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SYNTHESIS AND EMERGING IDEAS

Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland

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Abstract Wetlands are often highly effective nitrogen (N) sinks. In the Lake Waco Wetland (LWW), near Waco, Texas, USA, nitrate (NO_3^-) concentrations are reduced by more than 90% in the first 500 m downstream of the inflow, creating a distinct gradient in NO_3^- concentration along the flow path of water. The relative importance of sediment denitrification (DNF), dissimilatory NO_3^- reduction to ammonium (DNRA), and N_2 fixation were examined along the NO_3^- concentration gradient in the LWW. "Potential DNF" (hereafter potDNF) was observed in all months and ranged from 54 to 278 μmol N m^{-2} h^{-1}. "Potential DNRA" (hereafter potDNRA) was observed only in summer months and ranged from 1.3 to 33 μmol N m^{-2} h^{-1}. Net N_2 flux ranged from 184 (net denitrification) to −270 (net N_2 fixation) μmol N m^{-2} h^{-1}. Nitrogen fixation was variable, ranging from 0 to 426 μmol N m^{-2} h^{-1}, but high rates ranked among the highest reported for aquatic sediments. On average, summer potDNRA comprised only 5% (±2% SE) of total NO_3^- loss through dissimilatory pathways, but was as high as 36% at one site where potDNF was consistently low. Potential DNRA was higher in sediments with higher sediment oxygen demand ($r^2 = 0.84$), and was related to NO_3^- concentration in overlying water in one summer ($r^2 = 0.81$). Sediments were a NO_3^- sink and accounted for 50% of wetland NO_3^- removal ($r^2 = 0.90$). Sediments were an NH_4^+ source, but the wetland was often a net NH_4^+ sink. Although DNRA rates in freshwater wetlands may rival those observed in estuarine systems, the importance of DNRA in freshwater sediments appears to be minor relative to DNF. Furthermore, sediment N_2 fixation can be extremely high when NO_3^- in overlying water is consistently low. The data suggest that newly fixed N can support sustained N transformation processes such as DNF and DNRA when surface water inorganic N supply rates are low.

Keywords DNRA · Coupled nitrification–denitrification · Sediment N transformation · N_2 fixation · Sediment nutrient flux

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Introduction

The use of synthetically derived nitrogenous fertilizers has increased nitrogen (N) export from landscapes and enriched N in coastal waters. As a result, coastal ecosystems have experienced increased hypoxia (Rabalais et al. 2002) and harmful algal blooms (Paerl 1997). Although the effects of N enrichment in coastal waters are severe, only a fraction of bioavailable N (primarily as nitrate [NO_3^-]) introduced onto landscape via human activity actually enters coastal ecosystems. As much as 75% of this N is stored in the landscape or lost back to the atmosphere through balancing mechanisms, such as denitrification (Howarth et al. 1996).

Wetland ecosystems often are efficient N sinks (Bowden 1987). Consequently, wetlands have high potential capacities for controlling NO_3^- enrichment in surface waters and reducing N export to coastal ecosystems (Mitsch et al. 2001, 2005). Nitrate removal from wetland waters is mediated biologically and occurs rapidly (Whitmire and Hamilton 2005). Consensus among scientists is that NO_3^- transported over wetland sediments enters either the organic N pool following assimilation by microorganisms or re-enters the atmospheric N_2 pool following respiratory denitrification (DNF). Organic N remains available to organisms in a remineralization-uptake cycle, while atmospheric N_2 is unavailable to most organisms. Some alternative NO_3^- removal pathways exist, which may compete with DNF for NO_3^- and maintain N in a biologically available form (Burgin and Hamilton 2007). Dissimilatory NO_3^- reduction to ammonium (DNRA) is a bacterial-mediated heterotrophic process occurring in anaerobic environments. DNRA represents a potential positive feedback to ecosystems because it converts NO_3^- to ammonium (NH_4^+), a biologically available form of N (Brunet and Garcia-Gil 1996; An and Gardner 2002; Gardner et al. 2006). However, NH_4^+ generated by DNRA could also fuel nitrification (as suggested by Burgin and Hamilton 2007) and may simply provide an intermediate mechanism causing N loss to the atmosphere via coupled nitrification-DNF (Jenkins and Kemp 1984). Relative to abundant literature on DNF, DNRA has seldom been quantified in aquatic sediments. In a recent review of alternate NO_3^- removal pathways, Burgin and Hamilton (2007) highlight the lack of data describing when and where DNRA occurs and discuss how this information is imperative for understanding the importance of DNRA relative to other NO_3^- removal mechanisms. The authors propose a series of conditions that might favor DNRA as an important NO_3^- removal pathway in aquatic sediments, namely the availability of labile carbon, reduced sulfur, and reduced iron. Freshwater wetlands, in particular, have not been well studied with regard to diverse NO_3^- removal pathways, including DNRA.

The Lake Waco Wetland (LWW), near Waco, Texas, USA (Fig. 1) is a created freshwater marsh with enormous NO_3^- removal capability. The NO_3^- concentration in waters entering LWW is reduced by more than 90% in the first 500 m downstream of the inflow (Scott et al. 2005). As a result, microbial communities in the downstream areas of LWW are severely N limited (Scott et al. 2005, 2007; Scott and Doyle 2006). Therefore, LWW is an ideal system in which to study NO_3^- removal and N transformations at the sediment–water interface of freshwater wetlands.

