Seasonal effects of the zebra mussel (*Dreissena polymorpha*) on sediment denitrification rates in Pool 8 of the Upper Mississippi River

Denise A. Bruesewitz, Jennifer L. Tank, Melody J. Bernot, William B. Richardson, and Eric A. Strauss

Abstract: Zebra mussels (*Dreissena polymorpha*) have altered the structure of invaded ecosystems and exhibit characteristics that suggest they may influence ecosystem processes such as nitrogen (N) cycling. We measured denitrification rates seasonally on sediments underlying zebra mussel beds collected from the impounded zone of Navigation Pool 8 of the Upper Mississippi River. Denitrification assays were amended with nutrients to characterize variation in nutrient limitation of denitrification in the presence or absence of zebra mussels. Denitrification rates at zebra mussel sites were high relative to sites without zebra mussels in February 2004 (repeated measures analysis of variance (RM ANOVA), $p = 0.005$), potentially because of high NO_3^--N variability from nitrification of high NH_4^+ zebra mussel waste. Denitrification rates were highest in June 2003 (RM ANOVA, $p < 0.001$), corresponding with the highest NO_3^--N concentrations during the study (linear regression, $R^2 = 0.72$, $p < 0.001$). Denitrification was always N-limited, but sites without zebra mussels showed the strongest response to N amendments relative to sites with zebra mussels (two-way ANOVA, $p \leq 0.01$). Examining how zebra mussels influence denitrification rates will aid in developing a more complete understanding of the impact of zebra mussels and more effective management strategies of eutrophic waters.

Résumé : Les moules zébrées (*Dreissena polymorpha*) ont modifié la structure des écosystèmes qu'elles ont envahis et elles possèdent des caractéristiques qui laissent croire qu'elles peuvent influencer les processus écosystémiques, tels que le cycle de l'azote (N). Nous avons mesuré les taux saisonniers de dénitrification dans les sédiments sous-jacents aux colonies de moules zébrées récoltés dans la zone de barrage du bassin de navigation 8 du Mississippi supérieur. Les tests de dénitrification ont été amendés avec des nutriments afin de décrire la variation de la limitation de la dénitrification due aux nutriments en présence des moules zébrées et en leur absence. En février 2004, les taux de dénitrification aux sites contenant des moules zébrées étaient plus élevés que dans les sites sans moules zébrées (analyse de variance à mesures répétées (RM ANOVA), $p = 0{,}005$), peut-être à cause d'une forte variabilité de NO_3^--N due à la nitrification de l'élimination importante de NH_4^+ par les moules zébrées. Les taux de dénitrification étaient maximaux en juin 2003 (RM ANOVA, $p < 0{,}001$), ce qui correspond aux plus fortes concentrations de NO_3^--N durant l'étude (régression linéaire, $R^2 = 0{,}72$, $p < 0{,}001$). La dénitrification est toujours limitée par l'azote, mais les sites sans moules zébrées ont la plus forte réaction aux amendements à l'azote par comparaison aux sites contenant des moules zébrées (ANOVA à deux critères de classification, $p \leq 0{,}01$). L'examen de l'influence des moules zébrées sur les taux de dénitrification aidera à obtenir une compréhension plus globale de l'impact des moules zébrées et à élaborer des stratégies de gestion plus efficaces dans les eaux eutrophes.

[Traduit par la Rédaction]

Introduction

Since the introduction of zebra mussels (*Dreissena polymorpha*) to North America in the mid-1980s, they have spread to lakes and rivers throughout the Great Lakes and Mississippi River watersheds (Hebert et al. 1989; USGS 2003). Owing to their large filtering capacity and high densities (0.5–20 g shell-free dry mass·m^{-2}; Strayer et al. 1999), zebra mussels effectively harvest substantial quantities of phytoplankton and particulates from the water column (Caraco et al. 1997; Tuchman et al. 2004). Consumption of phytoplankton and the associated transfer of nutrients from the water column to the benthos has resulted in decreased phytoplankton productivity (Fahnenstiel et al. 1995; Caraco et al. 1997). Additionally, decreased water turbidity (Caraco et al. 2000), lower dissolved oxygen (DO) concentrations (Effler et al. 1996; Caraco et al. 2000), increased macrophyte production (Hebert et al. 1991; Caraco et al. 2000), and changes in benthic sediment characteristics (Klerks et al. 1996) have been documented in ecosystems invaded by zebra mussels.

Zebra mussels exert changes in sediment composition that likely cascade to affect the sediment redox environment and biogeochemical cycles of nitrogen (N) and carbon (C). They

Received 29 March 2005. Accepted 13 December 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 21 March 2006.
J18625

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 doi:10.1139/F06-002

directly influence the N cycle through waste production, with zebra mussel N excretion rates among the highest reported for any animal (Vanni 2002). Ammonium (NH_4^+) excretion by zebra mussels has been shown to enhance NH_4^+ mineralization (Gardner et al. 1995, 2001) and increase porewater NH_4^+ concentrations (Effler et al. 1996, 1997). Nitrification, the microbial oxidation of NH_4^+ to nitrate (NO_3^-), may increase in the presence of zebra mussels because of increased NH_4^+ availability (Lavrentyev et al. 2000). Further, because of anaerobic conditions and accumulation of C-rich pseudofeces deposited in sediments underlying zebra mussel beds, it is likely that denitrification (i.e., microbial reduction of NO_3^- to gaseous nitrous oxide (N_2O) and dinitrogen (N_2)) occurs at rates higher than in uninvaded sediments (Seitzinger 1988).

Increased N delivery to streams and rivers as a result of human activity has led to multiple environmental problems, including elevated groundwater NO_3^- concentrations that are dangerous to humans (i.e., methylhemoglobinemia, non-Hodgkin's lymphoma) and the development of coastal hypoxic zones (Seitzinger 1988; Rabalais 2002; Turner et al. 2003). Denitrification in stream and river sediments is thought to have great potential to remove reactive NO_3^--N from the environment (Alexander et al. 2000; Galloway et al. 2003). Therefore, understanding what factors constrain denitrification in streams and rivers and how invasive species may influence this potential sink for anthropogenic N is an important area of research.

Many ecosystems with elevated NO_3^- concentrations have also been invaded by zebra mussels; thus, it is important to understand the interaction between human-induced eutrophication and zebra mussel invasion of freshwaters. Zebra mussels may influence direct controls on denitrification (NO_3^-, organic C, and anoxia; Seitzinger 1988), as well as transfer nutrients to the sediment–water interface, potentially influencing downstream N flux. In this study, we examined seasonal patterns in denitrification rates across varying zebra mussel densities in Pool 8 of the Upper Mississippi River (UMR). The UMR has been impacted by both anthropogenically elevated N concentrations and zebra mussel invasion, providing an opportunity to examine the interaction between zebra mussels and denitrification in a high N system. Spatial and temporal patterns of N cycling are also known for part of this river system (Richardson et al. 2004; Strauss et al. 2004), providing a good basis to evaluate the effect of *D. polymorpha*.

We predict that zebra mussel invasion may influence sediment denitrification via several pathways, including (*i*) increasing NO_3^- availability via nitrification of NH_4^+ wastes (Effler et al. 1997; Lavrentyev et al. 2000), (*ii*) increasing labile C availability in sediments via the addition of feces and pseudofeces (Klerks et al. 1996), and (*iii*) decreasing sediment DO via respiration and microbial decomposition of the labile C (Caraco et al. 2000; Burks et al. 2002). Additionally, the effective filtering capacity of zebra mussels may alter sediment microbial processes simply via the physical transfer of dissolved and particulate materials from the water column to the benthos (Strayer et al. 1999). Our objective is to examine the influence of zebra mussels on sediment denitrification rates and to identify the relative importance of the potential mechanisms driving observed sediment denitrification rates on a large scale.

