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Variability and regulation of denitrification in an Upper Mississippi River backwater

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Abstract. Sediments in the backwaters of the Upper Mississippi River (UMR) are highly organic and provide an optimal environment for N removal. We monitored an 8.6-ha UMR backwater site near La Crosse, Wisconsin, for nearly 3 y to assess temporal variability, seasonal trends, and the factors regulating denitrification. We measured rates of unamended denitrification (DEN) and denitrification enzyme activity (DEA) rates at ambient temperature and DEA at 30°C (DEA30). Seasonal mean (±1 SE) DEN rates ranged from 0.041 ± 0.015 to 0.47 ± 0.23 μg N cm^{-2} h^{-1} and were highest in winter and lowest in autumn. Seasonal rates of DEA exhibited a different pattern with the highest rates in summer (25.6 ± 3.4 μg N cm^{-2} h^{-1}) and the lowest rates in winter (10.6 ± 2.1 μg N cm^{-2} h^{-1}). The overall mean DEA30 rate was 31.0 ± 1.9 μg N cm^{-2} h^{-1} but showed no significant seasonal pattern. Short-term (weekly) and seasonal variability exhibited by rates of DEN and DEA were best explained by water-column NO_3^- concentration and temperature, respectively. No environmental variables explained a significant amount of variability in DEA30. Our results suggest that nutrient (i.e., NO_3^-) availability and temperature are both regulators of denitrification, with NO_3^- concentration being the most important limiting factor in this system. The high DEN rates during winter were in response to elevated NO_3^- concentrations resulting from a chain reaction beginning with algal blooms creating oxic conditions that stimulated nitrification. Increasing hydrological connectivity in large rivers as a river management tool to reduce N flux to downstream areas may be beneficial.

Key words: denitrification, large river, backwater, nitrate, nitrogen cycle.

Rivers are an important link transporting large volumes of water containing suspended and dissolved materials from the terrestrial landscape to coastal marine environments. High concentrations of dissolved nutrients in fresh waters have been linked to several environmental conditions that have been deemed harmful, including toxic algal blooms, NO_3^- contamination, excessive macrophyte growth, fish kills, and reduction in species richness (Carpenter et al. 1998, Dodds 2002). Delivery of reactive nutrients, especially N, from large rivers into coastal systems also has been cited as a cause of coastal eutrophication (Nixon 1995). In the US, seasonal hypoxia observed in the bottom waters of certain coastal areas in the Gulf of Mexico is an indirect result of the high nutrient load deposited into the Gulf from the Mississippi River (Rabalais et al. 2002, Dagg and Breed 2003).

The Mississippi River drains ~41% of the conterminous US and supplies an annual N flux of nearly 1.56×10^6 Mg N to the northern region of the Gulf of Mexico (Goolsby and Battaglin 2001). It has been estimated that much of the N (>90%) present in the Mississippi River travels conservatively through the main stem and ultimately will be delivered to the Gulf of Mexico (Alexander et al. 2000). In an analysis of major world rivers, Caraco and Cole (1999) found that point and nonpoint loading could account for >80% of NO_3^- river flux. These analyses imply that instream N cycling

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processes such as denitrification (anaerobic reduction of NO_3^- to NO_2^-, N_2O, or N_2) may have little effect on N loads in large rivers such as the Mississippi River. However, several studies have observed relatively high rates of N cycling in large riverine systems (Sjodin et al. 1997, Garcia-Ruiz et al. 1998, Richardson et al. 2004, Strauss et al. 2004), and the apparently small effect of processing on total N export may simply be a result of the high N load overwhelming the high processing rates (Strauss et al. 2004).

Large floodplain rivers are complex systems that characteristically contain an array of aquatic habitats, including the main channel, secondary channels, riverine wetlands, and backwaters that are defined and regulated by hydrological connectivity through the system (Ward et al. 2002). Variability in nutrient cycling processes among these different aquatic habitats is significant because of the different physical and chemical attributes inherent to each of these habitats. For example, denitrification potential in backwaters of the St. Louis River in northeastern Minnesota was high but limited by NO_3^- availability because of low hydrological connectivity between the backwaters and the river water that contained high levels of NO_3^- (Johnston et al. 2001).

In a recent study in one reach of the Upper Mississippi River (UMR), we showed that backwaters (24% of areal extent) disproportionately denitrify more NO_3^- than most of the other aquatic habitats in the reach (Richardson et al. 2004). Backwater denitrification accounted for 33% of the total denitrification losses (~3% of the total annual NO_3^- load) in that reach. Denitrification losses would probably be greater, but backwaters typically have the lowest water-column NO_3^- concentrations of any of the contiguous aquatic habitats in the river because of their low hydrological connectivity with the NO_3^--rich main channel (Soballe et al. 2002). Much of the NO_3^- that is denitrified in backwaters is probably produced by nitrification (aerobic oxidation of NH_4^+ to NO_3^-) in oxic microsites. The NO_3^- is then transported by way of diffusion to adjacent anoxic areas where it is immediately denitrified, i.e., coupled nitrification–denitrification. The total N-loss potential of these backwaters is unknown, but increasing NO_3^- delivery into these systems probably would increase N losses from the system. In fact, increasing hydrological connectivity to riverine backwaters and wetlands has been proposed as a way to decrease N loads to coastal ecosystems because of the high denitrification potential in the backwaters and wetlands (Mitsch et al. 2001).

