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Seasonal effects of zebra mussels on littoral nitrogen transformation rates in Gull Lake, Michigan, U.S.A.

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SUMMARY

1. Zebra mussels (*Dreissena polymorpha*) are successful colonisers of lake littoral habitats and they interact strongly with littoral benthos. Previous research suggests that localised areas colonised by zebra mussels may be hotspots of nitrogen (N) cycling.
2. The effects of zebra mussels on nitrification and denitrification rates were examined approximately every other month for 1 year in Gull Lake, Michigan, U.S.A. Littoral sediment was collected from an area free of zebra mussels and distributed into shallow trays; rocks colonised with zebra mussels were placed in half of the trays, while uncolonised rocks were placed in the remaining trays. After an incubation period of 6–8 weeks in the lake, sediment and zebra mussels were collected from the trays, replaced with new sediment and zebra mussels, and placed in the lake for the next interval. In the laboratory, sediment nitrification and denitrification rates were measured for each tray.
3. Sediment nitrification rates did not increase in the presence of zebra mussels; instead nitrification rates were sensitive to changes in water temperature and increased with increasing exchangeable sediment ammonium. In contrast, denitrification rates increased in sediment trays with zebra mussels in the winter when nitrate (NO_3^-) availability was high and when *Chara* did not grow in the trays.
4. Sediment denitrification was NO_3^--limited in all seasons, regardless of zebra mussel treatment. However, sediment in the presence of zebra mussels responded less to NO_3^- addition, suggesting that NO_3^- limitation of denitrification can be reduced by zebra mussel activity. Zebra mussels have a seasonally variable impact on sediment denitrification rates, and this may translate into altered seasonal patterns of N cycling in localised areas of lakes where they are particularly abundant.

Keywords: denitrification, lakes, nitrification, seasonal dynamics, zebra mussel

Introduction

Since the discovery of zebra mussels [*Dreissena polymorpha* (Pallas)] in Lake St Clair in 1986 (Herbert, Muncaster & Mackie, 1989), they have expanded their range in North America to include lakes and rivers throughout the eastern U.S.A. (O'Neill & Dextrase, 1994; USGS, 2008), spreading as human activities continue to aid their movement between lakes (Kraft & Johnson, 2000; Johnson, Bossenbroek & Kraft, 2006; Karatayev *et al.*, 2007). In invaded lakes, zebra mussels are most frequently found in the littoral, where there is typically suitable temperature, food and substratum available (Ludyanskiy, McDonald & MacNeill, 1993). Zebra mussels have altered littoral sediments and associated aquatic communities

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(Griffiths, 1993; Klerks, Fraleigh & Lawniczak, 1996), which can scale-up to ecosystem-wide impacts (Strayer *et al.*, 1999; Vadebonncoeur, Vander Zanden & Lodge, 2002; Karatayev *et al.*, 2007). These affects include increasing the physical complexity of the substratum via shell accumulations (Botts, Patterson & Schloesser, 1996; Stewart, Miner & Lowe, 1998), altering sediment bacterial communities (Lavrentyev, Gardner & Yang, 2000; Lohner *et al.*, 2007) and increasing the densities of benthic invertebrates (Beekey, McCabe & Marsden, 2004; Ward & Ricciardi, 2007). Zebra mussels also often increase macrophyte growth in the littoral zone by decreasing phytoplankton populations in the water column, thus increasing clarity (Caraco *et al.*, 1997; Zhu *et al.*, 2006). As the productivity of the food web in invaded ecosystems shifts towards the littoral zone, fish communities also shift from open water to mainly littoral species (Vanderploeg *et al.*, 2002; Strayer, Hattala & Kahnle, 2004).

'Benthification' is a process by which zebra mussels increase deposition and retention of particles in the littoral (Mills *et al.*, 2003; Hecky *et al.*, 2004; Zhu *et al.*, 2006), and thereby shift nutrient cycling to littoral habitats (MacIsaac *et al.*, 1999; Hecky *et al.*, 2004). In fact, zebra mussels have been shown to alter nitrogen (N) dynamics in a number of invaded ecosystems, ranging from shifting N : P ratios (Arnott & Vanni, 1996; Vanderploeg *et al.*, 2001) to increasing N transformation rates (Gardner *et al.*, 1995; James, Barko & Eakin, 1997; Lavrentyev *et al.*, 2000; Bruesewitz *et al.*, 2006). A number of zebra mussel life-history traits may alter N dynamics in aquatic systems (Vanderploeg *et al.*, 2002; Hecky *et al.*, 2004; Bruesewitz, Tank & Bernot, 2008). Nitrification, the chemoautotrophic conversion of ammonium (NH_4^+) to nitrate (NO_3^-), may be increased by N-rich zebra mussel waste, or alternatively inhibited by the increased organic carbon (C) availability (Strauss, Mitchell & Lamberti, 2002) provided by decomposing zebra mussel wastes and bodies. Denitrification, the microbial anoxic respiration pathway that converts NO_3^- to di-nitrogen gas (N_2) and nitrous oxide (N_2O), increases in the presence of zebra mussels through a number of different mechanisms, including increased coupling to nitrification, increased filtering activity drawing NO_3^- to the benthos, or through altered redox conditions at the sediment–water interface (Bruesewitz *et al.*, 2008).

Seasonality is an important factor that may influence the interpretation of results from laboratory or field studies in determining the importance of zebra mussels to N cycling in invaded ecosystems. Our previous work on the influence of zebra mussels on denitrification rates in a large Mid-western river found seasonality to be important (Bruesewitz *et al.*, 2006). In the Upper Mississippi River, areas colonised by zebra mussels exhibited increased denitrification rates during the winter, when river discharge was low and water-column NO_3^- was less available to sediments outside the main channel of flow. To our knowledge, however, no previous studies have examined seasonal aspects of the influence of zebra mussels on N cycling in lakes.

There are sharp differences in seasonal patterns between lentic and lotic ecosystems that have implications for nutrient cycling. For example, lentic systems are strongly influenced by seasonal patterns of thermal stratification that limit nutrient availability in surface waters, followed by periods of whole-lake mixing of nutrient-rich hypolimnetic water. In contrast, lotic systems do not exhibit seasonal stratification patterns, but may instead experience seasonal periods of flooding and base flow conditions that control nutrient and organic matter delivery. We hypothesised that seasonal patterns of N cycling in lentic sediment would depend primarily on the availability of N and C. As in lotic ecosystems, the activity of zebra mussels could enhance the availability of N or C to localised areas of sediment at times of the year when it is otherwise less available. We expected that the magnitude of zebra mussel impact would be mediated by periods of stratification and mixing rather than changes in hydrology (as we found in a lotic system). Although lower winter temperatures might be expected to reduce the metabolic activity of zebra mussels, our previous work in the Mississippi River showed feeding activity during the winter (Bruesewitz *et al.*, 2006), which provided the impetus to conduct this year-round study in Gull Lake.

Our objective was to examine seasonal variation in the effect of zebra mussels on littoral sediment nitrification and denitrification rates in Gull Lake, Michigan. By comparing sediment underlying zebra mussels with sediment that had been isolated from zebra mussels, our seasonal survey examined the local effects of zebra mussels on littoral sediments in an

invaded lake to provide an understanding of the ways in which local benthic effects of zebra mussel invasion could result in ecosystem-level effects on N cycling.

Methods

Site description

Gull Lake is relatively large (7.02 km^2, 34 m maximum depth, 12.5 m average depth, water residence time 4.3 years) groundwater-fed lake with hard water in which phosphorus (P) limits productivity (Tessier & Lauff, 1992). It has approximately equal proportions of littoral (<10 m deep) and profundal (>10 m deep) areas (*c.* 47% as littoral and *c.* 53% as profundal). The lake is dimictic, with periods of mixing in both the spring and autumn, and stratification from May to early November (Tessier & Lauff, 1992) and, based on the average duration of ice cover from 1955 to 2006, the lake freezes an average of 72 day each year. Zebra mussels had colonised Gull Lake by 1994, and the mean zebra mussel biomass within the 0–10 m depth contour is 6 ± 1 g m^{-2}, an intermediate value for invaded lakes of North America (Wilson & Sarnelle, 2002). Quagga mussels [*Dreissena rostriformis bugensis* (Andrusov)] have not been found in Gull Lake.

