

# Response of Sediment Denitrification Rates to Environmental Variables in Streams Heavily Impacted by Agriculture

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## ABSTRACT

Environmental controls of sediment denitrification were examined at four agricultural, channelized streams in Eastcentral Illinois between June 2003 and February 2005. Differences in sediment properties, water temperature, and nitrate-N ( $\text{NO}_3\text{-N}$ ) supply varied temporally and spatially among the four streams. Sediment denitrification rates ranged from 0 to  $0.3 \mu\text{g N g dry mass (DM)}^{-1} \text{ h}^{-1}$  at stream sites characterized by low benthic organic matter content and coarse-textured sediments, 0 to  $1.5 \mu\text{g N (g DM)}^{-1} \text{ h}^{-1}$  at stream sites characterized by moderate benthic organic matter content and fine-textured sediments, and 0.2 to  $3.3 \mu\text{g N (g DM)}^{-1} \text{ h}^{-1}$  in emergent grass beds characterized by high benthic organic matter content and fine-textured sediments. Seasonally, sediment denitrification rates were greatest in the summer and least in the winter, with water temperature explaining 42 to 65% of the variation in sediment denitrification rate in three of the four streams. Benthic organic matter concentration explained 21 to 67% of the variation of sediment denitrification in all streams. Denitrification assays conducted on sediments amended with increasing concentrations of  $\text{NO}_3\text{-N}$  indicated that sediment denitrification was limited by N availability in fine-textured sediments but not in coarse-textured sediments when stream  $\text{NO}_3\text{-N}$  concentrations were  $<1 \text{ mg L}^{-1}$ , which was typical in late summer.

## INTRODUCTION

Understanding the mechanisms that control nutrient processes in aquatic ecosystems is of fundamental importance to aquatic ecology, particularly in regions where nutrient loading is great. Nutrient enrichment can cause eutrophication in freshwater and coastal waters leading to algal blooms, reduced oxygen ( $\text{O}_2$ ) concentrations, and alteration in ecosystem structure and function (Carpenter et al. 1998). In the upper Midwest, agricultural tributaries are significant contributors of nitrogen (N) to the Mississippi River. Ninety percent of nitrate-N ( $\text{NO}_3\text{-N}$ ) inputs to the Mississippi River basin are from non-point sources, and 74% of those sources have agricultural origins (Rabalais et al. 2002). In Illinois, intensive fertilization, row-crop agriculture, subsurface drainage, and stream channelization have contributed to high N exports as great as  $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  during wet years (David and Gentry 2000).

Nitrogen loads from agricultural regions in the Mississippi River basin, along with hydrology, correlate with the severity and size of a seasonal hypoxic zone that occurs in the Gulf of Mexico (Rabalais et al. 2001). Concerns regarding the impact of hypoxia in the Gulf of Mexico have led to an increasing interest in N processes within agricultural regions of the Mississippi River basin. Sediment denitrification is of particular interest because this microbial-driven process permanently removes  $\text{NO}_3\text{-N}$  from the water of lakes, rivers, and wetlands. David et al. (2006) estimated that a 25% larger load of  $\text{NO}_3\text{-N}$  would be exported by Illinois rivers to the Gulf of Mexico if it were not for sediment denitrification. In addition, nearly one-third of sediment denitrification rates measured by Royer et al. (2004) in Eastcentral Illinois exceeded  $>5 \text{ mg N m}^{-2} \text{ h}^{-1}$ , significantly greater

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than most other stream types (Inwood et al. 2005). Therefore, understanding the controlling factors of sediment denitrification in agricultural watersheds is necessary for developing better models that address N retention mechanisms.

A primary control of sediment denitrification is O<sub>2</sub> concentration within sediment pore water (Jenkins and Kemp 1984, Rysgaard et al. 1994). Jenkins and Kemp (1984) suggested that the presence of anoxic microenvironments in aerobic sediments have a substantial influence on sediment denitrification. Compared to coarse-textured sediments, fine-textured sediments further reduce O<sub>2</sub> diffusion into the streambed, which enhances anoxic microenvironments. Organic carbon (C) (Jansson et al. 1994, Wall et al. 2005) and NO<sub>3</sub><sup>-</sup> availability (Kemp and Dodds 2002, Inwood et al. 2005) also control sediment denitrification, as organic C is the electron donor and NO<sub>3</sub><sup>-</sup> is reduced to nitrous oxide (N<sub>2</sub>O) or dinitrogen (N<sub>2</sub>). An additional control of sediment denitrification is water temperature. Pfenning and McMahon (1996) have identified a positive correlation between temperature and denitrification activity. As temperature increases, enzyme activity is stimulated, resulting in greater rates of sediment denitrification.

In this study, we used a multi-year dataset to evaluate controls on sediment denitrification in agricultural streams. Our objective was to evaluate water column and sediment physical and chemical properties that regulate sediment denitrification where streamwater NO<sub>3</sub>-N concentrations are typically high. Our results expand on previous in-stream denitrification studies by Royer et al. (2004) and Schaller et al. (2004) conducted in these systems by concentrating on streambed properties and examining a broader range of environmental factors that control sediment denitrification in regions impacted by intensive agriculture. Sediment denitrification and physical stream measurements of two streams from Opdyke et al. (2006) were used in this study to more accurately determine controls of sediment denitrification.