Materials and methods

Site description

The UMR is composed of 27 navigation pools divided by low head navigation dams. Pool 1 is located near Minneapolis, Minnesota, and Pool 27 is located near St. Louis, Missouri. This study was conducted in Navigation Pool 8 (hereafter referred to as Pool 8), a 37.5 km stretch of the UMR near La Crosse, Wisconsin (Fig. 1). Pools in the UMR are categorized into distinct areas, including main channel, side channel, contiguous backwater, and impounded habitats (Koel 2001; Strauss et al. 2004). Pool 8 has an annual mean depth of 1.7 m, wetted area under baseflow conditions of 10 425 ha, and median discharge of 905 $m^3 \cdot s^{-1}$ (Strauss et al. 2004).

Most of the flowing water in Pool 8 flows through the deeper main channel and is characterized by sandy substrate (D. Bruesewitz, personal observation). Commercial navigation requires a minimum depth in the main channel of 3.8 m, which is maintained by the US Army Corps of Engineers through channel dredging, wing dams, and side channel closing structures that direct flow. Side channels and contiguous backwaters have minimal water exchange with the main channel for much of the year, with the exception of flood periods (Sparks et al. 1998). Backwaters are characterized by organic sediments and widespread macrophyte growth (Fischer and Claflin 1995). The impounded zone is a quasilentic section of open water created upriver of a low head dam (Fig. 1) and exhibits less seasonal hydraulic variability than other aquatic areas (Sparks et al. 1998).

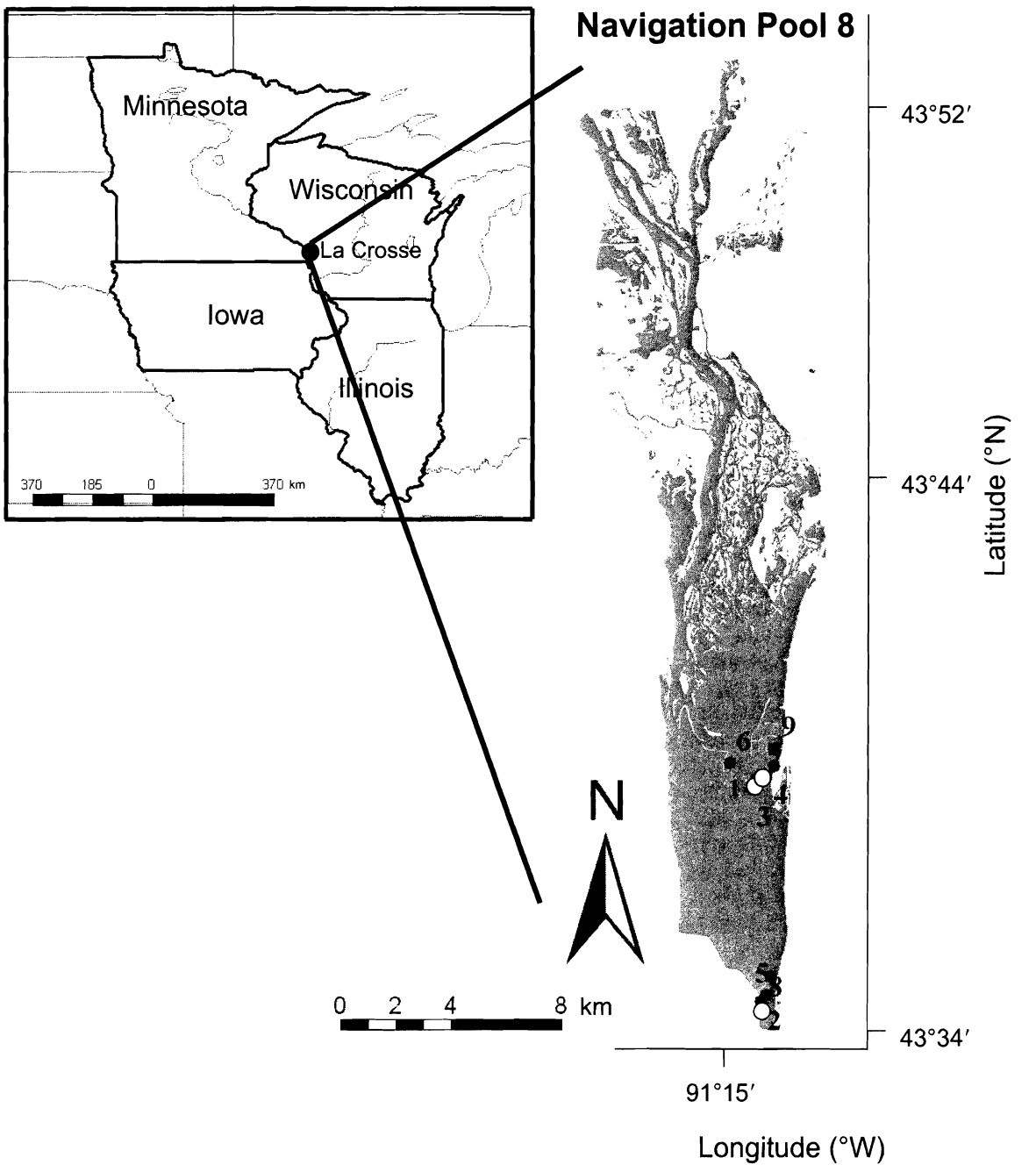
Zebra mussel distribution

Zebra mussels were first found in the UMR, including Pool 8, in 1991 and were introduced via commercial barge and recreational boat traffic (Cope et al. 1997). Because current in the main channel is potentially too powerful for zebra mussels to colonize in high densities, highest zebra mussel densities in Pool 8 occur in the impounded zone where quasilentic flow conditions are favorable and deeper waters potentially provide refugia from high water temperatures during summer or water level fluctuations (Smit et al. 1993; Stanczykowska and Lewandowski 1993). Sampling in Pool 8 conducted by the US Geological Survey (USGS) Long-term Resource Monitoring Program found that zebra mussel densities in the impounded zone peaked in 2001 (1609 individuals$\cdot m^{-2}$) and have declined since that time (Sauer 2003). Areas colonized by zebra mussels have been altered by the substantial buildup of shell material, and changes in the physical structure of benthic sediments has potentially increased habitat heterogeneity and altered sediment–water flow paths (Botts et al. 1996; Beekey et al. 2004).

Field sampling regime in Pool 8

Sediment and associated water column and porewater samples were collected approximately every other month from April 2003 to May 2004 from nine sites across a gradient of zebra mussel densities, with three sites in each cate-

Fig. 1. Map of Pool 8 of the Upper Mississippi River and the impounded zone of the pool, showing the nine study sites. Sites 1–3 (open circles) have no zebra mussels (*Dreissena polymorpha*, 0ZM sites); sites 4–9 (solid circles) have ~300–1000 mussels·m^{-2} (ZM sites).



gory: zero (0ZM), low (~500·m^{-2}, LZM), and high (~1000·m^{-2}, HZM) densities. Sampling sites were restricted to the impounded zone of Pool 8 to maintain similar flow conditions across all sites. There are two clusters of sites, each containing a subset of 0ZM, LZM, and HZM sites. Zebra mussel densities were quantified at each sampling point as the average number of live zebra mussels (determined visually) in six cores (2.54 cm diameter) taken from each site. Sediment for denitrification assays was collected from the top 5 cm of intact sediment cores. Sediment and surface water samples were stored at 4 °C and brought back to the laboratory until assays were performed within 14 h of collection. Water column temperature, conductivity, DO, and pH were measured prior to collection of sediment cores with a YSI 600XL multiparameter sonde at the water surface and at the sediment–water interface if there was DO stratification. Discharge data were obtained from the US Army Corps of Engineers St. Paul District Lock and Dam 8 (US Army Corps of Engineers 2006).

