COMMENTARY

Migrant solution to the anammox mystery

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Every night a massive migration takes place in ocean waters. Zooplankton and micronekton, a diverse group of organisms <1 mm to 10 cm in size, swim up from midwater depths (200–700 m) to the surface ocean to feed and return down to resting depths before daybreak. The importance of this diel vertical migration to marine biogeochemical cycles has been recognized for some time. Research has primarily focused on the migrant-mediated movement of elements including nitrogen (N) from the surface ocean to midwaters. Such an "active transport" involves feeding at night in the upper ocean and the consequent excretion of ammonium (1) and dissolved organic N (2, 3) at midwater depths during the day. However, within the midwater realm, the effect of such migratory behavior on elemental cycles is less well understood. This "twilight zone" hosts many of the reactions that drive global biogeochemical cycles, including the remineralization of particulate organic matter into nutrients such as nitrate and the production of dinitrogen (N_2) gas. In PNAS, Bianchi et al. (4) significantly advance our knowledge of the role of animals in midwater biogeochemistry by linking the activity of migrant zooplankton and micronekton to an important N transformation pathway, anammox.

Anammox and Ocean Nitrogen Sinks

Anammox (anaerobic ammonium oxidation with nitrite) refers to the conversion of ammonium and nitrite into N_2 gas, a process mediated by bacteria that are obligate anaerobes. Originally thought confined to wastewater treatment plants, anammox activity was more recently found to be significant in marine waters where poor ventilation and high levels of respiration result in severe oxygen depletion (5, 6). The documentation of this activity was a revelation for the scientific community. Previously, the major considered sink for N in such systems was denitrification, the oxidation of organic matter using nitrate as an electron acceptor. In this process, also mediated by marine microbes, nitrate is converted to nitrite and, ultimately, N_2 gas, which can then escape to the atmosphere. However, several lines of evidence point toward anammox as a major driver of N loss in the marine environment. In the first studies, "ladderane" lipids, a unique component of the membrane surrounding the anammoxosome (the compartment in anammox cells where ammonium is converted to N_2), and cells labeled with an anammox-specific oligonucleotide probe were found in suboxic waters (6). At the same time, anammox activity was demonstrated using ^{15}N isotope labeling techniques, i.e., with the addition of ^{15}N-labeled ammonium to incubations of anoxic water samples (5, 6). In these experiments, significant levels of $^{14}N^{15}N$ (i.e., N_2) gas were produced, indicating the added [^{15}N]ammonium had reacted with [^{14}N]nitrite with typical anammox stoichiometry. Such ^{15}N-labeling experiments have been repeated in suboxic and anoxic marine systems ranging from a coastal bay (Golfo Dulce, Costa Rica) to the ocean's major oxygen minimum zones (OMZs) in the Arabian Sea and eastern tropical Pacific and indicate the contribution of anammox can range from 6% to more than 80% of total N_2 production (5, 7–10). Thus, anammox, in addition to denitrification, is a major removal mechanism for fixed N (ammonium, nitrate, and nitrite) and contributes to the balance between marine N sources and sinks on a global scale.

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The Anammox Conundrum

Despite recognition of the importance of anammox to global biogeochemical cycles, a major conundrum soon became apparent to researchers. In many anoxic marine systems, ammonium concentrations in the water column are very low, and thus substrate (ammonium) availability has become a major question. Studies to date have suggested the coupling of anammox with N transformation processes that produce ammonium. Oxidation of organic matter via denitrification results in the production of both ammonium and nitrite, for example, which can further combine through anammox (5). Based on the stoichiometry of this reaction, anammox would be expected to contribute ~30% of total N_2 production, with denitrification accounting for the other 70%. Other pathways of ammonium production prevalent in anoxic waters include dissimilatory nitrate reduction to ammonium (DNRA) (9) and the remineralization of organic matter linked to sulfate reduction (11). However, evaluation of these alternate processes in several OMZs indicate insufficient production of ammonium to account for measured anammox rates (9, 11). Recently observed patchiness in the distribution of denitrification indicates this process could still play a significant role in ammonium production when averaged over large temporal or spatial scales (8). However, the source of ammonium for anammox in many systems has remained elusive (11).

A Migrant Animal Solution

All of the N transformation processes described above are tied to the activity of marine microbes, which until now have been thought to dominate N cycling in anoxic marine waters. However, Bianchi et al. (4) provide evidence that animals play a significant but unacknowledged role in the N dynamics of ocean OMZs and specifically could augment anammox in these regions. The authors focus on migrant zooplankton and micronekton, which in OMZs move between the surface ocean at night and resting depths within anoxic or nearly anoxic midwaters during the day (12). The major excretory product of these animals is ammonium, and Bianchi et al. (4) show that the contribution of this substrate is large enough to significantly enhance N_2 production by anammox compared with denitrification. Highlighted in this study is the importance of migrant behavior and our relatively poor

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understanding of migrant activity in midwaters. For example the exact depth at which migrators release ammonium will affect the contribution of anammox to N_2 production (4), but due in part to the limited sampling of migrant communities in midwaters, the vertical distribution of migrant metabolism is not well known. Similarly, a key parameter in the Bianchi et al. (4) model is the ratio of migrant N excretion (active transport) to total N entering the midwater zone (i.e., active transport + N sinking into this realm as particulate organic matter). Although current studies indicate a value of 0.2–0.4 (13), change in the composition of surface ocean plankton and migrant animal communities likely drives spatial and temporal variability in this ratio. Research on the scales needed to evaluate this variability is rare; however, given the demonstrated importance of migrant metabolic activity to N and other geochemical cycles (2–4, 13), it is urgently needed.

The findings of Bianchi et al. (4) support a unique role for migrant zooplankton and micronekton in the global N cycle, specifically in ocean OMZs. Multidecadal time series indicate these major zones of anoxic waters have expanded (14), and climate models suggest a further decline in ocean oxygen concentrations with global warming (15). The observed changes include not only decreasing oxygen levels in OMZ cores, but vertical expansion at the upper and lower boundaries. The implication is that migrant animals could spend more time in anoxic or nearly anoxic waters while swimming down to their daytime resting depths (12), potentially further enhancing their role in anammox and the marine N cycle. The effect of this expansion on migrant community structure and activity, with associated geochemical feedbacks, represents an additional unknown. Moreover, the importance of migrant–microbe dynamics is not limited to OMZs, but relevant throughout the ocean's deep midwaters where migrant-mediated introduction of metabolic waste products can be significant relative to organic inputs from sinking particles. Such presumably commensal migrant–microbe relationships are not well documented but must be addressed in future large-scale efforts targeting twilight zone processes and their impact on ocean biogeochemistry.

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