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

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Biologically available nitrogen limits photosynthesis in much of the world ocean. Organic matter (OM) stoichiometry had been thought to control the balance between the two major nitrogen removal pathways—denitrification and anammox—but the expected proportion of 30% anammox derived from mean oceanic OM is rarely observed in the environment. With incubations designed to directly test the effects of stoichiometry, however, we showed that the ratio of anammox to denitrification depends on the stoichiometry of OM supply, as predicted. Furthermore, observed rates of nitrogen loss increase with the magnitude of OM supply. The variable ratios between denitrification and anammox previously observed in the ocean are thus attributable to localized variations in OM quality and quantity and do not necessitate a revision to the global nitrogen cycle.

The processes that remove nitrogen (N) from the ocean are (i) anaerobic ammonium oxidation (anammox), the autotrophic oxidation of ammonium to N<sub>2</sub> by nitrite, and (ii) denitrification, a stepwise heterotrophic reduction of nitrate (the most abundant species of fixed N in the ocean) to N<sub>2</sub>. The existence of anammox was contemplated even before the anammox metabolism was discovered in bacteria (1, 2), based on the observation that ammonium, a by-product from the heterotrophic respiration of N-containing organic matter (OM), does not accumulate within strictly anoxic environments (3), such as the oxygen-deficient zones (ODZs). Because ammonium cannot be removed by oxidation via conventional aerobic nitrification in ODZs, the oxidation of the particulate organic matter (POM)-derived ammonium must occur through anammox. Building on previous work (4), we derived a balance between anammox and denitrification from generic OM stoichiometry rather than from average ocean POM by assuming (i) complete oxidation of POM to CO<sub>2</sub>, (ii) no accumulation of ammonium or nitrite, and (iii) that all ammonium liberated by the reduction of nitrate and nitrite must be oxidized via anammox in the absence of molecular O<sub>2</sub>. The equation stoichiometry is predicated on previous theoretical (4) and experimental (5) studies that determined the species coefficients, depending on the elemental composition of the POM (see the supplementary materials). From this theoretical balance, the fraction of total N loss via anammox is

$$f_{\text{amx}} = \frac{1.02 \frac{\text{N}}{\text{C}}}{0.4 + 0.1 \frac{\text{H}}{\text{C}} - 0.2 \frac{\text{O}}{\text{C}} + 0.456 \frac{\text{N}}{\text{C}}} \quad (1)$$

where the variables are the ratios of each of the major elements in the POM substrate with respect to C. From Eq. 1, it becomes apparent that the C/N ratio is the most important pa-

rameter in setting the balance, because (i) the N content is modified by the largest coefficient, and (ii) the H and O contents tend to compensate for each other. For the average labile POM stoichiometry in the ocean, C<sub>6.6</sub>H<sub>10.9</sub>O<sub>2.6</sub>N (6),  $f_{\text{amx}} = 28\%$ .

It is somewhat mysterious then that anammox and denitrification often do not appear to be stoichiometrically linked, given the generally conserved average composition (C/N = 6.6) of OM, but rather that one process or the other dominates the total N loss when measured discretely in space and time (7, 8). Spatiotemporal dynamics (7) and failure to include the effect of episodic POM supply in small-volume incubations are likely explanations, although so far unproven. Others have invoked alternative anaerobic mechanisms, such as dissimilatory nitrate reduction to ammonium (8), or benthic ammonium release (9), to provide ammonium for anammox in the absence of measurable denitrification, but these explanations cannot fully satisfy the anammox demand for ammonium. Recently, an extensive data set from the South Pacific showed that on a larger (areal) scale, anammox was 28% of the total N loss (10), as predicted (4) by the average composition of the OM (i.e., C/N = 6.6). However, most sites had low rates of anammox and even lower rates of denitrification, whereas a few discrete locations had very high denitrification rates. The balance between anammox and denitrification is important for understanding N cycling and the energy balance in suboxic zones, and because the two processes have different effects on C mineralization and on the production and consumption pathways of nitrous oxide, a major greenhouse gas and ozone-depleting agent (11), which is a significant intermediate in the denitrification pathway but not of anammox (12).

