Elusive marine nitrogen fixation

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Every year, the ocean is losing more than 200 Tg N from its bioavailable nitrogen pool through denitrification processes: that is, all of the processes that convert fixed nitrogen to nitrogen gas (1). If this loss wasn't compensated by N-inputs, the ocean would quickly lose its fertility, because fixed nitrogen is the most important nutrient fueling phytoplankton growth in the ocean (2). Thanks to the N_2 -fixers, a special group of marine plankton that live in the sunlit upper ocean and that are able to convert dissolved nitrogen gas into bioavailable nitrogen such as ammonium and organic nitrogen, a substantial part of this loss is compensated for (3). A question that has fascinated oceanographers for decades (4, 5) is how denitrification and N_2 fixation are coupled to each other; that is, how the ocean is ensuring that these two major sink and source processes are "dancing" with each other in step. Without such synchronization, the ocean would have experienced large swings in its fixed nitrogen inventory, potentially causing major disruptions to ocean life, something that Earth has not seen in its recent history. One hypothesis is that marine N_2 fixation occurs in close spatial association with the major regions of marine denitrification, such as the eastern tropical North and South Pacific (6) (Fig. 1). However, until recently, no one has looked for N_2 fixation in these regions (7), largely because this was against the prevailing view that N_2 -fixers thrive mostly in regions where their high iron demand is met by sufficient Fe deposition from the atmosphere, such as the subtropical North Atlantic (8). Knapp et al. (9) now report on the results from their journey to one of these major denitrification regions. Their goal was to find the purported high levels of N_2 fixation associated with these regions, and to test the hypothesis of the close spatial association between N_2 fixation and denitrification.

In 2010 and 2011, Knapp et al. (9) undertook two cruises to the eastern tropical South Pacific, one of the most intense oxygen-deficient zones of the world's ocean, which alone is responsible for about 40% of global water column denitrification (10). In the eastern tropical South Pacific, oxygen drops to very low oxygen levels (less than 1% of saturation) already at

depths as shallow as 100 m, and the ocean remains virtually oxygen-free down to several hundred meters depth, thus creating the conditions under which the denitrifiers thrive. Most of these organisms are heterotrophs that have the ability to switch from oxygen to nitrate as the oxidant to remineralize the organic matter that is being transported there from the surface ocean (1). Additional denitrification can occur through anaerobic ammonium oxidation (anammox) (1). Both processes lead to waters that are highly deficient in the concentration of nitrate relative to that of phosphate, although both nutrients remain abundant. When these waters come to the surface, they first induce a bloom of regular phytoplankton that consume nitrate and phosphate roughly with the Redfield ratio of 16:1 (3). This usually continues until all nitrate is exhausted, leaving an excess of phosphate behind. Knapp et al. (9) targeted these nitrate-poor but phosphate-rich surface waters because they should provide the ideal niche for N_2 -fixers. Under these conditions, the N_2 -fixers have ample light, which they need for photosynthesis, and thanks to regular phytoplankton being hampered in their growth—because of their being limited by nitrate availability—the N_2 -fixers have limited competition for the phosphate (and other nutrients, such as iron) they still need to take up from the water to fuel their growth.

During their two cruises, Knapp et al. (9) measured at a total of six stations the rates of N_2 fixation, as well as the contribution of the newly fixed nitrogen to the total export of organic nitrogen. The authors used a broad set of methods, including geochemical mass balances on the basis of the natural abundance of ^{15}N , direct incubation experiments with ^{15}N -labeled N_2 , and genomic analyses, with an emphasis on the quantification of the major genes encoding nitrogen fixation. Each of these methods has its strengths and weaknesses. The ^{15}N mass balance approach integrates N_2 fixation in time and space, and is therefore well suited to smooth out any short-term spatial and temporal variability. However, it estimates only the fraction of the exported organic nitrogen stemming from N_2 fixation, requiring an independent estimate of the magnitude of export production to convert this

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Fig. 1. Map of vertically integrated N_2 -fixation rates in the subtropical and tropical Pacific and western Atlantic ($\mu\text{mol m}^{-2} \text{d}^{-1}$). The color shading in the background are N_2 -fixation estimates inferred from surface nutrients through an inverse modeling approach (6). The rectangles show a gridded synthesis of directly measured N_2 -fixation rates (7), whereas the circles indicate the new measurements added by Knapp et al. (9). The green shaded areas denote the oxygen-deficient zones of the eastern tropical North and South Pacific, where the majority of the global ocean water column denitrification is taking place. Although the inverse modeling results would have suggested a close spatial association between N_2 fixation and denitrification in the eastern tropical Pacific, the new results by Knapp et al. (9) do not support this conclusion.

fraction into a N_2 -fixation estimate. Its application requires many assumptions: for example, steady-state, limited horizontal mixing and low export of dissolved organic nitrogen, as well as a good estimate of the ^{15}N content of the nitrate that is being supplied into the region of interest. As a result of these uncertainties, the ^{15}N mass balance approach has a detection limit that is quite high, preventing it from detecting pervasive, but low levels of N_2 fixation. Direct estimates provide relatively accurate point measurements of the biomass and rates of N_2 fixation, but due to their very limited number, the conclusions drawn from them are highly sensitive to unrecognized spatial and temporal variations.

