Landscape and Watershed Processes

Transport and Fate of Nitrate in Headwater Agricultural Streams in Illinois

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ABSTRACT

Nitrogen inputs to the Gulf of Mexico have increased during recent decades and agricultural regions in the upper Midwest, such as those in Illinois, are a major source of N to the Mississippi River. How strongly denitrification affects the transport of nitrate (NO_3–N) in Illinois streams has not been directly assessed. We used the nutrient spiraling model to assess the role of in-stream denitrification in affecting the concentration and downstream transport of NO_3–N in five headwater streams in agricultural areas of east-central Illinois. Denitrification in stream sediments was measured approximately monthly from April 2001 through January 2002. Denitrification rates tended to be high (up to 15 mg N m^{-2} h^{-1}), but the concentration of NO_3–N in the streams was also high (>7 mg N L^{-1}). Uptake velocities for NO_3–N (uptake rate/concentration) were lower than reported for undisturbed streams, indicating that denitrification was not an efficient N sink relative to the concentration of NO_3–N in the water column. Denitrification uptake lengths (the average distance NO_3–N travels before being denitrified) were long and indicated that denitrification in the streambed did not affect the transport of NO_3–N. Loss rates for NO_3–N in the streams were <5% d^{-1} except during periods of low discharge and low NO_3–N concentration, which occurred only in late summer and early autumn. Annually, most NO_3–N in these headwater sites appeared to be exported to downstream water bodies rather than denitrified, suggesting previous estimates of N losses through in-stream denitrification may have been overestimated.

THE POLLUTING OF North American coastal waters by river-borne nutrients, primarily N and P, during recent decades has resulted in coastal eutrophication and increased occurrences of harmful algal blooms, hypoxic zones, and fish kills (National Research Council, 2000; Boesch et al., 2001; Diaz, 2001). For example, the N load carried by the Mississippi River to the Gulf of Mexico has increased since the middle of the 20th century (Turner and Rabalais, 1991) and is now causing the development of seasonal hypoxia in an increasingly large area in the Gulf of Mexico (e.g., Rabalais et al., 2002). Nonpoint sources, particularly agricultural runoff, are often responsible for the increased nutrient loads carried by rivers to coastal waters (National Research Council, 2000; Boesch et al., 2001).

Streams in the agricultural regions of the upper Midwest are major contributors to N loads in the Mississippi River (Alexander et al., 2000; Goolsby et al., 1999). In the intensively agricultural areas of Illinois, watershed yields of total N are typically >15 kg N ha^{-1} yr^{-1} (Goolsby et al., 1999) and can exceed 40 kg N ha^{-1} yr^{-1} in wet years (David and Gentry, 2000). In contrast, yields from the western regions of the Mississippi River basin are typically <1 kg N ha^{-1} yr^{-1} (Goolsby et al., 1999; Alexander et al., 2000). The dissolved N pool in agricultural streams of the Midwest is dominated by NO_3–N, which can often exceed 10 mg NO_3–N L^{-1} in headwater streams (David et al., 1997; Goolsby et al., 1999).

As NO_3–N is transported downstream it is subject to retention via biotic and abiotic processes. The nutrient spiraling concept (Newbold, 1992) describes the downstream movement of N as it cycles between organic N retained in biomass and dissolved inorganic N in the water column. Of the processes affecting NO_3–N retention in streams, only denitrification results in a loss of N from the riverine system. Assimilatory uptake by vascular plants, algae, and microbes generally represents only short-term retention of NO_3–N because the organic N is eventually remineralized. Quantifying the role of in-stream denitrification and the fate of NO_3–N during stream transport will improve our understanding of the links between the agricultural areas of the Midwest and N loading to the Gulf of Mexico.

As stream size increases the likelihood of NO_3–N being denitrified declines sharply (Howarth et al., 1996; Alexander et al., 2000), suggesting that headwater streams are critical locations for processing N (Peterson et al., 2001). However, this generality appears not to hold in all cases. In-stream denitrification had no substantial effect on the annual export of NO_3–N from agricultural streams in Ontario (Hill, 1979) and Sweden (Jansson et al., 1994), or forested streams in the southern Appalachian Mountains (Martin et al., 2001). Conversely, mass-balance studies, although not measuring denitrification directly, often identify it as a major pathway for N loss (e.g., Howarth et al., 1996; Alexander et al., 2000; David and Gentry, 2000). For example, previous work in Illinois suggested that, state-wide, in-stream denitrification could be a substantial sink for N, potentially accounting for 132 000 Mg N yr^{-1} (Mg = 10^6 g) (David and Gentry, 2000). Similarly, Alexander et al. (2000) determined that for headwater streams in the Mississippi River basin, 45.5% of the N load was retained per day of travel time, presumably through in-stream denitrification.

We examined the role of in-stream denitrification as

Abbreviations: DOC, dissolved organic carbon.

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a mechanism for N retention in five headwater, agricultural streams in east-central Illinois. We hypothesized that rates of in-stream denitrification would be high and have a significant effect on the flux of NO_3–N from the streams. Our approach was to use the nutrient spiraling concept (Newbold, 1992) to assess how denitrification affected the transport of NO_3–N through the streams. Nutrient spiraling metrics are commonly used to describe the transport and uptake of N in streams with low available N (e.g., Peterson et al., 2001; Hall and Tank, 2003), but an application of nutrient spiraling to examine N processing in N-rich agricultural streams has not been conducted. Our specific objectives in this paper are to (i) estimate the distance traveled by NO_3–N in the streams before being denitrified; (ii) calculate the loss rates for NO_3–N due to denitrification; and (iii) describe environmental factors, such as nutrient limitation and hydrology, responsible for temporal patterns in how denitrification influenced the dynamics of NO_3–N in these streams.