To investigate the roles of OM stoichiometry and O<sub>2</sub> concentration in controlling N loss in the Eastern Tropical North Pacific ODZ, we performed incubation experiments using isotopically labeled nitrite as a tracer and various OM treatments at the top of the ODZ and the secondary nitrite maximum of two stations, one coastal and one offshore (fig. S1). Anammox and denitrification

rates increased by different proportions in response to specific OM additions, with higher proportions of anammox corresponding to greater N relative to C content (i.e., a lower C/N ratio) of the source substrate (Table 1). Experimental treatments included sterilized substrate organic compositions of C<sub>9.7</sub>H<sub>17.8</sub>O<sub>8.9</sub>NH<sub>3</sub> for the sucrose and ammonium mixture, C<sub>3.6</sub>H<sub>5.7</sub>ON for the casamino acids, and C<sub>6.8</sub>H<sub>11.2</sub>O<sub>2.7</sub>N for the sinking particulate OM (from the average measured POM C/N, with an inferred C/H/O from average POM stoichiometry) (6). Based on experiments at both depths of each of the two stations (Fig. 1), the predicted anammox proportions of N loss of 22, 45, and 27% (Eq. 1), respectively, match the observed values quite closely (Fig. 2). These data confirm that the proportions of the two N loss processes depend on the stoichiometry of the source OM.

The addition of fresh OM-induced changes in observed anammox and denitrification rates, indicating that the total fixed N loss must have been prevailingly limited by OM supply, probably because any ambient dissolved OM before the addition was highly refractory (13). This is confirmed by the measured increase of N loss rates from the addition of OM, regardless of stoichiometry, in 11 of 12 experiments; relative to no OM addition, these rates were stimulated by factors averaging 1.4, 2.4, and 7.0 (Fig. 1) by the addition of sucrose plus ammonium, casamino acids, and sinking POM, respectively. Furthermore, the same anammox rates were observed in the sucrose additions, compared with the no OM-added treatments at the same depths, so ambient OM contributed no ammonium during the incubations. The collected sinking POM induced a much greater increase in the rates as compared with the sucrose and casamino acids treatments, despite there being a lower amount of organic C amended. This indicates that a mixture of diverse organic compound classes in naturally occurring OM is more beneficial to cell growth, perhaps by lowering the energy requirements to build and maintain cells, as compared with a pure carbohydrate or amino acid diet.

The depth distribution of total N loss rates at both stations (Fig. 3), determined from separate experiments with only labeled nitrite tracer added, further supports the link between in situ rates and OM flux. The measured rates decreased as a function of depth, in accordance with a simple power scaling law (14) with an exponent of -1.3. The total areal flux (obtained by integrating the rates with respect to depth) must thus vary as a function of depth with an exponent one unit higher, or -0.3. The value of -0.3 matches that of the sinking POM flux commonly measured in suboxic basins (compared with a global average of -0.8) (14-18). This remarkable correspondence indicates that denitrification is responsible for the organic C remineralization, which in turn drives N loss in the anoxic depths of the water column.

Dissolved O<sub>2</sub> concentrations themselves constrain the domain within the global ocean where N loss can occur, due to the preferential use of O<sub>2</sub> over nitrate as a terminal electron acceptor

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in the remineralization of OM and the regulation by  $O_2$  of the enzymes catalyzing N loss (19). Marine zones where denitrification and anammox can occur have been defined variously as waters with an upper  $O_2$  concentration of 2 to 20  $\mu\text{mol liter}^{-1}$  (20–23). Although this uncertainty in threshold may seem trivial, when integrated over the volume of the ocean, the total volumes of subthreshold water decrease greatly as  $O_2$  concentrations approach zero (24), and therefore the actual threshold for anaerobic processes has important implications for modeling the global N budget.

The effect of  $O_2$  on N loss rates was distinct and apparently immediate: The addition of 3 or 8  $\mu\text{mol liter}^{-1}$  of  $O_2$  with 3  $\mu\text{mol liter}^{-1}$  of  $NH_4^+$  resulted in insignificant N loss rates, suggesting that the  $O_2$  tolerance limit for environmental assemblages is similar to that inferred from cultured organisms (25). Low levels of anammox consistently occurred in the 3  $\mu\text{mol liter}^{-1}$  of  $O_2$  treatment but not at 8  $\mu\text{mol liter}^{-1}$ , whereas denitrification was not consistently detected with either 3 or 8  $\mu\text{mol liter}^{-1}$  of  $O_2$ . This  $O_2$  tolerance is consistent with results from (i) the Baltic