Physiochemical analysis of porewater and surface water samples

Sediment porewater, as well as overlying river water, was filtered through a Whatman 0.45 μm glass fiber filter, imme-

diately stored on ice, frozen within 5 h of collection, and stored for analyses of NO_3^--N, NH_4^+-N, and dissolved organic carbon (DOC). Porewater NH_4^+-N samples were extracted with 1 mol·L^{-1} KCl, centrifuged, filtered, and frozen for subsequent nutrient analyses. NH_4^+-N analyses were conducted using the spectrophotometric phenol–hypochlorite method (Solorzano 1969), while water column NO_3^--N concentration was quantified using ion chromatography (USEPA 1993). DOC was analyzed according to standard methods on a Shimadzu TOC analyzer (APHA 1995). Minimum detection limits for water chemistry analyses were 0.01 mg N·L^{-1} for surface water NO_3^- and for surface and porewater NH_4^+. All water chemistry analyses were performed with frequent standard checks that did not exceed a 1.9% difference from standards.

Denitrification assays using chloramphenicol-amended acetylene inhibition method

Sediment denitrification rates were quantified within 24 h of field collection using the chloramphenicol-amended acetylene (C_2H_2) inhibition technique (Knowles 1990; Martin et al. 2001; Royer et al. 2004). Preliminary tests determined that cold storage of samples for <48 h did not influence measured denitrification rates (Inwood et al. 2005; D. Bruesewitz, unpublished data). Chloramphenicol was added to the assays at a concentration of 0.3 mmol·L^{-1} to suppress de novo denitrification enzyme production and more accurately estimate in situ denitrification rates (Murray and Knowles 1999). Chloramphenicol-amended sediment slurries yielded linear denitrification rates over our short incubation times (~4 h; Bernot et al. 2003; Royer et al. 2004; Schaller et al. 2004). The assays were conducted using ambient river water, without the addition of NO_3^--N or DOC (except for nutrient amendment experiments described below). Based on our results and those of other studies (Bernot et al. 2003; Richardson et al. 2004; Royer et al. 2004), we believe the C_2H_2 inhibition method was appropriate for our spatially and temporally replicated study design in Pool 8, providing a simple, cost-effective method for estimating denitrification while minimizing potential errors caused by simultaneous nitrification inhibition (Bernot et al. 2003). This method is also especially useful for measuring denitrification rates in a large system such as the Mississippi River (Richardson et al. 2004), where other techniques such as isotopic tracer releases are logistically impossible.

On each sampling date at each site, replicate samples of composite surface sediment (25 cm^3, top 5 cm) were combined with 25 mL of unfiltered river water in 353 mL Kerr™ canning jars modified with an *n*-butyl rubber septa in the lid (model 70610-00105, n = 3·treatment^{-1}·site^{-1}). Anoxic conditions were ensured in the jar headspace and sediment slurry by alternately purging the jars with ultrapure helium and placing them under a vacuum. Pure acetylene was added to the headspace of the jars (20 mL C_2H_2; 10 kPa final pressure), and bottles were shaken at 125 r·min^{-1} in a dark incubator. The incubator was either at room temperature for comparison of rates across seasons at a constant temperature or at in situ river water temperature to estimate more realistic seasonal denitrification rates and for comparison of sites on a single sampling date. Gas samples for analysis of nitrous oxide (N_2O) were collected from the headspace of each bottle 10 min after the addition of C_2H_2 and every subsequent hour for 4 h for a total of five gas samples. Headspace was replaced with a mixture of helium and 7% C_2H_2 after each gas sample was removed to maintain a constant partial pressure in the jars. Samples were analyzed for N_2O on a Hewlett-Packard model 5890 gas chromatograph equipped with a ^{63}Ni electron-capture detector. Denitrification rates were calculated from the linear increase in N_2O concentration over time (Smith and Tiedje 1979; Murray and Knowles 1999).

Sediment ash-free dry mass (AFDM) was quantified on subsamples from each denitrification replicate so that denitrification rates could be expressed per gram AFDM of sediment. Areal denitrification rate estimates were based on the sediment core area (5.07 cm^2). We reported denitrification rates on an areal basis to examine seasonal trends, to compare between site types, or to identify site physiochemical characteristics that may control denitrification rates. We expressed denitrification rates per gram AFDM of sediment when examining the finer-scale question of nutrient limitation. Sediment C and N content (as %) were analyzed on an Elementar VarioMax CN elemental analyzer. Sediment organic matter content (as %) was calculated as (sediment AFDM/sediment dry mass) × 100.

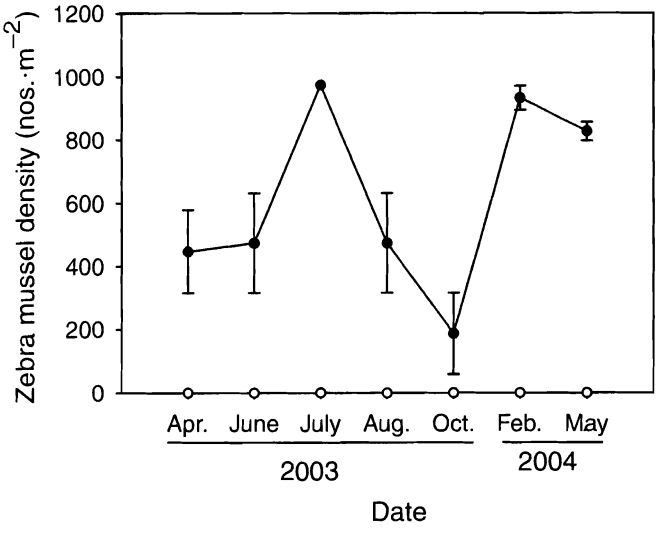
Nutrient amendment experiments of sediment denitrification

Nutrient amendments of sediment denitrification assays were conducted in April, August, and October of 2003 and in February and May of 2004 to determine if nutrient limitation of denitrification varied seasonally as a function of zebra mussel density. Additional sediment samples were collected from each site and amended with nitrogen (+N) as KNO_3^- to 6 mg N·L^{-1}, carbon (+C) as glucose to 30 mg C·L^{-1}, or both N and C (+N+C) to determine if sediment denitrification was limited by N or C availability. We consider glucose to be a representative labile source of C for denitrifiers, as has been shown by several recent studies (Dilly 2003; Garcia-Montiel et al. 2003; Murray et al. 2004). Amended bottles (three replicates per treatment per site) were incubated at room temperature for comparison with unamended bottles.

Statistical analyses

All statistical analyses were performed using SYSTAT (Version 10, SPSS Inc., Chicago, Illinois). Data were transformed to meet the assumption of normality, usually with a log transformation. Linear regression was used to identify site physiochemical characteristics that may control denitrification rates. The coefficient of variation (CV) was calculated for monthly denitrification rates. Patterns in sediment denitrification rates were analyzed with RM ANOVA with denitrification rates at each site type (0ZM or zebra mussel presence (ZM); see explanation in Results) as the repeated measure though time. Significant ANOVAs ($p < 0.05$) were followed by the Tukey–Kramer adjusted least square means test for post hoc multiple contrasts. Results from the nutrient amendment experiments were analyzed using a two-way ANOVA by presence or absence of each nutrient (+N or +C) to identify significant ($p < 0.05$) differences in denitrification rates among treatments (Tank and Dodds

Fig. 2. Zebra mussel (*Dreissena polymorpha*) densities at each site type (no zebra mussel sites presented in open circles; zebra mussel sites presented in solid circles) measured throughout the study with six replicate cores from each site (n = 3 for each site type). There is a significant difference at the α = 0.05 level between site types at each sampling date. All data are presented ±1 standard error.



