

New twist on nitrogen cycling in oceanic oxygen minimum zones

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Large uncertainties in the future of Earth's climate have intensified research on the global biogeochemical cycles on land and in the sea. The ocean ecosystem is critical in the carbon balance between the atmosphere and deep ocean, but also plays a large role in chemical and nutrient fluxes that maintain oceanic productivity and greenhouse gas fluxes to and from the atmosphere. Particularly relevant to ocean-atmosphere gas fluxes and control of nitrogen (N) availability are the ocean regions called oxygen minimum zones (OMZs). A few years ago, we believed that we knew the major components of the N cycle and were only challenged by how to extrapolate these processes to global scales. A new perspective on N cycling was initiated with the finding that a relatively newly discovered microbial transformation, anaerobic ammonia oxidation (anammox) (1) occurred in oceanic OMZs (2, 3) and was the major pathway for formation of N₂ in the Peruvian OMZ (4). This finding meant that previous notions of how, where, and when nitrogen was lost from the oceanic ecosystem had new uncertainties, not uncertainties of time and space, but of biological control of multiple competing biogeochemical pathways. In this issue of PNAS, Lam et al. (5) used a suite of approaches to tackle anammox in the Peruvian OMZ. They not only discovered how anammox requirements could be supported by other nitrogen transformations, but they added yet another nitrogen transformation to the oceanic nitrogen cycle mix: dissimilatory nitrate reduction to ammonia.

Anammox bacteria oxidize ammonia to obtain energy while simultaneously reducing nitrite as an electron acceptor, which results in the production of N₂ gas. This reaction leads to the loss of nitrogen from ecosystems similar to the better known "denitrification" reaction that also produces N₂ gas, but in the case of denitrification N₂ comes from reduction of oxidized nitrogen compounds coupled to the oxidation of organic matter ("heterotrophic denitrification"). Effectively, heterotrophic nitrate reduction to N₂ gas (or sometimes N₂O) and anammox are both denitrification reactions in the sense that they lead to the loss of fixed inorganic nitrogen from ecosystems to the atmosphere. The coupling of the loss of N from ecosystems to these 2 different pro-

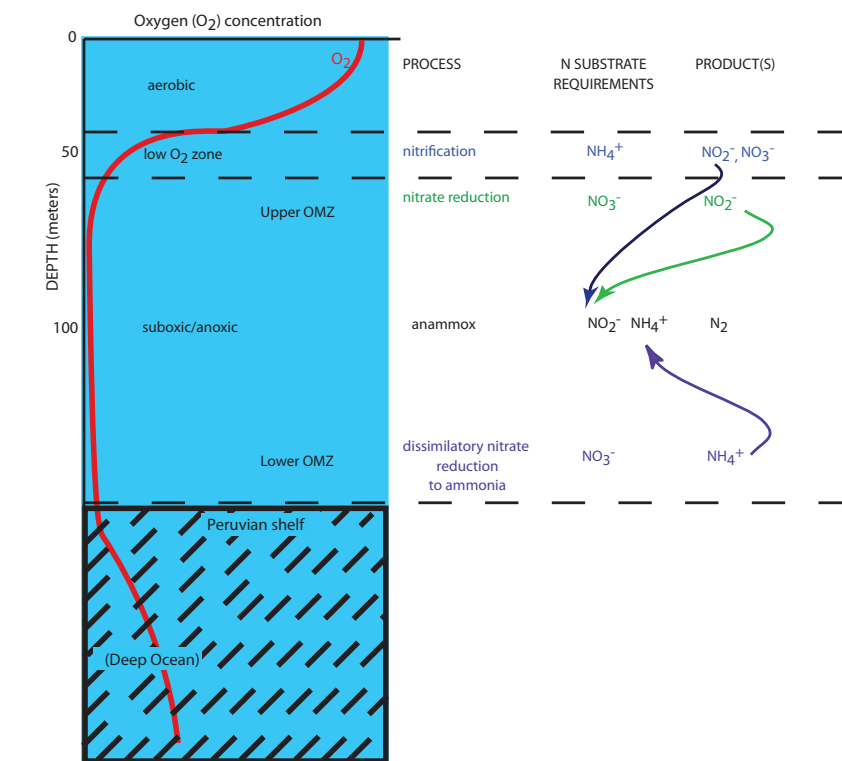


Fig. 1. Diagram of basic features of a typical OMZ, showing the zones of processes found to support anammox in the Peruvian upwelling zone (5). Denitrification is not shown because it was not significant in this system. The diagram shows how deep ocean water normally increases in oxygen concentration at depth (along with nitrate), but in this study the OMZ was underlain by sediments (cross-hatched area).

cess can have very different implications because of the difference in sources of substrates for the reaction, in other words, the source of the N that is lost from the ecosystem.

OMZs and sediment pore waters (or the bottom water near sediments) are the places in the oceans that provide the environmental conditions for denitrification and/or anammox, thus these regions are important in determining the overall balance of oceanic nitrogen gains and losses (6, 7). Disentangling the nitrogen cycle is difficult even in the surface waters of the oceans, but it is even more complex in the OMZs because of the multiple microbial transformations that occur (Fig. 1) and the complexity of the habitat itself. OMZ regions are formed by physical and biological phenomena that are dynamic and difficult to study. Although the definition of OMZs covers a range of oxygen concentrations (6), they generally have distinct vertical gradients of oxygen, which are associated with gradients in chemical spe-

cies of N compounds. The anammox conundrum arises because the concentrations of N compounds are not necessarily correlated with the N fluxes or transformations, making it difficult to associate compounds such as ammonium, which typically occur at very low concentrations, with processes such as anammox. To approach this problem, Lam et al. (5) used a combination of stable isotope pairing experiments and gene expression assays that made it possible to detect the organisms responsible for different microbial N transformations and the expression of genes that encode the enzymes for N transformations. This combination of biogeochemical and molecular biological approaches makes it possible to link observed biogeochemical transformations

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See companion article on page 4752.

