An assessment of nitrogen removal from headwater streams in an agricultural watershed, northeast Ohio, U.S.A.

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Abstract

The objective of this project was to assess in-stream nitrogen removal capacity in a fragmented agricultural landscape and to compare removal capacities in streams with agricultural or residential (hereafter referred to as agricultural streams) and forested riparian land use. We also identified what stream characteristics control nitrogen removal in these systems. We examined paired reaches (one agricultural and one forested reach) along five headwater streams in an agricultural watershed (Upper Sugar Creek Watershed) in northeast Ohio. Although denitrification rates were high ($<$0.1–17.2 mg N m^{-2} h^{-1}), annual nitrogen removal was most likely low because during spring and fall, when in-stream nitrogen loads were high, removal was low, and during summer when in-stream nitrogen loads were low, removal was high. Between the agricultural and forested reaches removal rates were similar in terms of loss rate and uptake velocity. Removal capacities were similar despite forested reaches having higher hydraulic residence times. Using a redundancy analysis we identified temperature, in-stream nitrate concentration, and relative transient storage as stream characteristics that affect nitrogen removal. Further analysis suggests that nitrogen removal via denitrification in these headwater streams was not limited by the availability of nitrate. In this fragmented agricultural watershed in-stream nitrogen removal was low and riparian land use had no effect on this process, most likely because of nitrate saturation.

Since the middle of the 20th century in the United States, reactive nitrogen inputs to landscapes have tripled as the result of agricultural fertilizer applications and cultivation of N-fixing crops (Galloway et al. 2003). Reactive N is considered by some researchers to be the third largest threat to our planet after biodiversity loss and climate change (Giles 2005). Excess N degrades habitat and limits biodiversity in aquatic ecosystems (Vitousek et al. 1997; Howarth et al. 2000). In the midwestern United States, agricultural watersheds are the dominant exporters of reactive N, particularly inorganic N (Goolsby et al. 1999), and have been linked to the eutrophication of the Gulf of Mexico (Rabalais et al. 2001). Because of these negative effects, several studies have examined N removal capacity and removal strategies over large spatial scales (Mitsch et al. 2001; Seitzinger et al. 2002). A crucial process responsible for the removal of N is denitrification. Denitrification is an important biogeochemical process because it removes a mobile, reactive form of N (nitrate)

from an ecosystem by converting it to an unreactive gas (dinitrogen). This reaction has been extensively studied since the early 1970s, yet questions still remain about its efficacy and the characteristics that control this process in stream ecosystems (Boyer et al. 2006).

In streams and rivers, the transport of nutrients has been coupled to the downstream movement of water by the nutrient spiraling theory (Webster and Patton 1979; Newbold et al. 1981). This theory provides quantitative metrics for nutrient retention and a framework for biogeochemists to compare data (Stream Solute Workshop 1990). The comparison of N retention in headwater streams has revealed some discrepancies. Headwater streams with inorganic N concentrations lower than 1 mg L^{-1} have been shown to retain substantial levels of N (Peterson et al. 2001). In contrast, low-order streams with inorganic N concentrations greater than 1 mg L^{-1} retain relatively little N (Royer et al. 2004; Bernot et al. 2006; Gücker and Pusch 2006). Retention of N in headwater streams appears to be highly variable and possibly dependent upon inorganic N concentration.

In accordance with the nutrient spiraling theory, biological, physical, and chemical processes interact to affect whole-stream N retention (Stream Solute Workshop 1990). Retention processes in natural aquatic systems include biotic assimilation, dissimilatory nitrate reduction to ammonia (DNRA), denitrification, and sorption. Chemical processes (i.e., sorption) and biological processes such as biotic assimilation and DNRA only temporarily retain N and eventually mineralize or transport N downstream as a different N-containing species. Denitrification is the only N retaining process that permanently removes N and has been reported as the dominant

retention mechanism in stream ecosystems (Jannson et al. 1994; Alexander et al. 2000; Saunders and Kalff 2001). Therefore, stream characteristics that affect N removal are limited to effects on denitrification and physical processes that affect the transport of nitrate (NO_3^-). For example, studies have found that in-stream N removal may not increase proportionately with NO_3^- availability because high concentrations of NO_3^- saturated denitrification (Bernot et al. 2006; Earl et al. 2006; Gücker and Pusch 2006), whereas other research identified a hydrologic variable, transient storage—a physical compartment in a stream channel that detains solutes in slow-moving water—as the key stream characteristic controlling N removal (Valett et al. 1996). The degree in which these two factors and other stream characteristics compete and interact with one another to affect N removal in an agricultural landscape is unclear.

The objective of our study was twofold. First, we quantified the overall N removal potential of headwater streams in a fragmented agricultural watershed. Second, we determined what stream characteristics control N removal in the study streams. To achieve this objective we examined headwater streams with two contrasting riparian land uses, agricultural or residential (hereafter referred to as agricultural) and forested. Although forested headwater streams were located in small, fragmented woodlots and most likely were affected by the surrounding agricultural land use, we hypothesized that hydraulic retention time (HRT) and transient storage would be higher in forested reaches. We expected to document this finding because forested streams commonly have higher in-stream geomorphic complexity such as riffle-pool complexes and debris dams. Because of higher HRT and transient storage, we also hypothesized that N removal would be higher in forested reaches compared with agricultural reaches. To our knowledge, only one study has examined the effect of transient storage on N removal in agricultural streams with high in-stream NO_3^- concentrations (Bernot et al. 2006). We expect this study to aid in the understanding of N cycling in headwater streams and to provide information regarding N processing in an agricultural landscape.

