

Saturation of NO_3^- uptake in prairie streams as a function of acute and chronic N exposure

Jonathan M. O'Brien¹ AND Walter K. Dodds²

Kansas State University, Division of Biology, Ackert Hall, Manhattan, Kansas 66506 USA

Abstract. We conducted a series of stepwise NO_3^- additions to investigate the response of NO_3^- uptake to short-term (acute) changes in N concentration in 3 prairie streams. Observed NO_3^- uptake rates increased with short-term elevations in NO_3^- concentration and were consistent with linear and Michaelis–Menten kinetics models. We compiled these data with uptake rates from additional published studies to calculate robust estimates of N uptake kinetics for prairie streams. Half-saturation coefficients based on compiled data were 6.7 $\mu\text{g/L}$ for NH_4^+ and 67 $\mu\text{g/L}$ for NO_3^- -N. This difference in half-saturation coefficients suggests that NH_4^+ is more efficiently assimilated than NO_3^- , indicating a preference for NH_4^+ as an N source. Similarly, ambient concentrations of NH_4^+ and NO_3^- were less than their respective half-saturation coefficients, and aerial uptake rates were generally <5% of the maximum, suggesting severe limitation of N uptake at ambient conditions. The observed pattern of uptake kinetics suggests that physiological constraints limit biotic N uptake in these low-N streams and contrasts with the pattern of uptake observed in streams with chronically elevated ambient NO_3^- concentrations.

Key words: nitrate uptake, biogeochemistry, Konza Prairie, Kings Creek.

Human land use and elevated atmospheric deposition of N can lead to increased concentrations of anthropogenic N in streams, particularly in the form of NO_3^- (Rabalais 2002). Headwater streams and small rivers might play a critical role in controlling transport of N from the landscape to the marine environment (Alexander et al. 2000). N transformations in streams, such as assimilative uptake, nitrification, and denitrification, are greatly influenced by N concentrations (Kemp and Dodds 2002a, Inwood et al. 2005, Mulholland et al. 2008). Theoretically, rates of N transformation should be stimulated by increased N concentrations only when N is the limiting nutrient for the process. Once N is no longer the limiting factor, rates of N transformation should become saturated and will no longer increase as a function of N concentration (Davis and Minshall 1999, Dodds et al. 2002).

The response of stream processes to increased N levels probably will depend on the duration of enrichment. Acute increases in N concentrations above normal conditions (median values) that persist

over hours to days and are caused by natural variation in dissolved N concentrations or pollution events might result in an immediate response by organisms to increased N availability. However, the ability of stream biofilms to take up N can be limited by physiological constraints (i.e., saturation of uptake enzymes). Chronic inputs of available N over longer periods (months to years) from point- or nonpoint source pollution might allow biotic communities to adjust their response to elevated concentrations by decreasing their N use efficiency. For the purposes of our study, we define chronic N loading as long-term and sustained increases in ambient stream-water dissolved inorganic N (DIN) concentrations above median and upper-quartile concentrations of regional or historical reference conditions (e.g., Smith et al. 2003, Dodds and Oakes 2004).

Bernot and Dodds (2005) predicted that assimilative uptake, denitrification, nitrification, and primary production would show Michaelis–Menten-based saturation in response to chronic N loading as the supply of available NO_3^- exceeded biological demand. Bernot et al. (2006) found that NO_3^- uptake was saturated in high-N agricultural streams, a result that supported this prediction. However, O'Brien et al. (2007) and Mulholland et al. (2008) found that NO_3^- uptake, nitrification, and denitrification did not

¹ Present address: University of Canterbury, School of Biological Sciences, Private Bag 4800, Christchurch, New Zealand. E-mail: jon.obrien@canterbury.ac.nz

² E-mail address: wkdodds@ksu.edu

saturate in streams across a wide gradient of chronic N loading. Instead, they found a pattern of efficiency loss as NO_3^- concentrations increased.

The functional response of biological activity to acute elevations in N concentration is often described by a Michaelis–Menten relationship in which processes increase hyperbolically with concentration until saturation is reached. Michaelis–Menten kinetic responses have been demonstrated for benthic stream organisms (Lohman and Priscu 1992, Borchardt et al. 1994, Kemp and Dodds 2002b) and often are attributed to enzymatic constraints that limit the rate of uptake (Borchardt 1996). Michaelis–Menten-based models also provide reasonably good fits to short-term acute NH_4^+ or NO_3^- additions in some streams (Dodds et al. 2002, O'Brien and Dodds 2008), but this response is not consistently observed across streams (Earl et al. 2006).

Parameters from the Michaelis–Menten models (particularly the half-saturation coefficient [K_s]) can provide a relative indication of ecosystem N limitation. If ambient concentrations are well below K_s , then we can infer that the stream is N-limited. Conversely, if N concentrations are well above K_s , then ecosystem processing rates are nearing saturation. Thus, the response of stream biota to short-term changes in streamwater N availability might yield information about overall N cycling in the stream ecosystem.

The objective of our study was to investigate the response of N cycling in undisturbed prairie streams to changes in N concentration. We used a series of stepwise, acute short-term NO_3^- additions to investigate the response of NO_3^- uptake to short-term changes in N concentration in 3 prairie streams. We also synthesized data from this and several recent studies and compared the relationship between N concentration and N uptake for NO_3^- and NH_4^+ in prairie streams to examine responses of stream biota to N enrichment across a range of N concentrations.

