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Denitrification associated with plants and sediments in an agricultural stream

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Abstract. Streams that drain agricultural watersheds in the midwestern US deliver large N loads to downstream water bodies. Denitrification is a potential sink for N in streams, but its importance in agricultural streams is unclear. Denitrification was examined in Big Ditch, a NO_3-rich tributary to the Sangamon River in east-central Illinois. Denitrification associated with benthic sediments and floating mats of algae and macrophytes was measured from May to November 2002. Daily NO_3-N loads were calculated for the 2002 calendar year to provide a context for the denitrification rates. Four other streams were sampled less intensively and the results indicated that Big Ditch was typical of agricultural streams in the region. During the growing season, plant biomass in Big Ditch was occasionally >200 g dry mass (DM)/m^2, but biomass declined sharply following scouring spates in June and August. Maximum rates of plant-associated and sediment denitrification were similar on a DM basis (4.2 and 3.7 μg N_2O [g DM]$^{-1}$ h^{-1}, respectively). However, denitrification rates in the sediments were more than an order of magnitude greater than the rates associated with plant material on an areal basis. Large floating mats of algae and macrophytes often covered much of Big Ditch, but were not major sites for denitrification. Denitrification rates in the sediments generally were higher than those reported from other streams, with a maximum value of 15.8 mg N m^{-2} h^{-1}, but daily NO_3-N loads in Big Ditch often were >5 Mg N/d (Mg = 10^6 g). Denitrification rates were high at times, but instream denitrification appeared not to substantially affect N losses from this agricultural watershed.

Key words: denitrification, nitrate, sediments, *Cladophora*, Illinois, Big Ditch, Sangamon River.

Elevated nutrient concentrations in agricultural streams of the midwestern US are a major water-quality problem. N loading to streams in the agricultural midwest affects local habitats and distant, downstream ecosystems (Alexander et al. 2000, Rabalais et al. 2002). Locally, elevated nutrients cause eutrophication and raise concerns regarding the quality of drinking water. In addition, nutrients, such as NO_3-N from agricultural fertilizers, are carried from fields into waterways leading to the Mississippi River and the Gulf of Mexico. Increased N loading to the Gulf of Mexico has caused harmful algal blooms, eutrophication, and hypoxia (Rabalais et al. 2002).

Headwater streams are critical sites for N processing, and these small streams may control N export from watersheds (Alexander et al. 2000, Peterson et al. 2001). However, studies of agricultural streams in Ontario (Hill 1979) and Sweden (Jansson et al. 1994) found that instream denitrification had only minor effects on N export. David and Gentry (2000) constructed an N mass-balance model for the state of Illinois and estimated that instream denitrification in Illinois could remove an average of 131,667 Mg N/y (Mg = 10^6 g). This quantity indicated that instream denitrification could be a large N sink. However, the degree to which denitrification controls N dynamics in streams is not clear and, despite its potential importance, few direct measurements have been made of denitrification in the streams of Illinois or the agricultural midwest in general.

Benthic sediments are likely to be the most important site for denitrification in streams, but denitrification also is associated with other sub-

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strates, such as algal mats and macrophytes, which tend to be abundant in nutrient-rich agricultural streams. Filamentous green algae, such as *Cladophora*, can form mats several centimeters thick, and anoxic zones in the mats support denitrifying bacteria (e.g., Kemp and Dodds 2002a). Vascular macrophytes also can support epiphytic denitrification, even when O_2 concentrations in the surrounding water are high (Eriksson 2001).

Denitrification in the streambed of several agricultural streams in Illinois did not affect the transport of NO_3-N except during periods of low discharge and low NO_3-N concentration, which occurred only in late summer and early autumn (Royer et al. 2004). However, Royer et al. (2004) did not examine denitrification in algal mats. Previous studies have found that sediments supported significantly greater areal rates of denitrification than did plants (Eriksson and Weisner 1997, Kemp and Dodds 2002b), but such comparisons have not been made in agricultural streams in Illinois. Thus, our goal was to compare denitrification in benthic sediments with that occurring in floating mats of algae and macrophytes in an agricultural stream in east-central Illinois. Standing stock of plant biomass was quantified throughout the growing season, so that denitrification in both habitats could be expressed as areal rates.

