Influence of organic carbon and nitrate loading on partitioning between dissimilatory nitrate
reduction to ammonium (DNRA) and N₂ production

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Abstract

Biologically available nitrogen is removed from ecosystems through the microbial processes of anaerobic ammonium oxidation (anammox) or denitrification, while dissimilatory nitrate reduction to ammonium (DNRA) retains it. A mechanistic understanding of controls on partitioning among these pathways is currently lacking. The objective of this study was to conduct a manipulative experiment to determine the influence of organic carbon and nitrate loading on partitioning. Sediment was collected from a location on the southern New England shelf (78 m water depth) and sieved. Half of the sediment was mixed with freeze-dried phytoplankton and the other half was not. Sediment was then spread into 1.5 mm, “thin discs” closed at the bottom and placed in large aquarium tanks with filtered, N_2/CO_2 sparged seawater to maintain oxygen limited conditions. Half of the discs received high nitrate loading, while the other half received low nitrate loading, resulting in a multifactorial design with four treatments: no C addition, low nitrate (-C-N); C addition, low nitrate (+C-N); no C addition, high nitrate (-C+N); and C addition, high nitrate (+C+N). Sediment discs were incubated in the tanks for 7 weeks, during which time inorganic N (ammonium, nitrate, and nitrite) was monitored, and sediment discs were periodically removed from the tanks to conduct ^{15}N isotope labeling experiments in vials to measure potential rates of anammox, denitrification, and DNRA. Temporal dynamics of inorganic N concentrations in the tanks were indicative of anoxic N metabolism, with strong response of the build up or consumption of the intermediate nitrite, depending on treatments. Vial incubation experiments with added $^{15}NO_2^- + ^{14}NH_4^+$ indicated significant denitrification and DNRA activity in sediment thin discs, but incubations with added

$^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ indicated anammox was not at all significant. Inorganic N concentrations in the tanks were fit to a reactive transport model assuming different N transformations. Organic C decomposition rates were inferred based on modeled rates as well as stoichiometric conversions of ammonium production in pre-incubated vials. Based on model results, partitioning between DNRA and N_2 production was positively linearly related to the ratio of C decomposition to NO_3^- reduction rates (C/NO_3^-) but not C decomposition alone. Based on vial results, partitioning was significantly related to C decomposition. Overall, this study supports the hypothesis that high organic C loading is a prerequisite for DNRA to be favored over denitrification but that N_2 production may still be significant when organic C is high depending on nitrate availability.

1. INTRODUCTION

Nitrogen (N) is a key limiting nutrient to primary production in marine ecosystems. Thus, processes that control N availability have potentially broad ecological impacts. Over the last century, human activities have more than doubled inputs of biologically available N in terrestrial ecosystems (Galloway et al., 2004). Much of this N finds its way into coastal estuaries and eventually the continental shelf, serving as significant net N sinks (Nixon et al., 1996). The reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and then N_2 via the anoxic processes of denitrification and anaerobic ammonium oxidation (anammox) is the primary means of N loss from ecosystems (Thamdrup, 2012). Alternatively, NO_3^- can be reduced to ammonium (NH_4^+) via dissimilatory NO_3^- reduction to NH_4^+ (DNRA), thereby retaining bioavailable N in coastal ecosystems (Tiedje, 1988). Controls of N loss and retention have important implications for understanding nutrient balance of ecosystems.

Denitrification occurs through the intermediates, NO_2^- , nitric oxide (NO), and nitrous oxide (N_2O), and the process is scattered throughout the phylogenetic tree of *Bacteria* and some *Archaea* and *Eukarya* (Zumft, 1997; Risgaard-Petersen et al., 2006). Anammox is the reduction of NO_2^- with NH_4^+ to form N_2 , and it is confined to a monophyletic group of *Planctomycetes* (Kartal et al., 2012). DNRA, like denitrification, is scattered throughout *Bacteria* and *Archaea*, and some *Eukarya* (Kamp et al., 2011; Welsh et al., 2014). Usually, only one of the pathways exists in any given type of microbe, but there are rare examples of two pathways present in one organism, either DNRA and denitrification or DNRA and anammox, but never all three (Kartal et al., 2006; Sanford et al., 2012). Although all three processes occur under anoxic conditions in

the presence of NO_3^- or NO_2^- , metabolic differences between denitrifying, anammox, and DNRA bacteria may lead to differences in their distribution. Anammox bacteria are anaerobic and autotrophic, depending on the anammox pathway for growth. In contrast, denitrification and DNRA are facultative, so these processes do not need to be active for growth. Most denitrifiers and some DNRA bacteria are aerobic, switching to NO_3^- and NO_2^- reduction under anoxic conditions, while many other DNRA bacteria are anaerobes that ferment organic C or reduce iron or sulfate (Tiedje, 1988; Welsh et al., 2014). Additionally, most denitrifying and DNRA bacteria can grow heterotrophically while some grow autotrophically.

Most studies that have examined environmental controls on these pathways have been observational, relying on correlations between rates and environmental factors (e.g., temperature, salinity, concentrations of NO_3^- , organic matter, hydrogen sulfide). Few studies have examined all three pathways simultaneously (Gardner and McCarthy, 2009; Jäntti et al., 2011; Song et al., 2013). Cumulatively, past studies indicate that organic C and NO_3^- loading appear to be key factors, albeit not the only factors, determining one pathway over another (Tiedje, 1988; Dalsgaard et al., 2005; Giblin et al., 2013). Along a productivity gradient, all else being equal, anammox might be expected to be favored at lowest C loading, denitrification at intermediate loads, and DNRA at the highest loads (Thamdrup and Dalsgaard, 2002; Gardner and McCarthy, 2009). However, some studies have found increased NO_3^- availability favors anammox or DNRA relative to denitrification (Rich et al., 2008; Dong et al., 2011; Kraft et al., 2014). In the environment, where NO_3^- availability co-varies along productivity gradients, due to differences in sediment nitrification rates or water column NO_3^- concentrations, the interactive effects between C and NO_3^- may significantly modify the prevalence among these three pathways, and thus the role of NO_3^- requires further investigation. Furthermore, processes that control

incomplete reduction of NO_3^- and build up of NO_2^- as an intermediate may be important for understanding partitioning (Trimmer et al., 2003).

In this study, we undertook an experimental approach to address uncertainty in controls of partitioning. We used the sediment, “thin disc” approach developed by others (Aller and Mackin, 1989; Sun et al., 1993; Kristensen and Holmer, 2001) to examine the interactive effects of organic C and NO_3^- availability on anammox, denitrification, and DNRA. The approach allows rapid equilibration between the sediment porewater and overlying water, providing advantages for manipulating porewater and modeling processes. We incubated sediment thin discs in large volume aquaria for 7 weeks and modeled sediment reaction rates based on inorganic N concentration changes in the overlying water. Sediment discs were sacrificed periodically during the experiment to measure rates of anammox, denitrification, and DNRA in vial incubations with added ^{15}N in order to examine effects of experimental treatments. Strong shifts in processes were observed during the course of the experiment, providing insights into how organic C and NO_3^- influence partitioning between DNRA and N_2 production.