Experimental design

We examined seasonal variation in the effects of zebra mussels on littoral sediment N transformation rates, using shallow plastic trays of lake sediment and zebra mussels. We collected Gull Lake sediment from the same area for each of the seven sampling periods, which was coarse sand devoid of zebra mussels or macrophytes. We collected the uppermost 10 cm of sediment, passed it through a 1 cm^2-mesh screen and placed the homogenised sediment to a depth of 4 cm in plastic trays (53 × 45.4 × 8 cm HDPE, item number 52055; US Plastic Corporation, Lima, OH, U.S.A.).

We collected rocks (typically *c.* 20 cm diameter) from the littoral that were colonised by mussels, and similar-sized rocks uncolonised by zebra mussels. In each tray, we placed three to four rocks with nine trays receiving zebra mussel rocks (+ZM) and the other nine receiving non-colonised rocks (−ZM). Zebra mussel rocks were submerged in a large tray of Gull Lake water for <30 min to minimise disturbance to the zebra mussels while we counted the approximate number of mussels per rock. The approximate counts were used to keep the mussel density consistent between replicate +ZM trays. Trays were then kept on the lake bottom near a dock, and then taken to the study site in the littoral zone. Our study site was *c.* 20 m from shore, *c.* 50 m from the dock, 3.5 m deep and relatively clear of macrophyte growth. We clustered the trays together at the site, 0.25–0.5 m apart, and attached a small float to each to mark its location.

We incubated the trays for the following seven periods: 21 April–8 June 2005, 8 June–20 July 2005, 20 July–1 September 2005, 1 September–13 October 2005, 13 October–4 December 2005, 4 December 2005–2 February 2006 and 19 April–26 June 2006. After each incubation period of 6–8 weeks we retrieved the trays, gently lifting each tray from the lake bottom and, while still underwater, slid it into a large plastic bag to minimise the loss of fine particles and brought it to shore for processing. We first assessed any new zebra mussel colonisation on the trays. We found new zebra mussels on the rocks in the −ZM trays only in July 2005 (1.3 individuals m^{-2}) and June 2006 (31.9 individuals m^{-2}). We occasionally found a small number (<25 individuals, each <5 mm) of new zebra mussels colonising the outer lip of both the +ZM and −ZM trays. All mussels, including the new colonisers, were removed from the rocks and the trays and brought back to the laboratory for counting and measuring. Additionally, we found that *Chara* spp. grew in the trays (as well as outside the trays) during June, July, September and October 2005 and in June 2006. We estimated *Chara* per cent coverage by eye and removed it from the trays. Approximate *Chara* coverage in each tray was put into categories of 0%, >5%, 10%, 25%, 50% or <75%. Sediment was collected after the rocks and plants were removed from the trays by taking three scoops of sediment down the length of each tray to the full 4-cm depth. After processing, we rinsed the trays, filled each with new sediment and new rocks from the same sources and returned them to the littoral for the next incubation period.

Winter conditions forced a slight modification in our tray deployment protocol from December to February. We used the −ZM and +ZM rocks in the trays from the December collection for the February sampling, due to difficulty of finding new rocks in the winter conditions, but +ZM rocks still had healthy zebra mussels.

On each retrieval date, we collected 1 L of unfiltered lake water for use in the N transformation assays, and filtered water samples [Pall A/E 1 μm nominal pore size (Pall Corporation, East Hills, NY, U.S.A.)] for determination of water column NO_3^-–N, NH_4^+–N and dissolved organic carbon (DOC). Water samples were frozen until analysis. Water temperature, dissolved oxygen (DO), pH and conductivity were also measured at each sampling. Exchangeable sediment NH_4^+–N and NO_3^-–N were measured in February and June 2006 using FIA Lachat QuikChem Methods 12-107-06-1-B and 12-107-04-1-B, respectively (Lachat QuikChem 8500, Lachat Instruments, Loveland, CO, U.S.A.). Water column NO_3^- was measured on the Lachat QuikChem 8500 using ion chromatography (Lachat QuikChem Method 10-510-00-1-A). We measured water column NH_4^+–N concentrations on a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) at 630 nm using the phenate method (Solorzano, 1969). We used a Shimadzu 5000A carbon analyser to measure non-purgeable organic carbon (i.e. DOC) from filtered water samples that were acidified to a pH of 2 (American Public Health Association (APHA) (1995).

We kept sediment and water samples on ice during transport to the laboratory and then stored them at 4 °C until assays could be performed, always within 24 h of sampling. To characterise the sediment, we quantified sediment dry mass (DM) and ash-free dry mass (AFDM) on three sub-samples from each tray. Each 5-mL sample was dried at 60 °C to a constant mass, weighed and ashed at 500 °C to obtain the ashed weight. Sediment organic matter was calculated as the ratio of AFDM to DM, expressed as a per cent.

Nitrification assays

We measured sediment nitrification rates using the nitrapyrin inhibition method (Strauss & Lamberti, 2000; Arango & Tank, 2008; Bruesewitz *et al.*, 2008). We collected two analytical replicates from each of the tray sediments. Each replicate consisted of two 125-mL Erlenmeyer flasks: one sediment slurry amended with nitrapyrin dissolved in dimethyl sulphoxide (DMSO) and one sediment slurry amended with DMSO only. Nitrification was inhibited in the nitrapyrin flask and allowed to occur in the DMSO flask. After a 2-day incubation on a shaker table at 3.4 *g*, to maintain aeration of the slurries, we determined exchangeable NH_4^+–N concentrations from 1N potassium chloride (KCl) extracts from each flask using the phenate method (Solorzano, 1969). We calculated nitrification rates from the decrease in NH_4^+ over time in the DMSO flask relative to NH_4^+ in the nitrification-inhibited flask, normalised to the area of the tray, and both the DM and AFDM of the sediment in the tray.

Denitrification assays

We measured sediment denitrification rates using the chloramphenicol-amended acetylene (C_2H_2) inhibition technique (Knowles, 1990; Inwood, Tank & Bernot, 2005; Bruesewitz *et al.*, 2006). For each replicate, we placed 30 mL of sediment from each subsample combined with 50 mL unfiltered mesocosm water in 125 mL media bottles with an *n*-butyl rubber septa in the lid (n = 3 replicates from each tray, and 18 trays from each sampling period). We did not amend water with additional NO_3^-–N or dissolved organic C, therefore these assays provide an index of sediment denitrification activity in the enclosure rather than a maximum denitrification potential (David *et al.*, 2006). We added chloramphenicol to the assays at a concentration of 0.3 mM to suppress *de novo* enzyme production in response to changes in oxygen availability or temperature (Murray & Knowles, 1999), and we purged sediment slurries with ultra pure helium for 10 min, swirling the slurries periodically to ensure anoxic conditions throughout the assay bottles. We added pure acetylene to the headspace of the bottles to prevent the conversion of N_2O to N_2 (15 mL) and collected gas samples for analysis of N_2O from the headspace hourly beginning 10 min after the addition of C_2H_2 for a total of 4 h (five gas samples per replicate). For each gas sample, we replaced headspace with a mixture of helium and 10% C_2H_2 to maintain constant pressure in the assay bottles. When the ambient lake water temperature was ±4 °C of the air temperature in the laboratory, assays were performed at laboratory air temperature, otherwise we kept the bottles at ambient lake water temperature throughout the incubation period by keeping sediment and bottles on ice or in a walk-in cooler (Wall *et al.*, 2005). We analysed gas samples for N_2O on a Varian 3600 gas chromatograph (Varian, Inc., Palo Alto, CA, U.S.A.) equipped with a Porapak Q column and electron capture detector (Agilent Technologies,

Santa Clara, CA, U.S.A.), and calculated denitrification rates from the linear increase in N_2O concentration over the 4 h incubations (Smith & Tiedje, 1979; Murray & Knowles, 1999). For all our samples, we found linear denitrification rates over our 4-h incubation times, which were normalised to the area of the tray, and both the DM and AFDM of the sediment in the tray. We measured sediment DM and AFDM from three sub-samples from each tray rather than directly from the assay bottles because the sediment was very homogeneous, with less than 8% and 5% variation in DM and AFDM replicate samples, respectively, during the study. Therefore, the measurement of sediment sub-samples from the trays accurately described the sediment sub-sampled from each tray for our bottle assays.