MATERIALS AND METHODS

Study Sites

The study was conducted from January 2001 through January 2002 in five headwater sites within three of the major river basins in east-central Illinois: the Sangamon, Embarras, and Kaskaskia Rivers. Each site was located in an area of extensive row-crop agriculture (Table 1). All but the fourth-order site are located in incised, channelized streams with grass as the only riparian vegetation; a description of channel alterations to the streams of east-central Illinois is given in Rhoads and Herricks (1996). The fourth-order site is located in a nonincised section of the Embarras River with a narrow corridor of hardwood forest, although all upstream tributaries are channelized and land use in the basin as a whole is dominated by agriculture. The study streams are low gradient and characterized by the flashy hydrology typical of streams in the area (David et al., 1997; Mitchell et al., 2000). However, floods rarely overtop the stream banks because of the incised channels. As is common in the Midwest, the fertilized cropland within these watersheds is underlain by extensive networks of tiles that drain excess water and associated solutes directly to headwater streams (David et al., 1997). Because of this, stream water concentrations of NO_3–N in east-central Illinois routinely exceed 10 mg L^{-1} and can approach 20 mg NO_3–N L^{-1} following heavy precipitation (David et al., 1997; Mitchell et al., 2000).

Monitoring Physiochemical Variables

To further characterize the streams and relate the denitrification measurements to annual patterns in water chemistry and hydrology, we measured several environmental variables at each of the sites during the study. Water chemistry was sampled intensively throughout the study with a combination of routine grab samples (approximately weekly) supplemented by automated samplers that collected samples more frequently during floods. Water samples were analyzed for major nutrients including NO_3–N, NH_4–N, organic N, soluble reactive phosphorus (SRP), total P, and dissolved organic carbon (DOC) using standard methods (American Public Health Administration, 1998) and a Dionex (Sunnyvale, CA) DX-120 ion chromatograph, Technicon (Tarrytown, NY) Autoanalyzer, or Dohrmann (Mason, OH) DC-80 carbon analyzer. Organic N was determined as the difference between total N and the dissolved inorganic fractions.

Water temperature in the streams was recorded hourly with HOBO temperature loggers (Onset Computer, Bourne, MA). Discharge was monitored by either installing a stilling well and stage recorder or locating the sites in proximity to existing gaging stations operated by the USGS or the Illinois State Water Survey. Size composition of the benthic sediments was determined twice during spring 2001 at each site by collecting sediment samples from the upper 5 cm of the streambed, drying the material, and sieving it into particle classes. Four classes were used: coarse particulate organic matter (>1 mm), gravel (>2 mm), sand (53 μm–2 mm), and fine sediment (<53-μm organic and inorganic particles). The relative abundance of each class was expressed as a percentage of the dry mass of the total sample.

Denitrification Rates

Rates of benthic denitrification were measured at the sites from April 2001 through January 2002. Sediment sampling was at times prevented by floods or ice cover, and occasionally during late summer the small streams would dry up. The sampling regime varied among the sites, but no site was sampled fewer than five times. We used the C_2H_2 inhibition method to estimate rates of benthic denitrification in sediment slurries (Knowles, 1990; Martin et al., 2001). Chloramphenicol, an antibiotic that suppresses de novo enzyme production but does not inhibit the action of existing enzymes, was added to the slurries at a concentration of 5 m*M*. The use of chloramphenicol in sediment slurries reduces bottle effects and improves estimates of in situ denitrification rates (Smith and Tiedje, 1979). The assays were limited in duration to 3 h and conducted at stream temperature without the addition of NO_3–N or DOC beyond that in the stream water (except for enrichment experiments described below). Based on our results and those of another study (Rudolph et al., 1991), we believe the C_2H_2 inhibition method was appropriate for these streams and did not bias the conclusions of the study (see Discussion).

For all assays, benthic sediments were collected from the upper 5 cm of the stream bed at several locations within a 10-m reach of the stream. The sediments were combined into a composite sample and taken immediately to the laboratory

Table 1. Site abbreviations, coordinates, and channel and watershed characteristics for the study sites on agricultural streams in east-central Illinois.

Stream site	Coordinates	Stream order	River basin†	Watershed area	Row-crop agriculture	Channel slope	Mean depth‡	Mean velocity‡
				km²	% land cover	%	m	m s⁻¹
BDT	**40°17′20″N, 88°17′37″W**	**1**	**1**	**13**	**80**	**0.3**	**0.31 (0.08)**	**0.19 (0.09)**
BLS	**39°57′07″N, 88°10′10″W**	**1**	**2**	**25**	**85**	**0.1**	**0.40 (0.15)**	**0.32 (0.08)**
BDO	**40°16′06″N, 88°19′35″W**	**2**	**1**	**101**	**80**	**0.1**	**0.28 (0.14)**	**0.36 (0.04)**
LFK	**39°50′09″N, 88°29′18″W**	**3**	**3**	**365**	**91**	**<0.1**	**0.53 (0.16)**	**0.17 (0.09)**
EMC	**39°47′29″N, 88°11′08″W**	**4**	**2**	**481**	**85**	**<0.1**	**0.58 (0.07)**	**0.15 (0.07)**

† 1, Upper Sangamon; 2, Upper Embarras; 3, Lake Fork Kaskaskia.

‡ Mean water depth and velocity reflect baseflow conditions during 2001. Values in parentheses are one standard deviation.

where 25 to 30 cm^3 of sediment was placed in 150-mL media bottles ($n = 4$ per date and site). Unfiltered stream water was added to bring the total volume of the sediment slurry to 75 mL. Oxygen in the headspace and slurry was removed by purging the media bottles with ultrapure helium; bottles were shaken periodically during the purging. We conducted enrichment experiments in August 2001 at two of the sites to test for limitation of denitrification by N or DOC. Three treatments were used: ambient, +NO$_3$–N, and +DOC. For the +NO$_3$–N treatment, 1 mL of stock solution (0.35 mg NO$_3$–N mL^{-1}) was added to the media bottles so that the final concentration in the bottles was approximately 5 mg NO$_3$–N L^{-1}, depending on the ambient concentration. Glucose was used for the DOC amendment and the final concentration in the bottles was approximately 30 mg L^{-1}.