Our study is an extension of our previous reports (Richardson et al. 2004, Strauss et al. 2004) on N cycling in the UMR. Those reports document denitrification and nitrification patterns measured over relatively broad spatial and temporal scales, but the studies were limited by infrequent sampling (once per season). We extrapolated across seasonal time scales to make generalized estimates of N cycling without knowing that denitrification was limited by variation in NO_3^- delivery (Richardson et al. 2004). Short-term variability (shorter than weeks) in river stage causes fluctuations in material transport into and out of backwater lakes (Heiler et al. 1995, Tockner et al. 2000, Hein et al. 2003, 2004). It is also likely that response times of denitrifying bacteria to NO_3^- fluxes are fairly rapid, so that seasonal sampling misses much of the dynamic linkage between variable environmental conditions and bacterially mediated N cycling (Tiedje et al. 1982). Our primary objective was to use frequent sampling in a single UMR backwater site to examine temporal variability of denitrification within seasons. Another goal was to assess factors that potentially regulate denitrification by comparing different denitrification metrics and relating the denitrification rates to environmental variables.

Methods

The UMR is the reach of the Mississippi River upstream of Cairo, Illinois, and much of that reach (the portion upstream of St. Louis, Missouri) is segmented into a series of navigation pools delineated by a series of locks and dams. This river architecture has resulted in hydrological patterns that have developed and maintained distinct aquatic habitats, including the main channel, side channels, impounded areas, and backwaters (Richardson et al. 2004, Strauss et al. 2004). Most of water flowing through this system travels within the navigation channel (Fig. 1), a part of the main channel that is dredged to maintain a minimum depth (2.7 m) to aid commercial navigation. Less water flows through the side channels, and water movement through backwaters is evident only during periods of high discharge or discharge fluctuations (i.e., backwaters release water to and receive water from the flowing channels during discharge decreases and increases, respectively). Backwaters in the UMR system generally have higher sediment organic C (27.2 g C/kg dry mass), total N (4.37 g N/kg dry mass), and macrophyte abundance relative to the other habitats (Strauss et al. 2004).

Field sampling

All samples in our study were collected from a single site (lat 43°52′0″N, long 91°14′57″W) in an 8.6-ha backwater in Navigation Pool 8 of the UMR (Fig. 1). The site was sampled 95 times over a 2.8-y period

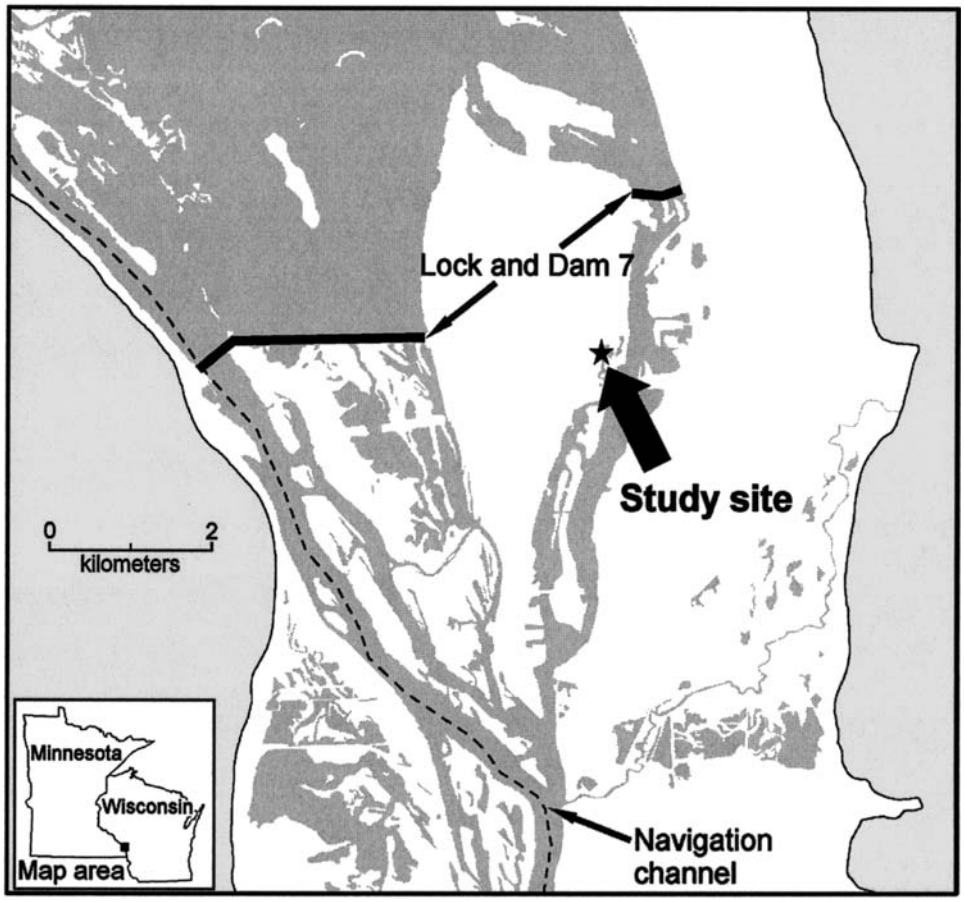

FIG. 1. Backwater study site in Navigation Pool 8 of the Upper Mississippi River.

between 22 February 2001 and 17 December 2003. Sampling frequency was determined by water temperature at the site (<5°C, sampled monthly; >5°C, sampled weekly). Sampling was less frequent during cold conditions because of the difficulty of sampling through ice and because previous studies indicated that denitrification activity was probably low under these conditions. The size of the site, ~6 m × 2 m, allowed us to systematically rotate among locations within the site for each sampling event to facilitate location recovery and avoid sampling the same location on consecutive sampling events. Sampling was conducted from a floating platform during ice-free conditions to prevent disturbance of the sampling site. Mean water depth at the sampling site during our study was 0.75 m and ranged from 0.04 to 3.33 m.

