Marine microorganisms and global nutrient cycles

Kevin R. Arrigo[1]

The way that nutrients cycle through atmospheric, terrestrial, oceanic and associated biotic reservoirs can constrain rates of biological production and help structure ecosystems on land and in the sea. On a global scale, cycling of nutrients also affects the concentration of atmospheric carbon dioxide. Because of their capacity for rapid growth, marine microorganisms are a major component of global nutrient cycles. Understanding what controls their distributions and their diverse suite of nutrient transformations is a major challenge facing contemporary biological oceanographers. What is emerging is an appreciation of the previously unknown degree of complexity within the marine microbial community.

To understand how carbon and nutrients, such as nitrogen and phosphorus, cycle through the atmosphere, land and oceans, we need a clearer picture of the underlying processes. This is particularly important in the face of increasing anthropogenic nutrient release and climate change. Marine microbes, which are responsible for approximately half of the Earth's primary production, play an enormous role in global nutrient cycling.

In this review, I will highlight the four exciting aspects of marine microbial ecology that are receiving a great deal of attention and may prove to be crucial to a revised understanding of marine and global nutrient cycling. The first involves the explanations for and consequences of the variable nutrient stoichiometry of phytoplankton. The second is the emerging concept that phytoplankton growth can be limited by more than one resource. The third concerns the upward revision of estimates of marine nitrogen fixation, and the fourth is the discovery that fixed nitrogen in the ocean can be lost through anaerobic ammonium oxidation (anammox) reactions. Although distinct, these topics all represent examples of the marine microbial community modulating the coupling between the cycles of nutrients and carbon. Consequently, they all have the capacity to fundamentally alter our perceptions of global nutrient cycles and their response to environmental change.

Non-Redfield behaviour of phytoplankton

In the early part of the twentieth century, Alfred Redfield noticed that the elemental composition of plankton was strikingly similar to that of the major dissolved nutrients in the deep ocean[1]. On the basis of these observations, Redfield proposed that the nitrate:phosphate (NO_3:PO_4) ratio of 16:1 in the sea was controlled by the requirements of phytoplankton, which subsequently release nitrogen (N) and phosphorus (P) to the environment at this ratio as they are broken down (remineralized). Redfield's initial observations have been confirmed numerous times, and the notion of a 'Redfield ratio' describing the stoichiometry of both phytoplankton and seawater remains a fundamental tenet shaping our understanding of marine ecology, biogeochemistry and even phytoplankton evolution. The Redfield ratio has been extended to include other elements, most notably carbon (C), and it links these three major biogeochemical cycles through the activities of marine phytoplankton.

Unfortunately, a clear mechanism explaining the observed magnitude of the Redfield C:N:P ratio of 106:16:1 for either phytoplankton or the deep ocean has been elusive. It has long been recognized that conditions exist under which phytoplankton stoichiometry diverges from the canonical Redfield ratio. Furthermore, a number of processes drive oceanic nutrient inventories away from the Redfield ratio, including changes in exogenous nutrient delivery[2] and microbial metabolism[3] (for example, nitrogen fixation, denitrification and anammox, see below). These processes are sometimes manifested as variations in N*, a measure of the degree of N deficit or excess relative to P for a given water mass[4]. What governs variations in phytoplankton nutrient stoichiometry, and, given that variation, why is the Redfield N:P ratio observed in the deep ocean so universal?

At the most basic level, the C:N:P stoichiometry of extant phytoplankton reflects the elemental composition retained from their early evolutionary history[5]. In the case of eukaryotic phytoplankton, the two major superfamilies differ markedly in their cellular C:P and N:P ratios, with the green superfamily exhibiting significantly higher ratios than the red (green, C:P $\approx$ 200 and N:P $\approx$ 27; red, C:P $\approx$ 70 and N:P $\approx$ 10). However, all observed C:N:P stoichiometries cannot be explained by the evolutionary lineage of an organism. The highly dynamic stoichiometry often exhibited by unicellular algae reflects their ability to store nutrients in internal pools, switch between enzymes with different nutrient requirements and modify osmolyte composition[6,7]. Lower-frequency variations in C:N:P stoichiometry are related to changes in the structural elements of the phytoplankton cell. A major breakthrough in our understanding of cellular C:N:P stoichiometry came with the realization that different cellular components have their own unique stoichiometric properties. Most notably, resource (light or nutrients) acquisition machinery, such as proteins and chlorophyll, is high in N but low in P, whereas growth machinery, such as ribosomal RNA, is high in both N and P[8,9]. Because these components make up a large proportion of cellular material, changes in their relative proportions have a marked effect on bulk cellular C:N:P stoichiometry. Why then might the proportions of these components change?

[1]Department of Geophysics, Stanford University, Stanford, California 94305-2215, USA.