Methods

Site description

Most of the study was conducted in Big Ditch, a 3rd-order tributary to the Sangamon River in east-central Illinois (Champaign County). Land use in the watershed is >80% row-crop agriculture, mostly corn and soy beans, and much of this land is heavily fertilized with N (David and Gentry 2000). As is typical of agricultural headwater streams, Big Ditch is extensively channelized and incised, and floods rarely overtop its banks. The stream receives agricultural runoff through subsurface tile-drains, but overland flow into the stream is rare. Channelization and tile-drains have created a hydrologically flashy system with short water-retention times (Rhoads and Herricks 1996). The riparian vegetation consists almost exclusively of grasses, and the stream has an open canopy throughout its length. The streambed consists of gravel and sand, with some accumulations of fine organic sediment (Royer et al. 2004). The stream lacks coarse allochthonous organic matter and inorganic substrates larger than gravel.

Field procedures

Five equidistant transects were established along a 50-m reach of Big Ditch at a site ~5 km above the confluence with the Sangamon River and 100 m upstream of a gauging station operated by the Illinois State Water Survey. Big Ditch was sampled 12 times between May and November 2002. On each sampling date, a grab sample for water chemistry was collected from the center of the stream at the most upstream transect. Temperature and dissolved O_2 (DO) were measured with an Orion probe (Model 835A) at midmorning on each date. In addition to the point measurements, continuous DO and temperature measurements were taken from 9 July to 12 July 2002 using a Hydrolab Mini Sonde.

Plant cover on the streambed was determined on each sampling date. On each transect, a meter tape was stretched across the stream. Wetted width and the distance along the transect (cm) covered by plant material were measured, and plant cover was expressed as % wetted width. At each transect, all plant material in a 314-cm^2 area that was completely covered by algae and macrophytes was collected, dried for 48 h at 60°C to determine the dry mass (DM), and expressed as DM/m^2. This value was then scaled by % cover at that transect to estimate total plant biomass on an areal basis (g DM/m^2). *Potamogeton* and *Cladophora* were not separated for estimates of plant cover and biomass because they grew in close association and were often intertwined. Plant cover and biomass for the study reach were calculated as the mean of the 5 transects.

On each sampling date, plant material was collected from an undisturbed area near the center of the stream on each transect for denitrification assays. Plant samples were taken from several individual plants or algal tufts, and all material for each transect was combined into a composite sample. A 28-cm^2 corer was used to sample the upper 5 cm of benthic sediment (~100 g) near or beneath where plant material had been collected. These samples were taken to the laboratory for use in the denitrification assays (described below). An additional sediment sample was collected at each transect for determination of standing stock of DM and ash-free dry mass (AFDM) in the streambed. AFDM was 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 postcombustion DM = AFDM). All samples were transported to the laboratory in stream water immediately after sampling.

The same methods were used to sample 4 other streams with similar land use and agricultural impacts to provide a frame of reference within which the Big Ditch results could be evaluated. The sites were in Black Slough, an unnamed tributary to the Kaskaskia River, and the West Okaw River in east-central Illinois, and Cobb Ditch in the Kankakee River watershed in northwest Indiana. Black Slough was sampled twice during the 2002 growing season, and the other sites were sampled once.

Water chemistry

Water samples were filtered through a 0.45-μm membrane, appropriately preserved (APHA 1995), and later analyzed for NO_3-N and dissolved organic C (DOC). NO_3-N concentrations were determined using a Dionex DX-120 ion chromatograph, at a detection limit (DL) of 0.01 mg NO_3-N/L. DOC concentrations were determined on a Dohrmann–Xertex DC-80 analyzer (DL = 0.5 mg/L). Unfiltered water samples were used to determine pH and specific conductance using a glass electrode and conductivity bridge, respectively.