Methods

Study sites—We identified paired 50-m reaches (one reach in agricultural land use and the other in forested land use) along five headwater streams in the Upper Sugar Creek Watershed in northeast Ohio (40°51′42″N, 81°50′29″W; Fig. 1). The Upper Sugar Creek Watershed is approximately 200 km², has over 80% agricultural land use that is mostly tile drained, and contains interspersed, fragmented woodlots (Ohio Environmental Protection Agency 2002; Fig. 1). The study reaches have a small drainage area (<5 km²) and a low topographic gradient (<0.02 m m⁻¹; Table 1). All sites, except the forested reach on Spring Creek, have low sinuosity (<1.1). Sediments were highly variable from stream to stream but were similar for each set of paired reaches (Table 1).

Field data collection—Sampling began in the summer of 2005 and was completed by fall of 2006. Sampling was
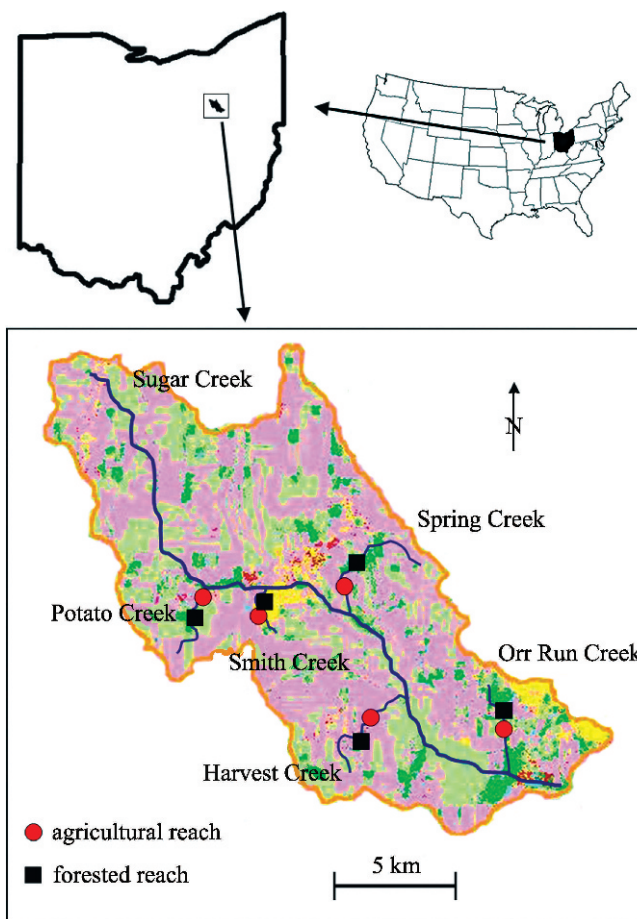

Fig. 1. Location of the Upper Sugar Creek Watershed (40°51′42″N, 81°50′29″W) within Ohio, U.S.A. The study sites are all located on primary headwater streams that drain directly into the main stem of the Sugar Creek. Land use patterns within the Upper Sugar Creek Watershed are as follows: pink and light green indicate pasture and row crop agriculture, dark green indicates forested land, yellow indicates residential land use, and red indicates industrial land use.

divided into three seasons: spring (May and June), summer (July and August), and fall (late September to November). During each season a reach was sampled twice, resulting in 10 sampling events per land use per season. During a routine sampling day, paired reaches along one stream were sampled, and all 10 sites were typically sampled within a 7-d period. At each site, water samples were collected and stored at 4°C for nutrient analysis and denitrification assays. Water quality parameters (i.e., temperature, dissolved oxygen, specific conductivity, and redox potential) were measured with a YSI 6600 multisonde probe. Rhodamine WT slug injections were then conducted to obtain a breakthrough curve, which was used to estimate HRT and transient storage (*see* below for methodology). After dye injection, stream velocity was measured using a SonTek acoustic Doppler velocimeter and discharge was calculated using the U.S. Geological Survey standard six-tenths method (Buchanan and Somers 1969). Finally, six to eight sediment cores (6.3 cm diameter) of the upper 5 cm of

Table 1. Stream characteristics for the paired reaches located along five headwater streams in the Upper Sugar Creek Watershed. Discharge measurements were made during rhodamine WT injections conducted twice per season for each reach ($n=6$). Water samples were taken periodically during the course of the experiment at each reach ($n=15$). Agr.=agricultural.