During each sampling event, surface-water temperature, pH, dissolved O_2 (DO), and conductivity were measured in situ with a 600XL YSI multiparameter probe prior to collection of water and sediment samples. Sediment temperature and pH were determined onsite with a Beckman Φ11 pH meter. A single surface-water grab sample and sediment sample were collected and returned to the laboratory for determination of NH_4^+ and NO_3^- concentrations in the water and pore water. Six additional sediment samples also were collected for denitrification rate analysis. Water samples were collected in low-density polyethylene bottles and sediment samples were collected and transported to the laboratory in polycarbonate cores (2.54 cm diameter × 5 cm depth).

Pore water was extracted from sediment through centrifugation (1366 × g, 12 min). Pore and surface water were filtered (Whatman GF/F), acidified (pH < 2 with H_2SO_4), and stored at 4°C until analysis. NH_4^+ and NO_3^- analyses were started within 30 d of collection and were done on a Bran+Luebbe TrAAcs 800 Continuous Flow Analysis System according to standard methods (APHA 1998).

Measurement of denitrification

Denitrification was assessed using 3 metrics: unamended denitrification rate (DEN), denitrification enzyme activity at ambient temperature (DEA), and

DEA at 30°C (DEA30). All 3 metrics are variants of the acetylene-inhibition technique and were measured using the methods described in detail in an earlier paper (Richardson et al. 2004). The DEN is a measure of ambient denitrification without amended nutrients, whereas DEA and DEA30 are measures of denitrification potential in the presence of added glucose, NO_3^-, and chloramphenicol (an enzyme synthesis inhibitor). All measurements were begun the same day as sediment collection. Samples consisted of 25 cm^3 of sediment suspended in 20 mL of unfiltered surface water in a 353-mL glass sample jar (standard 8-oz canning jar). The DEN samples received an additional 5 mL of surface water, whereas DEA and DEA30 samples received 5 mL of DEA solution (final concentrations: 12 mg glucose-C/L, 14 mg NO_3^--N/L, and 100 mg chloramphenicol/L). All 3 metrics were measured in duplicate from separate sediment samples during each sampling event for a planned total of 570 denitrification estimates during our study. Of the 570 planned estimates, only 534 were actually obtained because of compromised samples (e.g., sample loss during transport, spillage, gas leakage).

After the sediment slurries were prepared, the jars were sealed using standard canning jar lids and rings. Anaerobic conditions were initiated through subsequent evacuation and purging of sample jars with helium for 15 min. Atomic-absorption-grade acetylene (20 mL) was then added with a syringe through a septum on the top of each sample container. Slurries were incubated under constant agitation (175 rpm) at ambient surface-water temperature (DEN and DEA) or 30°C (DEA30) in a darkened incubator. Slurries were shaken to ensure equilibration of N_2O gas between slurry and headspace. Headspace gas (5 mL) was sampled at 30, 60, and 90 min for DEA and DEA30 and at 1, 4, and 24 h for DEN. Headspace N_2O concentrations were measured using a Hewlett–Packard model 5890 gas chromatograph equipped with an electron capture detector (ECD ^{63}Ni) and argon-5% methane (P5) was used as the carrier gas (Airgas, Inc., Radnor, Pennsylvania). Injection, column, and detector temperatures were 60, 60, and 340°C, respectively. The N_2O standard curve was generated using 1, 5, 10, 100, and 1000 ppm N_2O gas standards (Matheson Tri-Gas, Montgomeryville, Pennsylvania). Denitrification rates were measured from the linear rate of N_2O evolution according to the equations listed in Groffman et al. (1999).

Data analysis

SAS statistical software (version 8.02, SAS Institute, Cary, North Carolina) was used for all statistical analyses. Only sampling-event mean values from the duplicate measures of each denitrification metric were used in statistical analyses to improve estimate accuracy and to avoid the issue of pseudoreplication (Hurlbert 1984). Spearman correlations were calculated among physical/chemical variables measured during each sampling event and between the denitrification metrics to assess bivariate relationships. Seasonal denitrification means were calculated from values collected during September to November (autumn), December to February (winter), March to May (spring), and June to August (summer). Differences in seasonal denitrification metrics and certain physical/chemical variables were determined using nonparametric 1-way analysis of variance (ANOVA) on ranked data and pairwise comparisons were assessed using the Tukey method. This analysis is equivalent to the Kruskal–Wallis nonparametric *k*-sample test, however, the *F*-test produced by the ANOVA is often better than the χ^2 approximation used by the Kruskal–Wallis test.

Time-series regression models were developed to explain variability measured in DEN, DEA, and DEA30. Independent variables for initial time-series regression models were selected using the stepwise procedure. Significance criteria for all variables included in the models were set at $p < 0.05$. These initial regression models were then tested for time-series autocorrelation errors with the Durbin–Watson test and model corrections were made using Yule–Walker estimation if errors were found (autoreg procedure, SAS statistical software). The regression assumption of independent errors was considered irrelevant in this analysis because the autoreg procedure assumes this violation and corrects the regression estimates accordingly. The regression assumptions of error homeoscedasticity and normality of regression residuals were tested using the Portmanteau *Q*-test and by examination of normal probability plots, respectively.

