams that span a gradient of dissolved inorganic nitrogen (DIN) concentration. In each stream, spiraling metrics were independently quantified during a short-term  $\text{NO}_3\text{-N}$  amendment and under ambient conditions using a stable isotope ( $^{15}\text{NO}_3\text{-N}$ ) tracer. In order to compare how amendments overestimated ambient uptake lengths across a range of background and amendment concentrations, I standardized  $S_w':S_w$  to the ratio of  $C_{ADD}$  to  $C_{BKG}$  ( $C_{ADD}:C_{BKG}$ ). Finally, I used a multiple amendment approach to compare measured ambient uptake lengths and extrapolated ambient uptake lengths based on the method proposed by Payn et al. (submitted).

## Methods

I examined five first- or second-order headwater streams in the southern Appalachian Mountains of southwestern Virginia and western North Carolina, USA (Table 1). Study streams were selected to span a gradient of background DIN concentration. All streams had intact, forested riparian zones but land use within associated catchments was variable. Two streams (Sammy Creek and Hugh White Creek) were located within forested catchments relatively undisturbed by human activities since the 1920's. Vegetation included mixed deciduous forests with thick streamside canopies of rhododendron (*Rhododendron maximum* L.). Catchments of the other streams were

comprised of a mixture of forest, small farms, and residential areas. I selected a single study reach (50-388 m) in each stream for assessing  $\text{NO}_3\text{-N}$  uptake. Reach length depended on accessibility, streamwater travel time, and distance appropriate to properly measure uptake.

### *Solute addition experiments*

I conducted a series of short-term (3-5 h) nutrient releases in which streamwater  $\text{NO}_3\text{-N}$  concentration was successively elevated through experimental additions to the study reaches. Releases were generally conducted on consecutive days except at Greenbrier Creek where four releases were conducted over two days. Releases were planned for periods without storms in order to maintain generally similar climatic and hydraulic conditions.

Background samples (i.e., prior to the release) were collected from 4 to 7 transects along the study reach and analyzed for  $\text{NO}_3\text{-N}$ , bromide ( $\text{Br}^-$ ), and  $^{15}\text{NO}_3\text{-N}$ . A subset of the background samples was analyzed for ambient concentrations of ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and orthophosphate ( $\text{PO}_4\text{-P}$ ).

For the first release, a solution of  $\text{K}^{15}\text{NO}_3$  (98 atom %; Cambridge Isotope Laboratories, Andover, MA, USA),  $\text{NaBr}$ , and  $\text{NaCl}$  ( $\text{Br}^-$  and  $\text{Cl}^-$  to act as conservative tracers, sensu Bencala et al. 1990) was released at a constant rate designed to increase the streamwater  $\delta^{15}\text{NO}_3\text{-N}$  by 500‰. Tracer  $^{15}\text{NO}_3\text{-N}$  additions elevated streamwater  $\text{NO}_3\text{-N}$  concentrations by less than  $0.45 \mu\text{g L}^{-1}$ . Target concentrations for  $\text{Cl}^-$  and  $\text{Br}^-$  were  $2.00$  and  $0.30 \text{ mg L}^{-1}$ , respectively.

Three replicate samples were collected from each transect under well-mixed conditions (i.e., tracer plateau as indicated by steady-state conductivity in channel water). Samples for the analysis of  $^{15}\text{NO}_3\text{-N}$  were collected in acid-washed 4-L bottles, chilled until filtered (glass-fiber filters, Whatman GF/F, pore size =  $1.0 \mu\text{m}$ ), and refrigerated ( $\sim 4 \text{ }^\circ\text{C}$ ) for less than 1 week until analyzed. Additional samples ( $n = 3$  per transect) were collected for analysis of  $\text{NO}_3\text{-N}$  and  $\text{Br}^-$ , filtered (glass-fiber filters, Gelman A/E, pore size =  $1.0 \mu\text{m}$ ), and frozen that day.

Similar methods were employed for subsequent releases at each stream, however  $\text{NaNO}_3$ , instead of  $\text{K}^{15}\text{NO}_3$ , was added to the release solution in order to increase the

streamwater  $\text{NO}_3\text{-N}$  concentration. Enrichment concentrations were variable and ranged from  $\sim 40$  to  $600 \mu\text{g NO}_3\text{-N L}^{-1}$  above background.

Conductivity was continuously monitored at the downstream end of the reach by a data-logging Hydrolab Minisonde (Hydrolab, Inc., Loveland, CO, USA). Streamwater velocity was measured by analyzing conservative tracer curves using an advection-dispersion model with transient storage and inflow (Bencala and Walters 1983, Runkel 1998). Discharge ( $Q$ ) at each transect was determined by dilution gauging using the conservative tracer ( $\text{Br}^-$ ) and assumed to be temporally constant throughout background and plateau sampling. Widths and depths of the wetted channel were measured at 5-10 m intervals along the study reach prior to or following the series of releases.

#### *Laboratory methods*

Anions ( $\text{NO}_3\text{-N}$  and  $\text{Br}^-$ ) were analyzed on a Dionex DX500 Ion Chromatograph (Sunnyvale, CA, USA) with an AS4A anion column. Due to low  $\text{NO}_3\text{-N}$  concentrations ( $< 10 \mu\text{g L}^{-1}$ ), samples collected at Sammy Creek and Hugh White Creek were analyzed colorimetrically following reduction by Cd (Wood et al. 1967, APHA 1998) on a Technicon Autoanalyzer (Technicon, Emeryville, CA, USA). Samples were analyzed for  $\text{NH}_4\text{-N}$  using the phenol-hypochlorite method (Solorzano 1969, USEPA 1997a) and  $\text{PO}_4\text{-P}$  as soluble reactive phosphorus using the molbydate antimony method (Murphy and Riley 1962, USEPA 1997b).

$^{15}\text{NO}_3\text{-N}$  was measured by headspace diffusion according to Sigman et al. (1997). Ammonium was removed by boiling samples to a final volume of approximately 100 mL under basic conditions by adding MgO. Nitrate-nitrogen was converted to ammonia ( $\text{NH}_3$ ) by adding Devarda's Alloy. A precombusted, acidified ( $25 \mu\text{L } 2.5 \text{ M KHSO}_4$ ) glass-fiber filter (Whatman GF/D) encased by Teflon® tape was added to the sample immediately after adding the Devarda's alloy. Sealed samples were incubated at  $60 \text{ }^\circ\text{C}$  for 48 h and then gently shaken for 10 d during which time  $\text{NH}_3$  was captured by the acidified filter. The filters were then removed from the Teflon® sandwich, dried, tinned, and shipped to the UC Davis Stable Isotope Facility (Davis, CA) for analysis of  $^{15}\text{N}$  on a Europa Integra mass spectrometer (Sercon Ltd., Cheshire, UK).