2. MATERIALS AND METHODS

2.1. Experimental set up

Sediments consisting of very fine mud were collected from the, “Mud Patch” (Twichell et al., 1981) site (40.435N 70.483W, water depth 78 m) off the coast of Rhode Island in August 2011 using a box corer. Sediment in the box core was subsampled with individual polyvinyl chloride (PVC) tubes (7 cm diameter) and extruded into a cooler, with the vertical layers kept

intact. The sediment was covered with site water and brought back to the lab, where the top 3 cm section was passed through 2 mm and 1 mm sieves and divided in half. One half received 20 mg freeze-dried *Chlorella* algae (48% C, 10% N) per gram of wet sediment, making the +C treatment (see section 3.2 for resulting concentrations). Sediments for the -C treatment did not receive any organic C amendment. The sediment, with or without *Chlorella*, was mixed well, and 12 g of wet sediment was spread carefully into thin discs. These consisted of PVC molds, constructed of a thin ring mounted on a base to hold 1.5 mm thin sediment plugs (10 cm diameter, 11.8 mL volume), closed at the bottom and open at the top. Discs were placed carefully into aquarium tanks (122 cm length x 46 cm width x 33 cm height) filled with 155.7 L of 0.2 μm filtered Narragansett Bay seawater (salinity 30), bubbled vigorously and continuously with N_2 to make O_2 limited conditions and CO_2 to maintain pH at ~ 8 . The experiment and all rate measurements were conducted at room temperature ($\sim 21^\circ\text{C}$), in the dark. Tanks were covered with lids to maintain positive pressure inside them, and periodic monitoring of dissolved O_2 with a microelectrode (Unisense OX100) indicated fluctuations between 3 and 11 μM throughout the experiment, with no differences among tanks.

Aquarium tanks received two different NO_3^- loading rates delivered by a peristaltic pump from reservoirs containing 31 or 266 μM NO_3^- in filtered seawater (Table 1). This corresponded to a loading rate of 0.6 and 5 $\mu\text{mol NO}_3^- \text{ mL sediment}^{-1} \text{ day}^{-1}$, respectively. This resulted in four tanks, each corresponding to a treatment (-C-N, +C-N, -C+N, and +C+N), with 30 discs in each tank at the start of the experiment. Equal NH_4^+ loading across treatments was maintained by adding 20 μM NH_4^+ in reservoirs, corresponding to a loading rate of 0.4 $\mu\text{mol NH}_4^+ \text{ mL sediment}^{-1} \text{ day}^{-1}$. NO_3^- and NH_4^+ loading rates were kept constant throughout the experiment by decreasing flow rates proportionally as discs were sacrificed for vial incubation experiments.

Water was removed manually from the aquaria regularly to maintain water volume within 10% of the initial volume. The experiment was conducted for 47 days, during which time aquarium water samples (20 mL) were collected every other day and passed through a 0.45 μm filter into Whirlpak bags that were frozen (-20°C) until later analysis of NO_3^- , NO_2^- , and NH_4^+ concentrations.

2.2. ^{15}N incubations in vials to measure potential rates

Six sediment thin discs were removed from each tank and sacrificed at each of 5 time points during the experiment: Days 0, 4, 13, 31, and 47. Two discs from each tank were placed into separate glass jars and frozen at -80°C for elemental analysis. The remaining 4 discs were combined in pairs and homogenized, yielding two replicate samples of each treatment to conduct potential rate measurements, following previously published methods (Thamdrup and Dalsgaard, 2002; Risgaard-Petersen et al., 2004). The sediment was dispensed (1 mL) into glass vials (Exetainer, 5.9 mL, 3 mm butyl septum) containing an acid-cleaned glass bead, purged with He, and pre-incubated for 20 h to remove any residual $\text{NO}_3^-/\text{NO}_2^-$ from the experiment or O_2 during transfer to vials. After this pre-incubation, NO_2^- and NH_4^+ were added to the anaerobic vials in the following treatments: (1) $^{15}\text{NH}_4^+$, (2) $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$, (3) $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$ and (4) no addition. Stock solutions were made with $^{15}\text{NH}_4\text{Cl}$ (99.4 atom%), $\text{Na}^{15}\text{NO}_2$ (99.4 atom%), or the unlabeled (^{14}N) equivalent, and final concentrations in vials were $125\text{ nmol N mL}^{-1}$ sediment for each N compound. Half of the vials receiving ^{15}N were sacrificed immediately with addition of $100\text{ }\mu\text{L } 7\text{ M ZnCl}_2$, and the rest were incubated for 20 min and then sacrificed to measure production of $^{29}\text{N}_2$, $^{30}\text{N}_2$ and $^{15}\text{NH}_4^+$ during this time interval. The fraction of ^{15}N in the NO_2^- or

NH_4^+ pool was measured based on difference in concentration in the porewater before and after isotope addition. Vials used for this purpose were centrifuged and the porewater collected and frozen (-20°C) until analysis of NO_3^- , NO_2^- , and NH_4^+ . The fraction of ^{15}N -labeled NO_2^- (F_n) was always >0.99 , while for NH_4^+ (F_a), it was on average 0.24 in +C treatments and 0.61 in -C treatments.

The amount of ^{15}N - N_2 produced in ^{15}N incubations was measured in vial headspaces using a continuous flow isotope ratio mass spectrometer (Isoprime) in-line with an automated gas preparation unit (GV-Instruments). Production rates of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ were estimated by calculating the production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in excess of natural abundance during the vial incubation (Thamdrup and Dalsgaard, 2000). Lack of detectable $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production in vials with $^{15}\text{NH}_4^+$ alone ruled out any contamination of O_2 that might have stimulated coupled nitrification-denitrification. Anammox rates in vials receiving $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ were estimated by dividing the rate of $^{29}\text{N}_2$ production by F_a . In vials receiving $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$, F_n was >0.99 throughout our experiments, so $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production ($p^{29}\text{N}_2$ and $p^{30}\text{N}_2$, respectively) were nearly equivalent to anammox and denitrification rates that would be calculated using the equations of Thamdrup and Dalsgaard (2002). However, we do not report converted rates of $p^{30}\text{N}_2$ and $p^{29}\text{N}_2$ because results from our $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ treatment suggested anammox rates were not significant in most of the vial incubations (discussed further in section 3.4).

Measurements of DNRA were made for days 4, 13, and 47 on incubation vials receiving $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$ by extracting sediments with 3 volumes of 1M NaCl (25 mL). The sample extract and 10 μmoles of $^{14}\text{NH}_4^+$ as a carrier were sealed in a jar with MgO and the resulting NH_3 was trapped on acidified filters, following previously published methods (Holmes et al., 1998). Filters were then combusted for the analysis of ^{15}N using a continuous flow isotope ratio mass

spectrometer (Europa) coupled with an elemental analyzer (Europa). DNRA rates were estimated using the rate of $^{15}\text{NH}_4^+$ produced ($p^{15}\text{NH}_4^+$) in excess of natural abundance during the vial incubation.

2.3. N mineralization and organic C decomposition rates in pre-incubated vials

At the end of the 20 h pre-incubation, NH_4^+ concentration (C_t) was measured in the porewater of vials without any added N, as described above. This NH_4^+ would have been produced primarily by decomposition of organic matter, and not DNRA, as any residual NO_3^- or NO_2^- that may have been present initially in +C treatments would have been rapidly consumed and was very low compared to NH_4^+ concentrations in the porewater. Initial porewater concentrations in the pre-incubated vials were not measured directly but assumed to be equivalent to NH_4^+ concentration (C_0) measured in seawater in the tanks when discs were removed. Production rates of dissolved and exchangeable NH_4^+ (P) in pre-incubated vials were calculated using the equation, $P = (C_t - C_0) \times (1/t) \times \phi \times (K + 1)$, where t = pre-incubation time, ϕ = sediment porosity of 0.45, and K = NH_4^+ adsorption coefficient (Thamdrup and Canfield 1996). Porosity was measured by loss of water mass after drying a known volume of sediment (1 mL) in an oven overnight (65°C). We experimentally measured K in the sediments, with and without added *Chlorella*, as greater organic C content might be expected to increase K (Rosenfeld, 1979). Our empirically derived values of K were equivalent (1.4) for both cases, and thus, we used the same K value for all calculations of net NH_4^+ production. Organic C decomposition rates were determined by multiplying NH_4^+ production rates by a range of possible molar C/N ratios (r_{CN}) of organic matter being degraded in the experiment of 3, 5.88 and

10. The low value is slightly lower than the lowest values typically reported in the literature (Burdige, 1991; Kristensen and Hansen, 1995; Thamdrup and Canfield, 1996) while 5.88 was the r_{CN} of added *Chlorella*, and 10 was the upper bound of r_{CN} of unamended sediments.

