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Benthic metabolism and the fate of dissolved inorganic nitrogen in intertidal sediments

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ABSTRACT

We determined patterns of benthic metabolism and examined the relative importance of denitrification (DNF) and dissimilatory nitrate reduction to ammonium (DNRA) as sinks for nitrate (NO_3^-) in intertidal sediments in the presence and absence of benthic microalgal (BMA) activity. By influencing the activity of BMA, light regulated the metabolic status of the sediments, and, in turn, exerted strong control on sediment nitrogen dynamics and the fate of inorganic nitrogen. A pulsed addition of ^{15}N-labeled NO_3^- tracked the effect and fate of dissolved inorganic nitrogen (DIN) in the system. Under illuminated conditions, BMA communities influenced benthic fluxes directly, via DIN uptake, and indirectly, by altering the oxygen penetration depth. Under dark hypoxic and anoxic conditions, the fate of water column NO_3^- was determined largely by three competing dissimilatory reductive processes; DNF, DNRA, and, on one occasion, anaerobic ammonium oxidation (anammox). Mass balance of the added ^{15}N tracer illustrated that DNF accounted for a maximum of 48.2% of the $^{15}NO_3^-$ reduced while DNRA (a minimum of 11.4%) and anammox (a minimum of 2.2%) accounted for much less. A slurry experiment was employed to further examine the partitioning between DNF and DNRA. High sulfide concentrations negatively impacted rates of both processes, while high DOC:NO_3^- ratios favored DNRA over DNF.

1. Introduction

Nutrient regeneration in sediments and subsequent release to the overlying water column, commonly referred to as benthic-pelagic coupling, fuels a significant portion of water column production in shallow estuarine systems (Nixon et al., 1976). Water depth, turbidity, sedimentation rates, organic matter remineralization rates, bioturbation, and diffusive or advective transport influence the magnitude and efficiency of benthic–pelagic coupling (Aller, 1994; Jahnke et al., 2000). The presence and activity of a benthic microalgal community also strongly affects benthic–pelagic coupling (Joye et al., 1996; Jahnke et al., 2000). Benthic microalgae (BMA) occupy a μm to mm thick layer at the surface of intertidal and shallow subtidal sediments, and their activity affects both the oxygen (O_2) flux and the O_2 penetration depth in the sediment. By modulating O_2 dynamics, BMA alter the sediment redox status and influence rates and pathways of nutrient cycling. BMA also consume nutrients from both the water column and underlying sediments. Under oxic conditions, BMA-dominated sediments act as a nutrient sink, while under hypoxic or anoxic conditions the same sediments serve as a nutrient source to the water column (Joye et al., 1996; Eyre and Ferguson, 2002).

Oxygen production by BMA can stimulate nitrification, which may enhance dissimilatory nitrate (NO_3^-) sinks (An and Joye, 2001), e.g., denitrification (DNF) or dissimilatory nitrate reduction to ammonium (DNRA). Complete DNF is the reduction of NO_3^- to N_2 as described by (Zumft, 1992; Eq. (1)):

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \quad (1)$$

The reduction of NO_3^- to N_2 via DNF involves a $5e^-$ transfer and has a free energy yield of -560 kJ mole^{-1} NO_3^- (Zumft, 1992). DNF produces mainly gaseous end products (N_2O and N_2), which readily diffuse from a system and are not generally bioavailable (Howarth et al., 1988); thus, DNF represents a net loss of fixed nitrogen from a system. Conversely, the end product of DNRA is NH_4^+, which is readily assimilable and can support additional production. The DNRA reaction includes NO_3^- reduction to NO_2^- and then to NH_4^+ (Tiedje, 1994; Thauer et al., 1977; Eq. (2)):

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$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O \tag{2}$$

DNRA involves 8e$^-$ per NO_3^- reduced and has a free energy yield of −600 kJ mole^{-1} NO_3^- (Thauer et al., 1977). The interaction between DNF and DNRA is further complicated by recent evidence for a third pathway of NO_3^- reduction: anaerobic ammonium oxidation (anammox). Anammox involves the production of N_2 via the reduction of NO_2^- coupled to the oxidation of NH_4^+ (van de Graaf et al., 1995; Eq. (3)):

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O \tag{3}$$

Anammox has a free energy yield of −358 kJ mole^{-1} NO_2^- (van de Graaf et al., 1995). Like DNF, anammox generates N_2 and thus represents a net loss of nitrogen from the system.

DNF, DNRA, and anammox may compete for nitrogen oxides (NO_3^- or NO_2^-) in sediments in the absence of O_2 and influence the DIN form available for flux to the overlying water column. The relative importance of DNRA versus DNF as NO_3^- sinks in coastal systems is unclear; however, DNRA rates rival DNF rates in some environments (Koike and Hattori, 1978; An and Gardner, 2002). While no previous studies have examined the interaction between these three processes, a number of studies have examined the relative importance of DNF versus DNRA. The environmental controls on these two processes include the ratio of dissolved organic carbon (DOC) to NO_3^- (Tiedje et al., 1982), temperature (Ogilvie et al., 1997), NO_3^- concentration (Fazzolari et al., 1998), and hydrogen sulfide (H_2S) concentration (Brunet and Garcia-Gil, 1996). The controls on anammox are uncertain, and how anammox interacts with DNF and DNRA is not known. Available evidence suggests that anammox and DNF can occur simultaneously (Thamdrup and Dalsgaard, 2002; Risgaard-Petersen et al., 2003), and anammox bacteria may be capable of DNRA (Kartal et al., 2007).