During all assays, the bottles were kept in the dark in an incubator set at ambient stream temperature, and were not shaken except before sampling the headspace to equilibrate N$_2$O in the sediment and aqueous phases. Gas samples were collected from the headspace of each media bottle at the beginning of the assay and hourly thereafter. Samples were analyzed for N$_2$O on a Varian (Palo Alto, CA) 3600 gas chromatograph equipped with a Porapak Q column and a ^{63}Ni electron-capture detector (oven temperature = 70°C, flow rate = 30 mL min^{-1}). Following removal of the final gas sample, the sediment in each bottle was collected and the dry mass and ash-free dry mass (AFDM) measured by drying the sediment at 60°C, combusting the organics at 550°C, rewetting the sediment, drying at 60°C, and obtaining the final mass (difference between pre- and post-combustion mass = AFDM).

For each site and date, five quantitative sediment samples were collected, and the AFDM was measured in each to estimate the standing stock of AFDM in the upper 5 cm of the stream bed. To express the denitrification rates on an areal basis, the rates were multiplied by the mean standing stock (g AFDM m^{-2}) at each site.

Nutrient Spiraling Metrics

To determine the importance of in-stream denitrification as a mechanism for NO$_3$–N retention, we calculated the denitrification uptake length ($S_{\mathrm{w,dn}}$), defined as the average distance a NO$_3$–N molecule travels before being denitrified in the benthic sediments of a stream (Fig. 1). This term is similar to traditional uptake length (S_{w}) (Newbold, 1992) but differs in that it is a spiraling metric specific for denitrification. The term $S_{\mathrm{w,dn}}$ was calculated as:

$$S_{\mathrm{w,dn}} = vh/V_{\mathrm{f,dn}} \qquad [1]$$

where v is stream velocity (m s^{-1}), h is stream depth (m), and $V_{\mathrm{f,dn}}$ is the uptake velocity of NO$_3$–N due to denitrification in the benthic sediments. The term $V_{\mathrm{f,dn}}$ was calculated as:

$$V_{\mathrm{f,dn}} = U/C \qquad [2]$$

where U is the areal denitrification rate (mg N m^{-2} s^{-1}) in the sediments and C is NO$_3$–N concentration in the water column (mg m^{-3}). Short uptake lengths (approximately ≤1 km) indicate that NO$_3$–N is likely to be denitrified in the stream before traveling long distances downstream. Conversely, long uptake lengths indicate that denitrification has little influence on the export of NO$_3$–N from the watershed and that NO$_3$–N entering headwater streams is likely to reach larger rivers and downstream water bodies.

To compare our assessment of the role of denitrification with that presented by Alexander et al. (2000), we also calculated the loss rate, $-k$, for NO$_3$–N in the streams. Values for $-k$ were calculated as:

$$-k = V_{\mathrm{f,dn}}/h \qquad [3]$$

and then scaled from a fraction s^{-1} to % d^{-1}. This metric is an instantaneous measure of the percentage of the NO$_3$–N load lost to denitrification per day and indicates the effect that denitrification has on the concentration and load of NO$_3$–N in the stream water.

For the past 4 to 8 yr, intensive monitoring of NO$_3$–N concentrations has been conducted with a combination of flow-triggered automatic samplers and routine manual sampling at three of the sites (EMC, BDO, and LFK; see Table 1). This has allowed us to measure NO$_3$–N concentrations during short-term periods of high discharge when much of the annual export of NO$_3$–N occurs in these agricultural watersheds (David et al., 1997; Mitchell et al., 2000). Historic values of $S_{\mathrm{w,dn}}$ were estimated as described above (Eq. [1] and [2]) using mean depth and velocity determined from the gaging records,

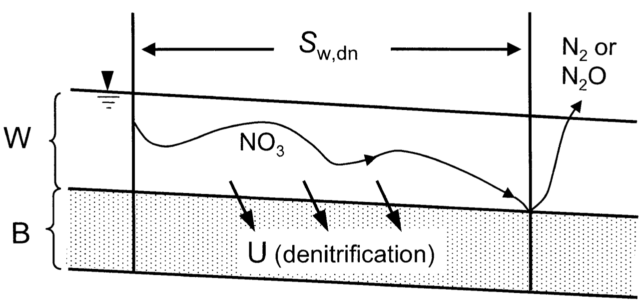

Fig. 1. Conceptual diagram of nutrient spiraling (modified from Newbold, 1992). The stream consists of two compartments, the water column (W) and the benthic sediments (B). In this model, uptake (U) of NO$_3$–N is due to denitrification, making the uptake length denitrification-specific.

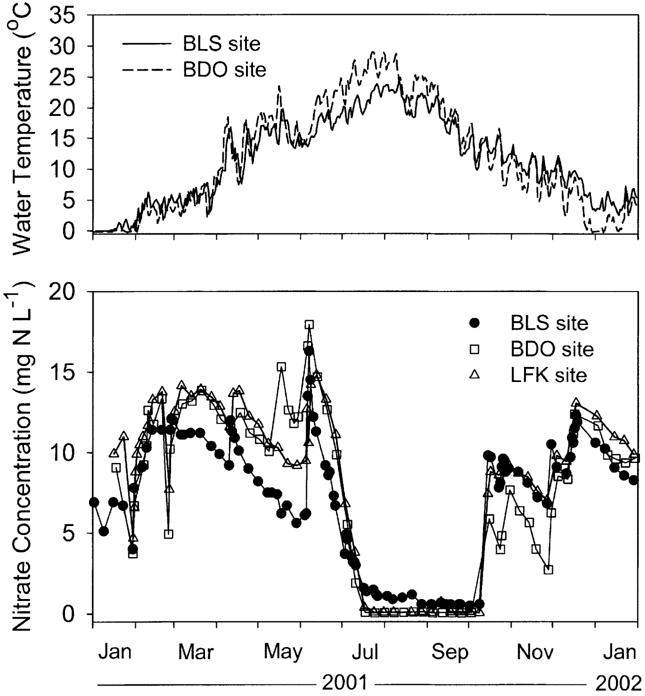

Fig. 2. Mean daily water temperature and stream NO$_3$–N concentrations at selected sites from January 2001 through January 2002. All sites showed similar patterns in temperature and NO$_3$–N, and therefore only selected sites are shown. (Site characteristics are given in Table 1.)