2.4 Measurements of dissolved inorganic N and total sediment organic C and N

$\text{NO}_2^- + \text{NO}_3^-$ concentrations in seawater from the aquarium tanks were measured manually on a Shimadzu UV-1800 spectrophotometer. NO_2^- was measured according to standard colorimetric methods (Parsons et al., 1984), and NO_3^- was measured after reduction to NO_2^- using spongy Cd (Jones, 1984). NH_4^+ concentrations were measured manually, following the standard colorimetric method using phenol and hypochlorite (Koroleff, 1983). Small volume porewater NO_2^- and NH_4^+ were measured colorimetrically, while $\text{NO}_3^- + \text{NO}_2^-$ was measured by vanadium reduction to NO on an NO analyzer (Garside, 1982). Sediments for total organic C and total N were freeze-dried, ground, homogenized, acidified to remove inorganic C, and analyzed on a NC2100 Elemental Analyzer.

2.5. Modeled rates

To further investigate N cycling processes in thin discs, we used a 0-D reactive-transport model to simulate NO_3^- , NO_2^- and NH_4^+ concentrations in the aquarium water. In the model, each experimental treatment was represented as a flow-through reactor containing a bulk water phase overlying a thin sediment layer. Only solute concentrations in the water column were modeled explicitly, as timescales of diffusion were much faster than the residence time of water in the

tanks. The timescale of diffusion is estimated according to l^2/D , where D is the diffusion coefficient and l is a characteristic length scale of the system. For this experimental system, we can estimate l to be the thickness of the sediment discs (1.5 mm) and D to be approximately $1 \text{ cm}^2 \text{ d}^{-1}$, which gives us the time scale of diffusion to be on the order of 30 min, while the residence time of water in the tanks is much longer, on the order of days.

Mass balance equations were constructed for dissolved NO_3^- , NO_2^- , and NH_4^+ measured in the tanks. In addition to experimentally manipulated transport into and out of the tanks, inorganic N was assumed to be influenced by a generic remineralization reaction (R_{nh4}), as well as specific respiratory reactions (Table 1). R_{nh4} represents breakdown of organic matter ($orgN$) to NH_4^+ and small carbon molecules ($r_{CN}\text{CH}_2\text{O}$), which can be used in N metabolism or other types of redox metabolism (i.e., aerobic respiration or sulfate reduction) not explicitly represented in the model. Reactions R_1 - R_5 are redox reactions describing N transformations that could potentially be active in the thin discs: NO_3^- reduction to NO_2^- (R_1), denitrification (R_2), DNRA (R_3), anammox (R_4), and, as low concentrations of O_2 occurred in the tanks, aerobic nitrification (R_5). Each metabolic process was assumed to be occurring in the sediment layer only. This assumption was supported a posteriori by agreement between modeled rates and vial incubations.

These reactions (R_{nh4} and R_1 - R_5) and transport into and out of the tanks resulted in the following system of ordinary differential equations,

$$\frac{[\text{NO}_3^-]}{dt} = \frac{Q(t)}{V} \left([\text{NO}_3^-]_{in} - [\text{NO}_3^-] \right) + \frac{A_{sed}(t)h_{sed}}{V} \left(R_5 - R_1 \right) \quad (1)$$

$$\frac{[\text{NO}_2^-]}{dt} = \frac{Q(t)}{V} \left([\text{NO}_2^-]_{in} - [\text{NO}_2^-] \right) + \frac{A_{sed}(t)h_{sed}}{V} \left(R_1 - R_2 - R_3 - R_4 \right) \quad (2)$$

$$\frac{[\text{NH}_4^+]}{dt} = \frac{Q(t)}{V} \left([\text{NH}_4^+]_{in} - [\text{NH}_4^+] \right) + \frac{A_{sed}(t)h_{sed}}{V} \left(R_{nh4} + R_3 - R_5 \right) \quad (3)$$

where V is the volume of water in each treatment tank, $[\text{NO}_3^-]_{in}$, $[\text{NO}_2^-]_{in}$, $[\text{NH}_4^+]_{in}$ are concentrations in the inflow water. $Q(t)$ is the flow rate into the tanks, $A_{sed}(t)$ is the combined surface area of all the sediments discs in a treatment tank and h_{sed} is the height of the sediment disks. $Q(t)$ and $A_{sed}(t)$ are treated as decreasing step functions to account for the removal of sediment discs and lowering of the flow rate throughout the experiment. Parameter values used for the simulations are given in Table 2. The system of differential equations was solved using the package ReacTran for the R software environment (Soetaert and Meysman, 2012; R Core Team, 2013).

The reaction rates were fitted to the measured concentrations by minimizing the sum of squared residuals, as performed using the fitting algorithm in the R package FME (Soetaert and Petzoldt, 2010). However, because the model contains 6 metabolic reactions and only 3 measured parameters, the model is unconstrained and therefore cannot be fit uniquely without some additional simplifying assumptions. First, R_{nh4} for each treatment was assumed to be equal to the time averaged rate of NH_4^+ production measured in pre-incubated vials (see section 2.6). We then made the assumption that DNRA was not active in -C treatments, which was supported with evidence from the vial incubations that showed low $p^{15}\text{NH}_4^+$ in -C treatments. We further assumed that anammox was not active in thin discs of any treatments, based on evidence from vial incubations with added $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ that showed lack of $^{29}\text{N}_2$ production. This leaves 3 unknown metabolic reactions (R_1 , R_2 , and R_5) to be solved in -C treatments. We further assumed that nitrification rates (R_5) in -C treatments provided an upper bound for nitrification rates in +C treatments and parameterized this nitrification rate in model runs of +C treatments. This leaves 3 unknown metabolic reactions (R_1 , R_2 , and R_3) to be solved in +C treatments. Additionally, the amount of C remineralization that is supported by R_1 , R_2 , or R_3 can be calculated for each

simulation according to, $R_{C-NOx} = 1/2R_1 + 3/4R_2 + 3/2R_3$, assuming these processes occur heterotrophically (Table 1). This can be compared to the C decomposition rate estimated from pre-incubated vials. The difference between the two provides an estimate of C remineralization by other modes of metabolism, i.e., sulfate reduction, that are not explicitly represented in the model. Rates for -C treatments were assumed to be constant throughout the duration of each treatment. Conversely, for +C treatments, the measured data were split into an early stage ($t \leq 10$ d) and a later stage ($t > 10$ d) and fits were performed on each stage independently. All model fits produced similarly low sum of squared residuals (data not shown) and highly significant p -values ($p < 0.0001$). The assumption of constant rates means that the fitted rates represent an average state for a time interval, but not necessarily short-term dynamics. The long residence time and large volume of water in the tanks dampens the influence of any short-term changes occurring in the sediments, such that the water N concentrations represent a similar time averaged value of the state of the system.

Net reaction rates of NO_3^- consumption, NO_2^- production and consumption, and NH_4^+ production or consumption, without assuming any metabolic processes, were also fit to inorganic N concentrations, taking into account transport into and out of the tanks. These rates reflect the actual net transformations occurring in the tanks and equal the sum of metabolic rates influencing one inorganic N species or another. For example, the sum of R_2 and R_3 is equal to the rate of NO_2^- consumption, and NO_2^- consumption is constant whether or not it is consumed by varying amounts of R_2 or R_3 .

2.6. Statistical analyses of rates

NH₄⁺ production rates in pre-incubated vials were fit to natural log of time. Based on these fits, a time-averaged NH₄⁺ production rate was calculated that corresponded to each stage of the experiment that was modeled (i.e., the full experiment for -C treatments or early and late stages for +C treatments). Partitioning of DNRA and N₂ production was expressed as %DNRA (i.e., %DNRA=100[DNRA/(DNRA+N₂ production)]), with total N₂ production in the model or $p^{30}N_2 + \frac{1}{2} p^{29}N_2$ in the vials accounting for the remainder. %DNRA measured in the vials was fit to C decomposition rates estimated in pre-incubated vials. %DNRA determined by the model was fit to the pre-incubated vial C decomposition rates and ratios of these C decomposition rates to the NO₃⁻ reduction rates (*R*₁) in the tanks (C/NO₃⁻). All relationships were assessed using JMP software and considered to be statistically significant at $p < 0.05$.

