

## Saturation of $\text{NO}_3^-$ uptake in prairie streams as a function of acute and chronic N exposure

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**Abstract.** We conducted a series of stepwise  $\text{NO}_3^-$  additions to investigate the response of  $\text{NO}_3^-$  uptake to short-term (acute) changes in N concentration in 3 prairie streams. Observed  $\text{NO}_3^-$  uptake rates increased with short-term elevations in  $\text{NO}_3^-$  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  $\text{NH}_4^+$  and 67  $\mu\text{g/L}$  for  $\text{NO}_3^-$ -N. This difference in half-saturation coefficients suggests that  $\text{NH}_4^+$  is more efficiently assimilated than  $\text{NO}_3^-$ , indicating a preference for  $\text{NH}_4^+$  as an N source. Similarly, ambient concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  (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  $\text{NO}_3^-$  exceeded biological demand. Bernot et al. (2006) found that  $\text{NO}_3^-$  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  $\text{NO}_3^-$  uptake, nitrification, and denitrification did not

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saturate in streams across a wide gradient of chronic N loading. Instead, they found a pattern of efficiency loss as  $\text{NO}_3^-$  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  $\text{NH}_4^+$  or  $\text{NO}_3^-$  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  $\text{NO}_3^-$  additions to investigate the response of  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and  $\text{NH}_4^+$  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).  $\text{NO}_3^-$  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  $\text{NO}_3^-$  concentrations of 1.3  $\mu\text{g/L}$  (0.7–3.25  $\mu\text{g/L}$  quartile range) and median  $\text{NH}_4^+$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  additions in 3 prairie reaches of Kings Creek between 2003 and 2005. On KC-South, we did 2 short-term  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{KNO}_3$  and a  $\text{NaBr}$  tracer into the stream at a steady rate. Once the concentration of  $\text{Br}^-$  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  $\text{NO}_3^-$  concentration colorimetrically within 24 h with the Cd-reduction method on a Technicon Auto-Analyzer (SEAL Analytical, Fareham, UK) (APHA 1995). We measured  $\text{Br}^-$  concentrations with an Orion  $\text{Br}^-$  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  $\text{NO}_3^-$  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 NO<sub>3</sub><sup>-</sup> concentrations, not uptake rates at ambient NO<sub>3</sub><sup>-</sup> concentrations.

We combined the results from the short-term NO<sub>3</sub><sup>-</sup> additions with data from <sup>15</sup>NO<sub>3</sub><sup>-</sup> isotope tracer additions done by O'Brien et al. (2007) in the same streams. These data were used to establish  $U_t$  at ambient NO<sub>3</sub><sup>-</sup> 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 <sup>15</sup>NO<sub>3</sub><sup>-</sup> and NaBr was added to the stream at a steady rate for 24 h. Duplicate samples for <sup>15</sup>NO<sub>3</sub><sup>-</sup> 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 <sup>15</sup>NO<sub>3</sub><sup>-</sup> content was determined by using a modified version of the method presented by Sigman et al. (1997). Uptake was measured based on <sup>15</sup>NO<sub>3</sub><sup>-</sup> 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 NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) 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 NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> 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 NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> 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 NO<sub>3</sub><sup>-</sup> uptake data with the results of O'Brien et al. (2007), who conducted a series of <sup>15</sup>NO<sub>3</sub><sup>-</sup> tracer additions in streams with a broad range of ambient NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> addition yielded  $U_t$  ranging from 1.0 to 9.9 µg m<sup>-2</sup> s<sup>-1</sup> (Fig. 1A). Based on the 5 NO<sub>3</sub><sup>-</sup> additions and 2 measures of <sup>15</sup>NO<sub>3</sub><sup>-</sup> (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<sup>-2</sup> s<sup>-1</sup> ( $\pm 12.6$  µg m<sup>-2</sup> s<sup>-1</sup> [SE]) and  $K_s$  was 184 µg/L ( $\pm 173$  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 NO<sub>3</sub><sup>-</sup> addition yielded  $U_t$  ranging from 0.7 to 4.3 µg m<sup>-2</sup> s<sup>-1</sup> (Fig. 1B). Based on the 5 NO<sub>3</sub><sup>-</sup> additions and 2 measures of <sup>15</sup>NO<sub>3</sub><sup>-</sup> (O'Brien et al. 2007), a significant fit of the Michaelis–