	Drainage area (km²)	Agriculture land use within subbasin (%)	Gradient (m m⁻¹)	Sediment size distribution			Bankfull conditions		Discharge (m³ s⁻¹) mean±SD	Median±SD (mg N L⁻¹)	
				>2 mm (%)	0.5–2 mm (%)	<0.5 mm (%)	Width (m)	Depth (m)		NO₃⁻	TN
Harvest Creek											
Agr.	3.5	92	0.007	86	10	4	1.5	0.3	0.013±0.011	14.2±5.6	16.2±4.6
Forest	3.2	91	0.008	82	6	12	3.9	0.2	0.010±0.008	18.8±4.1	19.0±3.8
Smith Creek											
Agr.	1.2	80	0.007	83	5	12	2.4	0.3	0.016±0.024	7.2±10.8	7.9±10.6
Forest	1.4	79	0.007	94	2	4	4.4	0.2	0.023±0.034	6.2±10.6	6.4±10.7
Potato Creek											
Agr.	2.7	94	0.009	87	4	9	1.4	0.3	0.013±0.010	12.7±3.3	12.6±2.9
Forest	1.3	96	0.019	73	14	13	3.3	0.2	0.004±0.002	12.2±2.5	12.5±2.5
Spring Creek											
Agr.	4.3	82	0.010	90	7	3	1.7	0.3	0.011±0.011	9.7±2.8	10.7±3.0
Forest	2.6	83	0.002	91	4	5	3.9	0.2	0.009±0.008	11.4±3.6	13.4±3.7
Orr Run Creek											
Agr.	0.7	95	0.006	2	46	52	2.3	0.4	0.038±0.053	6.5±3.2	7.0±3.4
Forest	0.7	95	0.003	4	3	93	3.3	0.2	0.023±0.030	7.0±3.8	7.3±3.8

sediments were collected from representative locations along the active channel of each reach. For example, a reach composed of approximately 50% riffle and 50% run habitat had four cores taken in riffles and four cores taken in runs. The top 5 cm of sediments were collected for the denitrification assays following previous work in similar aquatic systems (Royer et al. 2004; Inwood et al. 2005; David et al. 2006). These cores were homogenized and used for denitrification assays in the laboratory. Another four cores following the same sampling technique were taken and used to assess ash-free dry mass (AFDM) per unit area for each reach. All cores were stored at 4°C until processed in the laboratory.

Laboratory denitrification assays—Immediately after returning from the field, sediment samples were processed for denitrification assays. Denitrification was measured using the acetylene inhibition method on sediment slurries (Knowles 1990). Before slurry addition, chloramphenicol was added to each bottle to ensure that a final concentration of 5 mmol L⁻¹ was reached. The use of chloramphenicol reduces bottle effects by eliminating enzyme synthesis and allows denitrification to be measured over short incubation periods (Bernot et al. 2003). The use of chloramphenicol in combination with the acetylene inhibition method has produced rates that are comparable with other techniques such as membrane inlet mass spectrometry (Bernot et al. 2003) and has produced reliable estimates of in situ denitrification rates (David et al. 2006). Using unfiltered, unpreserved stream water and homogenized sediment, approximately 25 mL of slurry was added to a 150-mL borosilicate glass media bottle. Next, unfiltered, unpreserved stream water collected from each site was added to reach a final volume of 75 mL. The bottle was then capped with an open-top lid and the headspace was accessible via a replaceable butyl septum. Oxygen was purged from each bottle by continuously pumping helium at 35 mL min⁻¹ for 5 min and shaking the bottles every 30 s. Fifteen milliliters of acetylene was added to reach a final concentration of 10% and bottles were placed in a Torrey Pines Scientific temperature-controlled benchtop incubator at a constant temperature in the dark. The incubation temperature was the average between the water temperatures of the paired reaches sampled that day. After 20 min of equilibration time, 5 mL of gas sample was taken from the headspace of each bottle every hour for 3 h. Gas samples were stored at 4°C and analyzed for nitrous oxide (N_2O) within 7 d. Before sampling, bottles were shaken and given 3 min to equilibrate. Immediately after sampling, 5 mL of replacement gas (0.5 mL of acetylene and 4.5 mL of helium) was added. Gas samples were analyzed for N_2O on a Shimadzu gas chromatograph (Alltech Porapak-Q column, 80/100 mesh, 3.65 m × 0.32 cm × 0.22 cm; 40°C oven; 95:5 argon to methane carrier gas; flow rate: 35 mL min⁻¹) equipped with an electron-capture detector and a 1-mL sample loop. The slope of the regression of N_2O against time linear trend line was used as the denitrification rate. All linear regression lines in this study had regression coefficients (r^2) greater than 0.85 using at least three data points.

Sediment analysis—The sediment from each denitrification assay bottle was dried at 60°C for 24 h, weighed, combusted at 550°C for 6 h, and reweighed to determine AFDM. Denitrification rates were then expressed on the basis of mg N AFDM^{-1} time^{-1}. Four cores with a known surface area were also taken from each reach to determine AFDM using the methods described above. Values were averaged and a standing stock (AFDM m^{-2}) was estimated for each reach. By multiplying the denitrification rate expressed on an AFDM basis by the standing stock, we calculated a denitrification rate on an areal basis (U). A small subset of homogenized sediment was dried at 60°C for 24 h and analyzed for sedimentary carbon with a CE Instruments CHN analyzer.

For sediment particle size determination, eight cores of the top 5 cm of sediment were collected from each reach, dispersed in 0.02% sodium hexametaphosphate, and shaken for 1 h. Sediments were then passed through a 2-mm and 0.5-mm sieve and dried overnight at 60°C. Size fractions are based on dry mass.

N removal metrics—The areal denitrification rate (U) was used to calculate N removal metrics following Stream Solute Workshop (1990). Therefore, the N removal metrics in this study are entirely on the basis of denitrification and no other removal processes. The two N removal metrics we used were uptake velocity (v_f) and loss rate constant (k). Uptake velocity is represented in units of length per time and measures the efficiency of N removal by a stream ecosystem, whereas k is in units of percentage N removed (or fractional N removal rate if not multiplied by 100). Adopting equations from the Stream Solute Workshop (1990), we measured v_f and k as follows:

$$v_f = \frac{U}{C} \tag{1}$$

$$k = \frac{v_f}{h} \times 100 \tag{2}$$

where C is the concentration of in-stream NO$_3^-$ and h is stream depth.