NO_3–N concentrations from the intensive monitoring, and a denitrification rate of 15 mg N m^{-2} h^{-1}, the highest value measured during the study (see Results). By using a constant and high rate of denitrification, our long-term assessment describes the maximum role of denitrification as a N sink and gives conservative estimates of the distance NO_3–N traveled in these streams. We recognize that stream depths and velocities estimated from gaging records represent conditions at the gaging station, but not necessarily conditions throughout a stream reach. However, we believe this approach is robust enough to allow general conclusions regarding long-term temporal patterns in the effect of denitrification on NO_3–N dynamics in these streams.

RESULTS

Physiochemical Variables

Stream NO_3–N concentrations were high (5–15 mg NO_3–N L^{-1}) for the first 6 mo of 2001 and then declined dramatically during July to <1 mg NO_3–N L^{-1} before increasing again in October (Fig. 2, Table 2). Other forms of N had lower concentrations and a smaller range of values than did NO_3–N (Table 2). This pattern was consistent among all the study streams and is typical of the annual pattern for agricultural streams in east-central Illinois (David et al., 1997; Mitchell et al., 2000). During 2001, mean values of DOC among the sites ranged from approximately 2 to 7 mg L^{-1} with the lowest values occurring during autumn and early winter (Table 2). Stream concentrations of total P and soluble reactive P are affected by agricultural drainage and tended to be greatest during the first 6 mo of 2001 (Table 2). During floods, soluble reactive P concentrations exceeded 1 mg P L^{-1} in 2001, but during baseflow mean values were generally <75 µg P L^{-1} (Table 2).

Mean daily water temperatures during 2001 were <8°C until approximately April. From April on, temperatures increased steadily, reaching peaks of 25 to 30°C in late summer (Fig. 2). Benthic sediments in the streams were predominately sand and gravel with these two classes representing 95% or more of the sediment dry mass at all sites. Fine sediments (particles <53 µm), although visually abundant at each site, represented only 1 to 3% of the dry mass. Only EMC, the site with a narrow forest corridor, had measurable amounts (4%) of coarse particulate organic matter. Based on visual observations, the composition and texture of the sediments at each site did not change noticeably during the study period.

Denitrification Rates

Rates of in-stream denitrification ranged during the study from <0.1 to 15 mg N m^{-2} h^{-1} (>1000 µmol N m^{-2} h^{-1}) (Table 3). No consistent pattern was observed between stream size and rates of denitrification. Overall, denitrification rates were highly variable during the course of the study both within and among sites. There was no consistent relationship between concentrations of NO_3–N in the stream water and rates of denitrification in the benthic sediments. However, concentrations dropped below 5 mg NO_3–N L^{-1} only during July through September.

The two sites used for the enrichment study, BLS and BDO, had ambient nitrate concentrations of 1.05 and 0.36 mg NO_3–N L^{-1}, respectively, on the days the experiments were performed. Nitrate amendments to sediments from each site significantly increased denitrification rates above the rates measured under ambient conditions in August (Fig. 3; ANOVA, $P < 0.001$, Tukey's pairwise comparisons, $\alpha = 0.05$). Amendments with DOC did not affect denitrification rates in sediments from either site.

Table 2. Mean nutrient concentrations in the study sites in east-central Illinois from January 2001 through January 2002.

Site†	*n*	NO_3–N	NH_4–N	Organic N	Dissolved organic C	Soluble reactive P	Total P
		mg L^{-1}				µg L^{-1}	
				January 2001–mid-July 2001‡			
BDT	**33**	**12.6 (3.5)§**	**0.67 (2.20)**	**0.30 (0.52)**	**3.0 (2.4)**	**424 (1240)**	**505 (1379)**
BLS	**34**	**8.7 (2.7)**	**0.30 (1.06)**	**0.81 (2.44)**	**2.6 (2.3)**	**52 (87)**	**123 (273)**
BDO	**32**	**10.9 (3.7)**	**0.27 (0.61)**	**0.96 (2.61)**	**3.3 (2.3)**	**150 (320)**	**223 (419)**
LFK	**32**	**11.3 (2.6)**	**0.06 (0.07)**	**0.63 (1.30)**	**2.3 (1.2)**	**65 (127)**	**107 (157)**
EMC	**36**	**10.7 (2.7)**	**0.20 (0.60)**	**0.39 (0.43)**	**3.0 (2.3)**	**97 (144)**	**138 (178)**
Grand mean	**5**	**10.8 (1.39)**	**0.30 (0.23)**	**0.62 (0.28)**	**2.8 (0.4)**	**160 (150)**	**220 (170)**
				mid-July 2001–mid-October 2001			
BDT	**12**	**0.14 (0.07)**	**0.22 (0.22)**	**1.01 (0.62)**	**6.8 (1.7)**	**139 (83)**	**294 (197)**
BLS	**13**	**0.87 (0.37)**	**0.03 (0.02)**	**0.40 (0.17)**	**3.0 (1.0)**	**55 (16)**	**78 (29)**
BDO	**12**	**0.08 (0.02)**	**0.01 (0.01)**	**1.06 (1.07)**	**5.2 (1.0)**	**72 (56)**	**119 (62)**
LFK	**13**	**0.18 (0.19)**	**0.12 (0.11)**	**1.31 (0.50)**	**8.2 (1.4)**	**62 (33)**	**192 (66)**
EMC	**12**	**1.5 (1.9)**	**0.03 (0.05)**	**0.54 (0.20)**	**4.2 (0.7)**	**73 (18)**	**149 (89)**
Grand mean	**5**	**0.56 (0.63)**	**0.08 (0.09)**	**0.86 (0.38)**	**5.5 (2.0)**	**80 (30)**	**170 (80)**
				mid-October 2001–January 2002			
BDT	**20**	**10.6 (4.3)**	**0.03 (0.04)**	**0.34 (0.54)**	**2.9 (1.7)**	**48 (58)**	**78 (92)**
BLS	**21**	**9.3 (1.3)**	**0.06 (0.11)**	**0.34 (0.48)**	**1.9 (1.0)**	**45 (44)**	**71 (58)**
BDO	**19**	**8.0 (3.1)**	**0.05 (0.06)**	**0.49 (0.49)**	**3.1 (2.0)**	**30 (47)**	**70 (89)**
LFK	**19**	**9.8 (1.7)**	**0.03 (0.03)**	**0.22 (0.38)**	**1.9 (0.7)**	**21 (26)**	**52 (34)**
EMC	**21**	**10.3 (1.5)**	**0.04 (0.04)**	**0.36 (0.54)**	**2.8 (1.8)**	**73 (85)**	**114 (120)**
Grand mean	**5**	**9.6 (1.0)**	**0.04 (0.01)**	**0.35 (0.10)**	**2.5 (0.6)**	**40 (20)**	**80 (20)**