To determine whether sediment denitrification was limited by the availability of NO_3^- or C, we included additional denitrification replicates that were prepared identically to those above except that additional NO_3^- as potassium nitrate (KNO_3, 100 mg NO_3^-–N L^{-1}), C as glucose (120 mg C L^{-1}) or both NO_3^- and C together (hereafter referred to as +N+C) were added to the slurries in the assay bottles. These amended assay bottles were incorporated in our sampling on four selected sampling times to capture the variation between seasons (spring, 8 June 2005; summer, 1 September 2005; autumn, 13 October 2005 and winter, 4 February 2006). We measured nutrient limitation of denitrification with three +C replicates, three +NO_3^- replicates and three +N+C replicates from each sediment tray along with the three non-amended replicates from each tray. The denitrification potential was determined from the idealised conditions of the anoxic, +N+C-amended slurries (Groffman & Crawford, 2003; Strauss *et al*., 2006).

Statistical analyses

We performed statistical analyses with SPSS 11 (SPSS, Inc., Chicago, IL, U.S.A.). We determined differences between seasons or zebra mussel treatments of sediment-associated variables such as nitrification and denitrification rates using two-way ANOVA with main factors of season (four levels) and zebra mussel treatment (two levels). We used two-way ANOVA rather than repeated-measures ANOVA because sediment conditions were 'reset' at the start of each sampling period when newly homogenised sediment and new +ZM or −ZM rocks were placed in the trays at the start of each sampling period. Additionally, use of repeated-measures ANOVA on these data produced a similar outcome at the $\alpha = 0.05$ level. We determined nutrient limitation of denitrification with a two-way ANOVA based upon presence or absence of each amendment (+C or +NO_3^-) in the assay bottles to identify significant differences in denitrification rates among amendments (Tank & Dodds, 2003). Therefore, a significant P value for the NO_3^--amended denitrification indicates N limitation, and a significant $NO_3^- \times C$ interaction term indicates co-limitation by C and NO_3^- (Tank & Dodds, 2003). To meet the assumptions of parametric statistics, we tested all data for normality with KS Lillefors ($P > 0.05$), and transformed to meet the assumption of normality when necessary. We used linear regressions to determine environmental controls on nitrification and denitrification rates with significance determined at the $\alpha = 0.05$ level.

We used principal components analysis (PCA) to separate seasons and zebra mussel treatments based on differences in environmental variables that may control nitrification or denitrification rates (PC-ORD, version 4; MjM Software Design, Glenden Beach, OR, U.S.A.). We included temperature, DO, NH_4^+, NO_3^-, sediment organic matter and *Chara* coverage of the trays as environmental variables. We used PCA to examine autocorrelation among these variables and determine which combinations of these variables were most strongly impacting N transformation rates at different times of the year.

Results

Seasonal physicochemical characteristics

Physicochemical characteristics of our littoral site were typical of a hardwater, dimictic lake in the Great Lakes region. Lake temperature at the site on tray collection dates ranged from 1.7 °C in February 2006 to 28 °C in July 2005 (Fig. 1a) and we did not see stratification of the water at our site based on either temperature or DO (data not shown). There was no ice cover on the lake until after the December sampling date. Ice formed on the lake from 22 to 27 January 2006 and again from 9 February to 7 March 2006; our February tray collection took place between these two periods of ice cover.

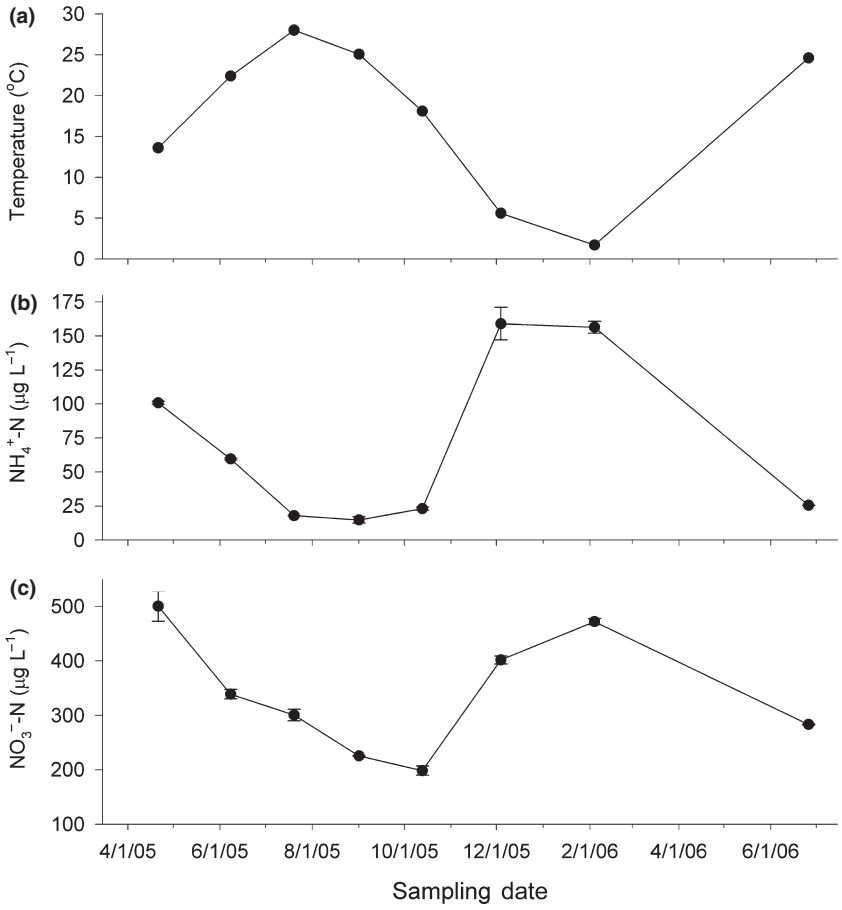

Fig. 1 Water column (a) temperature (°C), (b) ammonium (NH_4^+–N, μg L^{-1}) and (c) nitrate (NO_3^-–N, μg L^{-1}) concentrations of Gull Lake at our littoral site for each sediment tray collection date, from April 2005 to June 2006. NH_4^+–N and NO_3^-–N data are presented as the mean (±1 SE) of three analytical replicates.

Midday DO concentration ranged from a minimum of 6.0 mg L^{-1} (92% saturation) in June 2006 to 15.1 mg L^{-1} (110 % saturation) in February 2006. Spring DO averaged 10.3 mg L^{-1}; summer, 8.3 mg L^{-1}; autumn, 9.5 mg L^{-1} and winter, 13.5 mg L^{-1}, based on measurements made at the incubation site on tray collection days. Gull Lake conductivity ranged from 200 μS cm^{-1} in February 2006 to 383 μS cm^{-1} in June 2005.

Surface water NH_4^+ was highest in December and February at 160 μg NH_4^+–N L^{-1} and declined throughout the spring and summer to 15 μg NH_4^+–N L^{-1} in early September (Fig. 1b). Surface water NO_3^- followed a similar seasonal pattern (Fig. 1c). NO_3^- was highest in the winter and early spring (400–472 and 501 μg NO_3^-–N L^{-1}, respectively) and declined throughout the later spring, summer and early autumn to a minimum of *c.* 200 μg NO_3^-–N L^{-1} in October. Seasonally, DOC was not variable and ranged between 3 and 5 mg C L^{-1} (data not shown).