Methods

Study area

Our study was conducted on tributaries of Kings Creek on the Konza Prairie Biological Station (KPBS) in the Flint Hills region of northeastern Kansas, USA. Uptake measurements were done on 2 tributaries of Kings Creek, 1 on the north branch (KC-North, Watershed K2A, lat 39°06.008'N, long 96°34.454'W) and one on the south branch (KC-South, Watershed N4D, lat 39°05.271'N, long 96°35.067'W). NO_3^- uptake measurements also were done on a stream draining the adjacent Shane Creek watershed (lat 39°06.779'N, long 96°33.220'W). All 3 watersheds are entirely native

tallgrass prairie. Streams in the region typically have low concentrations of dissolved and total N and P (Dodds 2003). Headwater reaches of Kings Creek have median NO_3^- concentrations of 1.3 $\mu\text{g/L}$ (0.7–3.25 $\mu\text{g/L}$ quartile range) and median NH_4^+ concentrations of 11.4 $\mu\text{g/L}$ (4.1–27 $\mu\text{g/L}$ quartile range) (Konza Long-Term Ecological Research [LTER] data, Watershed N4D 1999–2001), but downstream reaches tend to increase in NO_3^- by a factor of 10 (Kemp and Dodds 2001). Stream reaches in our study were generally open (~35–55% shading from tree cover), and predominant biota at the time of the study were epilithic biofilms and filamentous algae (*Cladophora*) in Kings Creek (KC-North and KC-South), whereas large leaf packs and *Nostoc* mats were present in addition to epilithic biofilms and filamentous algae in Shane Creek.

Field and laboratory methods

We did short-term NO_3^- additions in 3 prairie reaches of Kings Creek between 2003 and 2005. On KC-South, we did 2 short-term NO_3^- additions in May 2003 (peak concentrations = 83 and 442 $\mu\text{g NO}_3^-$ -N/L, ambient = 8.6 $\mu\text{g/L}$) and 3 short-term NO_3^- additions in July 2004 (peak concentrations = 21, 22, and 33 $\mu\text{g NO}_3^-$ -N/L, ambient = 2.6 $\mu\text{g/L}$) under similar conditions. On KC-North, we did 1 short-term NO_3^- addition in July 2004 (peak concentration = 34 $\mu\text{g NO}_3^-$ -N/L, ambient = 0.9 $\mu\text{g/L}$) in July 2004 and 4 short-term stepwise NO_3^- additions 1 y later (peak concentrations = 17, 27, 68, and 169 $\mu\text{g NO}_3^-$ -N/L, ambient = 4.2 $\mu\text{g/L}$). On Shane Creek, we did 3 short-term stepwise NO_3^- additions in May 2005 (peak concentrations = 77, 220, and 411 $\mu\text{g NO}_3^-$ -N/L, ambient = 1.2 $\mu\text{g/L}$).

In each of the short-term additions, we pumped a solution of KNO_3 and a NaBr tracer 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 100-m reach, we collected 125-mL stream water samples at 10-m intervals from the point of addition to the end of the reach. We filtered samples in the field through Whatman GF/F glass fiber filters, cooled the samples on ice, and refrigerated them until analysis. We determined streamwater NO_3^- concentration colorimetrically within 24 h with the Cd-reduction method on a Technicon Auto-Analyzer (SEAL Analytical, Fareham, UK) (APHA 1995). We measured Br^- concentrations with an Orion Br^- specific electrode (Thermo Fisher Scientific, Waltham, Massachusetts), and all measurements were made at a consistent temperature and stirring rate. We analyzed data from short-term NO_3^- additions with

the standard method outlined by Webster and Ehrman (1996). We calculated uptake rate (U_t) and velocity (V_f) based on coefficient of uptake (k_x) as outlined by the Stream Solute Workshop (1990). U_t reported in our study represent uptake rates at the elevated NO₃⁻ concentrations, not uptake rates at ambient NO₃⁻ concentrations.

We combined the results from the short-term NO₃⁻ additions with data from ¹⁵NO₃⁻ isotope tracer additions done by O'Brien et al. (2007) in the same streams. These data were used to establish U_t at ambient NO₃⁻ concentrations and enabled us to calculate Michaelis–Menten uptake kinetics for each stream (see below). Tracer additions were done at KC-South in May 2003, KC-North in July 2004, and Shane Creek in May 2005. During tracer additions, a solution of ¹⁵NO₃⁻ and NaBr was added to the stream at a steady rate for 24 h. Duplicate samples for ¹⁵NO₃⁻ analysis were collected at 6 stations along the length of the reach at 0100 h and 1200 h (12 and 23 h after start of tracer addition), filtered in the field with Whatman GF/F glass fiber filters, and transported back to the laboratory where they were frozen or analyzed immediately. Stream water ¹⁵NO₃⁻ content was determined by using a modified version of the method presented by Sigman et al. (1997). Uptake was measured based on ¹⁵NO₃⁻ tracer loss from stream water over the length of the stream reach. A more detailed description of the methods used in these measurements is provided by O'Brien et al. (2007).

We fitted relationships between U_t and N concentration (C) in the individual streams with the Michaelis–Menten equation, $U_t = (V_{max}C)/(K_s + C)$, where V_{max} is the maximum rate of uptake and K_s is the half-saturation coefficient. We estimated Michaelis–Menten model parameters (V_{max} and K_s) with least-squares regression with the Levenberg–Marquardt algorithm. We used linear regressions to characterize the relationship between U_t and C at each stream.

We compiled data from isotopic tracer and short-term nutrient additions (for both NH₄⁺ and NO₃⁻) from previous studies (Dodds et al. 2000, 2002, O'Brien et al. 2007, O'Brien and Dodds 2008) to determine overall trends in stream N uptake with respect to concentration within the Kings Creek basin and to determine the extent of N uptake saturation.