NO_3-N load

Discharge was monitored continuously by the Illinois State Water Survey immediately downstream of our study reach. Water samples for NO_3-N analysis were collected at the monitoring station weekly from January through December 2002. During high discharge, samples were collected more frequently with an ISCO 2900 sampler. The concentration of NO_3-N in the water samples was determined as described above. Between measurements, daily concentrations of NO_3-N were estimated by interpolation. The NO_3-N load in Big Ditch was determined by multiplying mean daily discharge by the measured or interpolated daily NO_3-N concentration.

Denitrification rates

The acetylene block method (Tiedje et al. 1989, Knowles 1990) was used to measure denitrification (Martin et al. 2001). Subsamples of ~25 cm^3 of substrate (plant or sediment) from each transect were placed in 150-mL media bottles with a butyl septum in each lid. Plant samples were not rinsed to minimize disturbance to epiphyte communities. Following substrate addition, each bottle was filled to 75 mL with unfiltered stream water. Bottles containing plant material were covered with aluminum foil to inhibit photosynthesis. Chloramphenicol (5 mM concentration) was added to each bottle. This antibiotic prohibited de novo synthesis of proteins (Brock 1961), and it reduced bottle effects to give more accurate estimates of in situ denitrification rates (Smith and Tiedje 1979). Bottles were purged with He for 5 min to create a reducing environment of <1 mg O_2/L. Approximately 10% of the He in each bottle was replaced by adding 15 mL of pure C_2H_2 using gas-tight syringes (Martin et al. 2001). The bottles were then shaken, vented, and incubated at stream-water temperature. The headspace gas in each bottle was sampled 4 times during a 2-h period using gas-tight syringes. The sample volume was replaced with a mixture of 10% C_2H_2 and 90% He. Before headspace gas was sampled, bottles were shaken to release N_2O from the sediments and then allowed to equilibrate for 3 min.

The gas samples were analyzed on a Varian 3600 gas chromatograph with a ^{63}Ni electron-capture detector to quantify the concentration of N_2O. A serial dilution of high purity N_2O (Scotty II, Scott Specialty Gases, Plumsteadville, Pennsylvania) was used to create standards that were analyzed prior to analyzing samples from the denitrification assays. Denitrification rates were determined by regressing N_2O concentration in the bottles against time and correcting for the solubility of N_2O (Tiedje 1982). Denitrification rates were converted to areal rates by multiplying the rate by the standing stock of plant DM or sediment AFDM. Pearson product–moment correlation coefficients (Zar 1999) were used to examine relationships between NO_3-N, DOC, and denitrification rates in Big Ditch.

TABLE 1. Chemical and physical characteristics of Big Ditch during 2002. DOC = dissolved organic C.

Date	NO_3-N (mg/L)	DOC (mg/L)	Dissolved O_2 (mg/L)	Discharge (m^3/s)	Temperature (°C)
5 May	16.2	1.7	15.4	1.37	14.2
23 May	17.7	1.6	12.2	1.32	13.1
10 June	16.7	1.8	11.9	0.78	21.4
21 June	16.9	1.8	11.9	0.80	21.5
10 July	8.65	2.8	7.2	0.22	24.7
19 July	1.34	3.4	16.5	0.07	28.0
29 July	0.64	4.7	12.2	0.14	24.8
12 August	0.03	5.8	13.6	0.21	27.6
4 September	0.48	3.7	10.0	0.05	19.6
25 September	0.03	4.4	9.5	0.08	13.4
9 October	0.03	4.6	9.8	0.02	12.6
8 November	0.09	3.2	11.2	0.03	6.9

Results

Physical and chemical characteristics

Mean discharge was 0.44 m^3/s, but discharge was substantially higher during May and June than during late summer and autumn (Table 1). Width and depth of the stream averaged 7.4 m and 0.12 m, respectively. Water temperature ranged from ~7°C in November to 28°C in mid July, and DO ranged from ~7 to 16.5 mg/L (Table 1). Continuous DO measurements in Big Ditch from 9 to 12 July 2002 showed a minimum of ~3.8 mg/L (47% saturation) just prior to sunrise and a maximum of ~16.4 mg/L (>150% saturation) during midafternoon (data not shown). DOC ranged from 1.6 to 5.8 mg/L and was consistently greater from mid July through November than in May and June (Table 1). Specific conductance and pH averaged 582 μS/cm and 8.25, respectively (data not shown).

