

CONTROLS OF NITROGEN SPIRALING IN KANSAS STREAMS

by

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B.S., Southern Illinois University at Carbondale, 1999

M.S., Southern Illinois University at Carbondale, 2002

AN ABSTRACT OF A DISSERTATION

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Division of Biology
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ABSTRACT

We used a series of whole stream experiments to quantify the impacts of inorganic-nitrogen concentration on stream nitrogen cycling and transport in prairie streams. We conducted $^{15}\text{NO}_3^-$ stable isotope tracer experiments to measure the nitrogen cycling dynamics in 9 streams with a wide range (over 5 orders of magnitude) of nitrate concentrations. The major nitrogen-transforming processes, including uptake, nitrification, and denitrification, increased approximately 2 to 3 orders of magnitude and did not show signs of Michaelis-Menten type saturation across streams. Denitrification only accounted for a small proportion of total nitrate uptake. The observed functional relationships of biological nitrogen transformations and chronic nitrate concentration were best described by a Log-Log relationship. A series of inorganic-nitrogen addition experiments were conducted to quantify the impacts of acute nitrogen inputs on nitrogen cycling. These experiments showed that uptake saturated in response to short-term pulses of nitrogen. Ambient concentrations of ammonium and nitrate were less than their respective half-saturation coefficients, and uptake rates were less than 5% of V_{\max} , suggesting severe limitation of nitrogen. The saturation of uptake due to acute nitrogen inputs contrasts with uptake associated with chronic inputs of nitrate, which was not found to saturate. Chamber experiments and whole-stream ammonium addition experiments demonstrated that uptake and mineralization of ammonium varies spatially within the stream channel, occurring predominantly in riffles as opposed to pool habitats. The total transport distance of nitrogen and carbon within prairie streams was estimated based on field measurements and nutrient spiraling theory. Transport of organic nitrogen was dominant in prairie streams, as compared to inorganic nitrogen transport, both in terms of total concentration and transport distance. These results indicate that

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Major Professor
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INTRODUCTION

At present, the input of biologically available nitrogen (N) from human activities into the world's ecosystems is 10 times that of 1890, prior to wide spread use of fertilizers and fossil fuels (Galloway and Cowling 2002). A significant portion of this nitrogen is leached from the landscape and transported by streams and rivers to the ocean (Rabalas 2002, Donner et al. 2004). In coastal areas, this excess nitrogen has resulted in massive algal blooms, creating anoxic or hypoxic conditions in the water column resulting in destruction of the marine community (McIssiac et al. 2001, Rabelais 2002). A substantial portion of the N entering streams, however, is retained before transport to the sea. Retention of nitrogen in streams and rivers may be important in limiting such eutrophication effects (Alexander et al. 2000). Within the context of larger river networks, low-order streams can have a disproportionately large impact on the retention and attenuation of N due to the greater contact between dissolved N in the water column and benthic microorganisms (Alexander et al. 2000). A lingering question is, to what degree will increases in dissolved nitrogen concentrations impact the transport and retention of N by the streams?

Streams draining pristine watersheds typically have little dissolved inorganic nitrogen (Lewis 2002), however, human land uses such as urbanization and agriculture can greatly increase concentrations of nitrogen (N) (Torrecilla et al. 2005). These increases in N transport are often a result of increases in nitrate (NO_3^-) concentration (Bernot and Dodds 2005). The transport, retention, and removal of N from the landscape can be greatly influenced by biotic in-stream processes (Peterson et al. 2001, Mulholland 2004, Bernhardt et al. 2005).

N cycling in streams

Biological processes such as the assimilation (or uptake) of N by heterotrophic and autotrophic organisms can influence in-stream cycling of N (Peterson et al. 1999, Hall and Tank 2003), however, the influence of prolonged exposure to elevated N conditions is not fully understood. The growths of heterotrophic and autotrophic organisms are, in many instances, limited or co-limited by N and P (Tank and Dodds 2003, Niyogi et al. 2004). Conversely, Kemp and Dodds (2002b) demonstrated that uptake of N in prairie stream substrata often saturated in response to added NO_3^- and NH_4^+ .

Mineralization is the conversion of organic-N to ammonium (NH_4^+), primarily by the heterotrophic microbial community. The process of mineralization has not been intensively studied in streams, but the rate of mineralization relative to respiration is expected to increase as organic matter quality improves (e.g., lower C:N ratios) with greater N availability.

Nitrification is the microbially mediated conversion of NH_4^+ to NO_3^- . Nitrification was historically thought to be important only in streams with relatively high NH_4^+ concentrations, and its importance in relatively low-nutrient, pristine streams was not widely appreciated until recently (e.g., Peterson et al. 2001, Kemp and Dodds 2002a). Nitrification can be greater within agriculturally influenced streams (Kemp and Dodds 2000a), and is expected to increase as a function of NH_4^+ concentration.

Denitrification is the process by which NO_3^- is converted to di-nitrogen (N_2) and nitrous oxide (N_2O) gasses and is removed from the ecosystem entirely. Early estimates of denitrification in streams suggested that denitrification is an important process in the removal of N (Hill 1979, Jansen et al. 1998). Denitrification rate can also increase with concentration of NO_3^- in agricultural influenced systems (Kemp and Dodds 2002a, Inwood et al. 2005). More

recent research has demonstrated, however, that the rates of denitrification measured in small-scale stream sediment experiments may not be sufficient to have a large impact in the fluxes of N observed in agricultural streams (Royer et al. 2004, O'Brien and Williard 2006).

Bernot and Dodds (2005) argued that saturation of biological nutrient processing is inevitable under elevated nutrient concentrations because, at some point, other factors will begin to limit nutrient transformation rates. Additionally, N uptake and denitrification can exhibit saturation kinetics in response to added NO_3^- and NH_4^+ in sediment-scale experiments (Kemp and Dodds 2002b, Garcia-Ruiz et al. 1998), but NH_4^+ and NO_3^- uptake do not always saturate at the whole stream level (Dodds et al. 2002, Kemp and Dodds 2002). Also, even if short-term increases in N cause saturation, the response to chronic N increases may differ as the stream microbial and autotrophic communities have time to adjust. Most uptake experiments have been conducted in streams with short-term spikes of nutrients, but less is known about uptake across streams that have had time to acclimate to higher nutrient concentrations (chronic N loading).

N cycling at the whole-stream level

The transport and cycling of nutrients in streams and rivers is often referred to as 'spiraling' due to the downstream displacement of dissolved and particulate materials as the nutrient cycles between forms (Webster and Patten 1979). The length of the nutrient spiral can be characterized as the uptake length (S_w), the average distance that a dissolved atom travels before being taken up, and the particle transport distance (S_p) as the average distance traveled in the particulate form (Newbold et al. 1981). The total spiral length (S) is the sum of individual lengths of each compartment (e.g., S_w , S_p) multiplied by the probability that an atom will move through that compartment (Newbold et al. 1982).

Typically, the spiral lengths of N and P are described based on the dissolved inorganic forms, which have potentially the greatest availability to the autotrophic and heterotrophic microflora. Additionally, two other related parameters are often used to compare differences in nutrient spiraling characteristics (Stream Solute Workshop 1990). The flux rate per unit area of stream bottom is the uptake rate (U_t). The rate of movement of molecules out of the water column (V_f) is the mass transfer coefficient. These parameters are used to quantify changes in inorganic nutrient spiraling over time in a single stream (e.g., Simon et al. 2005) or to compare differences in nutrient spiraling among streams (e.g., Peterson et al. 2001). Streams with short spiral lengths are linked to increased short-term retention of N, while longer spiral lengths indicate less short-term retention.

The concept of nutrient spiraling was first used to describe export of Ca^{+2} and Mg^{+2} cations from forested watersheds (Webster and Patten 1979). Indices to describe nutrient spiraling such as spiral length, inorganic, and organic fluxes were developed by Newbold et al. (1981). These concepts were adapted and applied in their investigations of phosphorous cycling at Oakridge National Lab (Newbold et al. 1983, Mulholland et al. 1985, 1996) through the use of radioisotopes. Further studies demonstrated that the length of the inorganic P spiral was dependent on the concentration of PO_4^{-3} (Mulholland et al. 1985, Hart et al. 1993).

Nitrogen spiraling has been investigated at many sites through stable-isotope tracer studies and nutrient addition experiments. These studies have been conducted in relatively undisturbed headwater streams (e.g., Dodds et al. 2000, Hall and Tank 2003) as well as streams draining agricultural (Neogi et al. 2004, Bernot et al. 2005) and urban dominated catchments (Grimm et al. 2005). These studies provide a greater understanding of the mechanisms of

nitrogen uptake and retention in pristine systems and factors that control spiraling (Peterson et al. 2000, Dodds et al. 2002, Webster et al. 2003).

The two main objectives of this study were to 1.) quantify the impacts of land-use and N inputs on stream N cycling and 2.) use nutrient spiraling theory to characterize the transport of N in prairie streams. I addressed these objectives throughout this dissertation. In chapter 1, $^{15}\text{NO}_3^-$ stable isotope tracer experiments were conducted on 9 streams with varied intensities and types of human impacts in their watersheds to measure the effects of chronic N enrichments on NO_3^- cycling dynamics. In chapter 2, chamber experiments and short-term whole-stream ammonium addition experiments were used to determine the patterns of ammonium uptake and mineralization rates in Kings Creek, a prairie stream. In chapter 3, short-term N addition experiments were conducted in prairie streams to contrast the impacts of acute (pulsed) N enrichments on N cycling with those of chronic enrichments. In chapter 4, organic carbon spiraling theory was used to quantify the spiraling length of C and organic N, completing the total N spiral in four prairie streams.

CHAPTER 1-The saturation of N cycling in streams: ^{15}N experiments across a broad gradient of nitrate concentrations¹

¹ Modified from: O'Brien, J.M., W.K. Dodds, K.C. Wilson, J.N. Murdock and J. Eichmiller. The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentrations. *Biogeochemistry in review*

Abstract

We conducted $^{15}\text{NO}_3^-$ stable isotope tracer releases in nine streams with varied intensities and types of human impacts in the upstream watershed to measure nitrate (NO_3^-) cycling dynamics. Mean ambient NO_3^- concentrations of the streams ranged from 0.9 to 21,000 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$. Major N-transforming processes, including uptake, nitrification, and denitrification, all increased approximately 2 to 3 orders of magnitude along the same gradient. Despite increases in transformation rates, the efficiency with which stream biota utilized available NO_3^- decreased along the gradient of increasing NO_3^- . Observed functional relationships of biological N transformations (uptake and nitrification) with NO_3^- concentration did not support a 1st order model and did not show signs of Michaelis-Menten type saturation. The empirical relationship with the best fit was obtained when plotting log-transformed rates of cycling (uptake and nitrification) against log-transformed nitrate concentration. This log-log relationship was linear with a slope less than one. Denitrification increased linearly across the gradient of NO_3^- concentrations, but only accounted for ~1 % of total NO_3^- uptake. On average, 42% of stream water NO_3^- in water column was lost to denitrification per km, but the percentage removed was highly variable across streams. Although the rate of cycling was greater in streams with larger NO_3^- concentrations, the relative proportion of NO_3^- retained per unit length of stream decreased as NO_3^- concentration increased.

Introduction

Streams draining pristine watersheds typically export mostly organic nitrogen (N) and little dissolved inorganic N (Lewis 2002). Human land uses such as urbanization and fertilizing cropland can have large impacts on the concentrations of N transported in streams, particularly in the form of nitrate (NO_3^-). Increased NO_3^- transport occurs in part because the concentrations in the water columns are raised well above levels typical of pristine watersheds (Bernot and Dodds 2005; Torrecilla et al. 2005). Increased N in flowing waters can interfere with biotic integrity of streams (Dodds and Welch 2000), as well as cause downstream eutrophication in marine habitats such as the Gulf of Mexico (Rabelais 2002).

In-stream processes influence the transport, retention, and removal of N from the landscape (Peterson et al. 2001; Mulholland 2004; Bernhardt et al. 2005). Within the context of larger river networks, low-order streams can have a disproportionately large impact on the rate at which N is retained and attenuated within streams (Alexander et al. 2000). Nitrogen transport is governed by the rate of N cycling in the stream. Downstream transport is often referred to as N ‘spiraling’ due to the downstream displacement of N as it cycles from inorganic to organic forms and back again (Webster and Patten 1979).

Streams with short spiral length have relatively more short-term retention of N, while longer spiral lengths indicate less short-term retention. Long-term retention could be related to deposition of sediments over years or longer (Bernot and Dodds 2005) and will not be considered directly in this paper. Retention will be defined as short-term retention related to uptake processes for the purposes of our study.

Streams can also serve as an important site of denitrification (Hill 1979; O’Brien and Williard 2006), the process by which NO_3^- is converted to N gas (N_2) and nitrous oxide (N_2O)

gas and removed from the ecosystem entirely. The production of N_2O via denitrification in streams is also of environmental concern, because N_2O is a powerful greenhouse gas and can catalyze stratospheric ozone destruction.

Prairie streams were historically common in central North America, but today a large portion of the native prairie landscape has been converted to agriculture and urban uses (Dodds et al. 2004). Fertilizer and pesticide application within the watersheds has degraded the water quality of these formerly pristine prairie streams (Dodds and Oakes 2004). Human impacts on N retention in streams of this biome are relevant, because the areas historically covered with tallgrass prairie now contribute heavily to N transport into the Northern Gulf of Mexico (Alexander et al. 2000). We studied Central Plains streams under a range of anthropogenic N loadings to characterize spiraling and retention.

A lingering question, across all types of streams, is to what degree will anthropogenically driven increases in loading of dissolved N impact the transport, short-term retention and removal of N? Heterotrophic and autotrophic processes that influence in-stream spiraling of N are, in many instances, limited or co-limited by N and P (Tank and Dodds 2003; Niyogi et al. 2004). Nitrification and denitrification may be stimulated in N-rich agricultural sites, compared with N-poor prairie sites (Kemp and Dodds 2002a), and NO_3^- concentration can be the dominant predictor of denitrification in the sediments of agricultural streams (Inwood et al. 2005). Thus, increases in N concentration could result in concomitant retention or removal while increases in concomitant transport rates are negligible.

Bernot and Dodds (2005) argued that saturation of biological nutrient processing is inevitable under elevated nutrient concentrations because, at some point, other factors will begin to limit nutrient transformation rates. But phosphorus uptake or growth experiments in streams

(Bothwell 1989; Mulholland et al. 1990) and lakes (Dodds 1995) reveal half-saturation constants for uptake or growth for assemblages that are far greater than those exhibited by individual organisms in laboratory culture. The rates of ammonium (NH_4^+) uptake, NO_3^- uptake, nitrification, and denitrification do not always saturate in prairie streams (Dodds et al. 2002; Kemp and Dodds 2002b). Furthermore, even if short-term increases in N cause saturation, the influence of chronic N increases is not well characterized across systems. Most uptake experiments have been conducted in streams with short-term spikes of nutrients, but less is known about uptake across streams that have had time to acclimate to higher nutrient concentrations (chronic N loading).

Functional relationships between N processing and concentration

There are three models we consider most likely to describe the relationship between N processing rates and N concentration, based on previously published relationships. Each model has distinct implications for the functional relationships between spiraling characteristics, such as spiral length and uptake velocity, and N concentration in the water column of the stream (Fig. 1.1).

The first model is a 1st order response, in which biological process rates are tightly linked to available NO_3^- and are directly proportional to N concentration. In the 1st order model, process rates, such as uptake per unit area (U_t), nitrification, and denitrification, increase linearly with N concentration. Retention efficiency, as indexed by vertical uptake velocity (V_f) and uptake length (S_w), the average distance traveled before being taken up, are not expected to change in relation to increase in N load (Stream Solute Workshop 1990).

The second model takes the form of Michaelis-Menten uptake kinetics, and represents a clear saturation of processing rates as the supply of available NO_3^- exceeds biological demand (Bernot and Dodds 2005). In this model, U_t , nitrification and denitrification exhibit hyperbolic relationships, with increasing N concentration described by Michaelis-Menten type kinetics. As a result of saturation, S_w exhibits a linear increase with the increase in N load, where as V_f would be expected to dramatically decrease along the same gradient.

In the third model, the rate of N cycling by the biota increases with N availability, but efficiency of the process rates relative to concentration declines. An example is the relationship between NH_4^+ uptake and concentration in streams observed by Dodds et al. (2002). This model is described by a power relationship in which the exponent (or order) is less than one ($U_t = k \cdot [\text{N}]^m$, where $m < 1$). We will refer to this model as Efficiency Loss model. The model predicts that process rates will increase with increasing N load, S_w will increase nonlinearly with increasing nutrient concentration, and V_f will decrease nonlinearly along the same gradient.

We used $^{15}\text{NO}_3^-$ stable isotope injections on a series of 9 streams with various human impacts, along a gradient of NO_3^- concentration (~ 1 to $20,000 \mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$), to characterize cycling and retention. The streams in our study varied widely, including undisturbed tallgrass prairie streams; incised, silt-bottomed agricultural streams; and a concrete-lined urban ditch. The gradient of NO_3^- concentrations across the 9 streams in this study allowed us to test the hypothesis that the rate of NO_3^- cycling within the stream would exhibit saturation with increasing NO_3^- concentrations.

Methods

Study sites

This study was conducted in the Flint Hills region of Northeast Kansas, USA, which is home to the largest tract of remaining tallgrass prairie in the Great Plains. The area is characterized by rolling hills that are underlain by limestone and shale layers (Oviatt 1998). Because of the shallow, rocky soils in much of the region, the primary land use in the area is cattle grazing, although deeper lowland soils are suitable for row crop agriculture. Nine low-order streams in the vicinity of Manhattan, Kansas, were selected from among three general land use categories: prairie/reference, agriculture, and urban. Streams all had low discharge (were low order) and varied widely in NO_3^- concentration (Table 1.1). The watershed characteristics of each of the stream are provided and are in order of increasing mean water column NO_3^- concentrations (Table 1.2).

Four streams draining native vegetation provided a baseline of human impact and nutrient concentrations. Kings Creek-K2A, Kings Creek-N4D, and Shane Creek are located on the Konza Prairie Biological Station. The watersheds drained by these streams are dominated by native tallgrass prairie. Natalie's Creek drains an unfertilized, annually burned cattle pasture of predominantly prairie grasses. Kings Creek (and N4D in particular) has been heavily studied, including descriptions of the N cycle (Dodds et al. 2000), aquatic community (Gray and Dodds 1998) and hydrology (Gray et al. 1998).

Two agriculturally influenced streams were also included in this study. Ag North Creek drains a watershed with mixed land uses flowing through a straightened, incised stream channel in a reach surrounded by row-crop agriculture. Swine Creek also flows through a straightened, incised stream channel and drains a mixed watershed, including row-crop agriculture and several

livestock-rearing facilities. The riparian zones of both streams include grass buffer strips with occasional shrubs.

Additionally, three urban streams with varying levels of human impact were investigated. Little Kitten Creek drains a watershed dominated by a golf course (that has retained a portion of native vegetation) and recent housing development. Campus Creek drains a watershed on the campus of Kansas State University and exhibits elevated NO_3^- concentrations that probably originate from historic livestock holding facilities (now paved, with buildings, or with lawns) upstream from the point of the experiment. Finally, Wal-Mart Ditch is a former channel of the Big Blue River and is fed by the storm drainage system of the City of Manhattan, Kansas. The stream has been channelized and landscaped with 2 m high retaining walls on either side and the streambed is sealed with concrete.

Field methods

$^{15}\text{NO}_3^-$ isotope addition experiments were conducted on each of the streams between May 2003 and June 2005 as part of the Lotic Inter-site Nitrogen eXperiment II (LINX II) project. A solution of $^{15}\text{NO}_3^-$, along with a NaBr conservative solute, was injected into the stream at a steady rate (20 mL min^{-1}) for 24 hours. The amount of K^{15}NO_3 added to the release solution was scaled for each individual stream to produce a target δ enrichment of 20,000‰ of the NO_3^- in the stream water (Mulholland et al. 2004).

Pre-enrichment stream water samples (duplicate samples for $^{15}\text{NO}_3^-$) were collected to establish background isotopic ratios at six stations along the length of the stream reach before the release. Background samples of dissolved $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ were also collected at 10 stations along the length of the reach before the release.

During the $^{15}\text{NO}_3^-$ release, duplicate samples for $^{15}\text{NO}_3^-$ analysis were collected at six stations along the length of the reach (approximately 300 m) at 01:00 of the second day (12 h after the release began) and 12:00 of the second day (23 h after the release began) to determine day and night N uptake and spiraling metrics. Samples were filtered in the field through Whatman GF/F glass fiber filters (0.7 μm retention) and transported back to the laboratory where they were frozen or analyzed immediately.

Samples of dissolved gaseous N were collected at 10 locations along the length of the reach during the $^{15}\text{NO}_3^-$ releases coincident with water sampling. Water samples were collected in 60-mL (2003) or 140-mL (2004 and 2005) plastic syringes fitted with stopcocks, with care taken not to include bubbles with the samples. With the sample syringe submerged underwater, 20 mL of high purity He were added to each syringe. Syringes containing both He and stream water were shaken for 5 min to allow equilibration of dissolved N_2 gas into the He headspace. Again underwater, stream water was expelled from the syringes, and He headspace was collected in evacuated 12-mL exetainer vials (Vial Type 3, Labco, High Wycombe, Buckinghamshire, UK). The sample vials were stored in water-filled centrifuge tubes to avoid contamination from the atmosphere, and were analyzed for $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ by mass spectrometry (Mulholland et al. 2004; S. K. Hamilton unpublished).

Samples of hyporheic water were collected along the length of the stream reach with a “sipper” sampler. The “sipper” consisted of a hollow aluminum tube with a 1.25- cm^2 opening near the tip containing a 1.5-mm inner diameter TFE tube with a stainless steel 0.3-mm mesh fuel filter at the end (Hamilton, S.K., personal communication). The tip of the “sipper” was inserted 7 to 19 cm into the substratum and the sample was collected via syringe, after rinsing the

tubing and bottle with hyporheic water. Hyporheic water samples were placed on ice, filtered with 0.45- μm nylon filters, and analyzed for NO_3^- .

Standing-stock organic biomass for each stream was measured 24 hours after the release, at 10 stations along the reach. At each station, a 1- m^2 metal frame was placed without bias in the stream channel, and all coarse benthic organic matter (CBOM), macrophytes, and filamentous algae found within the frame were collected. Surface (material suspended with gentle agitation of water above the benthic surface) and deep (material from the surface to several cm deep suspended into the water with vigorous stirring) fine benthic organic matter (FBOM) were collected using a stovepipe corer device. Epilithic biofilm samples were collected by scraping a known surface area from 3 rocks selected without bias from each sampling station. Additional biomass samples were collected at each of the six water-sampling stations for ^{15}N analysis using the same techniques as biomass sampling.

Yellow Springs Instruments logging data sondes were deployed within the reach to take continuous (5-min interval) dissolved O_2 measurements for the duration of the ^{15}N experiments. These data were used to calculate whole stream metabolism, community respiration (CR), and gross primary production (GPP), using the one-station method (Bott 1996). Either propane or acetylene was used as a gas tracer, in conjunction with NaBr as a conservative hydraulic tracer, to determine the rate of reaeration along the stream reach. Gas was directly bubbled into the stream, along with a concurrent NaBr addition (to correct for dilution of gas). Water and dissolved-gas samples were taken at several locations downstream. Dissolved-gas samples were collected by drawing 5 mL of stream water into a 10-mL plastic syringe and transferring the water into a 15-mL He-filled exetainer.

Water samples were analyzed for bromide concentration using an ion-selective electrode. Dissolved gas samples were analyzed for propane and acetylene using a Shimadzu GC-14A gas chromatograph with a flame-ionization detector (Hayesep Q column, oven temperature = 50 °C, flow rate 25 mL/min). The dilution-corrected decline in propane or acetylene concentration was then used to calculate the longitudinal gas-exchange coefficient. This coefficient was then multiplied by a correction factor of 1.39 for propane (Rathbun et al. 1978) or 0.867 for acetylene to convert it to an O₂ exchange rate. Average velocity of the stream was then multiplied by the longitudinal O₂ exchange coefficient to determine the time-based reaeration coefficient (K₂₀).

The channel hydrologic parameters and transient storage zone sizes for each stream were measured using a step release of a hydrologically conservative tracer (NaBr) into the stream (Webster and Ehrman 1996). Stream Br⁻ concentrations were measured over time at a downstream station. The shape of the Br⁻ pulse at the downstream site was used to quantify the size of the transient storage zone using the OTIS-P model (Stream Solute Workshop 1990).

Laboratory methods

The ¹⁵N content of stream water NO₃⁻ was determined by using a modified version of the method presented by Sigman et al. (1997). Stream water samples of about 50 μg NO₃⁻-N were concentrated and NH₄⁺ was removed by adding 3.0 g MgO and 5.0 g NaCl and boiling. Stream water ¹⁵NO₃⁻ samples collected during the isotope release were spiked with additional NO₃⁻ (10× stream water NO₃⁻ concentration) in order to reduce δ¹⁵N below 2000‰ (the upper detection limit of the stable isotope laboratory). Samples were then transferred to 250-mL media bottles to which an additional 0.5 g MgO, 0.5 g Devarda's Alloy, and a Teflon filter packet were added. The Teflon filter packet was constructed by sealing a 10-mm Whatman GF/D glass-fiber filter,

acidified with 25 μL of 2.0 M KHSO_4 , within a packet made of a folded piece of 2.5-cm Teflon plumbing tape. The sample NO_3^- was reduced to NH_4^+ by reacting at 60 $^\circ\text{C}$ for 48 hr with DeVarda's alloy. Sealed bottles were then placed on a shaker for 7 days to allow for diffusion. The hydrophobic Teflon allowed for diffusion of NH_3 onto the acidified filter. The glass-fiber filter was then removed from the media bottle, dried, and analyzed for ^{15}N on a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and a Conflo II interface.

Stream water NO_3^- concentration was determined colorometrically by using the cadmium reduction method on a Technicon auto-analyzer (APHA 1995). Stream water NH_4^+ concentration was analyzed by using the indophenol method on a Technicon auto-analyzer. Stream water-soluble reactive phosphorus (SRP) was determined with the molybdate reduction method and analyzed on a Technicon auto-analyzer (APHA 1995). Total dissolved N (TDN) and total dissolved P (TDP) were determined by using a persulfate digestion, followed by analysis for NO_3^- or SRP, respectively (Valderrama 1981). Br^- hydraulic tracer concentrations were determined with a calibrated Orion 9435BN ion-specific electrode.

Standing-stock biomass was oven dried at 60 $^\circ\text{C}$ and weighed. Subsamples were ashed at 450 $^\circ\text{C}$ for 1.5 hr to determine the percentage of organic matter. Biomass samples for ^{15}N analysis were freeze-dried, ground, and analyzed for ^{15}N on a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and a Conflo II interface.

Calculations

Data from the plateau stream water $^{15}\text{NO}_3^-$ samples were corrected for background and spike $^{15}\text{NO}_3^-$ to determine total concentration of tracer $^{15}\text{NO}_3^-$ at each station. $^{15}\text{NO}_3^-$

concentration was then multiplied by discharge at each station to find the total $^{15}\text{NO}_3^-$ flux. Station-specific discharge was calculated by dilution of the Br^- hydraulic tracer. The flux of $^{15}\text{NO}_3^-$ at each station was ln-transformed, and slope of the decline in ln-transformed $^{15}\text{NO}_3^-$ flux over the length of the reach (k_m) was calculated using linear regression.

We calculated uptake length (S_w), from slope, k_m , as

$$S_w = -1 \times k_m^{-1} \quad (1)$$

for $^{15}\text{NO}_3^-$ during both plateaus measured at each stream. From S_w , we calculate the uptake rate (U_t):

$$U_t = \frac{F}{S_w \times w} \quad (2)$$

in which F is the flux of NO_3^- in stream water and w is average width of the stream. Mass transfer coefficient (V_f) was then calculated as:

$$V_f = \frac{U_t}{C} \quad (3)$$

in which C is the concentration of NO_3^- in the stream water (Stream Solute Workshop 1990).

