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Controls on Nitrogen Loss Processes in Chesapeake Bay Sediments

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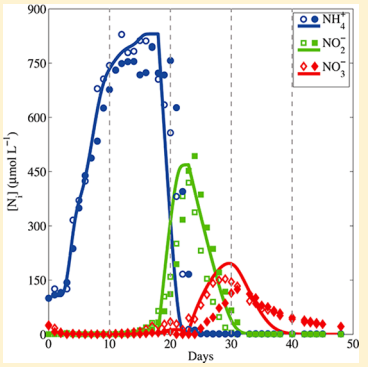
Supporting Information

ABSTRACT: The flux of fixed nitrogen into the marine environment is increasing as a direct result of anthropogenic nitrogen loading, but the controls on the mechanisms responsible for the removal of this increased supply are not well constrained. The fate of fixed nitrogen via mineralization and nitrogen loss processes was investigated by simulating a settling event of organic matter (OM) in mesocosms containing Chesapeake Bay sediments. Microorganisms rapidly transformed the OM during the course of a seven week incubation ultimately leading to nitrogen loss via denitrification and anaerobic ammonium oxidation (anammox). The microbial community responded quickly to the OM amendment suggesting that estuarine sediments can buffer the natural system against sudden injections of organic material. Two different levels of organic matter amendment resulted in different magnitudes of ammonium and nitrite accumulation during the incubation, but both treatments exhibited the same overall sequence of dissolved inorganic nitrogen (DIN) accumulation and removal. An inverse least-squares analysis coupled to a Michaelis–Menten prognostic model was conducted to estimate rates of nitrogen transformations from the measured DIN concentrations. Whereas the rates were higher at higher OM, the percentage of nitrogen lost via anammox was constant at 44.3 ± 0.3%. The stoichiometry of organic matter and the allochthonous supply of ammonium determined the relative contribution of anammox and denitrification to overall nitrogen loss. Further, in situ thermodynamics based on measured concentrations suggested that the energy favorability of denitrification and anammox plays a role in determining the timing of these processes as OM remineralization progresses.



INTRODUCTION

Humans have increased the amount of reactive (i.e., non-N_2) nitrogen (Nr) available for biological activity in the environment. Through anthropogenic practices such as the industrial Haber–Bosch process, preferential planting of leguminous crops, and fossil fuel combustion leading to NO_x emissions, anthropogenic nitrogen fixation is now comparable to natural nitrogen fixation.[1−3] Possible changes to the nitrogen budget due to this additional Nr need to be explored including how sedimentary nitrogen loss pathways might respond with consequences for individual watersheds, the coastal ocean, and the global cycling of nitrogen. Furthermore, the factors that limit these reactions should be determined to better assess the potential effects of the increased Nr.

Through the bacteria-mediated process of mineralization, organic nitrogen is transformed into dissolved inorganic nitrogen (DIN) beginning with ammonium. Under oxic conditions, this ammonium is typically oxidized to nitrite and then further to nitrate (i.e., nitrification). From nitrate, Nr can be removed from the system by reduction back to nitrogen gas under anoxic conditions. The two major biological nitrogen removal processes are heterotrophic denitrification, the stepwise reduction of nitrate to dinitrogen gas via oxidized intermediates, and anaerobic ammonium oxidation (anammox), the autotrophic synthesis of N_2 from ammonium and nitrite. In the case of heterotrophic denitrification, carbon dioxide is liberated during nitrate-based respiration of organic matter (OM), and nitrous oxide (N_2O) is a free intermediate in the penultimate reduction step. This N_2O can be partially lost before complete reduction to dinitrogen can occur. As a greenhouse gas with 300 times the global warming potential of carbon dioxide on a molar basis,[4] even minimal leakages of N_2O can have large climate impacts. Anammox, however, fixes inorganic carbon autotrophically via the acetyl-CoA pathway[5] and does not release any known greenhouse agent.[6] From a nitrogen inventory perspective, the impact on the nitrogen cycle of both processes is the same, that is the removal of Nr from the system; however, their different impacts on the carbon cycle and greenhouse gas budget may be important distinctions.

Anammox requires two reduced nitrogen species (ammonium and nitrite) that are not always available in the environment, and thus the process can be limited by the supply of these two ions. These ions have two possible origins: (1) autochthonous production by the first step of denitrification (reduction of nitrate to nitrite), mineralization during denitrification, and aerobic ammonium oxidation, or (2) diffusion or advection of ions produced allochthonously by these or other remineralization processes occurring elsewhere.