3. RESULTS

3.1. Inorganic N concentrations in tanks

Sediment thin discs had a strong influence on inorganic N concentrations in the overlying water in aquarium tanks (Fig. 1). The difference between expected concentrations based on inflows and outflows and actual concentrations in the tanks were used to generate net reaction rates of NO₃⁻ consumption, NO₂⁻ production and consumption, and NH₄⁺ production or consumption (Fig. 1 and Table 3). In -N treatments, NO₃⁻ was consumed at similar rates and remained <5 μM, irrespective of C addition (Fig. 1A, D). NO₂⁻ remained low although there was a small net production in the -C-N treatment (Fig. 1B, E). In +N treatments, NO₃⁻ concentrations increased, indicating NO₃⁻ loading rates exceeded capacity for uptake, even in +C+N treatment

(Fig. 1, G vs J). However, C addition had a strong influence on differences in NO_2^- dynamics in +N treatments. NO_2^- accumulated at a rate approaching NO_3^- consumption in -C+N treatment, while NO_2^- was nearly completely consumed in +C+N treatment (Fig. 1, H vs. K). This indicated that C addition had a disproportionately large stimulation of NO_2^- consumption compared to NO_3^- consumption (Table 3). Addition of C dramatically increased NH_4^+ production during the first 10 days of the experiment and then it leveled off, irrespective of NO_3^- loading rate (Fig. 1C, F, I and L).

3.2. Sediment organic C and N and net NH_4^+ production

Addition of *Chlorella* increased average total organic C from 1.3% to 2.1% (by dry weight), doubled sediment %N (from 0.15% to 0.30%) and lowered sediment C/N from 9.6 to 8.0. These parameters did not change significantly during the course of the experiment (Fig. S1).

Net NH_4^+ production, a proxy for mineralization, was measured during the 20 h pre-incubation of vials. NH_4^+ production was always higher in +C treatments, and it decreased logarithmically in both +C and -C treatments during the course of the experiment (Fig. 2). There was no difference in NH_4^+ production between +N or -N treatments within either C treatment (Fig. 2).

NH_4^+ production in pre-incubated vials was linearly related to NH_4^+ production measured in the tanks (Fig. 3). The slope of the relationship was significantly different than 1, reflecting higher rates of NH_4^+ production in the tanks compared to pre-incubated vials during the early stage of the experiment in +C treatments, and net NH_4^+ consumption in the tanks in -C treatments (Fig. 3, Table 3). NH_4^+ production rates were indistinguishable between tanks and

pre-incubated vials in the late stage of +C treatments, indicating good agreement of rates during this time.

3.3. Modeled rates in tanks

We used a 0-D reactive-transport model to fit net changes in dissolved inorganic N observed in the tanks. Rates of aerobic nitrification (R_5) were similar between -C treatments (+/- N), reflecting similar net NH_4^+ consumption rates (Table 3). In +C treatments, DNRA was completely dominant in the early stage of the +C-N treatment and less dominant in the later stage (Table 3). Denitrification was of primary importance in +C+N treatment, although DNRA also occurred during the early and late stages.

C remineralization based on R_1 , R_2 , or R_3 (R_{C-NOx}) was greatest in +C+N treatments, reflecting high rates of denitrification in this treatment (Table 3). Time-weighted NH_4^+ production rates in pre-incubated vials were used to determine potential C decomposition rates, assuming a range of different C/N ratios of decomposing organic matter, providing an upper and lower bound for C decomposition to compare to R_{C-NOx} (Fig. 4). In the +C-N early treatment, R_{C-NOx} was below the range of C decomposition estimated in pre-incubated vials (Fig. 4). Otherwise, R_{C-NOx} generally overlapped with the range estimated in pre-incubated vials.

3.4. ^{15}N transformations in vial incubations

In vial incubations with added $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$, $^{29}\text{N}_2$ production was detected only on day 0 and 4, and then was below detection for the rest of the experiment, in all treatments (Fig. 5).

376 More $^{29}\text{N}_2$ was detected in -C than +C treatments. After accounting for dilution of added $^{15}\text{NH}_4^+$,
 377 average ($\pm$ SD) anammox rates were $2.0 \pm 1.0 \text{ nmol N mL}^{-1} \text{ h}^{-1}$ in -C treatments and 0.6 ± 0.8
 378 $\text{nmol N mL}^{-1} \text{ h}^{-1}$ in +C treatments on day 0, but the treatment effect was no longer significant.
 379 However, the overall trend of disappearing anammox remained unchanged after the correction.
 380 Treatments had strong effects on potential rates measured in vials with added $^{15}\text{NO}_2^-$
 381 $+^{14}\text{NH}_4^+$. In +C treatments, $p^{15}\text{NH}_4^+$ was substantial, with greater rates in +C-N than +C+N
 382 treatments (Fig.6B, D, Table 3). In contrast, there was little or no $p^{15}\text{NH}_4^+$ in -C treatments (Fig.
 383 6A, C, Table 3). In +C treatments, $p^{30}\text{N}_2$ and $p^{29}\text{N}_2$ decreased between the beginning of the
 384 experiment and day 4 and remained low until day 13, after which time they increased, with
 385 greater increase in +C+N than +C-N treatments. At the beginning of the experiment, $p^{15}\text{NH}_4^+$
 386 exceeded $p^{30}\text{N}_2 + p^{29}\text{N}_2$ in both +C treatments, but by the end of the experiment, this was only true
 387 in the +C-N treatment. In -C treatments, $p^{30}\text{N}_2 + p^{29}\text{N}_2$ decreased over time but were always
 388 higher than $p^{15}\text{NH}_4^+$ (Fig. 6A, C). Across all treatments, $p^{30}\text{N}_2$ and $p^{29}\text{N}_2$ were positively linearly
 389 correlated, and based on the slope of the regression, $p^{29}\text{N}_2$ was on average 28% of $p^{30}\text{N}_2$ (Fig.
 390 S2).
 391 Anammox rates determined with added $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ were underestimated or not
 392 detected compared to $p^{29}\text{N}_2$ in the $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$ treatment. The discrepancy between
 393 incubation treatments was least prominent on day 0, when anammox rates were about 35% of
 394 $p^{29}\text{N}_2$. However, for the remainder of the experiment the discrepancy was more obvious.
 395 Detection sensitivity in $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ incubations was not an issue, so this does not explain
 396 the discrepancy. Most or all of $p^{29}\text{N}_2$ must have been produced by some other undefined process,
 397 but not anammox (see discussion). Therefore, in our analyses below, we focused on total ^{15}N -

labeled N_2 production from added $^{15}NO_2^-$ in the vials, or the sum of $p^{30}N_2$ and $\frac{1}{2}p^{29}N_2$, rather than anammox or denitrification explicitly.

3.5 Comparing model and vial rates of DNRA and N_2 production

Mean rates of N reduction (DNRA + N_2 production) were a similar magnitude between vials and model, except in the +C-N treatment (Fig. 7A, Table 3), where N reduction was much higher in the vials compared to the model. This exception was expected, as NO_2^- concentrations were not limiting rates in the vial incubation, while NO_3^- concentrations were limiting rates in the tank in the +C-N treatment. Mean DNRA, as a percent of N reduction, was higher in vials compared to the model (Fig. 7B, Table 3). However, across all treatments, there was a similar trend in the relationship between DNRA and N_2 production, with increasing relative importance of DNRA in +C treatments (Fig. 7B, Table 3).