In the two streams with background NO<sub>3</sub>-N concentrations < 80 µg L<sup>-1</sup> (Sammy Creek and Hugh White Creek; Table 1), background and plateau samples were spiked with a known amount of NO<sub>3</sub>-N to ensure a minimum mass (≥ 80 µg) of nitrogen (N) required to measure <sup>15</sup>NO<sub>3</sub>-N in a reasonable sample volume (i.e., ≤ 1 L). Thus, the target δ<sup>15</sup>NO<sub>3</sub>-N was elevated such that the sample δ<sup>15</sup>NO<sub>3</sub>-N would reflect an enrichment of 500‰ after spike addition.

#### *Data analysis*

<sup>15</sup>NO<sub>3</sub>-N fluxes were calculated at each transect at both background and plateau according to the following equation:

$$^{15}\text{NO}_3\text{-N flux}_i = \text{AF}_i [\text{NO}_3\text{-N}]_i Q_i \quad (3)$$

where <sup>15</sup>NO<sub>3</sub>-N flux<sub>i</sub> is the downstream transport (µg s<sup>-1</sup>) of <sup>15</sup>NO<sub>3</sub>-N, AF<sub>i</sub> (atomic fraction) is the ratio of <sup>15</sup>N:<sup>14</sup>N+<sup>15</sup>N, [NO<sub>3</sub>-N]<sub>i</sub> is the mean concentration (µg L<sup>-1</sup>) of NO<sub>3</sub>-N, and Q<sub>i</sub> is the discharge (L s<sup>-1</sup>) each at transect i. <sup>15</sup>NO<sub>3</sub>-N fluxes at plateau were corrected for background by subtracting background flux at each transect.

Uptake length was calculated for both NO<sub>3</sub>-N amendment (S<sub>w</sub>') and <sup>15</sup>NO<sub>3</sub>-N tracer (S<sub>w</sub>) releases by fitting an exponential decay model (SigmaPlot, SPSS, Inc., Chicago, IL, USA) to background and dilution corrected NO<sub>3</sub>-N and <sup>15</sup>NO<sub>3</sub>-N flux, respectively, versus distance downstream (Newbold et al. 1981). The standard error (SE) of uptake length was determined as the standard error of the uptake coefficient of <sup>15</sup>NO<sub>3</sub>-N (tracer) or NO<sub>3</sub>-N concentration (amendment) as described by a modified exponential decay model (equation 4). In (4), *a* is equal to the upstream <sup>15</sup>NO<sub>3</sub>-N flux (tracer) or dilution-corrected NO<sub>3</sub>-N concentration (amendment) at the upper-most transect and *x* is distance downstream.

$$\text{NO}_3\text{-N} = a \exp\left(\frac{x}{S_w}\right) \quad (4)$$

Uptake velocity (*v<sub>f</sub>*; mm min<sup>-1</sup>) was calculated following Stream Solute Workshop (1990):

$$v_f = \frac{d}{S_w} u \quad (5)$$

where  $u$  ( $\text{mm min}^{-1}$ ) is the average streamwater velocity, and  $d$  (m) is average stream depth. Uptake velocities estimated from amendment experiments ( $v_f'$ ) were calculated according to equation (5), substituting  $S_w'$  for  $S_w$ . Ambient areal uptake ( $U$ ) was calculated as the product of  $v_f$  and the background streamwater  $\text{NO}_3\text{-N}$  concentration ( $C_{BKG}$ ). Amendment-derived uptake ( $U_{EST}$ ) was calculated as the product of  $v_f'$  and  $C_{BKG}$ .

Linear regression was used to characterize relationships between  $S_w'$  and  $\text{NO}_3\text{-N}$  amendment and to extrapolate ambient uptake length based on these relationships. M-M kinetics ( $K_m$  and  $U_{max}$ ) were estimated from these regressions according to equations (6) and (7).

$$K_m = \left( \frac{\text{intercept}}{\text{slope}} \right) - C_{BKG} \quad (6)$$

$$U_{max} = \frac{ud(K_m + C_{BKG})}{\text{slope } K_m} \quad (7)$$

Linear regression (performed on SigmaStat, SPSS, Inc., Chicago, IL, USA) was used to compare  $S_w':S_w$  to amendment and background  $\text{NO}_3\text{-N}$  concentrations. The slopes of the relationships between  $S_w'$  and  $\text{NO}_3\text{-N}$  amendment were compared among sites by analysis of covariance (ANCOVA) using the PROC REG procedure in SAS (SAS, Inc., Cary, NC, USA). Significance level ( $\alpha$ ) was set to 0.05 for all statistical tests.

## Results

### *Stream characteristics*

Ammonium-nitrogen concentration in the five study streams was consistently low ( $\leq 5 \mu\text{g L}^{-1}$ ), whereas background  $\text{NO}_3\text{-N}$  concentrations ( $C_{BKG}$ ) at the time of the releases varied from 3 to 982  $\mu\text{g L}^{-1}$  (Table 1). The concentration of  $\text{PO}_4\text{-P}$  was generally low,

with the exception of Stonecrop Creek ( $20 \mu\text{g L}^{-1}$ ). Discharge of the study streams varied from 2 to  $31 \text{ L s}^{-1}$  at the time of the releases (Table 1).

#### *Comparison of tracer and amendment studies*

The lowest amendment concentration ( $C_{ADD}$ ) among streams varied from 35 to  $138 \mu\text{g NO}_3\text{-N L}^{-1}$ , representing a four-fold change in experimental amendment. However,  $C_{ADD}:C_{BKG}$  for these experiments ranged 570-fold and was highest in the low-N streams (Table 2). Both  $C_{ADD}$  ( $138 \mu\text{g NO}_3\text{-N L}^{-1}$ ) and  $C_{ADD}:C_{BKG}$  (57.0) were particularly high during a single amended release conducted at Sammy Creek in July 2003.

