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Nitrate reduction pathways in the presence of excess nitrogen in a shallow eutrophic estuary☆

Rebecca J. Domangue [a, b, *, 1], Behzad Mortazavi [a, b]

[a] The University of Alabama, Department of Biological Sciences, Tuscaloosa, AL 35487, USA
[b] The Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, AL 36528, USA

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ABSTRACT

The eutrophication of estuaries results from increasing anthropogenic nutrient inputs to coastal waters. Ecosystem recovery from eutrophication is partly dependent on the ability of a system to assimilate or remove nutrients, and denitrification and dissimilatory nitrate reduction to ammonium (DNRA) are important pathways for nitrogen (N) removal or retention. We measured rates of denitrification and DNRA over an annual cycle at two stations in Weeks Bay, AL, a shallow microtidal estuary receiving freshwater from two rivers with agricultural watersheds and high N inputs. We hypothesized that rates of DNRA would exceed denitrification in the sulfidogenic sediments in this estuary. Consistent with our hypothesis, we found that DNRA ($44.4 \pm 5.5\ \mu mol\ N\ m^{-2}\ hr^{-1}$) exceeded *in situ* denitrification ($0.9 \pm 2.3\ \mu mol\ N\ m^{-2}\ hr^{-1}$) and that even in the presence of abundant water column nitrate DNRA was favored over denitrification by a factor of two. DNRA is estimated to provide N to the water column at a rate equivalent to 15% of the N input that is retained within the estuary and is a significant component of the N budget in this highly impacted estuary. DNRA by retaining N in the system contributes to the N demand by primary producers and can impact this estuary through enhanced rates of primary production. Weeks Bay, like many coastal estuaries, experiences periods of hypoxia, blooms of harmful algae and fish kills. Future management efforts should focus on reducing nutrient input to this estuary without which the significant retention of N in this system through DRNA will contribute to the undesirable ecosystem attributes associated with eutrophication.

1. Introduction

Nearshore marine ecosystems are especially sensitive to anthropogenic nutrient inputs (Smith et al., 1999) with ecosystem structure and function markedly altered as a consequence (Cloern, 2001; Halpern et al., 2007; Harley et al., 2006). Anthropogenically-driven increases in N loads (primarily as nitrate, NO_3^-) to aquatic systems and associated water quality problems have focused attention on understanding the variables that affect processes within the N cycle, and more specifically the pathways of NO_3^- reduction within estuarine sediments. These processes include canonical denitrification, anaerobic ammonium (NH_4^+) oxidation (anammox), and dissimilatory nitrate reduction to NH_4^+ (DNRA). Denitrification is carried out by bacteria that reduce NO_3^- at low (0.2 mg/L) oxygen (O_2) concentrations and produces nitrous oxide (N_2O) and dinitrogen gas (N_2) (Knowles, 1982; Seitzinger et al., 2006). Anammox oxidizes NH_4^+ with NO_2^- as the electron acceptor to produce N_2, however, it generally accounts for only a minor fraction of the N_2 produced (Dalsgaard et al., 2005). As a result of DNRA, NO_3^- is reduced to NH_4^+ (Gardner et al., 2006; Kaspar et al., 1981). In contrast to denitrification and anammox that lead to the removal of N from the system, DNRA retains N as NH_4^+ (An and Gardner, 2002). In addition to N and phosphorus (P) regenerated through mineralization of sediment organic matter (Twilley et al., 1999) N retained through DNRA contributes to primary production in estuaries.

Understanding the factors that control how NO_3^- is cycled has implications for predicting the impact of excess nutrient inputs to nearshore marine systems (Christensen et al., 2003; Seitzinger et al., 2006). Indeed, anthropogenic N loading in the watershed and the fate of nutrients once they enter the estuary are primary

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* Corresponding author. The University of Alabama, Department of Biological Sciences, Tuscaloosa, AL 35487, USA.
E-mail address: rdomangue@mobilebaykeeper.org (R.J. Domangue).
[1] Present address: Mobile Baykeeper, 450C Government Street, Mobile AL 36602, USA.