Potential seasonal nutrient and temperature limitation of denitrification were assessed using the measured rates of DEN, DEA, and DEA30. An index of relative nutrient limitation (D_N) was calculated as DEA minus DEN, and an index of relative temperature limitation (D_T) was calculated as DEA30 minus DEA. The fact that chloramphenicol was amended to samples used for determination of DEA and DEA30 rates but not for DEN should not be an issue in this calculation because our preliminary studies (unpublished data) have suggested that measured rates of DEN with and without chloramphenicol do not vary significantly in this system. However, even if the absence of chloramphenicol did disproportionately

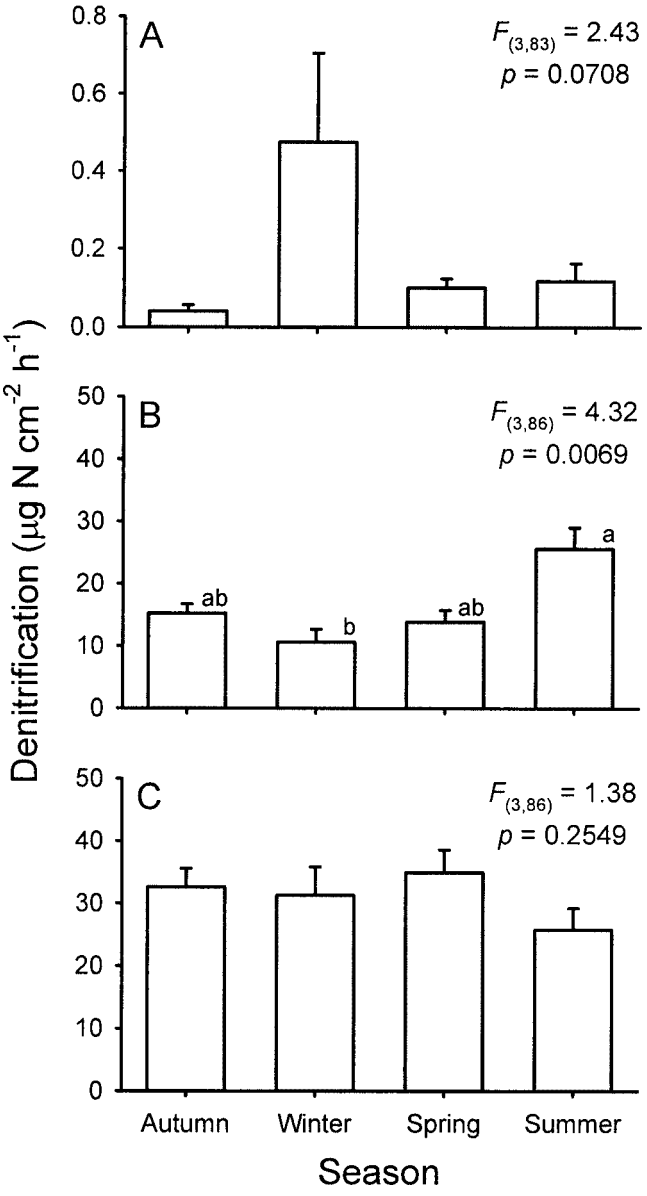

FIG. 2. Seasonal means (+1 SE) of denitrification rate measured at ambient temperature with no amendments (DEN, A), denitrification rate measured at ambient temperature with amended NO_3^- and glucose (DEA, B), and denitrification rate measured at 30°C with amended NO_3^- and glucose (DEA30, C) in a backwater site of the Upper Mississippi River. The seasonal *F*-statistic from each 1-way analysis of variance is shown in each panel. Seasonal means of DEA with the same lower-case letter are not significantly different ($p > 0.05$, Tukey pairwise comparison method).

elevate the measured DEN rate, the resulting assessment of nutrient limitation would only be a more conservative estimate. Differences among and between seasonal values of D_N and D_T were determined with nonparametric ANOVA as described above.

Results

The 3 denitrification metrics measured in this riverine backwater system had distinctly different temporal patterns, probably because each responded to different environmental variables. DEN rates (overall mean ± SE = 0.119 ± 0.026 μg N cm^{-2} h^{-1}) were the lowest of the 3 metrics ($p < 0.0001$) and ranged from 0 to 1.58 μg N cm^{-2} h^{-1}. Differences among seasonal DEN rates were marginally significant ($p = 0.07$), with the highest rates occurring during winter (Fig. 2A). DEN rates were most significantly correlated with concentrations of NO_3^- ($r = 0.43$, $p < 0.0001$; Fig. 3A) and DO ($r = 0.38$, $p = 0.0003$). DEA rates (overall mean ± SE = 17.87 ± 1.47 μg N cm^{-2} h^{-1}) did vary significantly among seasons ($p = 0.0069$) and were highest in summer, followed by autumn, spring, and winter, respectively (Fig. 2B). DEA rates were most significantly correlated with temperature ($r = 0.49$, $p < 0.0001$; Fig. 3B). DEA30 rates were the highest ($p < 0.0001$) of the 3 denitrification metrics (overall mean ± SE = 30.98 ± 1.87 μg N cm^{-2} h^{-1}), but had no discernable pattern of variability. As a consequence, no significant differences were found among seasonal DEA30 rates (Fig. 2C), nor were DEA30 rates correlated with any environmental variable.

Regression models predicting denitrification metrics included variables known to influence denitrification rates in the UMR (Table 1). The regression model that accounted for the most variability among the denitrification metrics was that for DEN and was dependant on the dissolved inorganic N (NO_3^- and NH_4^+) concentrations in the water column ($R^2 = 0.41$). DEA rates were best predicted by water-column temperature ($R^2 = 0.17$), and no variables could significantly account for the measured variability in DEA30 rates (i.e., a significant regression model could not be built using available data).

Denitrification rates were limited by temperature or nutrient availability during all seasons as evidenced by non-zero values of D_T or D_N (Fig. 4). Nutrient limitation varied seasonally ($p = 0.025$) with highest limitation occurring during summer and lowest limitation occurring during winter. Temperature limitation of denitrification also varied seasonally ($p < 0.0001$) with the lowest limitation occurring in summer and the greatest limitation occurring in autumn, winter, and spring. However, only during the summer did we detect one form of limitation that was more important than the other; during this season, limitation from insufficient nutrient supply was greater than that from low temperature ($p < 0.0001$).