Tray and sediment characteristics

We successfully manipulated zebra mussel density in the trays to obtain −ZM and +ZM treatments (Fig. 2a). A few small zebra mussels (<5 mm length) colonised rocks in the −ZM trays in July 2005 (1.3 m^{-2}) and June 2006 (31.9 m^{-2}), but densities were always well below those of the +ZM trays (two-way ANOVA, F_{ZM} = 1391.6, $P < 0.001$). The trays were commonly colonised by the aquatic macroalgae *Chara* spp. on all of our sampling dates except December and February (Fig. 2b). Using our per cent coverage estimates, we found more *Chara* growth in the +ZM trays than the −ZM trays (two-way ANOVA, F_{ZM} = 12.75, P = 0.001).

Sediment organic matter was consistently low, as expected in the sandy littoral zone of Gull Lake (Fig. 2c) ranging from an average of 0.69% in February −ZM trays to 1.8% organic in July +ZM trays. Although there were no significant differences between sediment organic matter in the −ZM and +ZM

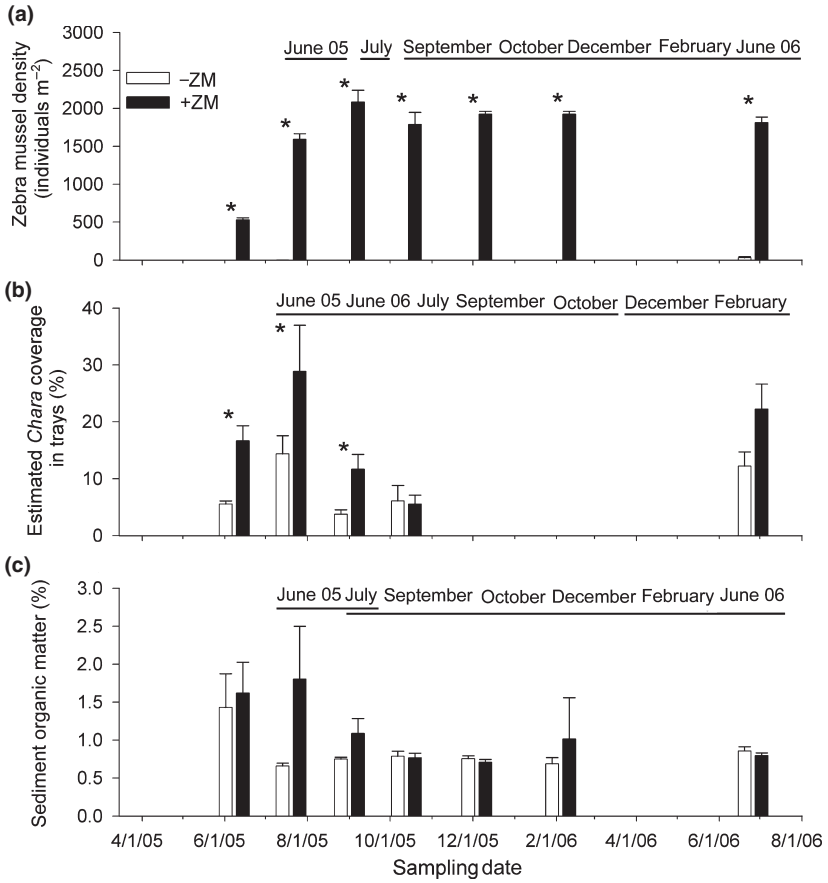

Fig. 2 Mean zebra mussel density (a) in the sediment trays (±1 SE), counted from rocks placed in each tray after each tray collection, mean (±1 SE) *Chara* coverage (b) in each tray and mean (±1 SE) sediment organic matter (c) throughout the year of the study. Brackets group similar means by month and zebra mussel treatments in the inset and significant differences between zebra mussel treatments are represented by an asterisk (*) (two-way ANOVA, $P < 0.05$).

trays (two-way ANOVA, $F_{ZM} = 2.35$, $P = 0.129$), +ZM sediments tended towards higher sediment organic matter in July and September 2005 (Fig. 2c). Sediment organic matter also varied by month (two-way ANOVA, $F_{month} = 3.32$, $P = 0.005$), with June and July 2005 exhibiting higher sediment organic matter than any other month of sampling.

Overall, exchangeable sediment NH_4^+ in June 2006 ranged from 803 to 1759 μg NH_4^+–N L^{-1} of sediment–water slurry and was higher then than in February (two-way ANOVA, $F_{season} = 75.43$, $P < 0.001$), when it ranged from 496 to 953 μg NH_4^+–N L^{-1} (Fig. 3a). Exchangeable sediment NH_4^+ was higher in the +ZM treatment relative to −ZM treatment in both February and June 2006, the two dates when these data were collected (two-way ANOVA, $F_{ZM} = 13.87$, $P = 0.001$, Fig. 3a). Sediment NO_3^- concentrations were much lower than sediment NH_4^+, ranging from 43.8 to 58.8 μg NO_3^-–N L^{-1} of sediment slurry, and were not significantly different between the two sampling dates (two-way ANOVA, $F_{season} = 0.47$, $P = 0.50$). In contrast to NH_4^+, exchangeable sediment NO_3^- concentrations varied between zebra mussel treatments only in February, with higher NO_3^- in +ZM sediment relative to −ZM sediment (two-way ANOVA, $F_{ZM} = 4.13$, $P = 0.056$, Fig. 3b).

Synthesis of environmental variables

A PCA of the environmental variables discussed above was used to show the inter-relatedness of the environmental factors that may control N transformation rates, and the potential importance of seasonality or zebra mussel treatments to these environmental variables. PC axis 1 clearly separated the winter (December and February) from other seasons, and was positively correlated with DO, NH_4^+–N and NO_3^-–N, and negatively correlated with temperature and *Chara* coverage (Fig. 4; Table 1). PC axis 1 explained *c.* 68% of the variation among environmental variables with an eigenvalue of 4.07 (broken-stick value of 2.45). PC axis 2 was negatively correlated with sediment organic matter (Fig. 4; Table 1). PC axis 2 explained 20% of the variation

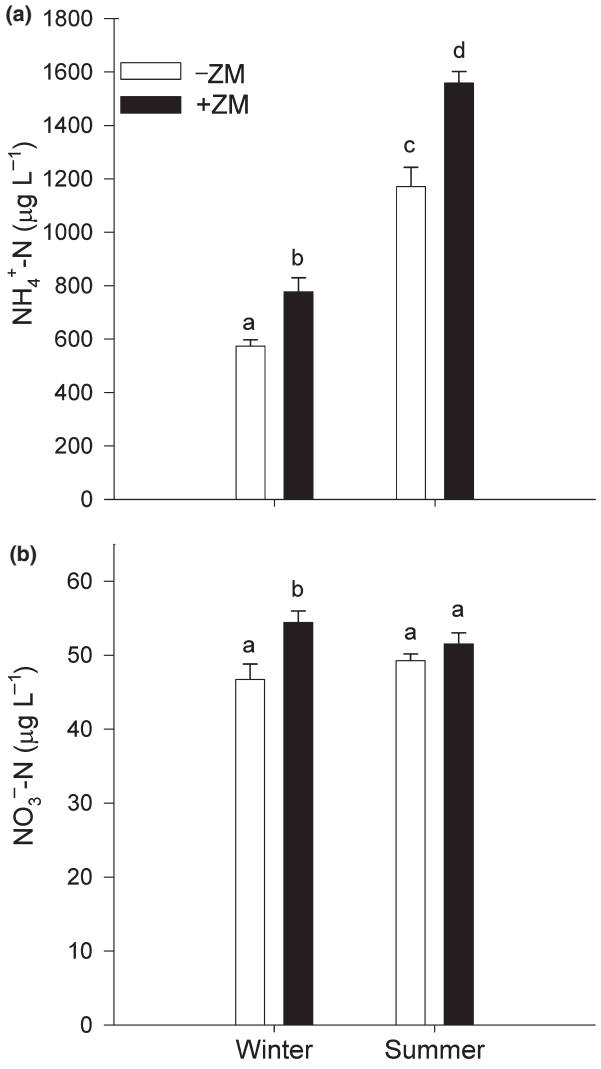

Fig. 3 Mean (±1 SE) exchangeable sediment ammonium (a) NH_4^+–N, μg L^{-1} of sediment–water slurry and (b) mean (±1 SE) sediment nitrate (NO_3^-–N, μg L^{-1} of sediment–water slurry) for −ZM and +ZM sediment in winter (4 February 2006, $n = 3$ for each treatment) and summer (26 June 2006, $n = 9$ for each treatment). Letters represent significant differences between treatments (two-way ANOVA, $P < 0.05$).

among environmental variables and had an eigenvalue of 1.20 (broken-stick value of 1.45).