In this study, we quantify potential rates of DNRA (hereafter potDNRA), DNF (net N_2 flux and potential denitrification; potDNF) and other N transformation processes in sediments along the NO_3^- concentration gradient in LWW. Furthermore, we quantify the rates of sediment NO_3^- flux and wetland NO_3^- removal to estimate the relative importance of sediment N transformations as an N sink. Specifically, we

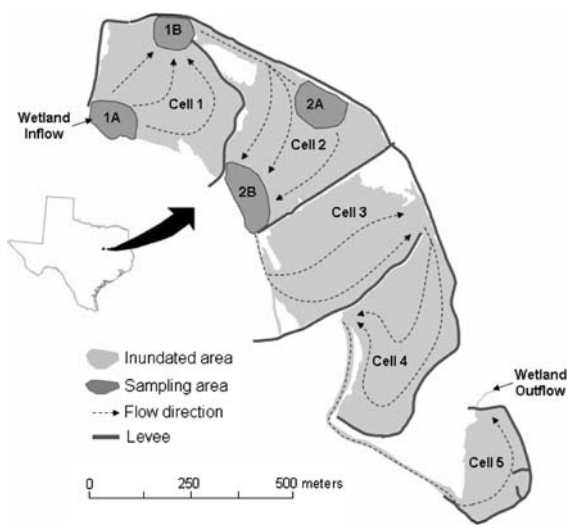

Fig. 1 Lake Waco Wetlands near Waco, Texas, USA. Intact sediment cores collected in area 1A, 1B, 2A, and 2B along the flow path of water in 2004 and 2005. Water chemistry monitored biweekly in areas 1A, 1B, and 2B

consider the following questions: 1. What are the relative rates of DNRA, DNF, and N_2 fixation seasonally? 2. Do N transformation rates vary spatially along the NO_3^- concentration gradient? 3. Are N_2 fixation rates sufficient to supplement N to sediments? 4. Is the coupling of nitrification-DNF correlated with DNRA? 5. To what extent are sediment NO_3^- fluxes correlated with ecosystem NO_3^- removal? Overall goals were to estimate the importance of DNRA relative to denitrification and other N transformation processes, and to examine the relative contribution of sediments to wetland NO_3^- removal.

Materials and methods

Site description

The Lake Waco Wetland is located near the inflow of the North Bosque River into Lake Waco, Texas, USA (Fig. 1). The wetland is an 80 ha created marsh that receives water pumped from the North Bosque River. Inflowing water meanders through five cascading wetland cells before flowing back to the North Bosque River and into Lake Waco. Cells 1 and 2 were originally flooded in January 2003 and the remainder of the wetland was flooded in November 2003. Aquatic vegetation began dispersing in spring 2003 and was well established by the initiation of this study in August 2004. The aquatic habitat within the wetland is primarily emergent marsh dominated by the macrophytes *Typha* sp., *Schoenplectus* sp., *Pontederia* sp., and *Sagittaria* sp. Deep areas (>1 m) of the marsh contain a variety of submerged and floating aquatic vegetation, such as *Najas* sp., the macroalgae *Chara* sp., *Nuphar* sp., and *Nymphaea* sp. A large proportion of both shallow and relatively deep open water area is inhabited by floating and submerged microbial mats.

Sediment nutrient flux and N transformations

Continuous-flow experiments with intact cores were used to quantify sediment nutrient and dissolved gas fluxes following the methods outlined by Gardner et al. (2006) and references therein. Three intact sediment cores (7.6 cm inner diameter; 10–20 cm depth) were collected from each of the four sampling areas along the nutrient availability gradient (Fig. 1) in January, April, and July 2005. Cores were collected in sampling areas 1A, 1B, and 2B in August 2004. Cores were collected with a coring device equipped with a one-way rubber valve to maintain structural integrity of the core and overlying water. Although cores did not include above ground biomass of emergent vegetation, roots and rhizomes of emergent plants were often included in cores. Dense benthic algal mats, which likely contained N_2 fixing cyanobacteria (Scott et al. 2005) were also included in some cores, particularly at station 2B where emergent vegetation was least dense. In addition to cores, approximately 20 l of site water was collected from each sampling area for nutrient analysis and sediment core incubations. Sediment cores were transferred the same day to the University of Texas Marine Science Institute in Port Aransas, Texas, and fitted with an adjustable flow-through plunger with O-ring seal and Teflon inlet and outlet tubes to create a continuous flow chamber (Lavrentyev et al. 2000). Water column depth over the sediment surface was maintained at ~5 cm in the chamber to give an overlying water volume of ~230 ml. Continuous flow chambers were incubated in a water bath at in situ temperature, and water from each sampling area was passed over the core surface at ~1.2 ml min^{-1}. Flow through water was aerated and maintained at ambient indoor temperature (21–26°C) before entering incubation chambers. Daily changes in N_2 concentration in flow through water never exceeded 0.1 μM N_2, which translated into a minimum detection level of approximately 5 μmol N m^{-2} h^{-1} for estimates calculated from N_2 flux. Incubations were conducted under ambient indoor lighting (photosynthetic active radiation ≈ 30 μmol m^{-2} s^{-1}) to represent the shaded conditions of a shallow marsh. Cores were "pre-incubated" for approximately 18 h, and after overnight stabilization, triplicate inflow and outflow samples were collected once daily from each chamber for dissolved gas analysis by membrane inlet mass spectrometry (MIMS). Dissolved N_2, O_2, and Ar were measured with MIMS using the method described by Kana et al. (1994) and modified by An et al. (2001). The N_2-scavenging effect observed by Eyre et al. (2002) was evaluated on the MIMS used in this study and was not significant (unpublished data with Soonmo An, Pusan National University, Korea).

Additional samples were collected from sediment core incubations to determine dissolved nutrients. On these samples, NO_2^- and NO_3^- were measured colorimetrically using a Lachat QuikChem 8000 flow injection autoanalyzer. Ammonium concentration and isotopic content were measured by HPLC (Gardner et al. 1995). Sediment flux for each compound was calculated as the concentration difference between inflow and outflow water divided by the flow rate and cross-sectional area (Lavrentyev et al. 2000) and final units were expressed as μmol N m^{-2} h^{-1}. After the second sampling day, inflow water was enriched with $^{15}NO_3^-$ (40 μmol l^{-1} final concentration in January and April 2005 and 100 μmol l^{-1} final concentration in August 2004 and July 2005). Production of mass-specific N_2 ($^{28}N_2$ from $^{14}NO_3^-$, $^{30}N_2$ from $^{15}NO_3^-$, and $^{29}N_2$ from $^{14}NO_3^-$ and $^{15}NO_3^-$) was used to estimate potential denitrification (potDNF), N_2 fixation, and the percentage of potDNF coupled to nitrification (Nielsen 1992; An et al. 2001; An and Gardner 2002). Anaerobic NH_4^+ oxidation (anammox; e.g., Mulder et al. 1995) was not quantified in this study, and the term "potDNF" is intended to encompass all microbial processes with N_2 as the end product. "Potential DNRA" rates were estimated from $^{15}NH_4^+$ production rates (An and Gardner 2002). Sediment oxygen demand (SOD; μmol O_2 m^{-2} h^{-1}) was determined from the consumption of O_2 by intact cores prior to $^{15}NO_3^-$ addition. Extensive replication was not possible because sediment core incubations are time consuming and expensive. Therefore, we present average spatial and temporal trends of sediment N transformations and SOD graphically, with appropriate error estimates. PROC GLM in SAS 9.1 was used to identify relationships between sediment N transformations, SOD, and water column nutrient concentrations.