Breakthrough curve analysis and hydraulic parameters—At the top of the 50-m reach, 100 mg of rhodamine WT was slug injected. Immediately after dye injection, a pole was used to mechanically mix the added rhodamine WT. At the bottom of the reach, polyvinyl chloride boards (<5 mm thick) directed flow to a YSI 6600 probe equipped with a rhodamine sensor. We ensured that both the top and bottom of each sampling reach were located in well-mixed runs. The concentration of rhodamine WT was measured every 0.5 s. The concentration–time breakthrough curve was obtained by measuring the rhodamine WT concentration immediately after dye injection and stopped when the in-stream concentration was 1% of the maximum concentration. The HRT for each 50-m reach was measured by locating the centroid of the breakthrough curve following Thomann and Mueller (1987)

$$\text{HRT} = \frac{\int_0^{T(1\%)} ct \times \mathrm{d}t}{\int_0^{T(1\%)} c \times \mathrm{d}t} \tag{3}$$

where c is the concentration of rhodamine WT and t is the time step. Integrations were done using the area-under-the-curve function on Sigma Plot 8.0. Transient storage was calculated by using the parameterized version of the one-dimensional transport with inflow and storage model (OTIS-P). Because inflow was negligible in our 50-m reaches, the model operated under the following two equations:

$$\frac{\partial c}{\partial t} = -u_x \frac{\partial c}{\partial x} + K_x \frac{\partial c}{\partial x} + \alpha(c_s - c) \tag{4}$$

$$\frac{\mathrm{d}c_s}{\mathrm{d}t} = \alpha \frac{A}{A_s}(c - c_s) \tag{5}$$

where u_x is velocity in the downstream direction, K_x is longitudinal dispersion, α is transient storage exchange rate, c is the concentration of solute in the main channel, c_s is the concentration of the solute in transient storage, A is the average cross-sectional area of the stream, and A_s is the transient storage area. The breakthrough curve, discharge, and cross-sectional area from each reach were input into OTIS-P. The model ran iteratively until the residual error between the observed breakthrough curve and the output was minimized (Runkel et al. 1998). Once convergence of the residual sum of squares was achieved, a value of transient storage was calculated. Transient storage (A_s) is frequently reported in literature as relative transient storage area by dividing it by the cross-sectional area (A).

Post hoc analyses of the OTIS-P outputs were run using a Damkohler index (DaI)

$$\text{DaI} = \frac{\alpha \left(1 + \frac{A}{A_s}\right) L}{u_x} \tag{6}$$

where L is stream length (Wagner and Harvey 1997). When DaI values are approximately 1, parameter uncertainty is minimized (Wagner and Harvey 1997). Values much greater than 1 have high parameter uncertainty because solute exchange rates between the storage zone and the main channel are high relative to stream water velocity (Wagner and Harvey 1997). Conversely, DaI values much lower than 1 have high parameter uncertainty because of low tracer interaction with the transient storage zone (Wagner and Harvey 1997).

Water analysis—Water samples were analyzed for N species on a Lachat QuikChem 8500 autoanalyzer and dissolved organic carbon (DOC) on a Dorhmann-Rosemont carbon analyzer. Samples analyzed for dissolved constituents were filtered through 0.45-μm membrane filters, preserved with H$_2$SO$_4$, and stored at 4°C until analysis. To determine total N, unfiltered, unpreserved samples were digested using an autoclave following the persulfate method (American Public Health Administration 1998) and then analyzed on a Lachat QuikChem 8500 autoanalyzer.

Statistical analysis—All data used in this study required log transformation to satisfy normality and heterogeneity of variances. Two-way ANOVAs were conducted to determine the effect of land use and the effect of season

on measured and derived stream variables and on N removal metrics using Sigma Stat 3.1. Post hoc analyses were conducted using Tukey tests with Sigma Stat 3.1. Statistical differences were noted at $p < 0.05$. To identify the stream characteristics that were important in controlling N removal, a redundancy analysis (RDA) was conducted using Canoco 4.5. All data input into the RDA were log transformed. The following stream parameters were used as independent variables: discharge, HRT, stream depth, DOC, sedimentary carbon, in-stream NO_3^- concentration, water temperature, ratio of water depth to HRT (h:HRT), and relative transient storage ($A_s : A$). Both metrics of N removal (v_f and k) were used as response variables in the RDA. All data were centered and standardized. Scaling was on the basis of interspecies distances. The results from the RDA were tested for significance by running a Monte Carlo bootstrapping method using 9999 permutations under the reduced model.

Results

Water temperature was similar in both the agricultural and the forested reaches (Table 2). Temperature varied seasonally with stream temperatures, increasing from fall to spring to summer (Table 2). Discharge ranged from less than 0.001 to 0.144 m³ s⁻¹ in the study sites and was similar in the agricultural and forested reaches (Table 2). Discharge was lowest during summer, whereas events during spring and fall were higher than summer yet similar to each other. Agricultural reaches were also deeper than forested reaches and overall were shallowest during summer compared with spring and fall (Table 2).