† For site characteristics, see Table 1.

‡ Data are divided into three time periods that reflect the seasonal variation in discharge and agricultural drainage.

§ Values in parentheses are one standard deviation.

Table 3. Discharge, stream water concentration of NO_3–N, and benthic denitrification rates in the study sites from April 2001 through January 2002.

Site†	Discharge‡	NO_3–N‡	Denitrification
	$m^3\ s^{-1}$	$mg\ L^{-1}$	$mg\ N\ m^{-2}\ h^{-1}$
April			
BDT	0.08	13.3	1.1
BLS	0.38	12.2	2.3
BDO	1.08	11.3	3.3
LFK	1.84	12.5	15.0
EMC	0.76	11.2	2.2
May			
BDT	0.08	13.8	1.1
BLS	0.13	7.5	13.0
BDO	0.12	10.3	3.6
LFK	0.87	9.6	6.3
EMC	1.29	11.2	0.2
June			
BDT	0.07	15.8	0.2
BLS	0.23	11.3	0.8
BDO	0.84	15.1	13.6
LFK	1.55	12.8	2.4
EMC	0.80	8.1	<0.1
July			
BDT	0.02	7.8	1.9
BLS	0.06	3.0	7.0
BDO	0.12	0.6	8.6
EMC	0.73	6.3	1.7
September–November			
BLS	0.02	0.6	8.8
BLS	0.10	8.7	1.5
BDO	0.24	7.5	5.4
LFK	0.56	8.1	3.0
December–January			
BDT	0.01	11.8	5.1
BLS	0.16	9.0	0.1
BLS	0.07	8.9	0.2
BDO	0.11	9.6	<0.1
LFK	0.85	10.7	3.9
EMC	0.93	10.7	<0.1

† For site characteristics, see Table 1.

‡ Discharge and nitrate concentrations correspond to the day the denitrification assays were conducted.

Nutrient Spiraling Metrics

Denitrification uptake velocity, $V_{f,dn}$, is a measure of the efficiency of denitrification in the stream bed relative to the availability of NO_3–N in the water column. Values for $V_{f,dn}$ were low, with all but two measurements being less than 0.04 mm min^{-1} (Table 4). The denitrification uptake lengths ($S_{w,dn}$) were generally long and often exceeded 200 km (Table 4). The only values less than 100 km occurred during July and September and corresponded with the highest values of $V_{f,dn}$. Overall, the long uptake lengths indicated that denitrification did not affect the movement of NO_3–N through these headwater streams during the study period.

Using historical data on stream flow, values of $S_{w,dn}$ were calculated for three sites for the past 4 to 8 yr. All sites showed a similar, cyclic pattern in which $S_{w,dn}$ was longest seasonally from winter through mid-summer, corresponding to the time of highest NO_3–N concentrations (Fig. 4). During this period, values of $S_{w,dn}$ were routinely >200 km and occasionally exceeded 3000 km. Extremely high values of $S_{w,dn}$ should not be interpreted as predictive of the actual transport distance for NO_3–N because of the changes in stream morphology, hydrology, and uptake rates that occur over such distances. Rather, $S_{w,dn}$ serves as an index of the importance of in-stream denitrification. In this regard, the past values of $S_{w,dn}$ indicate that when NO_3–N concentrations increased annually with the onset of agricultural drainage, in-stream denitrification did not influence the load of NO_3–N moving through the streams. For example, when NO_3–N concentrations were 5 mg NO_3–N L^{-1} or greater, the median values of $S_{w,dn}$ ranged from 280 to 532 km among the three streams and the 25th percentiles ranged from 162 to 262 km.

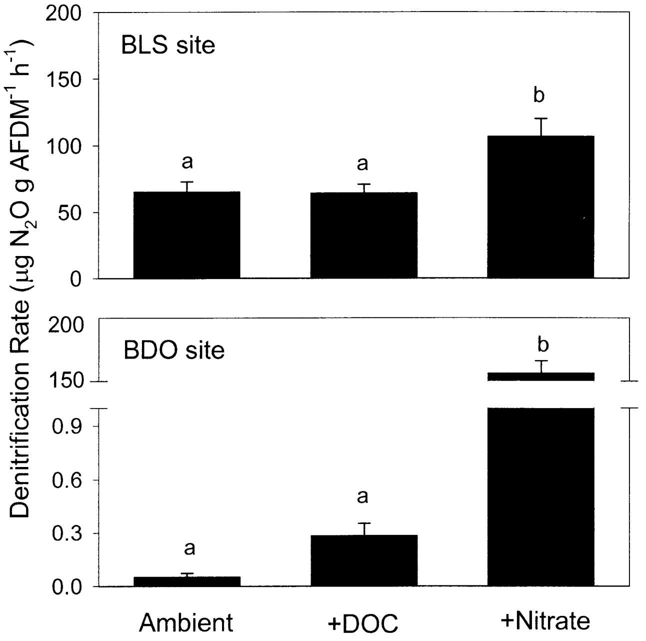

Fig. 3. Mean (plus one standard deviation) denitrification rates in August 2001 under ambient and amended conditions at two sites, BLS and BDO. (Site characteristics are given in Table 1.) Within a site, treatments with different letters are significantly different from each other ($p < 0.05$, Tukey's pairwise comparisons). AFDM, ash-free dry mass; DOC, dissolved organic carbon.