Sea (26), where an  $O_2$  level of 3  $\mu\text{mol liter}^{-1}$  inhibited anammox and denitrification by 90 and 100%, respectively; (ii) the Black Sea (21), where the anammox  $O_2$  tolerance was estimated to be between 8 and 13  $\mu\text{mol liter}^{-1}$ ; and (iii) the Eastern Tropical South Pacific (23), where samples from the core of the ODZ exhibited no anammox when amended with  $O_2$  greater than 3.5  $\mu\text{mol liter}^{-1}$ . The slight variation in the two  $O_2$  tolerances may allow anammox to be coupled to aerobic nitrification in water with low but nonzero  $O_2$ . But the magnitude of this difference and the steepness of the observed  $O_2$  gradient overlying the ODZ (4  $\mu\text{mol liter}^{-1}$  of  $O_2 \text{ m}^{-1}$ ) are such that the amount of anammox supported independently from denitrification in the ODZs must be negligible.

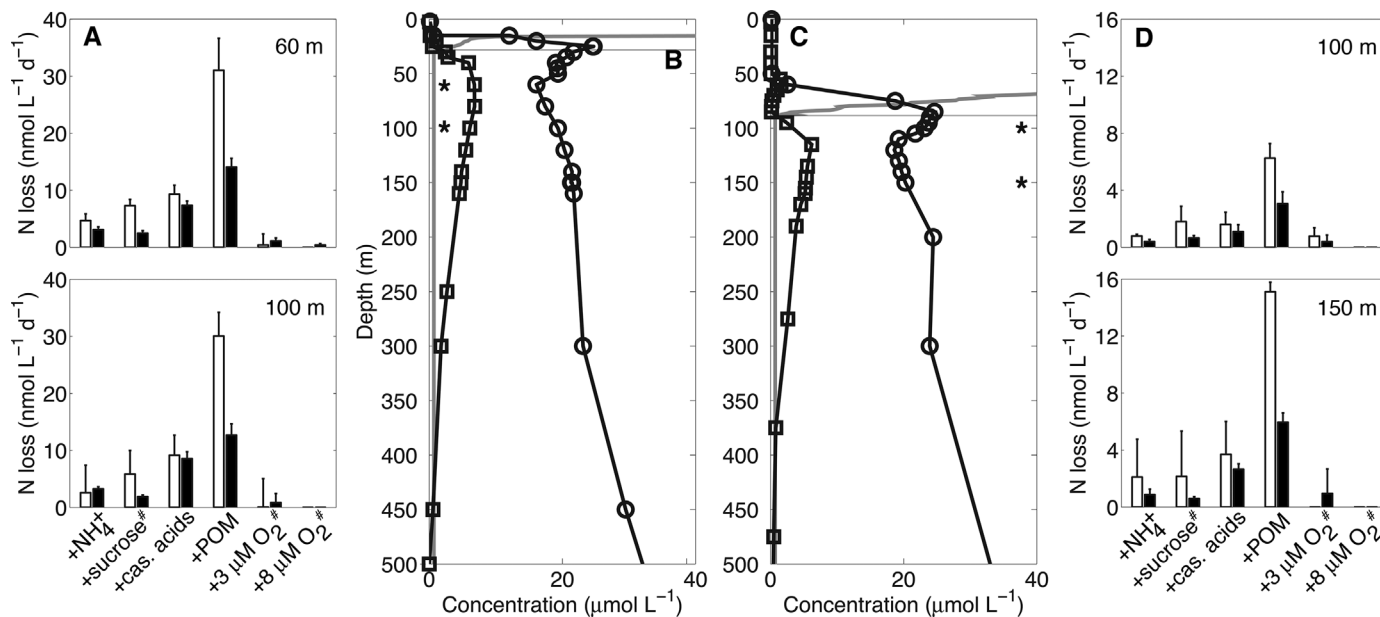
Rates of anammox and denitrification can be calculated based on the stoichiometry and rates of the OM flux, the exponent of the power law, and the depths bounding the anoxic zone. On the basis of this simple model, the particle fluxes of 24 and 10  $\text{mg of C m}^{-2} \text{ day}^{-1}$  measured in this study (Table S2) at the top of the coastal and off-