We used sediment flux core incubations and ^{15}N amendments to examine interactions between BMA activity and dissimilatory NO_3^- sinks in intertidal coastal sediments in Georgia and South Carolina, USA. We hypothesized that under diel, illuminated conditions, a pulse of DIN supplied to the water column would be assimilated by BMA. In contrast, under dark induced, anoxic conditions, DIN would be reduced by one of the three dissimilatory pathways. The relative importance of the dissimilatory processes has implications for the nitrogen budget of the system; thus, it is important to understand their regulation individually and collectively. Slurry experiments were used to investigate the impact of H_2S concentration and the DOC:NO_3^- ratio on the partitioning of dissimilatory NO_3^- reduction between DNF and DNRA. The results provide insight into the interactions between benthic photosynthesis, DNF, DNRA and anammox.

2. Methods

2.1. Study sites

Two tidal creek bank sites in Georgia and South Carolina, USA (Fig. 1) were sampled in January/February and August of 2002 and January of 2004 for benthic flux experiments, and in December 2005 for slurry experiments. At both sites, the adjacent saltmarsh was dominated by *Spartina alterniflora*. Creek bank sediments were macrophyte free but overlain by dense accumulations of benthic microalgae, mainly diatoms.

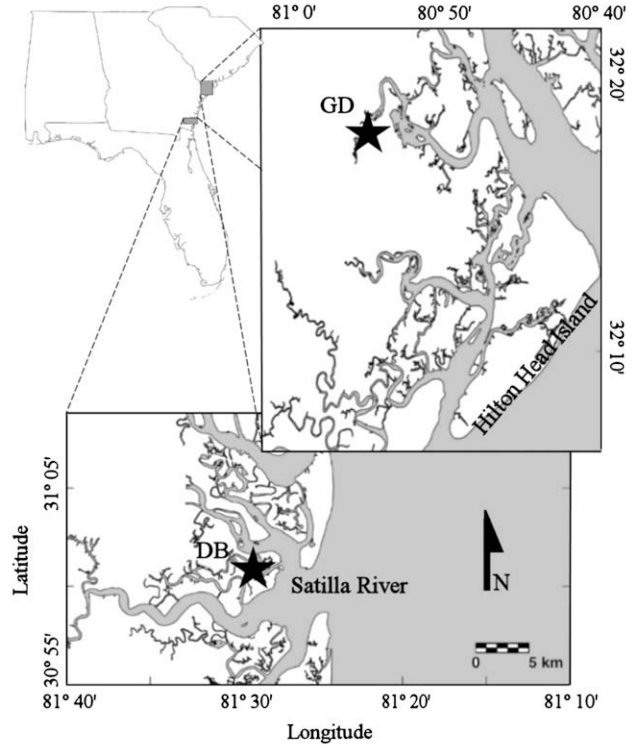

Fig. 1. Sampling locations in Georgia and South Carolina, USA. GD and DB denote Grave's Dock and Dover Bluff, respectively.

The Dover Bluff (DB) site, located on Umbrella Creek along the Satilla River (Georgia), has annual salinity and temperature ranges of approximately 12–30 and 15.5–25.6 °C, respectively. The site lies adjacent to a residential community and receives septic inputs from the developed upland (Weston et al., 2006). The Grave's Dock (GD) site, located within the Okatee River estuary (South Carolina), was characterized by near seawater salinity (Weston et al., 2006) and annual temperatures ranging from 15.5 to 26.8 °C. The Okatee watershed is heavily developed and nutrient inputs to the system are expected to increase in the coming years (Windom et al., 1998). Both sites had similar sediment characteristics, with sediment density in the range of ~1.1 to 1.3 g cm^{-3}, porosity in the range of 0.68–0.79, and bulk organic matter content (as loss on ignition) in the range of 5–10%. To contrast the two study sites, we include limited data from a previously published biogeochemistry study of these sediments (Table 1; see Weston et al., 2006 for detailed methods of pore water collection and analytical methods).

2.2. Benthic flux experimental design

Benthic fluxes were determined in re-circulating incubations of sediment cores ($n = 2$–3 cores per treatment per site). Clear acrylic core tubes with a diameter of 12 cm contained the top 20 cm of

Table 1
Integrated concentrations of pore water biogeochemical constituents in the upper 10 cm of the sediment (values taken from Weston et al., 2006). **Bold** values indicate significantly higher concentrations at DB relative to GD ($p < 0.05$).

Location	Date	NO_x[a]	NH_4^+[a]	DOC[a]	H_2S[a]	Cl^-[b]	Chl *a*[c]
DB	Jan-02	0.05	**7.37**	**63.66**	4.43	3.55	59.52
	Aug-02	0.01	**11.40**	**41.90**	**119.62**	3.78	45.53
	Jan-04	0.01	**10.34**	42.21	4.14	3.10	55.15
GD	Feb-02	n.d.	3.75	43.33	10.89	3.94	54.81
	Aug-02	0.01	2.21	31.92	4.71	3.84	56.28

[a] Units in μmol cm^{-2}.
[b] In mmol cm^{-2}.
[c] Chlorophyll concentration units are mg chl *a* m^{-2}.