3.6. Influence of C and NO_3^- loading on partitioning between N_2 production and DNRA

Regardless of which C/N value was used to estimate C decomposition rates in pre-incubated vials, %DNRA showed a non-linear relationship with C decomposition, with 50% DNRA at C decomposition rates $> \sim 50 \text{ nmol C mL}^{-1} \text{ h}^{-1}$ and 100% DNRA at C decomposition rates $> \sim 200 \text{ nmol C mL}^{-1} \text{ h}^{-1}$ (Fig. 8). In contrast, no significant relationship was found between %DNRA based on the model and C decomposition rates regardless of C/N of organic matter being decomposed (data not shown). However, based on the model, a significant linear

relationship was found between %DNRA and the ratio of rates of C decomposition to NO_3^- reduction (Fig. 9).

4. DISCUSSION

4.1. Influence of experimental conditions on rate estimates

Our study was a novel application of the thin disc approach to examine the influence of organic C and NO_3^- loading on partitioning between NO_3^- reduction pathways. This study is relevant to understanding NO_3^- reduction in coastal sediments, and more generally to other environments, such as anoxic N cycling in the water column. The approach we employed has been used in the past to infer reaction rates in sediments, or to investigate processes in the sediments themselves under well-defined experimental conditions (Sun et al., 1993; Dahllöf and Karle, 2005). It relies on the premise that equilibration time between sediment porewater and overlying water is rapid due to the thinness of the sediment layer (Aller and Mackin, 1989). We assumed in our model that changes in dissolved inorganic N in the tank water were influenced by microbes in sediment thin discs themselves, and not planktonic or tank surface attached microbes that might have proliferated during the experiment. If microbes not living in the sediments were contributing to rates, then our rates would be overestimates (Valdemarsen and Kristensen, 2005). We took precautions to limit the influence of tank water on measured rates by using filtered seawater in the experiment. Additionally, microbes outside of the thin discs did not appear to influence the results based on relatively good agreement between rates in the tanks and vials.

Conditions in the tanks were likely more relevant to in situ conditions than the vial incubations from the standpoint of simulating a NO_3^- reducing layer in a sediment profile. In the tanks, there was a continuous supply of low or high NO_3^- to the zone of NO_3^- reduction in the thin discs to simulate different supply rates of NO_3^- that might occur from varying sediment nitrification rates or water column NO_3^- concentrations. This allowed us to simultaneously test the influence of electron donor and acceptor availability on N dynamics. Conversely, in vial incubations, there was an anaerobic pre-incubation step, where the supply of NO_3^- was cut off for ~20 h, and then $^{15}\text{NO}_2^-$ introduced at non-limiting concentrations across treatments. This enabled us to test the influence of organic C availability on partitioning of NO_2^- reduction pathways, but not variation in a continuous supply of NO_3^- or NO_2^- on instantaneous activity.

4.2. Interpretation of N transformations occurring in tanks

Dynamics of NO_3^- and NO_2^- in the tanks were indicative of a system where anoxic dissimilatory N-oxide metabolisms were the dominant processes influencing NO_3^- and NO_2^- concentrations. Although NO_3^- reduction may be slightly less sensitive to O_2 contamination than NO_2^- reduction, NO_2^- can accumulate in anoxic environments, indicating that mechanisms other than O_2 sensitivity can explain NO_2^- accumulation (Tiedje, 1988; Thamdrup et al., 2012). We hypothesize that NO_2^- accumulated in the -C+N treatment of our experiment due to electron donor limitation, not differential sensitivity to O_2 of reduction steps. The pattern of NO_2^- accumulation we observed was similar to those commonly observed in studies of denitrifying or DNRA bacteria growing under electron donor limitation in pure cultures or wastewater reactors (Seitz and Heribert, 1986; Blaszczyk, 1993; Oh and Silverstein, 1999). It is also similar to

patterns observed in pelagic O₂ minimum zones, such as the Eastern Tropical South Pacific (ETSP) or Arabian Sea, where high NO₂⁻ in the water column overlaps with high NO₃⁻ deficits, but NO₃⁻ is never completely consumed, presumably because of C limitation (Lam and Kuypers, 2011).

Net NH₄⁺ consumption occurred in both -C treatments. Based on vial incubations and low levels of O₂ in the tanks, we interpreted this NH₄⁺ consumption as occurring due to aerobic nitrification and not anammox. Nitrifiers are generally microaerophilic and grow and oxidize NH₄⁺ under low O₂ tension, with measured half saturation constants (K_m) in the range of O₂ levels in the tanks (Laanbroek and Gerards, 1993; Kalvelage et al., 2011). However, O₂ concentrations were low enough and metabolic activity high enough that anoxic zones occurred in sediment thin discs, even in -C treatments (Fig. S3). Although anammox is inhibited by very low O₂, it is restored upon reintroduction of anoxic conditions (Strous et al., 1997; Babbin et al., 2014). As anoxic conditions would have persisted in the sediments, the presence of low levels of O₂ in the tank water does not explain the complete disappearance of anammox rates over time.

There was no apparent NH₄⁺ consumption in +C treatments. However, aerobic nitrification likely still occurred in these treatments, as assumed in our model. As NH₄⁺ mass balance is required, any consumption of NH₄⁺ through nitrification in +C treatments requires an equal rate of DNRA, thereby creating an internal N cycling loop between nitrification and DNRA. As a consequence, increasing nitrification in the model would increase NO₃⁻ reduction (R_1), DNRA (R_3) and %DNRA in +C treatments. However, our parameterization of nitrification in +C treatments is likely an upper bound, as O₂ consumption across treatments would have had to be similar and nitrifiers likely had greater competition for O₂ in +C compared to -C treatments.

Ammonification in +C treatments reflected two possible sources: DNRA (R_3) and breakdown of organic matter (R_{nh4}) through hydrolysis of polymers and subsequent deamination of monomers (i.e., amino acids or nucleic acids) during fermentation or respiration. R_{nh4} in the model was parameterized by rates measured in the pre-incubated vials. By definition in the model, ammonification rates greater than R_{nh4} were considered to be from DNRA (R_3). We assumed that R_{nh4} was equivalent in the vials and the tanks, which is supported by the general observation that choice of terminal electron acceptor does not influence overall rates of organic C decomposition in relatively labile material (Kristensen and Holmer, 2001; Sutton-Grier et al., 2011; Laverman et al., 2012).

4.3. Organic matter decomposition

To compare our experimental organic C addition to loading rates encountered in marine systems, we compared our anaerobic NH_4^+ production rates (R_{nh4}) to those measured in other studies under similar conditions. R_{nh4} in the early stage of +C treatments overlaps with those measured in the surface layers of near shore continental shelf sediments off Chile (40-100 nmol $\text{mL}^{-1} \text{h}^{-1}$), where exceptionally high decomposition rates were noted due to productive surface waters (Thamdrup and Canfield, 1996). A different study found that addition of a single pulse of fish food to estuarine sediments increased NH_4^+ production rates, integrated over a 30 day anaerobic incubation, up to $\sim 50 \text{ nmol N mL}^{-1} \text{h}^{-1}$ above unamended controls, which is in between R_{nh4} in early and late stages of +C treatments in our 47 day experiment (Holmer and Kristensen, 1994). In contrast, NH_4^+ production rates measured in the top layer of unamended Scandinavian shelf sediments (2-4 nmol $\text{NH}_4^+ \text{ mL}^{-1} \text{h}^{-1}$) overlap with our measured rates in -C treatments

(Blackburn and Henriksen, 1983; Canfield et al., 1993). Based on this comparison, our +C treatments likely represent the high end of the spectrum of C deposition in marine systems, while our -C treatments are more representative of typical continental shelf sediments.

We converted NH_4^+ production rates in pre-incubated vials to C decomposition rates, assuming a range of C/N ratios of decomposing organic matter. The C/N of decomposing organic matter is generally lower than the C/N of bulk sediment or of added amendments of organic matter, reflecting preferential degradation of proteins over carbohydrates (Burdige, 1991; Kristensen and Hansen, 1995; Thamdrup and Canfield, 1996). The C/N ratio of decomposing labile organic matter will therefore increase over time, likely on the time-scale of our experiment. In the early stage of +C treatments, the C/N ratio of decomposing substrate was likely less than the C/N of added substrate (5.88) but higher than 3, based on previously published studies. In contrast, in -C treatments, the C/N ratio of decomposing material was likely higher than in +C treatments, but not higher than 10, which was the upper bound of bulk C/N of the unamended sediments.