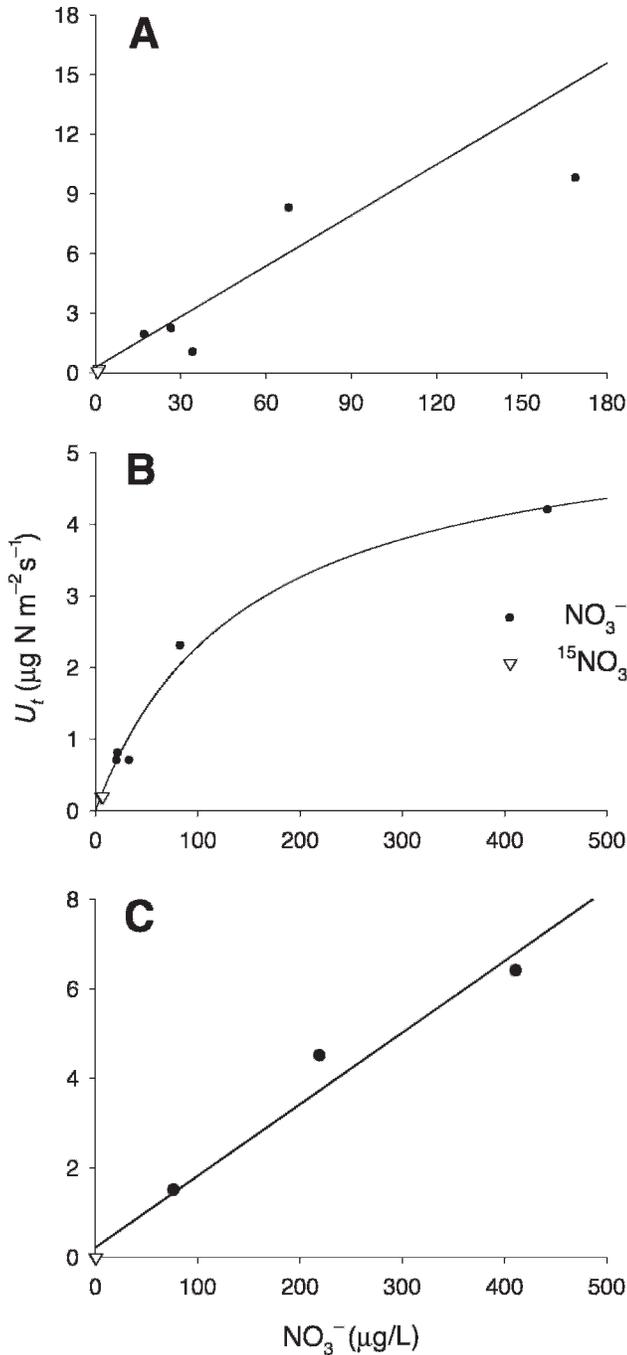


FIG. 1. Relationship between  $\text{NO}_3^-$  concentration and uptake rate ( $U_t$ ) for  $^{15}\text{NO}_3^-$  and short-term  $\text{NO}_3^-$  additions conducted on Konza Prairie Biological Station watersheds Kings Creek-North Branch (A), Kings Creek-South Branch (B), and Shane Creek (C). Linear 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  $\text{NO}_3^-$  additions yielded  $U_t$  ranging from 1.5 to  $6.4 \mu\text{g m}^{-2} \text{s}^{-1}$  (Fig. 1C). Based on  $\text{NO}_3^-$  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  $\text{NH}_4^+$  ( $R^2 = 0.37$ ,  $F_{1,18} = 11.6$ ,  $p < 0.001$ ) and for  $\text{NO}_3^-$  ( $R^2 = 0.34$ ,  $F_{1,25} = 18.8$ ,  $p < 0.001$ ). Uptake kinetics calculated by these models showed a greater  $V_{max}$  for  $\text{NO}_3^-$  ( $4.9 \pm 4.7 \mu\text{g m}^{-2} \text{s}^{-1}$ ) than for  $\text{NH}_4^+$  ( $1.7 \pm 1.3 \mu\text{g m}^{-2} \text{s}^{-1}$ ).  $K_s$  was greater for  $\text{NO}_3^-$  ( $67 \pm 74 \mu\text{g/L NO}_3^- \text{-N}$ ) than for  $\text{NH}_4^+$  ( $6.7 \pm 5.3 \mu\text{g NH}_4^+ \text{-N /L}$ ), but the variances around these estimates are considerable and the differences could not be confirmed statistically.