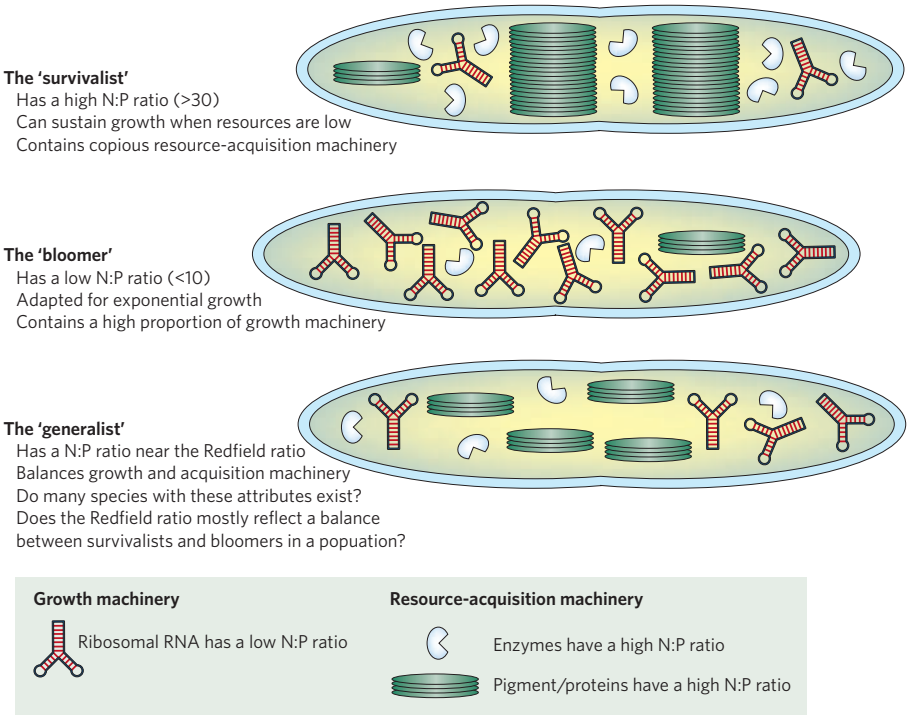


Figure 1 | Three different phytoplankton growth strategies and their resulting cellular N:P ratios. The allocation of resources and resulting N:P ratios for the 'survivalist' and the 'bloomer' are from the optimization model of Klausmeier *et al.*[10]. Interestingly, the model does not predict an optimal N:P ratio of 16 (our hypothetical 'generalist') under any of the environmental conditions tested. This indicates that the Redfield N:P ratio of 16 observed in nature is simply an average value that reflects an ecological balance between the 'survivalists' and 'bloomers' in a population.

This question was elegantly addressed recently using an optimization model[10] based on relative shifts in the cellular machinery described above (Fig. 1). It predicts that during exponential growth, bloom-forming phytoplankton optimally increase their allocation of resources toward production of growth machinery, reducing their N:P ratio to ~8, far below the Redfield value of 16. However, when resources are scarce, slow-growing phytoplankton that can synthesize additional resource acquisition machinery are favoured. This allocation of resources results in optimal N:P ratios ranging from 36 to 45, depending on which resource is limiting. Results of this model agree remarkably well with observed variations in N:P ratios reported for dozens of phytoplankton taxa growing under different environmental conditions[10]. The model is even able to explain the unusually high N:P ratio that has been measured for dinitrogen (N_2) fixers (>40). Rather than simply being the result of a ready-made N supply, it now appears that the high cellular N:P ratio of N_2 fixers is best explained by their need for large quantities of P-poor light-harvesting machinery required to drive N_2 fixation[11].

The key conclusion of the optimization model is that the canonical Redfield N:P ratio of 16 for phytoplankton is not a universally optimal value but instead represents an average for a diverse oceanic phytoplankton assemblage growing under a variety of different conditions and employing a range of growth strategies (Fig. 1). Consequently, the deep-sea NO_3:PO_4 ratio of ~16:1 simply reflects the stoichiometry of the current global phytoplankton community. This implies that, as environmental conditions change, the global mean phytoplankton nutrient stoichiometry could vary over time, potentially modifying current nutrient inventories. One change observed recently has been an increase in the flux of iron (Fe)-rich dust from an increasingly desertified Asian continent to the North Pacific ocean[12]. The response of the microbial community to this Fe enrichment has been enhanced particulate C:P and C:N ratios, reflecting increased export production and associated rates of remineralization[13]. Similarly, the N:P ratio of sinking particulate matter in the North Atlantic ocean has increased over the past 50 years, possibly because of increased N availability through atmospheric deposition of anthropogenically produced nitrous oxides[13].

In addition, if predictions by global climate models (GCMs) are correct and if surface waters in polar regions become more stratified[14], then the composition of phytoplankton species could be markedly altered[15]. Over time, a shift towards an increase in the abundance of diatoms, which prefer stratified waters and have a much lower N:P requirement[15] than the other major polar phytoplankton taxon, *Phaeocystis*[16], could deplete NO_3 relative to PO_4 and reduce the N:P ratio of subsurface waters, which are currently near the Redfield ratio[17]. NO_3 depletion may be further amplified by relatively high rates of P recycling in surface waters. Because waters formed in the Antarctic circulate globally[18], such long-term shifts in phytoplankton species composition and nutrient uptake characteristics could have implications for nutrient inventories in waters around the world.