Amendment-derived net uptake lengths ( $S_w'$ ) determined from the lowest amendment at each site varied from 104 to 4548 m and were consistently longer than ambient uptake lengths ( $S_w$ ) (23 to 3652 m) (Table 2). The longer net uptake lengths translated into consistently lower net uptake velocities ( $v_f'$ ) ( $0.06$  to  $0.89 \text{ mm min}^{-1}$ ) relative to ambient uptake velocities ( $v_f$ ) ( $0.09$  to  $2.80 \text{ mm min}^{-1}$ ) (Table 2). Because  $v_f'$  was consistently underestimated by amendment experiments,  $U_{EST}$  (the product of  $v_f'$  and  $C_{BKG}$ ) also underestimated ambient  $U$ . Amendment-derived  $U_{EST}$  underestimated  $U$  more than 8-fold at Hugh White Creek, however the difference was less than 2-fold at all other sites (Table 2).

Despite a substantial range in the disparity between ambient and amendment-derived spiraling metrics, there were no clear trends relating the discrepancies to either streamwater N concentration or the amendment concentration ( $C_{ADD}$ ). The ratio of  $S_w':S_w$  varied from 1.25 to 9.70 and was markedly higher at Hugh White Creek ( $S_w':S_w = 9.70$ ) relative to all other streams (Table 2). However,  $S_w':S_w$  was not significantly related to  $C_{BKG}$ ,  $C_{ADD}$ , or  $C_{ADD}:C_{BKG}$  ( $r^2 \leq 0.143$ ,  $P \geq 0.460$ ,  $n = 6$ ). Although Hugh White Creek appeared to be an outlier, regressions were still not significant when Hugh White Creek was omitted from the analyses ( $r^2 \leq 0.669$ ,  $P \geq 0.091$ ,  $n = 5$ ). The disparity between  $v_f'$  and  $v_f$  across streams mimicked the pattern of  $S_w':S_w$  and, as with uptake lengths, there were no discernable patterns relating the disparity between  $v_f'$  and  $v_f$  to either  $C_{BKG}$  or  $C_{ADD}$ . However, when  $S_w':S_w$  was standardized to  $C_{ADD}:C_{BKG}$ , the values varied from 0.04 to 12.14 (Table 2) and were significantly related to  $C_{BKG}$  ( $r^2 = 0.769$ ,  $P = 0.022$ ,  $n = 6$ ).

*Multiple amendments: extrapolating to ambient  $S_w$*

At least two releases at different amendment concentrations were conducted in each of the study streams except Sammy Creek in July 2003. Amendment concentrations ( $C_{ADD}$ ) among all releases varied from 35 to 588  $\mu\text{g NO}_3\text{-N L}^{-1}$  and  $C_{ADD}:C_{BKG}$  varied from 0.1 to 163.0 (Table 3, Figure 1). All measured uptake lengths were statistically significant except for the release corresponding to the final and highest amendment ( $C_{ADD} = 394 \mu\text{g NO}_3\text{-N L}^{-1}$ ;  $C_{ADD}:C_{BKG} = 2.2$ ) at Hagar Creek ( $P = 0.154$ ), thus results from this release were not included in the analyses.

Uptake length consistently increased with increasing  $\text{NO}_3\text{-N}$  amendment at all sites (Figure 1). A linear trend was generally apparent in streams when more than two amended releases were conducted (Hugh White Creek, Stonecrop Creek, and Greenbrier Creek) and a linear model described most of the variability ( $r^2 \geq 0.938$ ), although none of these relationships were statistically significant ( $P \geq 0.065$ ), reflecting the low power ( $n = 3$ ) associated with the multiple amendments in each stream.

Extrapolated uptake lengths were similar to ambient uptake lengths ( $S_w$ ) in two of the five experiments. The extrapolated uptake lengths at Sammy Creek in April/May (111 m) and at Stonecrop Creek (605 m) were well within the range of ambient  $S_w$  ( $\pm\text{SE}$ ) measured at those sites, 128 ( $\pm 21$ ) and 632 ( $\pm 24$ ) m, respectively (Table 3). However, the extrapolated uptake length at Hugh White Creek (189 m) was more than 8-fold higher than the ambient  $S_w$  (23  $\pm 2$  m). Extrapolated uptake lengths were negative at both Hagar Creek and Greenbrier Creek (-1342 and -12601 m, respectively) and were substantially different from ambient uptake lengths at both sites (Table 3). The extrapolated uptake length at Hagar Creek is based on a two-point regression (Figure 1), however, including the nonsignificant  $S_w'$  at the highest amendment concentration further decreased the extrapolated uptake length to -4249 m.

M-M kinetics ( $K_m$  and  $U_{\text{max}}$ ) varied across streams and were aberrant in several cases. Estimated  $K_m$  was negative at both Hagar Creek and Greenbrier Creek (-123 and -810  $\mu\text{g L}^{-1}$ , respectively) where extrapolated uptake lengths were also negative (Table 3). Similarly,  $U_{\text{max}}$  was also negative at Hagar Creek and Greenbrier Creek (-11  $\mu\text{g m}^{-2} \text{min}^{-1}$  in both streams). When Hagar Creek and Greenbrier Creek were excluded,  $K_m$  varied from 92 to 447  $\mu\text{g L}^{-1}$  and  $U_{\text{max}}$  varied from 47 to 129  $\mu\text{g m}^{-2} \text{min}^{-1}$  across streams.

Neither  $K_m$  nor  $U_{max}$  were significantly related to  $C_{BKG}$ ,  $C_{ADD}$ , or  $C_{ADD}:C_{BKG}$  ( $P \geq 0.057$ ), however, the limited number of data points ( $n = 3$ ) prohibits a rigorous analysis of these relationships.

The slopes of the relationships between  $S_w'$  and  $C_{ADD}$  were markedly higher at both Hagar Creek and Greenbrier Creek relative to the other streams (Figure 2). A statistical comparison of slopes carried out for those streams where the results of at least three releases were available (Hugh White Creek, Stonecrop Creek, and Greenbrier Creek) indicated that the slope of the relationship between  $S_w'$  and  $C_{ADD}$  was significantly greater at Greenbrier Creek than the slopes at both Stonecrop Creek and Hugh White Creek ( $P \leq 0.013$ ,  $\alpha = 0.017$  after Bonferroni correction). The slopes for Stonecrop Creek and Hugh White Creek were not significantly different ( $P = 0.719$ ).

## **Discussion**

### *Comparison of tracer and amendment studies*

Spiraling metrics measured in this study were well within the range of those reported in published studies. Those measured during the lowest amended release at each site are similar to other reported values for  $\text{NO}_3\text{-N}$  spiraling (Munn and Meyer 1990, Webster et al. 1991, Davis and Minshall 1999). While few studies have quantified  $\text{NO}_3\text{-N}$  spiraling under ambient conditions using stable isotopes, ambient values from this study are within the range of those reported by Webster et al. (2003) in a cross-biome comparison of 11 different streams.