The effects of chronic vs acute  $\text{NO}_3^-$  enrichment were tested by comparing the relationship between  $\log(x)$ -transformed  $U_t$  and  $\text{NO}_3^-$  concentrations found by O'Brien et al. (2007) with data from the compiled short-term  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  uptake among the 3 prairie stream reaches investigated in our study. Michaelis-Menten and linear models fit equally well for short-term  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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 <sub>3</sub> <sup>-</sup>	MM	21.5	184	–	–	–	–	0.94	45.3	<0.001
	Linear	–	–	0.058	0.27	–	–	0.95	102.6	<0.001
KC-South NO <sub>3</sub> <sup>-</sup>	MM	5.6	146	–	–	–	–	0.98	322.9	<0.001
	Linear	–	–	0.009	0.52	–	–	0.90	44.1	0.001
Shane Creek NO <sub>3</sub> <sup>-</sup>	MM	16.5	634	–	–	–	–	0.99	617.9	<0.001
	Linear	–	–	0.016	0.22	–	–	0.97	125.0	0.002
Overall NH <sub>4</sub> <sup>+</sup>	MM	1.7	6.7	–	–	–	–	0.37	11.6	<0.001
	Power	–	–	0.099	-0.23	–	–	0.20	4.4	0.05
Overall NO <sub>3</sub> <sup>-</sup>	MM	4.9	67	–	–	–	–	0.34	18.8	<0.001
	Power	–	–	0.087	-0.28	–	–	0.40	15.5	<0.001

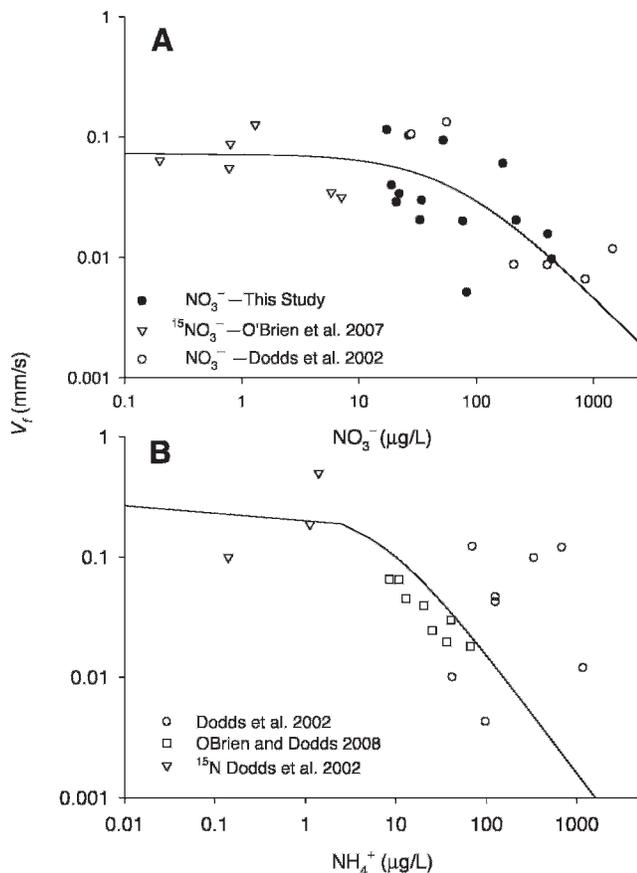


FIG. 2. Michaelis–Menten model fit to the N concentration and the uptake mass transfer coefficient ( $V_t$ ) data for NO<sub>3</sub><sup>-</sup> (A) and NH<sub>4</sub><sup>+</sup> (B) from the overall Kings Creek prairie stream data set.

this range of concentrations. Greater NO<sub>3</sub><sup>-</sup> concentrations (>1000  $\mu\text{g/L}$ ) might be necessary to saturate NO<sub>3</sub><sup>-</sup> uptake completely in these streams. The Michaelis–Menten model fit better than the linear model at KC-South, suggesting that saturation of NO<sub>3</sub><sup>-</sup> uptake occurred in this stream reach. The rate of increase in NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> additions. N demand in Shane Creek was very strong, so  $U_t$  did not change

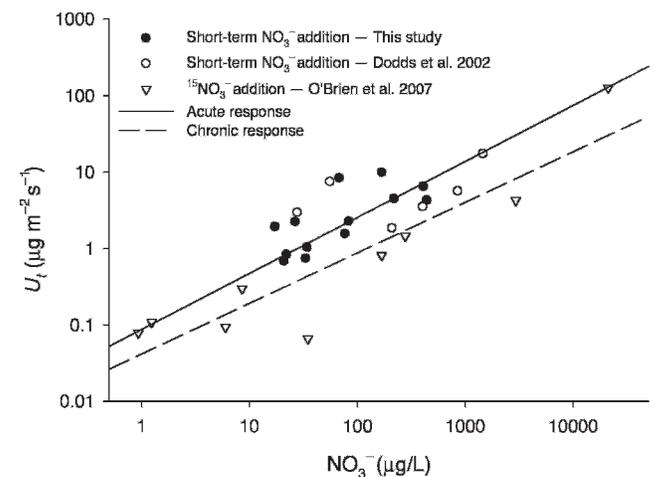


FIG. 3. A comparison of relationships between NO<sub>3</sub><sup>-</sup> concentration and uptake rate ( $U_t$ ) for streams in response to acute and chronic enrichments of NO<sub>3</sub><sup>-</sup>.

greatly (~20 m for all  $\text{NO}_3^-$  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  $\text{NH}_4^+$  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,  $\text{NH}_4^+$  uptake exhibits classic saturation in these streams. The overall  $\text{NO}_3^-$  data are consistent with a Michaelis–Menten model. However, the efficiency-loss model worked equally well. Uptake efficiency of  $\text{NO}_3^-$  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  $\text{NO}_3^-$  Michaelis–Menten model for the purposes of comparison with  $\text{NH}_4^+$ , but note that it is not the only model that can explain the data.