Flow and wetland NO_3^- and NH_4^+ flux estimates

Wetland inflow volume was estimated daily throughout the course of the study, and water chemistry was monitored weekly during each sampling month. Daily flow into the wetland (area 1A) was determined from the number of hours that two inflow pumps (rated at 865 m^3 h^{-1} each) were run each day. Outflow at sites 1B and 2B was estimated as the difference between inflow and the loss of water from wetland cell one and wetland cells one and two, respectively. Water loss was assumed to occur by two major mechanisms: evapotranspiration (ET) and infiltration of water into shallow groundwater. Monthly ET rates (mm $month^{-1}$) were estimated using the Thornthwaite model (Thornthwaite and Mather 1957; also described in Mitsch and Gosselink 2000) and multiplied by the area of each wetland cell to determine a monthly volumetric water loss from ET (m^3 $month^{-1}$). Infiltration losses were estimated by subtracting the measured outflow rate below cell 2 from the inflow rate when ET was at an annual low (December–February). Infiltration loss was normalized to total area of wetland cells 1 and 2. The measured loss during this period was 0.05 m^3 m^{-2} day^{-1}. Estimated infiltration loss was calculated for each wetland cell and summed with estimates of ET loss from each wetland cell to derive a total daily water loss and, ultimately, an outflow estimate (m^3 day^{-1}) at sites 1B and 2B. Infiltration losses were assumed to be constant across all seasons.

Water chemistry was monitored weekly at sites 1A, 1B, and 2B during each sampling month. Water chemistry samples were collected in acid-washed 1 l polyethylene bottles and returned to the laboratory for analysis of NO_3^- and NH_4^+. Nitrate was determined by colorimetry on a Beckman DU 650 spectrophotometer following cadmium reduction (Clesceri et al. 1998). Ammonium was determined colorimetrically on the same instrument using the phenate method (Clesceri et al. 1998). Surface water NO_3^- and NH_4^+ loading rates were estimated at sites 1A, 1B, and 2B by multiplying the average monthly concentration of each constituent by the estimated monthly flow rate at each site. Wetland areal NO_3^- and NH_4^+ fluxes were estimated as the difference between loading rates at the inflow and outflow of a wetland cell divided by the cell area. For example, the difference in NO_3^- loading rates between sites 1A and 1B was divided by the area of wetland cell 1 to estimate the ecosystem NO_3^- flux from wetland cell one. A negative value indicated the rate of NO_3^- or NH_4^+ retention in the wetland, and a positive value indicated the rate of NO_3^- or NH_4^+ release from the wetland.

Average sediment NO_3^- and NH_4^+ fluxes for each wetland cell were derived by averaging sediment nutrient fluxes (determined from continuous-flow

experiments with intact cores) from each sampling area within the respective wetland cells (i.e., sites 1A and 1B in cell 1, and sites 2A and 2B in cell 2). Because site 2A was not sampled in August 2004, sediment NO_3^- and NH_4^+ fluxes from site 2B were used as estimates for cell 2 during this month. We used PROC GLM in SAS 9.1 to explore the relationship between average sediment NO_3^- and NH_4^+ fluxes with nutrient flux from individual cells in LWW.

Results

Physical and chemical data at sediment coring sites

Table 1 provides physical and chemical data for sediment coring sites through the course of the study. Sites 1A and 2A were shallower (0.1–0.6 m) than sites 1B and 2B (0.3–1.0 m). Water temperature ranged from 7.4°C in January 2005 to 30.8°C in July 2005. Ammonium and NO_2^- concentrations were low compared with NO_3^- and showed little spatial or temporal variation. Nitrate concentration was always highest at site 1A and usually decreased sequentially at each downstream site. Nitrate concentration diminished by >95% from site 1A to site 2B in all months except January 2005, when NO_3^- concentration declined by 61%.

Sediment nutrient fluxes

Sediment nutrient fluxes for all sampling events are provided in Table 2. Ammonium was released from the sediments (positive flux) in every case except site 2A in July 2005, where NH_4^+ flux was not different from zero. Sediment NO_2^- flux was low and varied between positive (August 2004 and January 2005) and negative values (April and July 2005). Nitrate was consistently removed from the water column by sediments at relatively high rates, except in April 2005 at site 2A and July 2005 at site 2B, where sediment NO_3^- fluxes were small, but positive.

Sediment N transformations and SOD

Net N_2 flux ranged from 184 μmol N m^{-2} h^{-1} (net DNF) to −270 μmol N m^{-2} h^{-1} (net N_2 fixation). Potential DNF varied spatially and temporally (Fig. 2a) and ranged from 54 to 278 μmol N m^{-2} h^{-1}, with potDNF ranging from 2 to 8 times higher than DNF estimated from N_2 flux measurements alone.