We used a modified version of the Michaelis–Menten equation, $V_f = (V_{max})/(K_s + C)$, to determine the relationship between V_f and concentration across all prairie NO₃⁻ and NH₄⁺ data sets. We derived the equation by dividing both sides of the Michaelis–Menten equation by C ($V_f = U_t/C$). The Michaelis–Menten model produces a constant V_f while N

concentrations are $<K_s$, curving into a rapid decline in V_f as uptake saturates $>K_s$. We used V_f (instead of U_t) in our analysis because it provides a better metric of efficiency and allowed us to test the hypothesis of saturation across the data set. We estimated Michaelis–Menten model parameters with a least-squares regression with the Levenberg–Marquardt estimation algorithm.

An alternate representation of the relationship between V_f and C is described by efficiency-loss model described by O'Brien et al. (2007), which involves a continuous decrease in V_f with increasing C (following a power relationship with an exponent between 0 and -1). The efficiency-loss model appears as a linear decline on a plot of $\log(x)$ -transformed V_f and $\log(x)$ -transformed C . We did a linear regression on $\log(x)$ -transformed V_f and $\log(x)$ -transformed C to test for efficiency-loss across both the NO₃⁻ and NH₄⁺ data sets.

We tested the differences in responses to acute (short-term addition) and chronic (high ambient concentrations) high N concentrations by comparing the compiled prairie stream NO₃⁻ uptake data with the results of O'Brien et al. (2007), who conducted a series of ¹⁵NO₃⁻ tracer additions in streams with a broad range of ambient NO₃⁻ concentrations (0.9–20,000 µg/L) in the same geographical area as the current study. We used an homogeneity of slopes analysis of covariance (ANCOVA) model to test for differences between the 2 data sets in $\log(x)$ -transformed U_t and $\log(x)$ -transformed C . This ANCOVA model allowed us to compare slopes and intercepts of regressions between U_t and C for acute and chronic N data sets. All statistical analyses were done with the Statistica 6 (Statsoft, Tulsa, Oklahoma) statistical package.

Results

On KC-North, short-term NO₃⁻ addition yielded U_t ranging from 1.0 to 9.9 µg m⁻² s⁻¹ (Fig. 1A). Based on the 5 NO₃⁻ additions and 2 measures of ¹⁵NO₃⁻ (O'Brien et al. 2007) at KC-North, a significant fit of the Michaelis–Menten model existed between U_t and C ($F_{1,6} = 45.3$, $R^2 = 0.94$, $p < 0.001$; Table 1). V_{max} was 21.5 µg m⁻² s⁻¹ (± 12.6 µg m⁻² s⁻¹ [SE]) and K_s was 184 µg/L (± 173 µg/L). A significant linear regression was fit to these data, and the fit nearly matched the fit of the Michaelis–Menten model ($F_{1,6} = 102.6$, $R^2 = 0.95$, $p < 0.001$).

On KC-South, short-term NO₃⁻ addition yielded U_t ranging from 0.7 to 4.3 µg m⁻² s⁻¹ (Fig. 1B). Based on the 5 NO₃⁻ additions and 2 measures of ¹⁵NO₃⁻ (O'Brien et al. 2007), a significant fit of the Michaelis–

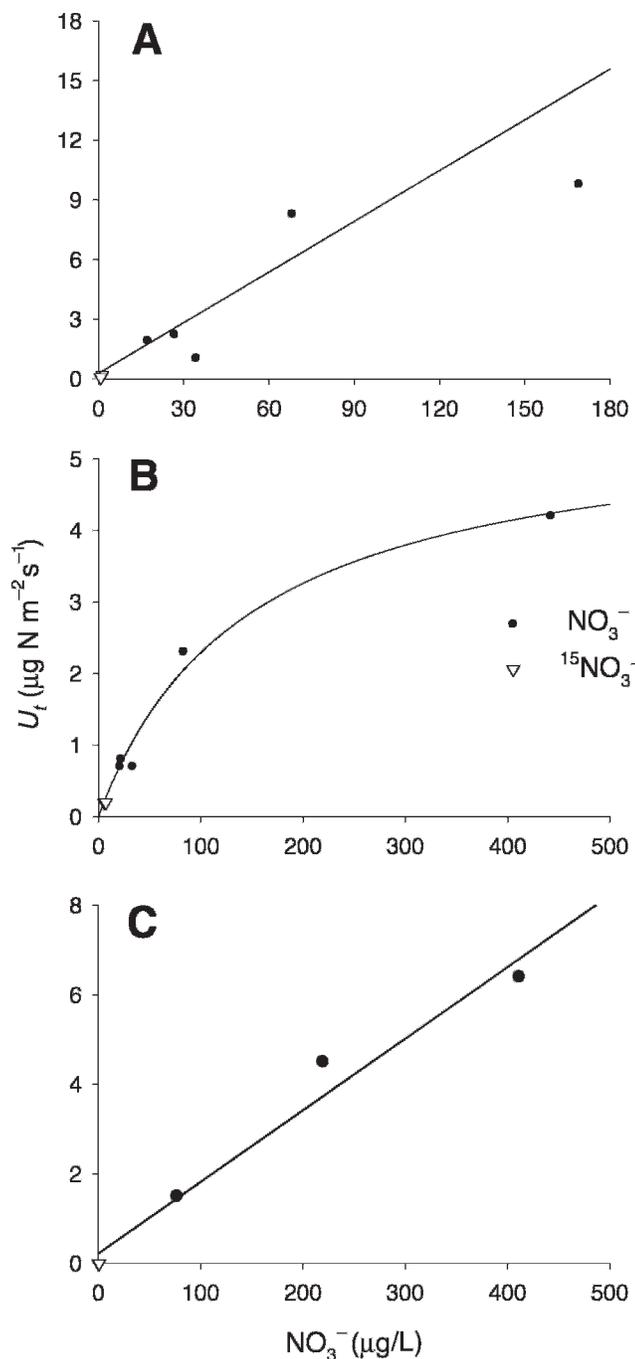


FIG. 1. Relationship between NO_3^- concentration and uptake rate (U_t) for $^{15}\text{NO}_3^-$ and short-term NO_3^- additions conducted on Konza Prairie Biological Station watersheds Kings Creek-North Branch (A), Kings Creek-South Branch (B), and Shane Creek (C). Linear regressions are presented for relationships in panels A and C, whereas Michaelis-Menten regression is presented in panel B. See Table 1 for regression statistics.