2003). Single nutrient limitation by either N or C is indicated by a significant F value for that treatment alone. This analysis also allows determination of colimitation by both N and C based on significant ($p < 0.05$) interaction terms (N × C). Denitrification response to nutrient addition was used to compare the relative differences in nutrient limitation of denitrification between site types for each sampling date. Denitrification response was calculated as log (nutrient-amended denitrification rate/control denitrification rate), after Tank and Dodds (2003). These data were also analyzed with a two-way ANOVA by presence or absence of each nutrient as described above.

Results

Zebra mussel densities and site physicochemical parameters

Initially we had designated three sites as 0ZM, three sites as LZM (~500 individuals·m^{-2}), and three sites as HZM (~1000 individuals·m^{-2}). However, zebra mussel densities fluctuated over time. Although LZM sites had generally lower densities of live zebra mussels than at HZM sites, densities were variable within sites, and live zebra mussel densities at the HZM and LZM sites were not substantially different (except for August 2003). We therefore combined the LZM and HZM treatments and considered categorical sites of 0ZM and ZM (Fig. 2). Sediments at 0ZM sites had the highest organic matter content measured with sediment AFDM (4.6% ± 0.3% standard error (SE); RM ANOVA: $F_{\text{site type}} = 8.03$, $p = 0.008$; Table 1), while the sediment from ZM sites had lower organic matter content (2.8% ± 0.3% SE). Additionally, sediment C/N was significantly higher in the ZM sites (17.7 ± 1.3 SE) than in the 0ZM sites (13.4 ± 1.7 SE; RM ANOVA: $F_{\text{site type}} = 5.02$, $p = 0.03$). Overall, ZM sites were characterized by a buildup of zebra mussel shells 2–20 cm deep on the river bottom as new mussels colonized shells of older mussels, resulting in sediment characterized by unconsolidated shell fragments with silt accumulation among the shells.

Table 1. Mean water and sediment variables with standard error (SE) values for each site type.

	Site type	
Variable	0ZM (±1 SE)	ZM (±1 SE)
Water		
NH_4^+ (μg N·L^{-1})	32.82 (18.90)	41.81 (16.70)
NO_3^- (μg N·L^{-1})	2118.24 (73.14)	2596.43 (58.6)
DOC (mg C·L^{-1})	7.09 (0.60)	6.87 (0.25)
DO (mg·L^{-1})	13.00 (1.25)	12.86 (0.67)
Sediment		
% organic matter	4.59* (0.29)	2.77 (0.30)
%N	0.24* (0.04)	0.12 (0.01)
%C	2.39 (0.36)	1.94 (0.22)
C/N	13.40* (1.74)	17.70 (1.29)
Porewater NH_4^+ (mg·L^{-1})	2.41* (0.12)	1.91 (0.13)

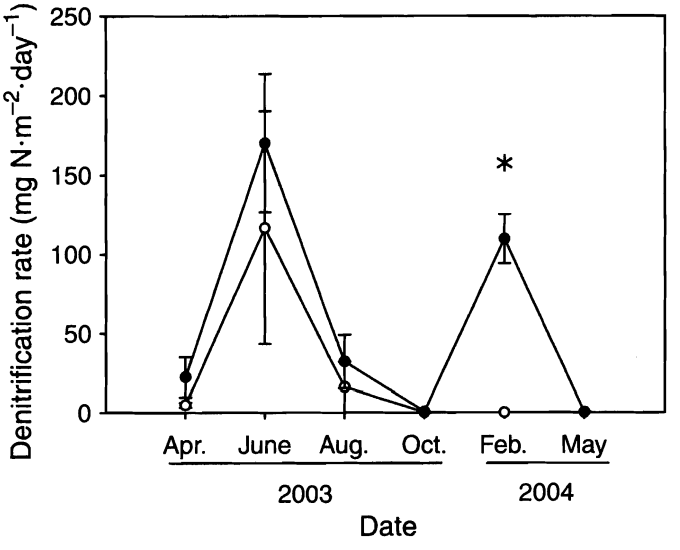
Note: Significant differences between site type means at the α = 0.05 level are indicated by an asterisk (*). 0ZM, no zebra mussels on site; ZM, zebra mussels present on site; DOC, dissolved organic carbon; DO, dissolved oxygen.

Water column NH_4^+-N, NO_3^--N, and DOC did not vary with zebra mussel density (RM ANOVA: $F_{\text{site type}} = 2.45$, 0.30, and 0.03, respectively, and $p = 0.13$, 0.59, and 0.88, respectively; Table 1). However, there were seasonal differences in mean nutrient concentrations at all sites (Table 1). NO_3^--N concentrations were highest in June (6.9 ± 2.6 mg NO_3^--N·L^{-1}; RM ANOVA: $F_{\text{time}} = 36.15$, $p < 0.001$) and lowest in October (0.21 ± 0.08 mg NO_3^--N·L^{-1}). In contrast, mean NH_4^+-N concentrations were highest in February (366 ± 49 μg NH_4^+-N·L^{-1}; RM ANOVA: $F_{\text{time}} = 36.38$, $p < 0.001$) and lowest in June (6.5 ± 0.6 μg NH_4^+-N·L^{-1}). DOC concentrations were significantly lower in February (4.5 ± 0.6 mg C·L^{-1}; RM ANOVA: $F_{\text{time}} = 6.74$, $p = 0.002$) compared with any other sampling date. Porewater NH_4^+ was significantly higher at 0ZM sites (2.41 ± 0.12 mg NH_4^+-N·L^{-1}; RM ANOVA: $F_{\text{site}} = 5.87$, $p = 0.02$) than at ZM sites (1.92 ± 0.13 mg NH_4^+-N·L^{-1}). Zebra mussel density did not affect DO concentrations in overlying water (RM ANOVA: $F_{\text{site}} = 0.03$, $p = 0.872$), although there were temporal trends in DO concentrations; DO was highest in October (16.5 ± 0.9 mg O_2·L^{-1}) and lowest in August (8.7 ± 0.3 mg O_2·L^{-1}; RM ANOVA: $F_{\text{time}} = 19.11$, $p < 0.001$). All RM ANOVA results reported in this section did not yield a significant time × site type interaction ($p > 0.05$).

Sediment denitrification rates

Overall, mean denitrification rates were lowest in February 2004 at 0ZM sites (0.02 ± 0.01 mg N·m^{-2}·day^{-1}) and highest in June 2003 at HZM sites (210 ± 52 mg N·m^{-2}·day^{-1}; RM ANOVA: $F_{\text{time}} = 9.76$, $p < 0.001$; Fig. 3). Denitrification rates in February 2004 at sites with zebra mussels were high despite low temperatures (0 °C) and similar to mean June 2003 denitrification rates, when river temperature was 22 °C. Denitrification rates measured for each site at ambient river water temperature and at room temperature were not signifi-

Fig. 3. Sediment denitrification rates (mg $N \cdot m^{-2} \cdot day^{-1}$) measured at ambient river water temperatures approximately every other month at each site type (no zebra mussel (*Dreissena polymorpha*) sites presented in open circles and zebra mussel sites presented in solid circles). Significant differences at the $\alpha = 0.05$ level between site types are represented by an asterisk (*). All data are presented ±1 standard error.



cantly different (paired t test: $t = 0.51$, $p = 0.61$). For example, in February 2004 at ZM sites denitrification rates measured at 0 °C were 95.2 ± 17.2 mg $N \cdot m^{-2} \cdot day^{-1}$ and those measured at 20 °C were 109.7 ± 14.8 mg $N \cdot m^{-2} \cdot day^{-1}$.