## METHODS AND MATERIALS

### *Site description*

Four headwater stream reaches (100 m lengths) were studied in Champaign County of Eastcentral Illinois. The stream reaches included a first-order stream, Black Slough (BLS, 39° 57' 06" N, 88° 10' 07" W), and two second-order streams, Embarras River (EMB, 39° 58' 56" N, 88° 12' 23" W) and East Branch Embarras River (EBE, 39° 56' 48" N, 88° 08' 29" W), located in the Embarras river basin, and a second-order stream, Camp Creek (CCR, 40° 07' 09" N, 88° 25' 49" W), located in the Sangamon River basin.

The BLS stream reach was divided into two compartments – the open, center channel (BLS<sub>C</sub>) and emergent grass beds (BLS<sub>V</sub>). The emergent grass beds were areas of dense perennial grasses that constrained both sides of the center channel, occupying 50 to 75% of the stream width year-round. At CCR, water milfoil (*Myriophyllum* spp.) covered 20 to 80% of the streambed, depending on time of year and flow. Less than 20% of the streambeds at BLS<sub>C</sub>, EMB, and EBE were covered by aquatic vegetation.

The channelized reaches were actively managed to maintain free-flowing subsurface drainage pipes, which drain an agricultural region consisting of >90% row-crop agriculture of predominantly corn and soybeans (David et al. 1997). Streambeds consisted of homogenous sediment properties of mainly gravel and sand. Perennial grasses were the only riparian vegetation located along the stream reaches. Dissolved organic carbon (DOC) was mostly allochthonous in origin from drainage tiles between late fall to early summer, switching to autochthonous from algal blooms when subsurface drainage flows declined or ceased between late summer to early fall (Royer and David 2005).

### *Field measurements*

Field measurements were taken approximately seven times each year between

June 2003 and February 2005 and included channel dimensions, velocity, discharge using a Marsh-McBirney Model 2000 Flo-Mate, and water temperature and dissolved oxygen (DO) concentrations using a YSI Model 55 DO meter. In addition, surface water grab samples were collected from the center of the streams for NO<sub>3</sub>-N and DOC analyses and were stored at freezing temperatures for NO<sub>3</sub>-N and acidified for DOC. Nitrate-N was analyzed with a Dionex (Sunnyvale, CA) DX-120 ion chromatograph, and DOC was measured with a Dohrmann (Mason, OH) DC-80 carbon analyzer.

Sediment composition was intensively measured in November 2004 at BLS<sub>C</sub>, EMB, EBE, and BLS<sub>V</sub>, and in January 2005 at CCR. Sediment grab samples were collected from the upper 5 cm of the streambed from three random locations within each stream reach. Samples were dried at 60 °C, organic matter was removed by combustion at 550 °C, and sediment aggregates were broken apart. Sediments were then sieved into six particle classes – coarse and medium gravel (>4 mm), fine gravel (4–2 mm), coarse sand (2–0.5 mm), medium sand (500–200 μm), fine sand (200–53 μm), and silt and clay (<53 μm).

#### *Sediment denitrification*

Sediment denitrification rates were measured concurrently with the collection of water samples. Benthic sediment was collected mid-morning and returned to the laboratory within 3 h for determining sediment denitrification rates.

Three parallel transects 50 m apart were established at BLS<sub>C</sub>, EMB, EBE, and CCR at least 100 m from any bridges. At each transect, three sediment 5-cm depth cores were collected equidistantly across the stream width and combined into a composite sample. The collective surface area of each composite sample was 21 cm<sup>2</sup>. Sediment from the emergent grass beds at BLS<sub>V</sub> was obtained by randomly collecting three 5-cm depth cores within the emergent grass beds and within 5 m of each transect.

Sediment denitrification rates were estimated using the acetylene inhibition technique (Tiedje et al. 1989, Knowles 1990) as modified by Royer et al. (2004). Although this method is inappropriate in sediments with low NO<sub>3</sub>-N or coupled nitrification-denitrification (Rudolph et al. 1991, Seitzinger et al. 1993), NO<sub>3</sub>-N ≥ 10 μM and the addition of chloramphenicol improve the estimates of measuring *in situ* denitrification rates (Smith and Tiedje 1979, Royer et al. 2004).

Representative subsample (25 cm<sup>3</sup>) of each composite sample, along with 75 mL of stream water, was placed in a 150-mL flask equipped with a butyl septum for gas sampling (n = 3 per site and date). Sediment particle sizes ≥4 mm were excluded from the subsamples placed in the flasks. Flasks were purged with helium, followed by the addition of acetylene gas (10% v/v), which blocks the transformation of N<sub>2</sub>O to N<sub>2</sub>. Flasks were incubated at stream temperature, and headspace gas samples were collected throughout a 2-h period.

