



Supplementary Materials for

Organic Matter Stoichiometry, Flux, and Oxygen Control Nitrogen Loss in the Ocean

Andrew R. Babbin,* Richard G. Keil, Allan H. Devol, Bess B. Ward

*Corresponding author. E-mail: babbin@princeton.edu

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Materials and Methods

Sites and sampling

Sampling was conducted aboard the *R/V Thomas G. Thompson* in the ETNP in March – April 2012 (Fig. S1). Two sites were chosen for intense study: coastal (20.15°N, 106.00°W; water depth 2200 m) and offshore (16.53°N, 107.11°W; water depth 3600 m). Nitrite and ammonium concentrations were determined by standard photometric methods (31). Ammonium was always $< 20 \text{ nmol L}^{-1}$ within the oxygen deficient zone. Water for incubations was collected from Niskin bottles into 300 mL ground glass stoppered bottles and transferred into a N_2 flushed glove bag for manipulations.

Sinking particulate organic matter (POM) was collected at 70 m (coastal) and 105 m (offshore) using a NetTrap (32), a large diameter (~2 m), free-drifting sediment trap, based on the design of a closing plankton net, capable of collecting large amounts of sinking POM ($> 50 \text{ }\mu\text{m}$ in size) in relatively short time periods (24 – 48 h). Contents of the cod end were used as the POM amendment. The POM was analyzed for C/N using an EA-IRMS at the UC Davis Stable Isotope Facility (33, 34).

^{15}N tracer experiments

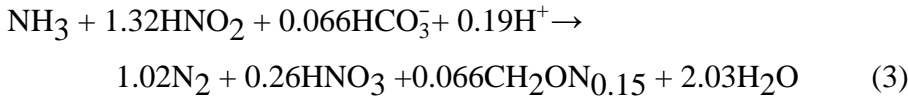
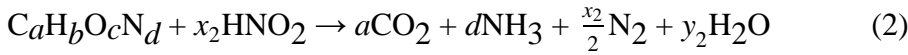
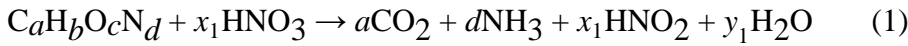
In addition to $3 \text{ }\mu\text{mol L}^{-1}$ (final concentration) ^{15}N -nitrite tracer, treatments for amendment experiments (Fig. 1, 2, Table 1) consisted of $3 \text{ }\mu\text{mol L}^{-1}$ ^{14}N -ammonium, $5.7 \text{ }\mu\text{mol C L}^{-1}$ (coastal) or $4.6 \text{ }\mu\text{mol C L}^{-1}$ (offshore) sinking POM, $37 \text{ }\mu\text{mol C L}^{-1}$ casamino acids, $29 \text{ }\mu\text{mol C L}^{-1}$ sucrose plus $3 \text{ }\mu\text{mol L}^{-1}$ ammonium, 3 or $8 \text{ }\mu\text{mol L}^{-1}$ O_2 plus $3 \text{ }\mu\text{mol L}^{-1}$ ammonium. All organic matter additions were either autoclaved (POM) or filter sterilized (sucrose and casamino acids; $0.2 \text{ }\mu\text{m}$ pore size). Furthermore, the POM was homogenized by pushing the solution repeatedly through a 23-gauge needle. The oxygen was added by equilibrating water from the same depth with air. 12 mL Exetainers (LabCo, UK) were filled with 8 mL of treatment-amended seawater for incubation and purged with He for 5 minutes. Air-equilibrated seawater was injected after He-purging using a gas-tight syringe. Similarly, a second set of experiments, where only $^{15}\text{NO}_2^-$ tracer (no $^{14}\text{NH}_4^+$) was added (Fig. 3B) was conducted using the same methods. Triplicate vials were poisoned with 50% (w/v) zinc chloride at 5 time points spanning 36-

48 hours after incubation at 10°C in the dark, and $^{29}\text{N}_2$ and $^{30}\text{N}_2$ accumulation were measured on a Europa 20/20 IRMS.