Peak NO_3-N concentrations in Big Ditch ranged from ~14 to ~18 mg NO_3-N/L and occurred from February through early July (Table 1, Fig. 1A). Concentrations decreased sharply during July as agricultural drainage ceased and generally remained <1 mg NO_3-N/L through the remainder of 2002 (Table 1, Fig. 1A). NO_3-N loads in Big Ditch ranged from <1 to >20 Mg N/d (Fig. 1B). The highest daily NO_3-N loads occurred from February through June. The other streams examined were generally similar to Big Ditch in physical and chemical characteristics (Table 2).

Plant biomass

Based on visual estimates, plant samples collected in May and June were ~50% *Potamogeton* and 50% *Cladophora* but, as the season progressed, the composition shifted to predominantly *Cladophora*. Plant cover ranged from 27 to 86% of the stream area in Big Ditch (Fig. 2A); the highest values occurred during periods of low flow, July through December. Three distinct peaks in plant biomass were observed: 212 g, 201 g, and 184 g DM/m^2 on 10 June, 12 August, and 24 September 2002, respectively. The first 2 peaks occurred just prior to scouring spates that reduced biomass substantially (Fig. 2B). Temporal patterns in plant biomass were not determined at the other sites because the sites were sampled only once; however, % cover was visually similar to that in Big Ditch.

Denitrification rates

On a biomass basis, plant-associated and sediment denitrification rates were similar, with maximum rates of 4.2 and 3.7 μg N_2O (g DM)$^{-1}$ h^{-1}, respectively, and an average rate of 1.2 μg N_2O (g DM)$^{-1}$ h^{-1} across both habitats (Fig. 3A, B). Temporal patterns for plant-associated and sediment denitrification rates were similar (Fig. 3A, B). In general, plant-associated and sediment denitrification rates at the other sites were similar to those in Big Ditch (Fig. 3A, B). On an areal basis, plant-associated denitrification rates (Fig. 4A) were lower than sediment rates (Fig.