Gas samples were analyzed for N<sub>2</sub>O on a Varian (Palo Alto, California) Model 3600 gas chromatograph equipped with an 80/100 mesh Porapak Q column and a <sup>63</sup>Ni electron-capture detector. Column and detector temperatures were 70 and 300 °C, respectively. Sediment denitrification rate was calculated as the linear rate of N<sub>2</sub>O accumulation over time multiplied by the Bunsen solubility coefficient (Tiedje 1982) and expressed per gram of dry mass (DM) of substrate. Upon completing sediment denitrification assays, subsamples and their parent composite sediment samples were dried at 60 °C to determine dry mass and combusted at 550 °C to determine ash-free dry mass (AFDM). This yielded total benthic organic matter for each composite sediment sample.

#### *Nutrient-amended denitrification assays*

Nutrient-amended sediment denitrification assays were conducted with additions

of NO<sub>3</sub>-N (N+) and NO<sub>3</sub>-N and glucose (N+C) on 11 August 2004 and increasing additions of NO<sub>3</sub>-N on 27 September 2004. Sediment samples were collected and measured for sediment denitrification as done for the ambient sediment denitrification rates, with the exception of N+ or N+C added to treatment flasks prior to sealing flasks for incubation.

On 11 August, we measured ambient sediment denitrification, N-amended sediment denitrification, and N+C-amended sediment denitrification at BLS<sub>C</sub>, EMB, EBE, and BLS<sub>V</sub> (n = 3 per site and treatment). Final NO<sub>3</sub>-N concentrations in N+ and N+C-amended flasks were ~19 mg L<sup>-1</sup>, and final DOC concentrations in N+C-amended flasks were ~15 mg L<sup>-1</sup>. Ambient stream NO<sub>3</sub>-N concentrations were 4.3 mg L<sup>-1</sup> at BLS<sub>C</sub>, EBE and BLS<sub>V</sub>, and 3.1 mg L<sup>-1</sup> at EMB; and ambient stream DOC concentrations were 1.8, 2.9, 2.7, and 1.9 mg L<sup>-1</sup> at BLS<sub>C</sub>, EMB, EBE, and BLS<sub>V</sub>, respectively. On 27 September, we amended the treatment flasks with different concentrations of NO<sub>3</sub>-N to obtain final NO<sub>3</sub>-N concentrations of ~2, 6, and 13 mg L<sup>-1</sup> in N-amended flasks. Ambient stream NO<sub>3</sub>-N concentrations were 0.6, 0.8, 0.6, and 0.1 mg L<sup>-1</sup> at BLS<sub>C</sub>, EMB, EBE, and BLS<sub>V</sub>, respectively. Ambient stream DOC concentrations were 3.1 mg L<sup>-1</sup> at EMB and 3.7 mg L<sup>-1</sup> at EBE. Stream DOC was not measured at BLS<sub>C</sub> and BLS<sub>V</sub>.

#### Statistical analyses

Statistical analyses were performed using SAS (version 8, SAS Institute, Cary, North Carolina). All data were initially tested for normality using the normal option in the PROC univariate procedure. Sediment denitrification rates did not meet the assumptions for parametric statistics and were therefore logarithmically transformed prior to statistical analyses. We did not transform data for the measured independent variables (discharge, water temperature, DO, stream NO<sub>3</sub>-N, stream DOC, benthic organic matter, and sediment composition) because they met the assumptions of parametric statistics.

Ambient sediment denitrification rates were grouped into four seasons (spring, summer, fall, and winter) when determining significance among study sites using one-way analysis of variance (ANOVA). We chose to group the data into four seasons because of close similarities among sediment denitrification rates within each season based on our data and previous data not published. Simple linear regression and multiple regression analyses organized by site were then used to examine relationships between sediment denitrification rates and measured independent variables, of which neither was grouped. When comparing sediment denitrification rates to all but benthic organic matter, we ran a simple linear regression analysis using the mean sediment denitrification rate per site and sampling date. Our results for the linear regression analyses are only shown if

Table 1. Mean nitrate-N and dissolved organic C concentrations (in mg L<sup>-1</sup> with mean differences between measurements in parentheses) at stream sites from June 2003 to February 2005 grouped by seasons.

	BLS	EMB	EBE	CCR
NO <sub>3</sub> -N				
April-May	10.6 (1.5)	6.7 (2.2)	13.5 (0.7)	12.3
June-July	8.9 (2.3)	8.1 (1.4)	10.5 (1.3)	10.7 (0.3)
August-September	1.7 (1.3)	1.3	1.9 (3.0)	-
October-February	5.3 (4.4)	4.2 (1.9)	9.1 (3.4)	7.9 (3.6)
Dissolved organic C				
April-May	1.6 (0.8)	2.8 (2.2)	1.7 (0.2)	1.9
June-July	2.5 (0.7)	2.3 (0.3)	2.1 (0.4)	2.9 (2.2)
August-September	2.4 (0.1)	3.4	3.4 (1.0)	-
October-February	1.6 (0.4)	2.3 (0.6)	1.8 (1.0)	1.7 (0.6)

significant relationships were found. When examining mean sediment denitrification rate and water temperature or DO, we excluded measurements taken when NO<sub>3</sub>-N concentrations were <1 mg L<sup>-1</sup>. When examining mean sediment denitrification rate and discharge, DOC, or benthic organic matter, we excluded measurements taken when NO<sub>3</sub>-N concentrations were <1 mg L<sup>-1</sup> and in February when water temperatures were <5 °C. From our data, we found that low NO<sub>3</sub>-N concentration and low water temperature limited sediment denitrification in our study and would otherwise have obscured relationships in the simple linear analyses if they were not removed. When examining relationships between sediment denitrification and benthic organic matter, we compared sediment denitrification and benthic organic matter of each composite sediment sample. For our examination of a relationship between benthic organic matter and sediment composition, we calculated the mean benthic organic matter concentration per site of all sampling dates and compared those means to the mean silt and clay content of each site, which was measured in November 2004 and January 2005.