ZM site denitrification rates were significantly higher than denitrification rates at 0ZM sites only in February 2004 (109.7 ± 15.5 and 0.02 ± 0.01 mg $N \cdot m^{-2} \cdot day^{-1}$, respectively; RM ANOVA: $F_{\text{site type}} = 8.71$, $p = 0.003$). Sediment denitrification rates were not significantly different ($p > 0.08$) between ZM and 0ZM sites for any other sampling date. Denitrification rates were significantly higher in June 2003 than on all other sampling dates (152 ± 36 mg $N \cdot m^{-2} \cdot day^{-1}$; RM ANOVA: $F_{\text{time}} = 9.76$, $p < 0.001$; Fig. 3), which was also the sampling date that showed the highest variability in denitrification rates, with a CV of 160%. In contrast, denitrification rates were least variable in May (CV = 0.8%). Ambient temperature denitrification rate RM ANOVA results did not yield a significant time × site type interaction ($F_{\text{sitextime}} = 2.10$, $p = 0.089$).

Sediment denitrification was positively related to water column NO_3^--N concentrations, and a linear regression explained 72% of the variation in rates (linear regression: $R^2 = 0.72$, $p < 0.001$; Fig. 4). Water column NO_3^--N concentrations were positively related to discharge in Pool 8 (linear regression: $R^2 = 0.31$, $p < 0.001$; Fig. 5) when connectivity between the high NO_3^--N main channel and the impounded zone increased with discharge (Strauss et al. 2004). The temporal relationship among discharge (measured upstream at Lock and Dam 7), NO_3^--N concentrations in the impounded zone, and sediment denitrification is plotted (Fig. 6). Note that peak denitrification rates occurred after June 2003 flooding and were generally lower during periods of low flow.

Fig. 4. Regression of denitrification rates (mg $N \cdot m^{-2} \cdot day^{-1}$) and water column NO_3^--N ($mg \cdot L^{-1}$) across all seasons and site types ($y = 1.85x - 0.45$; $R^2 = 0.72$; $p < 0.001$). Both denitrification rates and NO_3^--N concentrations are log + 1 transformed.

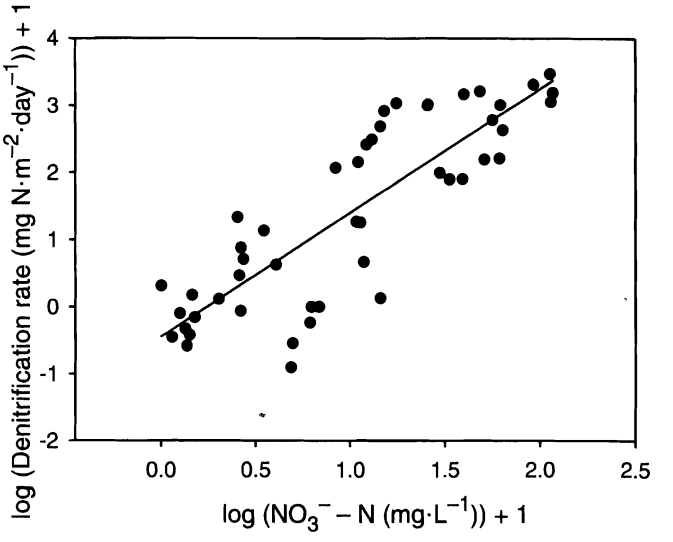
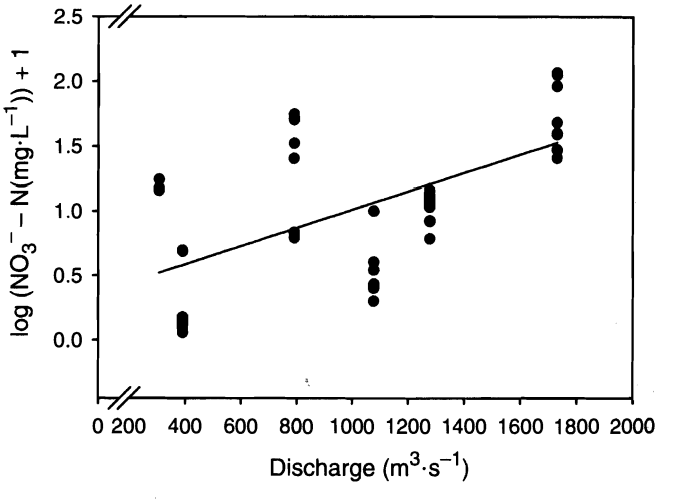


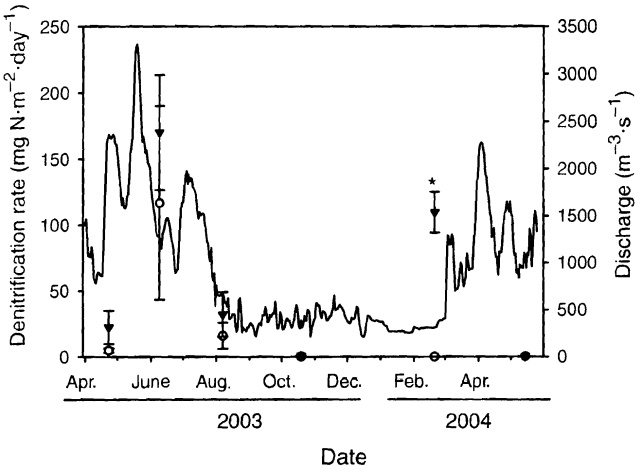
Fig. 5. Regression of log + 1 transformed water column NO_3^--N ($mg \cdot L^{-1}$) and Pool 8 discharge ($m^3 \cdot s^{-1}$) across all seasons and site types ($y = 0.0007x + 0.30$; $R^2 = 0.31$; $p < 0.001$).



Nutrient limitation of sediment denitrification

Sediment denitrification was N-limited at all sites and on all sampling dates, regardless of zebra mussel density (two-way ANOVA, $p \leq 0.01$; Table 2) as evidenced by increased sediment denitrification rates in the +N treatments compared with those with no nutrient amendment (Fig. 7). Amendment with NO_3^--N resulted in denitrification rates that increased by up to 4500% (May 0ZM sites). Sediment denitrification was never C-limited, nor was it colimited by N and C at any site or time during our sampling (two-way ANOVA, $p > 0.05$; Table 2). We rule out colimitation by N and C because the +N+C-amended denitrification rates were not significantly higher than the +N-amended denitrification rates and also because of the lack of significant interaction

Fig. 6. Pool 8 hydrograph for the period of the study and denitrification rates (mg $N \cdot m^{-2} \cdot day^{-1}$) measured over time at each site type ±1 standard error. 0ZM sites (open circles) have no zebra mussels (*Dreissena polymorpha*) and ZM sites (solid triangles) have ~300–1000 mussels·m^{-2}. Discharge is indicated by the black line. Significant differences at the $\alpha = 0.05$ level between site types are represented by an asterisk (*).



terms in the two-way ANOVA by presence or absence of +N or +C (Table 2).

In general, sediments from 0ZM sites exhibited a higher degree of N limitation than sites colonized by zebra mussels, and denitrification rates at 0ZM sites showed a higher response to the +N nutrient amendment compared with sediments from ZM sites in April, August, and October of 2003 and February 2004 (two-way ANOVA, $p < 0.01$; Fig. 8). In May 2004, sediment denitrification rates at the 0ZM site with the +N and +N+C treatments were the highest rates measured over the entire sampling period (131 and 172 μg $N_2O \cdot g$ $AFDM^{-1} \cdot h^{-1}$, respectively). The magnitude of difference in denitrification response between site types was greatest in February when the relative response to the N addition at 0ZM sites was three times higher than the response by sediments at the ZM sites. In contrast, the relative responses of 0ZM and ZM sites to N amendments in May were not significantly different (two-way ANOVA, $p > 0.05$; Fig. 8).