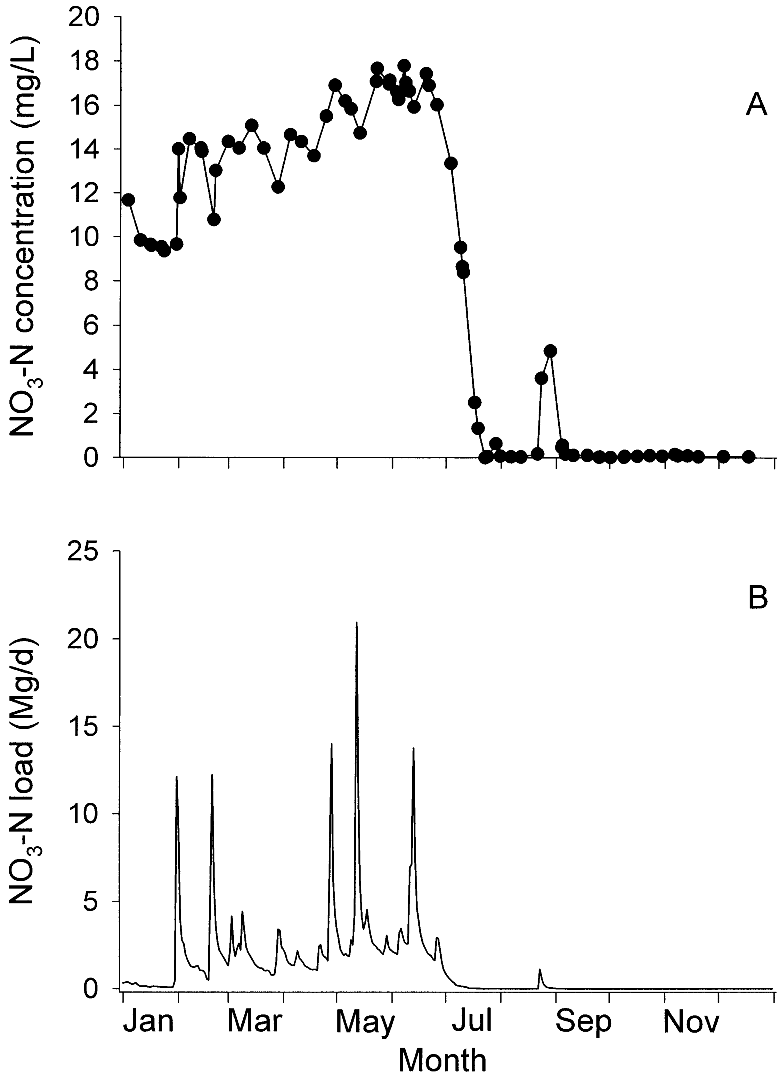

FIG. 1. Stream-water NO_3-N concentrations in Big Ditch from January to December 2002 (A), and daily NO_3-N loads in Big Ditch during the same period (B). Mg = 10^6 g.

TABLE 2. Chemical and physical characteristics of additional agricultural streams in Illinois and Indiana examined during 2002. DOC = dissolved organic C. – = no data.

Site	Date	NO_3-N (mg/L)	DOC (mg/L)	pH	Specific conductance (μS/cm)	Dissolved O_2 (mg/L)	Discharge (m^3/s)	Temperature (°C)
Cobb Ditch	15 July	0.04	2.5	8.0	856	10.3	1.01	21.4
Kaskaskia tributary	17 July	5.87	2.7	8.5	528	14.0	0.02	28.2
Black Slough	8 August	1.36	2.2	8.1	640	11.1	0.06	22.2
West Okaw River	16 August	0.04	–	–	–	15.3	0.03	29.2
Black Slough	22 August	3.02	2.4	8.0	665	8.6	0.05	23.4