The rate of nitrification occurring within the stream reach was calculated by mass balance of NO_3^- entering and leaving the reach. NO_3^- concentration was multiplied by discharge at stations 1 (most upstream) and 6 (most downstream) below each $^{15}\text{NO}_3^-$ addition site to achieve influx (IN) and out flux (OUT) rate measurements, respectively, of NO_3^- in each experimental stream reach. Groundwater NO_3^- flux rate (GW) inputs were calculated based on the change in stream discharge (Q) between stations 1 and 6, multiplied by average hyporheic NO_3^- concentration. Uptake flux (U) was calculated by multiplying U_t by the stream width and reach length. Nitrification was then calculated as the difference of inputs and outputs from the stream reach according to the mass balance equation:

$$\text{Nitrification} = \text{IN} + \text{GW} - \text{U} - \text{OUT} \quad (1.4)$$

The rate of denitrification occurring within the stream was determined by using longitudinal flux of tracer $^{15}\text{N}_2$ with the model presented by Mulholland et al. (2004). Flux of total $^{15}\text{N}_2$ was calculated by multiplying the mole fraction of ^{15}N in the N_2 gas at each station during the $^{15}\text{NO}_3^-$ experiment by the measured mass of dissolved N_2 in the water, multiplied by the stream discharge. Tracer $^{15}\text{N}_2$ was then calculated by correcting total $^{15}\text{N}_2$ for background by subtracting ambient $^{15}\text{N}_2$ flux as measured from the pre- ^{15}N addition samples.

The pattern of the longitudinal $^{15}\text{N}_2$ flux was then used to determine the coefficient of denitrification (K_{den}) by using the equation:

$$dC/dx = K_{\text{den}} \times A_0 \times e^{-(k_1) \times x - k_2 \times C} \quad (1.5)$$

in which C is flux of tracer $^{15}\text{N}_2$ ($\mu\text{g s}^{-1}$), A_0 is $^{15}\text{NO}_3^-$ flux at the injection location, k_1 is loss rate of $^{15}\text{NO}_3^-$ with distance (inverse of S_w), k_2 is N_2 air-water gas exchange coefficient, and x is downstream distance. Estimates of K_{den} was then calculated using an iterative approach that minimized the sum of squares between modeled and observed $^{15}\text{N}_2$ fluxes by using the Microsoft Excel Solver tool (Microsoft Excel 2000, Microsoft Corporation, Redmond, WA). Rate of denitrification was calculated by multiplying stream NO_3^- flux by K_{den} and dividing by average stream width.

Assimilatory uptake by biomass was calculated for each biomass compartment in the stream, based on total mass of ^{15}N tracer found in each biomass compartment (e.g., surface and deep FBOM, epilithon, leaves) at each station. Biomass-specific uptake was calculated as total molar mass of ^{15}N tracer per m^2 of each compartment divided by mole frequency of ^{15}N to ^{14}N in the NO_3^- of the overlying stream water.

Biological N demand was calculated for each stream according to the method and assumptions of Webster et al. (2003). Autotrophic demand was calculated for each stream, based on GPP measurements from stream metabolism estimates. Net primary production was estimated to be 70% of GPP (Graham et al. 1985, Hill et al. 2001). Photosynthetic quotient, or number of moles of CO₂ fixed per mole of O₂ released, was estimated to be 1.2 (Wetzel and Likens 2000). A carbon to nitrogen (C:N) molar ratio of 12 was then used to calculate N demand from CO₂ fixed. This ratio is the C:N of actively growing algal cultures, instead of measured C:N of the stream epilithon, which contains non-algal materials.

Heterotrophic N demand was then calculated using CR from the metabolism estimates (Webster et al. 2003). CR was corrected for autotrophic respiration (30% of GPP) and nitrification (2 mol O₂ per mol N) to determine heterotrophic respiration. A respiratory quotient of 0.85 was used to convert number of moles O₂ consumed to number of moles CO₂ evolved. Heterotrophic production was then assumed to be 28% of heterotrophic respiration, based on CO₂ evolution. Heterotrophic biomass was assumed to have a molar C:N of 5. Actual C:N of FBOM at each stream was used to calculate the proportion of heterotrophic N demand satisfied by consumption of FBOM. Total biotic N demand was the sum of autotrophic and heterotrophic N demand calculated from whole-stream metabolism measurements.

Statistical analyses

Linear regression was also used to test for a relationship between stream metabolism and transient storage zone size. An ANCOVA procedure was used to detect any consistent effect of day and night on NO₃⁻ spiraling metrics and process rates, with NO₃⁻ concentration used as the continuous covariate. All variables in the ANCOVA were similarly log transformed. Ratios of

U_t :nitrification were tested against a mean of 1 for day and night samplings by using a paired t-test.

Models of biotic response to increasing NO_3^- concentration were evaluated using the following statistical tests. The relationship between process rate and NO_3^- concentration was considered to be saturated if there was a significant fit with the Michaelis-Menten model (using least squares regression with Levenberg-Marquardt estimation algorithm, and non-log transformed data) and calculated K_s was within the range of experimental NO_3^- concentrations in the study. Linear regression was used to determine 1st order and Efficiency Loss models (df = 9 unless otherwise stated). In these regression analyses, dependent and independent variables were log transformed to satisfy regression assumptions of constant residual variance and normality of residuals and to reduce leverage effects of high- NO_3^- streams. Because of the log-transformations, the slope of any 1st order relationship between dependent and independent variables would be equal to one, which was determined using a t-test of the slope of the regression ($H_a: m = 1$). If the Efficiency Loss model was valid, then the slope of the regression would have to be between zero and one ($0 < m < 1$), which was likewise tested using t-tests ($H_a: m < 1$). Saturation was also tested using S_w by linear regression between S_w and stream NO_3^- concentration, in which the Michaelis-Menten model would be valid if there was a linear relationship between S_w and mean stream NO_3^- concentration, the 1st order model would be valid no relationship existed between S_w and mean stream NO_3^- , or the Efficiency Loss model would be valid if as significant power relationship was found in which $m < 1$. These relationships were tested using linear regression of non-log-transformed variables (linear relationship) and log-transformed variables (power relationship) with a t-test of the slope estimate. Censored regression (such as Tobit regression) was not used because of the small sample size, which may

bias the maximum likelihood estimation that forms the basis of such techniques (Helsel and Hirsch 2002). All statistical tests were conducted with Statistica 6 (Statsoft, Tulsa, OK, USA) software package.

Results

Stream chemical and physical parameters

Chemical and hydrologic conditions differed widely across the streams (Table 1.1). Nitrate concentration ranged across five orders of magnitude, from $0.9 \mu\text{g L}^{-1}$ to $21,000 \mu\text{g L}^{-1}$ NO_3^- -N. Concentrations of NH_4^+ ranged from below the detection limit ($1.0 \mu\text{g L}^{-1}$) to $32 \mu\text{g L}^{-1}$ NH_4^+ -N, and dissolved organic N concentration ranged from $85 \mu\text{g L}^{-1}$ to $472 \mu\text{g L}^{-1}$ N. Organic N was the dominant N fraction in low nutrient streams, whereas NO_3^- was the largest component in high NO_3^- streams (DON could not be detected in Campus Creek and Swine Creek using the presulfate-digestion method due to high background NO_3^- concentrations). Soluble reactive phosphorus concentrations ranged from $0.2 \mu\text{g L}^{-1}$ to $35.4 \mu\text{g L}^{-1}$.

All streams in this study were low-order, shallow and had discharges of 0.2 L s^{-1} to 26.3 L s^{-1} . Average water velocities ranged from 0.9 to 6.7 m min^{-1} . The relative size of transient storage zones (A_s/A , the ratio of transient storage zone area to stream channel area) also differed across streams, ranging from 0.06 in the channelized reach of Ag North to 1.54 in the natural prairie reach of Kings Creek -K2A. Exchange rates of these transient storage zones (α) differed across streams but were not correlated with size of the transient storage zone. We were unable to obtain estimates of transient storage for Natalie's Creek or Wal-Mart Ditch, due to flow disruptions that occurred during the conservative tracer release.

Stream metabolism and biomass

Whole-stream estimates of metabolism demonstrated that many of the streams had a strong autotrophic component (Table 1.3). Gross primary production (GPP) ranged from 0.62 g O₂ m⁻² day⁻¹ in Natalie's Creek to 12.5 g O₂ m⁻² day⁻¹ in Wal-Mart Ditch. Many of the streams were not shaded, leading to significant growths of filamentous algae, macrophytes, and epilithon. Community respiration ranged from 0.97 at Campus Creek to 7.02 g O₂ m⁻² day⁻¹ at Wal-Mart Ditch. The GPP and CR did not significantly co-vary along the gradient of increasing stream NO₃⁻ concentration. There was no statistically significant relationship ($p = 0.98$) between CR and the cross-sectional area of transient storage zone (A_s), as would be true if community respiration rates measured by O₂ changes in the water column were driven by activity within the hyporheic zone of these streams.

Surface and deep (0 – 10 cm) FBOM tended to be the largest standing-stock compartments across streams (Fig. 1.2). The exception to this was Wal-Mart Ditch, in which the thick algal biofilm was classified as epilithon and was the only biomass compartment found in the stream in substantial amounts. None of the standing-stock biomass compartments seemed to increase along the gradient of increasing NO₃⁻ concentration.

Nutrient dynamics

Nitrate uptake lengths (S_w) ranged from 17 m in Shane Creek to 2800 m in Little Kitten Creek, and increased along the increasing NO₃⁻ gradient (Fig. 1.3). There was large diurnal variability in S_w between the midnight and noon sampling, but the diurnal effect was not consistently greater in either day or night sampling across streams (ANCOVA, NO₃⁻ as

covariate, $F_{1,15} = 0.17$, $p = 0.69$). The efficiency of NO_3^- utilization, as indexed by V_f , decreased along the gradient of increasing NO_3^- (Fig. 1.3).

Nitrate uptake rates ranged from $0.01 \mu\text{g N m}^{-2} \text{s}^{-1}$ in Shane Creek to $192 \mu\text{g N m}^{-2} \text{s}^{-1}$ in Swine Creek, and increased along the gradient of streams with increasing NO_3^- concentration (Fig. 1.3). Nitrification ranged from $0.01 \mu\text{g N m}^{-2} \text{s}^{-1}$ at Kings Creek -K2A to $59.2 \mu\text{g N m}^{-2} \text{s}^{-1}$ at Swine Creek (Fig. 1.3). Nitrification increased with greater NO_3^- concentration in a similar manner to U_t . Nitrification and U_t were essentially balanced in the nine streams of this study, despite the dual increases in process rates that occurred along the NO_3^- gradient (Fig. 1.4). In addition, diurnal changes in uptake and nitrification often shifted the balance between U_t and nitrification. These ratios of U_t :nitrification did not significantly differ from one during either day or night samplings.

Total denitrification ranged from below detection to $2.0 \mu\text{g N m}^{-2} \text{s}^{-1}$ in Swine Creek (Fig. 1.5). Isotopic tracer was not detected in dissolved N_2 in Shane Creek, Ag North and Kings Creek- K2A, nor in dissolved N_2O in Shane Creek and Kings Creek- K2A. The total denitrification rates were greater at night than in the day in all of the high- NO_3^- streams. In nearly all of the streams, the dominant end product of denitrification was N_2 gas (Table 1.4). Production of N_2O in most cases was $<1\%$ for total denitrification. The exception to this was Ag North, in which there was no detectible $^{15}\text{N}_2$ production, while there was a relatively low N_2O production. With higher background concentration of N_2 , the detection limit for denitrification with N_2 as the end product still exceeded N_2O production rate in this stream.

Nutrient dynamics in relation to NO₃⁻ concentration

The largest and most important gradient across the 9 streams in this study was NO₃⁻ concentration, which allowed us to test the hypothesis that the rate of NO₃⁻ cycling within the stream would exhibit saturation with increasing NO₃⁻ concentrations. The statistical parameters used to test the criteria of the three functional relationship models can be found in Table 5.

We found a significant Michaelis-Menten model fit to the relationship between U_t and NO₃⁻, however, the half-saturation coefficient was several orders of magnitude higher than experimental concentrations ($K_s = 1.9 * 10^{10} \mu\text{g L}^{-1} \text{NO}_3^{-}\text{-N}$), suggesting that there was no saturation occurring within the broad range of concentrations encountered in this study. The high statistical significance of this Michaelis-Menten model was due to the high degree of leverage by the Swine Creek data. This leveraging was not an issue with log-transformed data. We found a significant log-log relationship ($R^2 = 0.84, p < 0.001$) between log U_t and log NO₃⁻ (Fig.1.6) with a slope of 0.66 (S.E. = 0.11). The slope of this relationship was significantly different from a slope 1, which excludes the 1st order model (Table 1.5). The Efficiency Loss model fit both required criteria ($m < 1, m > 0$).

The relationship between nitrification and NO₃⁻ concentration yielded significant Michaelis-Menten model fit, but due to a very large K_s relative to experimental concentrations ($K_s = 4.0 * 10^{10} \mu\text{g L}^{-1} \text{NO}_3^{-}\text{-N}$), no saturation of nitrification across the gradient of streams concentrations was evident. We found a significant relationship ($R^2 = 0.62, p = 0.012$) between log nitrification and log NO₃⁻ concentration across the streams in this study (Fig.1.6) with a slope of 0.54 (S.E. = 0.16). The slope of this relationship was significantly different from a slope 1, which again would have been true if the 1st order model were valid. The Efficiency Loss model fit both required criteria ($m < 1, m > 0$).

Log-transformed total denitrification demonstrated a marginally significant linear relationship with $\log \text{NO}_3^-$ concentration ($R^2 = 0.54$, $df = 7$, $p = 0.061$) (Fig.1.7). Although there was no evidence for saturation, the slope of the relationship was not significantly distinguishable from 1 and marginally distinguishable from zero, therefore the relationship fits the criteria for the 1st order model. Denitrification estimates from Kings Creek- K2A and Shane Creek were excluded from this analysis because denitrification could not be detected in these streams. Production of N_2O by denitrification was also related to NO_3^- concentration ($R^2 = 0.93$, $df = 7$, $p < 0.001$). The ratio of $\text{N}_2\text{O}:\text{N}_2$ production did not significantly change along the NO_3^- gradient.

A significant relationship ($R^2 = 0.60$, $p = 0.013$) existed between $\log S_w$ and $\log \text{NO}_3^-$ concentration across the streams in this study, indicating that the average distance traveled by NO_3^- increased as the quantity of NO_3^- increased. Linear regression of non-transformed S_w and NO_3^- concentration did not yield a significant relationship ($R^2 = 0.19$, $p = 0.23$). Uptake lengths also tend to be heavily influenced by the other factors such as discharge, which can be taken into account by examining stream NO_3^- flux (concentration \times Q). The relationship between $\log S_w$ and $\log \text{NO}_3^-$ flux was significant (Fig.1.6)($R^2 = 0.74$, $p = 0.003$).

The final NO_3^- cycling characteristic that we tested was V_f , which can be used as an index of the efficiency of uptake relative to the available NO_3^- . Again, if saturation were occurring as predicted by the Michaelis-Menten model, we would expect V_f to decrease in relation to NO_3^- concentration. We found a significant negative linear relationship ($R^2 = 0.58$, $p = 0.017$) between $\log V_f$ and $\log \text{NO}_3^-$ concentration across the streams in this study (Fig. 1.6).

Discussion

The relationship between spiraling and concentration

The goal of this study was to determine the influence of N concentration on in-stream N processing across a wide variety of stream types created by human activities, and to characterize saturation according to three potential models. We found that increased NO_3^- concentrations in the streams resulted in a stimulation of NO_3^- cycling rates and did not result in clear saturation of Michaelis-Menten form.

The 1st order model describes a relationship in which biological process rates are directly proportional to N concentration and will increase linearly with N concentration. The exponent (slope) of the increases in U_t and nitrification were less than one, which did not support the 1st order model. Also, S_w increased and V_f decreased as a function of NO_3^- concentration, which indicated that N spiraling was not following 1st order function across streams. Conversely, the relationship between denitrification and NO_3^- concentration did meet the criteria for the 1st order model.

According to the concept of saturation, NO_3^- concentration would reach a level at which biological capacity is saturated, and beyond which additional NO_3^- would no longer stimulate increased cycling, however, U_t , nitrification, and denitrification showed no sign of saturation ($K_s \gg$ experimental concentrations). Also if saturation were occurring as predicted by the Michaelis-Menten model, S_w would be expected to exhibit a linear relationship with increasing NO_3^- concentration (Fig. 1.1), which was not found to be true. The data from our study do not support the hypothesis of Michaelis-Menten saturation across streams as Bernot and Dodds (2005) had predicted.

Alternatively, according to an Efficiency Loss response, process rates of N cycling will increase with NO_3^- concentration, but at a slower rate than the increase in N concentration, resulting in a loss of N processing efficiency. Across the nine streams investigated, increases in U_t and nitrification were consistent with the Efficiency Loss model. Also as predicted by this model, V_f decreases along the NO_3^- gradient and S_w continued to increase with increasing NO_3^- concentration. Therefore, data from our study support the concept of Efficiency Loss response, in which the efficiency of N processing decreases with the increase in NO_3^- availability.

The results presented here represent the response to increased NO_3^- concentrations across a spatial gradient of streams receiving a presumably steady, chronic level of N input. This response may differ from responses to acute pulses of NO_3^- concentration, which may not follow the same pattern observed here. Dodds et al. (2002) reported a 1st order response to short NO_3^- additions on Kings Creek, while O'Brien (2006) observed saturation of NO_3^- uptake in response to short-term addition experiments in three prairie streams. Thus the response of an individual stream to temporal increases in NO_3^- may follow the Michaelis-Menten or 1st order models, but the response across streams with chronic NO_3^- inputs is best described with the Efficiency Loss model.

We hypothesize that diverse microbial communities that are exposed to elevated levels of NO_3^- over longer periods of time adjust to higher NO_3^- concentrations. This adjustment may occur as a result of changes in biochemical pathways, changes in microbial biomass or changes in community composition. Whereas short-term response to increased NO_3^- could be more likely to follow Michaelis-Menten type saturation, long-term response of the microbial community leads to the Efficiency Loss response we observed in this study. Further research is necessary to establish the exact mechanism for this form of saturation across a variety of stream types.

Denitrification and N₂O production

The increase in denitrification with NO₃⁻ concentration was consistent with a 1st order model across streams and did not show signs of saturation. Few studies have looked at the saturation kinetics of denitrification across whole streams. Denitrification rates saturate in laboratory experiments, with a wide range of half-saturation coefficients (K_s 180 $\mu\text{g L}^{-1}$ to 8900 $\mu\text{g L}^{-1}$) across a river continuum (Garcia-Ruiz et al. 1998). Kemp and Dodds (2002b) found that denitrification did not always saturate in prairie stream sediments, and predicted a linear response between denitrification rates and NO₃⁻ in their scaled ecosystem estimates.

Denitrification rate may eventually saturate at very elevated NO₃⁻, although this was not detected by our current study. Bernot and Dodds (2005) predicted saturation rates of 100 to 500 $\text{mg N m}^{-2} \text{day}^{-1}$. Only the denitrification rate observed in Swine Creek fell into this range. Denitrification is also highly variable within streams, as well as across streams, and NO₃⁻ concentration is certainly not the only controlling factor. Other factors influencing the rate of denitrification include the availability of labile carbon, dissolved oxygen availability, and microbial biomass (Cook and White 1987). All of these factors also directly influence community respiration.

Denitrification rates were considerably less than uptake and nitrification within each stream, and usually comprised about 1% of U_t . Denitrification was only a significant sink for NO₃⁻ in one stream (Wal-Mart Ditch). These results are similar to the findings of Royer et al. (2004), who observed that measured denitrification rates (which ranged from <2.4 to 360 $\text{mg m}^{-2} \text{day}^{-1}$) were much slower than commonly observed U_t leading to denitrification lengths (distance required for the average molecule of NO₃⁻ to be denitrified) substantially longer than S_w .

If the slope between log denitrification and log NO_3^- was greater than the slope of log U_t regression, then denitrification would become more important relative to U_t as NO_3^- concentration increased. Although the slope between log denitrification and log NO_3^- was not significantly different than one, it also could not be significantly differentiated from the slope of the U_t regression. Therefore, we are unable to determine if denitrification became more important relative to uptake as NO_3^- concentration increased.

Although denitrification was a small part of total NO_3^- uptake, an average of 41% of stream water NO_3^- per km in the water column was lost to denitrification while the median was 3%. The average was heavily skewed by the relatively high rate of denitrification at Wal-Mart Ditch (250% per km), and was highly variable among streams. If Wal-Mart Ditch is excluded from the analysis, the average of the remaining 8 streams was 14% of stream water NO_3^- flux lost per km to denitrification.

Production of N_2O by streams could be important because N_2O is a powerful greenhouse gas. N_2O production can be a byproduct of nitrification and denitrification. Production of isotopically labeled $^{15}\text{N}_2\text{O}$ measured in this study was considered to be primarily a product of denitrification, because NO_3^- was much more labeled than NH_4^+ . Our results indicate that N_2O production increased linearly with NO_3^- concentration along the NO_3^- gradient. This increase in overall N_2O production did not result from a change in ratio of $\text{N}_2:\text{N}_2\text{O}$ production by the microbial community, rather from a simple overall increase in the rate of denitrification. These data suggest that production of N_2O in streams will increase with NO_3^- concentrations and may contribute to greenhouse gas emissions.

Our ability to detect denitrification was limited by appearance ^{15}N label in dissolved N_2 or N_2O in a pattern that was conducive to modeling. Although the $\delta^{15}\text{N}$ of N_2 gas during the

plateau was higher than background in K2A and Shane Creek, there was no discernable pattern to the longitudinal flux data, thus any estimate of denitrification from these data would be highly questionable. Minimum limits of detection for denitrification were difficult to establish, because of a lack of $^{15}\text{N}_2$ flux pattern in these streams, probably related to rapid reaeration and slow denitrification rate. These streams were had NO_3^- concentrations in the water column that were just above detection, so we presumably rates were low.

Nitrification

Although nitrification was historically thought to be important only in streams with relatively high ammonium concentrations, our data suggest that nitrification provides an important source of NO_3^- in streams. Some research suggests that nitrification may be important in low-nutrient freshwater systems (Dodds and Jones 1987), but it was not widely appreciated until recently that nitrification could be an important flux in relatively pristine streams (e.g., Peterson et al. 2001; Kemp and Dodds 2002a).

The benefit of whole-stream NO_3^- isotope releases was that they showed that dilution of $^{15}\text{NO}_3^-$ that was not directly accounted for by groundwater dilution must be caused by nitrification. We measured significant nitrification even in our lowest- NO_3^- streams. Furthermore, uptake of NO_3^- was well correlated to nitrification rates (Fig. 1.4), suggesting that NO_3^- availability via nitrification controls uptake rates, at least over the short term. This coupling of nitrification and uptake confirms and extends the patterns observed by Kemp and Dodds (2002a) for four lower NO_3^- streams.

Biological N demand

One possible explanation for the lack of Michaelis-Menten saturation is that biological N demand also increased along the nutrient gradient. However, total biotic N demand calculated from the whole-stream metabolism estimates did not support this idea. Total biotic N demand did not co-vary with the NO_3^- gradient or with U_t (Fig. 1.8). Several assumptions were used to calculate assimilatory demand, so this comparison should be viewed with caution.

Increase in ^{15}N of the biomass collected from each stream is another piece of evidence that could potentially explain the increase in U_t along the gradient of NO_3^- concentration (Fig. 1.8), but biomass ^{15}N assimilation did not correlate with U_t , NO_3^- concentration, standing-stock of organic matter, or whole-stream metabolism. The lack of continuity between the observed assimilatory uptake in the biomass and the other measures of uptake may be due to the inherent errors associated with scaling these measurements up to the whole stream.

This decoupling of rate of label entering individual ecosystem compartments from concentration of NO_3^- in the water column is most likely due to the difficulties of scaling up small-scale measurements, such as those used to estimate ^{15}N assimilation, to ecosystem level (Schindler 1998). Heterogeneity in spatial distribution of stream biota and NO_3^- uptake by the biota made accurate estimates of NO_3^- incorporation into the biomass difficult due to the large number of samples required. A more intense sampling regime may be required to better quantify ^{15}N assimilated into stream biomass. Because of the reasons listed above, we consider U_t to be a more accurate estimate of whole-stream uptake than biomass ^{15}N assimilation scaled to the whole stream.

Wal-Mart Ditch

Being the most extreme example of a human-impacted stream of those we studied, Wal-Mart Ditch (a concrete ditch) merits its own explanation as to how it fits in the continuum of streams investigated in this study. Wal-Mart Ditch was an exceptionally wide and shallow “stream” relative to its discharge, which led to a large degree of interaction between the biota and the water column. The stream biota was a highly active, 0.5 cm thick, gelatinous biofilm consisting of diatoms and cyanobacteria at the time of our experiment. The concentration of NO_3^- was $670 \mu\text{g L}^{-1}$ at the head of the reach, and reduced to $<10 \mu\text{g L}^{-1}$ after only 300 m by the highly active biofilm. Much of this reduction in NO_3^- concentration was attributable to assimilation. The high primary production, respiration, and NO_3^- uptake in the Wal-Mart Ditch were similar to those of the concrete-lined stream documented by Kent et al. (2005).

During nighttime hours, the decrease in NO_3^- concentration along the stream reach was much less and was primarily attributable to denitrification. During the day, the biofilm was highly autotrophic, leading to rapid assimilation of available NO_3^- , while resulting in the release of large quantities of dissolved and particulate organic N. As a result, the quantity of N leaving the reach as organic N exceeded the amount of dissolved inorganic N assimilated in the stream reach. Thus, during the day, the stream did not serve as an N sink as the NO_3^- data suggest. At night, respiration from the biofilm was enormous, resulting in a rapid drop in O_2 concentration. It is very likely that large portions of the biofilm went anoxic and facilitated denitrification. Inclusion or exclusion of this site from the cross-stream analysis did not greatly alter the conclusions or model fits.

Conclusions

The relationship of N processing to stream water NO_3^- concentration did not saturate according to our hypothesis, rather, it conformed to an Efficiency Loss model in which the efficiency at which N is cycled and retained decreases with increasing nutrient concentration. In low- NO_3^- streams, available NO_3^- was spiraled very quickly and traveled a relatively short distance before being taken up again. As the concentration of NO_3^- increased, the magnitude and length of spiraling also increased. Although the rate of cycling between organic and inorganic forms increased along the NO_3^- gradient, the relative proportion of NO_3^- retained by the stream decreased, resulting in longer uptake lengths and a lower V_f .

For the most part, denitrification was not an important flux, composing only $\sim 1\%$ of U_t . The relative importance of both groundwater input and denitrification decreased along the gradient. NO_3^- flux increased faster, relative to increases in process fluxes characterized by an exponential decrease in the amount of N processed relative to N available. Overall NO_3^- retained by the stream reach, as a proportion of influx, decreased with increasing NO_3^- concentration.

Table 1. 1- Chemical and hydrologic parameters of the nine streams in this study during the $^{15}\text{NO}_3^-$ addition experiments. N.D.= Not detectable.

Stream Name	Date of experiment	NO_3^- -N ($\mu\text{g L}^{-1}$)	NH_4^+ -N ($\mu\text{g L}^{-1}$)	DON ($\mu\text{g L}^{-1}$)	SRP ($\mu\text{g L}^{-1}$)	Q (L s^{-1})	Width (m)	Depth (m)	Velocity (m min^{-1})	As/A	α (10^{-3} s^{-1})
Kings Creek -K2A	June 2004	0.9	6.7	85	1.9	26.3	2.5	0.09	6.7	1.34	5.46
Shane Creek	May 2005	1.2	4.7	98	1.0	4.4	2.36	0.08	1.3	0.50	0.20
Natalie's Creek	June 2004	6.0	3.1	156	2.4	1.3	1.2	0.04	1.6	N.A.	N.A.
Kings Creek -N4D	May 2003	8.6	0.0	196	0.5	13.4	2.4	0.07	5.0	0.09	0.02
Ag North	June 2003	35	31.7	111	0.2	0.2	0.77	0.02	1.1	0.06	0.19
Little Kitten Creek	June 2005	168	24.2	159	7.2	20.1	3.25	0.14	2.7	0.13	0.35
Wal-Mart Ditch	May 2004	277	28.3	472	35.4	1.6	2.1	0.05	0.9	N.A.	N.A.
Campus Creek	June 2003	2900	7.8	N.D.	4.0	2.9	2.56	0.08	0.9	0.07	0.17
Swine Creek	June 2005	21000	3.3	N.D.	15.5	5.4	1.57	0.07	2.8	0.23	0.36

Table 1.2- Location, watershed characteristics, and stream channel characteristics of the streams in this study. Burn frequency and livestock grazing refer to land-management practices in the native vegetation portions of the watershed. Stream shading refers to shading due to riparian vegetation.