Nitrate N loss rates, $-k$, represent the fraction of the NO_3–N load lost to denitrification per day. The values of $-k$ were variable spatially and temporally, ranging from <0.1 to 273% d^{-1} (Table 4). Across all sites, the mean value for April through June was 2.4% d^{-1} (standard deviation = 3.5% d^{-1}). The greatest values of $-k$ occurred during late summer and autumn, particularly when high denitrification rates coincided with relatively low stream NO_3–N concentrations. On two occasions in July and September, $-k$ exceeded 100% d^{-1}, indicating that demand by in-stream denitrification could potentially remove 100% of the NO_3–N load, in the absence of nitrification or other sources of NO_3–N.

DISCUSSION

Obtaining accurate rates of in-stream denitrification can be difficult and expensive, and so the role of denitrification as a N sink is often estimated by difference using a mass balance approach (e.g., Howarth et al., 1996; Alexander et al., 2000; David and Gentry, 2000). We used the commonly accepted C_2H_2 inhibition tech-

Table 4. Denitrification uptake velocity ($V_{f,dn}$), uptake length ($S_{w,dn}$), and nitrate N loss rate ($-k$) calculated for the study sites from April 2001 through January 2002.†

Site‡	$V_{f,dn}$	$S_{w,dn}$	$-k$
	mm min^{-1} × 10^{-2}	km	% d^{-1}
	April		
BDT	**0.1**	**>200**	**0.5**
BLS	**0.3**	**>200**	**0.8**
BDO	**0.5**	**>200**	**2.3**
LFK	**2.0**	**>200**	**5.9**
EMC	**0.3**	**>200**	**0.8**
	May		
BDT	**0.1**	**>200**	**0.7**
BLS	**2.9**	**172**	**13.0**
BDO	**0.6**	**>200**	**3.6**
LFK	**1.1**	**>200**	**2.4**
EMC	**<0.1**	**>200**	**<0.1**
	June		
BDT	**<0.1**	**>200**	**<0.1**
BLS	**0.1**	**>200**	**0.4**
BDO	**1.5**	**>200**	**4.8**
LFK	**0.3**	**>200**	**0.7**
EMC	**<0.1**	**>200**	**<0.1**
	July		
BDT	**0.4**	**189**	**2.7**
BLS	**3.8**	**104**	**24.0**
BDO	**22.7**	**11**	**273.0**
EMC	**0.4**	**>200**	**1.1**
	September–November		
BLS	**23.8**	**8**	**131.0**
BLS	**0.3**	**>200**	**0.9**
BDO	**1.2**	**>200**	**9.7**
LFK	**0.6**	**>200**	**2.2**
	December–January		
BDT	**0.7**	**166**	**2.6**
BLS	**<0.1**	**>200**	**<0.1**
BLS	**<0.1**	**>200**	**<0.1**
BDO	**<0.1**	**>200**	**<0.1**
LFK	**0.6**	**>200**	**1.8**
EMC	**<0.1**	**>200**	**<0.1**

† See text for equations and descriptions of the spiraling metrics.
‡ For site characteristics, see Table 1.

nique to measure denitrification in sediment slurries (Knowles, 1990). We recognize that the C_2H_2 inhibition technique can, in some situations, significantly underestimate denitrification rates (Seitzinger et al., 1993) and that it is particularly inappropriate when used in sediment cores with low available NO_3–N or coupled nitrification–denitrification (Rudolph et al., 1991; Seitzinger et al., 1993). Conversely, Rudolph et al. (1991) found that if NO_3–N was $\geq 10\ \mu M$, the C_2H_2 inhibition technique was acceptable for estimating in situ denitrification rates when used in sediment slurries, such as in our study. We also added chloramphenicol to the slurries (see above), which has been shown to improve estimates of in situ denitrification rates (Smith and Tiedje, 1979). We believe the C_2H_2 inhibition technique was appropriate for the streams we examined. The production of N_2O in the bottles was linear for all but three assays, and given the generally high denitrification rates we measured (see below) it is unlikely that the rates we report are underestimated to any great extent.

Among studies that have expressed in-stream denitrification rates in areal units, peak values are often <5 mg N m^{-2} h^{-1} (e.g., Seitzinger, 1988; Thompson et al., 2000; Kemp and Dodds, 2002), although rates up to 60 mg N m^{-2} h^{-1} have been reported (Howarth et al., 1996).