Both nitrogen removal processes are restricted to specific environments where the following conditions exist: (1) the organic matter flux is sufficiently high that aerobic remineraliza-

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tion consumes the ambient oxygen flux, (2) ventilation rates are slow enough that the resupply of oxygen is outpaced by its consumption, and (3) excess DIN flux allows dissimilatory conversion to N_2. These three constraints restrict fixed nitrogen loss in the marine environment to three systems: hemipelagic sediments (i.e., shallow continental shelves), stratified water columns overlying sulfidic bottom waters such as the Black Sea, and poorly ventilated water masses (the largest being the Eastern Tropical North and South Pacific and Arabian Sea oxygen deficient zones).[7] The contributions of and controls on the two nitrogen loss pathways in each environment under different conditions of DIN and organic matter supply, however, are not well understood.

Trimmer and Engström[8] reviewed the distribution of anammox in global marine sediments. The assembled data from numerous studies revealed a pattern of increasing anammox rates relative to total nitrogen loss rates (i.e., percent anammox) with increasing water depth even though the absolute rates of Nr removal generally decreased with depth. Dalsgaard et al.[9] hypothesized that this pattern derived from organic carbon loading because heterotrophic denitrifiers are dependent on a supply of organic matter. Deeper sediments should receive progressively less organic matter as more of the flux from above is remineralized in the overlying water column thus limiting the denitrification pathway in those sediments.

Whereas organic carbon supply might control the relative proportions of anammox and denitrification, the supply of ammonium and/or nitrite necessary for the anammox pathway may also constrain the rate of the process. These DIN species do not accumulate substantially in the oceanic oxygen deficient zones (ODZs). On the basis of this observation and on the basis of stoichiometric balance, Koeve and Kähler[10] concluded that anammox in the ODZ must be supported by ammonium released from heterotrophic denitrification. In hemipelagic sediments, however, where ammonium is supplied from below by manganese, iron, and sulfate reduction, anammox can be partially decoupled from denitrification provided nitrite is supplied by nitrification or nitrate reduction.

The experiments in this study were designed to investigate the relative contributions of denitrification and anammox to N_2 production and the role of organic matter in determining percent anammox and to address the capacity of sediments to ameliorate anthropogenic nitrogen loading by evaluating how nitrogen is transformed following a high-nitrogen discharge or settling event. Homogenized sediments from the Chesapeake Bay were incubated in mesocosms to evaluate changes to the nitrogen cycling that result from different OM inputs. Using observed changes in DIN concentrations, stoichiometric and thermodynamic controls on nitrogen cycle processes were also assessed.

METHODS

Sample Collection and Experimental Design. Sediments (consisting predominantly of clay and silt particles with a porosity of 0.8) were collected from the lower Choptank River in the Chesapeake Bay estuary system (station CT200, 38°37.197′ N 76°08.061′ W, station depth = 7.9 m, salinity = 14) in November 2009. A Plexiglas box core (30 × 20 cm^2) was used to obtain three 30 cm deep sediment cores, which were then homogenized in the field and placed in a cooler. Overlying water was collected by pumping into 20 L opaque carboys. The homogenized sediments were covered with site water and stored in the dark at room temperature until the experiment was begun a week later.

A layer of sediment slurry (2.5 kg, ~2.5 cm thick layer) was placed in the bottom of a 25 × 40 cm^2 covered plastic container and overlain with site water (18 L, depth of 18.5 cm). The overlying water was bubbled with air to mix the overlying water gently and to prevent sulfate reduction in the thin sediment column. The DIN (nitrate, nitrite, and ammonium) concentrations in the overlying water were monitored for six months during which time ammonium and nitrite were completely consumed and nitrate reached a constant low level <1 μM. This preincubation period was intended to allow the ambient labile organic carbon to be consumed.

The experimental design consisted of two mesocosms with low organic matter additions (L1 and L2) and two with a 10-fold higher addition (H1 and H2) in the form of fish food. Ground commercially available fish food flakes consisting of mostly algae and ground shrimp (TetraFin, Blacksburg, VA; 10% N by mass; C/N/P = 106:25:1) were combined with a small amount of distilled water to make a paste that was applied directly to the sediment surface and raked over the entire interface. The two low OM treatments received 0.4 mg cm^{-2} of fish food, whereas the two high OM treatments received 4.0 mg cm^{-2}. These applications were approximately equivalent to 2 days and 20 days respectively of natural Chesapeake Bay particulate OM sedimentation rate (~0.2 mg cm^{-2} d^{-1}).[11] The water volume of the mesocosms was replenished with approximately 0.5 L of Chesapeake Bay site water to replace the volume lost due to sampling and evaporation during the preincubation period. Ambient ammonium and nitrate concentrations in the overlying water were augmented to their ambient initial conditions, approximately 100 and 25 μM respectively using concentrated solutions of NH_4Cl and $NaNO_3$.