DO and nutrient concentrations also showed significant seasonal patterns and may explain patterns

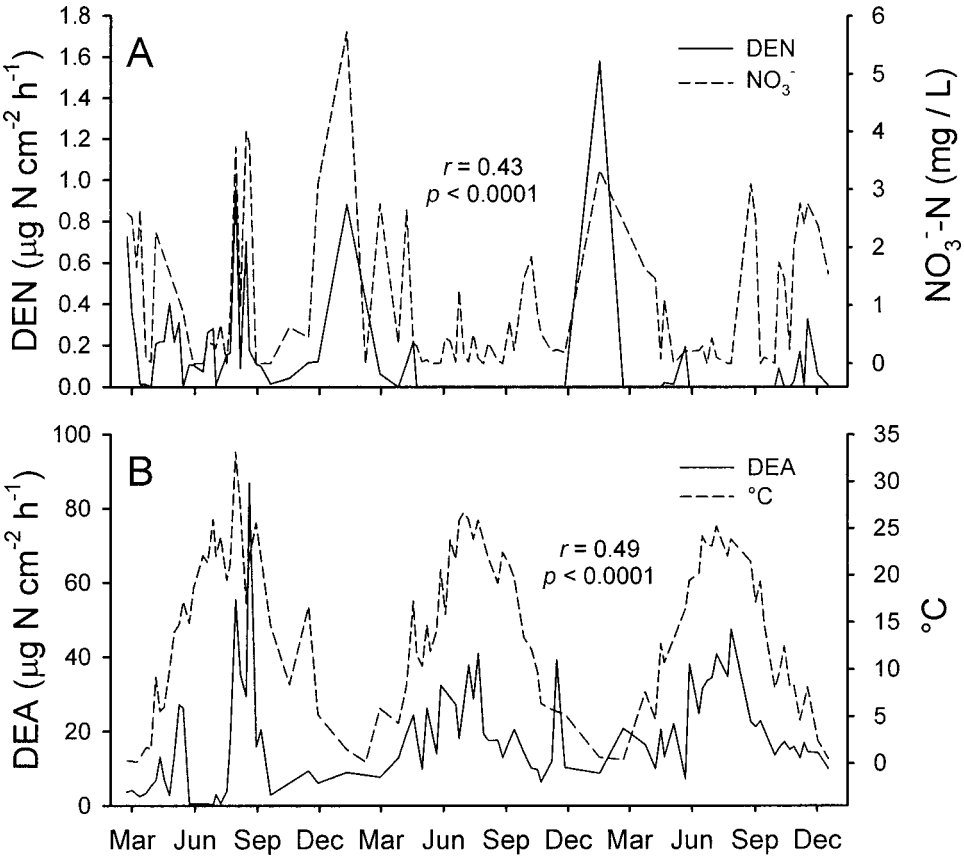

FIG. 3. Patterns of denitrification rate measured at ambient temperature without nutrient amendments (DEN) compared to NO_3^- concentration in the water column (A), and denitrification rate measured at ambient temperature with amended glucose and NO_3^- (DEA) compared to temperature (B) at a backwater site of the Upper Mississippi River. The dates on the x-axis range from 22 February 2001 to 17 December 2003.

observed in the denitrification metrics. Concentrations of DO were lowest in summer (mean ± SE = 5.5 ± 0.7 mg/L) and highest in winter (12.6 ± 3.3 mg/L) (Fig. 5A) despite the nearly constant ice cover present at the site during winter. Visual observations during field sampling suggest that high algal biomass (*Spirogyra* sp.) and production under the ice may be responsible for elevated winter DO. Porewater NO_3^- and water-column NO_3^- concentrations were also highest in winter (0.13 ± 0.04 and 2.6 ± 0.7 mg N/L, respectively) and lowest during summer (0.02 ± 0.01 and 0.7 ± 0.2 mg N/L, respectively; Fig. 5B, D). Conversely, porewater NH_4^+ concentrations were greatest in summer (7.8 ± 0.6 mg N/L) and lowest in winter (5.3 ± 1.2 mg N/L; Fig. 5C).

Discussion

Our results indicate that denitrification rates in this backwater system were highly variable, even on weekly or seasonal scales (Fig. 3). Despite this variability, our rates were comparable to rates measured in multiple backwater lakes in a separate study in Pool 8 of the UMR (Richardson et al. 2004). In that study, mean seasonal unamended denitrification rates in backwaters ranged from 0.03 to 0.40 μg N cm^{-2} h^{-1}; those rates are similar to our seasonal mean range of 0.04 to 0.47 μg N cm^{-2} h^{-1}. The DEA rates were also similar; our study found mean seasonal rates ranging from 10.6 to 25.6 μg N cm^{-2} h^{-1} and Richardson et al. (2004) reported preflood (a major 100-y flood in 2001 had a negative effect on denitrification rates) mean seasonal backwater rates ranging from 10.5 to 22.2 μg N cm^{-2} h^{-1}.

In our study, the rates differed significantly between the denitrification metrics (i.e., DEA30 > DEA > DEN) and reflect the expected responses to nutrient amendments and the different environmental conditions under which they were measured. The highest rates were expected and occurred in the DEA30 measurement because these samples were incubated under favorable conditions (i.e., amended with nutrients and incubated at a favorable temperature). The DEA rates

TABLE 1. Regression models predicting denitrification rates measured at ambient temperature without nutrient amendments (DEN), denitrification rate measured at ambient temperature with amended glucose and NO_3^- (DEA), and denitrification rate measured at 30°C with amended glucose and NO_3^- (DEA30) in a backwater site of the Upper Mississippi River. The regression variables NO_3^- and NH_4^+ are water-column concentrations (mg N/L). temp = water-column temperature (°C). A significant ($p < 0.05$) model predicting DEA30 could not be found.