Significant differences among treatments in the nutrient-amended sediment denitrification experiments were determined using ANOVA. When significance was found, we performed a non-linear regression using a Michaelis-Menten kinetics model to fit the best line for the response of sediment denitrification rates to increasing nutrient concentrations.

## RESULTS

### *Stream characteristics*

Water temperature was similar among all stream reaches, with lowest temperatures in February averaging 2.8 °C, temperatures in spring and fall averaging 10.9 °C, and highest temperatures in summer averaging 19.3 °C. Surface water DO concentrations were greater at BLS<sub>C</sub> and EMB than EBE and CCR by a factor of two, ranging from a mean low of 6.8 mg L<sup>-1</sup> in late summer to a mean high of 13.5 mg L<sup>-1</sup> in February. Stream width and water depth was consistently greater at EBE and CCR, averaging 7.1 and 0.66 m, respectively, compared to a mean stream width and water depth of 3.9 and 0.37 m at BLS and EMB, respectively. Nitrate-N concentrations at EBE and CCR were greater than at BLS and EMB (Table 1).

Compared to BLS<sub>C</sub> and EMB, stream sites at EBE, CCR, and BLS<sub>V</sub> had finer-textured sediments and greater concentrations of benthic organic matter in the streambeds (Table 2). No seasonal trends in benthic organic matter concentrations were observed.

Table 2. Sediment characteristics (in % with standard errors in parentheses) of stream sites. Sediment composition was measured in November 2004 and January 2005 and mean benthic organic matter from June 2003 to February 2005.

Site	Coarse gravel (>4 mm)	Fine gravel (4–2 mm)	Coarse sand (2–0.5 mm)	Medium sand (500–200 µm)	Fine sand (200–53 µm)	Silt + clay (<53 µm)	n	Organic matter
BLS <sub>C</sub>	45 (12)	26 (3)	24 (8)	2 (<1)	2 (<1)	1 (<1)	47	1.8 (0.1)
BLS <sub>V</sub>	8 (2)	9 (3)	40 (3)	13 (1)	15 (1)	15 (1)	24	4.6 (0.4)
EMB	30 (6)	15 (1)	43 (6)	8 (1)	3 (<1)	1 (<1)	47	1.7 (0.1)
EBE	7 (4)	7 (2)	60 (5)	18 (1)	5 (3)	3 (1)	48	2.6 (0.1)
CCR	9 (4)	8 (1)	31 (3)	20 (2)	19 (2)	13 (3)	24	3.0 (0.1)

### *Sediment denitrification*

Sediment denitrification rates ranged from 0 to 0.3 µg N g DM<sup>-1</sup> h<sup>-1</sup> at the coarse-textured sediment reaches at BLS<sub>C</sub> and EMB, 0 to 1.5 µg N g DM<sup>-1</sup> h<sup>-1</sup> at the fine-textured sediment reaches at EBE and CCR, and 0.2 to 3.3 µg N g DM<sup>-1</sup> h<sup>-1</sup> at the emergent grass beds at BLS<sub>V</sub>. Sediment denitrification rates at all stream reaches varied by season (Fig. 1). During summer, when water temperatures exceeded 15 °C, sediment denitrification rates were greatest. Mean sediment denitrification rates (± standard error)

during June through September, excluding measurements taken when  $\text{NO}_3\text{-N}$  concentrations were  $<1 \text{ mg L}^{-1}$ , were  $0.04 \pm 0.01 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{BLS}_C$  and  $\text{EMB}$ ,  $0.6 \pm 0.1 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{EBE}$  and  $\text{CCR}$ , and  $1.5 \pm 0.4 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{BLS}_V$ . Mean sediment denitrification rates ( $\pm$  standard error) during February, when temperatures were below  $5^\circ\text{C}$ , were  $<0.01 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{BLS}_C$ ,  $\text{EMB}$ , and  $\text{EBE}$ ,  $0.1 \pm 0.1 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{CCR}$ , and  $0.9 \pm 0.5 \text{ } \mu\text{g N g DM}^{-1} \text{ h}^{-1}$  at  $\text{BLS}_V$ . Sediment denitrification rates at  $\text{BLS}_C$  and  $\text{EMB}$  were similar, as were sediment denitrification rates at  $\text{EBE}$  and  $\text{CCR}$ ; but denitrification rates between the two paired stream sites were significantly different. Sediment denitrification rates at  $\text{BLS}_V$  were significantly greater than all stream sites throughout the study (ANOVA,  $P < 0.03$ ).