While  $C_{ADD}$  associated with the lowest amendment in each stream varied slightly, the amendment concentration relative to background ( $C_{ADD}:C_{BKG}$ ) varied considerably. During amendments, streamwater  $\text{NO}_3\text{-N}$  concentration was elevated to an extent sufficient to ensure that longitudinal changes in the concentration of the added nutrient could be readily detected. Because  $C_{ADD}$  was generally similar across streams,  $C_{ADD}:C_{BKG}$  fluctuated dramatically depending on background concentrations and was relatively higher in each of the low-N streams (Sammy Creek and Hugh White Creek). In contrast,  $C_{ADD}$  concentrations in the streams with relatively higher background concentrations (Hagar Creek, Stonecrop Creek, and Greenbrier Creek) translated into exceptionally low values of  $C_{ADD}:C_{BKG}$ . The range of  $C_{ADD}:C_{BKG}$  in this study is larger

than that reported by Mulholland et al. (2002). This is particularly true for the low end of the range. In this study, the lowest value for  $C_{ADD}:C_{BKG}$  was  $\sim 0.2$  compared to  $\sim 2$  indicated by Figure 4 of Mulholland et al. (2002). The considerably lower values of  $C_{ADD}:C_{BKG}$  in this study reflect substantially higher background  $\text{NO}_3\text{-N}$  concentrations in several N-rich streams relative to those in the study by Mulholland et al. (2002).

Amendment-derived uptake length ( $S_w'$ ) overestimated the ambient uptake length ( $S_w$ ) in all streams, consistent with patterns reported by Mulholland et al. (2002). Overestimates of  $S_w$  translated into proportionately similar underestimates of  $v_f'$  relative to  $v_f$ . These results confirm that the increase in  $\text{NO}_3\text{-N}$  uptake associated with amendments did not increase proportionately to the increase in  $\text{NO}_3\text{-N}$  availability, even in the low-N streams where N would likely be most limiting.

Although uptake quantified through amendment experiments reflects net uptake of the added nutrient, investigators have traditionally used this approach to approximate ambient uptake. Thus, the background streamwater nutrient concentration, in lieu of the added nutrient concentration, has been used to calculate  $U$  (i.e., the product of  $v_f'$  and  $C_{BKG}$ ; e.g., Bernhardt et al. 2002, and many others). Because the overestimation of  $S_w'$  results in an underestimate of  $v_f'$ , the product of  $v_f'$  and  $C_{BKG}$  results in an underestimate of  $U$  when this approach is taken. The extent to which  $U_{EST}$  underestimates  $U$  was generally proportional to  $S_w':S_w$ . In this study, uptake lengths derived from amendments were on average 3-fold longer than ambient uptake lengths, resulting in an average 3-fold difference between  $U$  and  $U_{EST}$ .

Despite a relatively large range of  $S_w':S_w$  across streams, the range primarily reflected the high  $S_w':S_w$  at Hugh White Creek and there was little variability among the other streams. It is unclear why  $S_w':S_w$  at Hugh White Creek was substantially higher than at the other streams, particularly Sammy Creek in April/May where  $C_{BKG}$ ,  $C_{ADD}$ , and  $C_{ADD}:C_{BKG}$  were similar between the two streams. There was a higher standing stock of organic matter at Hugh White Creek (S. R. Earl unpublished data) which may have contributed to the shorter  $S_w$  at Hugh White Creek relative to Sammy Creek in April/May, however that would not account for the longer  $S_w'$  at Hugh White Creek.

The ratio of  $S_w':S_w$  was not related to either streamwater DIN concentration or to the magnitude of amendment as either concentration ( $C_{ADD}$ ) or concentration relative to

background ( $C_{ADD}:C_{BKG}$ ). These results are contrary to those reported by Mulholland et al. (2002) in which  $S_w':S_w$  of ammonium was significantly related to the ammonium concentration increase (i.e.,  $C_{ADD}$ ). The absence of a similar relationship in this study likely reflects the limited variability of  $S_w':S_w$  across streams and observed enrichment effects and responses between low-N and high-N streams. The efficiency of  $\text{NO}_3\text{-N}$  uptake under ambient conditions, reflected in  $v_f$ , was distinctly higher in each of the low-N streams. Greater uptake efficiency reflects a high demand for N relative to availability. However, uptake efficiency measured under amended conditions ( $v_f'$ ) decreased dramatically in response to the substantial increase in N concentration relative to background concentrations. In contrast, ambient uptake efficiency ( $v_f$ ) was relatively lower in each of the high-N streams but amendments resulted in only slight increases in N concentration relative to background concentrations. Thus, uptake efficiency under amended conditions ( $v_f'$ ) reflected only a slight increase in N concentration relative to background concentration in the high-N streams. These results suggest that the extent to which nutrient amendment experiments overestimate ambient spiraling metrics is a combination of both background and amendment nutrient concentrations in addition to whole-stream nutrient demand. This interaction was also reflected in the significant relationship between  $S_w':S_w$  and  $C_{BKG}$  when  $S_w':S_w$  was standardized to  $C_{ADD}:C_{BKG}$ . I observed the highest ratio of  $S_w':S_w$  to  $C_{ADD}:C_{BKG}$  in the high-N streams, particularly at Hagar Creek and Greenbrier Creek, corresponding to weak limitation in these streams as indicated by the steeper slopes of the relationships between  $S_w'$  and  $C_{ADD}$ .

#### *Extrapolating ambient $S_w$*

Extrapolated ambient uptake lengths were similar to measured ambient uptake lengths in two of the five streams in which at least two amended releases were conducted. Extrapolated uptake lengths at Sammy Creek in April/May and Stonecrop Creek underestimated  $S_w$  by only 13 and 4%, respectively, and extrapolated uptake lengths were better predictors of  $S_w$  than  $S_w'$ . These results are consistent with Payn et al. (submitted) who reported that, compared to  $S_w'$ , back extrapolation gave a better estimate of ambient ammonium uptake for streams in Kansas and Tennessee, and a better estimate of nitrate uptake in a North Carolina stream. Although the extrapolated  $S_w$  was more than 8-fold

higher than the measured ambient  $S_w$  at Hugh White Creek, the extrapolated  $S_w$  was still a better predictor of ambient  $S_w$  than  $S_w'$ .