For the next seven weeks, the mesocosms were incubated with gentle bubbling of air at room temperature in the dark. The overlying water was sampled daily for DIN, filtered through a 0.22 μm filter, and stored frozen at −20 °C until analysis by standard methods.[12−14] At the beginning and end of the incubation period, sediments were collected by coring the full sediment column using a 1 cm diameter cutoff glass syringe. The collected sediment was homogenized, aliquoted into 1.5 mL Nalgene cryovials, and frozen at −80 °C for particulate organic nitrogen (PON) and carbon (POC) analyses. The homogenized sediments were rinsed three times with 10% HCl and washed with distilled water three times before being weighed into tin boats for combustion in an elemental analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific 20/20). The pH of the overlying water was monitored weekly with a Beckman Φ34 pH Meter.

Nitrogen Cycle Rate Modeling. A simple box model (Figure 1) was used to simultaneously evaluate rates of four possible transformation pathways of the measured inorganic nitrogen species − ammonification, ammonium oxidation, nitrite oxidation, and nitrogen loss from nitrate via denitrification. The model assumed removal of DIN from the measured pools was as N_2. A second, more sophisticated model (Figure 1, including boxed part) was also used to distinguish anammox from heterotrophic denitrification (where heterotrophic denitrification includes all nitrogen loss that ultimately is derived from organic material including the dissimilatory reduction of nitrate to ammonium process[10]). However, because of the measurement of only three parameters (DIN

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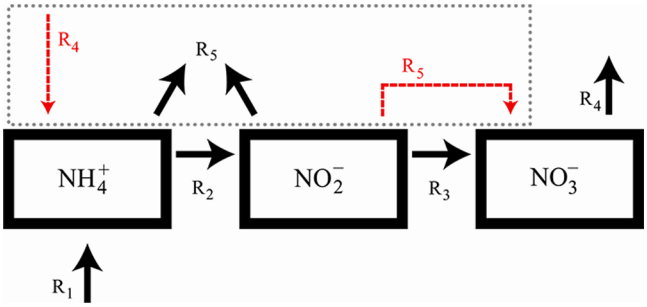

Figure 1. Least squares model used to solve for transformation rates. Inside the gray box are the additional processes included in the five-rate model. (R_1) ammonification, (R_2) aerobic ammonium oxidation, (R_3) nitrite oxidation, (R_4) denitrification, and (R_5) anammox are calculated. Solid black arrows show main transformations, whereas dashed red arrows represent ancillary impacts on nitrogen speciation.

pools), solving for additional variables without additional information results in an underconstrained system of equations. Therefore, a Michaelis–Menten parametrization of rates (R_i) as functions of substrate concentration (N_j) starting when ammonium begins to decrease was chosen a priori to fit the five-rate model output:

$$R_i = \frac{V^i_{max} \times N_j}{K^i_S + N_j} \quad (1)$$

This recursive structure increases the confidence in the inverse method because, instead of solving for independent rates at each point in time, only two parameters (V_{max} and K_S) are required to solve for each rate for which there are dozens of time points. Still, as organic matter concentration is not indicative of activity (Results and Discussion) in this system, ammonification rates through this experiment were determined directly from the box model. The Michaelis–Menten parameters were then incorporated into a prognostic model to predict the changes in DIN species that would occur in the high and low OM mesocosms.

Observed DIN data were empirically smoothed using a second-order local (loess) smoothing filter to minimize the higher frequency noise resulting from sampling. Numerical time derivatives were calculated and used in a non-negative least-squares analysis[15] of the rate equations:

$$\frac{dN_j}{dt} = \sum_i f_{ij} R_i \quad (2)$$

where R_i are the rates being optimized and N_j are the measured DIN species. The transformation equations used (Supporting Information) are based on the theoretical stoichiometry derived by Paulmier et al.[16] for denitrification (R_4) using fish food and the empirical stoichiometry for anammox (R_5) found by Strous et al.[17] The equations used for aerobic processes (R_1–R_3) were the simplified transformations (i.e., all coefficients = 1). A Monte Carlo simulation (n = 10 000) was run to assess rate errors based on the assumption that the observed data means and standard deviations well approximated normal distributions.

Thermodynamic Calculations. Independent, that is not dependent on the modeled nitrogen cycling, measures of in situ thermodynamic ΔG_{rxn} specific for the organic matter applied to the mesocosms were calculated on the basis of observed DIN concentrations, temperature, and pH. The inhibition effects of dissolved oxygen were excluded from the calculations, but comparisons among anaerobic processes can still be made. Whereas the possible differences in oxygen concentration thresholds of the anaerobic processes[18] may have ecological consequences, recent work has shown that neither anammox nor denitrification occurs at oxygen concentrations greater than a few nanomolar,[19] and therefore oxygen was not taken into account. As ΔG° and ΔG_{rxn} are dependent on the specific stoichiometries of the reactions they describe, the exact stoichiometries of the OM composition were used (Table 2), with ΔG_{rxn} calculated from:

$$\Delta G_{rxn} = \Delta G^\circ + RT \ln Q \quad (3)$$

where ΔG_{rxn} is the free energy released with ambient concentrations and pH, ΔG° the free energy at standard conditions, R the ideal gas constant, T ambient temperature, and Q the multiplicative ratio of product concentrations and reactant concentrations for a specific reaction.