In the study sites, NO_3^- was the dominant form of N (Table 1). Concentrations of NO_3^- were high, as the mean concentration from all sites was above 10 mg NO_3^--N L⁻¹ and peak concentrations reached 44.5 mg NO_3^--N L⁻¹ (Table 2). Concentrations of NO_3^- were not affected by riparian land use but varied seasonally (Table 2). During spring and fall, concentrations were similar to each other but twice the summer NO_3^- concentrations (Table 2). Concentration of NO_3^- was positively correlated with discharge ($y = 194.0x + 7.6$; $p < 0.001$), although the relationship was weak ($r^2 = 0.31$).

Concentrations of DOC were similar in the agricultural and the forested reaches (Table 2) and ranged from 0.2 to 9.7 and 0.6 to 9.8 mg C L⁻¹ in the agricultural and the forested sites, respectively. DOC concentrations were highest in spring compared with fall and summer (Table 2). Sedimentary carbon concentrations were also similar in the two reach types (Table 2) but had a much smaller range in the agricultural (2.0–4.2 mg C g sediment⁻¹) and forested (1.9–5.0 mg C g sediment⁻¹) sites than DOC. Sedimentary carbon concentrations were higher in summer compared with fall, whereas in the spring concentrations were similar to both summer and fall.

In forested reaches, HRT was almost twice as high as in agricultural reaches (Table 2). Seasonally, HRT was similar in spring and fall, but was almost twice as high during the summer (Table 2). Transient storage was similar in both the forested (0.22 m²) and the agricultural

Table 2. Measured and derived stream data and results from the two-way ANOVA testing for riparian land use ($n=30$) and seasonal effects ($n=20$). $A_s : A$ is the ratio of transient storage (A_s) to cross-sectional area (A). HRT=hydraulic residence time; Agr.=agricultural.

	Temperature (°C)	Discharge (m³ s⁻¹)	Depth (m)	NO_3^- (mg N L⁻¹)	DOC (mg C L⁻¹)	Sedimentary C (mg C g sed⁻¹)	HRT (min)	$A_s : A$
Agr.								
Mean±SD	13.8±5.1	0.018±0.027	0.16±0.06	10.4±8.5	3.9±2.0	3.2±0.7	13.0±8.8	2.4±2.7
Forest								
Mean±SD	13.5±4.9	0.014±0.021	0.10±0.04	11.1±8.6	3.9±2.3	3.5±1.1	22.9±15.9	1.6±1.4
Spring								
Mean±SD	12.8±2.3	0.026±0.038	0.14±0.06	14.5±11.1	5.6±2.3	3.4±0.9	12.7±8.9	1.5±1.1
Summer								
Mean±SD	19.3±3.1	0.005±0.004	0.10±0.05	5.4±4.8	3.0±1.9	3.6±0.8	27.3±16.0	3.4±3.0
Fall								
Mean±SD	8.3±1.7	0.018±0.010	0.15±0.06	12.3±5.4	3.1±0.7	2.9±0.8	13.9±10.4	1.1±0.9
Two-way ANOVA results								
Land use								
$F_{1,59}$	0.395	0.990	31.482	0.153	0.006	0.911	11.350	4.009
p value	0.532	0.324	<0.001	0.697	0.938	0.344	0.001	0.050
Season								
$F_{2,59}$	95.180	11.511	10.623	11.328	10.153	3.590	11.660	11.588
p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.034	<0.001	<0.001
Land use × season								
$F_{2,59}$	0.204	0.092	0.219	0.075	0.021	0.181	0.704	0.089
p value	0.816	0.912	0.804	0.928	0.979	0.835	0.499	0.915

Table 3.   Statistics for variables used to assess N removal capacity and two-way ANOVA results to determine differences between riparian land use ($n=30$) and season ($n=20$). Agr.=agricultural.

	NO_3^- load (kg N d^{-1})	Denitrification rate (mg N m^{-2} h^{-1})	K (% N removed d^{-1})	v_f (mm min^{-1})
Agr.				
Mean±SD	25.6±54.9	4.3±4.0	20.3±30.4	0.0164±0.0243
Forest				
Mean±SD	23.5±63.8	4.0±4.1	37.1±90.8	0.0216±0.0539
Spring				
Mean±SD	51.5±96.3	5.6±2.8	10.7±8.9	0.0091±0.0062
Summer				
Mean±SD	2.4±3.8	4.8±5.1	71.6±107.5	0.0435±0.0658
Fall				
Mean±SD	19.7±14.7	2.1±2.9	3.6±5.7	0.0045±0.0068
Two-way ANOVA results				
Land use				
$F_{1,59}$	0.105	0.014	0.914	0.094
p value	0.747	0.908	0.343	0.764
Season				
$F_{2,59}$	19.453	12.194	13.045	17.516
p value	<0.001	<0.001	<0.001	<0.001
Land use × season				
$F_{2,59}$	0.137	0.073	0.333	0.036
p value	0.872	0.930	0.718	0.965

(0.29 m²) reaches. In the agricultural reaches, $A_s:A$ was higher (Table 2). Relative transient storage was quite variable regardless of reach type and was recorded as high as 13.4. A seasonal effect was detected, as $A_s:A$ was highest during summer compared with spring and fall (Table 2). The majority of DaI values for the sites ranged from 0.4 to 7.8 (although two sites had DaI values of 12.5 and 22.4) and had a mean value close to 1 (DaI = 2.9 ± 3.2).