The PCA also identified environmental variation between zebra mussel treatments within a sampling period. This separation of −ZM from +ZM treatments occurred primarily along PC axis 2. June, July and September 2005 and February and June 2006 all showed separation between the zebra mussel treatments with −ZM points separating above +ZM points from the same sampling period along PC axis 2, in comparison to October and December 2005 where the

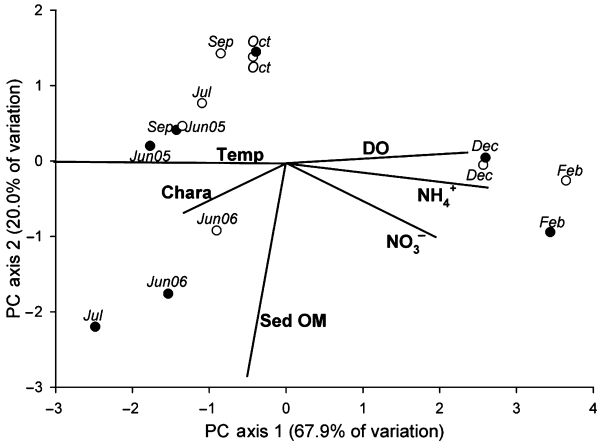

Fig. 4 Principal component analyses (PCA) of the environmental variables that are the potential controls on nitrification and denitrification rates. Environmental variables are represented as vectors, with longer vectors indicating a higher correlation with the closest axis. See Table 1 for the variables and their units. Open circles represent −ZM sediment, and filled circles represent +ZM sediment.

Table 1 Principal components analysis (PCA) for environmental variables that may control nitrification or denitrification rates

	Correlation coefficients	
Environmental variable	PC 1	PC 2
Sediment OM (%)	−0.23	−0.75
NO_3^-–N (μg L^{-1})	0.38	−0.50
NH_4^+–N (μg L^{-1})	0.46	−0.26
Chara coverage (%)	−0.40	−0.34
Temperature (°C)	−0.48	0.01
DO (mg L^{-1})	0.44	0.03

OM, organic matter; NO_3^-–N, nitrate; NH_4^+–N, ammonium; DO, dissolved oxygen.

−ZM and +ZM points were tightly clustered (Fig. 4). Based upon this distribution, we might have expected to see localised variation between −ZM and +ZM sediment in June, July and September 2005 and February and June 2006, as an environmental variable that can control N transformation rates (e.g. organic matter availability) differed between the zebra mussel treatments during these periods.

Sediment nitrification: seasonal trends and controls

The presence of zebra mussels did not influence sediment nitrification rates at any time (two-way ANOVA, $F_{ZM} = 0.23$, $P = 0.63$), but there were significant seasonal differences in nitrification rates, which

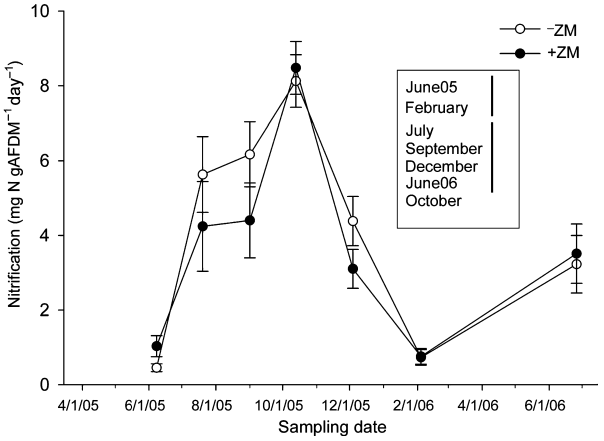

Fig. 5 Nitrification rates (mg N g AFDM^{-1} day^{-1}, ±1 SE) for each sampling period for –ZM (open circles) and +ZM (filled circles) sediments. Brackets group similar means by month in the inset, and zebra mussel treatments were never significantly different (two-way ANOVA, $P < 0.05$).

were highest in October at 8.3 ± 0.71 mg N g AFDM^{-1} day^{-1}, and lowest in June 2005, at 0.74 ± 0.19 mg N g AFDM^{-1} day^{-1} and February 2006, at 0.75 ± 0.21 mg N g AFDM^{-1} day^{-1} (two-way ANOVA, $F_{month} = 26.69$, $P < 0.001$, Fig. 5). Nitrification rate increased with increasing water temperature ($r^2 = 0.57$, $P < 0.01$, Fig. 6a), peaking at 18 °C in October. Although one would generally predict increasing nitrification with increasing NH_4^+, we did not find this; there was a negative relationship between water column NH_4^+ and nitrification rates ($r^2 = 0.14$, $P < 0.001$). Rather, nitrification rates increased with increasing exchangeable sediment NH_4^+ ($r^2 = 0.19$, $P < 0.01$, Fig. 6b).

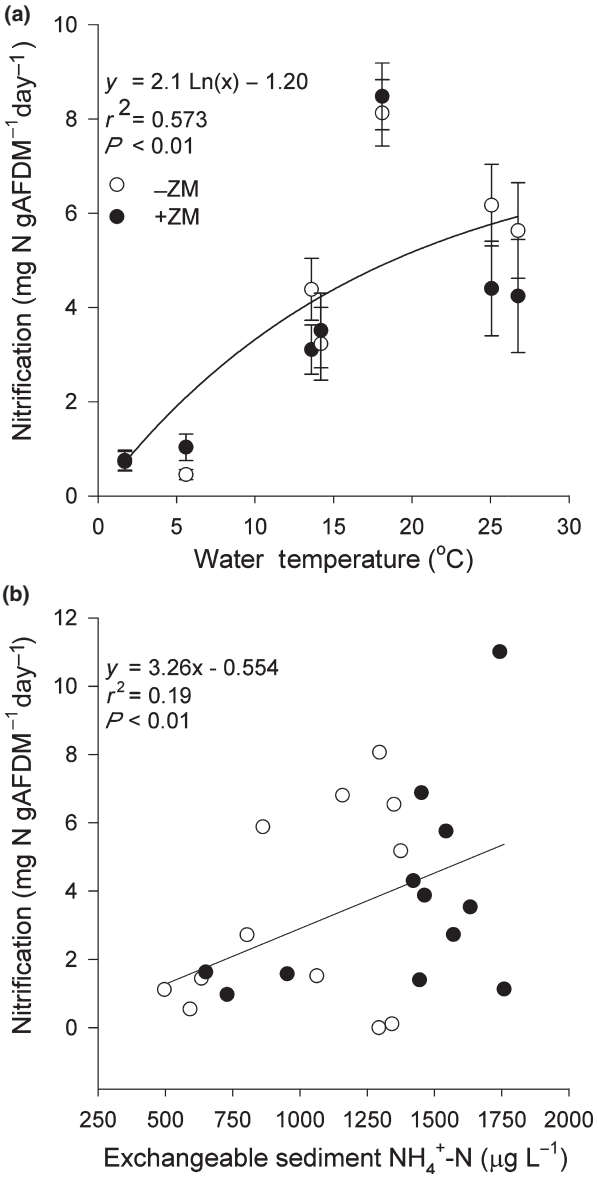

Fig. 6 Relationships between (a) water temperature (°C) and sediment nitrification rates (mg N g AFDM^{-1} day^{-1}) and (b) exchangeable sediment ammonium (NH_4^+–N, μg L^{-1}) and nitrification rates (mg N g AFDM^{-1} day^{-1}). –ZM sediment data are presented in open circles, and +ZM sediment data are presented in closed circles. Mean (±1 SE) nitrification rates are plotted in (a) for correspondence with water column data, and regressions incorporate both –ZM and +ZM data.