Stream Name	Latitude Longitude	% Native vegetation	% Row-crop Agriculture	% Urban	Burn Frequency	Livestock Grazing	% Stream Shading	Stream-bed Characteristics
Kings Creek-K2A	39° 06.008' N 96° 34.454' W	100%	0%	0%	2 year	no	37%	Cobble/bedrock
Shane Creek	39° 06.779' N 96° 33.220' W	100%	0%	0%	1 year	no	57%	Cobble
Natalie's Creek	39° 13.723' N 96° 39.530' W	100%	0%	0%	1 Year	yes	44%	Cobble/bedrock
Kings Creek-N4D	39° 05.271' N 96° 35.067' W	100%	0%	0%	4 year	yes	39%	Cobble
Ag North Creek	39° 12.741' N 96° 35.584' W	25%	30%	45%	N.A.	yes	0%	Silt/clay
Little Kitten Creek	39° 12.361' N, 96° 36.083' W)	0%	0%	100%	N.A.	N.A.	76%	Cobble
Wal-Mart Ditch	39° 11.135' N 96° 33.500' W	8%	0%	92%	N.A.	N.A.	0%	Concrete
Campus Creek	39° 11.577N 96° 34.722' W	0%	0%	100%	N.A.	N.A.	71%	Cobble/sand/clay
Swine Creek	39° 13.181' N 96° 35.271' W	30%	34%	36%	N.A.	yes	11%	Silt/clay

Table 1. 3- Whole-stream metabolism estimates during $^{15}\text{NO}_3^-$ release at each of the streams. Gross Primary Production (GPP) and Community Respiration (CR). P:R is the ratio of production to respiration. The O_2 exchange coefficient is the rate at which dissolved O_2 exchanges with the atmosphere.

Stream	GPP ($\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$)	CR ($\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$)	P:R ratio	O_2 exchange coefficient ($\times 10^{-3} \text{ min}^{-1}$)
Kings Creek -K2A	2.0	-3.6	0.5	18.9
Shane Creek	7.0	-6.2	1.1	18.0
Natalie's Creek	0.6	-2.7	0.2	24.5
Kings Creek -N4D	6.2	-3.9	1.6	25.2
Ag North	3.2	-2.7	1.2	5.1
Little Kitten Creek	4.3	-4.2	1.0	12.8
Wal-Mart Ditch	12.5	-7.0	1.8	18.5
Campus Creek	1.0	-1.0	1.0	1.6
Swine Creek	2.7	-4.4	0.6	9.2

Table 1. 4- Rates of denitrification observed in the nine streams, listed in order of increasing NO_3^- concentration. N_2O production due to denitrification composed only a small proportion of the total denitrification occurring in the streams, and is presented in smaller units than those for N_2 production.

	Total		
	Denitrification	N_2 production	N_2O Production
	($\text{mg N m}^{-2} \text{ day}^{-1}$)	($\text{mg N m}^{-2} \text{ day}^{-1}$)	($\mu\text{g N m}^{-2} \text{ day}^{-1}$)
Kings Creek -K2A	0.0	0.0*	0.0*
Shane Creek	0.0	0.0*	0.0*
Natalie's Creek	0.6	0.6	0.5
Kings Creek -N4D	0.2	0.2	0.4
Ag North	0.0	0.0*	0.4
Little Kitten Creek	12.8	12.8	55
Wal-Mart Ditch	43.6	43.6	27
Campus Creek	8.8	8.4	360
Swine Creek	221	219	2370

*below detection

Table 1. 5- Statistical parameters used to test the criteria of the three functional relationship models.

Model	Michaelis-Menten		Log-log regression					
	R ²	p	K _s	R ²	p	m (S.E.)	T (m=1)	p
Ut	0.99	<0.001	1.9 * 10 ¹⁰	0.84	<0.001	0.66 (0.11)	3.12	0.012
Nitrification	0.99	<0.001	4.0 * 10 ¹⁰	0.62	0.012	0.54 (0.16)	2.88	0.018
Denitrification	0.99	<0.001	1.4 * 10 ⁹	0.54	0.061	0.87 (0.36)	0.36	0.74

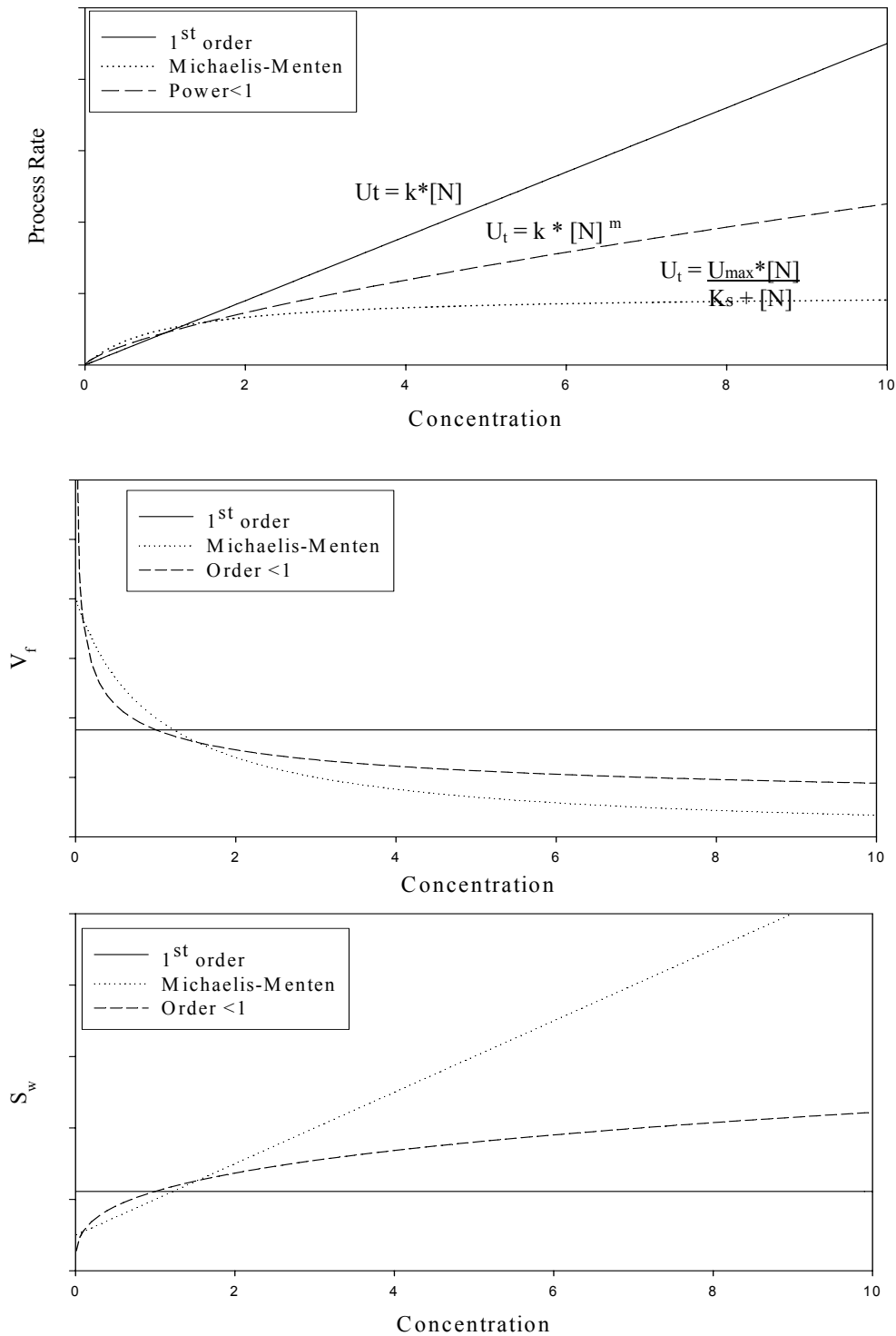


Figure 1.1- Comparison of the three potential models of the relationship between stream N processing and concentration. Scales are linear.

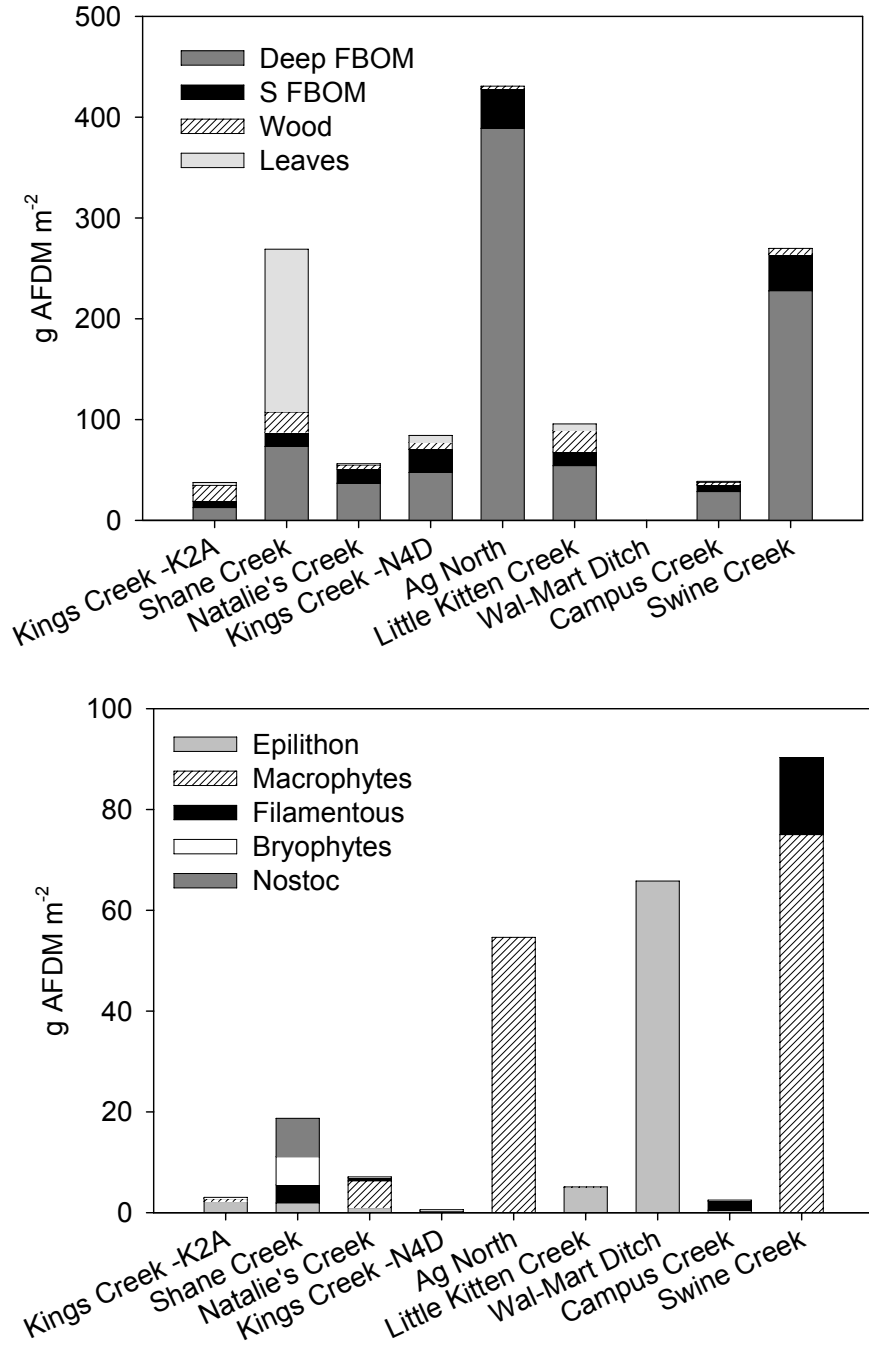


Figure 1.2- Average standing-stock organic matter compartments across the streams in this study.

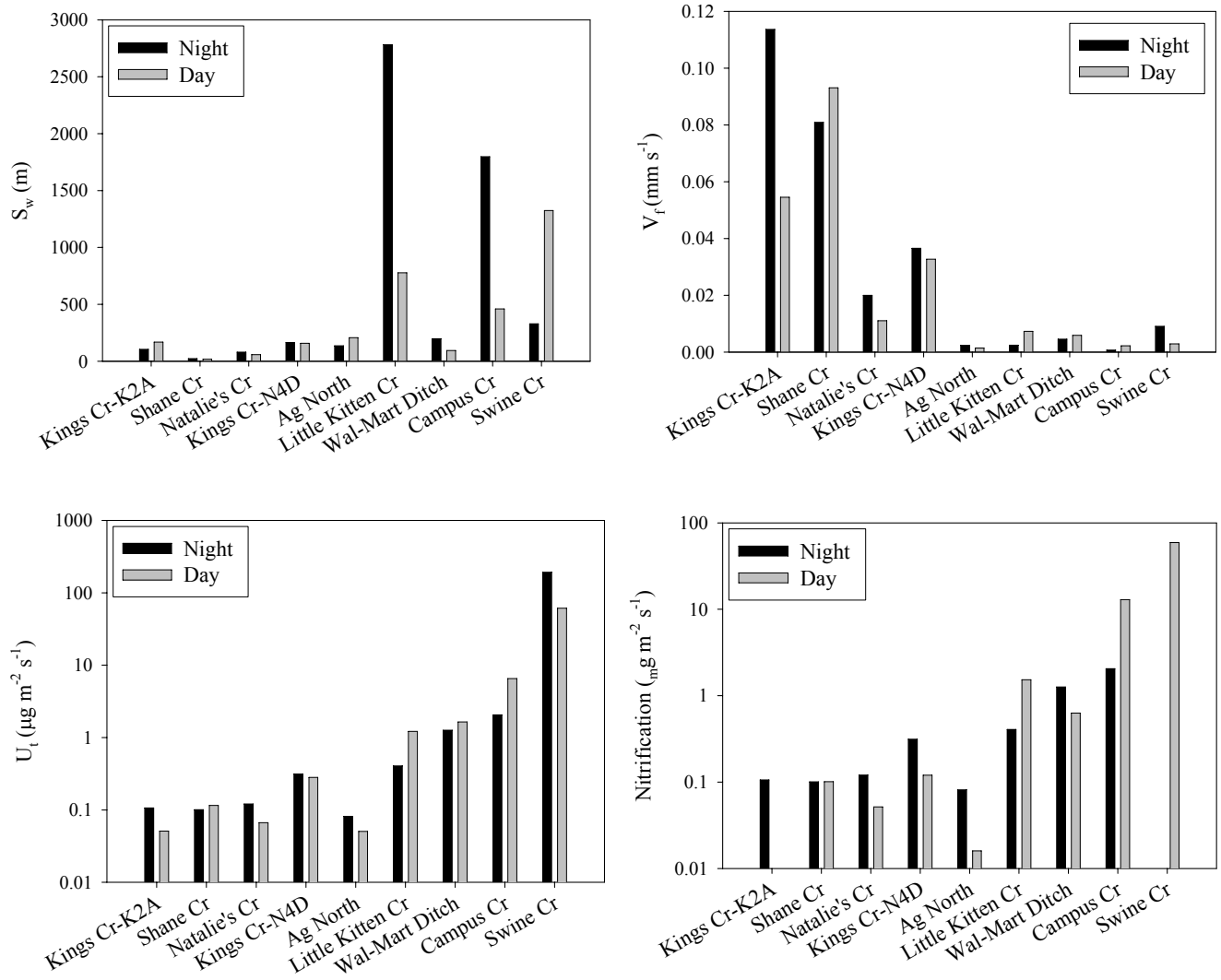


Figure 1.3- NO_3^- spiraling parameters and nitrification in day and night across the nine streams in this study. Streams are in order of increasing NO_3^- concentration.

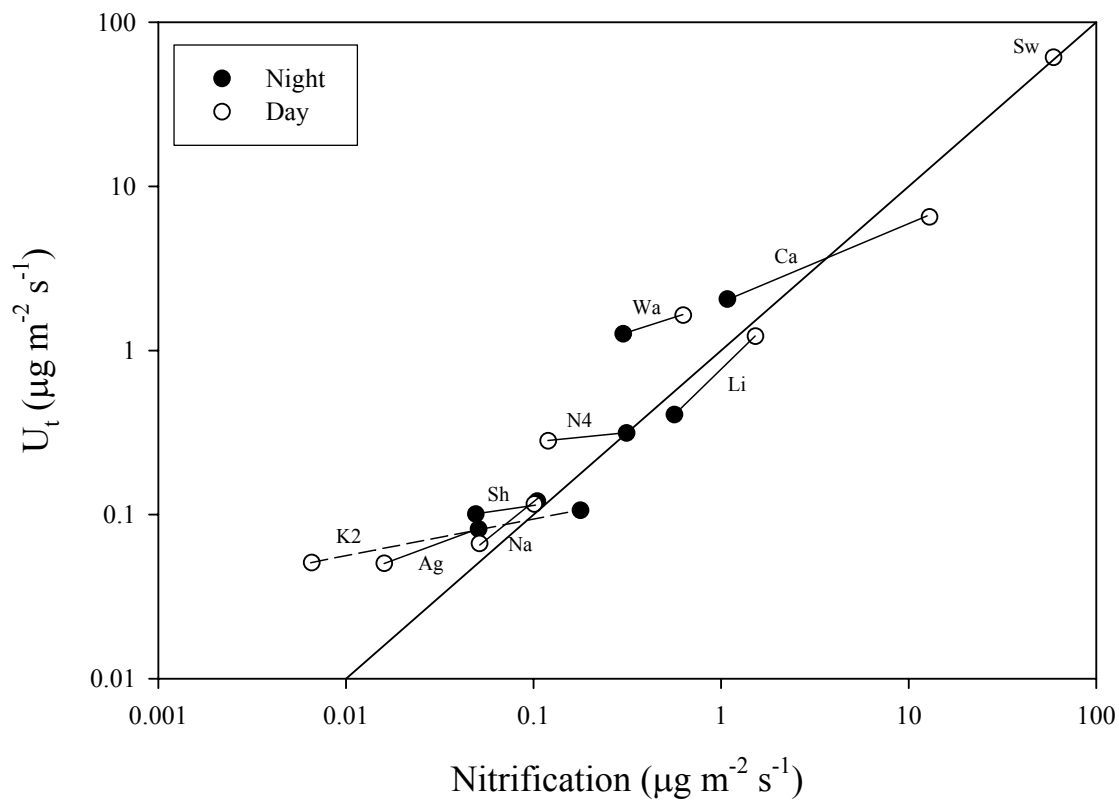


Figure 1.4- Relationship between U_t and nitrification for the nine streams in this study. Points to the left of the 1:1 line represent greater uptake than nitrification. The paired day and night U_t and nitrification points are linked with a solid line and are designated by the stream name: Kings Cr.-K2A: K2, Shane Cr.: Sh, Natalie's Cr.: Na, Kings Cr.-N4D: N4, Ag North: Ag, Little Kitten Cr.: Li, Wal-Mart Ditch: Wa, Campus Cr.: Ca, Swine Cr.: Sw.

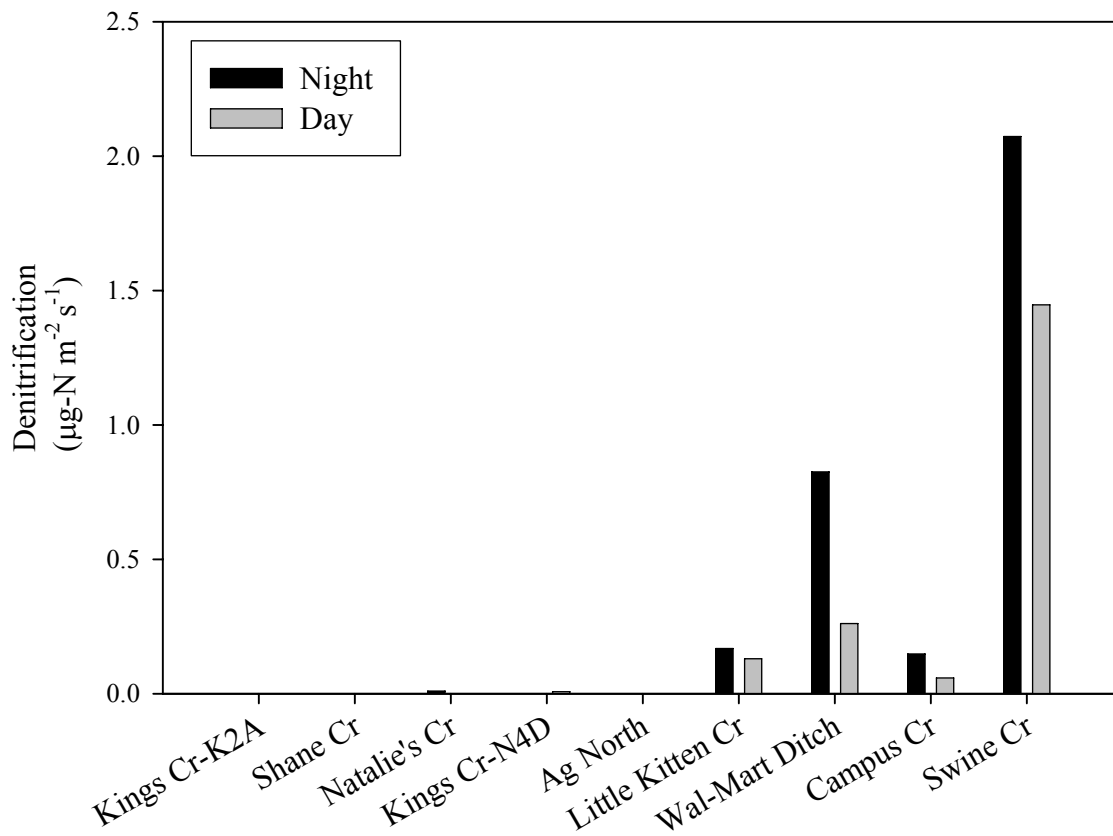


Figure 1.5- Denitrification in day and night samplings.

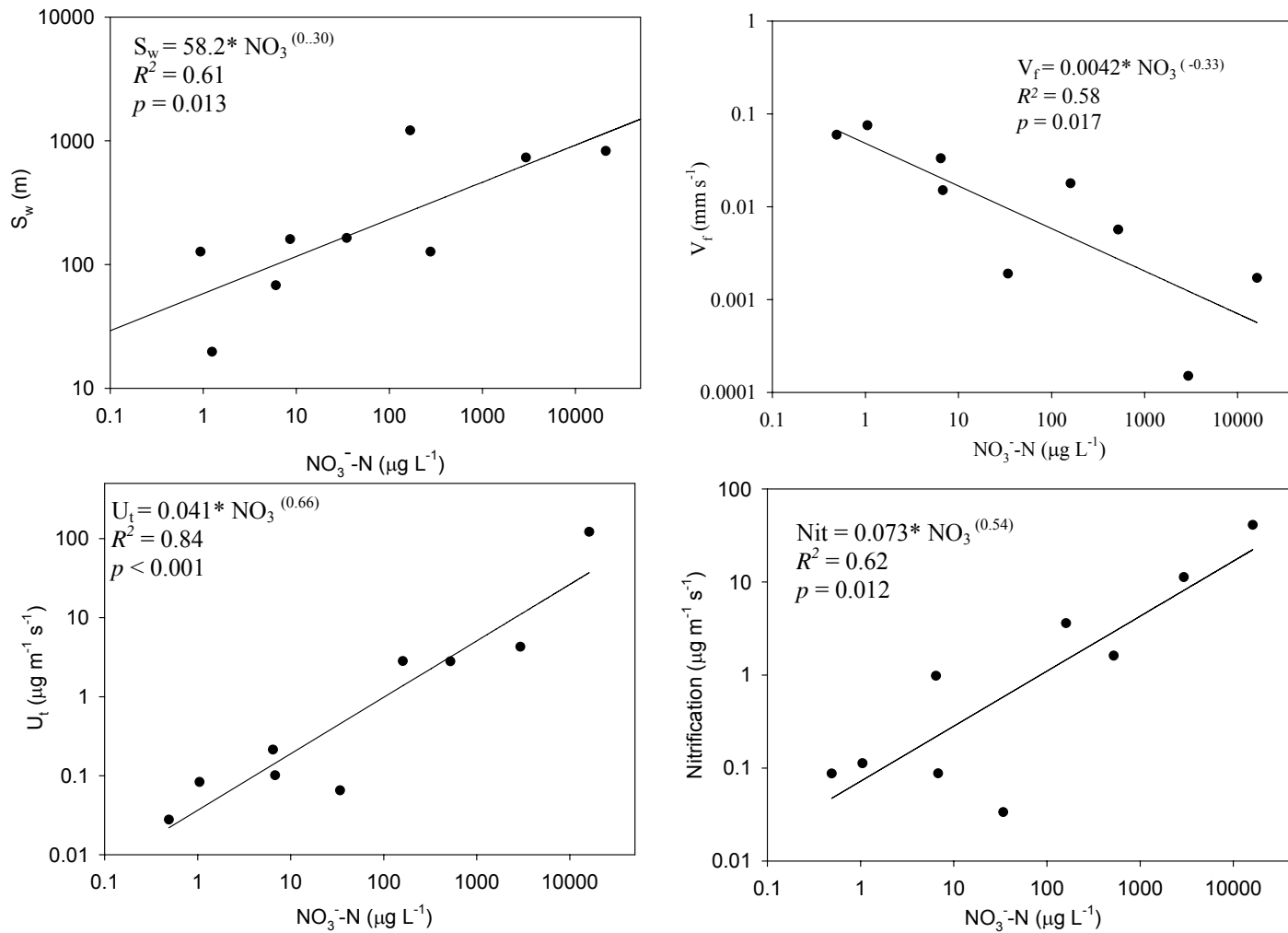


Figure 1.6- Log-log relationships between nitrate concentration and: uptake length (S_w); uptake velocity (V_f); uptake rate (U_t); and nitrification.

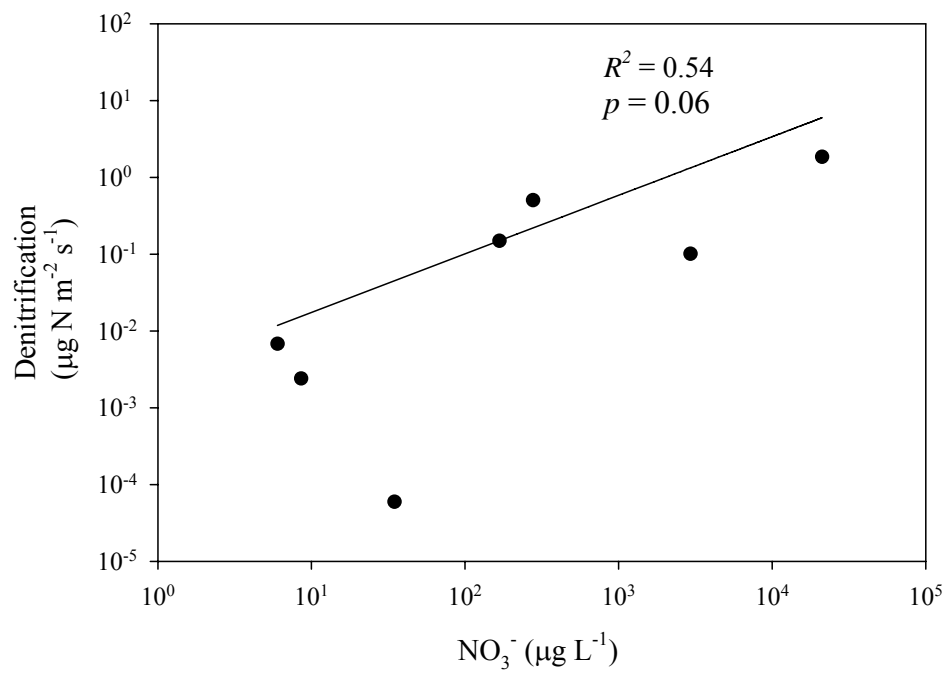


Figure 1.7- Denitrification rate as a function of NO_3^- concentration.