Table 1 Physical and chemical characteristics of water for each sampling site in all events

Date	Site	Depth (m)	Temp (°C)	NH_4^+ (μmol N l^{-1})	NO_2^- (μmol N l^{-1})	NO_3^- (μmol N l^{-1})
Aug 2004	1A	0.6	28.6	5.73	1.09	30.0
	1B	0.5	27.3	1.89	0.40	15.7
	2A					
	2B	0.4	28.9	1.45	0.28	0.04
Jan 2005	1A	0.1	10.1	0.75	0.29	25.4
	1B	0.4	8.0	0.44	0.12	20.7
	2A	0.3	7.4	0.89	0.20	7.45
	2B	0.7	8.1	1.14	0.44	9.86
Apr 2005	1A	0.1	21.4	1.87	0.44	36.8
	1B	0.3	19.6	1.38	0.26	8.86
	2A	0.3	20.9	0.31	0.05	0.22
	2B	0.5	20.0	2.18	0.10	0.73
Jul 2005	1A	0.1	30.8	2.71	0.68	18.6
	1B	1.0	27.1	0.53	0.13	4.38
	2A	0.5	28.1	BDL[a]	BDL[a]	0.09
	2B	0.8	29.2	0.95	0.13	0.81

[a] BDL, below detection limit

Table 2 Sediment nutrient flux rates

Date	Site	NH_4^+	NO_2^-	NO_3^-
Aug 2004	1A	626 ± 27.4	24 ± 2.3	−87.7 ± 117
	1B	328 ± 86.8	4.0 ± 1.9	−144 ± 10.7
	2A			
	2B	95.8 ± 25.5	1.5 ± 1.3	−1.68 ± 1.03
Jan 2005	1A	28.4 ± 8.70	3.1 ± 0.8	−61.8 ± 17.1
	1B	79.5 ± 30.7	4.4 ± 2.8	−30.6 ± 13.0
	2A	18.2 ± 5.06	0.7 ± 0.7	−30.1 ± 5.48
	2B	179 ± 50.3	1.0 ± 0.8	−28.2 ± 4.81
Apr 2005	1A	434 ± 184	5.9 ± 1.8	−213 ± 63.9
	1B	135 ± 46.2	−1.0 ± 0.6	−68.0 ± 11.0
	2A	3.19 ± 2.03	−1.4 ± 0.3	2.30 ± 1.25
	2B	75.5 ± 57.6	−0.3 ± 0.4	−5.57 ± 2.19
Jul 2005	1A	245 ± 28.8	−0.5 ± 1.2	−113 ± 3.85
	1B	27.1 ± 18.5	1.3 ± 1.0	−45.2 ± 7.41
	2A	−2.77 ± 4.67	−0.1 ± 0.1	−0.7 ± 0.24
	2B	26.8 ± 13.4	2.1 ± 1.6	3.2 ± 5.99

Positive values indicate flux out of sediments and negative values indicate flux from water column into sediments. All rates expressed as μmol N m^{-2} h^{-1}

In August 2004, April 2005, and July 2005, potDNF was higher at the wetland inflow (site 1A) than at the most downstream site (site 2B; Fig. 2a). A general pattern of decreasing potDNF was apparent between sites 1A, 1B, and 2A in January and July 2005. However, potDNF at site 2B was higher than potDNF at sites immediately upstream in these months, and site 2B had the highest potDNF in January 2005 (Fig. 2a). Potential DNRA varied temporally (Fig. 2b), ranging from 0 to 33 μmol N m^{-2} h^{-1}, with measurable rates occurring only in summers of both years. Although no spatial differences in potDNRA were apparent in August 2004, potDNRA did appear higher in wetland cell two (sites 2A and 2B) in July 2005. Coupled nitrification-DNF was highest (17–50% of total DNF) in January 2005 and April 2005, but relatively low (4–15%) in summer months of both years (Fig. 2c). A spatial pattern in net N_2 flux was apparent in August 2004 and April 2005, where net DNF was apparent near the wetland inflow, and net N_2 fixation was apparent at the most downstream site (Fig. 3a). A similar pattern was observed in July 2005, with the exception of site 2A, the only downstream site displaying net DNF (Fig. 3a). Potential N_2 fixation was highest at site 2B in both summers (Fig. 3b).

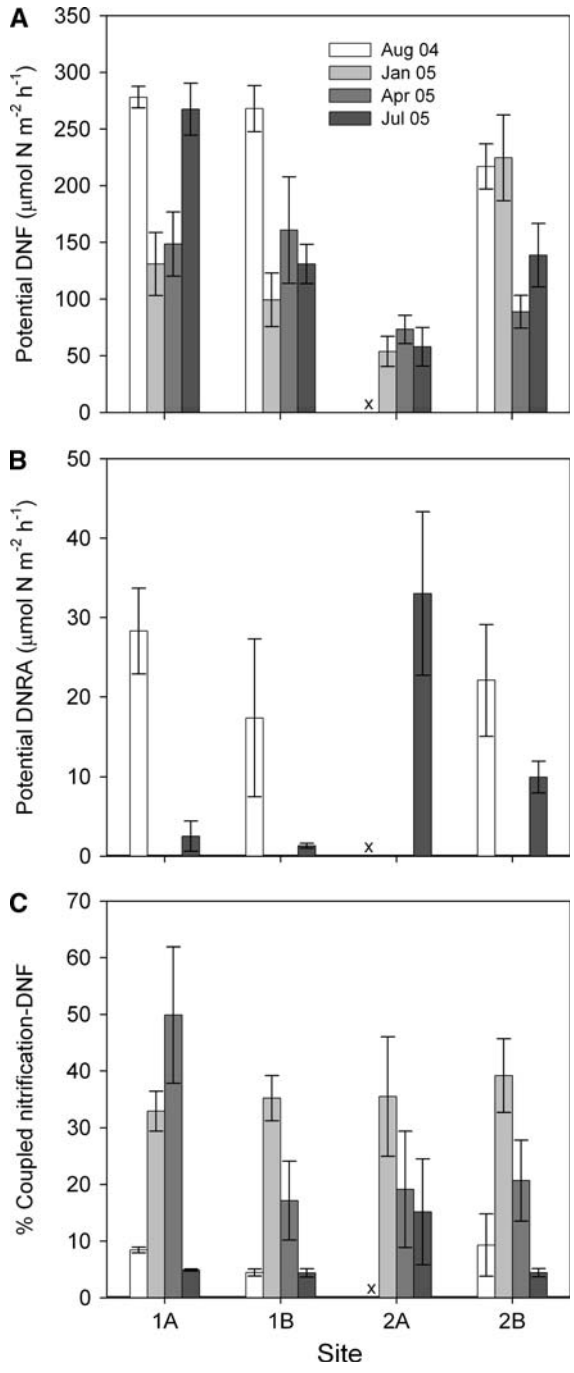

Fig. 2 Nitrogen transformations estimated along the NO_3^- gradient: (**a**) potential DNF, (**b**) potential DNRA, and (**c**) % DNF coupled with nitrification. The × indicates that site 2A was not sampled in August 2004. Error bars indicate standard error (SE)

Sediment oxygen demand was lowest in January 2005 and showed no consistent spatial pattern (Fig. 3c).