Sediment denitrification: seasonal trends and controls

The presence of zebra mussels significantly influenced denitrification rates (two-way ANOVA, $F_{ZM} = 11.47$, $P = 0.001$), although the effect of zebra mussels was not consistent across sampling periods (two-way ANOVA, $F_{ZM \times month} = 3.66$, $P = 0.002$). We found significantly higher sediment denitrification rates in zebra mussel trays in December and February, and significantly lower denitrification rates in June 2005 compared to –ZM trays (Fig. 7). Denitrification varied seasonally (two-way ANOVA, $F_{month} = 5.19$, $P < 0.001$), with the highest overall denitrification rates in June and February 2005, and the lowest in December 2005 (Fig. 7). In general, the winter months captured the range in denitrification for the entire study; we measured the lowest denitrification rates in –ZM December sediments at 0.31 mg N g AFDM^{-1} day^{-1} and the highest denitrification rates in +ZM February at 4.73 mg N g AFDM^{-1} day^{-1}. Denitrification rates were positively related to water column

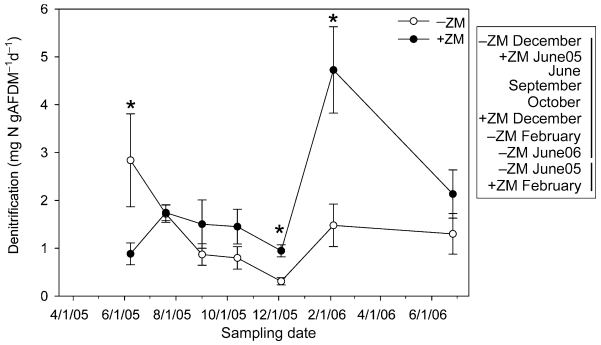

Fig. 7 Denitrification rates (mg N g AFDM^{-1} day^{-1}, ±1 SE) for each sampling period for –ZM (open circles) and +ZM (filled circles) sediments. Brackets group similar means by month and zebra mussel treatments in the inset and significant differences between Zebra mussel treatments are represented by an asterisk (*) (two-way ANOVA, $P < 0.05$).

NO_3^- ($r^2 = 0.32$, $P < 0.001$, Fig. 8a), with both increasing denitrification and increasing divergence between zebra mussel treatments at higher NO_3^- concentrations. Similarly, denitrification rate increased with increasing exchangeable sediment NO_3^-–N ($r^2 = 0.45$, $P < 0.001$, Fig. 8b). There was a positive relationship between nitrification and denitrification rates in +ZM trays in February ($r^2 = 0.52$, $P = 0.048$), which diminished when all of the sampling dates were combined (simple linear regression, $r^2 = 0.036$, $P = 0.04$, data not shown).

The growth of *Chara* in sediment trays may also be an important consideration, as *Chara* may assimilate and retain NO_3^- during the growing season. If we categorise our sampling periods based upon the periods when *Chara* grew in the trays, denitrification rates were 1.52 ± 0.4 mg N g AFDM^{-1} day^{-1} when *Chara* was in the trays, and there was no difference between zebra mussel treatments. However, when *Chara* was absent, denitrification rates increased from 0.89 ± 0.3 mg N g AFDM^{-1} day^{-1} in –ZM sediment to 2.84 ± 0.5 mg N g AFDM^{-1} day^{-1} in +ZM sediment (two-way ANOVA, $F_{ZM} = 6.83$, $P = 0.01$, and $F_{ZM \times Chara} = 9.95$, $P = 0.002$). It should also be noted that *Chara* was absent from the trays during the winter, when temperatures were low and water column and sediment NO_3^- was high.

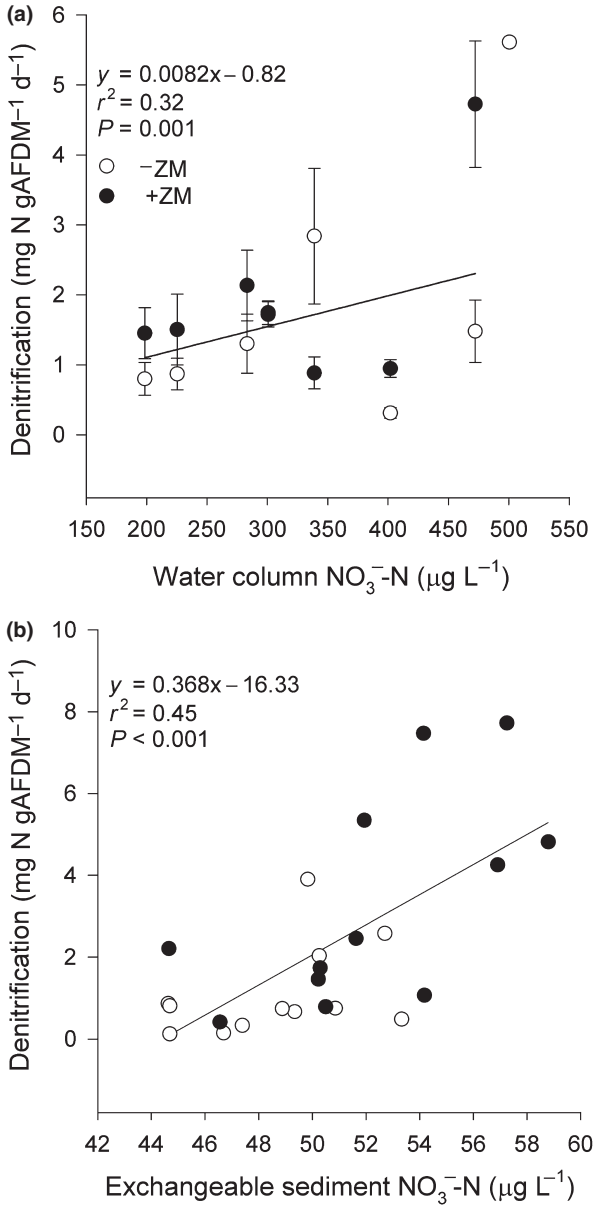

Fig. 8 Relationships between (a) water column NO_3^-–N and mean denitrification rates (mg N g AFDM^{-1} day^{-1}) and (b) exchangeable sediment nitrate (NO_3^-–N, μg L^{-1}) and denitrification rates (mg N g AFDM^{-1} day^{-1}) in summer (June 2006) and winter (February 2006), when porewater data were collected. –ZM sediment data are presented in open circles and +ZM sediment data are presented in closed circles. Mean (±1 SE) denitrification rates are plotted in (a) for correspondence with water column data, and regressions incorporate both –ZM and +ZM data.

Nutrient limitation of denitrification

Denitrification rates were consistently NO_3^--limited across all seasons (two-way ANOVA, $P < 0.001$: Table 2; Fig. 9). For each season, C-amended denitrification rate did not differ from the controls and NO_3^--amended denitrification rate did not significantly differ from +C+N denitrification rate (Table 2).

Table 2 Nutrient limitation of denitrification as determined by two-way ANOVA with +C, +N or +C+N amended denitrification assays

		Spring		Summer		Autumn		Winter	
Nutrient	d.f.	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value
N	1	85.051	**<0.001**	35.055	**<0.001**	211.854	**<0.001**	53.774	**<0.001**
C	1	0.291	0.592	1.401	0.25	3.615	0.062	1.878	0.178
N × C	1	0.985	0.324	1.784	0.197	2.199	0.143	0.004	0.951
Error	68								

Seasonal assays were performed on 8 June 2005 (spring), 1 September 2005 (summer), 13 October 2005 (autumn) and 4 February 2006 (winter). Significant *P*-values are shown in bold.