Table 1. Determined Michaelis–Menten Parameters for Maximum Community Rate (V_{max}, d^{-1}) and Half Saturation Coefficient (K_S, μM)[a]

		low OM			high OM		
rate	N_j	V_{max}	K_S	R^2	V_{max}	K_S	R^2
R_2	NH_4^+	35	40	0.96	170	40	0.96
R_3	NO_2^-	80	60	0.95	80	60	0.85
R_4	$NO_2^- + NO_3^-$	6	60	0.60	60	60	0.91
R_5	NH_4^+	15	25	0.88	50	25	0.77

[a]Ammonium oxidation (R_2) and anammox (R_5) are parameterized in terms of ammonium, nitrite oxidation (R_3) in terms of nitrite, and denitrification (R_4) in terms of the sum of nitrate and nitrite.

RESULTS AND DISCUSSION

DIN Time Series. The two low OM treatment mesocosms displayed identical trends in the DIN time series (part a of Figure 2) replicating all measured DIN species remarkably well (R^2 = 0.96, n = 147). Ammonium concentrations in the overlying water increased over the first 7 days of the experiment and then decreased sharply to very low levels (<1 μM) after about 15 days. The nitrite concentration reached a maximum of 12 μM at day 11. This nitrite concentration maximum was only 6% of the ammonium maximum, and nitrite decreased to the detection limit (<0.2 μM) by day 16. Concurrent with the decrease in ammonium, nitrate began to increase, achieving its maximum concentration (120 μM) at day 15 when ammonium and nitrite concentrations approached zero. Following this maximum, nitrate concentration decreased gradually until measurements ceased at day 48. The total DIN concentrations increased when ammonium increased (black line) and then decreased for the remainder of the experiment in an approximately exponential decay. Moreover, the small amount of nitrite accumulation implies that the cooperation between aerobic and anaerobic ammonium oxidizers previously reported for oxygen-limited wastewater engineering reactors also occurred in these sediments.[20,21]

The DIN patterns in the high OM treatment mesocosms were similar to those observed in the low OM treatments (part b of Figure 2) transitioning overall from ammonium to nitrate as the dominant DIN species. The measured DIN species in the

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Table 2. Averages and Standard Deviations (μmol L^{-1}) of Total Integrated Amount of Each Nitrogen Species Transformed for Both Models Used in This Study over the 48 Day Incubation[a]

		low OM		high OM	
rate	transformation	4-rate model	5-rate model	4-rate model	5-rate model
amendment	$NH_4^+ \rightarrow$	100 ± 0	100 ± 0	100 ± 0	100 ± 0
R_1	$OM \rightarrow NH_4^+$	65 ± 8	94 ± 7	768 ± 12	806 ± 7
R_2	$NH_4^+ \rightarrow NO_2^-$	231 ± 7	165 ± 6	1007 ± 13	779 ± 4
R_3	$NO_2^- \rightarrow NO_3^-$	233 ± 7	103 ± 4	1009 ± 13	502 ± 1
R_4	$NO_3^- \rightarrow {}^1/_2N_2$	226 ± 10	122 ± 4	989 ± 13	560 ± 1
	$OM \rightarrow NH_4^+$	39 ± 2	21 ± 1	169 ± 2	94 ± 0
R_5	${}^1/_2NH_4^+ + {}^1/_2NO_2^- \rightarrow {}^1/_2N_2$		98 ± 4		440 ± 5
	$NO_2^- \rightarrow NO_3^-$		13 ± 0		57 ± 1

[a]Anammox % is calculated as portion of N_2 produced from R_5 compared to $R_4 + R_5$ and equals 44.5 ± 0.1% and 44.0 ± 0.3% for the low and high OM treatments, respectively; R_i definitions are listed in Figure 1.

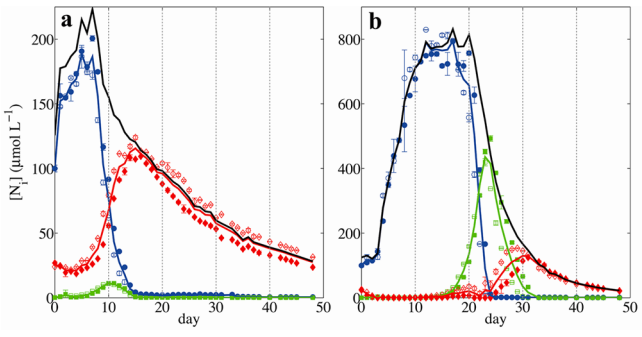

Figure 2. DIN time series for (a) low OM and (b) high OM mesocosms. Average species values (lines) between replicates (symbols) are shown for ammonium (blue), nitrite (green), nitrate (red), and total DIN (black).