Denitrification rates were calculated from the accumulation of $^{30}\text{N}_2$ and anammox by using the binomial distribution (7). In parallel experiments encompassing the same depths, $^{46}\text{N}_2\text{O}$ reduction rates, representing denitrification, were also measured (Fig. S2). Because these rates were not significantly different from the denitrification rates calculated from $^{15}\text{NO}_2^-$ reduction rates reported here, we conclude that dissimilatory reduction of nitrate to ammonium did not contribute significantly to the $^{30}\text{N}_2$ accumulation.

Derivation of f_{amx} equation

To derive the balance between anammox and denitrification given organic matter of a certain composition, we assume that all organic nitrogen and the nitrate used to oxidize it are converted to N_2 gas. We begin with the stoichiometries of three anaerobic metabolisms: dissimilatory nitrate reduction to nitrite (DNRN), denitrification, and anammox, using generic organic matter composition $C_aH_bO_cN_d$ for the first two heterotrophic reactions (4), and an empirical (and therefore slightly imbalanced) reaction of anammox (5).



Where $x_1 = 2a + \frac{b}{2} - c - \frac{3d}{2}$ and $x_2 = \frac{2x_1}{3}$. Water coefficients y_i are calculated to balance H and O and are also dependent on the organic matter stoichiometry, but are unimportant for this derivation.

We first balance NH_3 by multiplying Equation 3 by d , or the amount of ammonium released by the remineralization of one organic matter unit. HNO_2 is balanced by splitting the amount of organic matter used in Equation 1 from Equation 2 through multiplying them by $(1-F)$ and F respectively. The nitrite balance then results:

$$(1-F)x_1 = F x_2 + 1.32d \text{ or } F = \frac{x_1 - 1.32d}{x_1 + x_2}.$$

This means that the amount of N_2 lost via denitrification is $\frac{x_2}{2} \left(\frac{x_1 - 1.32d}{x_1 + x_2} \right)$, but by substituting in with $x_i = x_i(a, b, c, d)$, $N_2^{denit} = 0.4a + 0.1b - 0.2c - 0.564d$.

As $N_2^{amx} = 1.02d$, and normalizing to organic carbon content of the organic matter,

$$f_{\text{amx}} = \frac{N_2^{\text{amx}}}{N_2^{\text{amx}} + N_2^{\text{denit}}} = \frac{1.02d}{0.4a + 0.1b - 0.2c + 0.456d}$$

$$= \frac{1.02 \text{ N/C}}{0.4 + 0.1 \text{ H/C} - 0.2 \text{ O/C} + 0.456 \text{ N/C}}$$