The response of denitrification to NO_3^- amendment was highest in summer (two-way ANOVA, $F = 3.161$, $P = 0.034$), when −ZM sediment rate increased to 63 ± 8 mg N g AFDM^{-1} day^{-1} and +ZM sediment rate increased to 104.3 ± 42 mg N g AFDM^{-1} day^{-1}, each representing a rate that is over 1000% higher than the summer non-amended denitrification rate. Although in some instances there appeared to be faster denitrification in +C+N treatments (Fig. 9), these rates were not significantly higher than NO_3^--amended denitrification rates (Table 2).

We also calculated the denitrification response ratio (i.e. the ratio of the amended denitrification rate to the non-amended denitrification rate), where a value over 1 indicates positive response to the amendment. Analysis of the denitrification response ratio across seasons showed that the −ZM sediment responded more to additional NO_3^- than the +ZM treatments (two-way ANOVA, $F = 4.760$, $P = 0.035$), suggesting that sediments in the +ZM treatments were less NO_3^- limited than −ZM sediment. For example, the addition of NO_3^- to the denitrification assays in the spring resulted an average rate of 30.2 ± 10.7 mg N g AFDM^{-1} day^{-1}, or a 962% increase over the controls in rates for −ZM sediments, in contrast to 12.9 ± 4.8 mg N g AFDM^{-1} day^{-1}, or a 424% increase in rates for +ZM sediments. A similar trend was found in the autumn, when −ZM sediment denitrification increased 916% with NO_3^- amendment in comparison to a 364% increase in +ZM sediment denitrification.

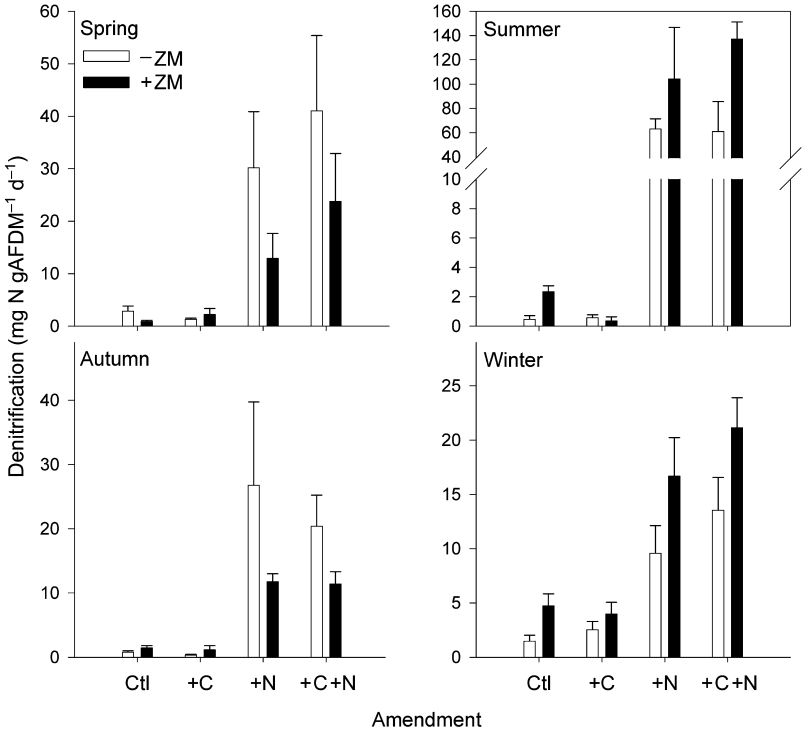

Fig. 9 Denitrification rates (mg N g AFDM^{-1} day^{-1}, ±1 SE) amended with carbon (C), nitrogen (as NO_3^-–N) and both N and C or non-amended controls (Ctl). Denitrification was N-limited in all seasons and with both zebra mussel treatments (two-way ANOVA, $P < 0.001$ for each).

Discussion

Seasonal patterns of sediment nitrification

Littoral sediment nitrification rates peaked in the autumn, and were the lowest in early spring and winter (Fig. 5). Lentic systems have been shown to have higher nitrification potential during seasonal mixing periods (Cavari, 1977). In particular, low winter temperatures can result in decreased nitrification and consequent accumulation of NH_4^+ (Luijn *et al.*, 1999). In fact, nitrification has been shown to be relatively sensitive to low temperature, with relatively higher Q_{10} values than other N transformation rates (Berounsky & Nixon, 1990). Our nitrification rates were also sensitive to temperature (Fig. 6a) and were highest in the autumn when water and sediment temperatures were both still high, and the water column was well mixed. In contrast, several studies of nitrification rates in lotic systems have found the fastest nitrification during late spring and early summer (Kemp & Dodds, 2002; Strauss *et al.*, 2004; Arango, 2007), driven by changes in temperature, oxygen and/or NH_4^+ availability. This difference in a spring/summer peak in lotic systems compared to an autumnal peak in nitrification in lentic systems is probably due to differences in seasonal oxygen, temperature and organic matter delivery patterns in lakes. Variations in oxygen profiles, temperature and organic matter delivery between littoral and profundal sediment due to seasonal stratification would also be an important consideration when determining seasonal nitrification patterns for a lake, and we expect that nitrification rates in profundal sediments would be more strongly regulated by seasonal stratification than those we measured in littoral sediments.

We used sediment standing stocks to scale our nitrification rates to an areal basis for comparison with previously measured nitrification rates in lake sediments; our nitrification rates ranged from 0.17 to 3.7 g N m^{-2} day^{-1}, with an average nitrification rate of 1.4 ± 0.28 g N m^{-2} day^{-1}, higher than nitrification rates measured in Michigan streams near Gull Lake (0.01–0.2 g N m^{-2} day^{-1}; Arango, 2007) and the Upper Mississippi River (0.1–0.4 g N m^{-2} day^{-1}; Strauss *et al.*, 2004). Compared to other lentic systems, Gull Lake nitrification rates were higher than rates measured in sediment of a lake in Denmark, which range from 0.0081 to 0.016 g N m^{-2} day^{-1} (Rysgaard *et al.*, 1993), in Onondaga Lake in the northeastern U.S.A. (0.21–0.42 g N m^{-2} day^{-1}), and in Hamilton Harbour, Lake Ontario, Canada (0.2 g N m^{-2} day^{-1}; Pauer & Auer, 2000). Nitrification rates in Hamilton Harbour were inhibited by low oxygen availability during the summer, but were also sensitive to low temperature. In summary, Gull Lake sediment nitrification rates were slightly higher than nitrification rates measured in other lakes, and higher than nitrification rates measured in other freshwater systems in a similar geographic area (e.g. Midwestern U.S.A. streams and rivers). This may be in part because we focused on the littoral zone. The sandy littoral sediment of Gull Lake is low in organic matter, high in sediment NH_4^+, and has comparatively higher oxygen availability and higher temperature than profundal sediments, all of which are good conditions for nitrification.

Seasonal patterns of denitrification

We measured relatively high sediment denitrification rates in June 2005 and February 2006 (Fig. 7). Many seasonal studies of denitrification have revealed high denitrification rates in winter (Hasegawa & Okino, 2004; Bruesewitz *et al.*, 2006; Strauss *et al.*, 2006; Arango, 2007), typically associated with high NO_3^- availability, and which is consistent with our results in Gull Lake (Fig. 1c). Higher NO_3^- availability to denitrifiers can be a result of increased nitrification-denitrification coupling (Strauss *et al.*, 2006), or less assimilatory biological demand during the winter. Additionally, in Gull Lake, sediment denitrification always responded positively to additional NO_3^- in our seasonal nutrient limitation assays (Fig. 9), and we saw a positive relationship between both water column and sediment NO_3^- and denitrification rates (Fig. 8a,b). In contrast to nitrifiers, it appears that denitrifiers are not necessarily inhibited by low temperature (Hasegawa & Okino, 2004; Wall *et al.*, 2005; Bruesewitz *et al.*, 2006). High denitrification rates in the winter may be an important, but often overlooked, contribution to overall annual N loss via denitrification.