two high OM tanks also replicated well (R^2 = 0.94, n = 147), but there were significant differences between the observed DIN magnitudes of the low and high OM treatments. The high OM mesocosms also displayed an increase in ammonium concentration, but this increase was more substantial than that in the low OM mesocosms, to ~800 μM over 15 days corresponding with an increased loading of labile organic nitrogen. In the high OM treatments, nitrite accumulated to a much larger extent (~450 μM at peak, ~60% of the ammonium peak) than in the low OM tanks and significant nitrite concentrations were detectable for 15 days. The maximum nitrite concentration was achieved as ammonium decreased to the detection limit (day 24). As the nitrite decreased, nitrate increased, reaching its own maximum of ~120 μM by day 29 of the experiment before decreasing until the end of the incubations, as in the low OM mesocosms.

The POC and PON concentrations in the sediments were high throughout the incubation in both treatments with no significant changes over the experiment (data not shown). The sediment PON reservoir was large averaging about 3000 μg PON g^{-1} dry sediment rendering the fish food amendments (20 and 200 μg PON g^{-1} dry sediment) for the low and high OM mesocosms respectively, which is small by comparison and barely measurable against the large residual PON background. The background organic material had an invariant C/N ratio of ~9, whereas the measured C/N of the fish food was 4.2 ± 0.2, which resulted in an addition of 0.4 and 4.0 μg N cm^{-2} to the low and high OM treatments, respectively. The increased response of the high OM treatment compared to the low OM treatment, when both began with the same initial DIN, implies that the background OM is recalcitrant by comparison. Labile OM was not exhausted when ammonium production rates declined as evidenced by the occurrence of heterotrophic denitrification later.

There were no significant trends detected in the pH (data not shown), which ranged between 8.1−8.2 for the low OM treatments and 8.0−8.2 for the high OM treatments over the course of the incubation.

Sediment Mesocosm as a Model System. The mesocosms reproduced the diagenetic sequence typical of both water- and sediment-based systems following an addition of organic matter, such as a bloom or wastewater discharge event in the pelagic environment,[22] the subsequent deposition of a bloom or settling event in the benthic environment,[23,24] or an inoculation of a bioreactor.[25,26] A response of increased rates with additional organic input, as observed in the mesocosms, would be expected, and, in fact, Law et al.[24] showed that ammonification and denitrification rates in a British estuary varied annually with rate maxima corresponding to a late spring input of new organic matter. Further, the initial stages of the mesocosm sequence also mimicked the sequence of ammonium−nitrite−nitrate observed in nitrification-only batch reactors presented in Anthonisen et al.[25] In a nitrification-only batch reactor in which nitrite oxidation was inhibited, nitrite accumulated significantly, similar to the nitrite accumulation observed in the high OM mesocosms suggesting an analogous inhibition occurred in the high OM treatments.

By preincubating the sediments to reduce the ambient labile organic matter, the diagenetic sequence was initiated by the addition of new labile organic matter, and the DIN transformation patterns differed in accord with the magnitude of organic matter addition. Because the DIN patterns were well replicated and consistent with observations of natural and engineered systems, these data can be used to model the relevant nitrogen transformation rates to understand analogous rate processes in the environment.

Sediment systems, like the experimental mesocosms used here, have been well-characterized as heterogeneous systems containing a range of particle types, both inorganic and organic, and pore spaces filled or semifilled with water that allow for the occurrence of both anoxic and microaerated zones within close spatial proximity.[27] Thus, processes are not typically stratified only in depth profile but occur wherever favorable conditions arise in the sediment/water matrix.[28] By measuring changes in the DIN species in the overlying water, net integrated rates over the entire spatial domain of the sediments may be determined regardless of the heterogeneous distribution of the substrates,

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oxygen, and processes in microzones in the sediments. The approach presented in this study allows the results of microbial organization to be observed while avoiding biases that might result from spatial variability of in situ measurements in the sediments.

The mesocosm approach also allows the codetermination of the major nitrogen transformations that are catalyzed by the full microbial community present in a natural environment but does not allow for the elucidation of small-scale processes, whose signals are swamped by the major changes or the mechanisms of specific pathways. The sequence and relative magnitudes of the dominant environmental processes, however, can be deduced in the mesocosm experiments.