Although back extrapolation accurately reflected ambient  $S_w$  in two of the five study streams and was moderately successful at Hugh White Creek, the nonsensical results at both Hagar Creek and Greenbrier Creek suggest that this technique is not broadly applicable in all cases. The underlying principle of the back extrapolation approach is that the relationship between nutrient uptake and nutrient concentration is described by an asymptotic function analogous to the M-M equation (Mulholland et al. 2002, Payn et al. submitted). Although  $S_w'$  increased linearly with increasing amendment concentration, as predicted given M-M kinetics, the negative  $K_m$  and  $U_{max}$  values estimated from the relationship suggest that uptake in these streams did not follow M-M kinetics. Dodds et al. (2002) reported that M-M kinetics described N uptake in some streams but not others. The slope of the relationship between  $S_w'$  and  $C_{ADD}$  is indicative of the severity of nutrient limitation, with higher slopes corresponding to streams that are less limited (Mulholland et al. 2002). The slopes of these relationships were relatively higher at both Hagar Creek and Greenbrier Creek, suggesting weak N limitation or, potentially, N saturation. Increased nutrient availability associated with amendments would result in only slight changes in uptake in those streams where that nutrient is weakly limiting or saturating. Further, there may be greater error in quantifying the downstream decline in the concentration of an added nutrient in a stream where there is little demand relative to supply (i.e., weak limitation or saturation), resulting in poor estimates of spiraling. Greater error associated with quantifying spiraling in weakly N-limited or -saturated streams was evident at both Hagar Creek and Greenbrier Creek. Error associated with spiraling metrics at each of these sites was reflected in large standard errors and low  $r^2$  values (data not shown) around estimates of both  $S_w$  and  $S_w'$ , and the nonsignificant  $S_w'$  during the highest amended release at Hagar Creek. Slight changes in uptake with increasing nutrient availability coupled to greater error around estimates of spiraling in weakly limited or saturated streams could lead to considerable inaccuracy using the back extrapolation method.

## Conclusions

My results agree with the findings of other studies that short-term nutrient amendments overestimate ambient uptake lengths in streams (Mulholland et al. 1990, 2000, 2002). The results of the multiple enrichment experiments, in which  $S_w'$  increased with increasing amendment concentration, also support the findings of Mulholland et al. (2002) that the magnitude of overestimating  $S_w$  is related to the concentration increase. However, contrary to the study by Mulholland et al. (2002), I did not see this pattern across streams unless  $S_w':S_w$  was standardized to  $C_{ADD}:C_{BKG}$ . Given the greater slopes of the relationships between  $S_w'$  and  $C_{ADD}$ , I would have expected to see the largest overestimation of  $S_w$  in the high-N streams as small changes in concentration result in relatively greater increases in  $S_w'$ . However, similar amendment concentrations ( $C_{ADD}$ ) across streams resulted in substantially larger amendments relative to background concentration in low-N streams. This substantial increase in supply relative to demand in low-N streams resulted in large increases in  $S_w'$ , similar to increases in  $S_w'$  in high-N streams with similar amendment concentrations.

Estimated ambient  $S_w$  using the back extrapolation technique outlined by Payn et al. (submitted) was a better predictor of ambient  $S_w$  than amendment-derived  $S_w'$  in three of the five study streams, and resulted in very accurate estimates of ambient  $S_w$  at two of those sites. The empirical examples cited in Payn et al. (submitted) generally occurred in low-N streams and, although the technique was successful, overestimation of ambient uptake lengths by amendment experiments did not cause considerable error. They suggested that the back extrapolation technique might be more useful in streams with higher background nutrient concentrations. However, my results indicate that when nutrients are weakly limiting or saturating, as would likely be associated with streams having higher background concentrations, increased error associated with quantifying  $S_w'$ , slight changes in uptake with increased nutrient availability, and apparent deviation from M-M kinetics result in considerable error that renders the back extrapolation technique less useful.

## Literature Cited

- American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater, 20th ed. APHA, New York.
- Bencala, K. E., and R. A. Walters. 1983. Simulation of solute transport in a mountain pool-and-riffle stream: a transient storage model. *Water Resources Research* 19:718-724.
- Bencala, K. E., D. M. McKnight, and G. W. Zellweger. 1990. Characterization of transport in an acidic metal-rich mountain stream based on a lithium tracer injection and simulations of transient storage. *Water Resources Research* 26:989-1000
- Bernhardt, E. S., R. O. Hall, Jr., 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.
- Davis, J. C., and G. W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia* 119:247-255
- 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. Morall, 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.
- Mulholland, P. J., A. D. Steinman, and J. W. Elwood. 1990. Measurement of phosphorus uptake length in streams: comparison of radiotracer and stable  $\text{PO}_4$  releases. *Canadian Journal of Fisheries and Aquatic Sciences* 47:2351-2357.
- Mulholland, P. J., J. L. Tank, D. M. Sanzone, J. R. Webster, W. M. Wollheim, B. J. Peterson, and J. L. Meyer. 2000. Ammonium and nitrate uptake lengths in a small forested stream determined by a  $^{15}\text{N}$  tracer and short-term nutrient enrichment experiments. *Verh. Internat. Verein. Limnol.* 27:1320-1325.
- 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 interbiome comparison study. *Journal of the North American Benthological Society* 21:544-560.
- Munn, M. D., and J. L. Meyer. 1990. Habitat-specific solute retention in two small streams: an intersite comparison. *Ecology* 71:2069-2082.
- Murphy, J., and J. P. Riley. 1962. Determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.

- Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 38:860-863.
- Payn, R. A., J. R. Webster, P. J. Mulholland, H. M. Valett, and W. K. Dodds. Submitted. Estimation of stream nutrient uptake from enrichment experiments. *L & O Methods*.
- Runkel, R.L. 1998. One dimensional transport with inflow and storage (OTIS): a solute transport model for streams and rivers: U.S. Geological Survey Water-Resources Investigation Report 98-4018.
- Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine chemistry* 57:227-242.
- Solorzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* 14:779-801.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95-119.
- U.S. Environmental Protection Agency (USEPA). 1997a. Method 349.0. Determination of ammonia in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis. Zhang, J. Z., P. B. Ortner, C. J. Fischer, and L. D. Moore *in* Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition. EPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- U.S. Environmental Protection Agency (USEPA). 1997b. Method 365.5. Determination of orthophosphate in estuarine and coastal waters by automated colorimetric analysis. Zimmerman, C. F., and C. W. Keefe *in* Methods for the determination of chemical substances in marine and estuarine environmental matrices – 2<sup>nd</sup> edition. EPA/600/R-97/072. National Exposure Research Laboratory, Cincinnati, OH.
- 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., J. D. D'Angelo, and G. T. Peters. 1991. Nitrate and phosphate uptake in streams at Coweeta Hydrologic Laboratory. *Internationale Vereinigung für Theoretische und Angewandte Limnologie, Verhandlungen* 24:1681-1686.