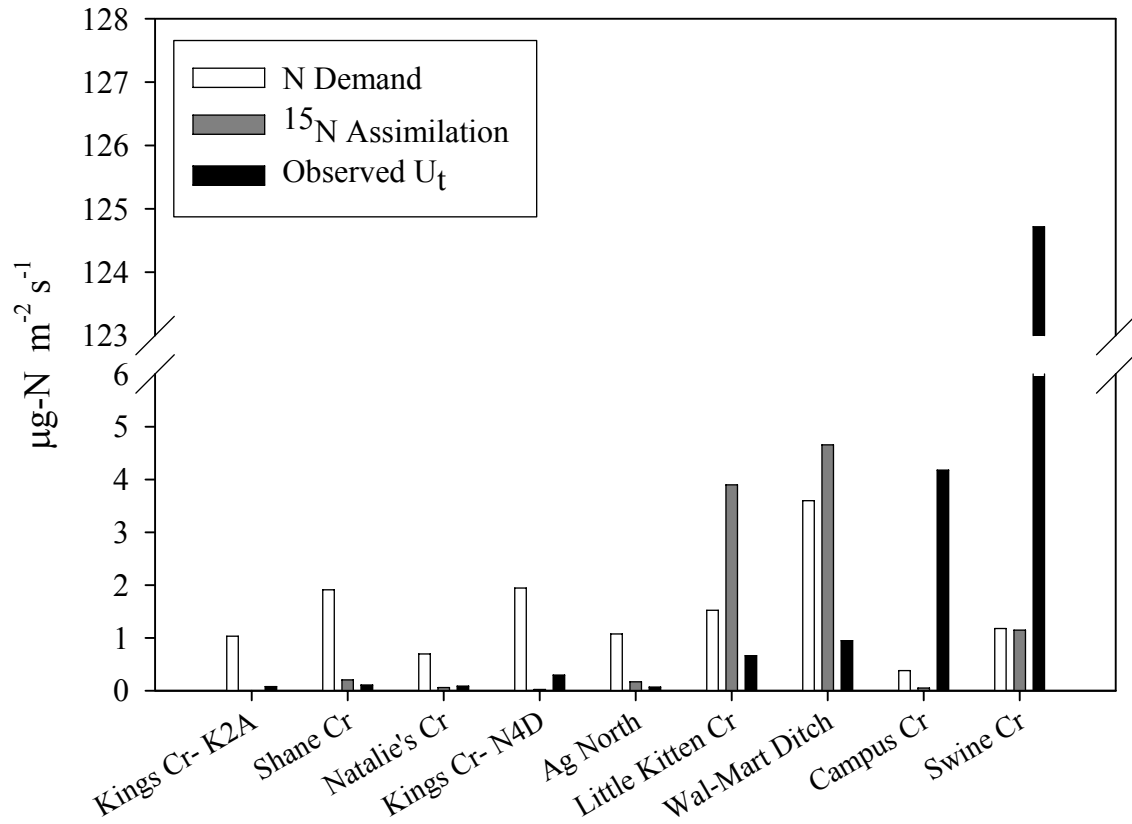


Figure 1.8- Biotic N demand and assimilatory NO_3^- uptake in the streams investigated.

**CHAPTER 2 - Predicting NH_4^+ uptake and mineralization in prairie streams
by using chamber incubation and short-term nutrient addition experiments²**

² Modified from: O'Brien, J.M. and W.K. Dodds. Predicting NH_4^+ uptake and mineralization in prairie streams by using chamber incubation and short-term nutrient addition experiments. *Freshwater Biology in review*

Abstract

We used two separate approaches to estimate ambient ammonium (NH_4^+) cycling in the north and south branches of Kings Creek, a prairie stream. Chamber experiments were conducted to determine ammonium uptake and mineralization rates associated with epilithic biofilms and filamentous algae collected from the streams. Short-term whole-stream ammonium addition experiments were also conducted to estimate the rate of uptake at ambient stream concentrations, based on the relationship between ammonium concentrations and uptake rates. Chamber experiments were scaled up to whole-stream levels, resulting in ambient gross uptake estimates of $0.08 \mu\text{g m}^{-2} \text{s}^{-1} \text{NH}_4^+ \text{-N}$ for the north branch and $0.16 \mu\text{g m}^{-2} \text{s}^{-1} \text{NH}_4^+ \text{-N}$ for the south branch. The substrata-specific estimates of mineralization were scaled up to whole-stream level, resulting in mineralization estimates that were higher than uptake in both streams. Short-term ammonium addition experiments show that uptake rates saturate with increasing ammonium concentrations. The observed saturation of uptake rates is consistent with a Michaelis-Menten relationship. Scaled estimates of uptake from the chamber experiments were similar to estimates of ambient ammonium uptake based on the whole-stream experiments, and were comparable with previous estimates of ammonium uptake and mineralization made by using stable isotope tracer methods in Kings Creek. The data on uptake as a function of ammonium concentration, coupled with ambient nutrient data, suggest that the rate of ammonium uptake can fluctuate 10-fold over periods of days to weeks in this prairie stream.

Introduction

Nutrient spiraling (Webster and Patten 1979) describes a key aspect of processing of available nutrients in streams (Newbold et al. 1981), and is a central component of stream ecosystem function. There are three interrelated parameters often used to quantify spiraling. The average distance a molecule travels downstream is indicated by the uptake length (S_w). The flux rate per unit area of stream bottom is the uptake rate (U_t). The rate of movement of molecules out of the water column (V_f) is the mass transfer coefficient (Stream Solute Workshop 1990). These parameters are used to quantify changes in nutrient spiraling over time in a single stream (e.g., Simon et al. 2005) or compare differences in nutrient spiraling among streams (e.g., Peterson et al. 2001).

Short-term nutrient addition experiments are commonly used to measure these parameters because they are much less expensive and are not as involved as isotopic tracers (e.g., Hall et al. 2002, Hall and Tank 2003, Niyogi et al. 2004). These experiments generally include release of an inorganic nutrient (NH_4^+ , NO_3^- , or PO_4^-) into a stream to elevate the concentration above the ambient. The resulting decline in nutrient concentration over distance downstream (net uptake) is considered to be proportional to the rate of uptake at ambient nutrient concentrations (gross uptake). If S_w does not change with concentration, net uptake and gross uptake will be linearly proportional. Extrapolation to ambient conditions with nutrient enrichment experiments typically underestimate ambient uptake rates (Dodds et al. 2002) because S_w tends to increase with elevations in concentration (Mulholland et al. 2002).

Although a single short-term nutrient addition may not accurately predict S_w at ambient conditions, these kinds of experiments can still be used to predict ambient S_w if the relationship between uptake and concentration is known (Dodds et al. 2002). Payne et al. (2004) proposed a

method in which the linear relation between S_w from multiple- or single-nutrient addition experiments and the concentration of added nutrient were used to project S_w at ambient stream conditions. Ambient gross uptake can also be projected if the relationship between U_t and concentration is known. It typically is assumed that the relationship between uptake and concentration is best described by the Michaelis-Menton equation (Stream Solute Workshop 1990, Mulholland et al. 2002). But, log-log relationships between nutrient concentration and uptake rate can also be useful in describing this relationship across streams (Dodds et al. 2002, Chapter 1).

In many streams, nutrient concentration is relatively stable over a time frame of hours, during baseflow conditions, and there is little net uptake. Under these conditions, gross uptake rate must be balanced by mineralization (Dodds 1993). Mineralization, the regeneration of NH_4^+ from the benthic zone to the water column, has been investigated in marine (Rysgaard et al. 2000), estuary (Anderson et al. 2003), and wetland (Zhu and Ehrenfeld 1999) ecosystems. Although uptake of NH_4^+ has been extensively studied in streams, mineralization has seldom been directly reported for lotic ecosystems. An exception is Peterson et al. (2001), in which regeneration (mineralization) was expressed as a proportion of ammonium uptake.

Sediment-scale assays also can predict whole-stream processes such as nitrification, denitrification, and uptake of ammonium and nitrate in streams (Kemp and Dodds 2002a, Kemp and Dodds 2002b). Sediment sample incubation assays are also commonly used in marine and wetland systems (e.g., Stanley and Ward 1997). The advantage of these assays is that they are relatively easy to conduct and replicate. The disadvantage is the difficulty in scaling measurements up to the whole system.

We conducted a series of short-term NO_3^- and NH_4^+ addition experiments in a whole stream and concurrently performed incubation experiments on substrata taken from that stream to determine the relationships between nitrogen uptake and concentration in prairie streams. We were interested in identifying the best approach to estimate ambient gross uptake and mineralization rates, as well as describing functional relationships between uptake and concentration (i.e., how variations in concentration influence net uptake kinetics).

Methods

Study area

Our study was conducted on tributaries of the Kings Creek watershed on the Konza Prairie Biological Station (KPBS), located in the Flint Hills region of Northeastern Kansas, USA. The experiments were conducted on streams draining two sub-watersheds, K2A (on the north branch, $39^\circ 06.008' \text{ N}$, $96^\circ 34.454' \text{ W}$) and N4D (on the south branch, $39^\circ 05.271' \text{ N}$, $96^\circ 35.067' \text{ W}$), of the Kings Creek system. Land cover in both watersheds is composed entirely of native tallgrass prairie. Streams in this region and land cover are typically oligotrophic, with low concentrations of dissolved and total N and P (Dodds 2003). Concentrations of NH_4^+ , however, are variable within and among years (Fig. 2.1). Kings Creek is an intensively studied system, and descriptions of the nitrogen cycling (Dodds et al. 2000), hydrology (Gray et al. 1998), and ecology (Gray and Dodds 1998) have been published.

The two streams we investigated had similar physical and biological characteristics, and their watersheds are burned every 2 or 4 years. The N4D watershed is grazed by bison and K2A is not, N4D was burned 3 years and K2A was burned 1 year before this study. The stream discharge was similar between N4D and K2A, and average width was the same for both streams

(Table 2.1). NH_4^+ concentrations were also similar between the two streams, with N4D having a slightly greater concentration than K2A. The predominant biota type in the two experimental reaches was epilithic biofilms that cover the cobble in the stream bottom. In K2A, there were streamers of filamentous algae (*Cladophora*) present in the riffle habitats.

Stepwise short-term whole-stream NH_4^+ addition experiments

A series of short-term NH_4^+ enrichment experiments were conducted on streams N4D and K2A in July 2004. The experiments consisted of four NH_4Cl enrichment treatments (with NaBr as a hydrologic tracer) per day at each stream, in which the NH_4^+ concentration was incrementally increased with each subsequent release. The enrichment was maintained at a constant rate until the concentration of the Br^- tracer reached a plateau (increased at less than 1% each 5 minutes, usually 1.5 hr for a 100 m reach) at the bottom of the reach. After plateau, samples were taken every 10 m along the length of the reach. After each sampling, the concentration added to the stream was increased. The stream was sampled again after the period of time required to reach plateau.

Transects were used to measure width, depth, and occurrence of biota in the stream. Transects were conducted every 5 m along the experimental reaches to measure wetted width, habitat, and benthic biota type (10 points per transect) in the stream ($n = 20$ transects or 200 points per stream). Average stream depth was measured every 10 m by using similar transects ($n = 10$ transects or 100 points per stream).

Water chemistry samples were returned to the laboratory in a cooler, filtered immediately with 47-mm Whatman GF/F glass fiber filters, and frozen until analysis. The NH_4^+ concentration of each sample was measured colorometrically using the phenol-hypochlorite

method on a Hitachi U-2000 spectrophotometer (Hitachi Ltd. Tokyo, Japan) equipped with a 5-cm cell (APHA 1995). The Br^- tracer concentration of each sample was measured with an Orion ion-specific electrode, with all samples measured at the same temperature as the standards and stirred at the same rate. Ammonium concentration for each sample was corrected for background NH_4^+ concentration (C_0) and dilution by correcting for the change in Br^- concentration. The slope of the ln-transformed concentration of added NH_4^+ over distance (k_x) was calculated by linear regression.

Chamber experiments

For sediment-scale investigations, dominant sediments/biota types were collected from each prairie stream. Cobbles were selected without bias from several pools and riffles within the reach. Cobbles were removed from the stream and transported in stream water to the laboratory for analysis within 2 hr. Cobbles collected from the stream were minimally disturbed during transport.

Substrata were incubated in the light (mixed florescent and Halogen sources with an average intensity of $11.75 \text{ mol quanta m}^{-2} \text{ day}^{-1}$) at 20°C with stream water in recirculating chambers (Dodds and Brock 1998) located inside controlled environmental chambers. Water velocity was held constant during and across the experiments and approximated average in-stream velocities. Stream water in the chambers was then diluted with de-ionized water amended with sodium bicarbonate and HCl (to approximate the osmotic balance and pH of ambient stream water) and the resulting change in NH_4^+ concentration was measured over time and used to establish the base concentration. Concentration of NH_4^+ was then increased to

approximately $25 \mu\text{g L}^{-1}$ by adding a spike of $5.0 \text{ mM NH}_4\text{Cl}$ to each chamber. The resulting decline in concentration after the spike was used to calculate the uptake coefficient.

This method assumes that, over the short period of the experiment in the closed chambers, a steady state NH_4^+ concentration will exist at which the rate of uptake equals the rate of mineralization. This steady state is referred to as the base concentration (C'). Raising the NH_4^+ concentration above C' will yield a net uptake over time (Fig. 2.3) because, over short periods of time, uptake is dependant on concentration and mineralization is not. Dilution of NH_4^+ below base concentration will result in net mineralization, because gross uptake will become less than mineralization. The resulting net mineralization will cause NH_4^+ concentration to return to base concentration. By identifying base concentration and rate of net uptake, rate of mineralization and uptake coefficient can be determined.

Uptake is assumed to be a first-order kinetic reaction, in the narrow range of concentrations in the chamber experiment, with K_t as the coefficient of uptake. Mineralization (M) is assumed to be independent of substrate (NH_4^+) concentration (C). Note that in this paper we define the solid rock and materials on the bottom of the streams that are colonized by organisms as substrata and the chemicals they metabolize as substrates. Net change in substrate concentration over time can be described by the equation:

$$dC/dt = -K_t * C + M \quad (2.1)$$

where t is time and K_t is chamber uptake coefficient. K_t was calculated as the slope of C' corrected, \ln -transformed, NH_4^+ concentrations over time after the spike. M was then calculated as:

$$M = K_t * C' \quad (2.2)$$

Chamber uptake rates at ambient stream conditions were calculated by the equation:

$$U_t = C_0 * V * K_t * A^{-1} \quad (2.3)$$

in which C_0 is ambient stream NH_4^+ concentration, V is volume of the chamber (in this experiment $V = 22.0$ L), and A is total substrata area (usually ~ 0.05 m²). Substrata-specific uptake rates were then weighted by the proportion at which each substrata type occurred in the stream. Substrata uptake rates were scaled up to whole-reach levels for comparisons with short-term nutrient addition experiments. The U_t for each stream was calculated as the sum of the weighted substrata-specific uptake rates.

Calculations of spiraling parameters

Using the k_x coefficient from the short-term N enrichment experiments, we calculated the appropriate parameters of nutrient spiraling for each addition experiment as outlined by the Stream Solute Workshop (1990). Uptake length (S_w) for each experiment was defined as the negative inverse of the linear uptake coefficient ($S_w = -1/k_x$). The area-weighted rate of N uptake (U_t) was calculated from S_w , NH_4^+ flux (F_w) and stream width (w):

$$U_t = \frac{F_w}{S_w * w} \quad (2.4)$$

Prediction of U_t at ambient NH_4^+ concentrations for each stream was based on the relationship between U_t and NH_4^+ concentration established by the stepwise short-term NH_4^+ addition experiments. Two different, yet potentially valid, models (Michaelis-Menten and Log-Log) were used to describe this relationship. Regression equations for both the Michaelis-Menten and Log-Log models were based on the pooled K2A and N4D data. Estimates of U_t at ambient conditions were made by solving the regression equations with the C_0 from each stream.

The Michaelis-Menten relationship was similarly used to estimate temporal variation in U_t based on temporal variation in NH_4^+ concentration at N4D.

Additional estimates of U_t at ambient concentrations, based on short-term NH_4^+ addition experiments, were made using the S_w projection method of Payne et al. (2004). In this method, linear regression is used to establish a relationship between S_w and the added NH_4^+ concentration ($C - C_0$). From this relationship, projected S_w was calculated for each stream by entering negative ($-1 * C_0$) ambient NH_4^+ concentration into the regression equation. The resulting S_w was used to calculate U_t by using equation 2.3.

Statistics

Uptake coefficients (K_t) in the chamber experiments were calculated by linear regression between ln-transformed added NH_4^+ ($C-C'$) and experimental time. Differences in substrata specific NH_4^+ uptake coefficients between streams (K2A and N4D) and habitats (pool and riffle) were tested by using a two-way factorial ANOVA. By examining uptake coefficients for the epilithon samples, we tested the hypotheses that NH_4^+ uptake coefficients differed between streams (K2A and N4D) and between habitats (pool and riffle). Uptake coefficients were used to test the difference in uptake rate between streams and habitats because they are not confounded by differences in ambient stream concentration that affect comparisons of specific uptake rate (U_t).

Differences in epilithic uptake and mineralization were also tested by using a two-way factorial ANOVA. Differences between substrata-specific uptake and mineralization rates were tested using an ANOVA with least-square means contrasts. Uptake coefficients (k_x) in whole-stream experiments were calculated by linear regression between ln-transformed added NH_4^+ ($C-$

C_0) and distance. The Michaelis-Menten model was fit to U_t and C using least squares regression with the Levenberg-Marquardt estimation algorithm. Linear regression was used to calculate Log-Log relationship by using log transformed U_t and log transform C . The relationship between S_w and added NH_4^+ concentration was fit using linear regression. All statistical analyses were performed with the Statistica 6 (Statsoft, Tulsa, OK, USA) statistical package.

Results

Chamber experiments

Chamber experiments yielded uptake coefficients (K_t) that ranged from 0.0027 min^{-1} in the N4D pool epilithon samples to 0.012 min^{-1} in the filamentous algae samples (Fig. 2.4). Based on the uptake coefficients, uptake rates at ambient stream NH_4^+ ranged from $0.042 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ in the N4D pool epilithon samples to $0.12 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ in the filamentous algae samples. On the basis of channel surveys, we scaled ambient gross uptake rates up to whole-stream level, with U_t estimates of $0.14 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ for N4D and $0.08 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ for K2A.

NH_4^+ mineralization rates ranged from $0.08 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ in K2A pool epilithon to $0.51 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ in N4D riffle epilithon (Fig. 2.5). From the channel surveys, we also scaled substrata-specific estimates of mineralization up to whole-stream level, with mineralization estimates of $0.24 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ for N4D and $0.20 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ for K2A. Average mineralization rates were greater than uptake in both streams (Fig. 2.5). Mineralization rates were significantly greater than uptake in riffle epilithon at both N4D ($p < 0.001$) and K2A ($p = 0.002$). Uptake and mineralization were not significantly different from each other in the pool epilithon or filamentous algae.

We found no significant differences between streams in NH_4^+ uptake coefficients; we did find a significant difference between NH_4^+ uptake coefficients for epilithon from pool and riffle habitats ($F_{1,8} = 29.5$, $p = 0.001$), with greater uptake in riffle habitats. We conducted a similar two-way factorial ANOVA to test for differences in mineralization rates between streams and habitats. Again, we found no significant differences in mineralization rates between streams. We did find a significant difference between NH_4^+ mineralization associated with epilithic biofilms from pool and riffle habitats ($F_{1,8} = 24.9$, $p = 0.001$), with greater mineralization in riffle habitats.

We used estimates of uptake from the chamber experiments to calculate habitat specific uptake rates and uptake lengths. Riffle habitats had greater U_t ($0.11 \mu\text{g-N m}^{-2} \text{s}^{-1}$ and $0.22 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for K2A and N4D, respectively) than the whole-stream average U_t . The greater U_t in the riffles resulted in a shorter, habitat-specific S_w (24 m and 27 m for K2A and N4D respectively) than the whole-stream S_w (42.9 m and 44.9 m, respectively). Pools had slower rates of habitat-specific U_t ($0.04 \mu\text{g-N m}^{-2} \text{s}^{-1}$ and $0.06 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for k2A and N4D respectively) resulting in longer uptake lengths (75 m and 108 m, respectively) than whole-stream S_w habitats.

NH_4^+ addition experiments

The short-term, whole-stream NH_4^+ addition experiments yielded peak NH_4^+ concentrations of 5.6, 10.0, 22.3, and 63.6 $\mu\text{g L}^{-1} \text{NH}_4^+\text{-N}$ at N4D and 10.0, 19.5, 35.5 and 39.4 $\mu\text{g L}^{-1} \text{NH}_4^+\text{-N}$ at K2A. Increases in concentration resulted in increases in U_t and S_w that were similar between the two streams.

Given similarities between the two streams in terms of biological (Fig. 2.4) and physical characteristics (Table 2.1), NH_4^+ spiraling data were pooled to establish a relationship between

uptake and NH_4^+ concentration. There were significant fits for the Michaelis-Menten ($R^2 = 0.69$, $F_{1,7} = 69.6$, $p < 0.001$) (Fig. 2.6A) and Log-Log models ($R^2 = 0.70$, $F_{1,7} = 14.1$, $p = 0.01$) (Fig. 2.6B). Using the Michaelis-Menten model, we calculated U_t estimates of $0.15 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for N4D and $0.03 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for K2A. Using the Log-Log model, we calculated ambient U_t estimates of $0.25 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for N4D and $0.11 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for K2A.

A significant linear regression was calculated between S_w and NH_4^+ concentration for the pooled K2A and N4D short-term addition experiments ($R^2 = 0.64$, $F_{1,7} = 10.89$, $p = 0.016$) (Fig. 2.7). According to the S_w projection method, ambient S_w was 41.4 m for N4D and 44.2 m for K2A. Based on the S_w projections, U_t was $0.14 \mu\text{g-N m}^{-2} \text{s}^{-1}$ for N4D and $\mu\text{g-N m}^{-2} \text{s}^{-1}$ for K2A.

We compared the projections of U_t based on the short-term NH_4^+ addition experiment MM relationship, Log-Log relationship, and S_w projection method with that of the chamber estimates. All of the methods of projection provided similar estimates of ambient U_t (Fig. 2.8), and the 95% confidence intervals greatly overlapped with the other methods of projection. We found no evidence to suggest that the projected U_t differed among the chamber estimates or the three short-term nutrient addition projection methods. Ambient uptake rates calculated from individual NH_4^+ addition experiments were similar to those from the chamber experiment using low concentrations, but uptake rates were under-estimated by experiments using higher concentrations of added NH_4^+ , as predicted by Mulholland et al. (2002).

Discussion

Can chamber experiments predict uptake at the whole-stream level?

The results of this study indicate that whole-stream ambient U_t predicted by the chamber experiments were not significantly different than projections made by short-term nutrient

addition experiments. A long-term $^{15}\text{NH}_4^+$ experiment on N4D conducted in the spring of 1998 found uptake rates ranging from $0.014 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ to $0.7 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ (Dodds et al. 2000, Dodds et al. 2002). These rates bracket the results of the chamber and short-term NH_4^+ addition experiments in the current study (0.042 to $0.12 \mu\text{g-N m}^{-2} \text{ s}^{-1}$). Kemp and Dodds (2002b) also reported whole-stream ecosystem estimates of NH_4^+ uptake kinetics generated by scaling up laboratory experiments of uptake kinetics of various substrata collected from N4D. Given their reported values of V_{max} and K_s , we would anticipate ambient uptake rates of $4.1 \mu\text{g-N m}^{-2} \text{ s}^{-1}$ and $1.8 \mu\text{g-N m}^{-2} \text{ s}^{-1}$, based on the NH_4^+ concentrations at N4D and K2A, respectively, which are significantly greater than the estimates of ambient uptake based on our data. The difference is due to a higher V_{max} reported by Kemp and Dodds (2002b).

There are inherent difficulties in scaling small-scale measurements, such as the results of the chamber experiments, to the whole aquatic ecosystem (Schindler 1998). It seems, however, that our selection of biomass compartments included the most important or representative components in the stream, or, at least we chose samples that best mimic the results of the whole-stream addition experiments. Cobbles collected from the stream represented filamentous algae, epilithic diatoms, and a fraction of the fine benthic organic matter (FBOM) components of the stream substrata. These substrata types were found to be the most active in terms of NH_4^+ uptake by Kemp and Dodds (2002b). These biota types were also in closest contact with overlying stream water, and most likely had the largest impact on short-term NH_4^+ releases.

Both experiments (chamber and short-term addition) focus primarily on the portion of the stream that rapidly interacts with stream water, and may miss important processes that are occurring elsewhere in the stream channel. For instance, transient storage areas and hyporheic zones can be very active areas of nutrient transformation (Mulholland et al. 1997, Valett et al.

1996) that could be missed or under-represented in these experiments. Chamber experiments to measure community respiration and primary production lead to underestimates when compared with whole-ecosystem methods (Uzarski et al. 2004, Wilson 2005). Epilithic biofilms and filamentous algae biota were in close physical contact with overlying stream water, were best positioned to have an immediate impact on stream NH_4^+ concentration, and most likely had the largest impact on short-term NH_4^+ releases.

The advantage of using whole substrata within recirculating chambers, as opposed to homogenized samples, is that they likely better mimic conditions in the stream. These chambers provide a better simulation of water flow and maintenance of diffusion boundary layers and turbulent mass transport than other methods. Bottle incubation methods, as used by Kemp and Dodds (2002b), involve the disruption of stream biota and a change in the availability of nutrients in stream water relative to the biota.

Mineralization

Our data indicate that riffle epilithon had greater rates of uptake and mineralization than pool epilithon. Based on our assumptions, mineralization was higher than uptake in stream conditions in all of the epilithic biofilms because base NH_4^+ concentrations in the chambers were higher than NH_4^+ concentrations in the stream. Uptake was greater than mineralization only in the filamentous green algae. On the basis of these findings, a net increase in NH_4^+ concentrations would be expected in the water of both stream reaches as it moved downstream.

A net increase in NH_4^+ concentration was observed at K2A, but this was not true at N4D (Fig. 2.2). Most of the net mineralization observed in the ambient NH_4^+ concentration at K2A (90% of the variation in NH_4^+ concentration) can be explained by differences in uptake and

mineralization rates from the chamber experiments and by dilution from incoming ground water. Based on previous samples of hyporheic water and samples from nearby springs, NH_4^+ of the groundwater is assumed to be at or below the detection limit. Conversely, the difference between measured uptake and mineralization could not explain the variation in ambient NH_4^+ in N4D. Other processes must therefore be compensating for the additional NH_4^+ generated by the net mineralization of epilithic biofilms in this stream.

Excess NH_4^+ not taken up by biofilm may move into slower turnover zones, such as the transient storage and hyporheic zones described above, or may participate in processes, such as nitrification, that were not explicitly measured. But these processes would have likely been accounted for by the whole-stream NH_4^+ addition experiments. An alternative explanation is that the biota types may have accounted for hot-spots of mineralization while missing biomass compartments with similar rates of uptake to those in the chamber experiments but with lower rates of turnover and mineralization (e.g., leaves or FBOM) (Dodds et al. 2004).

Rates of mineralization measured in this study are much higher than those estimated for Kings Creek by Peterson et al. (2001). Ammonium regeneration rates of $0.0046 \text{ mg m}^{-2} \text{ s}^{-1}$ were considered maximal for Kings Creek, based on $^{15}\text{NH}_4^+$ leaving the stream after the conclusion of an extended ^{15}N enrichment experiment. However, Peterson et al. (2000) noted that their Kings Creek estimate could have been inaccurate because of a low level of isotopic enrichment found in the NH_4^+ pool. Our rates of mineralization, however, do fall within the range of rates presented by the Peterson et al. (2001) study in other streams where a more significant labeling signal was obtained.