Michaelis–Menten Parameters. Examples of the Michaelis–Menten fits are provided in Figure 3, and the summary of

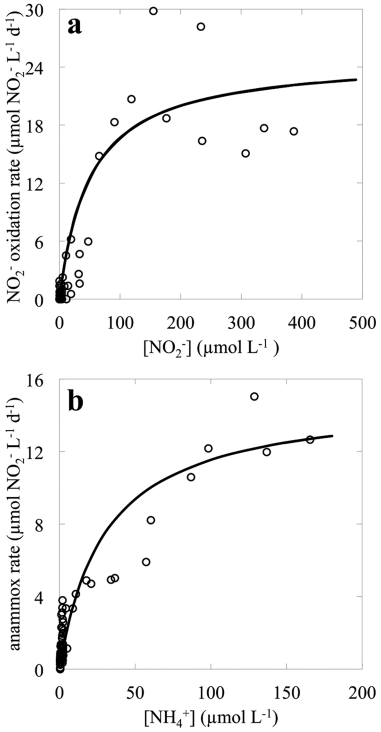

Figure 3. Example Michaelis–Menten fits for (a) nitrite oxidation in the high OM mesocosms and (b) anammox in the low OM mesocosms. Coefficients used are listed in Table 1.

all determined parameters are listed in Table 1. The prognostic model based on these values and the initial conditions in the mesocosms fit the observed data quite well (Figure 4, $R^2 > 0.97$, $n = 132$). The values for maximum community rates (V_{max}) increased with the higher OM treatment except for nitrite oxidation, which remained the same. The factor of increase ranged between 3 and 5. The half-saturation constants (K_S), however, were the same for all four modeled rates between treatments, as would be expected because the same types of organisms were initially present in both sets of mesocosms.

Still, the increase of community rates with an increase in mineralization of organic nitrogen makes sense as the growth of microorganisms (which was not explicitly accounted for in the models presented here) should increase the rates of metabolism. Thus, the increased amount of organic matter (and the reduced nitrogen it supplied) in the high OM

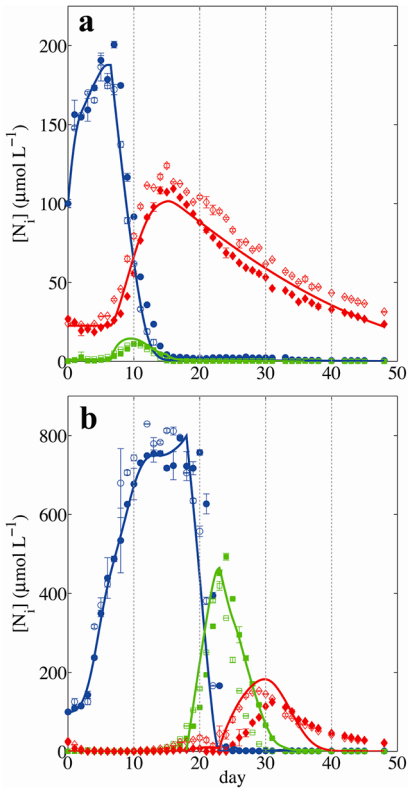

Figure 4. Observed replicate data (symbols) and prognostic Michaelis–Menten model (lines) for (a) low OM and (b) high OM mesocosm treatments. Ammonium (blue), nitrite (green), and nitrate (red) are shown.

treatment supported a more abundant community of aerobic and anaerobic ammonium oxidizers and denitrifiers.

Perhaps more interesting, however, is that nitrite oxidizers did not appear to respond in the same way as ammonia oxidizers and denitrifiers. In the low OM treatments, nitrite oxidation was apparently coupled directly with aerobic ammonium oxidation and in fact could be modeled using a Michaelis–Menten fit with an ammonium substrate (not shown), which is often observed in wastewater treatment systems and in the environment.[29] Furthermore, aerobic ammonium oxidation, anammox, and nitrite oxidation occurred in ratios that reduced the inorganic nitrogen load while preventing nitrite accumulation. In the high OM treatment, nitrite oxidation appeared to be inhibited by elevated ammonium concentrations allowing nitrite to accumulate to high levels. Nitrite oxidation later commenced, as seen from nitrate accumulation, after the ammonium concentrations were reduced. This is consistent with the findings of Anthonisen et al.[25] where, in aqueous nitrification batch reactors, non-permanent inhibition of the nitrite oxidizing *Nitrobacter* genus occurred at ammonium concentrations above 60 μM. Whereas the cause of this inhibition was not explicitly studied in this experiment, there is evidence to suggest that elevated hydroxylamine (an intermediate in the first ammonium oxidation step of nitrification) concentrations inhibit nitrite oxidation.[30]

Integrated Transformation Rates. The simpler least-squares analysis solving for four rates at each time point implied a balanced system (Table 2) where the ammonium liberated, ammonium oxidized, nitrite oxidized, and the nitrate lost were all equivalent for both mesocosm treatments. In separating

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anammox from denitrification, however, by adding a short circuit loss term that does not require complete nitrification, the integrated contribution of ammonium and nitrite oxidation to the overall cycling of nitrogen both decreased. Whereas the overall amount of ammonification, ammonium oxidation (sum of aerobic and anaerobic), and nitrogen loss remain similar, nitrite oxidation did decrease because some fraction of the ammonium pool was never oxidized to nitrite and a portion of the nitrite pool was reduced to N_2 rather than oxidized to nitrate.