Despite relatively large bounds, estimates of C decomposition in pre-incubated vials enabled us to compare to C remineralization rates generated by model fits (R_{C-NOx}). Greater C decomposition in the vials compared to R_{C-NOx} would indicate that other processes not explicitly in our model (e.g., iron or sulfate reduction) were contributing to C remineralization in the tanks. This was the case in +C-N treatment, suggesting that other processes, such as sulfate reduction, were important contributors to C remineralization in this treatment (Fig. 4). In contrast, in all other treatments, processes linked to NO_3^- or NO_2^- reduction were likely the primary pathways involved in remineralization.

4.4. Interpretation of ^{15}N vial incubation rates

There was a large discrepancy in $^{29}\text{N}_2$ production rates depending on whether $^{15}\text{NO}_2^-$ + $^{14}\text{NH}_4^+$ or $^{15}\text{NH}_4^+$ + $^{14}\text{NO}_2^-$ was added. Discrepancies between ^{15}N addition experiments have been reported in the literature (Engström et al., 2005; Song et al., 2013). In these cases, high background $^{14}\text{NH}_4^+$ has been suggested to explain inconsistencies. However in our study, this was not an issue, as we corrected for this background. Thus, we believe the discrepancy lies in an unaccounted for mechanism of $p^{29}\text{N}_2$ in the $^{15}\text{NO}_2^-$ + $^{14}\text{NH}_4^+$ incubation. In a recent study in the Arabian Sea, a novel mechanism of $^{29}\text{N}_2$ production was proposed, whereby unlabeled N (^{14}N) from organic N was directly incorporated into N_2 by combining with $^{15}\text{NO}_2^-$ (Trimmer and Purdy, 2012). Another possible explanation is the presence of intracellular NO_3^- in our experimental sediments, providing an unaccounted for source of $^{14}\text{NO}_3^-$ (Song et al., 2013). Foraminifera, diatoms, and filamentous sulfur oxidizing bacteria are all known to accumulate high concentrations of intracellular NO_3^- and reduce it to either N_2 or NH_4^+ (Risgaard-Petersen et al., 2006; Høglund et al., 2009; Kamp et al., 2011). Filamentous sulfur bacteria are easily damaged, leaking intracellular NO_3^- , while foraminifera require harsh treatment (decalcification and crushing) to extract it (Otte et al., 1999; Risgaard-Petersen et al., 2006). Any traces of NO_3^- or NO_2^- that we detected in porewater after centrifuging pre-incubated vials were always low ($<1\ \mu\text{M}$), not high enough to be of any consequence to measured $p^{29}\text{N}_2$. This suggested that if intracellular NO_3^- (or NO_2^-) was a factor, the organisms in question hung on to it tightly. Thus, we concluded that $p^{29}\text{N}_2$ was not due to anammox but some other mechanism that used an unaccounted for source of unlabeled N (^{14}N).

The vial incubations provided evidence for potential DNRA and denitrification activity, supporting the presence of these pathways in the tanks. However, incubation conditions in the vials differed from those in the tanks. This could account for some of the differences we observed between the model and vial incubations. For example, in the +C-N treatment, there was an initial high level of NO_2^- added during vial incubations, unlike in the tank. This led to much higher absolute rates in the vials compared to the tanks, as NO_2^- limitation was released in the vials in the +C-N treatment (Fig. 7A). In contrast, in the +C+N treatment, absolute rates of N reduction (DNRA + N_2 production) were similar between the model and vial incubations, suggesting similar lack of NO_3^- or NO_2^- limitation in the tank and vial incubations. However, during the early stage in this treatment, there was an important difference in partitioning, with much more DNRA in vial incubations compared to the model (Fig. 7B). This may have been due to a period of time during the pre-incubation in vials when NO_3^- or NO_2^- was absent, unlike in the +C+N tank. DNRA may have been favored over denitrification in the vials compared to the tank for several reasons, including anaerobic DNRA bacteria outcompeting denitrifiers in the absence of NO_3^- or NO_2^- during the vial pre-incubation or direct inhibition of denitrification and not DNRA due to fermentation of amino acids or sulfate reduction during the pre-incubation, leading to the build up of inhibitory substances, such as hydrogen sulfide or organic acids and low pH (Sorensen et al., 1980; Kristensen and Hansen, 1995; Brunet and Garcia-Gil, 1996). Denitrification rates in vials recovered towards the end of the experiment, with more rapid and stronger recovery in +C+N compared to +C-N treatments (Fig. 7). Presumably, this reflected more favorable conditions for denitrifiers in +C+N compared to +C-N tanks, providing conditions for denitrifying bacteria to have a competitive advantage over DNRA bacteria.

4.5. Effect of organic C and NO_3^- on partitioning between DNRA and N_2 production

Tiedje et al. (1982) hypothesized that DNRA would be favored over denitrification in highly reducing or C-rich environments, or in other words, environments with a high ratio of electron donor (e.g., organic C) to electron acceptor (e.g., NO_3^- , NO_2^-). Thermodynamic calculations, observational studies, and a recent modeling study also support Tiedje et al.'s original hypothesis (Tiedje et al., 1982; Christensen et al., 2000; Gardner and McCarthy, 2009; Algar and Vallino, 2014). For example, Christensen et al. (2000), found higher DNRA than denitrification rates in estuarine sediments under aquaculture fish cages, where O_2 consumption rates were very high, while denitrification was higher than DNRA outside the cages, where O_2 consumption was more typical of the region. However, there are exceptions to this pattern. For example, in several tropical estuaries, DNRA responded more readily to NO_3^- addition than denitrification (Dong et al., 2011). Despite these observations, experimental evidence for the influence of organic C or C/ NO_3^- ratio on partitioning is still limited (Porubsky et al., 2009; Kraft et al., 2014). Other factors such as temperature, sulfide concentrations and perhaps even Fe^{2+} availability are known to favor DNRA (Brunet and Garcia-Gil, 1996; Kelly-Gerreyn et al., 2001; Roberts et al., 2014).

In our study, we tested whether C decomposition rates, NO_3^- reduction rates (R_1), or the ratio of the two (C/ NO_3^-) were related to partitioning between DNRA and N_2 production. We assumed our C decomposition rates provided a measure of electron donor supply while R_1 provided a measure of electron acceptor supply, providing a test for Tiedje et al.'s (1982) original hypothesis. In vial measurements we found a strong relationship between %DNRA and C decomposition (Fig. 8). Regardless of assuming high or low C/N values of organic matter

being decomposed (Burdige, 1991; Kristensen and Hansen, 1995; Thamdrup and Canfield, 1996), DNRA was only favored over denitrification at high C decomposition rates. These rates are on the high end of the spectrum of C decomposition rates encountered in marine sediments, similar to those measured under fish cages, highly productive coastal upwelling systems, hypersaline photosynthetic mats, or salt marsh sediments with active macrophytes (Jørgensen, 1982; Thamdrup and Canfield, 1996; Christensen et al., 2000; Giblin et al., 2013).

To compare our empirical relationship between %DNRA and organic C decomposition shown in Fig. 8 to other published studies, we surveyed the literature for comparable field studies. Literature values were assembled from 11 coastal studies that reported whole core O₂ consumption, as a proxy for organic C decomposition, and denitrification and DNRA rates using the ¹⁵N isotope pairing technique under a range of temperatures (tropics to the Arctic), water depths (intertidal to 200 m shelf sediments), and salinities (oligohaline to marine). The published O₂ consumption rates range from those typical of temperate continental shelf sediments (i.e., 15-35 mmol O₂ m⁻² d⁻¹) to relatively extreme, as measured in tropical estuaries, shallow lagoons, or aquaculture impacted sediments (~100 mmol O₂ m⁻² d⁻¹) (Fig. S4). A similar range of organic C decomposition, as represented by O₂ consumption, is likely captured in our experiment. In general, %DNRA followed a similar trend as we showed in Fig. 8, with DNRA reaching nearly 100% at a few locations (Fig. S4). However, there is large variation in the relationship between %DNRA and O₂ consumption based on literature values, suggesting that other factors besides O₂ consumption, and by extension organic C decomposition, must be important in controlling %DNRA.