We did not directly measure nitrification (oxidation of ammonium to nitrate), but it may play a large role in the uptake of ammonium in these streams. Rates of nitrification reported for

N4D by previous studies are highly variable and make interpretation difficult in relation to our results. Dodds et al. (2000), in a $^{15}\text{NH}_4^+$ addition experiment, found nitrification to be low ($0.016 \mu\text{g m}^{-2} \text{s}^{-1}$) relative to NH_4^+ assimilation. Kemp and Dodds (2002a) found much greater rates (around $0.7 \mu\text{g m}^{-2} \text{s}^{-1}$) in N4D by scaling up substrata-specific nitrification assay rates, but they were still only a small fraction of the projected ecosystem NH_4^+ uptake rate (Kemp and Dodds 2002b). These rates of nitrification span the rates of uptake in our study, and it is likely that nitrification played a significant role in the transformation of ammonium in this stream.

Spatial heterogeneity in uptake

One advantage of the chamber method was that we were able to quantify some spatial heterogeneity in the stream system. We found that riffles were more active than pools in terms of both uptake and mineralization, resulting in shorter uptake lengths in this habitat type. This phenomenon is evident in several of the low-level NH_4^+ addition experiments at Kings Creek-K2A due to a large pool in the center of the reach (Fig. 2.2). Estimates of habitat-specific S_w from this release indicate that the uptake length in the pool segment (394 m) is 10 times greater than that in the riffles that precede (38 m) and follow it (40 m), however there are too few points to statistically test a difference in slopes. The estimated pool and riffle specific S_w are higher than those of the chamber experiments due to the elevated NH_4^+ concentrations. The effect of the pools and riffles is obscured in the Kings Creek-N4D reach, due to the lack of the spatial resolution in the sampling design as well as the relative size of the pools. Kings Creek-N4D lacked a pool large enough to encompass multiple sample points and demonstrate an effect of the pool environment.

The uptake length measured over a stream reach is dependent on the sizes and ratios of its pools and riffles. For expediency, researchers often avoid large pools when conducting whole stream addition experiments, because they have very long travel and residence times. By avoiding pools, however, researchers may be missing an important part of the stream and biasing the results of their study by overestimating uptake rates when their estimates are scaled to the whole stream.

Uptake lengths of other nutrients, such as NO_3^- and PO_4^{3-} , can differ with stream habitats, with higher velocity habitats such as rock outcrops having shorter uptake lengths (Munn and Meyer 1990). Riffles would be expected to be more active for uptake than pools for several reasons: a) higher water velocity in riffles may increase transport across the diffusion boundary layer (Dodds and Biggs 2002), b) algal photosynthesis may be stimulated by greater light penetration in shallower habitats, and c) grazers may have difficulty maintaining position in areas with high water velocity.

Temporal heterogeneity of NH_4^+ concentrations and uptake

Short-term addition experiments not only estimate the rate of uptake at a single concentration, but also provide a better understanding of how uptake changes in relation to changes in concentration. Accounting for variance in concentration is important because NH_4^+ concentration and uptake do not remain constant over time; instead, they can vary greatly over the year. Annual trends in ammonium concentration and U_t predicted by the Michaelis-Menten relationship (Fig. 2.1) demonstrate considerable variation within and between two years, and the associated variations in predicted rates of uptake (U_t). These predictions demonstrate that U_t can increase or decrease an order of magnitude in a matter of days. The first year (1999) exemplifies

an average year with a low level of variation over the spring and summer, and the second (2001) demonstrates an exceptionally variable year.

Variability in NH_4^+ concentration over days can have a large impact on rate of uptake in the ecosystem. These calculations demonstrate the ability of biota to rapidly respond to nutrient pulses naturally occurring in the stream environment. Nutrient pulses can have a large impact on the amount of nutrients available to the biota, especially in systems where N or P is limiting. Because N often limits autotrophs (alone or with P) and does limit algal biomass accrual in Kings Creek (Tank and Dodds 2003), and ammonium uptake is a substantial portion of N uptake in Kings Creek (Dodds et al. 2000), NH_4^+ uptake rates are relevant to ecosystem function.

In this temporal analysis, we assumed that the relationship between concentration and uptake by the stream biota does not change greatly over the flowing season. Uptake rates expressed in Fig. 2.1 should not be considered absolute, but they demonstrate the considerable variation in NH_4^+ concentration that occurs over time and the potential for rapid reaction of NH_4^+ uptake by stream biota in response to nutrient pulses.

Conclusions

Both the whole-stream addition experiments and chamber methods used in this study yielded comparable results for estimating the ambient rate of NH_4^+ uptake in the streams. Our results are comparable to $^{15}\text{NH}_4^+$ addition experiment results from previous studies on the same system. The results demonstrate the rapid cycling of N in these prairie streams.

The advantage of our experiments over isotopic methods is that they are easy to conduct and relatively inexpensive. Chamber methods provided data on spatial heterogeneity and how nutrient cycling changes along a stream. Short-term addition experiments provide a better understanding of how uptake changes in relation to increasing concentration, not just a snapshot

of uptake at current ambient concentration, and allow insight into temporal trends in uptake. Our methods could also provide a framework for experiments with inorganic P, which does not have stable isotopes available.

Table 2. 1- Hydrologic and N characteristics of the two streams in this study.

Stream	Discharge	Wetted width	Average depth	NH ₄ ⁺ -N	NO ₃ ⁻ -N	Burn Frequency
	L s ⁻¹	m	m	μg L ⁻¹	μg L ⁻¹	years
N4D	4.7	2.1	0.13	2.9	2.5	4
K2A	7.4	2.1	0.12	0.6	1.5	2

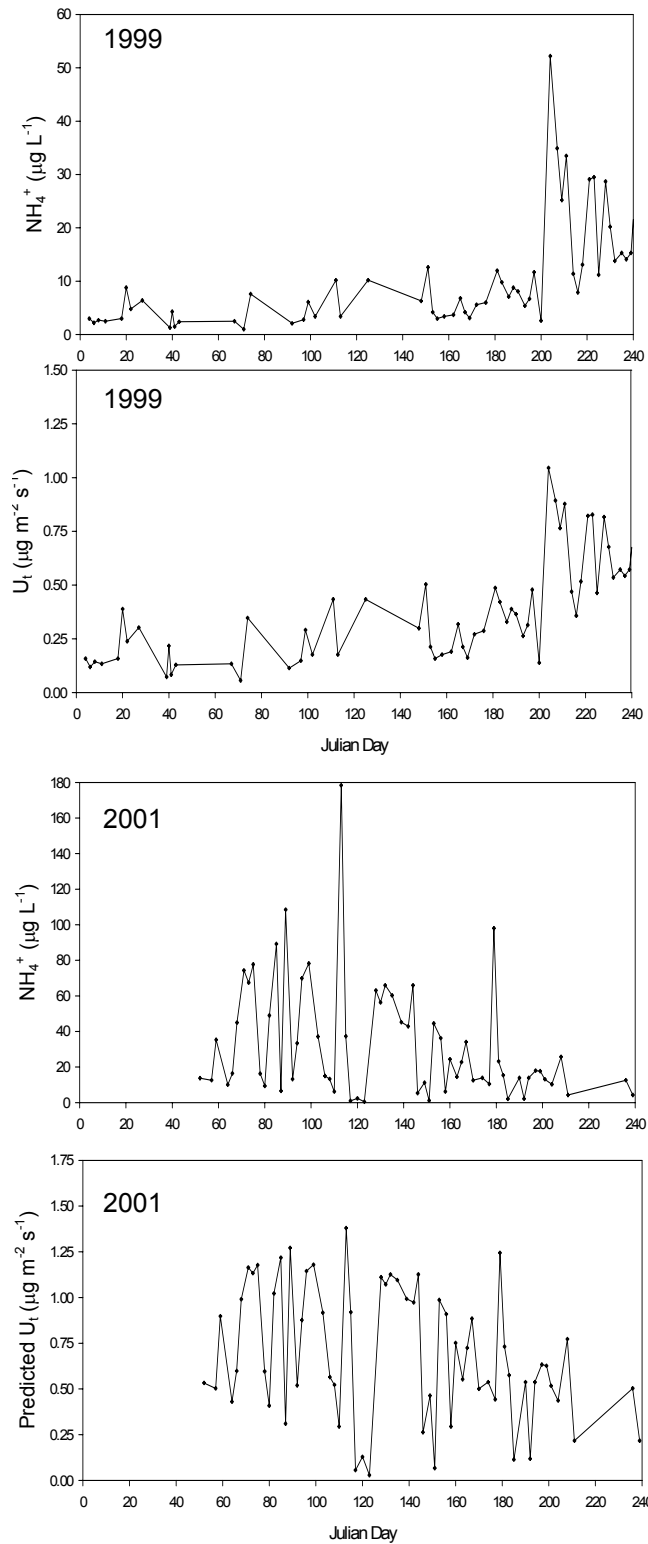


Figure 2.1- Variation in NH_4^+ concentration over a normal year (1999) and a highly variable year (2001) at N4D on the Konza Prairie Biological Station. Variation in U_1 is estimated based on NH_4^+ concentration

and the Michaelis-Menten relationship determined in this study.

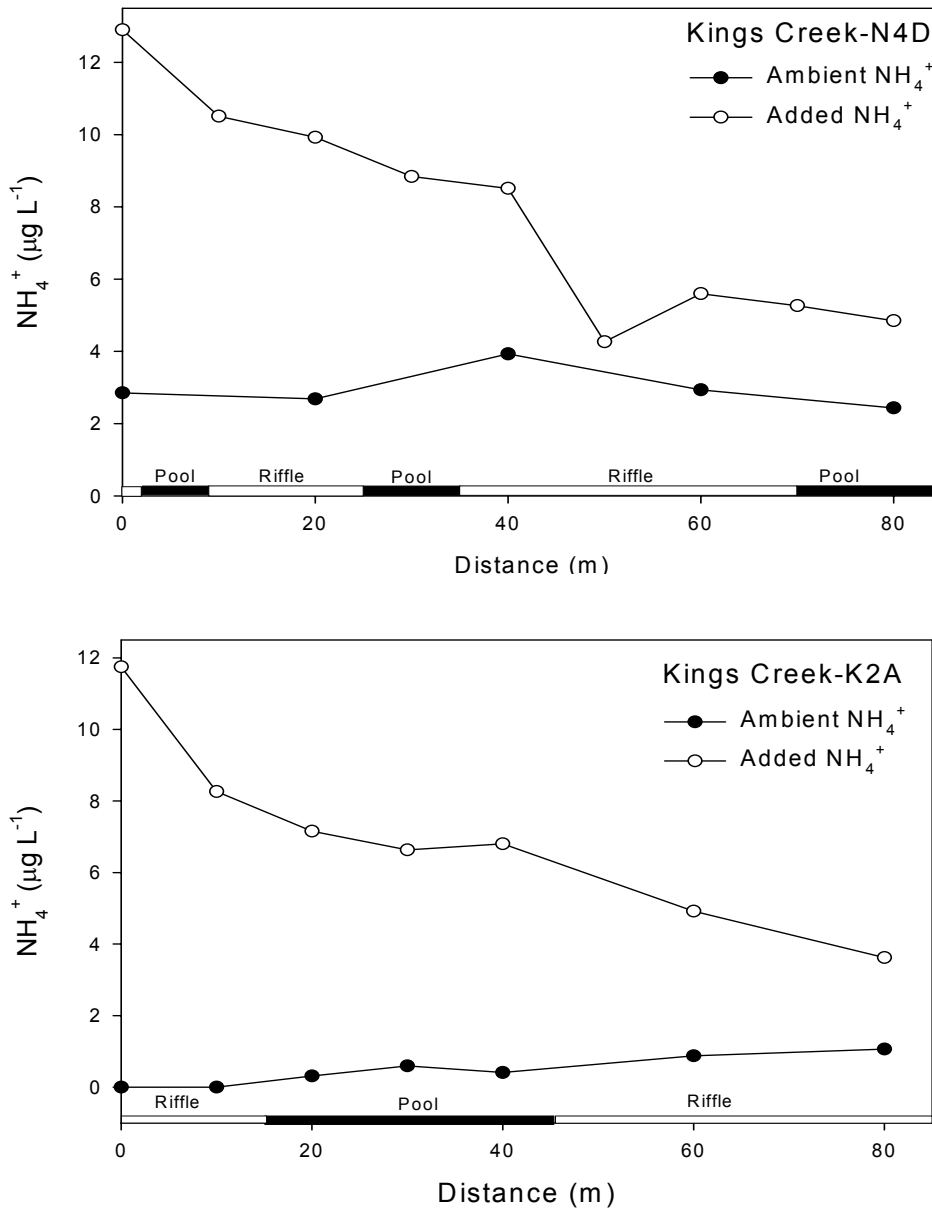


Figure 2.2- Variation in NH_4^+ concentration along the stream reaches at N4D and K2A before (●) and during (○) a NH_4^+ addition experiment. The ambient ammonium concentration at N4D did not show a net change with distance at ambient concentrations and showed no effects of pools in the experimental concentration. Ambient NH_4^+ concentrations at K2A demonstrate net mineralization with distance, and a reduction in net uptake in a large pool (15 m –45 m) during an experimental NH_4^+ addition.

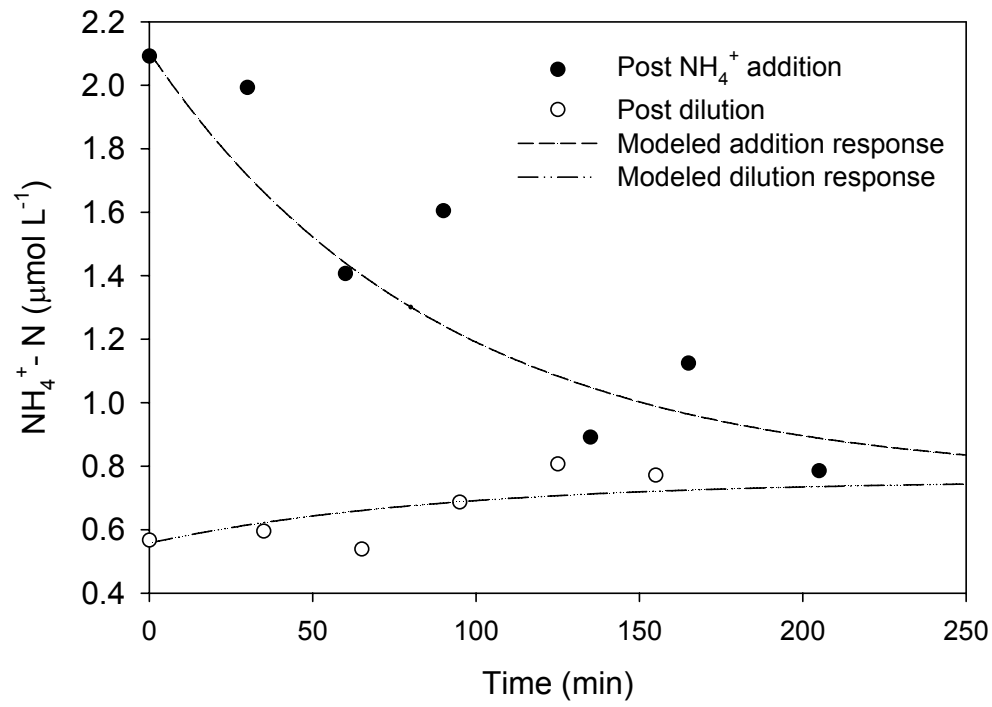


Figure 2.3- An example of the response of NH_4^+ concentration to dilution and addition of NH_4^+ in chamber experiments.

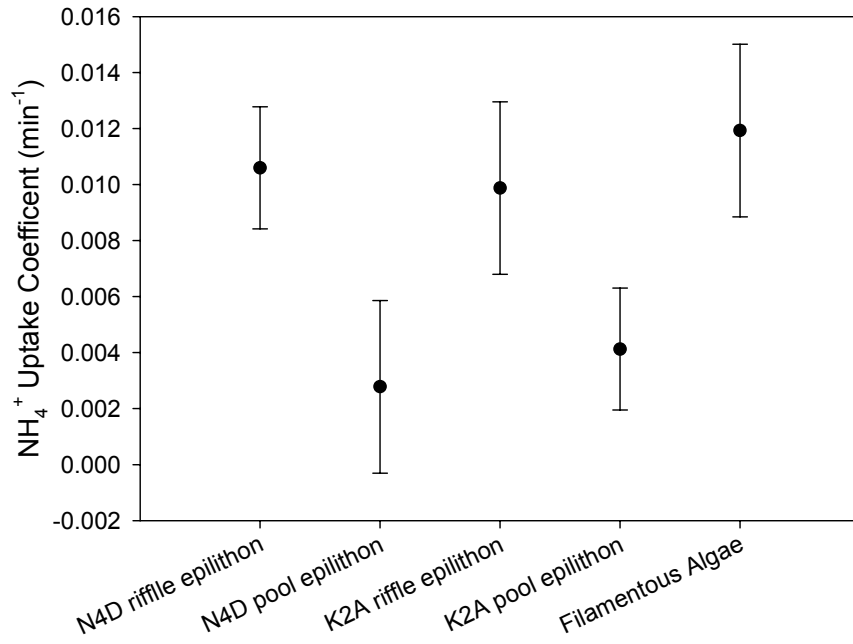


Figure 2.4- NH_4^+ uptake coefficients from chamber experiments by stream and substrata type. Error bars indicate 95% confidence intervals.

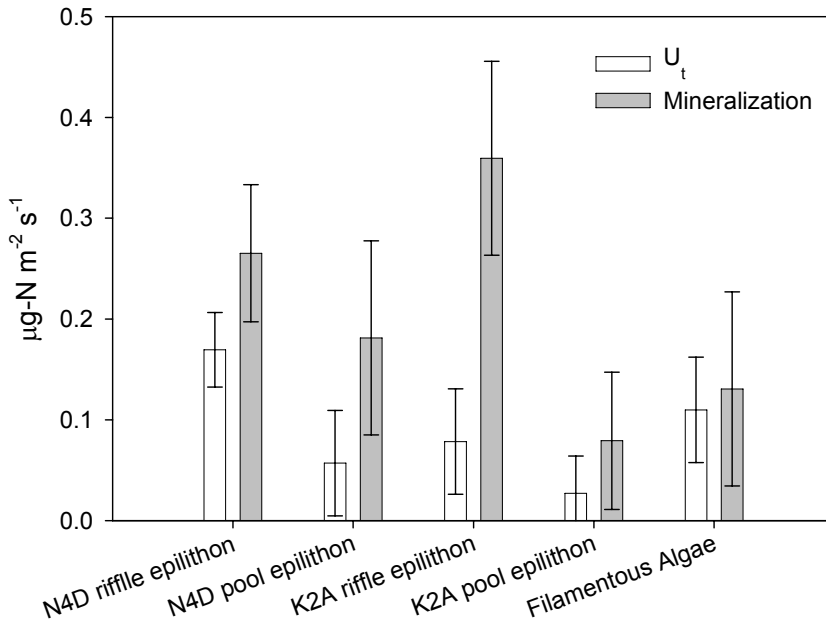


Figure 2.5- NH_4^+ uptake and mineralization rates from chamber experiments by stream and sediment type. Error bars indicate 95% confidence intervals.

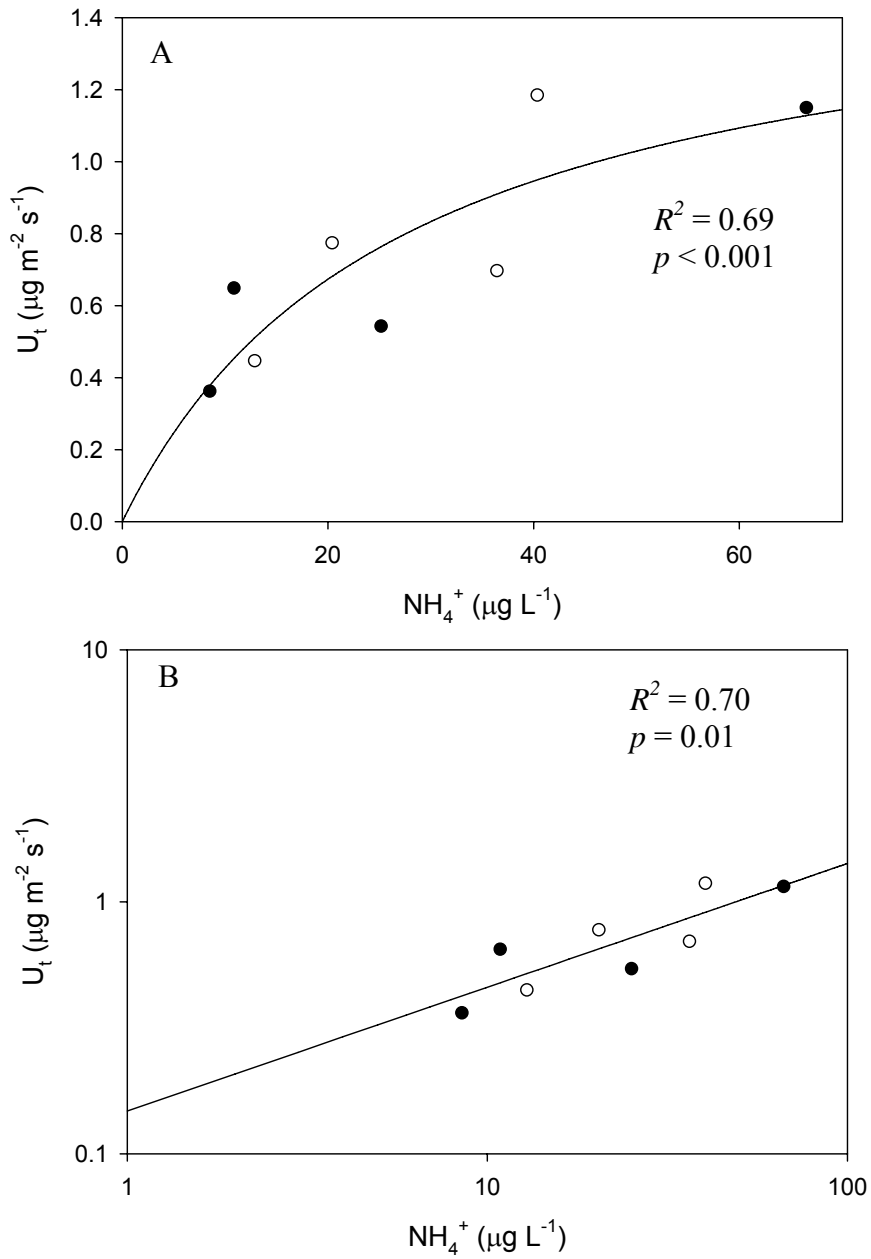


Figure 2.6- Relationship between NH_4^+ concentration and N uptake in K2A (○) and N4D (●) from short-term NH_4^+ addition experiments approximates both a Michaelis-Menten (A) and a Log-Log (B) model.

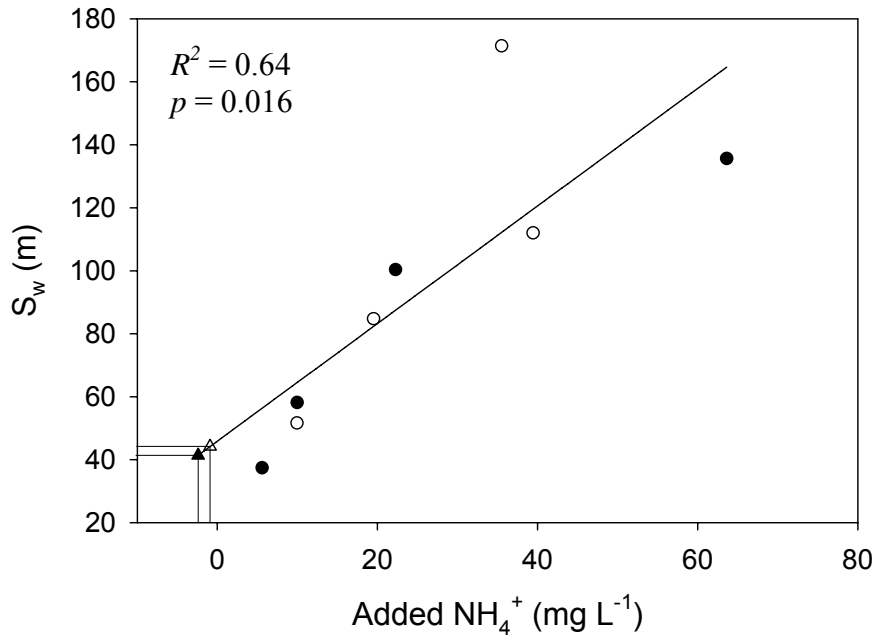


Figure 2.7- Prediction of ambient NH₄⁺ uptake length from K2A (Δ) and N4D (▲), based on linear regression of short-term NH₄⁺ addition experiments at both K2A (○) and N4D (●) according to the methods of Payne et al. (2004).

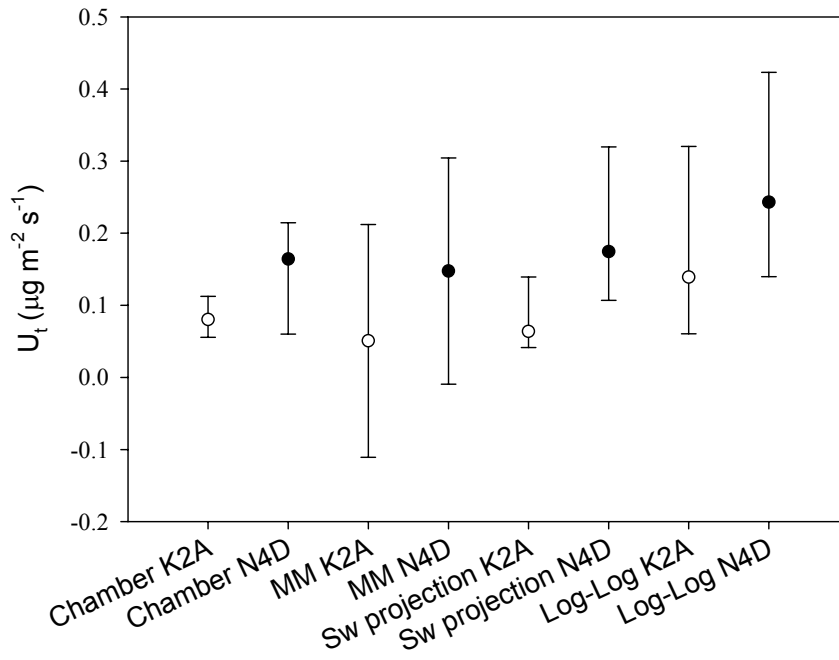


Figure 2.8- A comparison of predicted ambient U_t from the results of the chamber method and Michaelis-Menten, Log-Log, and S_w projection methods from short-term nutrient addition experiments for K2A (\circ) and N4D (\bullet). Error bars indicate 95% confidence intervals.

CHAPTER 3 - Chronic and acute saturation of N uptake in prairie streams

Abstract

We conducted a series of short-term N addition experiments in prairie streams to quantify the impacts of acute N enrichment on N cycling. In all 3 streams, uptake saturated in response to acute experimental NO_3^- enrichment. Half-saturation coefficients ranged between $146 \mu\text{g L}^{-1}$ and $634 \mu\text{g L}^{-1} \text{NO}_3^-$ -N, with V_{max} ranging from $5.6 \mu\text{g m}^{-2} \text{s}^{-1}$ to $21.5 \mu\text{g-N m}^{-2} \text{s}^{-1}$. We found similar results in response to experimental additions of NH_4^+ in the same streams, with a half-saturation of $6.7 \mu\text{g L}^{-1} \text{NH}_4^+$ -N and V_{max} of $1.6 \mu\text{g-N m}^{-2} \text{s}^{-1}$. Ambient concentrations of NH_4^+ and NO_3^- were less than their respective half-saturation coefficients, and uptake rates were less than 5% of V_{max} , suggesting severe N limitation. The saturation of uptake within streams contrasts with experiments conducted on streams with differences in levels of chronic enrichment of NO_3^- , which were found not to saturate. Compared across these streams, however, the acute additions are better described by using a Log-Log model, which provides better continuity with the results of the chronic N enrichment.