Menten model existed between U_t and C ($F_{1,6} = 322.9$, $R^2 = 0.98$, $p < 0.001$). V_{max} was $5.6 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\pm 0.4 \mu\text{g m}^{-2} \text{s}^{-1}$) and K_s was $146 \mu\text{g/L}$ ($\pm 25 \mu\text{g/L}$). A significant linear regression was fit to these data ($F_{1,6} = 44.1$, $R^2 = 0.90$, $p = 0.001$), but the R^2 was not as high as that of the Michaelis-Menten model.

On Shane Creek, short-term NO_3^- additions yielded U_t ranging from 1.5 to $6.4 \mu\text{g m}^{-2} \text{s}^{-1}$ (Fig. 1C). Based on NO_3^- additions on Shane Creek and $^{15}\text{NO}_3^-$ measurements from the stream (O'Brien et al. 2007), a significant fit of the Michaelis-Menten model existed between U_t and C ($F_{1,4} = 617.9$, $R^2 = 0.99$, $p < 0.001$). V_{max} was $16.5 \mu\text{g m}^{-2} \text{s}^{-1}$ ($\pm 3.3 \mu\text{g m}^{-2} \text{s}^{-1}$) and K_s was $634 \mu\text{g/L}$ ($\pm 192 \mu\text{g/L}$). A significant linear regression was fit to these data ($F_{1,4} = 125.0$, $R^2 = 0.97$, $p = 0.002$), and the fit was roughly equivalent to that of the Michaelis-Menten model.

N uptake data compiled from across Konza streams demonstrated an overall trend of saturation of N uptake in prairie streams (Fig. 2A, B). Significant Michaelis-Menten relationships existed between V_f and C for NH_4^+ ($R^2 = 0.37$, $F_{1,18} = 11.6$, $p < 0.001$) and for NO_3^- ($R^2 = 0.34$, $F_{1,25} = 18.8$, $p < 0.001$). Uptake kinetics calculated by these models showed a greater V_{max} for NO_3^- ($4.9 \pm 4.7 \mu\text{g m}^{-2} \text{s}^{-1}$) than for NH_4^+ ($1.7 \pm 1.3 \mu\text{g m}^{-2} \text{s}^{-1}$). K_s was greater for NO_3^- ($67 \pm 74 \mu\text{g/L NO}_3^- \text{-N}$) than for NH_4^+ ($6.7 \pm 5.3 \mu\text{g NH}_4^+ \text{-N /L}$), but the variances around these estimates are considerable and the differences could not be confirmed statistically.

The effects of chronic vs acute NO_3^- enrichment were tested by comparing the relationship between $\log(x)$ -transformed U_t and NO_3^- concentrations found by O'Brien et al. (2007) with data from the compiled short-term NO_3^- additions (Fig. 3). Slopes of relationships between U_t and C did not differ for chronic and acute N enrichment ($F_{1,25} = 0.88$, $p = 0.35$). Intercepts for the acute and chronic N enrichment differed significantly, indicating that short-term NO_3^- addition caused U_t to increase ($F_{1,25} = 5.92$, $p = 0.022$).

Discussion

We did not see a consistent pattern of saturation of NO_3^- uptake among the 3 prairie stream reaches investigated in our study. Michaelis-Menten and linear models fit equally well for short-term NO_3^- uptake in KC-North and Shane Creek. In both cases, estimated K_s values from the Michaelis-Menten model were higher than the highest peak NO_3^- addition concentration, suggesting a primarily linear relationship between uptake and concentration within

TABLE 1. Model parameters for the relationships between inorganic N concentrations and N uptake from Kings Creek-North branch (KC-North), Kings Creek-South branch (KC-South), and Shane Creek. Michaelis–Menten (MM) model parameters include the maximum rate of uptake (V_{max}) and half-saturation coefficient (K_s). Slope (m) and intercept (b) estimates are presented for linear ($U_t = mC + b$) and power (efficiency-loss, $U_t = be^{(mC)}$) models.