Significant relationships were found between water temperature (Fig. 2), benthic organic matter concentration (Fig. 3), and indirectly, silt and clay content of the sediments (Opdyke et al. 2006) and sediment denitrification rates; these were among the

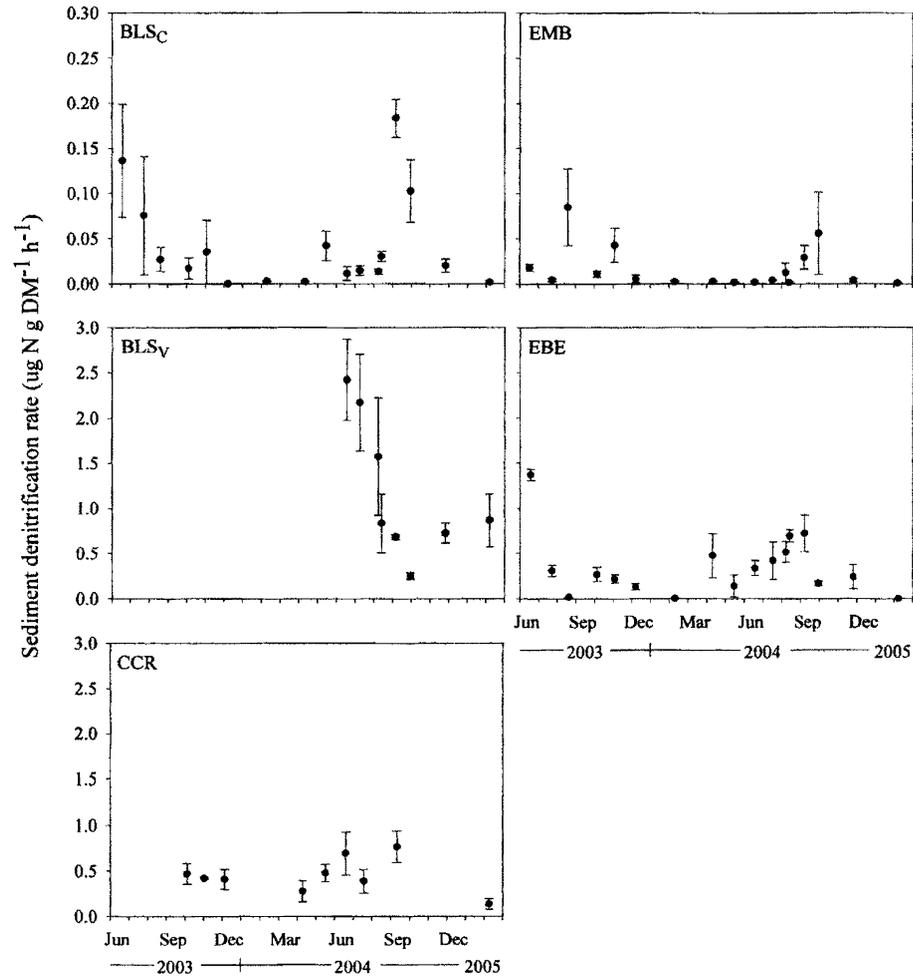


Figure 1. Mean sediment denitrification rates ( $\pm 1$  standard error) of stream sites from June 2003 to February 2005. The upper two graphs represent the coarse-textured sediment sites, and the lower three graphs represent the fine-textured sediment sites. Note factor of 10 difference in y-axis scale.

potential controls for denitrification tested in this study that also included thalweg velocity, discharge, surface water DO, and  $\text{NO}_3\text{-N}$  and DOC concentrations. A strong linear relationship between water temperature and sediment denitrification rate was present at EBE and CCR when excluding measurements taken when  $\text{NO}_3\text{-N}$  concentrations were  $<1 \text{ mg L}^{-1}$  (ANOVA,  $P \leq 0.01$ ). At all stream sites, we identified a positive linear relationship between sediment denitrification rate, excluding measurements taken in February of both years and when stream  $\text{NO}_3\text{-N}$  concentrations were  $<1 \text{ mg L}^{-1}$ , and benthic organic matter concentration from composite sediment samples (ANOVA,  $P \leq 0.01$ ). When measurements taken during low  $\text{NO}_3\text{-N}$  concentrations of  $<1 \text{ mg L}^{-1}$  were excluded, multiple regression analyses provided further evidence that water temperature and benthic organic matter were the master variables controlling sediment denitrification rates at  $\text{BLS}_C$  and EBE ( $P \leq 0.01$ ). Multiple regression was not useful when analyzing measurements from EMB and CCR because of the absence of any significant relationship between water temperature and sediment