shore ODZs, respectively, should support local  $N_2$  production rates at the top of the ODZ of 17 and 4  $\text{nmol of N liter}^{-1} \text{ day}^{-1}$ . These rates derived from the measured POM flux are comparable to those measured in the small-vial incubations of 22 and 4  $\text{nmol of N liter}^{-1} \text{ day}^{-1}$  near the depths of sediment trap deployment, and are well within errors associated by bridging two highly disparate types of measurements. The direct agreement between the calculated and measured rates provides evidence that N gas production is causally related to the organic C sedimentation flux and confirms that this simple model may be useful to estimate N loss rates in ODZs. Furthermore, using C flux data from the Mexican margin (27), we can predict the associated N gas production that occurs by differentiating with depth the organic flux driving this oxidation (Fig. 3A). The  $N_2$  production rates implied by these organic C data correspond closely to our measured rates, with rates highest near the surface and decreasing via a power law scaling with depth (Fig. 3B).

In addition to providing an explanation for the relative rates of anammox and denitrification

**Table 1. Anammox percent for each OM treatment.** Anammox percentages are included for both sampling sites at the base of the oxycline and at the depth of the secondary nitrite maximum (SNM).

Treatment	OM stoichiometry	N/C	C oxidation state	Site				Average $\pm$ standard error
				Coastal		Offshore		
				Oxycline 60 m	SNM 100 m	Oxycline 100 m	SNM 150 m	
$NH_4^+$ only	—	—	—	39.8%	55.8%	33.5%	42.5%	$42.9 \pm 4.5\%$
Sucrose + $NH_4^+$	$C_{9.7}H_{17.8}O_{8.9}NH_3$	0.10	0	25.3%	24.5%	26.5%	21.8%	$24.5 \pm 1.0\%$
Sinking POM	$C_{6.8}H_{11.2}O_{2.7}N$	0.15	-0.41	31.2%	29.7%	32.8%	28.3%	$30.5 \pm 1.0\%$
Casamino acids	$C_{3.6}H_{5.7}ON$	0.28	-0.19	44.1%	48.3%	40.7%	41.7%	$43.7 \pm 1.8\%$



**Fig. 1. Measured N loss rates.** Biogeochemical parameters (center panels) measured at coastal (A and B) and offshore (C and D) sites. Nitrate (circles), nitrite (squares), and  $O_2$  (dark-gray line) concentrations are shown. The horizontal light-gray line indicates the depth of the onset of anoxia. Asterisks

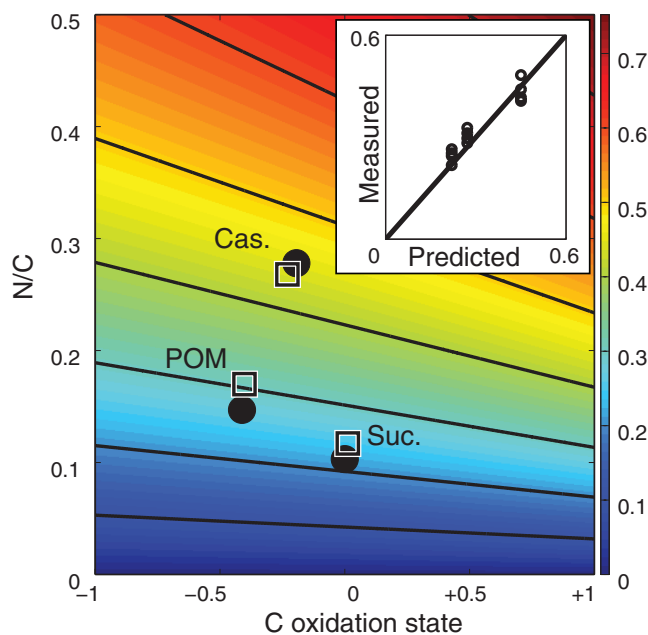
indicate depths where experiments were performed, and hash marks in (A) and (D) indicate additions supplemented by  $^{14}NH_4^+$ . Denitrification (open bars) and anammox (solid bars) rates are shown. Error bars indicate the standard deviation of rates derived from a linear fit to five time points measured in triplicate.

in ODZs and a quantitative relationship between N loss and the flux of OM, the results presented here also have the potential to explain much of the variability in the relative contributions of anammox and denitrification rates observed in the ODZs. Although the average OM C/N in the ocean is 6.6, large variations are observed, based on the phytoplankton community composition or the state of remineralization of the OM (14, 28). For instance, newly formed OM rich in amino acids can stimulate higher relative anammox rates due to the preferential remineralization of N-rich compounds (16). Older recalcitrant OM, poor in organic N, however, will result in a smaller proportion of N loss catalyzed by anammox bacteria. Yet, integrated over both space and time, the balance between anammox and denitrification must be constrained by the flux and C/N ratio of the OM in and out of the anoxic zone.