Gaining a better understanding of microbial C:N:P stoichiometry is essential because of the predominant role these largely biologically mediated relationships play in coupled elemental cycles. These cycles modulate, and are themselves modulated by, processes operating at scales ranging from algal photosynthesis to the global climate. Unfortunately, although the amount of atmospheric carbon dioxide removed by the ocean in GCMs is very sensitive to the stoichiometric relationships between phytoplankton and nutrients, so far, few models account for its observed variability. From a more human perspective, stoichiometric relationships also determine how the marine environment responds to increasing anthropogenic inputs of limiting nutrients, for example, the increase in nitrogenous fertilizers[19]. Finally, as will be discussed below, the balance between nutrient inventories and the stoichiometric requirements of cells controls fundamental aspects of marine microbial ecology and biogeochemistry, such as where and how much N_2 fixation, denitrification and anammox take place and what nutrients limit phytoplankton growth (Fig. 2).

Multiple resource co-limitation

For many decades, Liebig's law of the minimum[20], which states that only a single resource limits plant growth at any given time, was a dominant theory shaping how oceanographers viewed phytoplankton ecology and its impact on nutrient cycles. During the past decade, this simple view has been replaced by the realization that in some parts of the world's ocean multiple resources simultaneously limit phytoplankton growth. Resource co-limitation is a phenomenon observed most commonly in oligotrophic oceans (Fig. 2), where nutrients are low but are nevertheless responsible for a large

fraction of global net primary production[21].

Although resource co-limitation has been recognized for only a short time, it has already been attributed to a variety of situations. Hence, its precise definition remains unclear. Resource co-limitation has been invoked when phytoplankton growth is stimulated either by the simultaneous addition of two or more different resources (both resource A and B are limiting) or by the addition of different individual resources (either resource A or B is limiting). Furthermore, resource co-limitation has been attributed to responses ranging from the cellular to the community level. Below I use examples gleaned from the literature to define three distinct categories of resource co-limitation that apply most often in the marine environment (Fig. 3).

The simplest case of resource co-limitation exists when two or more nutrients are reduced to levels too low for cellular uptake. This can happen, for example, when luxury uptake by phytoplankton preferentially depletes the more abundant nutrient or when N_2 fixers draw down PO_4 to growth-limiting levels when N is already limiting. In situations like these, referred to here as multi-nutrient co-limitation, adding all of the limiting nutrients is required for phytoplankton growth. Multi-nutrient co-limitation was observed in the nutrient-depleted waters of the Baltic Sea, where addition of both N and P was required to stimulate phytoplankton growth[22]. Similarly, Si and P were found to be co-limiting the growth of diatoms in the South China Sea near the Pearl River estuary[23]. More recently, the simultaneous addition of both P and Fe was required to stimulate growth of N_2-fixing cyanobacteria in the tropical North Atlantic[24].

A fundamentally different type of resource co-limitation arises when phytoplankton growth is stimulated by the addition of one of a number of different resources (the addition of either resource A or B increases phytoplankton growth). Here, although both resources may be limiting, only one is required to elicit a growth response. This can happen under one of two conditions (Fig. 3). In the first, referred to here as biochemical co-limitation, addition of one limiting resource may facilitate the uptake or assimilation of another (previously) limiting resource. In the second, called community co-limitation, one segment of the phytoplankton population may respond to an increase in one resource whereas another segment may respond to the increase in a different resource.

Figure 3 | A breakdown of the three types of resource co-limitation. In this simplest of examples, there are two resources (A and B) and two members of the phytoplankton community. Resource co-limitation has been invoked in cases when the addition of resources A and B are both required for phytoplankton growth (multi-nutrient co-limitation) and in cases when addition of either resource A or resource B stimulates phytoplankton growth. In the latter case, either the presence of resource A facilitates the assimilation of resource B (biochemical co-limitation) or one member of the phytoplankton community responds to resource A and the other member to resource B (community co-limitation).

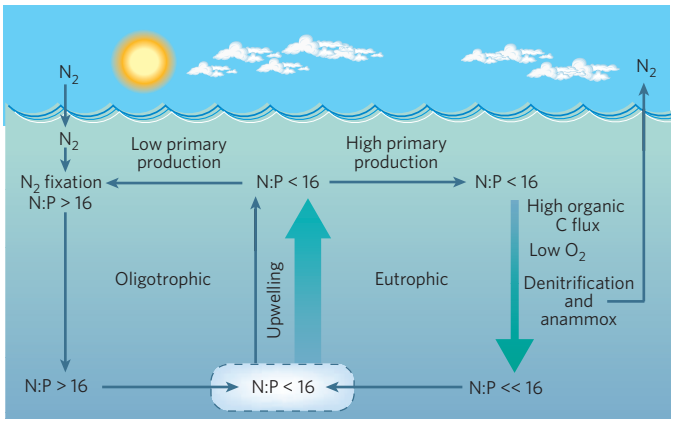
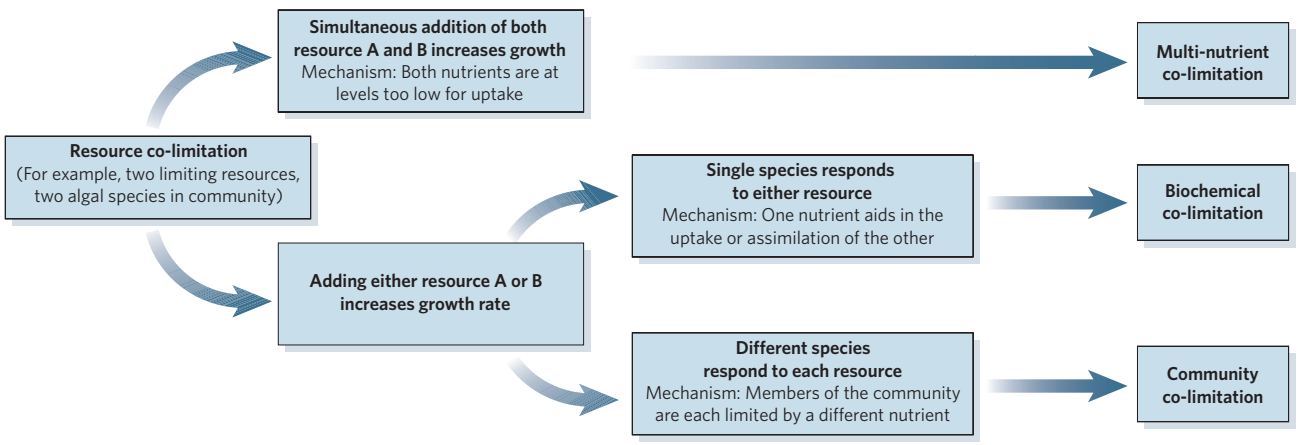