Denitrification metric ($\mu g\ N\ cm^{-2}\ h^{-1}$)	Regression model	n*n*	R^2	Overall *p*
DEN	$0.111(NO_3^-) + 0.087(NH_4^+) - 0.038$	80	0.41	<0.0001
DEA	0.826(temp) + 6.622	89	0.17	<0.0001

were lower than DEA30 because these samples received a nutrient amendment, but were incubated at generally lower temperatures. The lowest rates were in the DEN samples that did not receive amended nutrients and were incubated at ambient temperatures.

The technique we used to quantify all of our denitrification metrics (acetylene inhibition) also inhibits the nitrification process (i.e., NO_3^- production). Inhibition of nitrification can cause underestimation of denitrification rates when NO_3^- concentrations are low and denitrification is coupled with NO_3^- production generated by nitrification in the same system that is sampled (Knowles 1990). Nitrification rates are high in backwater sediments of the UMR and denitrification is probably coupled with nitrification in these areas (Richardson et al. 2004, Strauss et al. 2004). Thus, our DEN rates are probably underestimates of actual denitrification rates. Inhibition of nitrification was not important for DEA measurement because NO_3^- was added at a rate higher than could have been supplied through nitrification, but these rates should be considered only as a denitrification potential or the upper limit of actual denitrification. Therefore, actual rates of denitrification in this system are probably somewhat greater than the DEN rates but less than the DEA rates we reported.

Limitation of denitrification

Temperature limitation and nutrient limitation are both important in this backwater system as evidenced

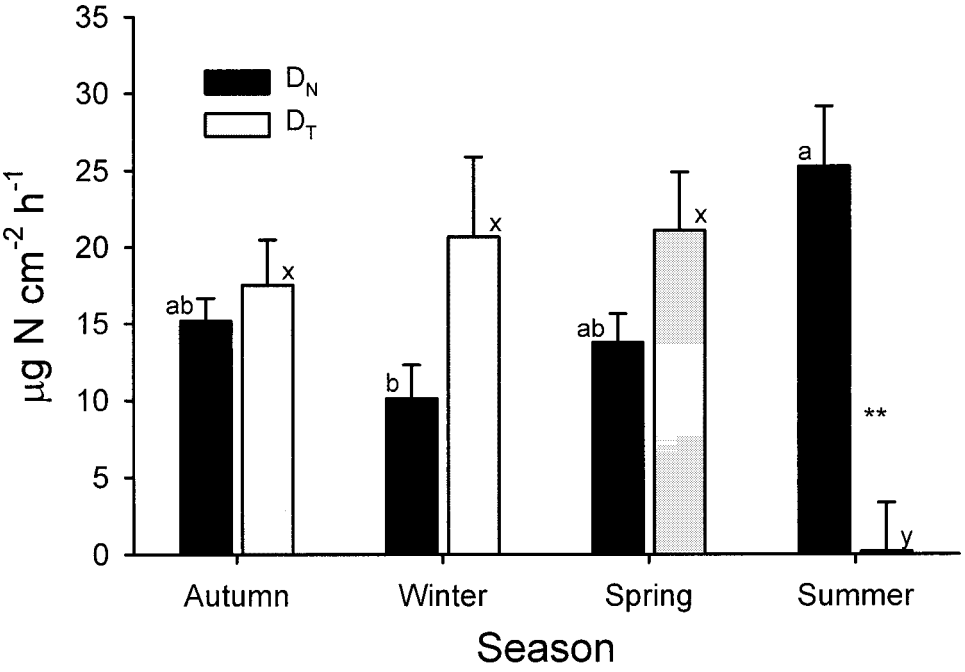

FIG. 4. Seasonal mean (+1 SE) indices of relative nutrient (D_N) and temperature (D_T) limitation of denitrification measured in a backwater site of the Upper Mississippi River. Values with the same lower-case letter (a and b for D_N; x and y for D_T) are not significantly different ($p > 0.05$, Tukey pairwise comparison method). ** indicates a significant difference ($p < 0.0001$) between seasonal values of D_N and D_T.