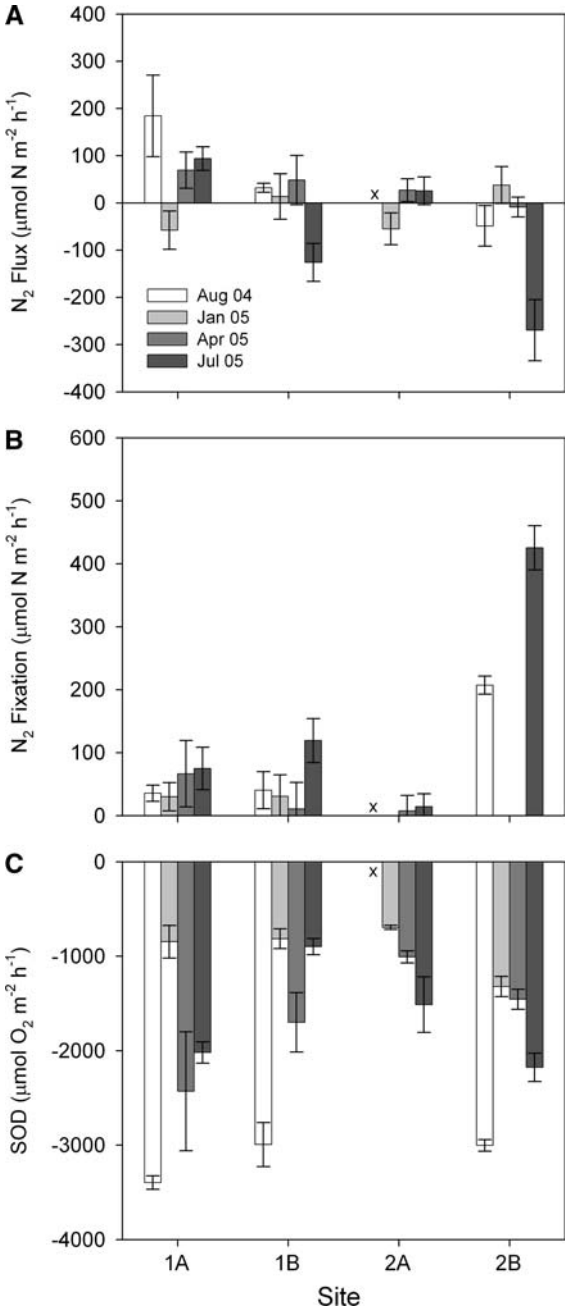

Fig. 3 Nitrogen transformations and sediment oxygen demand (SOD) along the NO_3^- gradient: (**a**) net N_2 flux in sediment cores prior to $^{15}NO_3^-$ addition, (**b**) N_2 fixation, and (**c**) SOD. The × indicates that site 2A was not sampled in August 2004. Error bars indicate standard error (SE)

Flow and wetland nutrient flux

Flow rates, NH_4^+ and NO_3^- import and export rates, and areal NH_4^+ and NO_3^- fluxes for wetland cells 1 and 2 are provided in Table 3. The average monthly inflow rate into the wetland ranged from 0.23 to 0.37 $m^3\ s^{-1}$. Evapotranspiration and infiltration resulted in a 19% (±1.8% SE) reduction in the flow below cell 1 and another 27% (±4.8% SE) reduction in flow below cell 2. Of this water loss, only 1–10% was from ET, and 90–99% was from infiltration. Ammonium import into the wetland was low relative to NO_3^-. With the exception of cell 2 in January and April 2005, NH_4^+ import generally exceeded export, resulting in negative NH_4^+ fluxes for both wetland cells. Even though the sediments were often a substantial source of NH_4^+ (Table 2), sediment NH_4^+ production was correlated negatively with wetland NH_4^+ flux (Fig. 4a). Nitrate import always exceeded export in both wetland cells in all months (Table 3), resulting in high NO_3^- removal (i.e., large negative sediment NO_3^- flux). The rate of NO_3^- diffusion from the water column into the sediments accounted for 50% of wetland NO_3^- removal ($m = 2.03$; Fig. 4b).

Discussion

potDNRA, potDNF, N_2 fixation, and spatial variability along the NO_3^- gradient

We sought to quantify the importance of DNRA as a NO_3^- removal pathway in a created freshwater wetland. In particular, one of our questions was "what are the relative rates of DNRA and DNF seasonally?" The results suggest that potDNRA contributes to NO_3^- removal in LWW, particularly in summer months, but the relative importance of this NO_3^- removal pathway is minor. Summer potDNRA rates varied from 1.3 to 33 µmol N $m^{-2}\ h^{-1}$, and resembled rates reported in several Texas estuaries (An and Gardner 2002; Gardner et al. 2006), a coastal wetland on Lake Erie (McCarthy et al. 2007a), and a subtropical lake in China (McCarthy et al. 2007b) obtained with similar methodology. However, potDNRA comprised only 5% (±2%, SE) of the total NO_3^- loss through dissimilatory pathways (potDNF and potDNRA combined). The lower contribution of potDNRA to total dissimilatory NO_3^- removal in freshwater sediments appears to be driven by relatively high potDNF rates in freshwater systems. The contribution of DNRA to NO_3^- removal was 50% or more in estuaries in Texas (An and Gardner 2002;