Based on Fig. 9, we demonstrated the importance of C/NO₃⁻ ratio as a factor influencing partitioning between DNRA and N₂ production. This suggests a direct physiological mechanism

influencing partitioning based on energetics, with DNRA becoming progressively more favorable at higher ratios. An indirect effect due to NO_3^- limitation and buildup of sulfides that favored re-oxidation of sulfide by DNRA bacteria instead of denitrifiers is also possible (Brunet and Garcia-Gil, 1996). However, a recent study found that sulfide was not a selective force in the competition between DNRA and denitrifying bacteria, supporting a more general role for the influence of electron donor/acceptor ratio instead of indirect effects (Kraft et al., 2014).

Few studies have experimentally demonstrated or quantified a relationship between %DNRA and C/NO_3^- ratio (Porubsky et al., 2009; Kraft et al., 2014). Porubsky et al. (2009), found C/NO_3^- ratios between about 50 and 200 favored DNRA over denitrification, much higher than in our study. Perhaps, differences in experimental approaches explain the difference in results, as they added NO_3^- and acetate to washed sediment slurries and DNRA was measured based on changes in unlabeled NH_4^+ . Our results are more similar to those found in a recent modeling study by Algar and Vallino (2014), who found DNRA exceeded N_2 production at C/NO_3^- ratios of ~ 3 . Although more research is required, we propose that our study provides a testable hypothesis as to the quantitative nature of the relationship between partitioning and the ratio of electron donor to acceptor supply in the NO_3^- reducing zone of intact sediments.

4.6. Conclusions

Elucidating controls on N loss or retention in coastal sediments is critical to predicting the N balance of coastal ecosystems and ultimately ecosystem level processes, such as primary production. In our study, dissolved inorganic N concentrations in the tanks were characteristic of those driven by anoxic N cycling, with the build up of the intermediate NO_2^- responding strongly

to organic C limitation. We used two approaches to quantify partitioning; the first was based on fitting a model to changing inorganic N concentrations in the tanks and the second was based on directly measuring processes in ^{15}N incubation experiments in vials. Due to the disappearance of anammox rates in vial incubations, we were not able to assess anammox rates in this experiment. However, our experiment suggested that other processes besides anammox could contribute to a portion of N_2 production (Trimmer and Purdy, 2012; Chang et al., 2014). Both the model and vial incubations indicated that DNRA was only substantial under high C loading, while based on the model, N_2 production could be important under low or high C loading, depending on NO_3^- levels. Overall, our study demonstrated that taking C/ NO_3^- ratio into account might aid in predicting the fate of NO_3^- in anoxic marine environments.

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Figure captions

Fig. 1. Concentrations of inorganic N in aquarium tanks containing sediment thin discs, corresponding to different experimental treatments: -C-N (A-C), +C-N (D-F), -C+N (G-I), and +C+N (J-L). The dashed line (inflow only) corresponds to what inorganic N concentrations would have been if there was only experimentally manipulated transport into and out of the tanks. The solid line corresponds to concentrations of inorganic N generated by net reaction rates shown in Table 3 (model), while closed circles (obs.) are the measured concentrations. The difference between the dashed line and solid line is due to biological activity. Note that the scale of y-axes differ between graphs.

Fig. 2. NH_4^+ production in vials during 20 h pre-incubation of sediment thin discs removed from tanks (mean $\pm$ 1 SE for two replicates), with lines representing mean equations for -C treatments of $Y = -1.12 \pm 0.19(\text{Ln}X) + 6.62 \pm 0.54$ ($r^2=0.67$, $p<0.0001$) and for +C treatments of $Y = -15.56 \pm 1.03(\text{Ln}X) + 78.49 \pm 2.92$. ($r^2=0.93$, $p<0.0001$).

Fig. 3. Net NH_4^+ production in pre-incubated vials and tanks containing sediment thin discs (mean $\pm$ 1 SE), with the solid line representing the equation of $Y = 1.96 \pm 0.13(X) - 21.49 \pm 5.75$ ($r^2=0.98$, $p<0.0001$). The slope of the solid line is statistically different from 1 ($p=0.002$). Points corresponding to early and late stages of the experiment are indicated. Negative values in the tank indicate net NH_4^+ consumption.

Fig. 4. Organic C decomposition rates estimated in vials and the model. In -C treatments, the open box represents the lower, middle, and upper bound of mean C decomposition estimated in vials, assuming a C/N of 3, 5.88, and 10 for decomposing organic matter, respectively, while the error bars represent the SE around the mean for the lower and upper bounds. In +C treatments, the open box represents the lower and middle bound of mean C decomposition estimated in the vials, assuming a C/N of 3 and 5.88 for decomposing organic matter, respectively, while the error bars represent the SE around the mean, assuming a C/N of 3 and 5.88, respectively (the upper bound represented by a C/N of 10 is not shown). The closed symbols represent the mean ± 1 SE of R_{C-NOx} determined with the model. The values in the graph correspond to those in Table 3.

Fig. 5. Potential anammox rates based on production of $^{29}N_2$ from added $^{15}NH_4^+ + ^{14}NO_2^-$ in vial incubation experiments of sediment thin discs removed from tanks (mean ± 1 SE for two replicates).

Fig. 6. $p^{29}N_2$, $p^{30}N_2$, and $p^{15}NH_4^+$ from added $^{15}NO_2^- + ^{14}NH_4^+$ in vial incubation experiments of sediment thin discs removed from the tanks (mean ± 1 SE for two replicates). $p^{30}N_2$ reflects denitrification and $p^{15}NH_4^+$ reflects DNRA of added $^{15}NO_2^-$. $p^{29}N_2$ is assumed to primarily not reflect anammox rates based on results in Fig 5. Note that the scales of y-axes differ between graphs.

Fig. 7. Mean absolute (A) and relative rates (B) of DNRA (gray) and N_2 production (black) based on the vial incubations and model. In +C treatments, t_{early} corresponds to the first 10 days

in the model or the mean of day 4 and 14 from vial incubations. t_{late} corresponds to after day 10 in the model and day 47 from vial incubations. The values in the graph correspond to those in Table 3.

Fig. 8. Relationship between C decomposition rates and %DNRA. C decomposition rates were estimated in pre-incubated vials, while %DNRA was measured in the same vials after the pre-incubation step. The solid line represents C decomposition rates assuming C/N of 5.88, with the corresponding equation $Y = 27.69 \pm 2.76(\ln X) - 69.82 \pm 12.06$ ($r^2=0.83$, $p<0.0001$). Dotted lines represent C decomposition rates assuming a C/N of 3 (upper) or 10 (lower).

Fig. 9. Relationship between the ratio of C decomposition to NO_3^- reduction (R_1) rates (C/NO_3^-) and %DNRA. C decomposition rates were estimated in pre-incubated vials, while R_1 and %DNRA were determined in the model. The solid line represents C decomposition rates assuming C/N of 5.88, with the corresponding equation $Y = 12.19 \pm 0.44(X) - 2.01 \pm 1.71$ ($r^2=0.99$, $p<0.0001$). Dotted lines represent C decomposition rates assuming C/N of 3 (upper) or 10 (lower).

Table 1. Metabolic N cycling reactions included in the reaction-transport model.