The NO_3^- loads were similar in the agricultural and the forested reaches (Table 3); during summer, NO_3^- loads were lower than in fall and spring (Table 3). The denitrification rates ranged from <0.1 to 17.2 mg N m^{-2} h^{-1} and were similar in the agricultural and the forested reaches (Table 3). A seasonal effect was detected and denitrification rates were highest during summer and spring and lowest during fall (Table 3). We used two different metrics to measure N removal capacity in our study sites. Nitrogen removal was similar in agricultural and forested reaches in terms of k and v_f (Table 3). Loss rate constants and v_f were highest during summer and spring and lowest during fall (Table 3). To examine overall N removal capacity we compared k against in-stream NO_3^- load in the spring, summer, and fall (Fig. 2). During spring and fall k was low while NO_3^- loads were high, and during summer k was high while NO_3^- loads were low.

A RDA was used to determine which specific stream characteristics correlated with the N removal metrics (i.e., k and v_f). Eigen analysis of the first two principal component axes determined that 57.1% of the variance was explained. A Monte Carlo test indicated that the overall model was significant ($F_{2,57} = 8.479$, $p < 0.001$). Both removal metrics were strongly aligned with principal axis 1 (Fig. 3). Stream characteristics with high correlations with axis 1 were considered important variables affecting N removal. The top three variables in decreasing correlation with axis 1 were temperature ($r^2 = 0.67$), in-stream NO_3^- concentration ($r^2 = -0.46$), and $A_s:A$ ($r^2 = 0.44$). All other stream characteristics had correlations of less than 0.35 with principal axis 1 and were considered not important in explaining N removal. Regression analysis between the top three variables and k revealed significant relationships but the relationship with in-stream NO_3^- had the highest regression coefficient (Fig. 4).

Discussion

Nitrogen removal capacity—Denitrification rates measured in our study were similar to rates measured in other low-order streams with high in-stream NO_3^- concentrations (Royer et al. 2004; Schaller et al. 2004; Smith et al. 2006). When comparing N removal in streams with variable geomorphology, v_f should be used because this metric factors out variation in hydraulic transport variables (e.g., stream velocity) that can dominate other removal metrics such as spiraling length (Stream Solute Workshop 1990; Davis and Minshall 1999). Compared with other low-order streams our values of v_f were low (Hall and Tank 2003; Bernot et al. 2006; Earl et al. 2006). The maximum in-stream NO_3^- concentration measured in these other studies was ~3 mg NO_3^--N L^{-1}, whereas in our sites the maximum in-stream NO_3^- concentration measured was >40 mg NO_3^--N L^{-1}. As NO_3^- concentrations increase, v_f responds by decreasing exponentially until reaching a constant value near zero (Davis and Minshall 1999; Earl et al. 2006; Mulholland et al. 2008). Elevated concentrations of NO_3^- most likely suppressed N removal in our streams, resulting in v_f being low compared with other studies.

We used k to assess N removal capacity in our streams relative to concentrations in the overlying water. Alexander
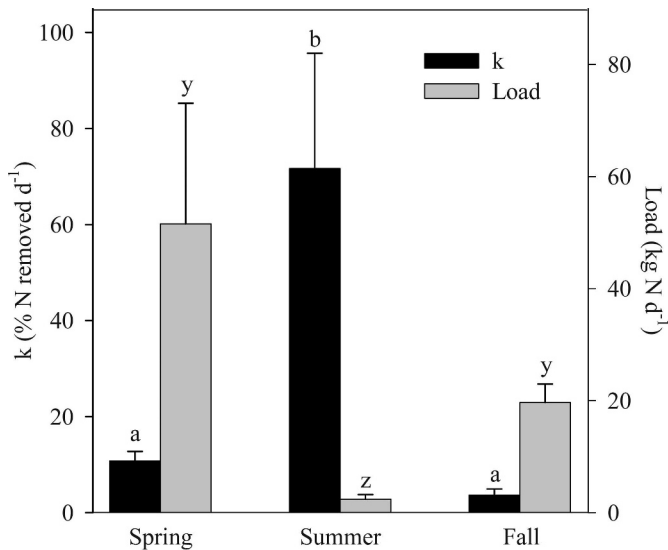
Fig. 2. Seasonal comparison of the nitrate (NO_3^-) loss rate constant (k) and NO_3^- load. Since there were no differences between agricultural and forested reaches for k and NO_3^- load, data are from all sites combined. During summer, k was highest while NO_3^- load was lowest, indicating a strong N removal potential during this period. However, during spring and fall, k was low while NO_3^- load was substantially higher, indicating a period of high N export. Bars with similar letters are not significantly different (*see* Tables 2 and 3 for statistics).

et al. (2000) modeled stream systems in the Mississippi River Basin and determined that k = 45% N removed per day for low-order streams (i.e., mean depths less than 1 m). Over the seasons we sampled, the headwater streams in the Upper Sugar Creek were underperforming (k = 28.7% N removed per day) compared with the rate assumed by Alexander et al. (2000). During summer, k was high but this corresponded to the period when NO_3^- loads were low. Conversely, during spring and fall when k was low, NO_3^- loads were high. Therefore, annual N removal was low in our study sites and N export from these streams was most likely high. This finding is similar to agricultural streams in Ontario (Hill 1979) and Illinois (Royer et al. 2004) in which substantial N removal occurred during brief periods in summer when discharge and NO_3^- concentrations were low, but annually these systems exported significant amounts of in-stream N.