Stream	Type	V_{max}		K_s		b	m	R^2	F	p
		$\mu\text{g m}^{-2} \text{s}^{-1}$	$\mu\text{g/L}$	$\mu\text{g m}^{-2} \text{s}^{-1}$	$\mu\text{g/L}$					
KC-North NO ₃ ⁻	MM	21.5	184	–	–	–	–	0.94	45.3	<0.001
	Linear	–	–	0.058	0.27	–	–	0.95	102.6	<0.001
KC-South NO ₃ ⁻	MM	5.6	146	–	–	–	–	0.98	322.9	<0.001
	Linear	–	–	0.009	0.52	–	–	0.90	44.1	0.001
Shane Creek NO ₃ ⁻	MM	16.5	634	–	–	–	–	0.99	617.9	<0.001
	Linear	–	–	0.016	0.22	–	–	0.97	125.0	0.002
Overall NH ₄ ⁺	MM	1.7	6.7	–	–	–	–	0.37	11.6	<0.001
	Power	–	–	0.099	-0.23	–	–	0.20	4.4	0.05
Overall NO ₃ ⁻	MM	4.9	67	–	–	–	–	0.34	18.8	<0.001
	Power	–	–	0.087	-0.28	–	–	0.40	15.5	<0.001

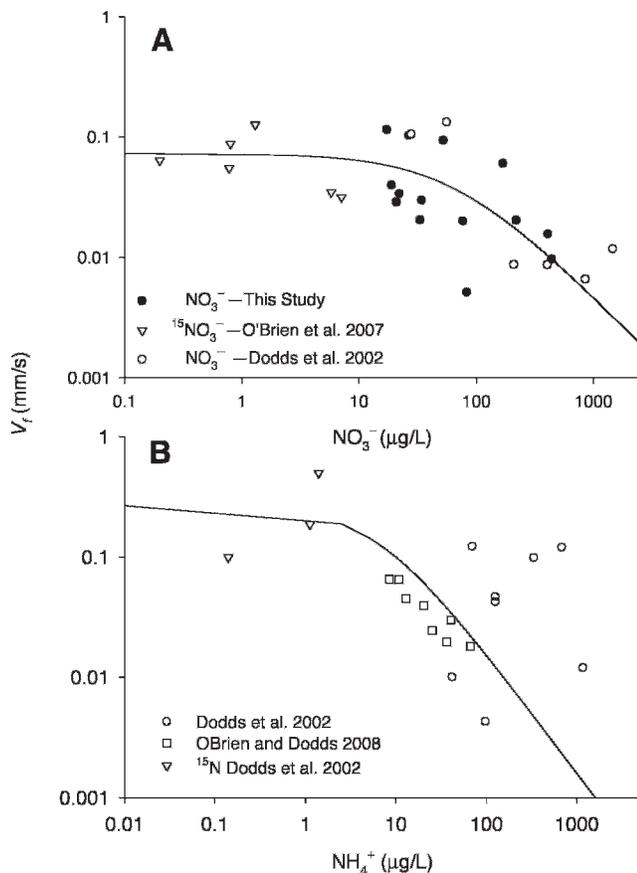


FIG. 2. Michaelis–Menten model fit to the N concentration and the uptake mass transfer coefficient (V_t) data for NO₃⁻ (A) and NH₄⁺ (B) from the overall Kings Creek prairie stream data set.

this range of concentrations. Greater NO₃⁻ concentrations (>1000 $\mu\text{g/L}$) might be necessary to saturate NO₃⁻ uptake completely in these streams. The Michaelis–Menten model fit better than the linear model at KC-South, suggesting that saturation of NO₃⁻ uptake occurred in this stream reach. The rate of increase in NO₃⁻ uptake was faster (greater slope in linear model) in KC-North than in the other 2 streams, possibly because of greater biofilm development later in the season when these measurements were made and because of the development of *Cladophora* mats in the stream reach. Similarly, Shane Creek had very high standing stocks of benthic organic matter that could have led to the linear response to the stepwise NO₃⁻ additions. N demand in Shane Creek was very strong, so U_t did not change

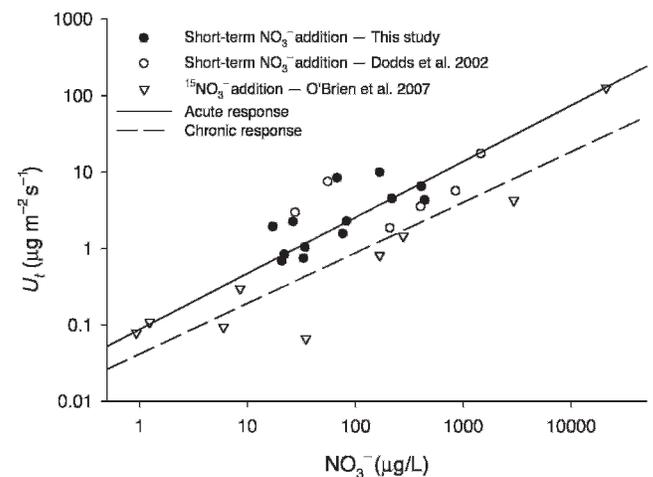


FIG. 3. A comparison of relationships between NO₃⁻ concentration and uptake rate (U_t) for streams in response to acute and chronic enrichments of NO₃⁻.

greatly (~ 20 m for all NO_3^- levels) despite an increase in concentration of 2 orders of magnitude.

The decline in V_f with increasing N concentration across the entire data set of N uptake measurements from Kings Creek indicates a loss of uptake efficiency. The observed loss of NH_4^+ uptake efficiency was more consistent with the Michaelis–Menten model than the efficiency-loss model. The relative strength of the Michaelis–Menten model suggests that, overall, NH_4^+ uptake exhibits classic saturation in these streams. The overall NO_3^- data are consistent with a Michaelis–Menten model. However, the efficiency-loss model worked equally well. Uptake efficiency of NO_3^- declines with increasing concentration, but the pattern is not strong enough to differentiate between Michaelis–Menten and efficiency loss. Below, we will consider the estimates of K_s from the NO_3^- Michaelis–Menten model for the purposes of comparison with NH_4^+ , but note that it is not the only model that can explain the data.