Figure 2 | The global ocean balance between N_2 fixation and the loss of fixed N through anammox and denitrification. Waters upwelling to the surface are generally slightly depleted in N relative to P (below Redfield). Where upwelling of N and P is substantial (eutrophic regions), primary production is high, resulting in the sinking of large amounts of organic matter. As this organic matter is broken down and its N and P are solubilized, a large fraction of the available O_2 is consumed. In these suboxic waters, anammox and denitrification converts NH_4 and NO_3 to N_2, resulting in a loss of biologically reactive N from the system and a marked decrease in the deep ocean N:P ratio. In contrast, where upwelling of N and P is low (oligotrophic regions), primary production is reduced and often dominated by N_2-fixing cyanobacteria, which are favoured in low-nutrient, N-depleted surface waters. N_2 fixation increases the N:P ratio of the organic matter to values above the Redfield ratio, which is eventually remineralized to produce waters enriched in N. However, global rates of N_2 fixation are not sufficient to balance the losses of fixed N to anammox and denitrification. Consequently, the contemporary ocean has a mean N:P ratio slightly less than the Redfield ratio.

In the case of biochemical co-limitation, the assimilation of resource A depends upon its abundance within the environment and the amount of cellular machinery available for its assimilation or transport into the cell. Resource B (often a trace metal cofactor) is integral to the functioning of the machinery required to assimilate or transport resource A. Hence, adding either resource increases phytoplankton growth. An important characteristic of biochemical co-limitation is that it operates entirely at the cellular level.

An early example of biochemical co-limitation involves the interaction between light and Fe. During culture[25] and field experiments[26], phytoplankton growth was enhanced either by increasing the light level or by adding Fe, an essential component of the additional photosynthetic units required for absorption of low light. Furthermore, the degree of biochemical co-limitation was shown to vary with cell size[27]. Small-celled *Chaetoceros* species in the Southern Ocean were less sus-



ceptible to light limitation at low Fe concentrations owing to their high surface area to volume ratio and, thus, their superior ability to take up Fe. Larger *Chaetoceros* species could not acquire sufficient Fe to grow under similarly low light and only bloomed when Fe and light were abundant.

A more recent example of biochemical co-limitation[28] involves PO_4 and the trace element zinc (Zn). In waters with low inorganic P, some phytoplankton can access the more abundant dissolved organic P (DOP) pool[29]. This is achieved through the activity of alkaline phosphatase (AP), an enzyme that cleaves the P-containing moiety from DOP so that it can be taken into the cell. However, AP requires a Zn cofactor. Consequently, the ability of phytoplankton to exploit DOP is compromised at reduced Zn concentrations. Experiments have shown that under such conditions, the rate of phytoplankton growth can be increased either through DOP enrichment, with the DOP being cleaved more rapidly at higher concentrations by AP, or by adding more Zn, which facilitates the production of more AP, thereby allowing the cells to more efficiently access the available DOP[28].

In the case of community co-limitation, adding one nutrient increases the growth of one segment of the population, whereas adding a different nutrient increases the growth of another. Thus, community co-limitation can occur even if individual members of the community are each limited by only a single nutrient. The best-known example of community co-limitation is found in Fe-replete oligotrophic waters where N_2-fixing cyanobacteria are abundant and N and P are scarce. Addition of NO_3 to these waters stimulates non-N_2 fixers in the population capable of assimilating DOP using AP[30]. Addition of P stimulates cyanobacterium such as *Trichodesmium* that support their high N requirement through N_2 fixation[31,32]. Community co-limitation can even involve assemblages where some species are multi-nutrient co-limited whereas others are limited by a single nutrient. For instance, in the tropical north Atlantic, addition of both P and Fe is required to stimulate N_2 fixers[24]. Addition of N alone stimulates non-N_2-fixing picoplankton, such as *Prochlorococcus* and *Synechococcus*, which may be more effective than the N_2 fixers at using the low P and Fe concentrations found in these waters.