Stream characteristics that control N removal—Recent studies have indicated that headwater streams may be effective at removing in-stream NO_3^- (Alexander et al. 2000; Peterson et al. 2001) and retaining N in general (Tank et al. 2000; Peterson et al. 2001; Hall and Tank 2003). This research, with the exception of Alexander et al. (2000), has been conducted in forested headwater streams with low in-stream NO_3^- concentrations. Our study and other research in agricultural headwater streams have indicated that these particular streams are ineffective at N removal because of NO_3^- saturation (Haggard et al. 2001; Royer et al. 2004; Bernot et al. 2006). Studies in a variety of stream types have identified several factors to be important in removing N, such as organic carbon supply (Groffman et al. 2005),

NO_3^- concentration (Bernot et al. 2006; Gücker and Pusch 2006; Herrman et al. 2008), ratio of depth to HRT (Seitzinger et al. 2002), discharge (Peterson et al. 2001), stream depth (Alexander et al. 2000), and relative transient storage (Valett et al. 1996). Our research was unique because it determined the importance of in-stream transport (i.e., transient storage and HRT) and several other stream characteristics on N removal in headwater streams with high NO_3^- concentrations and variable riparian land use.

The forested headwater reaches in our study had high HRT and high in-stream NO_3^- concentrations, whereas the agricultural reaches had low HRT and high in-stream NO_3^- concentrations. Both stream types had high transient storage as indicated by $A_s:A$ values being greater than 1, although relative transient storage was higher in the agricultural reaches. Transient storage and HRT are two hydraulic variables that slow the transport of solutes and allow for greater opportunities for removal. Carbon availability to support denitrification was similar in terms of sedimentary carbon and DOC in the two reach types over all seasons. Other research in fragmented agricultural landscapes found similar results and determined that high stream velocities throughout the watershed did not allow for carbon to preferentially accumulate in any specific reach type (Sweeney et al. 2004).

Overall, the agricultural and forested reaches had similar N removal capacities despite differences in HRT. Because land use did not affect N removal capacity, we examined other predictors of N removal. On the basis of RDA, temperature, NO_3^-, and $A_s:A$ were identified as key variables affecting N removal. Regression analysis revealed that NO_3^- had the strongest relation with k and the exponential relationship suggests that these systems were NO_3^- saturated (Davis and Minshall 1999; Bernot and Dodds 2005; Earl et al. 2006). Temperature and relative transient storage were also identified as important variables, but on the basis of their weak correlations with k we concluded that these variables only had a potential effect on N removal. Most likely, NO_3^- saturation was reducing the efficiency of in-stream N removal regardless of land use and in-stream transport mechanisms. Although hydrological transport variables may be important in other headwater streams, in our agricultural streams the high concentrations of inorganic N overwhelmed removal capacity.

Implications—As previously stated, Alexander et al. (2000) estimated k = 45% N removed per day for headwater streams in the Mississippi River Basin. In our sites, k was high at low NO_3^- concentrations, and as the concentration increased, k declined exponentially until reaching a constant value near 5% N removed per day (Fig. 4b). Closer examination of our data reveals that when $k \sim$ 45% N removed per day the in-stream NO_3^- concentration was approximately 2 mg NO_3^--N L^{-1} (Fig. 4b). This concentration represents the threshold above which the study sites increasingly become saturated with NO_3^- and export substantial N. When NO_3^- concentrations are at or below this threshold, the streams
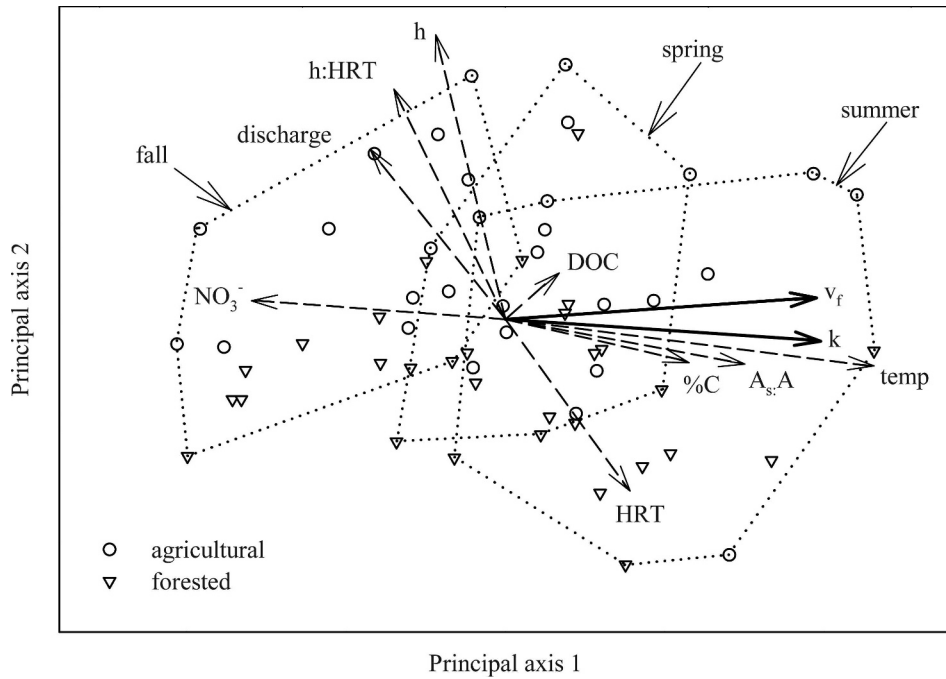
Fig. 3.   Redundancy analysis (RDA) ordination diagram showing agricultural and forested sites. A Monte Carlo test found the overall model to be significant ($p < 0.001$) and eigen analysis determined that 57.1% of the variance in the data set was explained by the first two principal component axes. We used two metrics of N removal, loss rate constant (k) and uptake velocity (v_f) as response variables in the RDA and these are represented by the solid arrows. The dashed arrows are independent variables; we identified temperature, NO_3^-, and relative transient storage ($A_s : A$) to be highly correlated with N removal. Summer appeared to have the highest N removal capacity compared with fall and spring. HRT is hydraulic residence time, %C is sedimentary carbon, DOC is dissolved organic carbon, and h is water column depth.