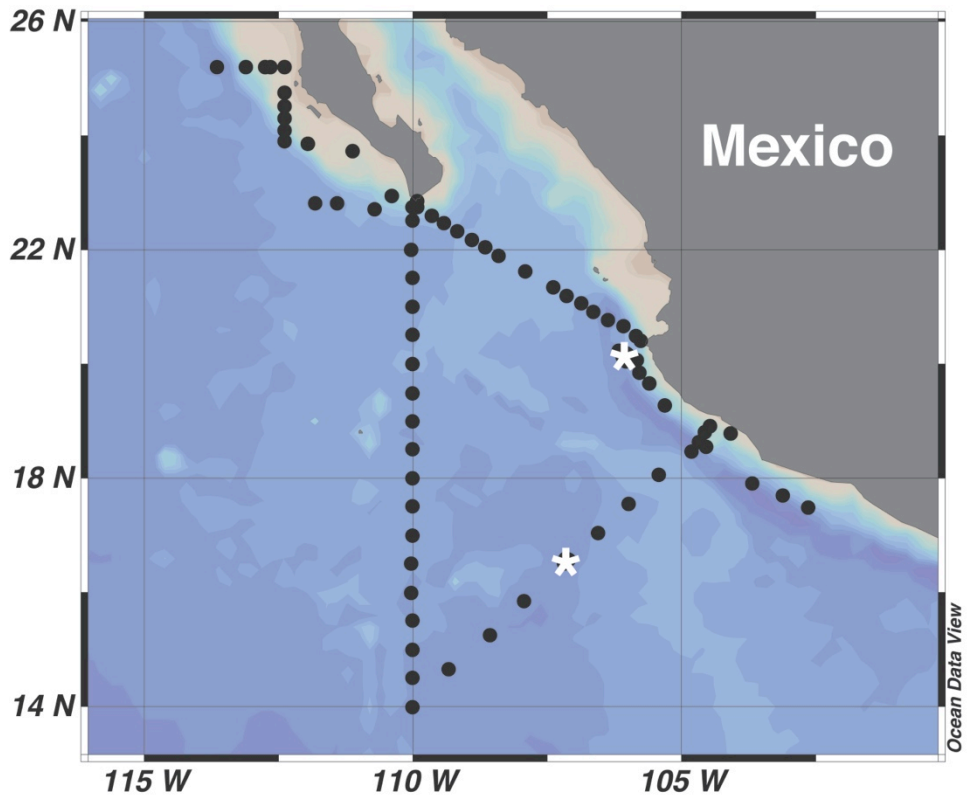
Next, by assuming oxidation states of H (+1), O (-2) and N (-3), we can calculate the oxidation state of C in the organic molecule:

$$C_{\text{ox}} = -\text{H/C} + 2 \text{ O/C} + 3 \text{ N/C}$$

This expression can lastly be substituted into the equation for f_{amx} , giving the final equation plotted in Figure 2:

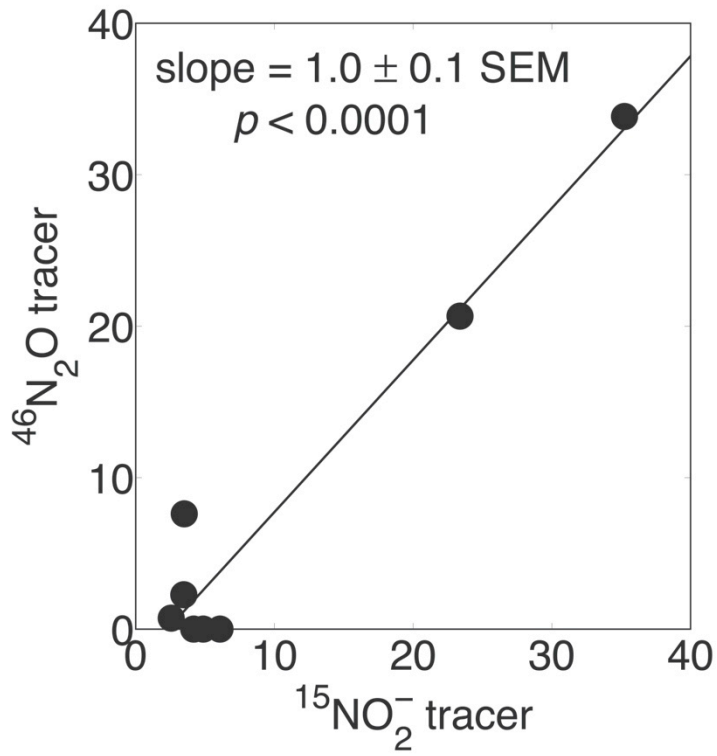
$$f_{\text{amx}} = \frac{1.02 \text{ N/C}}{0.4 - 0.1C_{\text{ox}} + 0.756 \text{ N/C}}$$