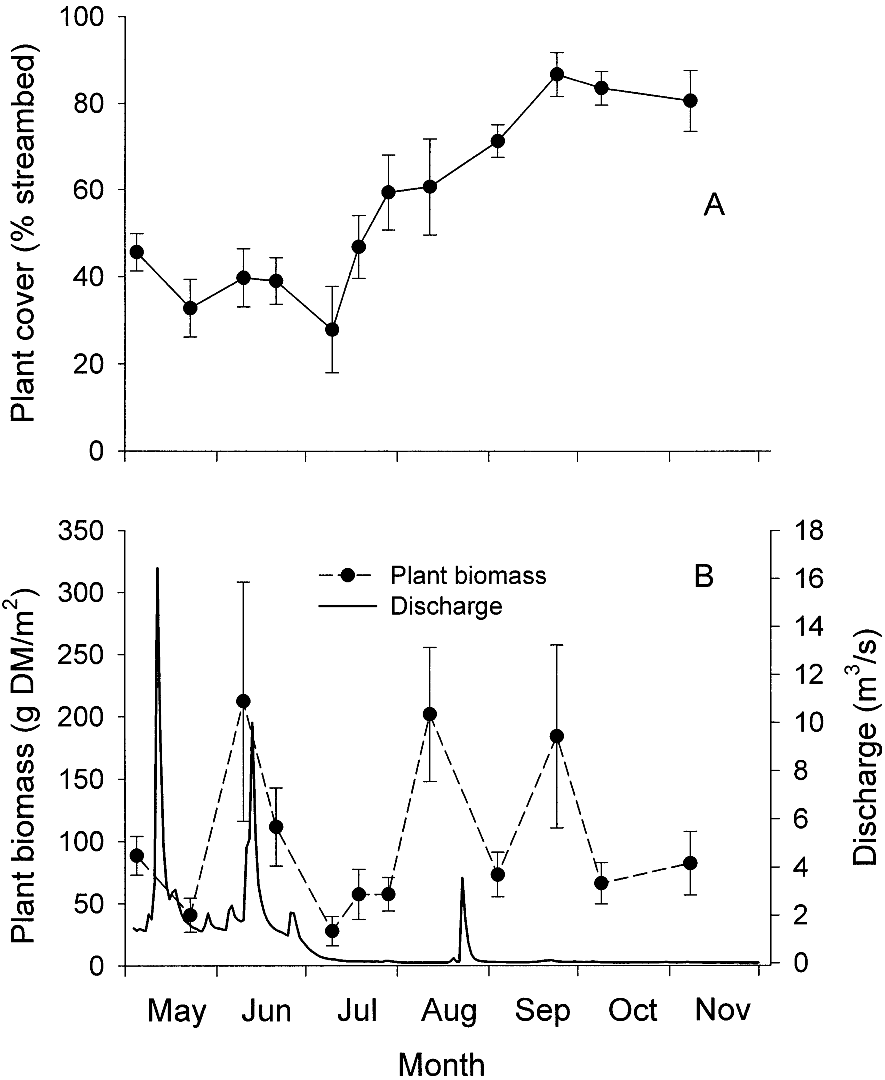

FIG. 2. Mean (±1 SE, $n = 5$) % plant cover on the streambed of Big Ditch from May to November 2002 (A), and mean (±1 SE, $n = 5$) areal biomass of plant material and discharge in Big Ditch during the same period (B). DM = dry mass.

4B) by at least an order of magnitude. The maximum plant-associated rate was 0.29 mg N m^{-2} h^{-1} (Fig. 4A), whereas the maximum sediment rate was 15.8 mg N m^{-2} h^{-1} (Fig. 4B).

Plant-associated denitrification in Big Ditch was correlated with NO_3-N concentration ($r = 0.648$, $p < 0.05$), but denitrification in the sediment was not ($r = 0.216$, $p > 0.05$). Both plant-associated and sediment denitrification were inversely correlated with DOC ($r = -0.600$, $p < 0.05$ and -0.337, $p < 0.05$, respectively).

Discussion

NO_3-N concentrations in Big Ditch ranged from 0.03 to >17 mg NO_3-N/L. This large range is typical of tile-drained agricultural streams in east-central Illinois, where NO_3-N concentrations change seasonally in relation to agricultural drainage and discharge (David et al. 1997). Daily NO_3-N loads declined markedly during late summer and autumn because both NO_3-N concentration and stream discharge were lower