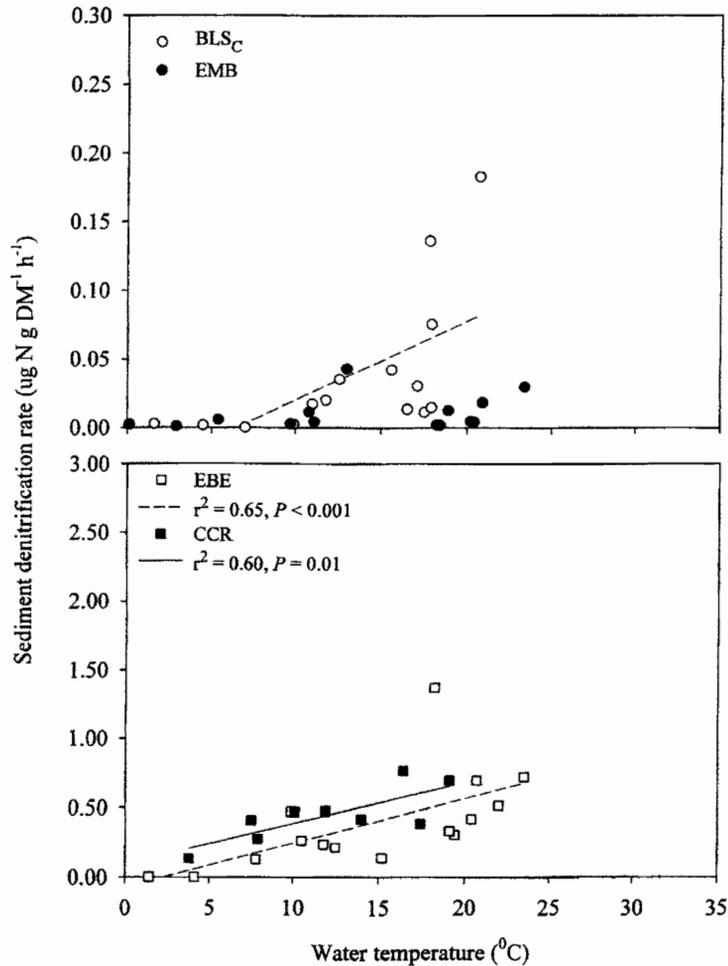


Figure 2. Sediment denitrification rate plotted against stream water temperature. Rates measured when stream  $\text{NO}_3\text{-N}$  concentrations were  $<1 \text{ mg L}^{-1}$  are excluded. The upper graph represents the coarse-textured sediment sites, and the lower graph represents the fine-textured sediment sites. Note factor of 10 difference in y-axis scale.

denitrification rate at EMB and limited measurements at CCR. Similarly, a positive relationship existed between mean benthic organic matter concentration of composite samples measured per stream site and silt and clay content in sediments measured in November 2004 and January 2005 (ANOVA,  $P = 0.04$ ) (Opdyke et al. 2006).

*Nutrient-amended denitrification assays*

There was no significant relationship between ambient sediment denitrification rate and stream  $\text{NO}_3\text{-N}$  concentration. However, in the nutrient-amended assays conducted on 27 September 2004, sediment denitrification rate responded significantly to increasing concentration of  $\text{NO}_3\text{-N}$  in the N-amended experiments with sediments from EBE and  $\text{BLS}_V$  (ANOVA,  $P < 0.05$ , Fig. 4). A Michaelis-Menten kinetics model was applied, suggesting that sediment denitrification rates using sediments from EBE and  $\text{BLS}_V$  reached a maximum of approximately  $0.88$  and  $0.67 \mu\text{g N g DM}^{-1} \text{h}^{-1}$ , respectively. No significant relationship existed between sediment denitrification rate and increasing  $\text{NO}_3\text{-N}$  concentration using the coarser sediments from  $\text{BLS}_C$  and EMB. The N+C-

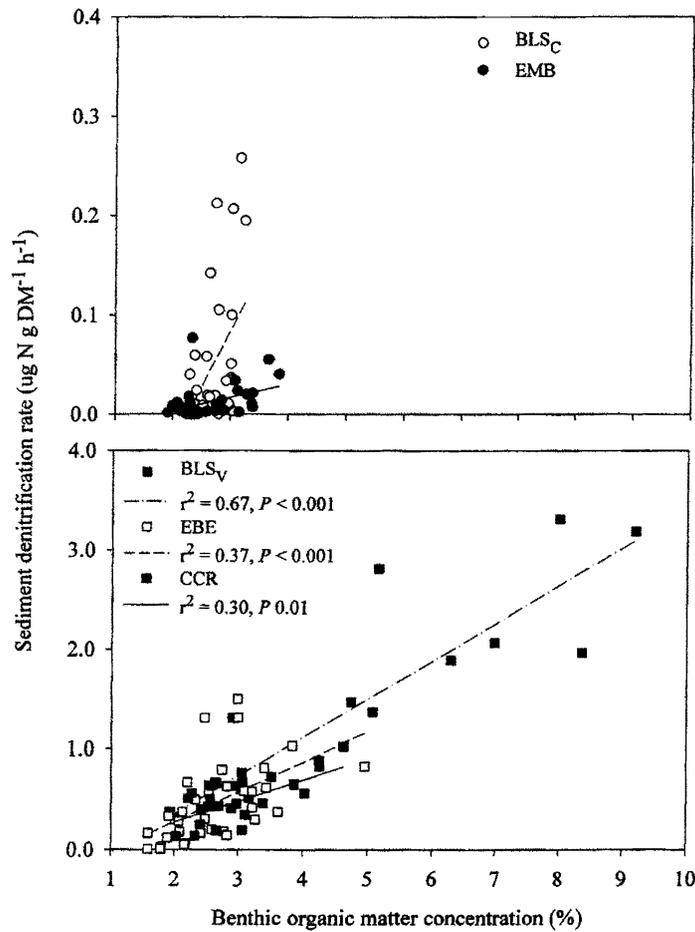


Figure 3. Sediment denitrification rate plotted against benthic organic matter. Rates measured in February and when stream  $\text{NO}_3\text{-N}$  concentrations were  $< 1 \text{ mg L}^{-1}$  are excluded. The upper graph represents the coarse-textured sediment sites and the lower graph represents the fine-textured sediment sites. Note factor of 10 difference in y-axis scale.