N-limited uptake at ambient conditions

K_s for NO_3^- and NH_4^+ suggest that organisms in Kings Creek were influenced by N limitation because ambient concentrations of NO_3^- and NH_4^+ were less than their respective values of K_s (Tilman 1982). Degree of N limitation also can be inferred from ambient U_t relative to total potential uptake (V_{max}). In N-saturated systems, ambient U_t should be $\approx V_{max}$. Conversely, if N is limiting, 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. O'Brien and Dodds (2008) found that ambient U_t for NH_4^+ averaged only 6% of V_{max} for Kings Creek. Thus, the capacity for N uptake is much greater than the actual rate of assimilation, indicating a strong degree of limitation at ambient concentrations. This N limitation of uptake is consistent with bioassay data that previously indicated that primary producers are colimited by N and P at the research sites (Tank and Dodds 2003, Johnson et al. 2009). Median and interquartile NH_4^+ concentrations at Kings Creek (at N4D, median $\text{NH}_4^+ = 11 \mu\text{g/L}$ and 75% quartile = $27 \mu\text{g/L}$) suggest that NH_4^+ concentrations often exceed the mean K_s and that uptake of NH_4^+ might occasionally become saturated.

In Kings Creek, K_s for NH_4^+ occurred at a lower concentration than for NO_3^- , a result suggesting that NH_4^+ is assimilated more efficiently than 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 reflects lower energetic requirements for using NH_4^+ than 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 on respective V_{max} , K_s , and normally low concentrations of NO_3^- and NH_4^+ . Some evidence indicates that NO_3^- might be favored by photoautotrophs in streams (e.g., Fellows et al. 2006), and given the open nature of prairie streams, one might expect NO_3^- uptake to be the dominant N assimilation pathway. However, although Kings Creek is a relatively high-light stream, it is still net heterotrophic and N uptake is evidently influenced by the heterotrophic compartment. The lower K_s (showing greater preference) and the higher average U_t under ambient conditions lead us to conclude that NH_4^+ is the primary source of DIN to the microflora in the headwater reaches of Kings Creek.

Until now, only a few studies have reported estimates of K_s at the whole-stream level. However, the observed K_s values for NH_4^+ and NO_3^- uptake in our study fall within the range of values that have been reported in the literature. Dodds et al. (2002) reported a K_s of $64 \mu\text{g NH}_4^+\text{-N/L}$ for a prairie reach of Kings Creek, whereas Kemp and Dodds (2002b) projected whole-stream K_s of $12.3 \mu\text{g/L}$ for NO_3^- and $6.7 \mu\text{g/L}$ and NH_4^+ based on uptake kinetics of different substratum types within the stream. Payn et al. (2005) reported K_s in forested streams of $6 \mu\text{g NH}_4^+\text{-N/L}$ for Ball Creek, North Carolina (USA), and $14 \mu\text{g NH}_4^+\text{-N/L}$ for Walker Branch, Tennessee (USA). In both streams, reported K_s was higher than ambient NH_4^+ concentrations (3.0 and $2.7 \mu\text{g/L}$, respectively), results suggesting these streams are N limited. Earl et al. (2006) reported K_s ranging from $90\text{--}447 \mu\text{g NO}_3^-\text{-N/L}$ for NO_3^- in forested streams, again higher than ambient NO_3^- concentrations in the streams studied. Simon et al. (2005) reported K_s of only 1.2 to $19 \mu\text{g NO}_3^-\text{-N/L}$ in a grassland stream in New Zealand based on repeated NO_3^- additions over the course of a year. In this case, mean stream concentrations were higher than K_s for NO_3^- but not NH_4^+ , a result suggesting that the streams varied in their degree of N limitation and uptake characteristics throughout the year.

Responses to acute and chronic N increases

Acute N enrichment represents short-term increases in DIN concentration above long-term median DIN concentrations that take place over a period of minutes to days. Such pulses might be caused by short-term disturbances occurring upstream or within

the watershed (e.g., short-term disturbance, spill or pollution event, bison or cattle activity in stream). In N-limited streams, these pulses might be a boon for microbes and algae who suddenly find themselves awash in a limiting nutrient. Microorganisms should be adapted to take advantage of pulses by increasing their U_t . If the concentration of the pulse is large enough, the elevated U_t might be constrained by the organisms' physiology, i.e., the saturation of uptake enzymes and storage capacity.

Chronic N loadings represent long-term increases in DIN concentration above the historic or regional median of N concentrations and persist over a period of months to decades. Such increases can be caused by changes in the landscape that result in point- or nonpoint pollution of N, such as via agricultural or urban development. Estimates of historic concentrations can be derived from reference watersheds or through regional analysis of stream concentrations (Smith et al. 2003, Dodds and Oakes 2004). On Konza Prairie, headwater streams typically have NO₃⁻-N and NH₄⁺-N concentrations of <10 µg/L. Concentrations of NO₃⁻-N tend to increase down stream, and the median concentration in lower Kings Creek is 27.2 mg/L (15–52 µg/L interquartile range) before it enters a zone of agricultural influence. Using lower Kings Creek as a reference, we suggest that streams in the region that consistently exhibit NO₃⁻ concentrations >25–50 µg/L have chronic NO₃⁻ enrichment. Five of the 9 streams studied by O'Brien et al. (2007) fit this category.

Generally, stream ecologists have thought that stream N uptake would respond in a similar manner to acute and chronic increases in concentration, albeit via different mechanisms. Theoretically, uptake should saturate in response to short-term pulses because of physiological (enzyme) constraints. Likewise, uptake should saturate in response to chronic N loads as N becomes a nonlimiting resource and supply overwhelms biological N demand. In Kings Creek, evidence exists that N uptake begins to saturate with short-term increases in N. Saturation occurred for NH₄⁺ uptake, but the pattern of saturation of NO₃⁻ uptake is less clear. Recent studies of NO₃⁻ uptake at the local (O'Brien et al. 2007) and continental (Mulholland et al. 2008) scale have demonstrated that NO₃⁻ uptake does not saturate as previously expected, even across streams with NO₃⁻ concentrations that are many orders of magnitude above ambient reference conditions. Instead, a consistent loss in uptake efficiency occurs because of elevated NO₃⁻ concentrations.