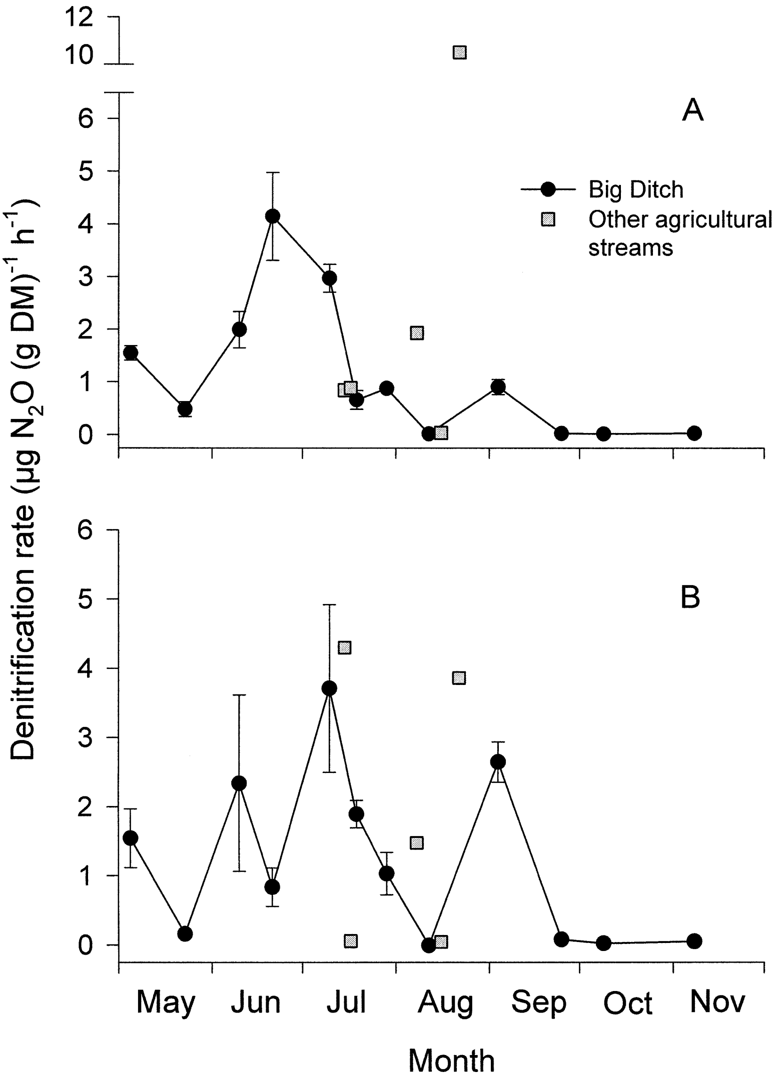

FIG. 3. Mean (±1 SE, n = 5) biomass-based denitrification rates for plant material (A) and benthic sediments (B). Error bars are present for all dates but may be encompassed by the symbol. DM = dry mass.

than earlier in the year (Fig. 1). This seasonal pattern in NO_3-N loads is typical of streams in the N-fertilized watersheds of east-central Illinois (David et al. 1997).

Benthic sediments supported denitrification rates as high as 15 to16 mg N m^{-2} h^{-1} in Big Ditch. Howarth et al. (1996) reported denitrification rates up to 60 mg N m^{-2} h^{-1}, but other studies from a range of stream types typically reported rates <5 mg N m^{-2} h^{-1} (Seitzinger 1988, Thompson et al. 2000, Kemp and Dodds 2002b). Five of the 12 measurements in Big Ditch were >6 mg N m^{-2} h^{-1} (Fig. 4B), indicating the site often supported high rates of sediment denitrification. Plant-associated and sediment denitrification rates in Big Ditch were similar when expressed on the basis of biomass (Fig. 3A, B). However, on an areal basis, sediment rates were often 1 to 2 orders of magnitude greater than denitrification in mats of *Cladophora* and *Potamogeton* (Fig. 4A, B), suggesting sediments represent the greater N sink in Big Ditch. Moreover, photosynthesis can prevent high rates of denitrification in periphyton dur-