Introduction

Changes in human land use and elevated atmospheric deposition of N can lead to the increased transport of anthropogenic N in streams. Streams or small rivers may play a role in controlling transport of N from the landscape to the marine environment (Alexander et al. 2000). The manner in which in-stream processes, particularly uptake and retention, respond to increases of N in these streams must be understood if we are to establish the biogeochemical consequences of increased anthropogenic N loading.

Increases in availability and concentration of N and P can alter the trophic state (both autotrophic and heterotrophic) of stream ecosystems (Dodds 2006). In many instances, the heterotrophic and autotrophic processes that influence in-stream spiraling of N are limited or co-limited by N and P (Tank and Dodds 2003, Niyogi et al. 2004). Nitrogen transformation processes such as nitrification and denitrification can also be influenced by greater nitrogen concentrations in agricultural streams (Kemp and Dodds 2002a, Inwood et al. 2005). The stimulatory effect of increased N on cycling rates should only last to the point at which N is no longer a limiting factor for determining process rates. Once this point is reached, the rates of biotic uptake, nitrification, and denitrification will no longer increase as a function of N concentration, a condition referred to as saturation (Bernot and Dodds 2005).

Bernot and Dodds (2005) predicted that assimilative uptake, denitrification, nitrification, and primary production would exhibit Michaelis-Menten-based saturation as the supply of available NO_3^- exceeded biological demand in response to chronic N loading. Bernot et al. (2006) conducting stable-isotope tracer experiments in 6 high-N agricultural streams and found that uptake of NO_3^- was saturated. O'Brien (Chapter 1) found that NO_3^- uptake, nitrification, and

denitrification did not saturate across nine streams ranging from pristine prairie to those with chronic high levels of N loading.

Nutrient enrichment can be described, in terms of the duration of enrichment, as chronic or acute. Chronic N input is a continuous enrichment of available nitrogen over long periods. An example of this is the input of consistently N-enriched groundwater. Acute enrichment are increases in N concentration over the course of hours to days, which may result in the immediate response of organisms to the increase in N availability. An example of acute response is the response of the stream biota to rapid fluctuations or pulses in N concentration (Chapter 2). Short-term nutrient-addition experiments are commonly used to measure nutrient uptake in stream systems, to assess the responses of the biota to acute increases in nutrient concentration. The effects of chronic N are usually investigated with isotopic tracers (Bernot et al. 2006, Chapter 1).

Functional responses of biological activity to elevated N inputs can be described by two models, the Michaelis-Menten and Log-Log models. On the organismic level, the relationship between nutrient uptake and nutrient concentration can be approximated with Michaelis-Menten kinetics for benthic stream organisms (Lohman and Priscu 1992, Borchardt et al. 1994, Kemp and Dodds 2002b). Michaelis-Menten-based models also provide reasonably good fits to enrichment experiments in some streams (Dodds et al. 2002, Payne et al. 2004, Simon et al. 2005), but other streams have illustrated more linear relationships between uptake and concentration.

An alternate model that has been used to describe the interaction between N processing and concentration across streams is a Log-Log model. It has been used to describe the relationship between NH_4^+ uptake and concentration in streams from a variety of biomes (Dodds

et al. 2002). This model has also been used to describe the connection between N transformation rates and NO_3^- concentration across a range of human-impacted streams (Chapter 1).

We used a series of stepwise short-term NO_3^- addition experiments, in addition to recently published data, to determine whole-stream N-uptake kinetics for 3 prairie streams. We also compiled literature data for prairie streams to compare the responses of stream biota to chronic versus acute N enrichment.

Methods

Study area

Our study was conducted on tributaries of the Kings Creek watershed on the Konza Prairie Biological Station (KPBS), located in the Flint Hills region of northeastern Kansas, USA. The experiments were conducted on streams draining 2 sub-watersheds, Kings Creek-K2A (on the north branch, $39^\circ 06.008'$ N, $96^\circ 34.454'$ W) and Kings Creek-N4D (on the south branch, $39^\circ 05.271'$ N, $96^\circ 35.067'$ W), of the system and on a stream draining the adjacent Shane Creek ($39^\circ 06.779'$ N, $96^\circ 33.220'$ W) watershed. Land use in both watersheds is composed entirely of native tallgrass prairie. Streams in this region are typically oligotrophic, with low concentrations of dissolved and total N and P (Dodds 2003). Kings Creek and Shane Creek serve as reference sites in this study, used to test the effects of both chronic and acute N enrichment. Kings Creek is an intensively studied system, and descriptions of the nitrogen cycling (Dodds et al. 2000), hydrology (Gray et al. 1998), and ecology (Gray and Dodds 1998) have been published.

The 2 streams investigated have similar physical and biological characteristics, and their watersheds are burned every 2 or 4 years. The Kings Creek-N4D watershed is grazed by bison and quadrennially burned (N4D was burned 3 years before this study). The Kings Creek-K2A

watershed is ungrazed and biennially burned (burned 1 year before the experiment). The Shane Creek watershed is ungrazed and biennially burned (burned 1 year before the experiment). The predominant biota types were epilithic biofilms and filamentous algae (*Cladophora*) in Kings Creek (N4D and K2A). Large leaf packs and *Nostoc* mats were also present Shane Creek in addition to epilithic biofilms and filamentous algae.

¹⁵NO₃⁻ Experiments

A series of ¹⁵NO₃⁻ addition experiments was conducted on Shane Creek and two tributaries of Kings Creek (N4D and K2A) as part of the LINX II project (Chapter 1). In these experiments, a solution of ¹⁵NO₃⁻, along with a NaBr conservative solute, was dripped into the stream at a steady rate for 24 hours. Samples for ¹⁵NO₃⁻ analysis were then collected at 6 stations along the length of the reach at 1:00 am and 12:00 pm. The decline in ¹⁵NO₃⁻ flux over the length of the reach was used to calculate gross uptake of NO₃⁻ at ambient concentrations.

The ¹⁵N content of the stream water NO₃⁻ was determined using a modified version of the method presented by Sigman et al. (1997). Stream water samples were concentrated, and NH₄⁺ was removed by boiling with 3.0 g MgO and 5.0 g NaCl. The samples were then transferred to 250-mL media bottles, to which 0.5 g Devarda's alloy, a Teflon filter packet, and an additional 0.5 g MgO were added. The sample NO₃⁻ was reduced to NH₄⁺ using Devarda's alloy. The Teflon filter packet, constructed by sealing a 10-mm glass-fiber filter, acidified with 25- μ l of 2.0 M KHSO₄, within a folded piece of 2.5 cm Teflon plumbing tape, allowed for diffusion of NH₃, followed by the collection of NH₄⁺ onto the acidified filter. Samples were incubated at 60 °C for 48 hr to activate the DeVarda's alloy and then placed on a shaker for 7 days to allow for diffusion. The glass-fiber filter was then removed from the media bottle, dried, and analyzed for

^{15}N on a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and a ConFlo II interface.

Data from the plateau stream water $^{15}\text{NO}_3^-$ samples were corrected for background and spike $^{15}\text{NO}_3^-$ to determine the total concentration of tracer $^{15}\text{NO}_3^-$ at each station. The $^{15}\text{NO}_3^-$ concentration was then multiplied by the discharge at each station to find the total $^{15}\text{NO}_3^-$ flux ($\mu\text{g}\cdot^{15}\text{N s}^{-1}$). The station-specific discharge was calculated by the dilution of the Br^- hydraulic tracer. The flux of $^{15}\text{NO}_3^-$ at each station was ln-transformed, and the slope of the decline in ln-transformed $^{15}\text{NO}_3^-$ flux over the length of the reach (k_m) was calculated using linear regression.

Short-term N addition experiments

Several short-term NO_3^- experiments were conducted on a prairie reach of Kings Creek-N4D in May 2003 (several days before the $^{15}\text{NO}_3^-$ addition) and July 2004. Stepwise short-term NO_3^- addition experiments were conducted on Kings Creek-K2A in July of 2005 under conditions similar to those of the $^{15}\text{NO}_3^-$ experiment one-year prior. Stepwise short-term NO_3^- addition experiments were conducted on Shane Creek several days before the $^{15}\text{NO}_3^-$ experiment. In each of the experiments, a solution of KNO_3 , along with a NaBr tracer, was pumped into the stream at a steady rate. Once the concentration of Br reached a steady-state plateau (increase of $<1\%$ over 5 min) at the end of the reach, samples for NO_3^- were collected at 10-m intervals along the length of the reach.

A series of short-term NH_4^+ enrichment experiments were also conducted on Kings Creek-N4D and Kings Creek-K2A in July 2004, and are described in greater detail in Chapter 2. The experiments consisted of a series of four NH_4Cl enrichments in each stream, with NH_4^+ concentration incrementally increased with each subsequent release. Once the concentration of

Br⁻ tracer arrived at plateau at the bottom of the reach, samples were taken at 10-m increments along the length. Ammonium concentration for each sample was corrected for background NH₄⁺ and dilution using the change in Br⁻ concentration.

Stream water NO₃⁻ concentration was determined colorometrically using the cadmium reduction method on a Technicon auto-analyzer (APHA 1995). Stream water NH₄⁺ concentration was analyzed using the indophenol method on a Technicon auto-analyzer (APHA 1995). Bromide concentrations were determined with an Orion Br⁻ specific electrode. Care was taken that all samples were at the same temperature and were stirred at the same rate during measurements.

The ¹⁵NO₃⁻ experiments, conducted in May 2003, June 2004, and May 2005 for Kings Creek-N4D, Kings Creek-K2A, and Shane Creek, respectively, were used in conjunction with the short-term NO₃⁻ addition experiments to determine uptake kinetics in each of the streams. Both of the diurnal measurements from these ¹⁵NO₃⁻ experiments were used to establish uptake at ambient condition (gross uptake) in each of the streams, and the values are indicated in Figure 1. The results of the stable-isotope experiments represent a direct measure of gross uptake. The NO₃⁻ addition experiments are actually measures of net uptake.

We compiled data from isotopic and short-term nutrient addition experiments, for both NH₄⁺ and NO₃⁻, from previous studies (Dodds et al. 2002, Chapters 1, 2) in order to determine the overall trends in stream N cycling with respect to concentration within the King's Creek basin. The data set was then fit with modified Michael-Menten and Log-Log models to determine the extent of saturation over the entire data set.

Calculations

Short-term nutrient-addition experiments assume that the concentration of added nutrient will decline asymptotically toward the ambient stream-nutrient concentration at a rate proportional to the amount of added nutrient. This is best described by the first-order equation:

$$\ln (C_x - C_0) = \ln C_i + k_x * x \quad (3.1)$$

in which C_x is the concentration at some downstream location x meters from the addition point, C_i is the increase in concentration due to the addition, C_0 is the background nutrient concentration, and k is the uptake coefficient. All of the nutrient spiraling metrics were then calculated based on this coefficient of uptake (k_x), as outlined by the Stream Solute Workshop (1990).

We calculated uptake length (S_w) as:

$$S_w = -1 \times k_x^{-1} \quad (3.2)$$

$^{15}\text{NO}_3^-$ during both plateaus measured at each stream. From S_w , we calculated the uptake rate (U_t):

$$U_t = \frac{F}{S_w \times w} \quad (3.3)$$

in which F is the flux of NO_3^- in the stream water and w is the average width of the stream. The mass transfer coefficient (V_f) is:

$$V_f = \frac{U_t}{C} \quad (3.4)$$

in which C is the concentration of NO_3^- in the stream water (Stream Solute Workshop 1990).

Relationships between U_t and N concentration (C) in the individual streams were fit with the Michaelis-Menton equation:

$$U_t = \frac{V_{\max} * C}{(K_s + C)} \quad (3.5)$$

in which V_{\max} is the maximum rate of uptake and K_s is the half-saturation coefficient. A modified version of this equation was used to determine the relationship between uptake mass transfer coefficient and concentration:

$$V_f = \frac{V_{\max}}{(K_s + C)} \quad (3.6)$$

Statistics

The Michaelis-Menten model was fit to U_t and N concentration by using least squares regression with the Levenberg-Marquardt estimation algorithm. Linear regression was used to calculate Log-Log relationship using log transformed U_t and log transform C. Michaelis-Menten model parameters for the entire prairie NO_3^- and NH_4^+ data sets were estimated by using a least squares regression with the Levenberg-Marquardt estimation algorithm. The differences between acute and chronic N enrichment were tested between log-transformed U_t and log-transformed C by using a homogeneity of slopes ANCOVA model. All statistical analyses were performed with the Statistica 6 (Statsoft, Tulsa, OK, USA) statistical package.

Results

$^{15}\text{NO}_3^-$ and short-term N addition experiments

One short term NO_3^- addition was conducted on Kings Creek-K2A the day before the $^{15}\text{NO}_3^-$ experiments (peak concentration of $34.4 \mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$), and an additional four short-term NO_3^- addition experiments were conducted in July 2005 as stepwise-increase experiments

(peak concentrations of 17.4, 26.8, 68.2, and 169.1 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$) (Fig. 1A). Based on the five NO_3^- addition experiments and two measures of $^{15}\text{NO}_3^-$, there was a significant fit of the Michaelis-Menten model ($F_{1,6} = 24.8$, $R^2 = 0.81$, $p = 0.004$) (Table 3.1). From this model, we calculated a maximum rate of uptake (V_{max}) of $21.5 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\text{SE} = 12.6 \mu\text{g m}^{-2} \text{s}^{-1}$) and a half-saturation coefficient (K_s) of $184 \mu\text{g L}^{-1}$ ($\text{SE} = 173 \mu\text{g L}^{-1}$).

Two short-term NO_3^- additions were conducted on Kings Creek-N4D 2 days before the $^{15}\text{NO}_3^-$ experiments, and yielded peak NO_3^- concentrations of 83.2 and 442.1 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$ (Fig. 1B). Three more short-term NO_3^- addition experiments were conducted in July 2004 on the same stream, and yielded peak concentrations of 21.0, 22.2, and 33.4 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$. Uptake kinetics were calculated based on the five NO_3^- addition experiments and two measures of $^{15}\text{NO}_3^-$. There was a significant fit of the Michaelis-Menten model ($F_{1,6} = 21.5$, $R^2 = 0.98$, $p = 0.006$), and we calculated a V_{max} of $5.6 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\text{SE} = 0.4 \mu\text{g m}^{-2} \text{s}^{-1}$) and a K_s of $146 \mu\text{g L}^{-1}$ ($\text{SE} = 25 \mu\text{g L}^{-1}$).

Three short-term NO_3^- additions were conducted in a stepwise manner several days before the $^{15}\text{NO}_3^-$ experiments on Shane Creek, and yielded peak NO_3^- concentrations of 77.2, 220 and 411 $\mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$ (Fig 1C). Based on the NO_3^- addition experiments and $^{15}\text{NO}_3^-$ measurements, there was a significant fit of the Michaelis-Menten model ($F_{1,4} = 141.0$, $R^2 = 0.97$, $p = 0.001$), and we calculated a V_{max} of $16.5 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\text{SE} = 3.3 \mu\text{g m}^{-2} \text{s}^{-1}$) and a K_s of $634 \mu\text{g L}^{-1}$ ($\text{SE} = 192 \mu\text{g L}^{-1}$).

The short-term NH_4^+ addition experiments yielded peak NH_4^+ concentrations of 5.6, 10.0, 22.3, and 63.6 $\mu\text{g L}^{-1} \text{NH}_4^+ \text{-N}$ at Kings Creek-N4D and 10.0, 19.5, 35.5, and 39.4 $\mu\text{g L}^{-1} \text{NH}_4^+ \text{-N}$ at Kings Creek-K2A. Given the similarities between the two streams in terms of biological and physical characteristics, NH_4^+ uptake data were pooled to establish a relationship between uptake

and NH_4^+ concentration. There was a significant Michaelis-Menten fit ($R^2 = 0.69$, $F_{1,7} = 69.6$, $p < 0.001$) to the data. From this model, we calculated a V_{\max} of $1.6 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\text{SE} = 0.5 \mu\text{g m}^{-2} \text{s}^{-1}$) and a K_s of $27.2 \mu\text{g L}^{-1}$ ($\text{SE} = 17 \mu\text{g L}^{-1}$).

Overall trends

The data from this study were combined with that of another study of concentration and whole-stream uptake of NO_3^- and NH_4^+ in prairie streams (Dodds et al. 2002) to establish the overall trends in N uptake in this system. We found significant Michaelis-Menten relationships between V_f and concentration for NH_4^+ ($R^2=0.37$, $F_{1,18}= 11.1$, $p<0.001$) and for NO_3^- ($R^2= 0.34$, $F_{1,25}= 13.2$, $p<0.001$) (Fig. 2). Uptake kinetics calculated by these models showed a greater V_{\max} for NO_3^- ($5.0 \mu\text{g m}^{-2} \text{s}^{-1}$) than for NH_4^+ ($1.6 \mu\text{g m}^{-2} \text{s}^{-1}$). Half-saturation coefficients were also greater for NO_3^- ($65 \mu\text{g L}^{-1} \text{NO}_3^- \text{-N}$) than for NH_4^+ ($6.0 \mu\text{g L}^{-1} \text{NH}_4^+ \text{-N}$). In addition, we found significant Log-Log relationships between U_t and concentration for NO_3^- ($R^2= 0.86$, $F_{1,25}= 150.8$, $p<0.001$) and for NH_4^+ ($R^2= 0.73$, $F_{1,25}= 35.8$, $p<0.001$).

The effects of chronic vs. acute NO_3^- enrichment were tested by comparing the relationship between log transformed U_t and NO_3^- concentrations found by O'Brien (Chapter 1) with the Log-Log relationship derived by the short-term addition experiments in this study and Dodds et al. (2002) (Fig. 3). There were no differences between the slopes of the relationships between uptake and concentration for chronic and acute N enrichment ($F_{1,25} = 0.88$, $p = 0.35$). Uptake rates were higher on average, however, in the acute enrichments than in the chronically enriched streams ($F_{1,25} = 5.92$, $p = 0.022$).

Discussion

Nitrate uptake demonstrated Michaelis-Menten saturation in all three streams in response to acute additions of NO_3^- . Ammonium uptake likewise demonstrated saturation in response to acute NH_4^+ enrichment. We found significant general trends of Michaelis-Menten saturation for both NO_3^- and NH_4^+ across all the uptake data collected from Kings Creek.

The observed half-saturation coefficients for NH_4^+ occurred at a smaller concentration than those of NO_3^- in Kings Creek. This suggests that NH_4^+ is more efficiently assimilated when scarce than is NO_3^- . This strong affinity for NH_4^+ relative to NO_3^- is consistent with data published for freshwater plankton (Dodds et al. 1991) and is reflective of the lesser energetic requirements for using NH_4^+ than for NO_3^- . Observed V_{max} for NH_4^+ was less than that of NO_3^- in Kings Creek, suggesting that NO_3^- uptake is the predominant form of inorganic N assimilation when NO_3^- is available at high concentrations. We would expect NH_4^+ uptake to be greater than NO_3^- uptake, based the V_{max} and half-saturation coefficients for NO_3^- and NH_4^+ , and the normally low ambient stream concentrations of NO_3^- and NH_4^+ (Fig. 3). Projections of uptake based on the variation of inorganic N concentrations during the period of stream flow in 2004 demonstrate that NH_4^+ is the dominant N compound in N uptake when both NO_3^- and NH_4^+ are present.

Half-saturation coefficients for NO_3^- and NH_4^+ suggest that organisms in Kings Creek were exhibiting N limitation because ambient concentrations of NO_3^- and NH_4^+ were less than their respective values of K_s (Tilman 1982). The degree of limitation can also be inferred from the ambient uptake relative to total potential uptake (V_{max}). In N-saturated systems, ambient U_t would be expected to be approximately equal to V_{max} . Conversely, if N is limiting in the system, U_t would be only a small proportion of V_{max} . Ambient U_t ranged between 0.3% and 5% of V_{max} for NO_3^- in the 3 streams. Ambient U_t averaged only 6% of V_{max} for NH_4^+ in N4D and K2A

(Chapter 2). Thus, the capacity for N uptake is much greater than the actual rate of assimilation, indicating a strong degree of limitation. This N limitation is consistent with bioassay data that indicated that primary producers are co-limited by N and P at the research sites (L. Taylor, personal communication).

Few studies have documented the uptake kinetics of N in benthic organisms (Borchardt 1996). Lohman and Priscu (1992) measured NO_3^- and NH_4^+ uptake kinetics in *Cladophora*, finding half-saturation coefficients of 7.3-15.2 $\mu\text{g L}^{-1}$ for NO_3^- and 17.4-41.9 $\mu\text{g L}^{-1}$ for NH_4^+ . Kemp and Dodds (2002b) characterized uptake kinetics of different substratum types and, based on these, projected uptake kinetics for NO_3^- ($V_{\text{max}} = 0.07 \mu\text{g m}^{-2} \text{s}^{-1}$ and $K_s = 12.3 \mu\text{g L}^{-1}$) and NH_4^+ ($V_{\text{max}} = 15.8 \mu\text{g m}^{-2} \text{s}^{-1}$ and $K_s = 6.7 \mu\text{g L}^{-1}$) for the whole stream. Half saturation coefficients of NO_3^- at the ecosystem scale were much greater than the half saturation coefficients reported in studies of benthic organisms (Lohman and Priscu 1992, Kemp and Dodds 2002b). Half-saturation coefficients of NH_4^+ reported here from the short-term addition experiments were greater than those reported by Kemp and Dodds (2002b), but are within the range reported by Lohman and Priscu (1992).

Relatively few studies have directly measured whole-stream uptake kinetics. Dodds et al. (2002) reported a half-saturation coefficient of 64 $\text{NH}_4^+\text{-N } \mu\text{g L}^{-1}$ for a prairie reach of Kings Creek. Payn et al. (2004) reported half-saturation coefficients in forested streams of 6 $\mu\text{g NH}_4^+\text{-N L}^{-1}$ for Ball Creek, North Carolina, and 14 $\mu\text{g NH}_4^+\text{-N L}^{-1}$ for Walker Branch, Tennessee. Simon et al. (2005) reported half-saturation coefficients of only 1.2 $\mu\text{g NO}_3^-\text{-N L}^{-1}$ to 1.4 $\mu\text{g NO}_3^-\text{-N L}^{-1}$ in a grassland stream in New Zealand based on repeated NO_3^- addition experiments over the course of a year. The half saturation coefficients for NH_4^+ reported in our study fall within the range of those reported in the literature.

Acute versus chronic enrichments

Nitrate uptake showed signs of Michaelis-Menten saturation in all three streams in response to added NO_3^- . Ammonium uptake likewise demonstrated saturation in response to acute NH_4^+ enrichment. This saturation indicates, as predicted by substrata incubation experiments (Kemp and Dodds 2002b), that N uptake by biota in these reference streams will be less responsive to acute N enrichment at relatively large concentrations. The disparity between acute and chronic saturation leaves us with the question: why is saturation evident in acute N enrichments, yet not evident in chronic N enrichments?

For a single cell, uptake will be governed by Michaelis-Menten enzyme kinetics, because the physical act of moving ions, such as NO_3^- and NH_4^+ , into the cell is an enzymatically-mediated process. This same relationship seems to scale up to stream microbial communities in response to short pulses of nutrients in the entire ecosystem. These diverse microbial communities may adjust to higher NO_3^- concentrations when they are exposed to elevated levels of NO_3^- over longer periods of time. This adjustment may occur as a result of changes in biochemical pathways or changes in community composition and abundance. Kemp and Dodds (2002a) demonstrated that nitrification associated with transplanted substrata rapidly responded to new nutrient regimes. Further research is necessary to establish the exact mechanism for this form of saturation across a variety of stream types.

Acute enrichment was measured within streams, whereas comparisons of chronic effects are, by necessity, conducted across streams. During stepwise nutrient-addition experiments within a stream, hydrologic and biological conditions were likely to vary only minimally over the course of the experiment (or the experiment would fail). Hydrologic and biological

conditions differ from stream to stream. It is this difference, particularly in biotic conditions, that may give rise to the lack of evident saturation across streams.

Another possible reason for the discrepancy is the model used to describe saturation. The Michaelis-Menten model may not be appropriate for comparisons across streams, due to the changes in hydrology, biotic community, and adjustments of the biota to the conditions within the stream. The fit of this model to the Kings Creek NO_3^- and NH_4^+ data was not especially robust, explaining only 37% of the variation in V_f with respect to concentration for the NO_3^- , and explaining only 34% of the variation for NH_4^+ . Conversely, the Log-Log model explained 84% of the variation in overall U_t with respect to N concentration for NO_3^- , and explained 74% of the variation for NH_4^+ . The Michaelis-Menten model did not fit the cross-stream chronic enrichment data presented by either O'Brien (Chapter 1) or Bernot et al. (2006).

The Log-Log model demonstrates greater uptake rates for acute NO_3^- enrichment than for chronic N enrichment at the same concentrations, due to a significantly higher intercept. This may be because these prairie streams are N limited (Tank and Dodds 2003) and may possess a biological N deficit. Thus, they have a greater uptake rate in response to acute enrichment of N. Streams with chronic N enrichment probably have less N deficit and, thus, may not have lower uptake rates in response to the same concentrations.

Conclusions

Nitrogen uptake rates saturated in three pristine streams in response to acute experimental NO_3^- and NH_4^+ enrichments. Uptake rates of NH_4^+ and NO_3^- at ambient conditions were only a small proportion of the V_{max} for these compounds, suggesting severe limitation of N within these streams. The form of saturation exhibited within streams in response to acute N enrichment is in contrast to the form of saturation observed in streams with different levels of chronic enrichment

of NO_3^- . Although within-stream experiments can saturate in a Michaelis-Menten fashion, this model of saturation does not seem to extend across streams.

Acute additions are better described by a Log-Log model when compared across streams, making the relationship between uptake and NO_3^- concentration nearly identical to that of the streams receiving chronic enrichment of N. Although enzyme uptake kinetics dictate that individual organisms exhibit Michaelis-Menten uptake, the complex communities found in stream benthic habitats, coupled with diffusion effects, evidently lead to a situation in which a Log-Log model describes the functional response of microphytobenthos to inorganic nitrogen concentrations in the water column.

Table 3.1- Michaelis-Menten model parameters for the relationships between inorganic N concentrations and N uptake for NO_3^- from $^{15}\text{NO}_3^-$ stable-isotope tracer experiments and short-term NO_3^- addition experiments at N4D, Kings Creek-K2A, and Shane Creek, as well as from short-term NH_4^+ addition experiments at Kings Creek-N4D and Kings Creek-K2A.