Discussion

Sediment denitrification rates in Pool 8 of the UMR

Measurement of sediment denitrification rates in large rivers is rare because of difficulties associated with sampling (but see Garcia-Ruiz et al. 1998; Baker and Vervier 2004; Richardson et al. 2004). Sediment denitrification rates we measured in the impounded zone of Pool 8 in the Mississippi River ranged from 0.02 ± 0.01 to 210 ± 52 mg $N \cdot m^{-2} \cdot day^{-1}$ and were slightly lower than rates reported for other large rivers. Richardson et al. (2004) measured denitrification rates using the same field and laboratory methods as reported here ranging from 4.8 to 960 mg $N \cdot m^{-2} \cdot day^{-1}$ over 2 years (2000–2001) of seasonal sampling of all aquatic areas in Pool 8. These aquatic areas included a roughly equal number of sampling sites in the impounded zone (where all our sampling sites are located) as well as the main channel, side channels, and backwater areas of Pool 8. Zebra mussels were rarely found at their sampling sites throughout the 2 years of their study (W.B. Richardson, personal communication). Assuming these values represent mean denitrification rates in areas of Pool 8 without zebra mussels, our data suggest that although there is substantial variation, there are periods of the year where sediments under zebra mussel beds do exhibit higher denitrification rates than the mean for the impounded zone. Specifically, spring denitrification rates in HZM sites averaged 210 mg $N \cdot m^{-2} \cdot day^{-1}$ in this study, whereas Richardson et al. (2004) measured an average denitrification rate of 84 mg $N \cdot m^{-2} \cdot day^{-1}$. However, it is important to note that Richardson et al. (2004) also measured substantial interannual variation in denitrification rates, so comparing rates between years is confounded by this variation. The impounded zone in Pool 8 is likely a habitat conducive for high denitrification rates because of high NO_3^- water coming from the main channel combined with C-rich sediments (Richardson et al. 2004). The invasion of zebra mussels in the impounded zone could enhance conditions for denitrification in this area that is already favorable for higher denitrification rates.

Pool 8 has denitrification rates within the range reported for marine, estuarine, coastal, and lake sediments (Seitzinger 1988; Gilbert et al. 1998; Risgaard-Petersen 2003), and only wetland habitats exhibit higher denitrification rates than measured in Pool 8 (Xue et al. 1999). Higher sediment denitrification rates were observed when estimated in other large rivers using mass balance approaches, ranging from 122–135 mg $N \cdot m^{-2} \cdot day^{-1}$ in the Potomac River, Maryland, to 96–200 mg $N \cdot m^{-2} \cdot day^{-1}$ in the Delaware River (Seitzinger 1988). In general, sediment denitrification rates in this study were variable but within the expected range.

Zebra mussels and denitrification

In this study, zebra mussels were found to influence sediment denitrification rates primarily in the winter, when water temperatures and discharge were low. During other periods, the presence of zebra mussels did not significantly alter rates of denitrification. The presence of zebra mussels may have increased denitrification rates in February because the low flow conditions resulted in less exchange at the sediment–water interface. During low flow periods and potentially low vertical hydraulic exchange, zebra mussel wastes may remain in contact with sediments for a longer period of time, allowing for the nitrification of NH_4^+-N rich wastes, thereby stimulating denitrification. During periods of high flow, high NH_4^+-N zebra mussel wastes are likely quickly flushed downstream. Also, during periods of high flow and high hydraulic connectivity, there may be increased NO_3^--N delivery, likely confounding any effect that zebra mussels might have. In general, NO_3^--N availability is likely the primary control of sediment denitrification rates in Pool 8 of the UMR.

We found denitrification rates were not influenced by measuring denitrification either at ambient river water temperature or at room temperature. Likewise, Richardson et al. (2004) found equally high denitrification rates during winter and summer in Pool 8 and concluded that nitrification-

Table 2. Results from a two-way analysis of variance (ANOVA) of nutrient limitation experiments for each season.

	Treatment					
	N		C		N × C	
Month	$F_{[2,32]}$	p	$F_{[2,32]}$	p	$F_{[4,32]}$	p
April 2003	107.4	**<0.001**	0.21	0.648	0.07	0.793
August 2003	33.95	**<0.001**	0.02	0.878	0.015	0.904
October 2003	442	**<0.001**	3.62	0.064	2.47	0.124
February 2004	34.62	**0.017**	2.01	0.166	1.30	0.263
May 2004	11.64	**0.002**	3.41	0.074	3.42	0.074

Note: Significant p values (bold) indicate limitation of denitrification (significant N effect, N limited). The nonsignificant p values for the +C and +N+C treatments show a lack of C limitation or N and C colimitation.

Fig. 7. Denitrification rates (μg N_2O·g $AFDM^{-1}$·h^{-1}; AFDM = ash-free dry mass) in response to nutrient additions of (from left to right) no amendment (open bars), nitrate (+N, solid bars), carbon (+C, shaded bars), or both N and C (+N+C, hatched bars) for each season and site type. The seasonal data are displayed as follows: (*a*) April 2003, (*b*) August 2003, (*c*) October 2003, (*d*) February 2004, and (*e*) May 2004. In all cases, the +N treatment denitrification rates are significantly higher than the control denitrification rates. The amended denitrification rates for +N and +N+C are never significantly different from each other (no significant interaction terms). These data were analyzed with two-way analysis of variance at $\alpha < 0.05$. All data are presented +1 standard error.