- Webster, J. R., Mulholland, P. J., J. L. Tank, H. M. Valett, W. K. Dodds, B. J. Peterson, W. B. 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, W. M. Wollheim. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology* 48:1329-1352.
- Wollheim, W. M., B. J. Peterson, L. A. Deegan, J. E. Hobbie, and B. Hooker. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnology and Oceanography* 46:1-13.
- Wood, E. D., F. A. J. Armstrong, and F. A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom* 47:23-31.

Table 1. Characteristics of study streams. Nitrate-nitrogen (NO<sub>3</sub>-N), ammonium-nitrogen (NH<sub>4</sub>-N), and orthophosphate (PO<sub>4</sub>-P) are reach average means of background concentrations prior to all releases. Nutrient concentrations < 3 µg L<sup>-1</sup> were below detectable limits (bdl).

Stream	Location	Experiment dates (all dates in 2003)	NO <sub>3</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P	Specific	Discharge
			µg L <sup>-1</sup>			conductance	
						µS cm <sup>-1</sup>	
Sammy Creek (April/May)	Giles County, VA	29 April – 2 May	5	5	bdl	21	7
Sammy Creek (July)	Giles County, VA	15 – 16 July	3	bdl	bdl	20	6
Hugh White Creek	Macon County, NC	8 – 11 October	3	3	bdl	12	7
Hagar Creek	Montgomery County, VA	25 – 28 August	182	bdl	7	62	15
Stonecrop Creek	Giles County, VA	10 – 13 September	298	5	20	262	2
Greenbrier Creek	Roanoke County, VA	14 – 15 August	983	bdl	5	145	31

Table 2. Ambient and amendment-derived spiraling metrics.  $C_{ADD}$  is the  $\text{NO}_3\text{-N}$  amendment concentration above background and  $C_{ADD}:C_{BKG}$  is the ratio of  $C_{ADD}$  to the background streamwater  $\text{NO}_3\text{-N}$  concentration ( $C_{BKG}$ ). Metrics determined by  $^{15}\text{NO}_3\text{-N}$  tracer ( $S_w, v_f, U$ ) reflect spiraling under ambient conditions. Metrics determined by amendment ( $S_w', v_f', U_{EST}$ ) reflect spiraling metrics derived from the lowest amendment concentration in each stream and  $U_{EST}$  is the product of  $v_f'$  and  $C_{BKG}$ .

Stream	$C_{ADD}$	$C_{ADD}:C_{BKG}$	$S_w'$ (SE)	$S_w$ (SE)	$S_w':S_w$	$\frac{S_w':S_w}{C_{ADD}:C_{BKG}}$	$v_f'$	$v_f$	$U_{EST}$	$U$
	$\mu\text{g L}^{-1}$		m				mm min <sup>-1</sup>		$\mu\text{g m}^{-2} \text{min}^{-1}$	
Sammy Creek (April/May)	42	8.5	168 (37)	128 (21)	1.31	0.15	0.89	1.57	4.4	7.8
Sammy Creek (July)	138	57.0	104 (8)	42 (3)	2.48	0.04	0.62	2.09	3.4	5.1
Hugh White Creek	35	10.8	223 (5)	23 (2)	9.70	0.89	0.29	2.80	1.0	8.8
Hagar Creek	41	0.2	1088 (217)	587 (81)	1.85	8.19	0.24	0.46	42.4	84.0
Stonecrop Creek	68	0.2	1047 (158)	632 (24)	1.66	7.21	0.06	0.09	16.4	26.5
Greenbrier Creek	101	0.1	4548 (na)	3652 (1037)	1.25	12.14	0.18	0.23	179.1	222.4

Table 3. Extrapolated uptake lengths and Michaelis-Menten kinetics. The range of amendment concentration for each stream is presented as the amendment concentration above background ( $C_{ADD}$ ) and the ratio of  $C_{ADD}$  to the background streamwater concentration ( $C_{ADD}:C_{BKG}$ ). Extrapolated uptake lengths were estimated by regressing  $S_w'$  measured from multiple amendments in a given stream to the  $\text{NO}_3\text{-N}$  amendment concentration ( $C_{ADD}$ ) and extrapolating to the negative background concentration. Tracer-derived ambient uptake lengths ( $S_w$ ) are presented for comparison. Only one amended release was conducted at Sammy Creek in July, thus an extrapolated  $S_w$  and Michaelis-Menten kinetics could not be determined.

Stream	Amendments		Range of $S_w'$ m	Extrapolated $S_w$ m	$S_w$ (SE) m	$K_m$ $\mu\text{g L}^{-1}$	$U_{\max}$ $\mu\text{g m}^{-2} \text{min}^{-1}$
	$C_{ADD}$	$C_{ADD}:C_{BKG}$					
Sammy Creek (April/May)	42 – 80	8.5 – 16.1	168 – 214	111†	128 (21)	92	129
Sammy Creek (July)	138	57.0	104	na	42 (3)	na	na
Hugh White Creek	35 – 533	10.8 – 163.0	223 – 981	189	23 (2)	125	47
Hagar Creek	41 – 394	0.2 – 2.2	1088 – 8467	-1342†	587 (81)	-123	-11
Stonecrop Creek	68 – 555	0.2 – 1.9	1047 – 1719	605	632 (24)	447	75
Greenbrier Creek	101 – 588	0.1 – 0.6	4548 – 11973	-12601	3652 (1037)	-810	-11