#### *N-limited uptake at ambient conditions*

$K_s$  for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  suggest that organisms in Kings Creek were influenced by N limitation because ambient concentrations of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  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  $\text{NO}_3^-$  in the 3 streams. O'Brien and Dodds (2008) found that ambient  $U_t$  for  $\text{NH}_4^+$  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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  concentrations often exceed the mean  $K_s$  and that uptake of  $\text{NH}_4^+$  might occasionally become saturated.

In Kings Creek,  $K_s$  for  $\text{NH}_4^+$  occurred at a lower concentration than for  $\text{NO}_3^-$ , a result suggesting that  $\text{NH}_4^+$  is assimilated more efficiently than  $\text{NO}_3^-$ . This strong affinity for  $\text{NH}_4^+$  relative to  $\text{NO}_3^-$  is consistent with data published for freshwater plankton (Dodds et al. 1991) and reflects lower energetic requirements for using  $\text{NH}_4^+$  than  $\text{NO}_3^-$ . Observed  $V_{max}$  for  $\text{NH}_4^+$

was less than that of  $\text{NO}_3^-$  in Kings Creek, suggesting that  $\text{NO}_3^-$  uptake is the predominant form of inorganic N assimilation when  $\text{NO}_3^-$  is available at high concentrations. We would expect  $\text{NH}_4^+$  uptake to be greater than  $\text{NO}_3^-$  uptake, based on respective  $V_{max}$ ,  $K_s$ , and normally low concentrations of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Some evidence indicates that  $\text{NO}_3^-$  might be favored by photoautotrophs in streams (e.g., Fellows et al. 2006), and given the open nature of prairie streams, one might expect  $\text{NO}_3^-$  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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and  $6.7 \mu\text{g/L}$  and  $\text{NH}_4^+$  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  $\text{NH}_4^+$  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  $\text{NO}_3^-$  in forested streams, again higher than ambient  $\text{NO}_3^-$  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  $\text{NO}_3^-$  additions over the course of a year. In this case, mean stream concentrations were higher than  $K_s$  for  $\text{NO}_3^-$  but not  $\text{NH}_4^+$ , 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 NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N concentrations of <10 µg/L. Concentrations of NO<sub>3</sub><sup>-</sup>-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 NO<sub>3</sub><sup>-</sup> concentrations >25–50 µg/L have chronic NO<sub>3</sub><sup>-</sup> 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 NH<sub>4</sub><sup>+</sup> uptake, but the pattern of saturation of NO<sub>3</sub><sup>-</sup> uptake is less clear. Recent studies of NO<sub>3</sub><sup>-</sup> uptake at the local (O'Brien et al. 2007) and continental (Mulholland et al. 2008) scale have demonstrated that NO<sub>3</sub><sup>-</sup> uptake does not saturate as previously expected, even across streams with NO<sub>3</sub><sup>-</sup> concentrations that are many orders of magnitude above ambient reference conditions. Instead, a consistent loss in uptake efficiency occurs because of elevated NO<sub>3</sub><sup>-</sup> concentrations.

NO<sub>3</sub><sup>-</sup> uptake efficiency decreases with increasing NO<sub>3</sub><sup>-</sup> concentration within streams (because of short-

term additions of NO<sub>3</sub><sup>-</sup> concentration) and across streams (gradient of low NO<sub>3</sub><sup>-</sup> to high ambient NO<sub>3</sub><sup>-</sup> concentration). The slopes of the regressions between  $U_t$  and NO<sub>3</sub><sup>-</sup> concentration were the same (regression lines parallel) for chronic and acute exposure to elevated NO<sub>3</sub><sup>-</sup>, a result suggesting that no functional difference exists between responses to acute or chronic elevations in NO<sub>3</sub><sup>-</sup> concentration. Had higher concentrations of NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> at moderate concentrations (10–1000 µg/L). However, this conclusion is based on only a few <sup>15</sup>N 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 NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> 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 <sup>15</sup>N studies and reported  $U_t$  of NO<sub>3</sub><sup>-</sup> 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.

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