amended experiments conducted on 11 August 2004 yielded no significant differences among ambient sediment denitrification rate, sediment denitrification rate, and treatments that included N- and N+C-amendments.

#### DISCUSSION

Among the environmental variables examined in this study, water temperature, benthic organic matter, and abiotic sediment composition had the greatest influences on sediment denitrification rate. Water temperature explained 42 to 65% of the variation in mean sediment denitrification rate at BLS<sub>C</sub>, EBE, and CCR when measurements taken during low NO<sub>3</sub>-N concentrations (<1 mg L<sup>-1</sup>) were removed. Sediment denitrification rates were greatest when water temperatures exceeded 15 °C and least when water temperatures were below 5 °C, with a deviation from these findings occurring when NO<sub>3</sub>-N concentrations were <1 mg L<sup>-1</sup> in late summer. These results are consistent with other studies that have observed responses of sediment denitrification rates to water temperature fluctuations (Seitzinger 1988, Pfenning and McMahon 1996). Greater water temperature results in elevated microbial metabolism, causing denitrifying bacteria to metabolize NO<sub>3</sub><sup>-</sup> faster in the summer than winter (Richardson et al. 2004).

Benthic organic matter explained 21 to 67% of the variation of sediment denitrification rate at all stream sites, excluding measurements taken in the winter and during low stream NO<sub>3</sub>-N concentrations when water temperature and NO<sub>3</sub>-N availability were more important in controlling sediment denitrification rate. The stream sites with the greatest benthic organic matter concentration had the greatest sediment denitrification rates. Sediment denitrification rates were between 2 and 1000 times greater at BLS<sub>V</sub>, where dense emergent grass beds contributed to high benthic organic matter concentrations compared to stream sites at BLS<sub>C</sub> and EMB, which had low benthic

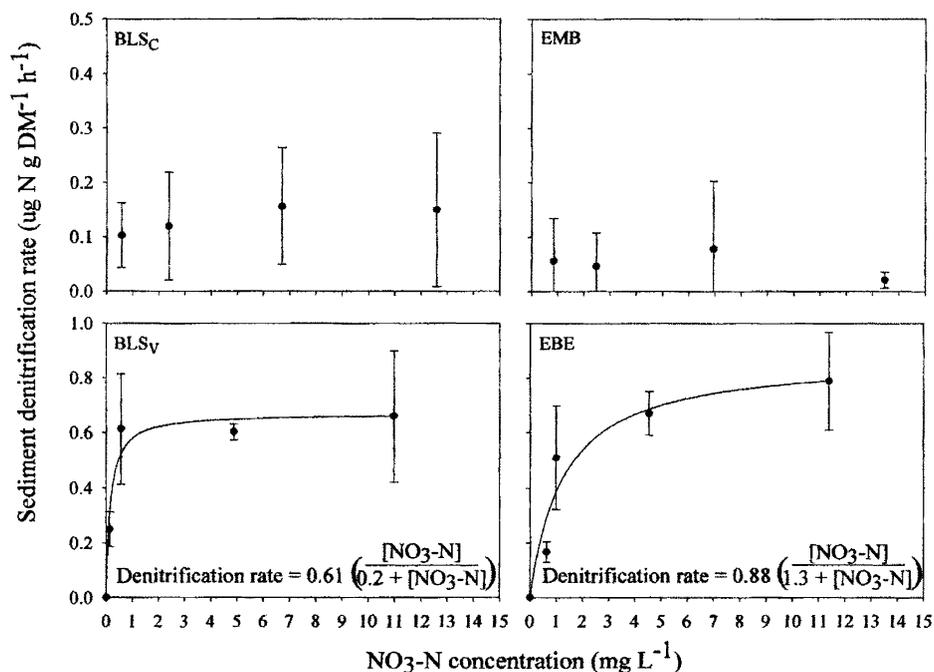


Figure 4. Sediment denitrification response ( $\pm 1$  standard error) to increasing N amendments conducted on 27 September 2004 when stream NO<sub>3</sub>-N concentrations were <1 mg L<sup>-1</sup>. Note difference in y-axis scale.

organic matter concentrations and subsequently low sediment denitrification rates. Several studies (Garcia-Ruiz et al. 1998, Kemp and Dodds 2002, Richardson et al. 2004) have provided supportive evidence that benthic organic matter is an important factor controlling sediment denitrification. Organic C derived from benthic organic matter donates electrons to denitrifying bacteria, enabling the bacteria to complete its metabolism of  $\text{NO}_3^-$  under reducing conditions.