Table 3 Flow rates, NH_4^+ and NO_3^- import and export rates, and areal NH_4^+ and NO_3^- flux rates for wetland cells 1 and 2 are provided for each of the months when sediment cores were sampled

Date	Wetland cell	Area (ha)	Inflow (cms)	Outflow (cms)	NH_4^+ in (mol h^{-1})	NH_4^+ out (mol h^{-1})	NO_3^- in (mol h^{-1})	NO_3^- out (mol h^{-1})	Wetland NH_4^+ flux (μmol m^{-2} h^{-1})	Wetland NO_3^- flux (μmol m^{-2} h^{-1})
Aug 2004	1	9.32	0.32	0.26	4.5	1.1	23	4.9	−35.9	−190
Jan 2005	1	9.32	0.23	0.18	0.4	0.3	33	18	−1.67	−156
Apr 2005	1	9.32	0.30	0.24	1.2	0.7	39	6.0	−5.44	−354
Jul 2005	1	9.32	0.37	0.32	3.5	1.1	29	5.1	−25.7	−254
Aug 2004	2	10.4	0.26	0.19	1.1	0.6	4.9	0.1	−5.02	−46.5
Jan 2005	2	10.4	0.18	0.11	0.3	0.4	18	2.5	1.00	−149
Apr 2005	2	10.4	0.24	0.18	0.7	1.4	6.0	1.1	6.93	−46.6
Jul 2005	2	10.4	0.32	0.27	1.1	1.0	5.1	1.0	−0.71	−39.3

Gardner et al. 2006) and France (Rysgaard et al. 1996; Bonin et al. 1998), but lower and more variable in freshwater environments (this study, McCarthy et al. 2007a, b). However, DNF in these and other freshwater systems (Tomaszek et al. 1997; Poe et al. 2003) were 2–10 times greater than the highest DNF rates measured in the estuarine environments. One exception to the relatively low contribution of potDNRA to total dissimilatory NO_3^- removal in LWW was at site 2A in July 2005, where potDNRA accounted for 36% of total NO_3^- loss through dissimilatory pathways (Fig. 2). This site was also characterized by lower water column NO_3^- concentrations (Table 1) and low potDNF throughout the course of the study (Fig. 2a). Also worth noting, but not explicitly tested in this study, was that site 2A appeared to have the most dense *Typha* stands of any of the four sampling areas in the wetland.

Burgin and Hamilton (2007) distinguish between two DNRA pathways in aquatic sediments [chemolithoautotrophic DNRA (Brettar and Rheinheimer 1991; Brunet and Garcia-Gil 1996) or fermentative DNRA (Tiedje 1988)]. They further suggest conditions that each of these pathways may exist and compete with DNF as important NO_3^- removal pathways in carbon rich environments, such as freshwater wetlands. Chemolithoautotrophic DNRA may be favored in high carbon systems with relatively high reduced sulfur concentrations due to sulfide inhibition of DNF. Fermentative DNRA may be favored over DNF in high carbon systems with little or no reduced sulfur, particularly when the carbon to NO_3^- ratio is high. Unfortunately, we have no data to describe reduced sulfur conditions in LWW. However, DNRA in both summers was related to sediment oxygen demand (Fig. 5a). This result suggests that stronger reducing conditions, and by implication a greater proportion of reduced sulfur, may have been related to the contribution of potDNRA to dissimilatory NO_3^- removal observed in LWW. Therefore, we conclude that chemolithoautotrophic DNRA may have been the primary DNRA pathway. However, the relationship between potDNRA and SOD observed at site 2A in July 2005 was inconsistent with most data on potDNRA and SOD (Fig. 5a). This inconsistency may be related to the availability of NO_3^- at that site, suggesting some importance of fermentative DNRA.

This observation leads to the second question "do DNRA, DNF, and other N transformation rates vary spatially along a NO_3^- concentration gradient?" Although potDNRA showed no spatial heterogeneity along the NO_3^- concentration gradient in August 2004, substantial spatial variation was observed in July 2005 (Fig. 2b). Furthermore, potDNRA in that month was negatively correlated with NO_3^- concentration (Fig. 5b). This relationship was largely driven by high potDNRA at site 2A in July 2005, which also had the lowest NO_3^- concentration observed during this sampling event. Although we can only be 90% confident that this relationship was meaningful ($p = 0.0988$; Fig. 5b), the observation supports the hypothesis that a high carbon to NO_3^- ratio may favor fermentative DNRA over DNF in less reduced or non-sulfidic sediments (Burgin and Hamilton 2007). King and Nedwell (1985) and more recently Laverman et al. (2006), found that DNRA accounted for a greater proportion of NO_3^- reduction in marine