Between the high and low OM treatments, total amounts of DIN consumption increased by factors of 4.7 ± 0.2 despite a factor of 9 increase in total ammonification (similar to the 10-fold increase in labile OM provided). This is due to the fact that allochthonous ammonium was provided to both treatments such that the total increase in ammonium supply (ammonium and OM) differed only by a factor of 4.7 ± 0.2 between low and high treatments. Because of these similar increases in all factors between treatments, the net percentage of nitrogen lost by denitrification and anammox remained similar in both the low and high OM treatments (44.5 ± 0.1% and 44.0 ± 0.3%, respectively; Table 2). The Michaelis−Menten model resolves the source of additional organic matter to support all of the nitrogen transformations through denitrification resulting in a full inorganic nitrogen mass balance despite not being explicitly required by the model. Attempts to measure the labile component of organic matter were unsuccessful, however, due to the overwhelming (>10−100 fold) signal of refractory organic nitrogen present at the start of the incubations.

Controls on Anammox and Denitrification. On the basis of thermodynamics of the actual mesocosm conditions, ΔG_{rxn} were calculated at each time point for both anaerobic nitrogen loss processes and for the first step of denitrification separately (Figure 5). Whereas anammox is generally thought to be less thermodynamically favorable than denitrification because it is an autotrophic metabolism,[31] anammox can be favored during times of high ammonium concentrations. Indeed this occurred in the high OM mesocosms, albeit only for a short period of time (days 15 to 21). A consistent finding across all mesocosms, however, was that when anammox became less favorable than the first of the sequential denitrification steps (days 15 and 30 for low and high OM treatments, respectively), nitrate stopped accumulating and began to decrease suggesting a possible thermodynamic control on denitrification starting with nitrate reduction.

A model similar to that of Koeve and Kähler[10] was used to calculate the relative contributions of anammox and denitrification in the mesocosms assuming they were constrained stoichiometrically and that ammonium and nitrite do not accumulate in the system. Because ammonium released by denitrifiers must be removed (i.e., it is not observed to accumulate), anammox rates should be directly proportional to those of denitrification. If the available OM has Redfield stoichiometry (C/N/P of 106:16:1), anammox should account for 29% of nitrogen loss in the marine environment[32] as was observed in the eastern tropical South Pacific ODZ.[19] However, because a nitrogen-enriched source of organic matter with a C/N/P of 106:25:1 was added to the mesocosms, the percentage of nitrogen loss attributed to anammox in the model (43%) is higher than that predicted by Redfield stoichiometry to balance ammonium and nitrite sources and sinks. Indeed, this predicted value is very similar to the percent anammox in all mesocosms regardless of treatment indicating that the stoichiometry of

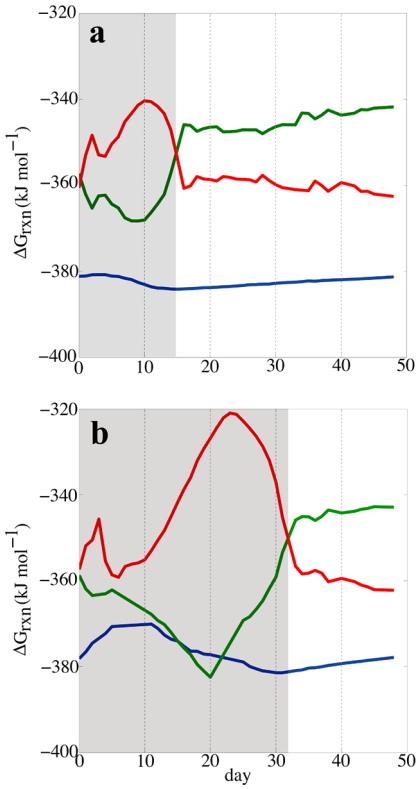

Figure 5. Calculated free energies for denitrification (blue), anammox (green), and nitrate reduction (red) for (a) low OM and (b) high OM mesocosms. The period of time when anammox is more energetically favorable than nitrate reduction is marked with a gray box.

organic matter supporting the remineralization scheme, rather than the sheer amount of this organic matter, drives the relative proportion of the two processes. Thus, the partitioning of Nr loss between anammox and denitrification depends directly on the C/N of the organic matter supply.