Identifying the significance of benthic organic matter controlling sediment denitrification rates at all of our stream sites suggests that it is a greater control than water temperature. More organic matter associated with a greater density of aquatic plants in streams may increase  $\text{NO}_3\text{-N}$  removal by denitrification, which could be important in agricultural regions during the winter and spring months when increased flows carry higher  $\text{NO}_3\text{-N}$  loads and colder temperatures limit sediment denitrification rates in channelized streams lacking any aquatic plants. Given a 200 m stream reach at Black Slough, with 50 to 75% of the reach covered by emergent grass beds,  $\text{NO}_3\text{-N}$  removal by sediment denitrification averaged 650 and 210  $\text{g d}^{-1}$  in the emergent grass beds from April to July and August to February, respectively. In the area of the channel not covered by emergent grass beds,  $\text{NO}_3\text{-N}$  removal averaged 3.2, 11, and 1.2  $\text{g d}^{-1}$  from April to July, August to September, and October to February, respectively. Although the percent of stream N load removed by sediment denitrification was less than 1.0% during all sampling periods, except in August and September when reduced flows led to loss rates of 13%, greater than 95% of the N removed from the stream occurred in the emergent grass beds. However, the presence of drainage-tile outlets, which convey N-rich subsurface water, occur at many locations along headwater streams in Eastcentral Illinois, adding N to these streams from numerous sources. These contributions overwhelm the capacity of sediment denitrification to remove a significant percentage of the total  $\text{NO}_3\text{-N}$ .

A positive relationship between benthic organic matter concentration and percent silt and clay in the sediments suggests that abiotic sediment composition may have an additional influence on sediment denitrification rates (Opdyke et al. 2006). It should be noted that Opdyke et al. (2006) did not directly examine the influence of abiotic sediment composition to sediment denitrification rates and that organic content and abiotic sediment composition are linked variables. In our stream sites, benthic organic matter was positively correlated with finer sediments, which is typical of many streams. Fine-textured sediments have greater particle density and reduced porosity compared to coarse sediments. Therefore, finer sediments may inhibit  $\text{O}_2$  diffusion from the water column into the underlying sediment, enhancing anoxic microenvironments. Since oxygen inhibits denitrification through competition for electrons with denitrifying enzymes (Knowles 1982) and headwater streams are typically aerobic systems, anoxic microenvironments in sediments may play an important role in influencing sediment denitrification. In addition, finer sediments have greater surface areas providing more substrate to support a larger population of denitrifying bacteria. At EBE and CCR, where benthic organic matter and finer sediments were greater than at EMB,  $\text{NO}_3\text{-N}$  removal rates by sediment denitrification averaged 850, 940, and 250  $\text{g d}^{-1}$  from April to July, August to September, and October to February, respectively. During those same periods,  $\text{NO}_3\text{-N}$  removal rates at EMB averaged 8.3, 58, and 5.0  $\text{g d}^{-1}$ , respectively.

Results from our  $\text{NO}_3\text{-N}$  addition experiment provide further support of the influence of benthic organic matter and abiotic sediment composition on sediment denitrification rates. Compared to the coarser sediments collected from  $\text{BLS}_C$  and EMB, sediments from EBE and  $\text{BLS}_V$  had greater benthic organic matter concentrations and finer sediments, resulting in greater sediment denitrification rates with an increase in  $\text{NO}_3\text{-N}$  concentrations. In addition, the N+C-amendment study found that neither of the four stream sites were limited by labile organic C availability. Thus, sediment

composition could be controlling sediment denitrification at BLS<sub>C</sub> and EMB because coarser sediments would allow greater O<sub>2</sub> diffusion deeper into the sediments and provide less surface area for denitrifying bacteria to colonize.

Royer et al. (2004) conducted a similar nutrient amendment study on sediments from BLS<sub>C</sub> and identified a significant difference in sediment denitrification rates between an ambient NO<sub>3</sub>-N concentration of 1.1 mg L<sup>-1</sup> and a spiked NO<sub>3</sub>-N concentration of approximately 5.0 mg L<sup>-1</sup>. We believe the differences in our results from Royer et al. (2004) for examining N limitation in sediments from BLS<sub>C</sub> is due to year-to-year variations in sediment characteristics. This suggests that environmental variables that influence sediment denitrification rate can change in a stream over time. Other nutrient amendment studies have reported variations in NO<sub>3</sub>-N limitation of sediment denitrification relating to differences in substrate characteristics (Martin et al. 2001, Kemp and Dodds 2002, Wall et al. 2005).

This study demonstrated that sediment denitrification in headwater, agricultural streams in an intensive agricultural region was linked to sediment properties and water temperature. Sediment properties were more significant in controlling sediment denitrification rates when focusing on the spatial diversity of streams in an agricultural region, where N limitation is not a factor throughout most of the year. High NO<sub>3</sub>-N loads in agricultural streams may limit the effectiveness of sediment denitrification at reducing NO<sub>3</sub>-N, but understanding the controlling factors of denitrification in agricultural regions provides information that can be used to focus efforts on enhancing NO<sub>3</sub>-N removal.

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