Stream	Type	V_{\max} $\mu\text{g m}^{-2} \text{s}^{-1}$	K_s $\mu\text{g L}^{-1}$	R^2	F	P
K2A	NO_3^-	21.5	184	0.94	45.3	<0.001
N4D	NO_3^-	5.6	146	0.98	322.9	<0.001
Shane Creek	NO_3^-	16.5	634	0.99	617.9	<0.001
K2A and N4D	NH_4^+	1.59	27.2	0.69	69.6	<0.001
Overall	NH_4^+	1.7	6.7	0.48	11.6	<0.001
Overall	NO_3^-	4.9	67	0.34	18.8	<0.001

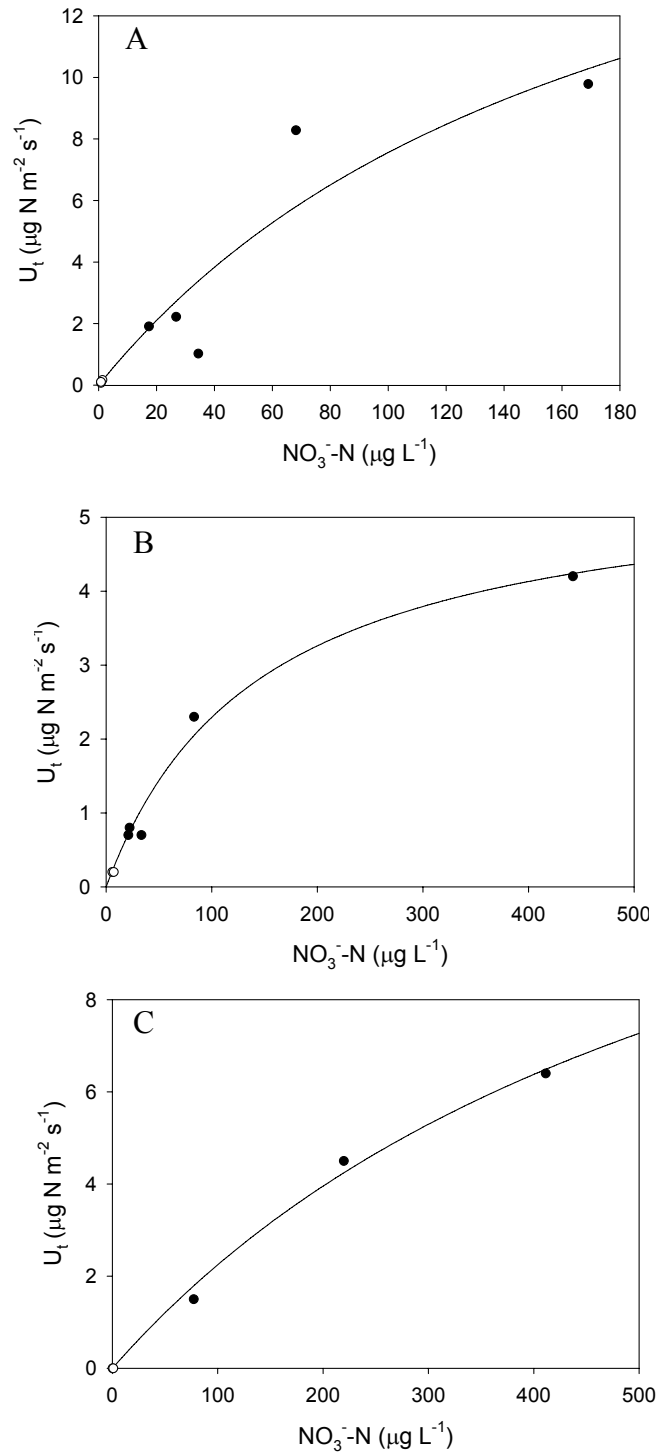


Figure 3.1- Relationship between NO_3^- concentration and U_1 for $^{15}\text{NO}_3^-$ and short-term NO_3^- addition experiments conducted on KPBS watersheds Kings Creek-K2A (A), Kings Creek-N4D (B), and Shane Creek (C).

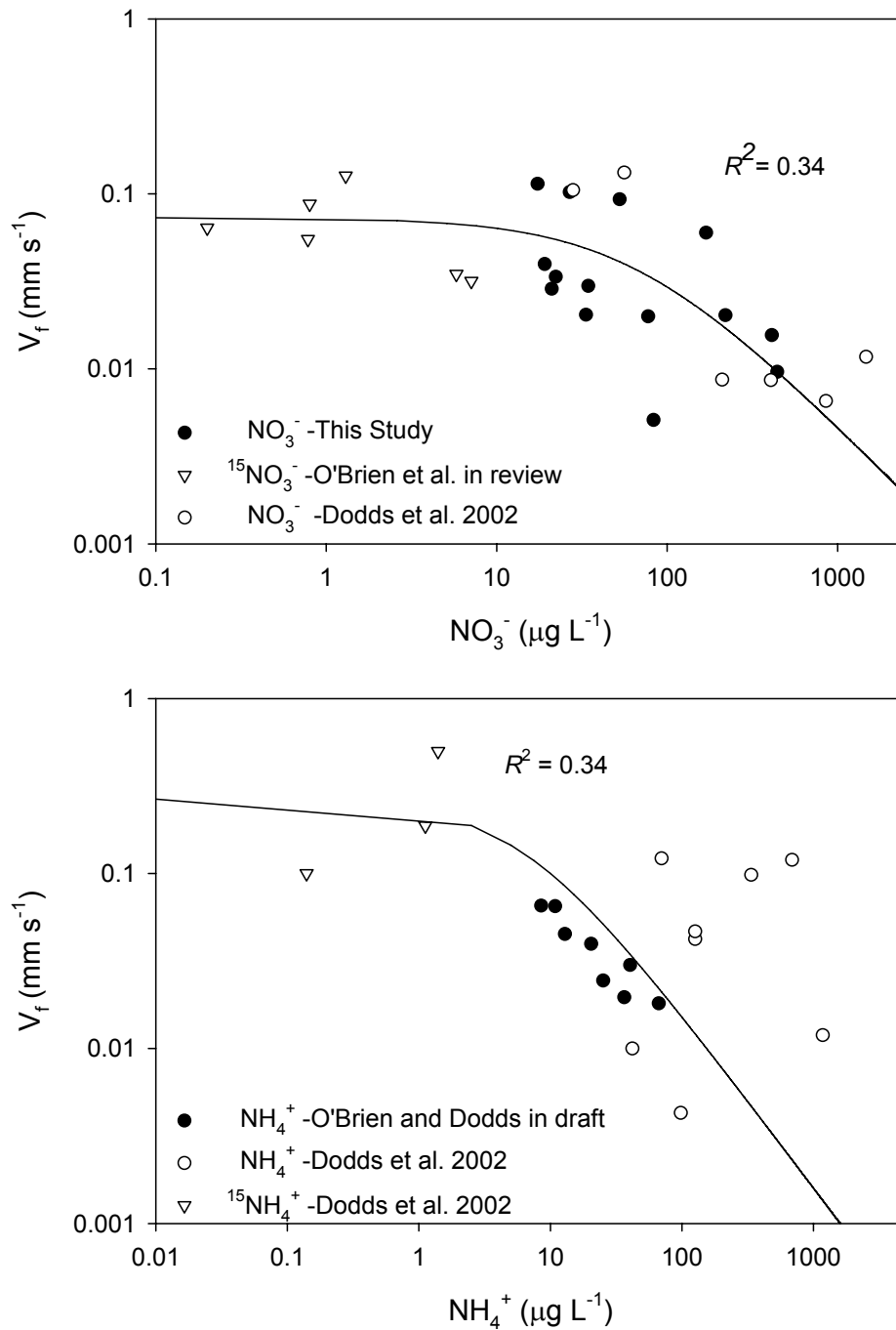


Figure 3.2- Michaelis-Menten model fit to the N concentration and V_f data for NO_3^- and NH_4^+ from the overall Kings Creek prairie stream data set.

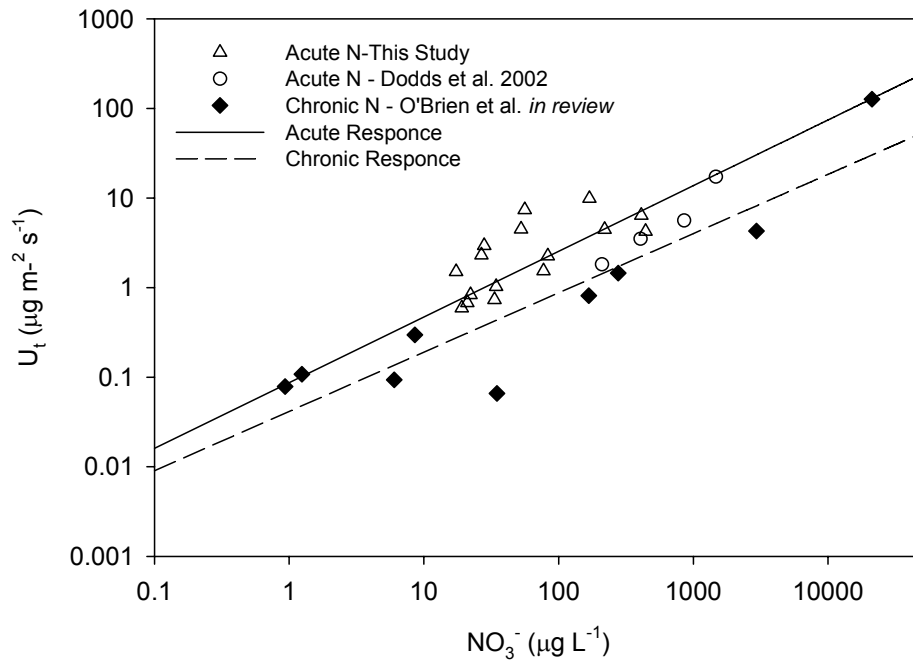


Figure 3.3- A comparison of Log-Log relationships between NO_3^- concentration and U_1 for streams with acute enrichment of NO_3^- and chronic enrichment of NO_3^- .

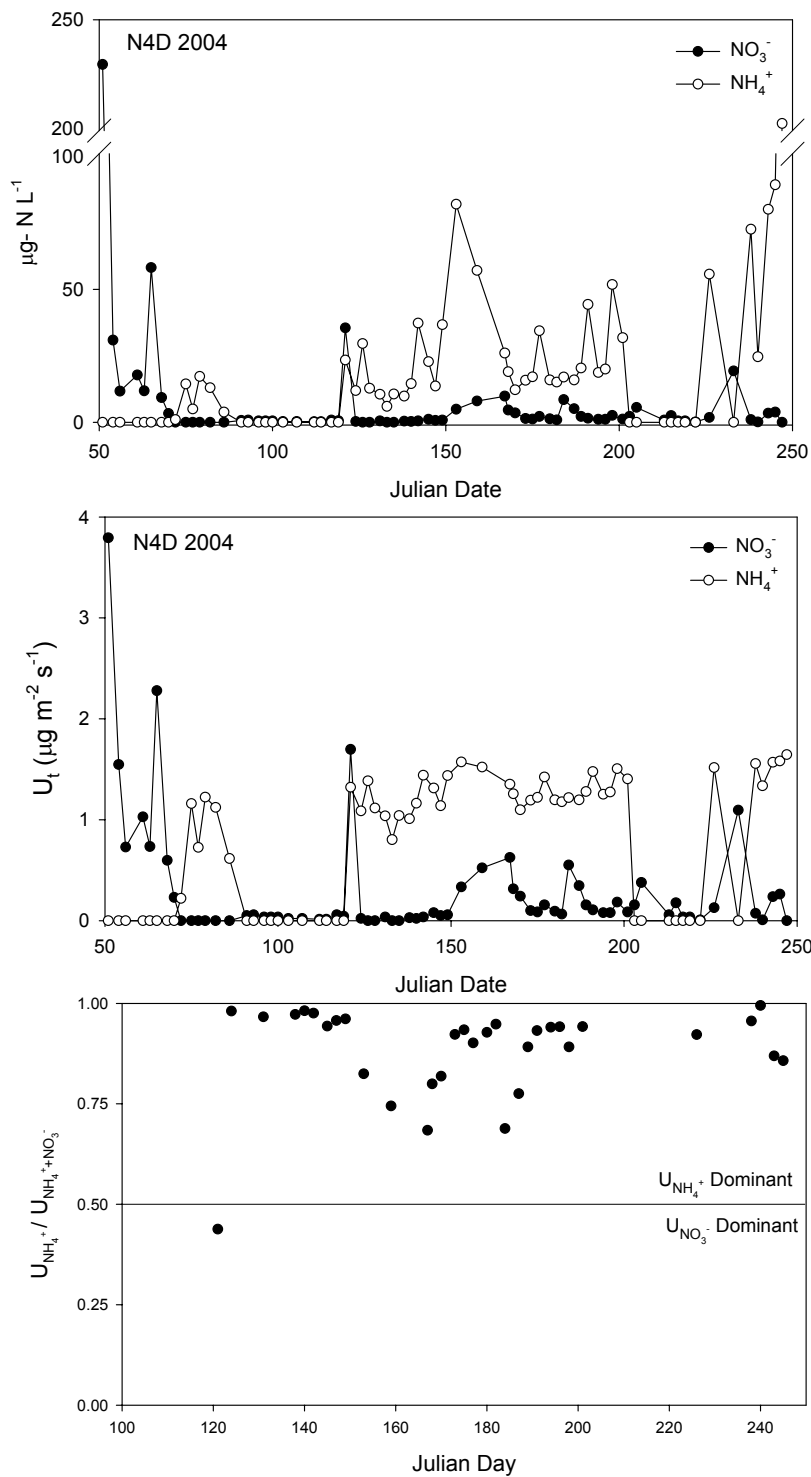


Figure 3.4- Mean NH_4^+ and NO_3^- concentrations in 2004 at Kings Creek-N4D, predicted NH_4^+ and NO_3^- U_t , as predicted by the Michaelis-Menten model, and the proportion of $U_{t-\text{NH}_4}$ to total uptake ($U_{t-\text{NH}_4 + \text{NO}_3}$) on dates when both are present.

CHAPTER 4 - Organic carbon and nitrogen spiraling in prairie streams

Abstract: We measured organic carbon (C) and nitrogen (N) spiral lengths in four headwater prairie streams. Organic C spiraling was calculated from total benthic C, flux of dissolved and suspended C, and rate of respiration within the stream channel. Organic N spiraling was similarly calculated from benthic organic N, flux of dissolved and suspended N, and estimated rate of N mineralization. Carbon spiraling lengths in the streams ranged from 24 m to 400 m. Spiral length for organic N ranged from 240 m to 2656 m and was approximately 10-fold that of C in most of the prairie streams. In contrast, the average uptake length of inorganic N ranged from 28 m to 174 m. Total spiral length, or average distance between mineralization events, was then calculated as the sum of inorganic and organic N spirals, and ranged from 260 m to 2830 m. The C:N of suspended and dissolved organic matter was less than the average C:N of benthic organic matter; therefore, the ratio of organic N in the water column to total benthic N was greater than the ratio of water column C to benthic C. This difference led to the longer spiraling distances observed for organic N. Transport of organic N was more prominent in this system than inorganic transport, both in terms of total N concentration and transport distance. These results indicate that, although organic C and inorganic N were highly conserved in the headwater streams, organic N was much more readily transported.

Introduction

Streams play an important role in transporting nutrients and organic matter from the terrestrial habitats of watersheds they drain to downstream aquatic habitats. Transport of organic C from headwater streams is an important subsidy to food webs of downstream water bodies (Vanotte et al. 1980). In-stream processes can alter timing and quantity of nutrients and materials transported within the stream (Bernhardt et al. 2003, Bernhardt et al. 2005, Mulholland et al. 2005) and their availability for organisms in the stream channel.

Transport of nutrients in streams is governed by rate of nutrient cycling in the stream ecosystem, often referred to as 'spiraling' because of the linear downstream movement associated with dissolved and suspended forms as they cycle (Webster and Patten 1979). Spiraling of nutrients such as N and P can be described by spiral length (S), the distance necessary to cycle between organic and inorganic forms. Spiral length is the sum of uptake length (S_w), average distance that a dissolved molecule travels before being taken up, and particle transport distance (S_p), which is the average distance traveled in particulate form (Newbold et al. 1981). These parameters can be used to describe the degree to which a nutrient is conserved and retained in a stream. Processing of organic C during transport has also been described using spiraling theory (Newbold et al. 1982). Carbon spiraling length (S_C) is described as the average distance traveled by C in organic form.

Investigators typically focus on spiraling dynamics of dissolved N, usually in inorganic form (e.g., Simon et al. 2005, O'Brien 2006). The advantage of studying the inorganic portion of the N cycle is that inorganic fractions of a stream are most biologically available to primary producers and heterotrophic bacteria, and have clearly defined chemistries that make them easy to measure and manipulate. Availability of inorganic nutrients such as N and P often influences

ecologically important processes such as primary production and heterotrophic growth (Tank and Dodds 2003), thus the exchange of inorganic nutrients with stream water is an important part of ecosystem function. Focusing on the inorganic spiral, however, may provide an incomplete picture of the true dynamics of N transport within the stream because it does not account for dissolved organic and particulate N forms.

Spiraling of C within streams primarily involves movement of organic C. Inorganic C (as CO_2) readily exchanges with the atmosphere when utilization or regeneration lead to sub- or super-saturation in the water column. Thus, spiraling of inorganic C is generally not considered (Newbold et al. 1982). Measurements of C spiraling in streams are based on three important factors: 1) total standing stock of organic C in the stream channel 2) amount of organic C dissolved or suspended in stream flow, and 3) rate at which organic C is being respired within the stream channel (Thomas et al. 2005). Two main components of organic C spiral length (S_C) can be calculated from measurements: mean downstream velocity of C (V_{OC}) and rate coefficient of C respiration (K_{OC}) (Newbold et al. 1982). Like the S_w in the N and P spiral, S_C can be used as an index of retention or export. A shorter S_C indicates that the stream is more retentive of organic C. A longer S_C indicates that stream organic C is retained less efficiently and the stream is exporting more of its organic C to downstream reaches and water bodies.

By definition, spiral length is the sum of individual lengths of each compartment, (e.g., S_w , S_p) multiplied by the probability that an atom will move through that compartment (Newbold et al. 1982). Inorganic N spiral length represents the average distance between mineralization and assimilation. Dissolved inorganic N (DIN) spiral is composed of the weighted average of assimilative NH_4^+ S_w (average distance traveled by NH_4^+ before being assimilated) and the sum of nitrification length (S_{Nit}) and NO_3^- S_w (distance traveled by NH_4^+ before being nitrified and

subsequently traveled as NO_3^- before being assimilated or denitrified). If nitrification uses locally mineralized NH_4^+ (Kemp and Dodds 2002), then S_{Nit} is very small and DIN spiral is simply equal to the concentration-weighted average of $\text{NH}_4^+ S_w$ and $\text{NO}_3^- S_w$.

By applying C spiraling theory to organic N, we are attempting to integrate spiraling lengths of all organic N compartments (benthic N, dissolved inorganic N (DON), and particulate N) into a single spiral length, namely distance that the average atom of organic N will travel before being mineralized. The sum of the organic N spiral and inorganic spiral will represent an entire nitrogen spiral length (S_N), or the average distance between mineralization events.

Prairie streams were historically a significant part of the Mississippi River drainage area; today however, a large portion of the native prairie landscape has been converted to agriculture and urban uses (Dodds et al. 2004). Relatively pristine prairie streams are still present in some regions of the Great Plains, such as the Flint Hills of eastern Kansas. Areas historically covered with tallgrass prairie now are very important to downstream N transport into the Northern Gulf of Mexico (Alexander et al. 2000), and we are just beginning to understand the N and C transport that serves as the historic baseline for much of the Mississippi River basin.

We used in-stream measurements of benthic, dissolved, and suspended particulate N and C, in conjunction with measurements of inorganic N cycling and whole stream metabolism, to calculate organic C and N spiraling dynamics in four prairie streams. Goals of this study were to determine the degree to which cycling of organic C and N are coupled and to determine if organic N is as tightly retained as inorganic N in this ecosystem. We measured the degree of N limitation as a potential indicator of coupling between C and N cycles.

Methods

Study sites

Our study was conducted on four streams draining native tallgrass prairie in the Flint Hills region of Kansas, USA. Watersheds that drain the four streams in this study are tallgrass prairie, which is dominated by native C₄ grasses. Gallery forests are found in the riparian areas of some streams, which provide the streams with some degree of riparian shading and small amounts of wood and leaf materials. Large woody debris is usually absent from these headwater streams. The stream bottom is characterized by cobble and bedrock. Primary producers within the stream channel are dominated by epilithic diatoms and filamentous green algae (*Cladophora* and *Spirogyra*). Headwater reaches are often intermittent and typically only flow 4 to 6 months a year.

Grassland management practices, such as watershed burn frequencies and grazing by large herbivores in the watershed, can influence N export in prairie streams (Dodds et al.1996). Burn frequencies and grazing patterns were variable across study streams (Table 4.1). Kings Creek-K2A, Shane Creek, and Natalie's Creek were all burned one year prior to the experiment, while Kings Creek-N4D was burned 2 years before the experiment. The watersheds of Kings Creek-K2A and Shane Creek are ungrazed, Natalie's Creek is grazed by cattle, and Kings Creek-N4D is grazed by native grazers (*Bos bison*).

Inorganic N cycling data

Releases of $^{15}\text{NO}_3^-$ were conducted on Natalie's Creek, Shane Creek, and two tributaries of Kings Creek (N4D and K2A), as part of the LINX II project (O'Brien 2006). A solution of $^{15}\text{NO}_3^-$, along with a NaBr conservative solute, was dripped into the stream at a steady rate for 24

hours. Samples for $^{15}\text{NO}_3^-$ analysis were then collected along the length of the reach. ^{15}N content of the stream water NO_3^- was determined using a modified version of the method presented by Sigman et al. (1997). The slope of the decline in ln-transformed $^{15}\text{NO}_3^-$ flux over the length of the reach was calculated by linear regression, and was used to calculate NO_3^- spiraling metrics.

The rate of nitrification occurring within the stream reach was calculated by mass balance of NO_3^- fluxes entering and leaving each experimental reach, as well as uptake and groundwater inputs. NO_3^- flux was measured at stations at the top and bottom of the stream reach. Groundwater NO_3^- flux was measured from groundwater discharge and average hyporheic NO_3^- concentration. Uptake flux was calculated based on uptake rate (U_t) measured by the $^{15}\text{NO}_3^-$ addition experiment. Nitrification rate was calculated as the dilution of $^{15}\text{NO}_3^-$ not accounted for by groundwater NO_3^- .

Uptake of NH_4^+ was not directly measured during the $^{15}\text{NO}_3^-$ experiments, however, uptake rates were estimated based on stream NH_4^+ concentration and an empirical log-log relationship between NH_4^+ uptake and concentration developed by O'Brien (2006):

$$\log(U_t) = 0.779 * \log(\text{NH}_4^+) - 1.0414 \quad (4.1)$$

$$R^2 = 0.7416$$

Briefly, a log-log relationship was developed between NH_4^+ concentration and uptake from the results of $^{15}\text{NH}_4$ and short-term NH_4^+ addition experiments conducted on prairie streams (Fig. 4.1).

Mineralization of NH_4^+ was estimated from the predicted uptake rate of net change in NH_4^+ concentration over the stream reach and known rate of nitrification. In all four streams, there was no net uptake or mineralization of NH_4^+ along the study reach, suggesting that uptake

and mineralization were approximately equal (O'Brien 2006). Nitrification along the stream reach was measured by dilution of added $^{15}\text{NO}_3^-$ not accounted for by groundwater (O'Brien 2006). Nitrification has been closely linked to mineralization in stream sediments (Kemp and Dodds 2002). The source of NH_4^+ for nitrification in these streams was assumed to be locally mineralized N, and to be independent of estimated NH_4^+ uptake from the water column.

Standing stocks

Standing-stock organic biomass for each stream was measured 24 hours after the $^{15}\text{NO}_3^-$ addition experiment, at 10 stations along the reach. At each station, a 1-m² metal frame was placed without bias in the stream channel, and all coarse benthic organic matter (CBOM), macrophytes, and filamentous algae found within the frame were collected. Surface (material suspended with gentle agitation of water above the benthic surface) and deep (material from the benthic surface to several cm deep suspended into the water with vigorous stirring) fine benthic organic matter (FBOM) were collected with a stovepipe corer device. Epilithic biofilm samples were collected by scraping a known surface area from rocks (3 rocks from each m²) selected without bias. Additional biomass samples were collected at each of the six water-sampling stations for ^{15}N analysis by the same techniques as biomass sampling.

Samples for particulate analysis were collected along the experimental stream reaches the day after the 24-hr $^{15}\text{NO}_3^-$ addition experiment. Additional suspended particulate organic matter (SPOM) samples were collected on subsequent days after the experiment, during various flow conditions. Stream water was collected in 4-L cubitainers, transported to the laboratory in coolers, and kept refrigerated (4 °C) until filtering. A sub-sample of 2 L of stream water was filtered within 12 h using a pre-weighed, ashed 47-mm Whatman GF/F glass fiber filter. The

filter was then oven dried for 24 hr at 60 °C and weighed to determine particulate dry mass. Sub-samples were also filtered on an ashed 25-mm Whatman GF/F glass fiber filter with a microanalysis filtration apparatus. The filter was then dried, stuffed in a tin capsule, and analyzed for %C and %N content on a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and a Conflo II interface.

Carbon cycling

Whole-stream metabolism, community respiration (CR), and gross primary production (GPP), were calculated using the two-station, open-system diurnal-oxygen-curve method (Marzolf et al. 1994, Bott 1996, Marzolf et al. 1998, Young and Huryn 1998). Yellow Springs Instruments logging data sondes were deployed within the reach to take continuous (5-min interval) dissolved O₂ measurements for 24 hours. The rate of reaeration along the stream reach was measured using acetylene as a gas tracer, in conjunction with NaBr as a conservative hydraulic tracer. Gas was directly bubbled into the stream, along with a concurrent NaBr addition (to correct for dilution of gas) and water/dissolved-gas samples were taken at several locations downstream. Dissolved-gas samples were collected by drawing 5 mL of stream water into a 10-mL plastic syringe and transferring the water into a 15-mL He-filled exetainer. Gas samples were analyzed for acetylene using a Shimadzu GC-14A gas chromatograph with a flame-ionization detector (Hayesep Q column, oven temperature = 50 °C, flow rate 25 mL/min).

Measures of whole-stream O₂ metabolism were converted to CO₂. The photosynthetic quotient, or number of moles of CO₂ fixed per mole of O₂ released, was estimated to be 1.2 (Wetzel and Likens 2000). A respiratory quotient of 0.85 was used to convert the number of moles O₂ consumed to the number of moles CO₂ evolved.

Nutrient deficiency

Nutrient-diffusing substrata (60-mL, nutrient-enriched, agar-filled cups containing 0.5 M KNO₃, 0.5 M NaH₂PO₄, both, or neither, covered with Whatman GF/F glass fiber frits) were placed in the stream for 2 weeks and analyzed for accumulation of algal chlorophyll-a by standard fluorometric techniques (APHA 1995). These data were used to calculate an index of N limitation for each stream. The index of N limitation was calculated from nutrient enrichment bioassays according to the relationship described by Dodds et al. (2004):

$$\text{N deficiency index} = \frac{(B_{N+P} - B_P)}{B_C} \quad (4.2)$$

where B_{N+P} = mean biomass in the N+P diffusion treatments, B_P = mean biomass in the P diffusion treatments, and B_C = mean biomass in control treatments. This index reflects N deficiency without the confounding effects of P deficiency, and assumes that even secondary limitation by N can alter uptake and retention of N.

Organic C and N spiraling

Indices of organic C and N spiraling were calculated with equations developed by Newbold et al. (1982) and applied by Thomas et al. (2005). Net longitudinal velocity of organic C (V_{OC} , m day⁻¹) was calculated as:

$$V_{OC} = \frac{TOC \times Q}{BOC \times w} \quad (4.3)$$

where TOC = total transported organic C concentration (g m⁻³), Q = discharge (m³ day⁻¹), BOC = benthic standing stock of organic C (g m⁻²), and w = mean wetted width (m). Biotic turnover rate (K_{OC} , day⁻¹), the proportion of organic C respired in a day, was calculated as:

$$K_{OC} = \frac{CR_{het}}{BOC + (TOC \times h)} \quad (4.4)$$

where CR_{het} = heterotrophic respiration rate ($\text{g-C m}^{-2} \text{ day}^{-1}$) and h = mean stream depth (m).

Organic C turnover length, or average distance traveled by C in the organic form, was calculated as:

$$S_C = \frac{V_{OC}}{K_{OC}} \quad (4.5)$$

Spiraling indices of organic N were calculated in a manner similar to calculations of C. Net longitudinal velocity of organic N (V_{ON} , m day^{-1}) was calculated according to equation 4.3 by substituting transported organic N (TON) and benthic organic N (BON) for TOC and BOC, respectively. Biotic turnover rate (K_{ON} , day^{-1}), the proportion of organic C respired in a day, was calculated as:

$$K_{ON} = \frac{M}{BON + (TON \times h)} \quad (4.6)$$

where the estimated rate of NH_4^+ mineralization (M), is substituted for heterotrophic respiration.

Organic N turnover length (S_{ON}) was calculated according to equation 4.5.

Results

Dissolved organic N comprised the largest proportion of total N in stream water of all four streams, about 80% of total N (Table 4.2). In contrast, DIN (NO_3^- and NH_4^+) was the smallest compartment of N in the water column and composed only 5% of total N. Particulate N made up the remaining 15% of total N. Dissolved organic C, likewise, was 60% to 90% of organic C in the water column. Average C:N ratio of dissolved organic matter was 7.5, and average C:N of SPOM was 15.