Fig. S1



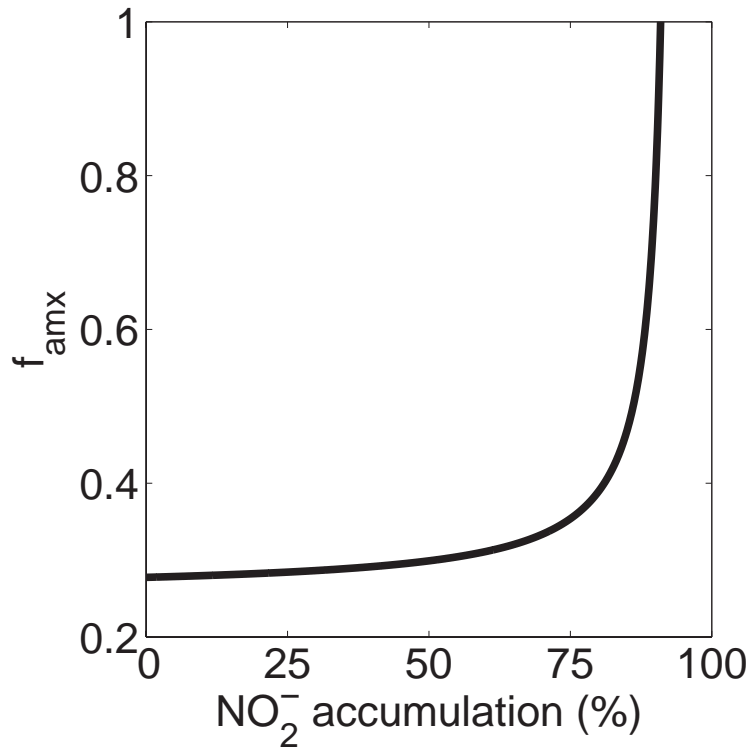
Cruise track of the *R/V Thomas G. Thompson* cruise number 278 (black circles). Asterisks denote the locations at which incubations reported here were performed.

Fig. S2



Rates of denitrification ($\text{nmol N L}^{-1} \text{d}^{-1}$) as measured by $^{15}\text{NO}_2^-$ and $^{46}\text{N}_2\text{O}$ tracers (no $^{14}\text{NH}_4^+$ addition) at the coastal station

Fig. S3



Changes to the fraction of nitrogen loss attributed to anammox given nitrite accumulation as a percentage of the net reduction from nitrate to N₂. The relationship breaks down at 91% accumulation, when there is not enough nitrite to oxidize ammonium via anammox and still have denitrification. This has been done for average marine POM stoichiometry (6).

Table S1.

Treatment	Coastal oxycline (60 m)	Coastal SNM (100 m)	Offshore oxycline (100 m)	Offshore SNM (150 m)
NH ₄ ⁺ only	4.7 / 3.1	2.6 / 3.3	0.8 / 0.4	2.1 / 0.9
Sucrose + NH ₄ ⁺	7.3 / 2.5	5.9 / 1.9	1.8 / 0.7	2.2 / 0.6
Casamino acids	9.3 / 7.4	9.2 / 8.6	1.6 / 1.1	3.7 / 2.7
Sinking POM	31.1 / 14.1	30.1 / 12.7	6.3 / 3.1	15.1 / 6.0
3 μmol L ⁻¹ O ₂ + NH ₄ ⁺	0.4 / 1.1	0.1 / 0.9	0.8 / 0.4	0 / 0.9
8 μmol L ⁻¹ O ₂ + NH ₄ ⁺	0 / 0.4	0 / 0	0 / 0	0 / 0

Measured rates of denitrification / anammox (nmol L⁻¹ d⁻¹) for each treatment at both stations at the depths of the oxycline and secondary nitrite maximum (SNM).

Table S2.

Property	Coastal site	Offshore site
Depth of deployment (m)	70	105
Time of deployment (hr)	24	48
Mass collected (mg m ⁻²)	88	87
Weight % organic C	27	22
Organic C sedimentation rate (mg m ⁻² d ⁻¹)	24	10
C/N (mol mol ⁻¹)	6.16	7.51

Properties of particles collected via sediment traps at the two sampling sites.

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