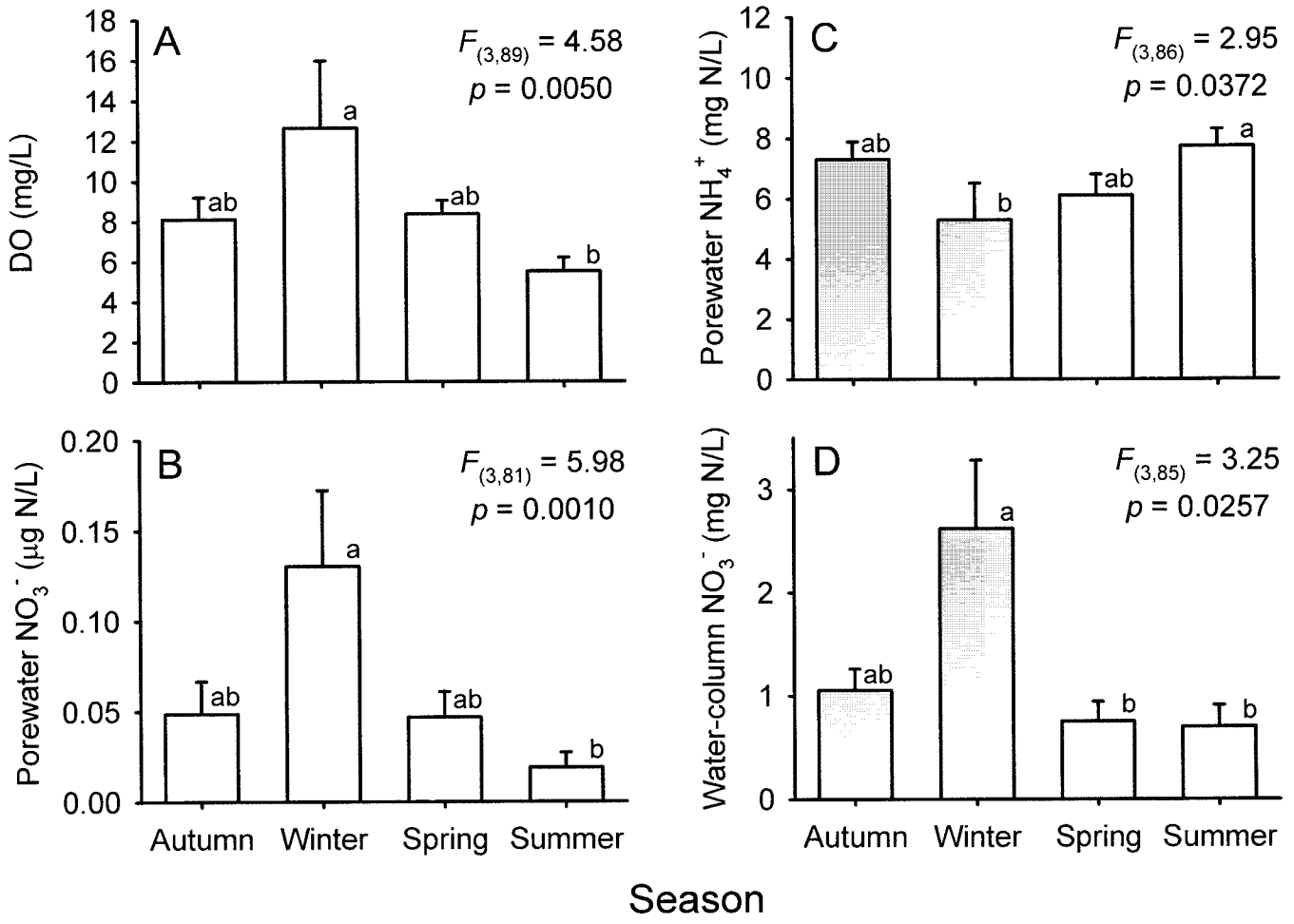

FIG. 5. Seasonal mean (+1 SE) dissolved O_2 (DO; A), porewater NO_3^- (B), porewater NH_4^+ (C), and water-column NO_3^- (D) concentrations measured in a backwater site of the Upper Mississippi River. The seasonal *F*-statistic from each 1-way analysis of variance is shown in each panel. Means with the same lower-case letter are not significantly different ($p > 0.05$, Tukey pairwise comparison method).

by the relatively large values of D_T and D_N (Fig. 4) compared to the rates of DEN (Fig. 2A). However, our estimate of temperature limitation was obtained under nonlimiting levels of nutrients. Thus, the effect of temperature without added nutrients is unknown and cannot be assessed with the current data. Our data also cannot distinguish between NO_3^- limitation, organic C limitation, or colimitation by both NO_3^- and organic C. However, the DEN regression model (Table 1), the significant correlation between NO_3^- concentration and DEN (Fig. 3A), and denitrification limitation studies conducted in similar areas by Richardson et al. (2004) all suggest that denitrification in this backwater system is limited primarily by the availability of NO_3^-. Many other studies have also noted that denitrification rates are positively related to NO_3^- concentrations in sediments and in a variety of other substrates (Kana et al. 1998, White and Reddy 1999, Kemp and Dodds 2002). When nutrients are not limiting, denitrification is most influenced by temperature as evidenced by the regression model for DEA (Table 1) and the significant positive correlation between DEA and temperature (Fig. 3B). Temperature limitation reflected the positive relationship that typically occurs between temperature and bacterial activity and was predictably least important during summer because of the warmer water during this period.

Seasonal patterns of denitrification

Studies that include year-round measurements of denitrification in temperate ecosystems are uncommon, but the pattern observed in our study (highest unamended denitrification rates occurring in the winter) was somewhat unusual compared to other systems. Most studies that measure denitrification at

various times throughout a year report peak denitrification during the warmer nonwinter months (e.g., Thompson et al. 1995, White and Reddy 1999, Martin et al. 2001). However, high annual unamended denitrification rates during the winter have been documented in some studies. For example, in the sediments of Lake Suwa, Japan, and in the sediments of a lagoon on the French Mediterranean coast, the highest denitrification rates were observed in winter and spring, whereas the lowest rates occurred in summer (Gilbert et al. 1997, Hasegawa and Okino 2004). In both of these studies and in our study, the winter denitrification peaks also coincided with elevated NO_3^- availability (Fig. 3A).

Sources of NO_3^- in riverine systems are restricted to intrasystem NO_3^- production (i.e., nitrification), groundwater inputs, and loading from upstream or tributary sources. We did not measure nitrification rates directly, but reduced levels of porewater NH_4^+ and elevated levels of DO, porewater NO_3^-, and water-column NO_3^- during winter (Fig. 5) suggest that nitrification was probably responsible for the increased surface-water NO_3^- concentrations also observed during winter. Furthermore, we know from previous studies that nitrification can be a significant source of NO_3^- production in UMR backwaters (Strauss et al. 2004). Using the mean winter UMR backwater nitrification rate from Strauss et al. (2004), we calculate that it would take ~40 h to raise the NO_3^- concentration 1 mg N/L at this site (assuming no uptake or loss of NO_3^-). This rate of NO_3^- production is adequate to account for the NO_3^- concentrations observed.