NO₃⁻ uptake efficiency decreases with increasing NO₃⁻ concentration within streams (because of short-

term additions of NO₃⁻ concentration) and across streams (gradient of low NO₃⁻ to high ambient NO₃⁻ concentration). The slopes of the regressions between U_t and NO₃⁻ concentration were the same (regression lines parallel) for chronic and acute exposure to elevated NO₃⁻, a result suggesting that no functional difference exists between responses to acute or chronic elevations in NO₃⁻ concentration. Had higher concentrations of NO₃⁻ been used in the short-term additions, we might have seen greater saturation of uptake, and the regression slopes might have differed. More research will be needed to determine which trend holds at high NO₃⁻ concentrations (1000–10,000 µg/L). We did find that the intercepts of the regressions were different. This result suggests that, on average, U_t was higher in short-term N additions than in streams with chronically enriched streams with ambient NO₃⁻ at moderate concentrations (10–1000 µg/L). However, this conclusion is based on only a few ¹⁵N data points, and the results might be unduly influenced by ≥1 low values of U_t . A potential explanation for the difference in U_t between the acute and chronic enrichment is that prairie streams are N-limited or co-N limited and, therefore, organisms might have a biological N deficit. Microbial biofilms in low-N streams could engage in luxury uptake when short term pulses of NO₃⁻ or NH₄⁺ are available, and this response might result in higher U_t than is necessary for metabolic or stoichiometric requirements. Streams with chronic N loading have lower N deficit and, thus, might have lower U_t in response to the same N concentrations.

Potential problems exist when comparing U_t values that were measured with different methods. Uptake lengths measured with short-term nutrient addition techniques (S_w') overestimate the actual uptake length (S_w) at ambient conditions (Mulholland et al. 1990, 2002, Earl et al. 2006). Ambient U_t estimates from S_w' will underestimate the actual ambient U_t as measured with stable isotope tracers (Dodds et al. 2002), and multiple additions are required to extrapolate ambient S_w and U_t (Payn et al. 2005, O'Brien et al. 2008). We anchored ambient uptake parameters with published ¹⁵N studies and reported U_t of NO₃⁻ at the elevated addition concentrations experienced during the addition.

Prairie streams in this region typically have low DIN concentrations and rapid uptake of available DIN, which leads to greater in-stream N retention. From a management perspective, the high degree of N retention is an ecosystem service. Our results suggest that short-term pulses of N added to the stream (from natural or anthropogenic sources) would be sequestered quickly by stream biota. If the

concentration of an N pulse is too great, then the removal of DIN by the biota will saturate, limiting the proportion of the pulse that is retained by the stream. Consistently high loading of N to the stream will lead to elevated N-cycling rates in the stream, but the efficiency of N-cycling will be lower, decreasing the stream's capacity to retain N. Because of this loss of efficiency with increased N load, in-stream processing should not be viewed as a primary mechanism of N remediation. Therefore, strategies that reduce chronic loading of N to the stream, including protection of wetlands and riparian areas, are encouraged.

Acknowledgements

This research was supported by the National Science Foundation (project DEB-0111410), as part of the lotic intersite N experiment (LINX) project, and by the Konza Prairie LTER grant. This paper is contribution #06-296-J from the Kansas Agriculture Experiment Station.