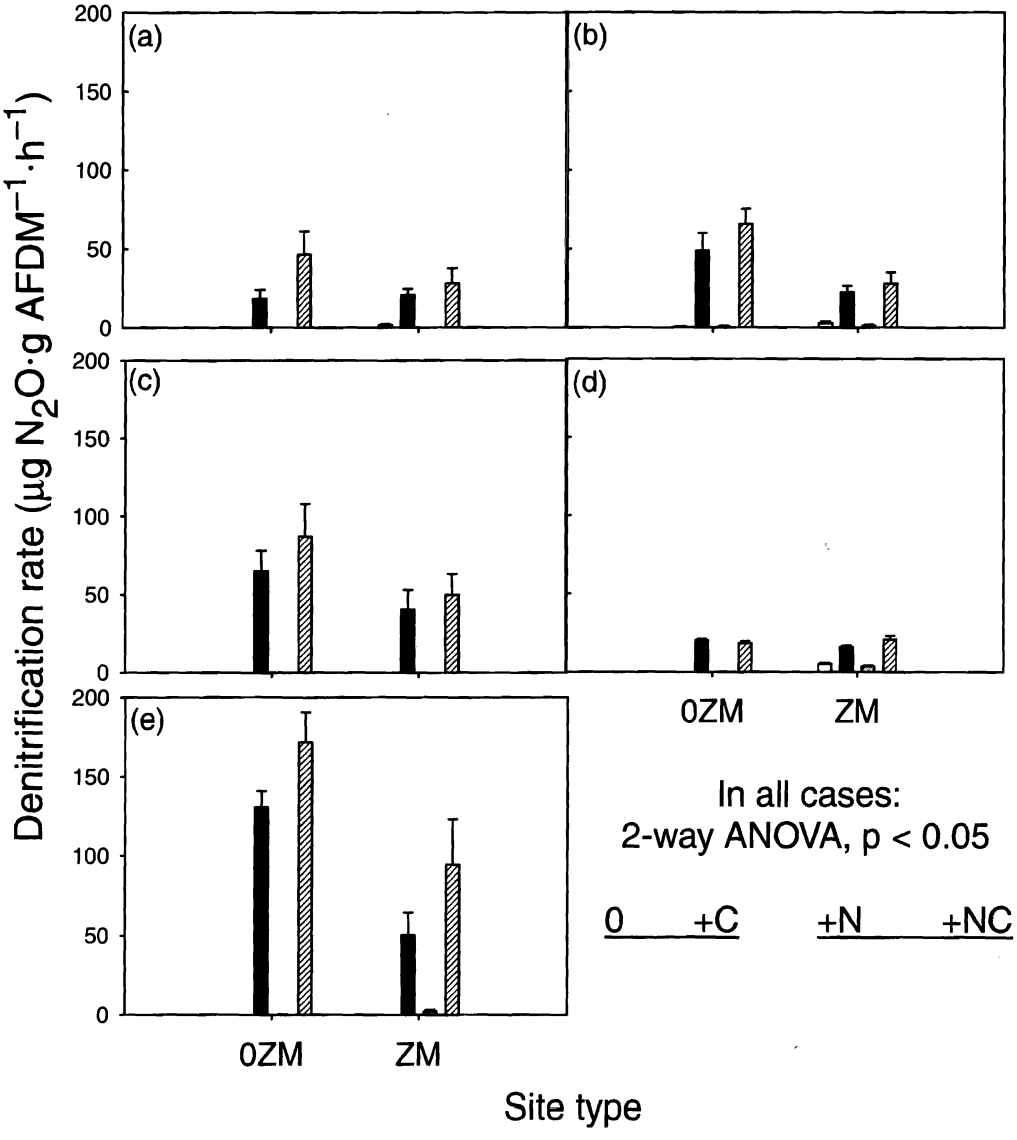
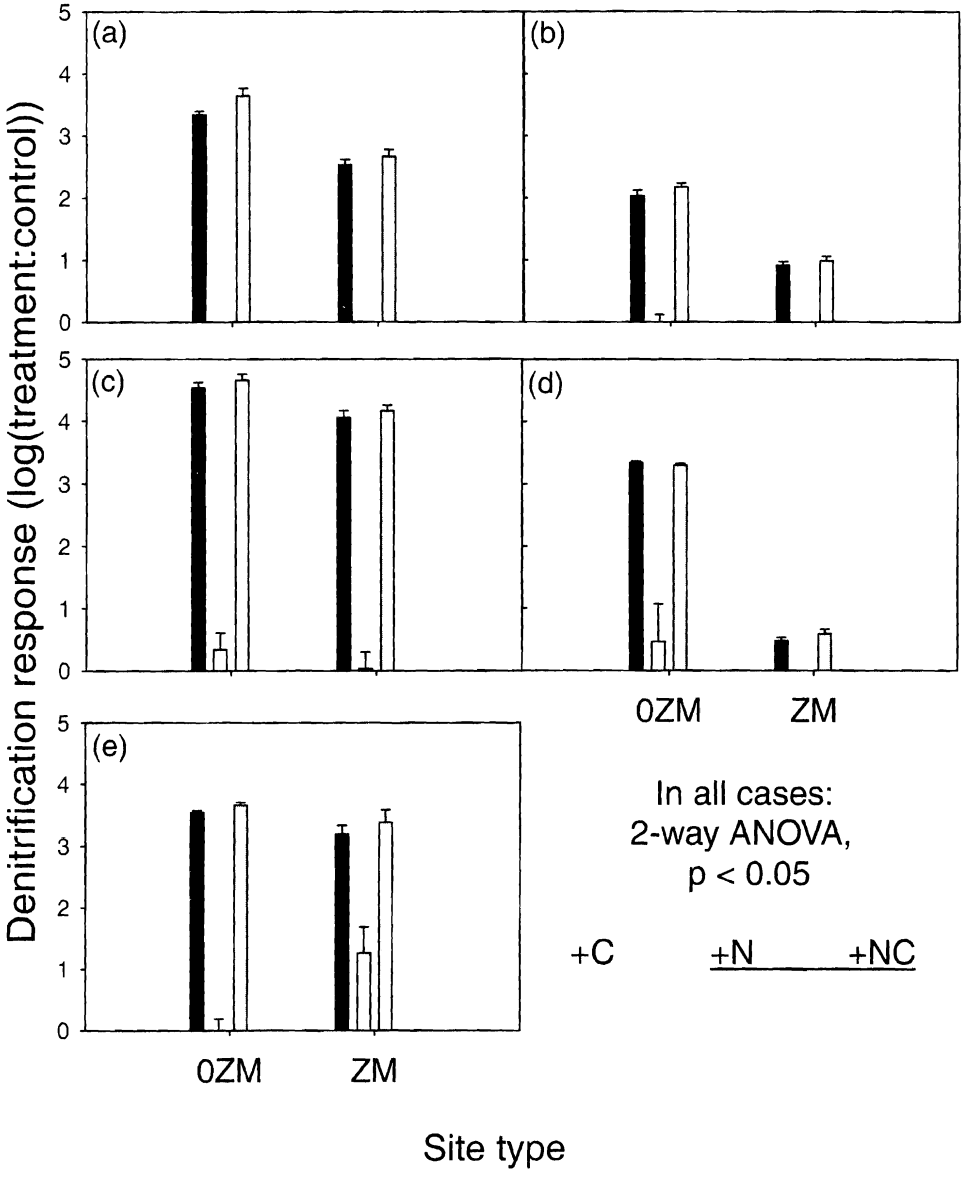


Fig. 8. Denitrification response, calculated as the log of the treatment denitrification rate/control denitrification rate, for each season and site type. The seasonal data are displayed as follows: (*a*) April 2003, (*b*) August 2003, (*c*) October 2003, (*d*) February 2004, and (*e*) May 2004. Denitrification response to nitrate is shown with solid bars, to carbon with open bars, and to both nitrate and carbon in shaded bars. These data were analyzed with two-way analysis of variance at $\alpha < 0.05$. All data are presented +1 standard error.



derived NO_3^- was more important than temperature in controlling sediment denitrification rates in backwaters. Similarly, the highest rates of denitrification in a seasonal study of Lake Suwa, Japan, were measured in the winter and the spring (Hasegawa and Okino 2004). It has been suggested that activity of microbial communities may not respond directly to temperature because of complications of the history of substrate availability (Updegraff et al. 1998). We suggest that annual estimates of N loss via denitrification must incorporate denitrification during winter months despite lower temperatures.

Factors regulating denitrification: N and C availability

Nutrient limitation of denitrification has been shown previously in many different systems, with up to 50-fold increases in denitrification rates with the addition of nutrients (Hunter and Faulkner 2001; Martin et al. 2001; Storey et al. 2004). Denitrification rates in sediments from a prairie stream exhibited a 200%–300% increase when amended with NO_3^--N (Kemp and Dodds 2002). Similarly, denitrification rates increased 70%–1000% when sediments from agricultural streams in Illinois were amended with NO_3^--N (Royer et al. 2004). Richardson et al. (2004) measured strong NO_3^--N limitation of sediment denitrification in Pool 8, with slight colimitation by C only in main channel sediments. In this study, NO_3^--N-amended sediment denitrification rates were up to 180% greater than measured ambient denitrification rates and were similar to sediment denitrification rates measured in an agriculturally influenced reservoir (Wall 2003). Results from our nutrient amendment experiments demonstrated that Pool 8 sediments have the potential for high denitrification rates, depending on delivery of water column

NO_3^--N. The coupling of nitrification–denitrification likely induces the production of bacterial denitrification enzymes even during periods of low ambient NO_3^--N concentrations (Richardson et al. 2004; Strauss et al. 2004).