Irrespective of the methods, Knapp et al. (9) found at all stations undetectable-to-low rates of N_2 fixation. With the isotopic mass balance approach, they were able to detect N_2 fixation at only two of the six stations and the rates ranged from 0 to $23 \mu\text{mol N m}^{-2} \text{d}^{-1}$ when they used the sediment trap fluxes to multiply the fractional estimates of nitrogen fixation to obtain rates of N_2 fixation. Higher rates would be obtained if alternative export estimates were used, pushing the upper range to values of $200 \mu\text{mol N m}^{-2} \text{d}^{-1}$ or more. However, these alternative export estimates are fraught with high uncertainties. Nevertheless, the direct incubation experiments also tended to give higher N_2 -fixation rates than the mass balance values suggest. In particular, the direct measurements showed measurable rates at all stations, yielding on average rates of about $50 \mu\text{mol N m}^{-2} \text{d}^{-1}$. Although sizeable, all of these rates are much lower than what is generally observed in the subtropical North Atlantic (order of $200 \mu\text{mol N m}^{-2} \text{d}^{-1}$ and more); that is, in a region far away from the place that generates the nitrate deficits thought to stimulate N_2 fixation (Fig. 1). The estimates are also much smaller than N_2 -fixation rates inferred from surface nutrients through an inverse modeling approach (6): that is, from the study that gave rise to the spatial-coupling hypothesis. Thus, the new data by Knapp et al. (9) put the hypothesis of a close spatial coupling between N_2 fixation and denitrification into serious question.

However, before fully rejecting this hypothesis, it is worthwhile to consider potential reasons for this discrepancy. The high level

Accepting for the moment that N_2 fixation is low in the nitrate-poor and phosphate-rich waters of the eastern tropical South Pacific, the question emerges: what prevents the N_2 -fixers from excelling there? Knapp et al. (9) suggest that the N_2 -fixers are limited by iron, which is scarce in this part of the ocean as a result of very low atmospheric input. This is a very feasible explanation, given the substantial iron requirements of the N_2 -fixers, but Knapp et al. did not make the necessary measurements and incubation experiments to support this statement. If iron was indeed the missing ingredient in the eastern tropical and subtropical Pacific, then one expects the western subtropical and tropical Pacific (i.e., the regions closer to the continental sources of iron) to host the majority of the N_2 -fixers in the Pacific. More recent model simulations and the available N_2 -fixation rate observations indeed support such a distribution, but the available data are too sparse to constrain this statement well.

Thus, if N_2 fixation and denitrification are, after all, spatially much more separated than hypothesized, then our quest to

understand the synchronization between the two dance partners (i.e., how N_2 fixation and denitrification are coupled to each other to ensure a global homeostasis of the marine nitrogen cycle) remains unsolved. Although laboratory studies are very important to better understand the factors that control the growth of individual N_2 -fixers, it is only a vast increase in the coverage of marine N_2 -fixation measurements that will permit us to better determine how nitrogen fixation is controlled in the environment. Thus, the search for the elusive N_2 -fixers needs to continue. The journey remains exciting, though, because the range of marine organisms that have the capability to fix nitrogen is rapidly expanding and already spans several clades, including symbiotic and parasitic organisms, as well as heterotrophs (12). At the same time, the marine nitrogen cycle is becoming an ever faster moving target of study. Ocean warming, ocean acidification, and deoxygenation, together with a massive increase in the input of fixed nitrogen through rivers and the atmosphere, are pushing the marine nitrogen cycle and its organisms strongly out of balance (5).

- 1 Devol AH (2008) Denitrification including Anammox. *Nitrogen in the Marine Environment*, eds Capone DG, Bronk DA, Mullholand MR, Carpenter EJ (Academic, San Diego), pp 263–301.
- 2 Moore CM, et al. (2013) Processes and patterns of oceanic nutrient limitation. *Nat Geosci* 6:701–710.
- 3 Gruber N (2008) The marine nitrogen cycle: Overview and challenges. *Nitrogen in the Marine Environment*, eds Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (Academic, San Diego), pp 1–50.
- 4 Redfield AC (1958) The biological control of chemical factors in the environment. *Am Sci* 46:205–221.
- 5 Gruber N, Deutsch CA (2014) Redfield's evolving legacy. *Nat Geosci* 7:853–855.
- 6 Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP (2007) Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 445(7124):163–167.
- 7 Luo Y-W, Lima ID, Karl DM, Deutsch CA, Doney SC (2014) Data-based assessment of environmental controls on global marine nitrogen fixation. *Biogeosciences* 11:691–708.
- 8 Falkowski PG (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. *Nature* 387:272–275.
- 9 Knapp AN, Casciotti KL, Berelson WM, Prokopenko MG, Capone DG (2016) Low rates of nitrogen fixation in eastern tropical South Pacific surface waters. *Proc Natl Acad Sci USA* 113:4398–4403.
- 10 Deutsch CD, Gruber N, Key RM, Ganachaud A, Sarmiento JL (2001) Denitrification and N_2 fixation in the Pacific Ocean. *Global Biogeochem Cycles* 15:483–506.
- 11 Altabet Ma, et al. (2012) An eddy-stimulated hotspot for fixed nitrogen-loss from the Peru oxygen minimum zone. *Biogeosciences* 9:4897–4908.
- 12 Sohm JA, Webb EA, Capone DG (2011) Emerging patterns of marine nitrogen fixation. *Nat Rev Microbiol* 9(7):499–508.