Groundwater inputs of NO_3^- in this area are negligible (R. J. Hunt, US Geological Survey, personal communication). The other mechanism for increasing NO_3^- concentrations in riverine backwaters is through increased river discharge, which increases hydrological connectivity between the NO_3^--rich main channel and backwaters (Heiler et al. 1995, Tockner et al. 2000, Richardson et al. 2004). However, increased connectivity was probably not the reason for higher winter NO_3^- concentrations because fluctuations in discharge typically were small during winter, and NO_3^- concentrations and water depth (a surrogate for discharge) were not significantly correlated during winter.

The apparent increase in nitrification and the subsequent increase in NO_3^- concentration during winter were probably in response to increased DO concentrations associated with the observed blooms of metaphyton (i.e., *Spirogyra* sp.). Algal growth was probably stimulated by the presence of clear ice and low snow cover. Increased coupling between nitrification and denitrification fueled by benthic primary production has been documented in estuarine sediments (Dong et al. 2000, An and Joye 2001). However, this phenomenon may be limited to high-NH_4^+ sediments (like the sediments in UMR backwaters) because benthic algae may out-compete nitrifying bacteria for available NH_4^+ when sediment NH_4^+ concentrations are low, thereby decreasing the coupling between nitrification and denitrification (Risgaard-Petersen 2003).

Even though DEA rates were probably higher than actual denitrification rates, the seasonal pattern of DEA (summer > autumn > spring > winter) was probably indicative of the actual seasonal denitrification pattern that exists in the system. Clearly, denitrification is NO_3^- limited and responds positively to NO_3^- loading. However, our DEN measurement cannot account for internal NO_3^- production by way of nitrification because the acetylene gas used to measure DEN also inhibits nitrification. If nitrification is an important source of NO_3^- in this system (i.e., if nitrification and denitrification are coupled), then patterns of actual denitrification would be more similar to those of nitrification and DEA rather than the patterns exhibited by DEN. Our earlier study documented high nitrification rates in a pattern similar to our DEA rates: highest nitrification rates were found in summer followed by spring, autumn, and winter, respectively (Strauss et al. 2004). Furthermore, our previous studies also showed that UMR nitrification rates are greater than DEN, but less than DEA (Richardson et al. 2004, Strauss et al. 2004), suggesting that denitrification is limited by NO_3^- availability and can reduce all of the NO_3^- produced by nitrification. Therefore, it is reasonable to conclude that internal NO_3^- loading and, subsequently, denitrification follow the same temporal pattern.

Our measured rates of DEA30 were useful for documenting temperature limitation, but the absence of a seasonal pattern exhibited by these rates also indicated that the pool of viable denitrification enzymes present in the sediment was relatively constant throughout the year. We cannot comment on the seasonal variance in relative abundance of populations of denitrifying bacteria or the richness of the total denitrification community, but it is clear that the capacity of the denitrifying bacteria community to reduce NO_3^- did not change significantly at any time throughout the study. Regardless of when the sediments were collected, denitrification rates were relatively constant when they were incubated at a favorable temperature with adequate substrates. One explanation could be that enzyme synthesis occurs even when denitrification is low, producing a large pool of enzymes that can be used quickly when

environmental conditions favoring denitrification arise (Parsons et al. 1991).

River management

One of the river management tools proposed to alleviate N discharge into coastal regions includes diverting a portion of the NO_3^--rich flowing water into wetland and backwater habitats that have high denitrification potential (Mitsch et al. 2001, Hey 2002). Our results support this hypothetical means of reducing N flux in the UMR. We did not observe high connectivity between our study backwater and flowing water, but we did document high denitrification potential, NO_3^- limitation of denitrification, and the propensity of denitrification in the backwater to respond positively to increases in NO_3^- availability. Higher connectivity would increase transport of NO_3^- and increase circulation of oxygenated water in the backwaters, resulting in a larger proportion of oxic sediments and, ultimately, in more tightly coupled nitrification–denitrification. Preliminary results from a study examining connectivity in UMR backwaters showed that controlled flows through backwater lakes are capable of removing an average of 40% of the total NO_3^- load, with denitrification accounting for ~35% of this removal (W. F. James, US Army Corps of Engineers, personal communication).

Natural hydrologic connectivity in many backwater systems creates periodic fluctuations in water level that move water into and out of the backwaters, and this transient process may be important for maintaining ecologic functioning of backwater systems (Tockner et al. 2000). Increasing hydrological connectivity in highly modified systems may include removing channel-training structures like revetments, closing dams, and wing dams, but may also include construction of flow diversions and flow-through structures. Many of these actions would return a river to a more natural state, and the river itself would restore natural habitat heterogeneity and hydrologic patterns (Stanford et al. 1996). However, increasing water movement and flow through backwaters will increase NO_3^- delivery, but it may have side effects that could limit potential N loss. First, flow will decrease water residence time in the backwater and, thereby, reduce the time available for denitrification. Second, flowing water could alter the biological and physical characteristics of the backwater system that produce high denitrification potential (e.g., erosion of the thick layer of organic sediment). It is difficult to address the effect of these potential side effects on N dynamics because they have received little attention, but the effect probably depends on the level of flow and the geomorphology of the system. Nevertheless, thoughtful river management designed to increase hydrologic connectivity should have a positive effect on N loss from large river ecosystems.

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