Our data indicate that zebra mussels may have the potential to alleviate N limitation of denitrification. Denitrification was not limited by C (as glucose) at any site; therefore, the presence of zebra mussels did not impact denitrification via this potential control mechanism. In every season sampled (except May 2004), sites colonized by zebra mussels showed less N limitation of denitrification than sites without zebra mussels. This relationship could have important implications for invaded ecosystems that exhibit N-limited denitrification. While the presence of zebra mussels may increase ecosystem capacity for N removal via denitrification, if the ecosystem also has anthropogenically elevated N concentrations, the N removal via denitrification enhanced by zebra mussels may not surpass the increased N loads. The balance between N inputs and N removal is system specific and should consider not only the impact of zebra mussels, but other characteristics such as hydrology and watershed land use (Richardson et al. 2004; Inwood et al. 2005). Alternatively, zebra mussels in other ecosystems have the potential to alter the other primary controls of denitrification (i.e., anoxia and C availability). This is more likely to occur in ecosystems with larger zebra mussel populations than seen in Pool 8. Dense zebra mussel colonies such as those in portions of the Great Lakes could likely induce anoxia and concentrate organic matter in sediments below the colonies. Increased denitrification rates as a result of any of these pathways could decrease the problems associated with excess N.

Conceptual model of the influence of zebra mussels on denitrification

Zebra mussels may influence denitrification rates via several mechanisms, including increasing labile C availability in sediments, decreasing sediment DO, or increasing NO_3^- availability. While all these mechanisms are plausible, a few can be ruled out as a mechanism in Pool 8. Sediment organic matter or sediment C did not increase in sites with zebra mussels. In fact, sites with zebra mussels tended to have lower sediment organic matter and C in comparison with sites without zebra mussels, mainly because zebra mussels prefer rocky areas for colonization. Additionally, low sediment organic matter at zebra mussel sites is likely due to the accumulation of shell material as zebra mussel colonies have grown. Zebra mussel shells, which are primarily inorganic C, have come to replace soft, organic sediments as the upper layer of river bottom in some areas of the impounded zone. It is possible that the overall sediment organic matter or C content has increased since colonization by zebra mussels, but we do not have pre-invasion data to evaluate this prospect. However, changes in sediment organic matter content are unlikely to affect denitrification rates because denitrification is not C-limited in this system.

We were unable to directly measure sediment DO concentrations in undisturbed sediments at our sampling sites because collecting sediment via coring undoubtedly altered sediment DO profiles. However, Beekey et al. (2004) measured in situ DO in soft sediments similar to sediments found at our sites and found that while sediments colonized by zebra mussels had decreased DO in comparison with bare sediments, sediments colonized by zebra mussels were not anoxic. Other studies have shown that sediments exhibit microscale variability in DO, and anoxic microsites are generally present even in oxic sediments (Rysgaard et al. 1994; Kemp and Dodds 2001). Therefore, even if the sediment at our sites was oxic, a lack of anoxic microsites most likely was not the primary inhibitor of denitrification in these sediments. However, we cannot explicitly rule out the role of DO in denitrification at our sites.

The most likely mechanisms for increased denitrification with zebra mussels in this study are increased nitrification rates and the movement of nutrients via a "biological pump". Although we did not measure nitrification rates at these sites, Strauss et al. (2004) measured high rates of nitrification in Pool 8, particularly in the impounded areas, where nitrification rates averaged 1.38 $\mu g\ N \cdot cm^{-2} \cdot h^{-1}$ over the course of their study. Nitrification of high NH_4^+-N zebra mussel wastes would provide a consistent source of NO_3^- for denitrification , alleviating N limitation of denitrification by increasing porewater NO_3^--N. This mechanism is particularly relevant because of N limitation of denitrification and the extremely high porewater NH_4^+-N concentrations ($mg \cdot L^{-1}$) observed at all our sites.

Increased delivery of nutrients to the benthos via high filtration rates of zebra mussels could also be important in Pool 8 (i.e., the biological pump). This mechanism alleviates N limitation of denitrification by delivering N to the benthos where denitrification occurs and could explain the observed increase in denitrification rates. Burks et al. (2002) measured over a 100% increase in NO_3^--N concentrations in interstitial waters of zebra mussel colonies in comparison with surface waters in Lake Michigan, showing that the zebra mussel colonies create vertical gradients in water quality through their filtering activity and waste production. Significantly higher denitrification rates measured at zebra mussel sites in February corresponded with an increased density of live zebra mussels. Increased filtering activity in February could have increased nutrient delivery to the sediments resulting in increased denitrification rates. Our future work will focus on examining these potential mechanisms to determine their relative importance. It is likely that several of these mechanisms are acting in concert to increase denitrification rates.

Invasive species and ecosystem processes

Recent surveys show that zebra mussels are now established in major rivers, including the Mississippi, Illinois, Hudson, Ohio, Arkansas, and Tennessee rivers and the St. Lawrence Seaway. They are also established in all the Great Lakes and inland waters of 19 states and two Canadian provinces (USGS 2003). It is likely that zebra mussels will continue to spread across North America into hardwater lakes and rivers >30 m wide (Strayer 1991). Much of the research done on invasive species such as zebra mussels focuses on predicting the future distribution and pathways of invasion (Kolar and Lodge 2001) or preventing the spread of invasive species (Sharov and Liebhold 1998). Research that focuses on understanding the indirect ways that invasive species may alter invaded systems is equally important (Vitousek et al.

1996; Strayer et al. 1999; Bohlen et al. 2004) but currently understudied (but see Raikow et al. 2004).

In Pool 8 of the Mississippi River, we found zebra mussels to increase rates of denitrification during certain times of the year. It is also important to note that while these findings imply a benefit to humans by alleviating problems associated with N pollution, zebra mussel invasions are associated with many negative biological and economic impacts (Strayer 1999; Caraco et al. 2000; Leung et al. 2002). The effect that zebra mussels will have on denitrification is in part dependent on the nutrient status of the system, because the presence of zebra mussels may relieve N limitation of denitrification. The effects of zebra mussels on N cycling in a system with a more stable and dense population of zebra mussels than seen in Pool 8 of the UMR would likely be greater and more prolonged than measured here. Land use changes such as urbanization and agricultural activities increase total N export from ecosystems (Groffman et al. 2004). The invasion and spread of zebra mussels is also most prevalent in these human-impacted systems, such as systems used for recreational boating or commercial barge traffic (Johnson and Padilla 1996). Management plans to minimize the effects of eutrophication or the development of hypoxic waters should account for the presence of zebra mussels, because systems invaded by zebra mussels may have increased potential for denitrification. However, this potential for increased denitrification will certainly vary between different ecosystems and within an ecosystem over seasonal shifts. Therefore, examining how zebra mussels influence N cycling rates in the different types of invaded systems (i.e., lakes and rivers or high and low nutrient systems) will be important for both the management of eutrophic waters and for a more complete understanding of the impact of zebra mussel invasions.

Acknowledgements

This research was supported by the Graduate School at the University of Notre Dame and the US Geological Survey. We thank Lynn Bartsch, Robert Kennedy, Becky Kreiling, Jennifer Cavanaugh, Heidi Imiker, Tim Hoellein, and Kathryn Docherty for assistance in the field and in the laboratory. We thank Jennifer Sauer for zebra mussel distribution data in Pool 8. We also thank Jake Beaulieu for insightful review of the manuscript. We thank the USGS Upper Midwest Environmental Sciences Center for the generous use of field equipment and laboratory space.

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