Gull Lake denitrification rates per unit surface area ranged from 25.7 to 420.7 mg N m^{-2} day^{-1}, with an annual average of 133 ± 26 mg N m^{-2} day^{-1}. A review of denitrification studies reported the annual average denitrification rates from various aquatic

ecosystems including: rivers (80.93 ± 41.04 mg N m^{-2} day^{-1}) and estuaries (12.27 ± 11.89 mg N m^{-2} day^{-1} (Piña-Ochoa & Álvarez-Cobelas, 2006). They also report lake sediment denitrification rates that are lower than in Gull Lake, ranging from 3.84 to 142.68 mg N m^{-2} day^{-1}, and with an average denitrification rate in lakes of 77.86 ± 45.64 mg N m^{-2} day^{-1}. A study of littoral sediment denitrification rates in an oligotrophic lake measured littoral denitrification rates of 37 mg N m^{-2} day^{-1}, which is within the range of our study (Saunders & Kalff, 2001). Lake Michigan has considerably lower denitrification rates than those of Gull Lake, with an average denitrification rate of 0.36–1.22 mg N m^{-2} day^{-1} (Gardner, Nalepa & Malczyk, 1987). Overall, we measured relatively high denitrification rates in the littoral zone of Gull Lake. It is worth noting that the majority of lake sediment denitrification studies were conducted using profundal sediments, and in many cases littoral denitrification rates may be higher (Saunders & Kalff, 2001). This, along with the relatively high NO_3^- concentrations in Gull Lake, may explain in part why our denitrification rates are at the high end of the reported range for lakes.

Our use of slurries to measure sediment N transformation rates has the disadvantage of disturbing redox gradients in the sediment. However, for comparing our experimentally manipulated treatments of −ZM and +ZM, sediment slurries were an efficient way to replicate strongly in our experimental design. Slurries are also advantageous in that variation among sediment sub-samples is minimised by mixing prior to sub-sampling. Other studies have successfully used sediment slurries to make similar comparative measurements (Groffman *et al*., 2006). Additionally, the use of the antibiotic chloramphenicol in denitrification assays prevented denitrifiers from producing new enzymes in response to favourable changes to their environmental conditions in the anoxic slurries, so the measured denitrification rates reflect their capacity for denitrification at that particular sampling period (Murray & Knowles, 1999). Lastly, our rates are comparable to other rates measured in aquatic ecosystems with a variety of methods, as discussed above. For these reasons our sediment slurry methods are a reliable way to determine the differences in N transformation rates between our −ZM and +ZM treatments as seasonal environmental conditions changed.

The effect of zebra mussels on littoral N transformations

'Benthification' as a result of zebra mussel activity has the potential to alter many sediment characteristics that enhance nutrient cycling (Hecky *et al*., 2004; Zhu *et al*., 2006). Our PCA of environmental variables in Gull Lake suggested that the zebra mussel treatment generated environmental variability on many of our sampling dates (Fig. 4), and based on this we expected that there may be differences in nitrification and denitrification rates between zebra mussel treatments in June, July and September 2005 and February and June 2006. We also measured higher exchangeable sediment NH_4^+ and NO_3^- concentrations in the +ZM compared to −ZM sediment (Fig. 3), which may be related to zebra mussel activity increasing N availability in the littoral sediment. However, these differences did not result in localised increases in sediment nitrification rates in the presence of zebra mussels (Fig. 5). As discussed above, Gull Lake nitrification rates were high in comparison to many other lakes where nitrification has been measured, and zebra mussel activity may have played a role in the overall high nitrification rates we measured throughout the littoral zone.

In contrast, we measured comparatively higher denitrification rates in the presence of zebra mussels during both of our winter sampling dates (Fig. 7), and in fact we measured one of the highest overall denitrification rates of the entire study in February +ZM sediments. Zebra mussels continue to feed during the winter, though both temperature and food quantity are low. The quality of zebra mussel food can often be high during winter, with high P availability and dominance of algae with higher polyunsaturated fatty acid concentrations (Sterner *et al*., 1997; Wacker & Elert, 2004; Naddafi, Pettersson & Eklov, 2007). Zebra mussels actively feed and can even grow during the winter (Thorp *et al*., 1998; Cope, Bartsch & Hightower, 2006), although their filtration rate slows (Sprung, 1995b; Diggins, 2001). As a result, this feeding and waste production continues to deposit N to the sediment during the winter (Sprung, 1995a; Naddafi *et al*., 2007). A seasonal study of another filter feeding mollusc, the slipper limpet [*Crepidula fornicata* (Linnaeus)], found that NH_4^+ excretion rates were not correlated with seasonal declines in respiration; these molluscs continued to have high NH_4^+ excretion rates during

the winter despite seasonal changes in respiration (Martin *et al.*, 2006).

In addition to zebra mussel waste production during winter, there may also be less competition for sediment N among biota in the winter; specifically *Chara* spp. and benthic algae were not growing in any of our sediment trays at that time (Fig. 1b). When *Chara* spp. is present, it is able to take up and store N from both interstitial water and from the sediment–water interface (Kufel & Kufel, 2002). *Chara* and zebra mussels are often found in the same areas (Lowe & Pillsbury, 1995; Vanderploeg *et al.*, 2002) because of their similar requirements, including high calcium concentrations and littoral habitats. *Chara* could use N made available to littoral sediment by zebra mussel excretion during the growing season (Vanderploeg *et al.*, 2002). We did not measure increased denitrification rates in +ZM sediment during spring, summer or autumn when *Chara* was present (Fig. 7), although the PCA suggested there was environmental variability between zebra mussel treatments during these periods (Fig. 4). The lack of higher denitrification rates in the +ZM treatment may have been due in part to the demand for N by *Chara* spp. When *Chara* was absent from the sediment trays, N availability was higher, which occurred during winter when we also measured higher sediment denitrification rates in the presence of zebra mussels.

Local and ecosystem-wide effects of zebra mussels

Our seasonal survey was conducted in a lake ecosystem that has been invaded by zebra mussels for more than a decade (they have been present in Gull Lake since at least 1994; Wilson & Sarnelle, 2002). We created local −ZM and +ZM treatments by isolating a known area of sediment in association with a known density of zebra mussels, but we could not isolate the sediment from the potentially more widespread influence of zebra mussels in Gull Lake. Waves and storms may be re-distributing zebra mussel wastes within the littoral zone, and our sediment trays would have been exposed to such wastes. Zebra mussel faeces and pseudofaeces can be suspended (Roditi, Strayer & Findlay, 1997; Vanderploeg *et al.*, 2002; Hecky *et al.*, 2004) and redistributed away from areas colonised by zebra mussels. In an attempt to avoid the effects of resuspension, we never retrieved trays on the day of or on the day immediately following a large wind event.

Considering the fact that zebra mussels potentially produce lakewide effects in Gull Lake, our study results should be considered only in the context of localised effects of zebra mussels on sediment N transformations. The differences we measured between −ZM and +ZM sediments therefore represent a minimum level of impact that zebra mussels have on denitrification. Our measurement of the local effects of zebra mussels on sediments surrounding zebra mussel colonies provides a critical component in the development of a more complete understanding of the localised and ecosystem-scale effects of zebra mussels. The seasonal aspect of our study provides insight into an additional level of complexity that is important when considering the ecosystem-scale effects of zebra mussels.

Though it is difficult to determine the impact of *D. polymorpha* on N transformation rates in Gull Lake, due to the complications of studying a lake that has already been invaded, we have shown that the mussels seasonally increased sediment denitrification rates. In comparison to other lake ecosystems where nitrification and denitrification have been measured, Gull Lake had relatively high rates of nitrification and denitrification in littoral sediments and zebra mussel activity may have enhanced the role of littoral sediments in N transformation rates.

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