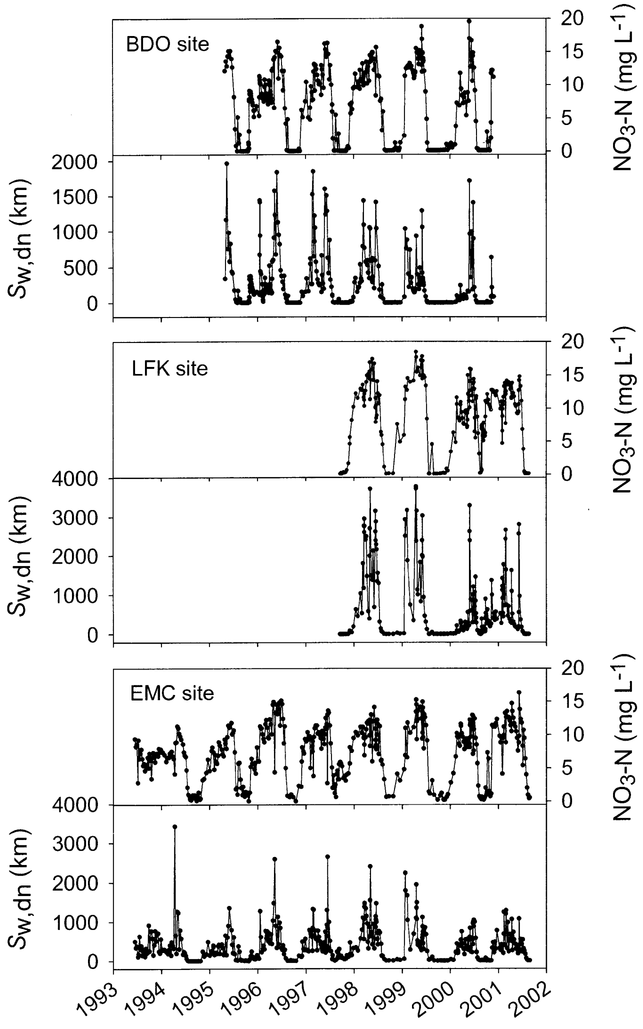

Fig. 4. Calculated denitrification uptake length ($S_{w,dn}$) and measured stream water concentrations of NO_3–N at sites BDO, LFK, and EMC, 1993–2002. (Site characteristics are given in Table 1.) The term $S_{w,dn}$ was calculated using stream gaging records and an in-stream denitrification rate of 15 mg N m^{-2} h^{-1}.

Using an innovative whole-stream technique, Laursen and Seitzinger (2002) made three estimates of denitrification in the Iroquois River system in Illinois and reported rates of 3.8, 47.6, and 118.6 mg N m^{-2} h^{-1} and suggested that rates in this range may be typical of low-gradient, agricultural streams. Likewise, denitrification rates tended to be high in the sites we examined, with nearly one-third of the measurements greater than 5 mg N m^{-2} h^{-1} and the highest value reaching 15 mg N m^{-2} h^{-1}. Our rates are in the range reported by Laursen and Seitzinger (2002) and demonstrate that benthic sediments in the agricultural streams of east-central Illinois are capable of supporting high rates of denitrification for much of the year. The rates we measured were highly variable spatially and temporally, as has also occurred in similar studies (Garcia-Ruiz et al., 1998; Thompson et al., 2000; Kemp and Dodds, 2002). A large degree of spatial and/or temporal variability may be a characteristic of denitrification in agricultural streams, particularly those in which discharge and NO_3–N loads are closely tied to precipitation and tile drainage.

The enrichment experiments showed that nitrate additions to sediment slurries increased denitrification rates in late summer, whereas rates did not respond to additions of labile DOC. Other workers have reported the same result for streams with low available N (Holmes et al., 1996; Martin et al., 2001). This pattern suggests that the availability of NO_3–N can at times limit denitrification in stream sediments, even in streams that are nitrate-rich for much of the year. For our sites, the period of nitrate limitation appeared to be late summer and early autumn when discharge and NO_3–N concentrations were low. An alternative explanation is that denitrification rates in late summer and autumn are driven by coupled nitrification–denitrification, rather than by NO_3–N in the water column. Our methods do not allow us to conclude whether denitrification in late summer was limited by NO_3–N concentrations in the water column or simply coupled at that time to nitrification. Regardless, late summer to early autumn is a period of low discharge and low NO_3–N concentrations for streams in east-central Illinois, making the time period relatively unimportant in terms of annual N export (David et al., 1997; Mitchell et al., 2000).

Uptake velocity is a measure of demand relative to concentration and reflects the efficiency of processes within the stream at removing a nutrient from the water column (Davis and Minshall, 1999). Other studies have reported uptake velocities for NO_3–N in streams that are two to three orders of magnitude greater than those we measured (Davis and Minshall, 1999; Peterson et al., 2001; Hall and Tank, 2003). This difference could have resulted from the fact that we examined only one of the processes (denitrification) that affects the uptake velocity of NO_3–N, although it is assumed to be the primary mechanism of N retention in streams of the Mississippi River basin (Alexander et al., 2000). Our $V_{\mathrm{f,dn}}$ results indicate that, although the rates were generally high, denitrification in the stream beds was not an efficient N sink relative to the concentration of NO_3–N in the water column. Because benthic denitrification did not affect water column concentrations of NO_3–N, the distance that NO_3–N was estimated to travel before being denitrified ($S_{\mathrm{w,dn}}$) tended to be long. The fate of NO_3–N in the headwater sites we studied appeared to be export to downstream water bodies, rather than denitrification.

In their assessment of N transport and retention in the Mississippi River basin, Alexander et al. (2000) used a mean annual loss rate ($-k$) of 45.5% d^{-1} for headwater streams. In the headwater streams we examined, $-k$ was $<5\%$ d^{-1} except during July through September. During July through September, in-stream denitrification did have a strong influence on NO_3–N movement through some of the streams (see Table 4), and this is probably the typical condition for late summer and autumn when agricultural drainage has ceased and NO_3–N concentrations often drop to <0.5 mg L^{-1}. In relation to annual load, however, high rates of NO_3–N loss during late summer and autumn are misleading, because by that time of the year most of the NO_3–N export has already occurred (David et al., 1997). On the basis of our results, we cannot rule out substantial in-stream retention of N at the scale of the Mississippi River basin, but in the headwater sites we examined denitrification in the benthic sediments did not appear to be a significant N sink relative to the annual load of N.

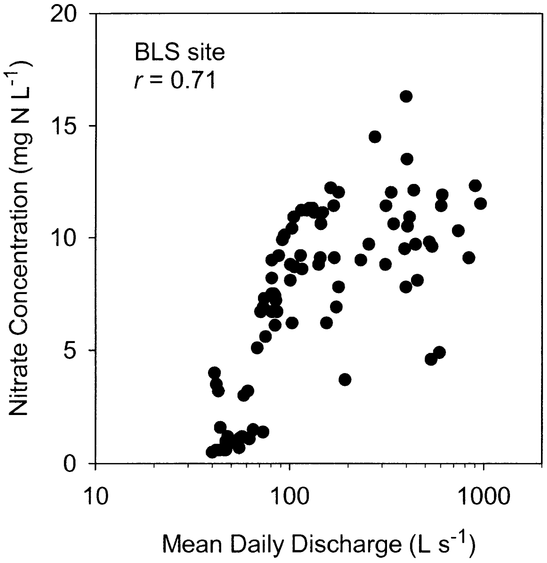

Fig. 5. Relationship between mean daily discharge and stream NO_3–N concentration for January 2001 through January 2002 in a headwater agricultural stream in Illinois. (Site characteristics are given in Table 1.)