It is becoming increasingly clear that the old notion of a single nutrient (or other resource such as light) limiting the growth of marine phytoplankton must give way to a more complex view that allows for limitation by multiple nutrients, both at the level of the individual cell and at the level of the entire community. Because nutrient inventories in seawater are profoundly influenced by the activities within the microbial community on both short and long timescales, it is essential that we understand how interactions between multiple resources can influence community composition and the nature of nutrient cycling between particulate and dissolved phases. This will prove particularly important for understanding and predicting how a non-steady-state ocean[13,31] responds to further anthropogenic influences that alter the nutrient stoichiometry of surface waters.

A new paradigm for nitrogen fixation

The balance between cellular nutrient stoichiometry and oceanic nutrient inventories often shapes phytoplankton community composition, with N_2-fixing phytoplankton being favoured in well-lit tropical and subtropical waters depleted of inorganic N[33]. N_2 fixation by these organisms fuels new production and helps determine the amount of C and N that can be consumed by other organisms or exported to depth. The importance of N_2 fixation for the marine N cycle has received considerable attention in the past decade, prompted in part by recent estimates of N demand in the euphotic zone that exceed NO_3 fluxes from depth[34]. This upward revision in N demand should be balanced primarily by N_2 fixation, although increased atmospheric N deposition or non-Redfield stoichiometry may also play a role[35]. However, early survey work suggested that marine N_2 fixers were relatively rare and N_2 fixation was of minor importance[36,37], amounting to only 10–20 Tg N yr^{-1}, far below the 90–130 Tg N yr^{-1} fixed in the terrestrial environment[38]. How then can this increased N-demand be satisfied if N_2 fixation is so low? The likely answer is that the abundance of the N_2 fixers such as *Trichodesmium*, the most common representative, has been severely underestimated[39] and that N_2 fixation is a more important component of the marine N cycle than previously realized.

Over timescales of hundreds to thousands of years[40,41], the amount of biological N_2 fixation approximately balances the slightly greater losses of fixed N due to microbial processes such as denitrification (Fig. 2). Although absolute rates of these two processes may be controlled through complex climate feedbacks involving changes in Fe availability[42], the steady-state oceanic balance between N_2 fixation and denitrification is maintained primarily through the N:P ratio of phytoplankton (Fig. 2). Simply put, if rates of denitrification were to increase because of elevated Fe input, waters welling up to the surface would be depleted in N relative to P, favouring the growth of N_2 fixers until P became depleted[43]. If rates of denitrification were to drop, N_2 fixation would cease once the N:P ratio of upwelled waters was again equal to the N:P requirements of the phytoplankton and N was no longer limiting.

N_2 fixation requires the Fe-rich nitrogenase enzyme complex[11,41], and so N_2 fixers were thought to have Fe requirements orders of magnitude greater than phytoplankton growing on ammonium (NH_4)[44]. Consequently, N_2 fixation was considered to be possible only in surface waters receiving large aeolian Fe input[32,45]. This notion was consistent with observed correlations between areas with high dust fluxes and *Trichodesmium* distributions[39]. However, newer evidence, gathered under trace-metal-clean conditions[46,47], suggests that Fe requirements of N_2 fixers are actually only about two to five times greater than for phytoplankton growing on NH_4 and only slightly higher than phytoplankton growing on NO_3. Accordingly, the notion that N_2 fixation is universally controlled by Fe availability is being replaced by a more complex view that allows for limitation by other nutrients such as PO_4 (ref. 46), or even co-limitation by P and Fe[24]. This is particularly true in areas such as the oligotrophic Pacific[31] and Atlantic[48] and the northern Red Sea[49], where aeolian dust deposition raises surface Fe concentrations to ~1 nM, levels generally non-limiting to N_2 fixation.

Although *Trichodesmium* is considered the dominant N_2 fixer in the ocean[50], other N_2 fixers also deserve consideration. Most notably, the diatom genera *Rhizosolenia* and *Hemiaulus* contain the endosymbiotic N_2-fixing cyanobacteria *Richelia intracellularis*[51], which is capable of extremely high N_2-fixation rates. During one study in the western tropical north Atlantic, nearly 70% of N demand in surface waters was met by N_2 fixation during an extensive bloom of these organisms[52]. In addition, it has recently been discovered that some species of unicellular cyanobacteria and proteobacteria also fix N_2 (ref. 53). However, neither the distributions nor the N_2-fixation rates of these organisms are well known[54], although molecular tools are being developed to better quantify the abundance of N_2 fixers in a phytoplankton population[55]. In some regions, their contribution to total N_2 fixation is low and in others, such as the oligotrophic North Pacific, it can exceed that of *Trichodesmium*[56].

Extrapolating from daily N_2-fixation rates to obtain global annual values has been difficult because of the large geographic area over which N_2 fixation is important and the relatively small number of direct measurements. Nevertheless, recent efforts are encouraging. Annual rates of N_2 fixation by *Trichodesmium* in the north Atlantic have been estimated to range between 22 and 34 Tg N yr^{-1} (ref. 34). These values are consistent with geochemical proxies suggesting that N_2 fixation adds an average of 28 Tg N yr^{-1} to the oceanic N inventory[4,34]. Applying this type of analysis to the global ocean yields annual N_2-fixation rates in the range of 100–200 Tg N yr^{-1} (refs 4,41). Although there are still large uncertainties in this value[57], it is clear that rates of oceanic N_2 fixation are much greater than the 10–20 Tg N yr^{-1} estimated previously[36,37] and that they are at least as high as those measured for the terrestrial environment[38]. In the subtropical and