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with the organisms and biochemical pathways responsible. This study (5) is one of a few that has shown the power of linking genes and gene expression to biogeochemistry. It undoubtedly will be a classic example of the application of molecular ecological approaches to marine biogeochemical cycles.

The simplified overall anaerobic ammonia oxidation reaction is $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}$ (8); anammox requires ammonium and nitrite and suboxic to anoxic conditions. This is the reason for the paradox of the anammox reaction in the marine environment. The conditions for anammox are rather unique, requiring an environment with a supply of ammonium and nitrite and low oxygen. Generally, nitrite is found in restricted zones within the ocean, because it is biologically active and quickly oxidized or reduced. Thus, in the complex OMZ regions it can be difficult to determine how an observed metabolism, such as anammox, is supported by the required substrates, given the different sources and fluxes of compounds in the complex gradients of the OMZ (Fig. 1). Lam et al. (5) were able to determine the sources of substrates for anammox by labeling NH_4^+ and NO_3^- with the stable isotope ^{15}N and examining the $^{15}\text{N}/^{14}\text{N}$ ratio in various products, including N_2 . They found several surprising things. First, a process called nitrate reduction (without full reduction to the gaseous products N_2 or N_2O) was a major process in the OMZ. Although commonly known in the microbiological literature, this process was not fully recognized as a major pathway in marine, in particular the oceanic, habitats. It could account for a major fraction of the NO_2^- needed to support anammox. Second, a process unrecognized except in sediments and more recently in bottom waters, called dissimilatory nitrate reduction to ammonium (9), was also detected in the Peruvian OMZ and could explain a large fraction of the ammonium flux necessary for anammox activity in the Peruvian shelf OMZ waters.

Some of the most important implications of the Lam et al. (5) study are not the findings with respect to anammox or nitrogen cycling within the OMZ, but the

broader nutrient balancing effects on the ocean environment. It was estimated that much ($\approx 50\%$) of the source of substrates for the anammox reaction (ammonium and nitrite) was derived from N recycled within the OMZ waters itself, instead of from the deep nitrate-rich water beneath. This finding has implications for the cycling of N, but more importantly for the cycling of N relative to other nutrients (P and Fe) needed for growth in the mixed layer. Biological growth, fueled by photosynthesis in marine unicellular algae (phytoplankton), depends on the supply of elements needed for growth in the stoichiometric ratios needed for biomass. These nutrients are lost to the deep ocean and recycled by physical upwelling or advection of deepwater. Thus, processes such as anammox or denitrification that remove N preferentially to other nutrients lead to the limitation of growth by N and can lead to a selection for N_2 -fixing microorganisms (10). Deutsch et al. (10) hypothesized that N_2 fixation should be coupled to OMZs for this reason. The finding that most of the N that is lost from the ecosystem is lost from N that is recycled within the OMZ has implications for the nutrient ratios in OMZ water that supports surface water phytoplankton production.

This study represents one of the most comprehensive N-cycle investigations in the OMZs, but questions remain. This and related studies in the Peruvian upwelling zone present convincing evidence for the dominance of anammox over the heterotrophic denitrification pathway, but it may still ultimately be found that anammox does not dominate in all suboxic regions (11). The spatial and temporal variability of microbial processes in dynamic ocean systems is difficult to assess, given the logistics of ocean sampling. It may yet be demonstrated that there is an interchange between roles of heterotrophic denitrification and anammox (12). If anammox is the major pathway for N loss, then the pathways for organic matter degradation in OMZs need to be re-evaluated. Perhaps the most intriguing questions relate to how the exchange of anammox for canonical denitrification

affects the nutrient and energy balance of the sea: anammox organisms fix CO_2 while removing N, whereas heterotrophic denitrifiers metabolize organic carbon (releasing CO_2).

Even with the elegant measurements of isotope uptake and gene expression used in this study, there are still details to be resolved. Isotope additions often cannot be at tracer concentrations, and thus, can stimulate the rates of microbially-mediated reactions. Gene expression is an excellent way of determining that microorganisms are actively using the genes that encode enzymes, but there is a fundamental cellular disconnect between gene transcription and enzyme activity. We simply do not know the regulatory pathways and the genetic variability in gene expression to be able to hope to relate gene expression to metabolic activity.

With certain large-scale perturbations of atmospheric gases and resultant perturbations of Earth's gas-liquid biosphere, the fluxes of chemical components relevant to physical and biological processes are critical to identify and quantify. OMZ regions are believed to be expanding (13), volume and effect on the N cycle have likely been underestimated (6), and N_2 formation in these areas may be of the same order of magnitude as sediment activity (7). There are large uncertainties in the balance of N production (N_2 fixation, the reduction of gaseous dinitrogen, N_2 , gas to biologically available ammonium) and N losses (return of N to gaseous states N_2 or N_2O) (7, 14), and human activities have significantly perturbed the global N cycle (14). In the past decade, partially because of new technologies including molecular, genomic, and isotopic techniques, new discoveries of N cycling have been made at an increasingly rapid pace (1, 15–17). These discoveries are exciting, and we have a new appreciation for the nitrogen cycle, but the new information also presents new challenges for understanding how the nitrogen cycle operates, has operated, and will operate in the future (7). There is still much to learn about the N cycle of the oceans. Undoubtedly, more new discoveries are on the horizon.

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