Using the site with the most complete data set as an example (BLS), we show in Fig. 5 and 6 the relationship between hydrology (discharge), NO_3–N concentrations, and in-stream retention of NO_3–N by denitrification, as occurred in an agricultural stream in east-central Illinois. There is a strong relationship between increasing discharge and stream NO_3–N concentrations (Fig. 5) because most of the flow originates from agricultural drainage (David et al., 1997; Mitchell et al., 2000). Figure 6 shows the relationships between the concentration, $-k$, and mean daily load of NO_3–N from the watershed. Increases in discharge lead to corresponding increases in both NO_3–N concentrations and water depth, thereby reducing the ability of denitrification in the stream bed to affect the NO_3–N load. Alternatively, during late summer, when discharge and NO_3–N are both low, the stream bed is effective at removing NO_3–N from the water column and controlling the transport of NO_3–N through the stream.

Hydrology is a major factor influencing the retention of NO_3–N (and other nutrients) in streams. Meyer and Likens (1979) identified two distinct conditions for streams regarding nutrients: a processing-retention mode and a through-put mode. During periods of high discharge, nutrient inputs to the stream are quickly exported with little retention or processing (i.e., through-put mode). We suggest that hydrology is the primary control on the export of NO_3–N from headwater agricultural streams and that the streams are in a through-put mode (Meyer and Likens, 1979) for all but a few weeks in late summer and autumn. The switching between through-put and processing modes is shown clearly by the estimates of

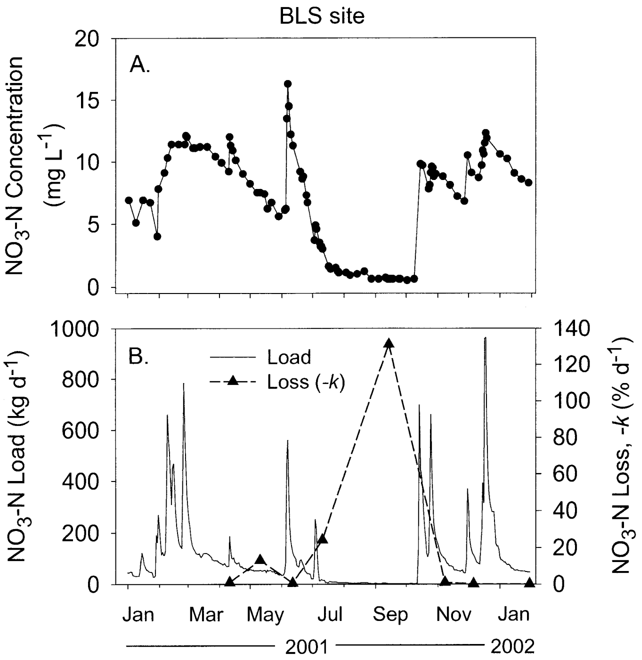

Fig. 6. (A) Nitrate concentrations at site BLS, January 2001 through January. (B) Mean daily nitrate load and measured rates of nitrate N loss, $-k$ (% d^{-1}), for site BLS. (Site characteristics are given in Table 1.)

past $S_{\mathrm{w,dn}}$ values (Fig. 4). Hill (1979) also concluded that during critical times of the year (i.e., high discharge, high NO_3–N concentrations) the export of NO_3–N from agricultural streams in Ontario was not affected by denitrification.

We suggest that the through-put mode that occurs in the streams we examined is due largely to the drainage and channelization that has occurred in the watersheds (see Rhoads and Herricks, 1996). With the extensive network of subsurface tile drains now in place throughout the Midwest, precipitation and solutes are quickly drained into channelized headwater streams, bypassing riparian buffer zones (Hill, 1976). The dynamics of N cycling and the role of denitrification in Illinois streams before agricultural development are unknown. However, an undisturbed prairie stream in Kansas showed substantial retention of inorganic N (Dodds et al., 2000), suggesting the headwater streams in east-central Illinois are less retentive of N now than they were before European settlement and conversion of the native prairie and wetlands to agriculture.

CONCLUSIONS

The nutrient spiraling model (Newbold, 1992) was used to assess the role of in-stream denitrification in affecting the concentration and transport of NO_3–N in headwater agricultural streams in east-central Illinois. Nutrient spiraling has been used successfully to examine nutrient cycling in relatively undisturbed, low-nutrient streams (e.g., Hall and Tank, 2003), but we believe this study is the first to apply the concept to nitrogen-rich, agricultural streams. The results indicate that denitrification in the stream sediments did not affect the transport of NO_3–N or represent a substantial N sink in the sites we examined. Although the areal denitrification rates tended to be high, the concentrations of NO_3–N in the streams also were high and resulted in an overall low efficiency of NO_3–N removal.

There are more than 20 million ha of drained cropland in the Mississippi River basin, much of it concentrated in the Corn Belt (Goolsby et al., 1999). Our study was limited to five headwater streams in east-central Illinois, but we believe the sites are typical of headwater streams in the Corn Belt that receive tile drainage. If the results obtained from our sites are representative of headwater agricultural streams throughout the Corn Belt, previous studies of N transport in Illinois (David and Gentry, 2000) and the Mississippi River basin (Alexander et al., 2000) may have overestimated the loss of N through denitrification in headwater streams. In the northeastern United States, headwater streams appear to be the major habitat for N removal (Seitzinger et al., 2002). In the agricultural Midwest, we suggest habitats such as reservoirs or floodplain wetlands may support greater N removal than do headwater streams, particularly those streams in tile-drained watersheds.

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