†extrapolated  $S_w$  based on a two-point regression

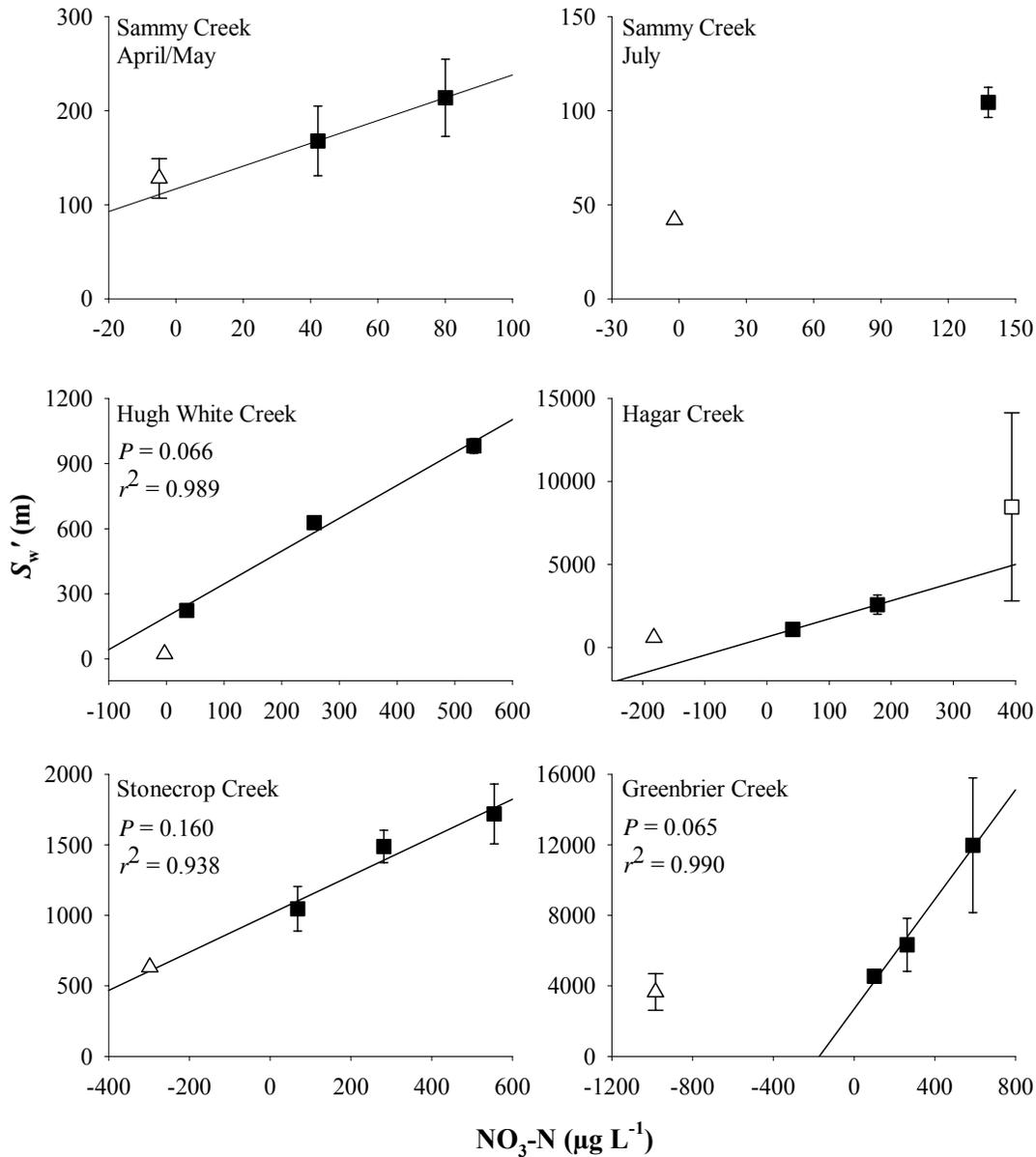


Figure 1. Extrapolations to ambient uptake lengths. Filled squares are  $S_w \pm \text{SE}$  from multiple amendment experiments plotted at the corresponding  $\text{NO}_3\text{-N}$  amendment concentration ( $C_{ADD}$ ). Open triangles are tracer-derived ambient uptake lengths ( $S_w$ ) plotted at the negative ambient streamwater  $\text{NO}_3\text{-N}$  concentration. Standard error of the first amended release at Greenbrier Creek could not be determined. The nonsignificant  $S_w'$  at Hagar Creek (open square) was not included in the analyses. Linear regression results ( $P$ ,  $r^2$ ) are presented for those streams in which more than two releases were included in the analyses. Extrapolation was not possible at Sammy Creek in July where only one amended release was conducted.

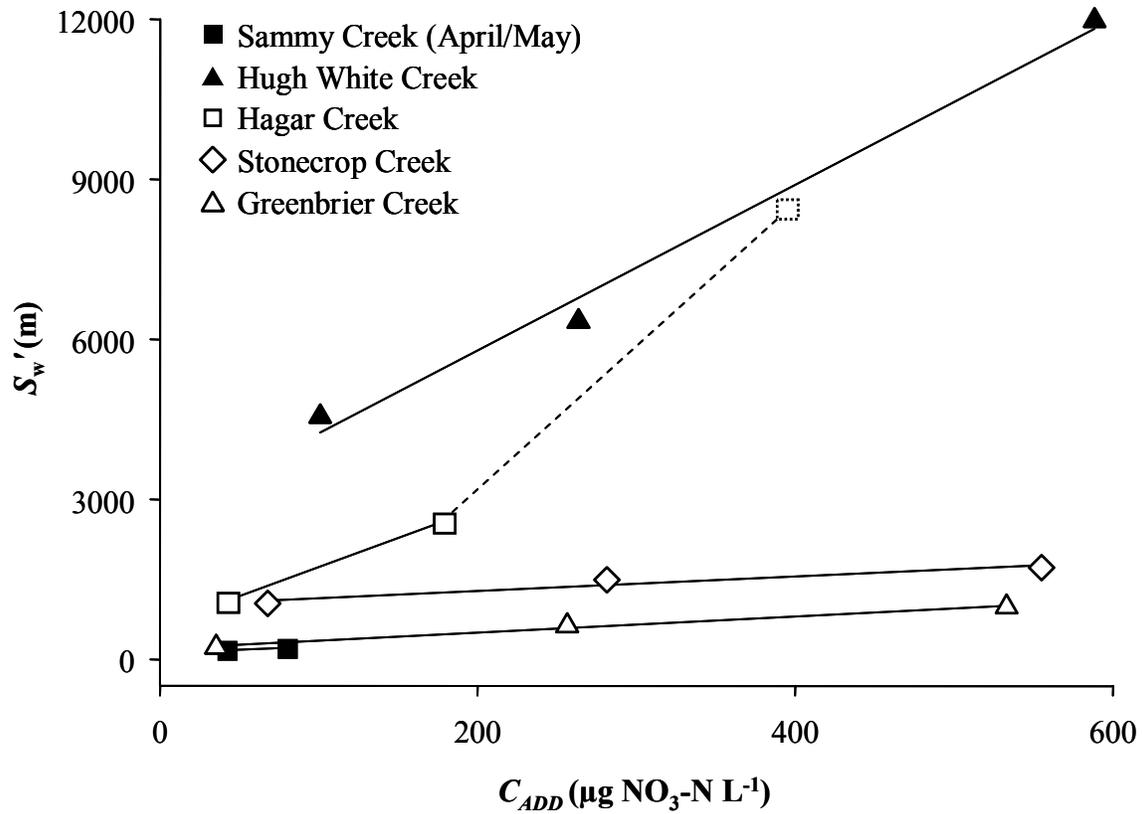


Figure 2.  $\text{NO}_3\text{-N}$  amendments ( $C_{ADD}$ ) and  $S_w'$  for all releases. The July 2003 release at Sammy Creek was omitted as only one amended release was conducted at that time. The open, dashed square and line correspond to the nonsignificant  $S_w'$  at Hagar Creek. Lines represent best-fit linear regression at sites where more than two releases were conducted (except Hagar Creek).