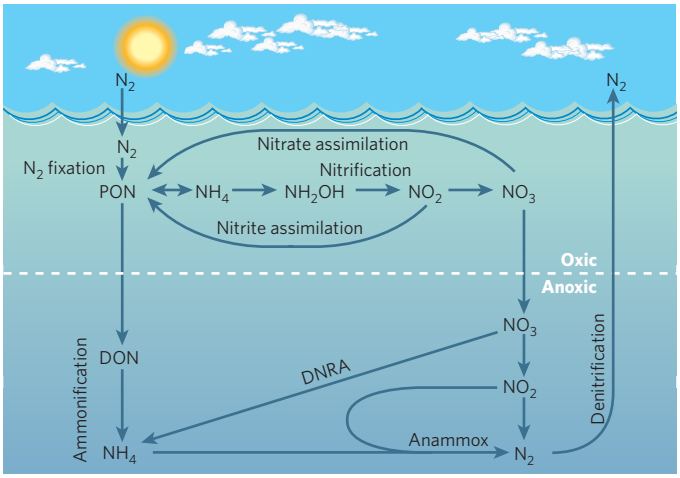


Figure 4 | Marine N cycle, including losses of ammonium and nitrite as N_2 owing to anammox. PON, particulate organic nitrogen, including phytoplankton; DON, dissolved organic nitrogen, DNRA, dissimilatory nitrate reductase to ammonium.

tropical Atlantic and Pacific oceans, N_2 fixation accounts for approximately 36–50% of the total N demand by the microbial community[52,58]. It has been estimated that N_2 fixation is equivalent to 50–180% of the flux of NO_3 into the euphotic zone[31,34], demonstrating that a large fraction of the new production in these waters is actually fuelled by N_2 fixation, rather than by movement of NO_3 into the euphotic zone.

As our understanding of the global importance of N_2 fixation grows, other aspects of this unique process are coming to light. For example, oceanic rates of N_2 fixation may not be at steady state and can vary in intensity with changes in climate[31,41]. These changes may include increased upper-ocean stratification, which could enhance N_2 fixation and shift organic matter export from being mostly particulate to mostly dissolved[31]. Consequently, changes in N_2 fixation can markedly alter the N inventory of the ocean and, hence, the stoichiometric balance between C, N and P available as nutrients. Yet despite its obvious importance, few large-scale biogeochemical models that include the tropics and subtropics include the process of N_2 fixation. More work in this area is necessary if we are to fully understand the future biogeochemical role of biologically fixed N_2 under the expected conditions of enhanced upper-ocean warming and stratification and, possibly, changes in dust fluxes.

Microbial N metabolism in low-oxygen environments

Denitrification is a microbial process thought to be responsible for the global loss of 175–450 Tg fixed N yr^{-1} within oxygen (O_2)-depleted environments[4,59]. It is a crucial component of the marine N (Fig. 4) and C cycles because it reduces the N content of waters upwelling to the surface (Fig. 2), favouring the growth of N_2 fixers in oligotrophic waters. Years ago, Richards[60] noticed that most of the NH_4 that should be produced during the anaerobic remineralization of organic matter was unaccounted for. He proposed that the missing NH_4 was anaerobically oxidized to N_2 by some unknown microbe using NO_3 as an oxidant. Because there was no known biological pathway for this transformation, biological anaerobic NH_4 oxidation received little further attention.

By the mid-1990s, work with bioreactors designed to remove NH_4 from wastewater provided direct evidence for anaerobic ammonium oxidation, and the process was termed 'anammox'[61]. In this reaction, NH_4 is oxidized to N_2 using nitrite (NO_2) as an oxidant[62], consistent with Richards' suggestion 30 years earlier. During anammox (Fig. 4), N_2 is formed through pairing of one N atom from both NO_2 and NH_4 (ref. 63), clearly distinguishing anammox from denitrification, which combines N from two NO_3 molecules to form N_2. A few years after the discovery of anammox, the microbes responsible for the reaction were identified as members of the bacterial order Planctomycetales[64]. This discovery reinvigorated efforts to determine whether anammox is an important process controlling N distributions in the marine environment.

Anammox activity in the ocean was first investigated in anoxic sediments using ^{15}N-labelled NH_4. The approach is based on the fact that because anammox combines N from NO_2 with NH_4 to form N_2, the anammox reaction can be recognized by the production of singly labelled N_2 gas ($^{15}N^{14}N$, with the ^{14}N coming from NO_2 and the ^{15}N coming from NH_4). Surprisingly, this study[63] found that anammox accounted for 24–67% of the total N_2 production in the continental shelf sediments that were studied. (Denitrification accounted for the rest.)

Soon after, some of the first evidence of the anammox reaction in suboxic waters (rather than sediments) came from the Gulfo Duce, Costa Rica[65]. Using the ^{15}N-labelling technique, researchers showed that the observed NH_4 deficiency in low-O_2 waters was due to coupling between denitrification and anammox, with denitrification providing the NO_2 required by the anammox reaction. During this study, anammox accounted for 19–35% of total N_2 production, and as much as 58% at some depths. Anammox in oxygen minimum zones (OMZ) was estimated to account for 10–15% of the loss of fixed N from the world's oceans. It was suggested that anammox may be even more important in regions such as the Peruvian and Chilean upwelling zones, where anoxic NO_3-rich waters contact sediments that produce significant amounts of NH_4.