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management concerns (Paerl et al., 2014). Denitrification has empirically been shown to vary as a function water column NO_3^- concentration, the water column residence time, (Nixon et al., 1996; Seitzinger et al., 2006), as well the overall rate of sediment organic matter mineralization (Fennel et al., 2009). With higher water residence time and elevated NO_3^- concentrations, primary production is enhanced which leads to higher inputs of organic matter to the sediment and leads to higher denitrification rates (Middelburg et al., 1996). However, the same factors, namely NO_3^- availability and organic matter content of the sediments (Tiedje, 1988), have also been shown to influence DNRA (Christensen et al., 2000; Dong et al., 2011). The ratio of NO_3^- to organic matter content is a primary factor that determines if NO_3^- is lost through denitrification or retained in the system through DNRA (Burgin and Hamilton, 2007). Other variables such as the presence of reduced sulfur in the sediments also influence denitrification and DNRA. The presence of sulfides in sediments lead to reduced denitrification (Tobias et al., 2001) and coupled nitrification-denitrification (Christensen et al., 2003), though autotrophic denitrification coupled to reduced sulfur compounds is noted (Batchelor and Lawrence, 1978). But DNRA can proceed chemolithoautotrophically through oxidation of reduced sulfur species (Brunet and Garcia-Gil, 1996; Dalsgaard and Bak, 1994), and in the presence of sulfides a larger fraction of the available NO_3^- can be retained in the system as opposed to lost from the systems through denitrification (Christensen et al., 2000, 2003). These complexities make it challenging to predict how excess NO_3^- delivered to the coast will be processed.

We determined rates of denitrification and DNRA in Weeks Bay, AL, USA, a shallow (1.4 m depth) microtidal (0.4 m) estuary in the northern Gulf of Mexico that is part of the National Estuarine Research Reserve System. Weeks Bay is fringed with a variety of wetland habitats receiving freshwater from the Fish and Magnolia Rivers that both have highly agricultural watersheds with dissolved inorganic nitrogen (DIN) concentrations in the rivers exceeding at times 140 μM (Lehrter, 2008). Caffrey et al. (2013) reported total N inputs into Weeks Bay of 10 mol N m^{-2} yr^{-1}, which is one of the highest rates of N loading to an estuary in the northern Gulf of Mexico estuaries. Previous studies in Weeks Bay found high porewater sulfide concentrations (Caffrey et al., 2007), significant sediment uptake of NO_3^- and high NH_4^+ fluxes and concurrent low net denitrification rates (Mortazavi et al., 2012; Riggs, 2010). Therefore, we hypothesized that DNRA is the significant reduction pathway for NO_3^- in Weeks Bay and because of the sulfidogenic sediments, DNRA would also be a significant NO_3^- reduction pathway in the presence of excess NO_3^-. Periods of anoxia are common occurrences in Weeks Bay (http://cdmo.baruch.sc.edu/), as are blooms of harmful algae (Canion et al., 2013) and fish kills and understanding the fate of nutrients in this system has management implications.

2. Methods

2.1. Field collections

Intact sediment cores and water column samples for experiments were collected quarterly from bare sediments by hand near the mouth and in the mid bay area of the Weeks Bay National Estuarine Research Reserve (hereafter referred to as MidBay and Mouth stations) between December 2011 and October 2013 (Fig. 1). At both sites, we measured temperature, salinity, pH, and dissolved oxygen (DO) with a YSI Model 556 Multiparameter Meter. Water column samples for nutrient analysis were collected by hand, filtered in the field (GF/F, 0.7 μm) and frozen until DIN and phosphate (PO_4^{3-}) analyses. All nutrient concentrations from the field and experimental samples described below were measured with a Skalar SAN$^+$ Autoanalyzer. Total nitrogen and carbon content were measured in triplicate from the top 1 cm of sediment. Samples were acidified to remove carbonates (Harris et al., 2001) and total C and N were analyzed with an elemental combustion analyzer (Costech Instruments, model ECS 4010). Based on the ASTM C136-06 standard, grain size distribution was determined by sieve analysis using sieves #10, #60, and #230 from a haphazard sediment grab of approximately 2 kg at each site (ASTM C136-06, 2006).