# Curriculum vitae

## Stevan R. Earl

### 11 October 2004

#### Education

**Virginia Polytechnic Institute and State University, Blacksburg, Virginia**  
Ph.D., Biology, October 2004

**Northern Arizona University, Flagstaff, Arizona**  
M.S., Biology, May 1999  
B.A., Environmental Science, December 1995

#### Professional and Research Experience

**Virginia Polytechnic Institute and State University, Blacksburg, Virginia**  
*Research Assistant – 2001 to 2004*

- Responsible for coordinating field components of several stream ecology research projects
- Managed technical staff of approximately 12 employees
- Oversaw day-to-day lab operations, including maintaining lab resources and equipment

*Doctoral Candidate - 1999 to 2004*

- Examined the relationships between nitrogen availability and stream structure and function

*Teaching Assistant - 1999 to 2001*

- Instructed several lab courses, including General and Honors Biology, and Freshwater Ecology
- Independently developed and implemented curriculum for Honors Biology lab course
- Guest lecturer for Ecology, Freshwater Ecology, and Groundwater Ecology courses

**Northern Arizona University, Flagstaff, Arizona**

*Master of Science - 1995 to 1999*

- Investigated the influence of fire-ash runoff on water quality and algal and macroinvertebrate assemblages in southwestern USA streams
- Monitored the water quality of the Gila River through the Gila River Bird Habitat Area, Gila National Forest, New Mexico

*Research Assistant - 1995 to 1999*

- Assisted with data collection and analyses on several stream and river ecology research projects
- Maintained the freshwater algae culture collection

## **Publications**

Earl, S. R., and D. W. Blinn. 2003. Effects of wildfire ash on water chemistry and biota in South-Western U.S.A. streams. *Freshwater Biology* 48:1015-1030

Simmons, G. W., J. R. Webster, S. R. Earl, and C. L. Burcher. 2001. Methods for use in freshwater ecology. Department of Biology, Virginia Tech

Earl, S. R., and D. W. Blinn. 1999. Implications of forest fires, land use, and discharge on water chemistry and biota along the Gila River, New Mexico. U.S. Department of Agriculture, Gila National Forest, Silver City, New Mexico

Blinn, D. W., F. R. Govedich, and S. R. Earl. 1996. Preliminary report on an aquatic ecosystem assessment of four major tributaries in the Gila Wilderness Area, New Mexico: role of fire on stream ecosystems. U.S. Department of Agriculture, Gila National Forest, New Mexico

## **Presentations**

Earl, S. R., H. M. Valett, and J. R. Webster. 2004. An analysis of nitrogen saturation in stream ecosystems. 89th annual meeting of the Ecological Society of America. Portland, Oregon, USA

Earl, S. R., H. M. Valett, and J. R. Webster. 2004. An analysis of nitrogen saturation in streams. 52nd annual meeting of the North American Benthological Society. Vancouver, B.C., Canada

Earl, S. R., H. M. Valett, and J. R. Webster. 2003. Nitrogen uptake and retention in nutrient enriched streams. 51st annual meeting of the North American Benthological Society. Athens, Georgia, USA

Webster, J. R., R. A. Payn, E. N. J. Brookshire, S. R. Earl, O. S. Starry, H. M. Valett, and P. J. Mulholland. 2003. Estimation of ambient nutrient uptake from nutrient enrichment experiments. 51st annual meeting of the North American Benthological Society. Athens, Georgia, USA

Earl, S. R., H. M. Valett, and C. G. Peterson. 2002. Nitrogen retention in streams spanning a gradient of ambient stream water nitrogen concentration. 50th annual meeting of the North American Benthological Society. Pittsburgh, Pennsylvania, USA

Earl, S. R., and D. W. Blinn. 2000. Implications of forest fires on water quality and biota of streams in the Gila National Forest, New Mexico. 48th annual meeting of the North American Benthological Society. Keystone, Colorado, USA

Earl, S. R., and D. W. Blinn. 1998. Role of fire ash on nutrients and food web dynamics in a Southwestern USA forest stream. 52nd annual meeting of the Phycological Society of America. Flagstaff, Arizona, USA

Earl, S. R., and D. W. Blinn. 1998. Fire and its effects on water quality. Gila Monster: Interstate Watershed Management Program. Glenwood, New Mexico, USA

## **Grants and Awards**

An analysis of nitrogen saturation in streams, Travel Fund Award, Graduate Student Assembly, Virginia Polytechnic Institute and State University, \$220.00, June 2004

Nitrogen uptake and retention in nutrient enriched streams, Travel Fund Award, Graduate Student Assembly, Virginia Polytechnic Institute and State University, \$200.00, May 2003

Nitrogen retention in streams spanning a gradient of ambient stream water nitrogen concentration, Travel Fund Award, Graduate Student Assembly, Virginia Polytechnic Institute and State University, \$300.00, June 2002

Dissertation research: Quantifying nitrogen spiraling in anthropogenically enriched stream ecosystems, Doctoral Dissertation Improvement Grant (DDIG), Ecosystems Studies Program, Division of Environmental Science, National Science Foundation, Arlington, VA, \$9985.00, April 2002

Quantifying nitrogen spiraling in anthropogenically enriched stream ecosystems, Graduate Research Development Grant (GRDP), Graduate Student Assembly, Virginia Polytechnic Institute and State University, \$500.00, October 2001

Quantifying nitrogen spiraling in anthropogenically enriched stream ecosystems, Integrating applied and basic research grant, The Proctor and Gamble Co., \$500.00, May 2001

Quantifying nitrogen spiraling in anthropogenically enriched stream ecosystems, Virginia Academy of Science research grant, \$1250.00, May 2001

Outstanding Master's Thesis, 2000 Graduate Awards, Northern Arizona University

## **Workshops**

Ecological Circuitry Collaboratory - August 2002 to August 2003  
(NSF sponsored workshop to train ecologists in quantitative and simulation modeling)

## **Professional Affiliations**

North American Benthological Society

Ecological Society of America