Literature Cited

- ALEXANDER, R. B., R. A. SMITH, AND G. E. SCHWARZ. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758–761.
- AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 1995. Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- BERNOT, M. J., AND W. K. DODDS. 2005. Nitrogen retention, removal and saturation in lotic ecosystems. *Ecosystems* 8:442–453.
- BERNOT, M. J., J. L. TANK, T. V. ROYER, AND M. B. DAVID. 2006. Nutrient uptake in streams draining agricultural catchments of the mid-western United States. *Freshwater Biology* 51:499–509.
- BORCHARDT, M. A. 1996. Nutrients. Pages 184–228 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). *Algal ecology: freshwater benthic ecosystems*. Academic Press, San Diego, California.
- BORCHARDT, M. A., J. P. HOFFMANN, AND P. W. COOK. 1994. Phosphorus uptake kinetics of *Spirogyra fluviatilis* (Charophyceae) in flowing water. *Journal of Phycology* 30:403–417.
- DAVIS, J. C., AND G. W. MINSHALL. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia* (Berlin) 119:247–255.
- DODDS, W. K. 2003. The misuse of inorganic N and soluble reactive P to indicate nutrient status of surface waters. *Journal of the North American Benthological Society* 22:171–181.
- DODDS, W. K., M. A. EVANS-WHITE, N. GERLANC, L. GRAY, D. A. GUDDER, M. J. KEMP, A. L. LÓPEZ, D. STAGLIANO, E. STRAUSS, J. L. TANK, M. R. WHILES, AND W. WOLLHEIM. 2000. Quantification of the nitrogen cycle in a prairie stream. *Ecosystems* 3:574–589.
- DODDS, W. K., A. J. LÓPEZ, W. B. BOWDEN, S. GREGORY, N. B. GRIMM, S. K. HAMILTON, A. E. HERSHEY, E. MARTÍ, W. B. McDOWELL, J. L. MEYER, D. MORRALL, P. J. MULHOLLAND, B. J. PETERSON, J. L. TANK, H. M. VALETT, J. R. WEBSTER, AND W. WOLLHEIM. 2002. N uptake as a function of concentration in streams. *Journal of the North American Benthological Society* 21:206–220.
- DODDS, W. K., AND R. M. OAKES. 2004. A technique for establishing reference nutrient concentrations across watersheds impacted by humans. *Limnology and Oceanography Methods* 2:333–341.
- DODDS, W. K., J. C. PRISCU, AND B. K. ELLIS. 1991. Seasonal uptake and regeneration of inorganic nitrogen and phosphorus in a large oligotrophic lake: size-fractionation and antibiotic treatment. *Journal of Plankton Research* 13:1339–1358.
- EARL, S. R., H. M. VALETT, AND J. R. WEBSTER. 2006. Nitrogen saturation in stream ecosystems. *Ecology* 87:3140–3151.
- FELLOWS, C. S., H. M. VALETT, C. N. DAHM, P. J. MULHOLLAND, AND S. A. THOMAS. 2006. Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems* 9:788–804.
- INWOOD, S. E., J. L. TANK, AND M. J. BERNOT. 2005. Patterns of denitrification associated with land-use in 9 Midwestern headwater streams. *Journal of the North American Benthological Society* 24:227–245.
- JOHNSON, L. T., J. L. TANK, AND W. K. DODDS. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. *Canadian Journal of Fisheries and Aquatic Sciences* 66:1081–1094.
- KEMP, M. J., AND W. K. DODDS. 2001. Spatial and temporal patterns of nitrogen in prairie streams. *Biogeochemistry* 53:125–141.
- KEMP, M. J., AND W. K. DODDS. 2002a. Comparisons of nitrification and denitrification in pristine and agriculturally influenced streams. *Ecological Applications* 12:998–1009.
- KEMP, M. J., AND W. K. DODDS. 2002b. The influence of ammonium, nitrate, and dissolved oxygen concentration on uptake, nitrification, and denitrification rates associated with prairie stream substrata. *Limnology and Oceanography* 47:1380–1393.
- LOHMAN, K., AND J. C. PRISCU. 1992. Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. *Journal of Phycology* 28:443–448.
- MULHOLLAND, P. J., A. M. HELTON, G. C. POOLE, R. O. HALL, S. K. HAMILTON, B. J. PETERSON, J. L. TANK, L. R. ASHKENAS, L. W. COOPER, C. N. DAHM, W. K. DODDS, S. FINDLAY, S. V. GREGORY, N. B. GRIMM, S. L. JOHNSON, W. H. McDOWELL, J. L. MEYER, H. M. VALETT, J. R. WEBSTER, C. ARANGO, J. J. BEAULIEU, M. J. BERNOT, A. J. BURGIN, C. CRENSHAW, L. JOHNSON, B. R. NIEDERLEHNER, J. M. O'BRIEN, J. D. POTTER, R. W. SHEIBLEY, D. J. SOBOTA, AND S. M. THOMAS. 2008. Excess nitrate from agricultural and urban areas reduces

- denitrification efficiency in streams. *Nature* 452: 202–205.
- MULHOLLAND, P. J., A. D. STEINMAN, AND J. W. ELWOOD. 1990. Measurement of phosphorus uptake length in streams – comparison of radiotracer and stable PO₄ releases. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 2351–2357.
- MULHOLLAND, P. J., J. L. TANK, J. R. WEBSTER, W. B. BOWDEN, W. K. DODDS, S. V. GREGORY, N. B. GRIMM, S. K. HAMILTON, S. L. JOHNSON, E. MARTÍ, W. H. MCDOWELL, J. L. MERRIAM, J. L. MEYER, B. J. PETERSON, H. M. VALETT, AND W. M. WOLLHEIM. 2002. Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. *Journal of the North American Benthological Society* 21:544–560.
- O'BRIEN, J. M., AND W. K. DODDS. 2008. Predicting NH₄⁺ uptake and mineralization in prairie streams by using chamber incubation and short-term nutrient addition experiments. *Freshwater Biology* 53:102–112.
- O'BRIEN, J. M., W. K. DODDS, K. C. WILSON, J. N. MURDOCK, AND J. EICHMILLER. 2007. The saturation of N cycling in Central Plains streams: ¹⁵N experiments across a broad gradient of nitrate concentrations. *Biogeochemistry* 84:31–49.
- PAYN, R. A., J. R. WEBSTER, P. J. MULHOLLAND, H. M. VALETT, AND W. K. DODDS. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. *Limnology and Oceanography: Methods* 3:174–182.
- RABALAIS, N. N. 2002. Nitrogen in aquatic ecosystems. *Ambio* 31:102–112.
- SIGMAN, D. M., M. A. ALTABET, R. MICHENER, D. C. McCORKLE, B. FRY, AND R. M. HOLMES. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry* 57:227–242.
- SIMON, K. S., C. R. TOWNSEND, B. J. F. BIGGS, AND W. B. BOWDEN. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. *Journal of the North American Benthological Society* 24:1–18.
- SMITH, R. A., R. B. ALEXANDER, AND G. E. SCHWARZ. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science and Technology* 37:3039–3047.
- STREAM SOLUTE WORKSHOP. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95–119.
- TANK, J. L., AND W. K. DODDS. 2003. Responses of heterotrophic and autotrophic biofilms to nutrients in ten streams. *Freshwater Biology* 48:1031–1049.
- TILMAN, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey.
- WEBSTER, J. R., AND T. P. EHRMAN. 1996. Solute dynamics. Pages 145–160 in F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. Academic Press, San Diego, California.

Received: 9 February 2009

Accepted: 16 February 2010