2.2. Denitrification and DNRA from intact sediment cores with N enrichment

In a darkened environmental chamber set to site temperature, denitrification and DNRA at the sediment-water interface were measured on sediment cores with N enrichment (9.5 cm inner diameter; 19 cm sediment with 5 cm overlying water; 3 per station in 2011 and 2012; 6 per station in 2013) set up in a flow-through system. Site water was filtered (0.7 μm) and amended to ~100 μM $Na^{15}NO_3^-$ (99 atom %) representing similar N concentrations reported by Lehrter (2008), and used as the inflow water at a continuous flow rate (1.2 mL min^{-1}) into each core. The outflow from each core was collected in a reservoir. Inflow and outflow samples for dissolved gas and nutrient analysis were collected at 36 h to allow the systems to approach steady-state conditions (Eyre et al., 2002). Benthic flux calculations were calculated according to: $(C_o - C_i) * f/a$, where C_o and, C_i are the outflow and inflow concentration in μmole L^{-1}, f is the flow rate (0.072 L h^{-1}), and a is the sediment surface area (0.00708 m^{-2}) (Lavrentyev et al., 2000).

Samples for dissolved gas analysis were collected in 12 mL Exetainers and preserved with 250 μL of 50% (w/v) $ZnCl_2$ before analysis on a membrane inlet mass spectrometer (MIMS) (Kana et al., 1998) fitted with a copper column heated to 600 °C to remove O_2 (Eyre et al., 2002). Following the Isotope Pairing Technique (IPT) (Nielsen, 1992), denitrification rates were calculated under ambient environmental conditions (D_{14}) (which can be further portioned as ambient $^{14}NO_3^-$ from the water column (D_w) and coupled nitrification-denitrification (D_n)) and amended denitrification rates ($D_{14} + D_{15}$), calculated as the sum of denitrification rates of ambient NO_3^- (D_{14}) and denitrification stimulated by the added labeled $^{15}NO_3^-$ (D_{15}), and hereafter will be referred to as the denitrification capacity. Denitrification was explicitly calculated from the $^{29}N_2$ and $^{30}N_2$ fluxes calculated directly from dissolved $^{29}N_2$:$^{28}N_2$ and $^{30}N_2$:$^{28}N_2$ measured with a MIMS. Sediment-water interface gas flux (μmol m^{-2} hr^{-1}) greater than zero indicates a release from the sediments to the water column. All rates and fluxes pertaining to N species are expressed on N atom basis.

After sample collection for denitrification, approximately 1 L of inflow reservoir water and outflow water from each core were collected for DNRA analysis. Samples and standards for $^{15}NH_4^+$ were prepared according to Holmes et al. (1998) and as described in Bernard et al. (2015). ^{15}N analysis was performed at Utah State University's Stable Isotope Lab. DNRA was determined from the production rate of $^{15}NH_4^+$ ($p^{15}NH_4^+$) according to Christensen et al. (2000), assuming that (i) DNRA takes place in the same sediment layers as denitrification and (ii) that the $^{15}NO_3^-$ that was reduced to NH_4^+ is similar to that of the $^{15}NO_3^-$ that was reduced to N_2 (Christensen et al., 2000).

2.3. Anammox from slurry assays

Following intact sediment core collection, sediments (n = 3) at each site were collected by hand with a sediment core (9.5 cm ID) and the top 5 cm were combined and homogenized. At each sampling event, anammox rates were determined with ^{15}N (99 atom %,