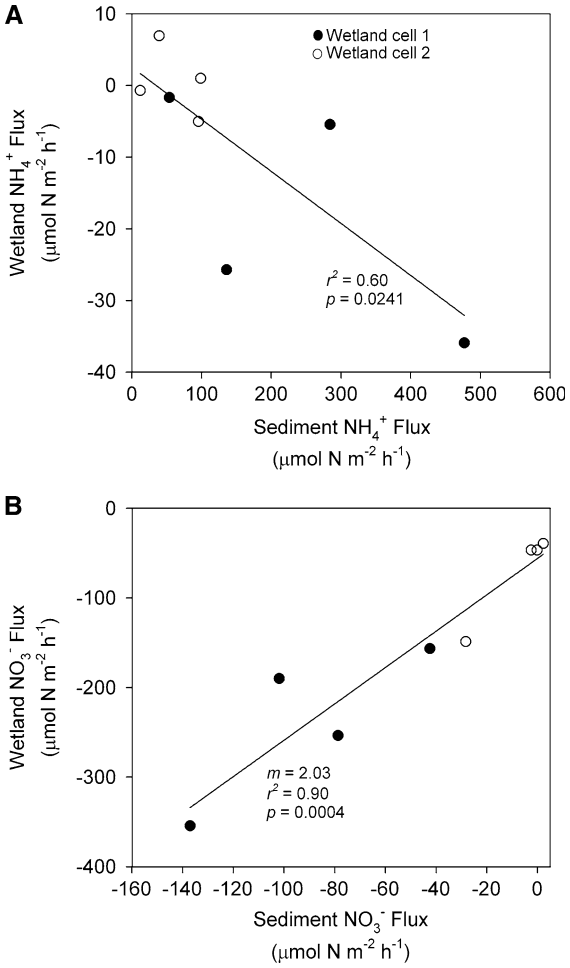

Fig. 4 Relationship between sediment nutrient flux and wetland nutrient flux: (**a**) sediment NH_4^+ production vs. wetland NH_4^+ flux, which was generally negative indicating net NH_4^+ removal, (**b**) sediment NO_3^- uptake vs. wetland NO_3^- removal. In both plots, filled circles indicate measurements from wetland cell 1 and hollow circles indicate measurements from wetland cell 2

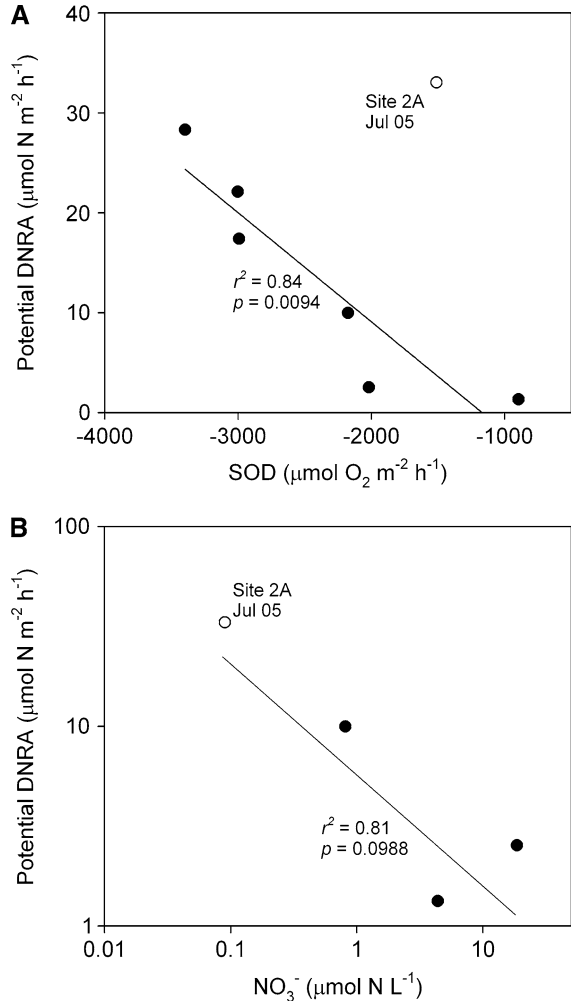

Fig. 5 Potential controls on DNRA: (**a**) DNRA exhibited a negative linear relationship with SOD, with the exception of site 2A in July 2005 (hollow circle), (**b**) DNRA was negatively correlated with NO_3^- concentration (log – log scale) in July 2005, largely due to the influence observed at site 2A (hollow circle)

sediments when NO_3^- was limiting. Megonigal et al. (2003) hypothesized that fermentative DNRA might be favored over DNF when NO_3^- is in short supply relative to potential electron donors in anoxic sediments. Collectively, higher potDNRA observed in this study appeared related to SOD (Fig. 5a), suggesting that chemolithoautotrophic DNRA is the more important pathway in this system. Therefore, chemolithoautotrophic DNRA in sulfidic sediments may be the most common DNRA pathway in LWW, but fermentative DNRA may occur in more oxic sediments when NO_3^- is low in overlying waters. More research is needed to better understand conditions by which chemolithoautotrophic or fermentative DNRA may be favored over DNF in freshwater sediments.

Other N transformations demonstrated more consistent spatial variation with NO_3^- concentrations. In spring and summer months, net N_2 flux often decreased from high positive values to negative ones along the NO_3^- gradient (Fig. 3a). However, potDNF decreased from sites 1A to 2A, but then increased again at site 2B (Fig. 2a). This increase was not associated with a substantial increase in NO_3^- concentration at site 2B (Table 1), nor was site 2B characterized by high coupled nitrification-DNF

(Fig. 2c). N_2 fixation was much higher at site 2B than at upstream sites (Fig. 3b), consistent with decreased NO_3^- availability (Table 1). It is possible that N_2 fixation at both the sediment-water interface and in attached and floating microbial communities, which was previously demonstrated as an important source of N in this wetland (Scott et al. 2005, 2007), increased N sufficiently to stimulate DNF at this site.

The N_2 fixation rates measured in both summers at site 2B are among the highest reported for aquatic sediments (Howarth et al. 1988). Another recent study, which used similar methods to our study, found even higher rates in coastal marine sediments (Fulweiler et al. 2007). Significant N_2 fixation was expected at site 2B because NO_3^- and NH_4^+ concentrations at this site were consistently and substantially lower than concentrations near the wetland inflow. In fact, only 6.2 and 9.8% of the dissolved inorganic N entering the wetland during these summers left the wetland as DIN in surface water. Therefore, sediments near the wetland outflow (site 2B) received a much lower proportion of inorganic N, which is apparent in the consistently lower sediment NO_3^- flux at this site (Table 2). Unlike site 2A where *Typha* stands were very dense, site 2B also had large expanses of shallow open water with low emergent macrophyte densities, which favored the proliferation of dense microphytobenthic mats. Measurements on periphyton and floating metaphyton communities in this system revealed substantial N_2 fixation (Scott et al. 2005, 2007), suggesting that cyanobacteria in sediment-bound microphytobenthos may have contributed to high N_2 fixation estimates observed in this study. Even low ambient light at which sediment core incubations were conducted ($\sim$30 μmol m^{-2} s^{-1}) may have been sufficient to stimulate cyanobacterial N_2 fixation in these experiments. We asked "are N_2 fixation rates sufficient to supplement N to sediments?" The results suggest that N_2 fixation in LWW may provide an important feedback of "new" N to fuel production and possibly other N transformation processes, particularly in downstream areas where water column NO_3^- concentrations are low.