The effect of allochthonous DIN (and its relative magnitude compared with the amount of organic nitrogen introduced to the systems) was muted due to the increase in nitrification, but it is worth noting that Engström et al.[33] observed an average anammox percent of 40−42% in sediments in the Cascadia Basin due to an ammonium supply derived from sulfate reduction in deeper anoxic sediments. In this way, the additional reduced nitrogen (as ammonium) had the same effect as a lowered C/N ratio of the organic matter; that is, anammox rates increased proportionally over denitrification to remove the excess nitrogen. This effect can be enhanced when OM fluxes to deeper sediments are decreased even if the organic matter is more degraded (i.e., higher C/N) because the allochthonous ammonium supply from below more than compensates. The trend of increasing percent anammox with decreasing organic matter loading[9] may be explained by a balance of organic matter flux to ammonium supply rather than depending upon the magnitude of organic matter flux alone. In the ocean, however, given the absence of allochthonous ammonium sources the proportion of nitrogen loss attributed to anammox should be more closely constrained to that predicted by Redfield stoichiometry.

Implications for the Environment. The rapid response of microorganisms in the mesocosms to a sudden addition of organic matter and inorganic nutrients indicates that the sediments were preconditioned to labile OM processing − they

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were lacking only substrates. Once the substrates were provided, the ambient microbial assemblages quickly responded and increased their rates accordingly. In fact, both treatments processed the OM and DIN amendments in the same length of time suggesting an elastic dynamic variability in the time course whereby the sediment microbes increase the rates at which they process the nitrogen loading − typical of a rate response as a function of substrate concentration. Whereas the amount of residual organic matter in the sediments at the beginning of the experiment greatly exceeded the mass of the organic amendment, the organic matter amendments nevertheless elicited dramatic responses in DIN fluxes and microbial transformation rates. Thus, it is clear that total OM content of sediments is not an indicator or predictor of microbial activity. Rather, it is the composition, timing, and magnitude of the episodic influxes of labile OM that matter to the sediment community and drive microbial processes. Because of this constraint, seasonal cycles and even stochastic pulses of organic and inorganic nitrogen likely influence the pathway of nitrogen loss both in sediments and in ODZs.

These mesocosm treatments might simulate a sewage discharge or fertilizer runoff event to which estuarine sediment microbial communities are commonly subjected. The microbes were able to accommodate both treatment levels adjusting their transformation rates accordingly to remove the added nitrogen in a similar time frame. In all four mesocosms, the initial (125 ± 5 μM) and end (25 ± 5 μM) DIN concentrations were indistinguishable (p = 0.30). The addition of organic matter greatly increased the total amount of nitrogen loss that occurred. This is consistent with estimates that estuarine processes can up-regulate in response to high nitrogen loading to remove 65% of the total nitrogen that would pass into the coastal ocean.[34]

The background C/N of ∼9 after the preincubation sediments was very similar to measured sediment C/N values from previously studied estuarine systems.[24,35,36] The mesocosms thus appropriately simulated the natural estuarine system: the labile fraction of pulses of organic material is quickly remineralized leading to loss as nitrogen gas after which the sediment C/N returns to a steady state C/N ratio dictated by the large reservoir of recalcitrant OM. This experiment showed that even a quiescent community of microorganisms is able to respond rapidly to an input of both organic matter and ammonium despite not receiving pulses of new organic matter or inorganic nitrogen for six months.

■ ASSOCIATED CONTENT

Supporting Information

Details of the model parameters used in the calculations of rates in the article. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Supporting information for

Controls on nitrogen loss processes in Chesapeake Bay sediments

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This supporting information comprises Tables **S1**: Dissimilatory nitrogen transformations modeled and **S2**: Stoichiometric coefficients used in the model

Table S1.

Dissimilatory nitrogen transformations modeled; stoichiometric coefficients are in Table S2.

Process	Reaction	Equation
R_1	ammonification	$\mathrm{orgN} + \mathrm{O_2} \rightarrow \mathrm{NH_4^+}$
R_2	aerobic ammonium oxidation	$\mathrm{NH_4^+} + \mathrm{O_2} \rightarrow \mathrm{NO_2^-}$
R_3	nitrite oxidation	$\mathrm{NO_2^-} + \mathrm{H_2O} \rightarrow \mathrm{NO_3^-}$
R_4	denitrification	$\mathrm{orgN} + \mathrm{NO_3^-} \rightarrow \mathrm{N_2} + \mathrm{NH_4^+}$
R_5	anaerobic ammonium oxidation	$\mathrm{NH_4^+} + \mathrm{NO_2^-} \rightarrow \mathrm{N_2} + \mathrm{NO_3^-}$

Table S2.

Stoichiometric coefficients used in the model, normalized to the highest magnitude coefficient of each process for the equations listed in Table S1.

		Transformation process				
		R_1	R_2	R_3	R_4	R_5
Nitrogen species	NH_4^+	1	-1	0	0.1685	-0.7935
	NO_2^-	0	1	-1	0	-1
	NO_3^-	0	0	1	-1	0.2063
	orgN	-1	0	0	-0.1685	0.0079
	N_2-N	0	0	0	1	1.5455