Name	Symbol	Reaction
rem mineralization	R_{nh4}	$\text{orgN} \longrightarrow \text{NH}_4^+ + r_{CN} \text{CH}_2\text{O}$
nitrate reduction	R_1	$\text{NO}_3^- + 1/2\text{CH}_2\text{O} \longrightarrow \text{NO}_2^- + 1/2\text{HCO}_3^- + 1/2\text{H}^+$
denitrification	R_2	$\text{NO}_2^- + 3/4\text{CH}_2\text{O} + 1/4\text{H}^+ \longrightarrow 1/2\text{N}_2 + 3/4\text{HCO}_3^- + 1/2\text{H}_2\text{O}$
DNRA	R_3	$\text{NO}_2^- + \text{H}^+ + 3/2\text{CH}_2\text{O} + 1/2\text{H}_2\text{O} \longrightarrow 1/2\text{CO}_2 + \text{HCO}_3^- + \text{NH}_4^+$
anammox	R_4	$\text{NO}_2^- + \text{NH}_4^+ \longrightarrow \text{N}_2 + 2\text{H}_2\text{O}$
nitrification	R_5	$\text{NH}_4^+ + 2\text{O}_2 \longrightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+$

Table 2. Parameter values used during simulations of each treatment.

Parameter	Symbol	Units	-C-N	+C-N early	+C-N late	-C+N	+C+N early	+C+N late
Tank volume	V	L	155.7	155.7	155.7	155.7	155.7	155.7
Disc height	h_{sed}	mm	1.5	1.5	1.5	1.5	1.5	1.5
Input NO ₃ ⁻ conc.	[NO ₃ ⁻] _{in}	μM	31.0	31.0	31.0	266.0	266.0	266.0
Input NO ₂ ⁻ conc.	[NO ₂ ⁻] _{in}	μM	0.0	0.0	0.0	0.0	0.0	0.0
Input NH ₄ ⁺ conc.	[NH ₄ ⁺] _{in}	μM	20.0	20.0	20.0	20.0	20.0	20.0
N min. rate	R_{nh4}	nmol N mL ⁻¹ h ⁻¹	4.0	70.5	24.6	3.2	71.6	27.7

Table 3. Average (± 1 SE) rates of N transformations (nmol N mL^{-1} sediment h^{-1}) and C decomposition (nmol C mL^{-1} sediment h^{-1}) in sediment thin discs.

	Treatment					
	-C-N	+C-N early ^a	+C-N late	-C+N	+C+N early	+C+N late
<i>Measured net rates in tanks</i>						
NO ₃ ⁻ consumption	26.4 $\pm$ 1.9	30.8 $\pm$ 5.5	24.4 $\pm$ 1.5	122.7 $\pm$ 5.5	192.0 $\pm$ 6.1	166.4 $\pm$ 4.9
NO ₂ ⁻ production	3.0 $\pm$ 1.0	0.2 $\pm$ 1.7	0.9 $\pm$ 2.8	103.5 $\pm$ 4.9	4.1 $\pm$ 12.2	10.8 $\pm$ 4.1
NO ₂ ⁻ consumption ^b	23.4 $\pm$ 2.1	30.6 $\pm$ 5.8	23.5 $\pm$ 3.2	19.2 $\pm$ 7.4	187.9 $\pm$ 13.6	155.6 $\pm$ 6.4
NH ₄ ⁺ production ^c	-11.3 $\pm$ 0.8	129.9 $\pm$ 7.3	25.3 $\pm$ 3.8	-13.2 $\pm$ 0.1	108.4 $\pm$ 9.2	27.3 $\pm$ 2.4
<i>Rates in pre-incubated vials^d</i>						
NH ₄ ⁺ production (R_{nh4})	4.0 $\pm$ 3.6	70.5 $\pm$ 7.1	24.6 $\pm$ 10.1	3.2 $\pm$ 1.8	71.6 $\pm$ 7.4	27.7 $\pm$ 10.6
C decomposition (C/N=3) ^e	12.0 $\pm$ 10.7	211.5 $\pm$ 21.2	73.8 $\pm$ 30.2	9.7 $\pm$ 5.5	214.9 $\pm$ 22.2	83.0 $\pm$ 31.7
C decomposition (C/N=5.88)	23.6 $\pm$ 21.0	414.6 $\pm$ 41.5	144.6 $\pm$ 59.2	19.0 $\pm$ 10.9	421.3 $\pm$ 43.5	162.7 $\pm$ 62.0
C decomposition (C/N=10)	40.1 $\pm$ 35.8	705.0 $\pm$ 70.6	245.9 $\pm$ 100.6	32.4 $\pm$ 18.5	716.4 $\pm$ 74.0	276.6 $\pm$ 105.5
<i>Modeled rates in tanks</i>						
NO ₃ ⁻ reduction (R_1)	42.3 $\pm$ 0.9	49.2 $\pm$ 2.2	40.4 $\pm$ 1.4	125.5 $\pm$ 10.2	208.0 $\pm$ 5.2	183.8 $\pm$ 5.2
Denitrification (R_2)	39.3 $\pm$ 1.0	0.0 $\pm$ 0.0	23.0 $\pm$ 2.8	7.9 $\pm$ 0.7	151.1 $\pm$ 5.2	131.8 $\pm$ 10.0
DNRA (R_3)	0	51.1 $\pm$ 2.1	16.5 $\pm$ 1.5	0	52.7 $\pm$ 12.6	15.7 $\pm$ 8.2
Nitrification (R_5)	15.8 $\pm$ 0.9	16 ^f	16	16.3 $\pm$ 4.3	16	16
% DNRA ^g	0	100.0 $\pm$ 0.0	41.8 $\pm$ 12.9	0	26.0 $\pm$ 13	10.7 $\pm$ 10
NO _x ⁻ linked C remineralization (R_{C-NOx}) ^h	50.6 $\pm$ 1.9	101.4 $\pm$ 4.3	62.3 $\pm$ 5.7	68.6 $\pm$ 4.8	296.4 $\pm$ 30.4	228.0 $\pm$ 19.6
<i>Potential rates in vialsⁱ</i>						
$p^{30}\text{N}_2 + \frac{1}{2}p^{29}\text{N}_2$	10.9 $\pm$ 2.3	1.0 $\pm$ 0.1	53.6 $\pm$ 3.8	14.9 $\pm$ 4.6	12.8 $\pm$ 3.1	132.4 $\pm$ 17.4
$p^{15}\text{NH}_4^+$ (DNRA)	2.1 $\pm$ 0.7	249.7 $\pm$ 28.3	75.4 $\pm$ 10.9	0.7 $\pm$ 0.5	172.7 $\pm$ 53.6	28.9 $\pm$ 1.6
% DNRA ^j	16.5 $\pm$ 4.5	99.6 $\pm$ 0.1	58.2 $\pm$ 1.8	7.3 $\pm$ 6.4	91.9 $\pm$ 2.9	18.1 $\pm$ 1.1

^aEarly corresponds to the first time period of the experiment ($t \leq 10$ d) and late to the second time period ($t > 10$ d) or as defined under superscript, “i” below.

^bNO₂⁻ consumption is the difference between NO₃⁻ consumption and NO₂⁻ production.

^cNegative values indicate NH₄⁺ consumption.

^dRates in pre-incubated vials are the time weighted average.

^eC decomposition was determined by multiplying NH₄⁺ production by the C/N ratio shown.

^fNitrification was a fixed parameter in model runs of +C treatments.

^g%DNRA for the model is defined as $\%DNRA = 100(R_3/(R_2+R_3))$.

^h NO_x^- linked C mineralization is defined as, $R_{C-NO_x} = 1/2R_1 + 3/4R_2 + 3/2R_3$.

ⁱPotential rates in vials correspond to days 4, 13, and 47 in -C treatments and day 4 and 13 in early phase and day 47 in late phase of the +C treatments.

^j%DNRA of vial rates is defined as $\%DNRA = 100(p^{15}NH_4^+ / (p^{15}NH_4^+ + 1/2p^{29}N_2 + p^{30}N_2))$.

Figure

Fig. 1

Fig. 2

Fig. 3

Fig. 4.

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9