Although anammox had been shown to be a potentially important process for removal of fixed N in O_2-deficient marine waters, it had yet to be determined whether the organisms responsible were the same planctomycetes identified in sediments. This breakthrough was first achieved during a study of the suboxic and anoxic zones of the Black Sea[3]. Again using the ^{15}N-labelling technique, direct evidence of anammox activity was found below the oxic zone. Moreover, ladderane lipids were found in association with the zone of high anammox activity. These marker lipids are unique to the bacterial anammoxosome, the cellular structure where anammox reactions take place[66]. This observation provided strong evidence that anammox bacteria, similar to those found living in bioreactors, were responsible for the anaerobic NH_4 oxidation observed in the Black Sea. Additional evidence was provided by the use of molecular probes that are specific for planctomycetes[67]. These probes detected anammox bacteria in suboxic waters of both the central basin and the shelf break. The activity of these anammox bacteria was sufficient to oxidize all the NH_4 diffusing up into the suboxic zone and to consume $>40\%$ of the fixed N sinking into anoxic waters of the Black Sea.

More recent studies have reported anammox activity in marine ecosystems as diverse as polar sea ice[68] and the OMZ of the Benguela upwelling system[69]. Bottom waters in the Benguelan OMZ become suboxic because of O_2 decomposition of sinking organic matter after the highly productive upwelling season[70]. The low levels of fixed N relative to PO_4 in these suboxic waters have generally been attributed to denitrification[71]. However, the low NO_3 and NH_4 in waters with reduced O_2 suggest that anammox may play a role. This role was recently confirmed by ^{15}N-labelling, which detected significant anammox activity[69]. Additional analyses demonstrating the presence of both ladderane lipids and the 16S ribosomal RNA of a form of planctomycetes that is closely related to known anammox bacteria, verify that the loss of fixed N was due to anammox bacteria[69].

Among the most startling findings of the Benguela ONZ study came from the ^{15}N labelling experiments that showed that very little N_2 was produced by denitrifiers. The role of the denitrifiers in these waters seemed to be limited to reducing NO_3 to NO_2, with anammox bacteria completing the process by converting NO_2 to N_2 during the oxidation of NH_4. The authors of that study[69] boldly point out that they are aware of no direct evidence from ^{15}N labelling experiments that denitrifiers in OMZs anywhere in the world can convert NO_3 to N_2. One possible reason is that nobody has bothered to look, given that,

until very recently, denitrification was the only known pathway capable of converting NO_3 to N_2. However, if their suggestion is true, then anammox, perhaps coupled to denitrification, may be the dominant process in the loss of 80–150 Tg N yr^{-1} from the world's OMZs[4,59].

Studies of anammox are still in their infancy. What is becoming clear, however, is that this process is turning up in an ever-increasing number of low-O_2 marine environments. If it is prevalent in the Black Sea and the Benguela upwelling system, might it also be important in other areas favourable for denitrification, such as the west coasts of India and Central and South America[72]? What about the recently discovered 'dead zones' near the coast of the northwest United States[73] or the anoxic waters of the Gulf of Mexico? Current estimates suggest that globally anammox may be responsible for 30–50% of N_2 production in the ocean[72] and, if Kuypers *et al.*[69] are correct, much more. One important issue that needs to be addressed is the extent to which anammox represents an as-yet unquantified loss of fixed N. Will anammox alter existing N budgets because it consumes NH_4 rather than NO_3 or is it already accounted for in estimates of denitrification? It will be interesting to watch as the anammox story continues to unfold.

Future directions

The past decade has seen profound shifts in some of the fundamental theories of biological oceanography, particularly as they relate to microbe–nutrient interactions. The simple concepts of a uniform Redfield stoichiometry for phytoplankton, single resources universally limiting phytoplankton growth, low levels of marine N_2 fixation and the pre-eminence of denitrification in the loss of fixed N are all being replaced by more complex conceptual models. It is also becoming increasingly clear that these seemingly disparate biological/biogeochemical concepts are really linked through the activity and elemental composition of marine microbes (Fig. 2). The C:N:P stoichiometry of phytoplankton ultimately controls the nutrient ratios of the deep ocean, which are subsequently modified by microbial reactions such as anammox and denitrification. The abundance and elemental ratio of nutrients returning to the surface determine the activity and composition of the phytoplankton, favouring rapid-growing taxa where nutrients are in abundance and N_2 fixers in nutrient-poor waters with a low N:P ratio (Fig. 4). These interactions produce a self-regulating biogeochemical system that maintains quasi-stable oceanic nutrient inventories over both short and long timescales.

Although our understanding of the roles of microbes in global nutrient cycles has increased markedly, the recent discovery of a whole new N biochemistry through anammox aptly illustrates that there is still much to be done. For instance, global biogeochemistry models that currently assume constant Redfield ratios may have to be reformulated to take into account a time-varying, taxon-specific non-Redfield stoichiometry. N budgets will need to be adjusted as we re-evaluate the balance between N_2 fixation and fixed N losses, as well as the relative magnitudes of denitrification and anammox.