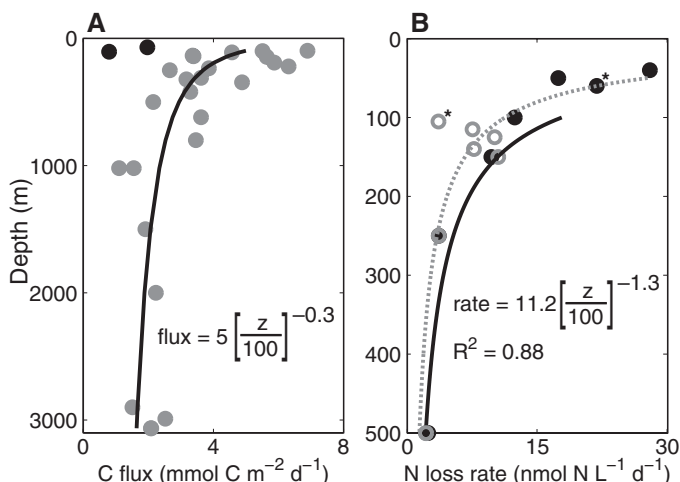
It is also worth noting that the occurrence of nitrite accumulation in the ODZs does not significantly alter the balance between anammox and denitrification, because of the requirement that a large percentage of nitrate reduction proceeds no further than nitrite before a noticeable enrichment in fraction anammox results (fig. S3).

Furthermore, these constraints have applicability to estimating future fixed N loss. The model developed here, predicting water column denitrification rates using POM fluxes and C/N ratios, can be integrated into global biogeochemical models to provide robust constraints on present and past fixed N losses in marine suboxic regions. It is additionally helpful in evaluating the effects of ODZ expansion on future climate (29), potential shifts in average C/N ratios with changing atmospheric CO<sub>2</sub> concentrations (30), and negative feedbacks to global primary production via N loss.

**Fig. 2. Theoretical  $f_{amx}$  from OM stoichiometry.** Fractions of N loss attributed to anammox ( $f_{amx}$ ) are calculated by a modified version of Eq. 1 (supplementary materials text) are shown in color contours. Cas, casamino acids; Suc, sucrose. Predicted (circles) and measured (squares)  $f_{amx}$  for each OM treatment are overlaid. The inset shows these values in comparison to the expected 1:1 line.



**Fig. 3. Organic C-dependence of N loss.** (A) Organic C flux previously measured (gray symbols) by benthic lander incubations (27) and collected in this study (black symbols) by sediment traps. The derived power law fit (black line) for the benthic lander study is included for reference. (B) Total N loss rates for both coastal (solid symbols) and offshore (open symbols) stations from +<sup>15</sup>NO<sub>2</sub><sup>-</sup>-only experiments. The power law best fit of these points (dashed gray line) and the theoretical rates (solid black line) driven by and derived from the organic C flux in (A) are also shown. Asterisks denote the rate experiments closest to the sediment trap deployment depths. z, depth; R<sup>2</sup>, correlation coefficient.



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**Acknowledgments:** We thank the captain and crew of the R/V *Thomas G. Thompson* for assistance in sampling; C. Buchwald and A. Morello for nutrient measurements; and B. X. Chang, D. Bianchi, and D. M. Sigman for useful discussions of this work. F. M. M. Morel provided invaluable advice in honing and improving this paper. The data presented in this paper are available in the supplementary materials. This study was supported by an NSF grant to A.H.D. and B.B.W. and a National Defense Science and Engineering Graduate Fellowship to A.R.B. A.R.B., A.H.D., and B.B.W. designed and conducted the experiments; R.G.K. collected and analyzed sediment trap material; and A.R.B. and B.B.W. wrote the manuscript with input from all authors.

Supplementary Materials

www.sciencemag.org/content/344/6182/406/suppl/DC1  
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11 November 2013; accepted 26 March 2014  
Published online 10 April 2014;  
10.1126/science.1248364