potDNRA and coupled nitrification-DNF

Ammonium production from DNRA could potentially fuel coupled nitrification-DNF in aquatic sediments by providing nitrifying bacteria a source of NH_4^+ (Burgin and Hamilton 2007). We asked if the coupling of nitrification-DNF was correlated with DNRA? Although this link cannot be explicitly ruled out, results of this study suggest that DNRA supplies little, if any, NH_4^+ for nitrification in LWW. Coupled nitrification-DNF estimates were high throughout LWW in January and April 2005 (Fig. 2c) and potDNRA was undetectable during all these sampling events (Fig. 2b). Furthermore, mean summer potDNRA for the entire wetland was 16 μmol N m^{-2} h^{-1} (±4.7 μmol N m^{-2} h^{-1} SE), but the mean summer sediment NH_4^+ production rate for the wetland was 190 μmol N m^{-2} h^{-1} (±86 μmol N m^{-2} h^{-1} SE). Therefore, the proportion of sediment NH_4^+ production appeared to be dominated by assimilative N uptake and regeneration rather than DNRA. However, summer potDNRA was positively correlated with the percentage of coupled nitrification-DNF (Fig. 6), suggesting that this link may occur, but only be minimally important in summer months.

Coupled nitrification-DNF is often stimulated near aquatic macrophyte roots (Risgaard-Petersen and Jensen 1997), but may be stimulated (An and Joye 2001) or suppressed (Risgaard-Petersen 2003) by microphytobenthic assemblages. Microphytobenthos are an important group of primary producers in LWW

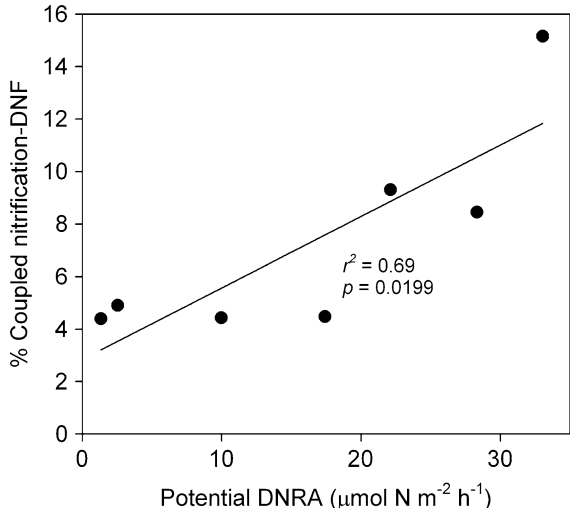

Fig. 6 The percent DNF coupled with nitrification was positively correlated with DNRA using data from August 2004 and July 2005. This relationship was only apparent in summer months when DNRA was measurable, but coupled nitrification-DNF was low relative to cooler months

(Scott et al. 2007) and can be dense in cooler months when emergent vegetation is dormant (J.T. Scott, personal observation). This observation suggests that microphytobenthos may stimulate coupled nitrification-DNF in LWW, but more work is needed to quantify this relationship.

Sediment and wetland NO_3^- removal

The final question was "to what extent are sediment NO_3^- fluxes correlated with ecosystem NO_3^- removal?" The rate of sediment NO_3^- removal from overlying water observed in this study was similar to rates in freshwater wetland systems in Michigan (Whitmire and Hamilton 2005) and North Carolina (Poe et al. 2003). Mean NO_3^- diffusion into sediments accounted for 50% of the wetland NO_3^- removal rate (Fig. 4b), suggesting that the other 50% of NO_3^- removal was driven by loss via water infiltration and/or microbial uptake above the sediments (Scott et al. 2007). Although quantifying the fate of NO_3^- entering wetland sediments is difficult, a substantial NO_3^- demand must exist in LWW sediments. The addition of $^{15}NO_3^-$ to overlying water in sediment core incubations always stimulated NO_3^- diffusion into sediments (data not shown). The presence of measurable N_2 fixation at the sediment–water interface, found using the isotope pairing method used in this study, confounded our ability to estimate whether or not DNF and DNRA were limited by the availability of NO_3^-. However, other studies have found that increased NO_3^- can stimulate DNF in freshwater wetland sediments (White and Reddy 1999). We suggest that NO_3^- reduction pathways in LWW may be limited by available NO_3^-. The system may have greater capacity for NO_3^- removal through dissimilatory pathways in response to increased NO_3^- supplies.

Rates of sediment NH_4^+ production in LWW were higher than rates for lentic freshwater systems reviewed by Seitzinger (1988), but were similar to more recently derived estimates for a freshwater wetland (Tomaszek et al. 1997), freshwater lake (Nowlin et al. 2005), and Texas estuaries (Gardner et al. 2006). Interestingly, almost none of the sediment-generated NH_4^+ flows out of LWW (Fig. 4a). The sediments, therefore, may be an important source of NH_4^+ to submersed and floating microbial communities in this system.

Conclusions

Dissimilatory NO_3^- reduction to NH_4^+ occurred in freshwater sediments, and rates resembled those observed in marine environments. However, the relative proportion of potDNRA to potDNF in this freshwater system was small, because freshwater sediments experience less sulfide inhibition of DNF. Sediment N_2 fixation was an important source of N to sediments when NO_3^- concentrations in the overlying water were low. Newly fixed N may have also supported sustained potDNF and NH_4^+ flux from sediments, because these rates displayed little spatial variation along the NO_3^- concentration gradient.

Our results also support the idea that created wetlands function as N sinks. The efficacy by which N may be removed or retained in wetland ecosystems may decrease the N load to downstream systems, particularly coastal systems, impacted by accelerated eutrophication. Increasing wetland acreage in areas such as the Mississippi drainage basin could reduce the amount of N loading to receiving waters and aid in alleviating some downstream eutrophication issues, such as Gulf of Mexico hypoxia.

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