Future work will almost certainly be driven by the development of improved tools for more comprehensive observation and quantification of microbial processes. Among other things, these new tools will facilitate an expansion of the *in situ* genomic work that is currently underway (see the review in this issue by DeLong and Karl, page 336) and that has allowed us to identify microbial genes governing a wide array of functions within a large number of marine habitats. For example, the widespread incidence of the *nrfA* genes that code for NO_2 reductase suggest that this pathway may be another important, yet largely unaccounted for, component of the N cycle[74]. New tools also will improve characterization of marine microbial communities using satellite remote sensing technology. Currently, we use satellites to estimate phytoplankton biomass and productivity, and in some cases, distributions of taxa such as *Trichodesmium* and coccolithophores. As both sensor performance and our bio-optical characterization of different phytoplankton taxa improves, so will our ability to monitor changes in phytoplankton biomass and community structure. Finally, new modelling tools will be able to incorporate our increasing understanding of the role of marine microbes in global nutrient cycling. Because many biological and biogeochemical processes involve complex interactions and multiple feedbacks, predicting their response to environmental perturbations is difficult, but essential, if we are to accurately characterize our ever-changing world. ■

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Acknowledgements I would like to thank S. Rysgaard, T. Dalsgaard, and M. Kuypers for assistance with the anammox section of this manuscript. D. Capone, E. Carpenter, and A. Subramaniam provided valuable insight about N_2 fixation. I would like to thank M. Davey and G. Tarran for providing their unpublished flow cytometry data from their North Atlantic work. Thanks also to M. Mills, G. van Dijken, A. Tagliabue, R. Labiosa, T. Reddy, S. Pabi, L. Kropuenske and B. Saenz for comments on earlier drafts of this manuscript.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to K.R.A. (arrigo@stanford.edu).

CORRIGENDUM

doi:10.1038/nature04289

Eocene bipolar glaciation associated with global carbon cycle changes

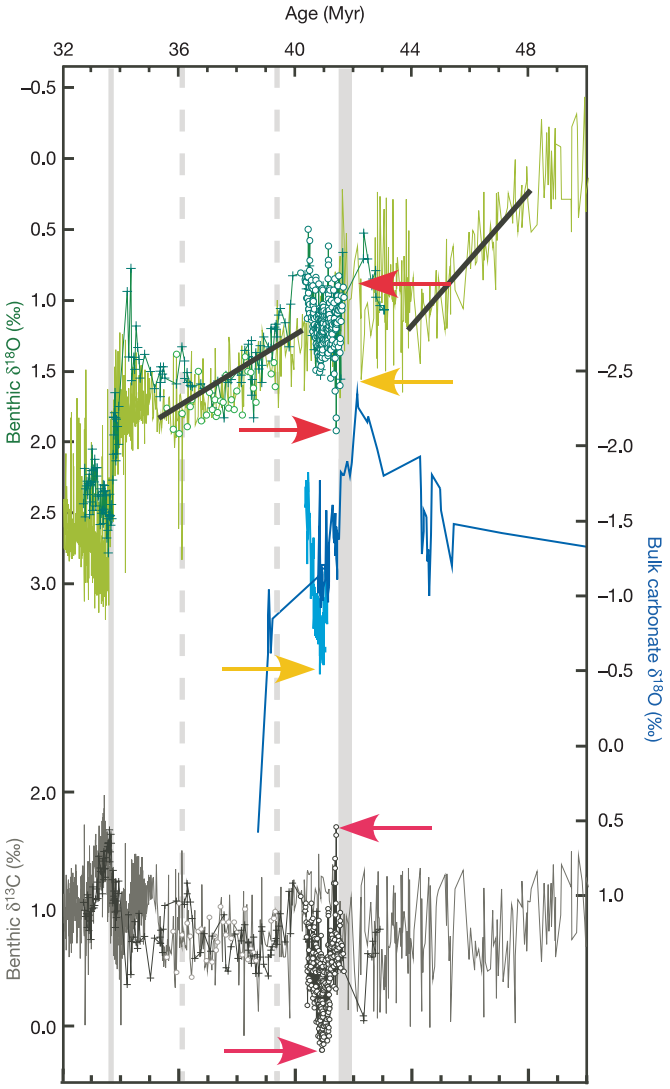
Aradhna Tripati, Jan Backman, Henry Elderfield & Patrizia Ferretti

Nature 436, 341–346 (2005)

We wish to clarify that mass accumulation rates in this Article were calculated using dry bulk densities from Michael Vanden Berg (personal communication) and that the method used for calculating the calcite compensation depth (CCD) in Fig. 1a (linear extrapolation of the CCD) is detailed in a forthcoming publication[1].

Also, the dark green and dark grey lines in Fig. 2 of the Article should have contained symbols to distinguish between data from different laboratories for site 1218, and a revised version of Fig. 2 is accordingly shown here. Benthic foraminiferal data from our study are now indicated by open circles (dark colours, site 1218; light colours, site 1209) and published data[2] for site 1218 are represented as crosses.

Our conclusions remain unchanged.



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ERRATUM

doi:10.1038/nature04265

Marine microorganisms and global nutrient cycles

Kevin R. Arrigo

Nature 437, 349–455 (2005)

In this Review, the digital object identifier (DOI) number was incorrectly given as doi:10.1038/nature04158. The correct DOI number for this Review is doi:10.1038/nature04159.