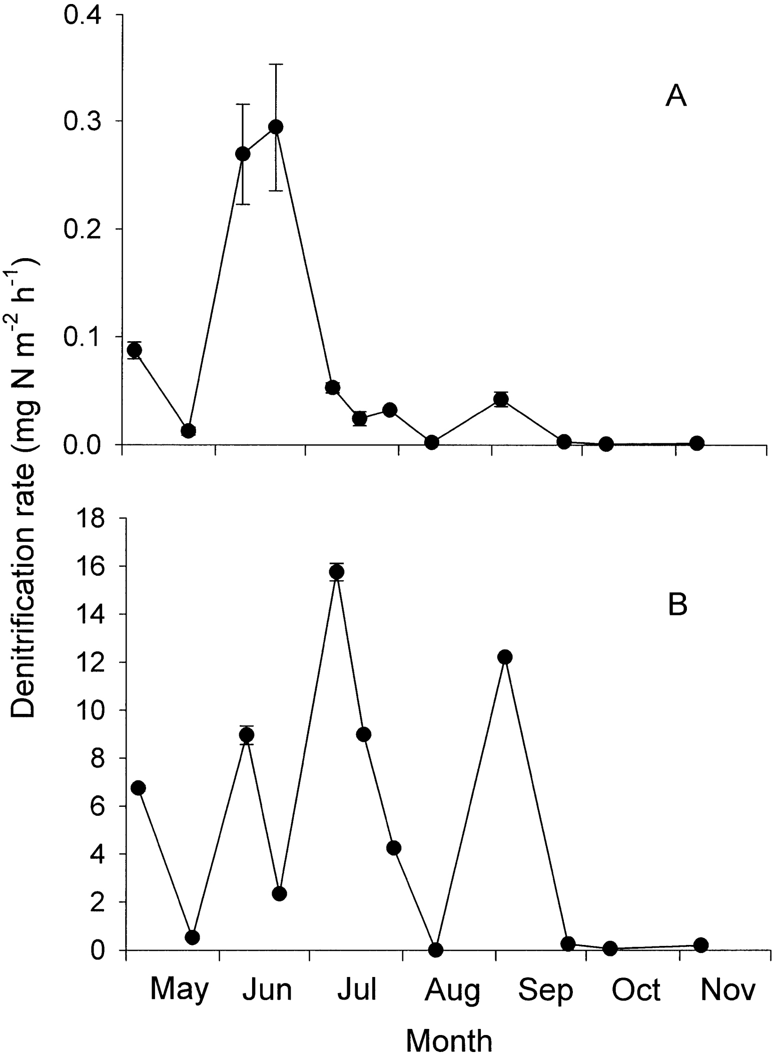

FIG. 4. Mean (± 1 SE, n = 5) areal denitrification rates for plant material (A) and benthic sediments (B). Error bars are present for all dates but may be encompassed by the symbol.

ing daylight (Triska and Oremland 1981), reducing the importance of plants as sites for denitrification. Other comparisons of plant-associated and sediment denitrification have shown that sediments supported much greater areal denitrification rates than plants (Eriksson and Weisner 1997, Kemp and Dodds 2002b). Thus, large mats of macrophytes and *Cladophora* are visually impressive in many agricultural streams, but they may play a relatively minor role as sites for N removal by denitrification.

The denitrification rates in Big Ditch were within the range of values measured at our other sites (Fig. 3), indicating that the range of spatial and temporal variability in denitrification rates in Big Ditch probably is typical of tile-drained, agricultural streams. Variability in sediment denitrification is often related to NO_3-N or DOC concentrations (e.g., Cooke and White 1987, Garcia-Ruiz et al. 1998). Denitrification in the sediments of Big Ditch was not strongly related to NO_3-N in the water column and was inversely related to DOC, suggesting other factors were involved in controlling sediment denitrification rates. NO_3-N did appear to control denitrification associated with mats of *Cladopho-*

ra and *Potamogeton*, suggesting the factors controlling denitrification might vary between habitats within a stream reach.

Denitrification requires anaerobic conditions and may be strongly influenced by diel patterns in DO caused by photosynthesis (Eriksson 2001). Continuous measurements made in July showed that DO concentrations became supersaturated under full sunlight but declined to <50% saturation at night. The effect of fluctuating DO on denitrification rates in Big Ditch was not addressed directly in our study, but it is plausible that temporal patterns of denitrification may be cyclic in the same manner as DO in streams. Eutrophic streams often display large diel changes in DO (e.g., Wiley et al. 1990), so extrapolating denitrification rates over time periods as short as 24 h may give misleading results on the importance of denitrification as a NO_3-N sink. As with NO_3-N, the effect of DO on denitrification might vary between habitats, such as algal mats and benthic sediments, within a stream.

A complete N budget was not constructed for Big Ditch, but our study indicated that denitrification in floating mats of *Cladophora* and *Potamogeton* contributed relatively little to total denitrification. Moreover, NO_3-N concentrations and loads during the first part of 2002 suggested that total instream denitrification did not substantially reduce NO_3-N export from the Big Ditch watershed, despite high rates of sediment denitrification. Peterson et al. (2001) examined relatively undisturbed headwater streams and found that instream processes strongly influenced N losses from watersheds. Royer et al. (2004) examined several agricultural streams, including Big Ditch, and found the transport of NO_3-N was unaffected by instream denitrification, particularly during periods of high NO_3-N concentrations. Overall, our research indicates that agricultural streams, such as Big Ditch, can support high rates of denitrification at times, but the effect of denitrification on NO_3-N dynamics is likely to be greater in streams less disturbed by agricultural practices than those in our study.

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