Standing stock of benthic C ranged from 35 g m^{-2} to 142 g m^{-2} , and benthic N ranged

from 1 g m^{-2} to 5 g m^{-2} (Table 4.3). The average C:N ratio of benthic standing stock was 37. Fine benthic organic matter made-up the largest fraction of organic C (77% across all three streams) and had a C:N ratio of 31. Coarse benthic organic matter accounted for 20% of standing stock of benthic C, and had an average C:N ratio of 45. Aquatic primary producers and bryophytes composed a small fraction of benthic C (2.5% and <1%, respectively) and had low C:N (20 and 25), but most likely had rapid turnover rates, based on mass, in relation to rates of primary production.

C and N cycling rates

Whole-stream metabolism measurements indicated that all four streams were net heterotrophic during the experiment (Fig. 4.3). Gross primary production ranged from 0.02 g C m^{-2} at Kings Creek-K2A to 0.89 g C m^{-2} at Kings Creek-N4D. Community respiration ranged from 0.9 g C m^{-2} at Natalie's Creek to 3.9 g C m^{-2} at Kings Creek-K2A. All four streams were net heterotrophic, with P:R ratios <1. Community respiration did not co-vary across streams with BOC standing stock, but did increase across streams with increases in TOC.

Estimated NH_4^+ uptake rates were higher than NO_3^- uptake rates in three of the streams, the exception being Kings Creek-N4D, in which the NH_4^+ concentration was below detection (Fig. 4.2). Nitrification ranged from $6 \text{ mg N m}^{-2} \text{ day}^{-1}$ to $86 \text{ mg N m}^{-2} \text{ day}^{-1}$. None of the streams demonstrated significant longitudinal net uptake in the manner described by O'Brien (2006); therefore, gross mineralization estimates were based on ammonium uptake and the nitrification rate. Estimates of gross mineralization ranged from $26 \text{ mg N m}^{-2} \text{ day}^{-1}$ to $86 \text{ mg N m}^{-2} \text{ day}^{-1}$, and bracketed rates measured by (Chapter 2). On the basis of whole-stream community respiration rates, and an average C:N ratio of 37 found in the standing stock benthic organic matter in the

streams, we predict mineralization rates ranging from $25 \text{ mg m}^{-2} \text{ day}^{-1}$ to $105 \text{ mg m}^{-2} \text{ day}^{-1}$. These rates are similar to gross mineralization rates predicted from NH_4^+ uptake and nitrification.

The ratio of C respiration to N mineralization (R:M ratio) ranged from 9.8 at Natalie's Creek to 123.8 at Kings Creek-K2A, and greatly exceeded the range of C:N of benthic organic matter. R:M ratios of the other two streams (17.4 at Kings Creek-N4D and 44.4 at Shane Creek) were closer to C:N ratios observed in benthic standing stock. Nutrient-limitation bioassays indicate that N was limiting or co-limiting with P in all four streams. The N deficiency index ranged from 0.10 in Natalie's Creek to 3.00 in Kings Creek-K2A. Moderate levels of N deficiency were found at Kings Creek-N4D (0.96) and Shane Creek (1.49).

Organic C and N transport metrics

Net longitudinal organic C velocity ranged across two orders of magnitude, from 0.2 m day^{-1} to 21.9 m day^{-1} (Table 4.4). Net longitudinal velocity of organic N tended to be longer than that of C, with velocities ranging between 1.4 m day^{-1} and 82 m day^{-1} . Organic C turnover was rapid in the streams, ranging from 1% to $11\% \text{ day}^{-1}$. Organic N turnover rates were similar to those of C, but only ranged from 1% to $3\% \text{ day}^{-1}$. Carbon spiraling distances for the streams ranged from 24 m to 400 m. Calculation of organic C spiraling lengths in these streams by the method of Young and Huynh (1997), which only takes dissolved and suspended C into account, did not differ greatly from those presented here. Spiral length for organic N was approximately 10 times that of C, ranging from 250 m to 2656 m in three of the streams. The exception was Natalie's Creek, in which C and N spiraling distances were nearly identical.

The average DIN uptake length was calculated as the average of the NH_4^+ and NO_3^- uptake lengths weighted by their relative concentrations in stream water, and ranged from 28 m

in Shane Creek to 174 m in Kings Creek-K2A. Total nitrogen spiral length was then calculated as the sum of organic and inorganic spiral lengths, and ranged from 260 m in Natalie's Creek to 2830 m in Kings Creek-K2A (Fig. 4.4).

Discussion

Spiraling distance was much shorter for organic C than for organic N, indicating a decoupling of transport of C and N in these streams. Organic matter turnover rates were not greatly different between C and N, which would suggest that spiraling lengths should be the same. The difference between C and N is that the benthic compartment has a much larger C:N ratio than water-column organic matter. Thus, the proportion of organic N currently in motion (water column) relative to benthic standing stock is larger than the proportion of C in the water column relative to benthic standing stock. Hence, there is a lot of C-rich organic matter on the stream bottom whereas lower C:N organic matter is rapidly exchanging with the water column.

Carbon and nitrogen spiraling lengths take into account organic matter moving in the water column, as well as benthic organic matter with which it is presumably exchanging. Dissolved organic C can be an important resource for stream microbial communities (Hall and Meyer 1998), but the degree to which the water column and benthic organic matter exchange is not entirely certain. Dissolved organic C and N may be predominantly represented by recalcitrant forms entering the stream from groundwater, and may not readily interact with the microbial community.

Stoichiometry of C respiration and N mineralization rates did not necessarily reflect C:N of respired substrate. This is because uptake and mineralization rates in this study do not represent total gross rates, but gross exchanges between the water column and benthic biomass.

Local transformations of N in the benthic environment, such as rapid uptake and assimilation or nitrification of mineralized N between closely associated microbes, may be missed in this analysis. The gross exchanges presented here, however, are ideal for calculating transport of N, which relies on exchanges between the water column and benthic biomass.

The trend of increasing R:M with increasing N limitation (N deficiency index) (Fig. 4.6) supports the hypothesis that products of gross mineralization may be immediately utilized and not allowed to interact with the water column. Under highly N-limiting conditions, mineralized N is more likely to be assimilated locally in the biofilm, before reaching the water column where it can be detected. As a result, the apparent mineralization rate will be much lower than would be expected from respiration rate and C:N of the substrata.

Organic C spiraling lengths measured in this study are much shorter than in similar studies, such as Thomas et al. (2005) and Young and Huryn (1997 1999). The other studies investigated larger streams with greater discharges, which have been demonstrated to influence C spiral length (Young and Huryn 1999). Minshall et al. (1992) reported C spiraling lengths of 3743 m for a similarly sized stream in the Salmon River headwaters, which is longer than the spiral lengths calculated in this study, due primarily to higher rates of respiration and organic C turnover in prairie headwater streams. To our knowledge, no other study has applied this technique to investigate organic N spiraling.

Organic N cycling

The method used in this study provides an integrated measure of N transport in prairie streams and an estimate of total N spiral length, however the length reported for organic N processing should be viewed as more of index of retention than a physical distance. Organic N

moving in the stream are predominantly in the form of dissolved compounds and fine suspended particulates each of which may be physically traveling different distance and mineralizing at different rates due to differences in the recalcitrance of the organic matter.

The distance traveled by organic matter in particulate form is dependent on variables such as discharge, channel characteristics, and particle size (Thomas et al. 2001; Georgian et al. 2003). Additionally, particulates may undergo repeated re-suspension events and travel multiple S_p before being mineralized (Cushing et al. 1993, Newbold et al. 2005).

Dissolved organic N is often a large portion of N export in many streams (Dodds et al. 1996, McHale et al. 2000), but few studies have looked at DON transport because it is composed of a diverse array of compounds of potentially differing biotic availability, and it is difficult to accurately measure. In addition, some forms of DON, such as amino acids, may cycle rapidly, but others may remain recalcitrant and interact slowly with the biota over long distances. Brookshire et al. (2005) found rapid uptake and short uptake lengths of labile DON compounds, such as glutamic acid and urea, in a headwater stream. Conversely, Kaushal and Lewis (2005) found that only 40% of the DON in a mountain stream was biologically available to sediment microbes. Although these types of studies give some idea of the rates of exchange of organic components into and out of the water column, they do not necessarily quantify the rate at which N is returned to inorganic form, which is necessary for completion of the spiral.

These measurements of organic matter spiraling provide a snapshot of transport of organic C and N in the prairie stream and represent the biota during the most biologically active times of the year. The method also averages over a wide range of organic matter sources with various levels of recalcitrance, and for faster rates of turnover in some compartments than other methods. The approach presented in this paper provides a general understanding of the

processing and turnover of C and N as a whole in this system. According to our results, organic C does not move very far at baseflow conditions, compared with movement of organic C in other streams. Export to downstream segments may be restricted to periods of elevated discharge and during scouring spates. Such spates can remove a large portion of the standing stock of organic matter in this system.

Dissolved inorganic N (NO_3^- and NH_4^+) repeatedly has been shown to be biologically limiting and tightly retained in prairie streams based on short spiraling distances (Dodds et al. 2000, O'Brien 2006). Likewise, the short spiraling distances of organic C observed in this study indicate a high degree of retention relative to other systems (Newbold et al. 1982). Conversely, spiraling distance of organic N is much longer than that of either DIN or organic C in this system and is not being retained as efficiently in these prairie streams.

Table 4.1- Locations, riparian shading, and grassland management characteristics in the watersheds of the four streams in this study. Riparian shading can have an impact on carbon cycling in streams. Watershed burn frequencies and the presence of large herbivores in the watershed can influence N export.

Stream Name	Latitude	% Stream	Burn	Large Herbivore
	Longitude	Shading	Frequency	Grazing
Kings Creek-K2A	39° 06.008' N	37%	2 year	no
	96° 34.454' W			
Shane Creek	39° 06.779' N	57%	1 year	no
	96° 33.220' W			
Natalie's Creek	39° 13.723' N	44%	1 Year	yes
	96° 39.530' W			
Kings Creek-N4D	39° 05.271' N	39%	4 year	yes
	96° 35.067' W			

Table 4.2- Concentration of dissolved and particulate C and N during ¹⁵N addition experiments. *SPOM-N concentration was estimated from the dry weight of SPOM measured at the site and the average %N from the other sites.

<i>Stream Name</i>	NO ₃ ⁻ -N (μg L ⁻¹)	NH ₄ ⁺ -N (μg L ⁻¹)	DON ¹ (μg L ⁻¹)	SPOM-N ² (μg L ⁻¹)	DOC ³ (mg L ⁻¹)	SPOM-C ⁴ (mg L ⁻¹)	
Kings Creek -K2A	0.9	6.7	85	14.2	0.77	0.20	¹ Dissolved organic nitrogen
Shane Creek	1.2	4.7	98	14.5	0.54	0.06	
Natalie's Creek	6	3.1	156	35.4*	1.56	0.53	Suspended particulate nitrogen
Kings Creek -N4D	8.6	0	196	24.3	0.73	0.43	

³ Dissolved organic carbon

⁴ Suspended particulate carbon

Table 4.3- Physical parameters and organic standing stock in four streams.

Stream Name	Q	Width	Stream Velocity	Depth	Benthic C	Benthic N
	L s ⁻¹	m	m min ⁻¹	m	g C m ⁻²	g N m ⁻²
Kings Creek-K2A	24	2.5	6.7	0.09	34.5	1.0
Shane Creek	1.6	2.4	1.3	0.08	142	4.8
Natalie's Creek	1.3	1.2	1.6	0.04	71.8	2.4
Kings Creek-N4D	13	2.4	5	0.07	77.1	2.4

Table 4.4- Summary of organic C and N turnover metrics in four streams.

Stream Name	V_{OC}^1 (m day ⁻¹)	K_{OC}^2 (day ⁻¹)	S_C^3 (m)	V_{ON}^4 (m day ⁻¹)	K_{ON}^5 (day ⁻¹)	S_{ON}^6 (m)
Kings Creek-K2A	21.9	0.11	195	82.0	0.03	2656
Shane Creek	0.2	0.01	20	1.4	0.01	251
Natalie's Creek	2.5	0.01	207	7.5	0.04	210
Kings Creek-N4D	6.4	0.02	264	41.5	0.03	1412

¹ Mean downstream velocity of carbon (V_{OC})

² Rate coefficient of carbon respiration (k_{OC})

³ Carbon spiral length (S_C)

⁴ Mean downstream velocity of nitrogen (V_{ON})

⁵ Rate coefficient of nitrogen mineralization (K_{ON})

⁶ Nitrogen spiral length (S_{ON})

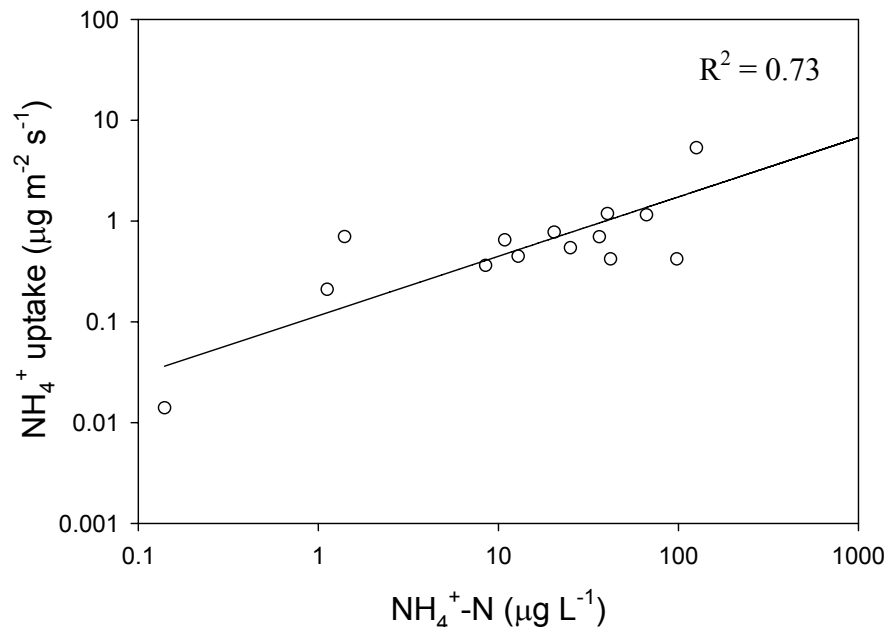


Figure 4.1- The relationship between $\text{NH}_4^+\text{-N}$ concentration and uptake in prairie streams (Chapter 3) used to estimate NH_4^+ uptake in this study.

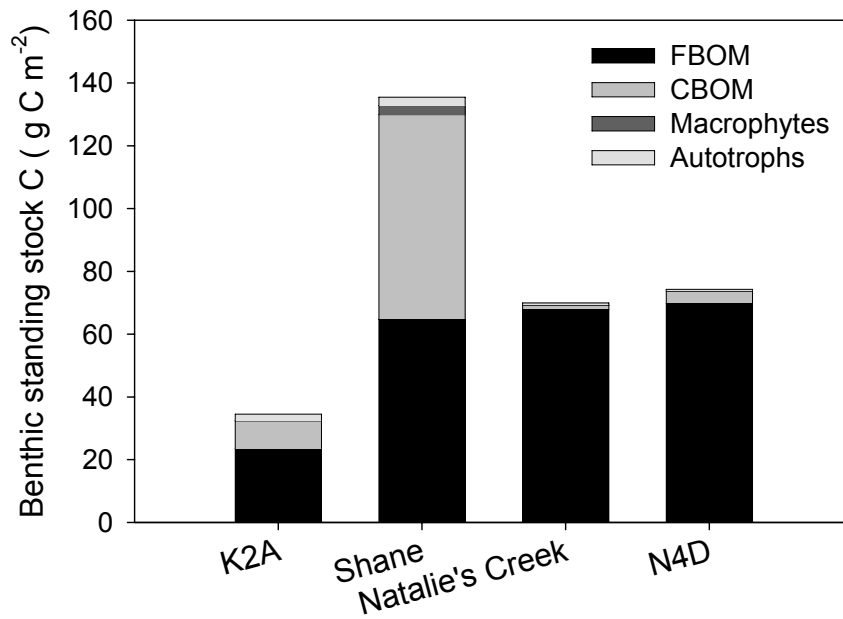


Figure 4.2- Benthic standing stock of C in four streams partitioned into four compartments: FBOM (Fine Benthic Organic Material), CBOM (Coarse Benthic Organic Material), aquatic autotrophs, and bryophytes.

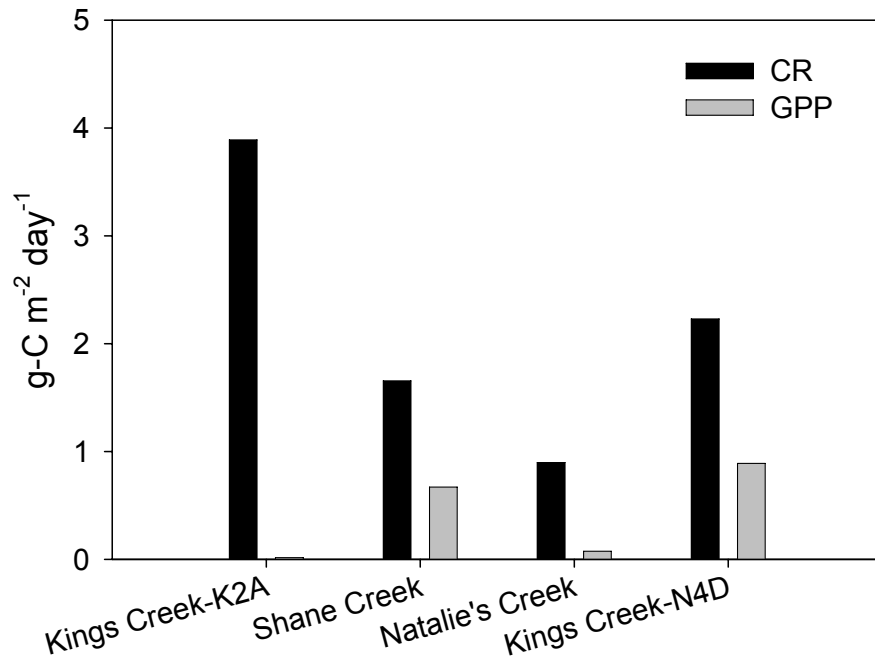


Figure 4.3- Whole stream metabolism (gross primary production (GPP) and community respiration (CR)) rates measured in the four streams.

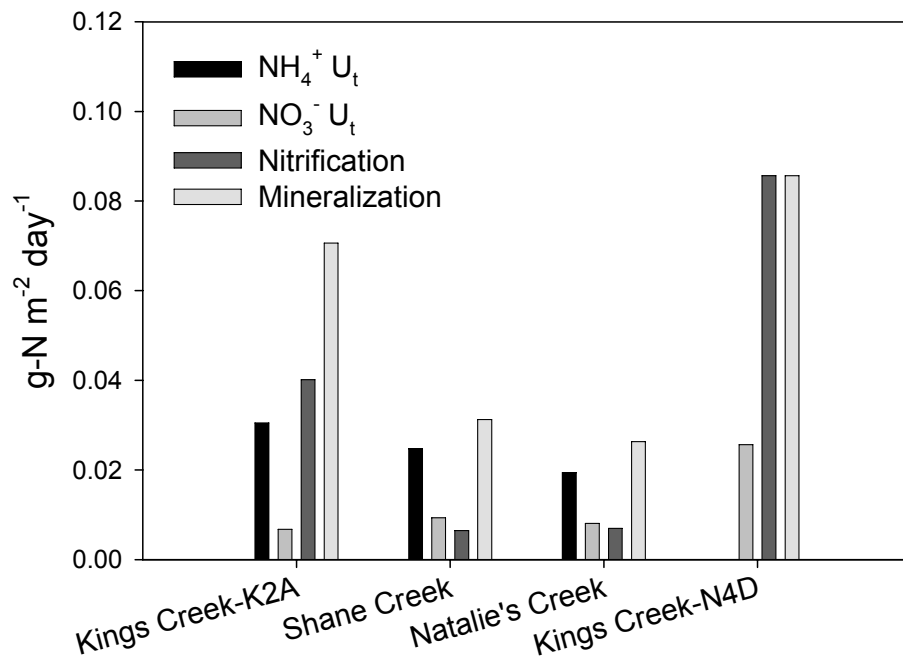


Figure 4.4- Whole stream N transformation rates in four streams including NH_4^+ uptake, NO_3^- uptake, nitrification and mineralization.

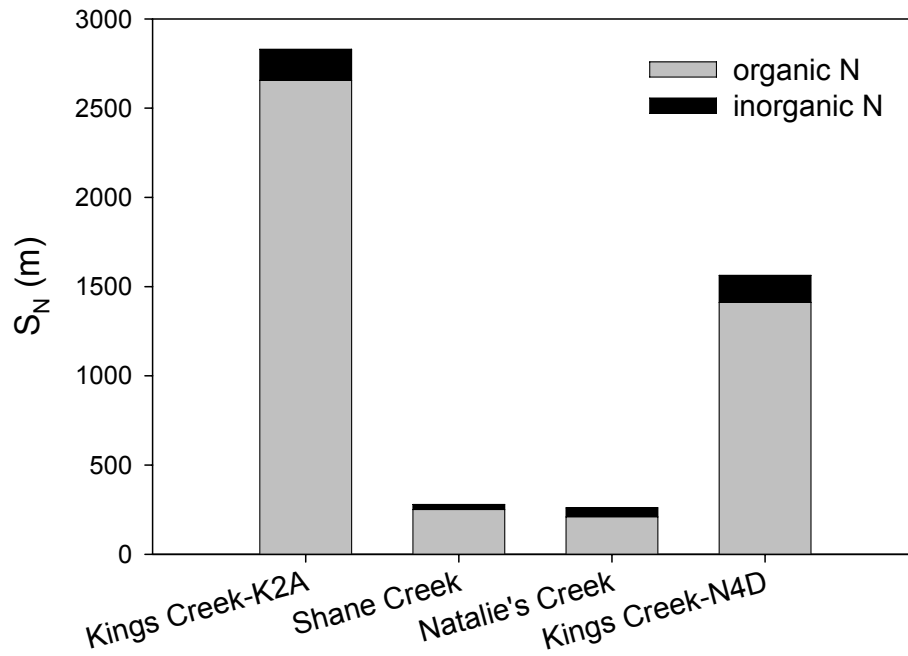


Figure 4.5- Components of N spiral length (S_N) for four streams, comprised of inorganic uptake length (S_w) and organic transport length (S_{ON}).

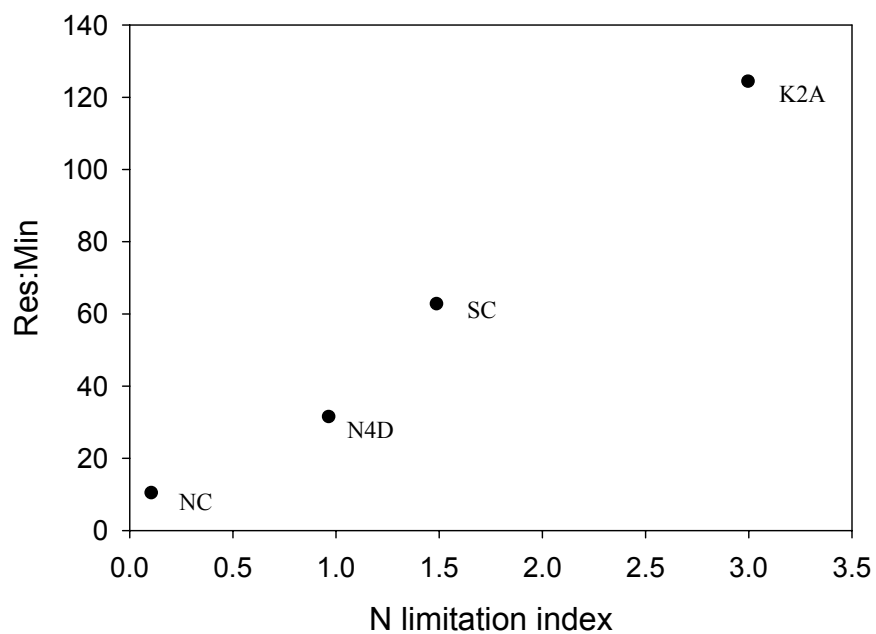


Figure 4.6- The ratio of respiration to mineralization (R:M) increased with N limitation index in the four streams ($R^2=0.98$, $p=0.007$): Kings Creek-K2A (K2A), Kings Creek-N4D (N4D), Natalie's Creek (NC), and Shane Creek (SC).

SUMMARY AND CONCLUSIONS

The underlying question in this research was, to what degree do increases in dissolved nitrogen concentrations impact the transport and retention of N by streams? Our approach to this question was, first, to examine the impacts of land-use and N inputs on stream N cycling and, second, to characterize the transport and retention of N in prairie streams.

In the first set of experiments, a stable isotope tracer was used to measure the nitrogen dynamics in 9 streams with a wide range of NO_3^- concentrations. We found that the major N-transforming processes that influence NO_3^- spiraling, namely uptake (which removes NO_3^- from the water column) and nitrification (which introduces NO_3^- to the water column), increased as a function of NO_3^- concentration across the set of streams. Despite expectations, uptake and nitrification did not show signs of Michaelis-Menten type saturation across streams, instead showing a form of reduced efficiency as the concentration of NO_3^- increased. With the exception of Wal-Mart Ditch, none of the streams demonstrated a net retention of NO_3^- . Denitrification did not saturate along the gradient of increasing NO_3^- , yet it only accounted for a small proportion of total NO_3^- uptake. The rates of denitrification in these streams were not sufficient to greatly reduce the transport of NO_3^- by these streams.

In another series of experiments, short-term inorganic-N addition experiments were conducted to test the response to acute N enrichments. These experiments demonstrated that uptake did saturate in response to short-term pulses of N. The saturation of uptake due to acute N enrichments contrasts with the pattern of uptake associated with chronic enrichments of NO_3^- , which was not found to saturate when assessed across a range of different streams.

The cycling and transport characteristics of inorganic-N in prairie streams were also measured using a series of chamber and whole-stream NH_4^+ and NO_3^- addition experiments. The

chamber experiments demonstrated that uptake and mineralization of NH_4^+ varies spatially within the stream channel, occurring predominantly in riffles as opposed to pool habitats. The inorganic-N addition experiments allowed us to measure the whole-stream uptake kinetics of NO_3^- and NH_4^+ . Ambient concentrations of NO_3^- and NH_4^+ were less than their respective half-saturation coefficients, suggesting severe limitation of N (Mulholland et al. 2002), which supports the findings of previous research in prairie streams (Kemp and Dodds 2002b, Tank and Dodds 2003).

Finally, we attempted to characterize all components of the N spiral by estimating total transport distance of organic-N and carbon within prairie streams. The results indicate that the organic-N transport was dominant in prairie streams, as compared to inorganic-N transport. Organic-N transport was larger both in terms of total concentration and transport distance. These results indicate that although carbon and inorganic-N were highly conserved in these headwater streams, organic-N was much more readily transported.

Taken as a whole, the results of this study lead to a better idea of N cycling and retention in prairie streams. We can now make better predictions about how N spiraling changes as a function of increasing N load. In prairie streams, inorganic-N is tightly conserved; NH_4^+ and NO_3^- are spiraled very quickly and traveled a relatively short distance before being taken up again. Organic N is the chief form of export, both in terms of mass and spiraling distance. As N load increases, there will be a switch between the importance of inorganic- and organic-N transport and retention. Bernot and Dodds (2005) demonstrated that increases in total nitrogen (TN) of stream water are mostly due to increases in NO_3^- , rather than increases in ammonium or organic-N. We have demonstrated that uptake lengths of nitrate increases with nitrate concentration, while retention decreases. Organic-N transport lengths may remain unchanged or

decrease as TN loads increase. This prediction is based on the observations that the benthic standing stock of N does not greatly increase with increasing TN load, suspended and dissolved organic-N also does not generally keep pace with increase with increasing TN load, and mineralization is likely to increase as a function of TN load, as evidenced by the increase in nitrification rate. As TN load increases, inorganic-N transport will therefore become more important relative to organic-N transport.

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