may remove significant amounts of N via denitrification. We also postulate that for hydrological factors to affect N removal, in-stream NO_3^- concentrations need to be at or below this threshold. Increased transient storage and longer HRT will result in greater contact time with denitrifiers, but if the microbial community is saturated with NO_3^-, slowing the transport of solutes will have less effect on N

removal in lotic systems. Thus, our study is in agreement with the growing body of research stating that the most important objective in reducing N export from stream ecosystems is to decrease the amount of inorganic N inputs from the surrounding landscape (Bernot and Dodds 2005; Earl et al. 2006; Gücker and Pusch 2006; Mulholland et al. 2008).
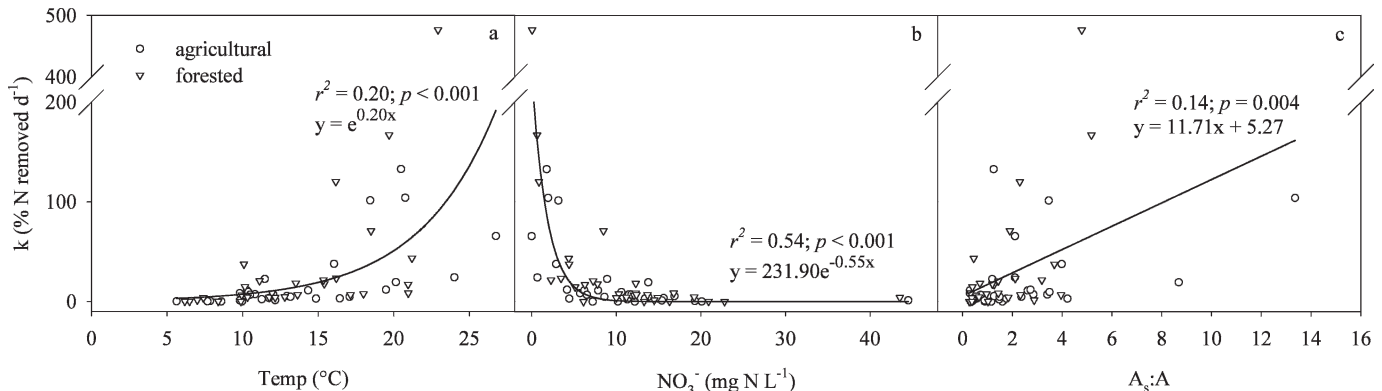

Fig. 4.   Regression analysis of the NO_3^- loss rate constant (k) on (a) temperature, (b) in-stream NO_3^- concentration, and (c) relative transient storage ($A_s : A$). No differences were observed between k in agricultural and forested sites. Therefore, best-fit trend lines were created using all data. In-stream NO_3^- best predicted k and this exponential decay indicates that streams became increasingly NO_3^- saturated as NO_3^- concentrations increased. It appears that when NO_3^- concentrations were above 2 mg NO_3^--N L^{-1}, N removal was minimal relative to concentrations and the streams were dominated by N export.

Our assessment of N removal in the Upper Sugar Creek Watershed revealed that these headwater streams remove relatively little in-stream N because of NO_3^- saturation. Only during summer months when NO_3^- concentrations were low was substantial N removal observed. In this fragmented agricultural watershed, high NO_3^- loads were observed during spring and fall. During these periods N removal mechanisms were overwhelmed and this most likely resulted in high amounts of N export. Although other studies have identified hydrologic factors to be highly correlated with N removal, we were unable to find such relationships. This was most likely due to the saturating concentrations of NO_3^- in our study streams regardless of riparian land use.

The implications of this study are not only important for understanding N cycling in headwater streams, but also to provide insight into stream restoration and management in eutrophic watersheds. To understand N removal capacities stream studies must consider all seasons, as hydrology and microbial activity vary temporally. Further, the forested reaches with high HRT and high transient storage removed similar magnitudes of N compared with agricultural reaches with low HRT and high transient storage. Stream restoration will sometimes attempt to increase HRT and transient storage by remeandering streams in addition to improving stream habitat. Our study found that streams with high in-stream NO_3^- will not be effective at removing N even when they have high HRT and high transient storage. We suggest that hydrologic transport variables do not affect N removal until NO_3^- falls below a threshold concentration of 2 mg NO_3^--N L^{-1}. We do acknowledge that our study was not designed to test such restoration efforts and stress that our results only provide evidence for such conclusions.

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